

CHAPTER III

MODIFICATION OF DISPOSABLE SCREEN-PRINTED CARBON ELECTRODE SURFACES WITH CONDUCTIVE ELECTROSPUN NANOFIBER FOR BIOSENSE APPLICATIONS

3.1 Abstract

Amperometric disposal electrodes have been used as an enzyme-based biosensor to detect hydrogen peroxide (H_2O_2), which is not only an important analyte in clinical, food, pharmaceutical and environmental analysis but also a key product of the catalytic reaction of various oxidase enzymes. In this work, we describe an alternative approach to the surface modification of screen-printed carbon electrodes (SPCEs) to fabricate a polypyrrole/polyacrylonitrile multiwall carbon nanotube-modified screen-printed carbon electrode (PPy/PAN-MWCNT/SPCE) using a two-step process. First, polyacrylonitrile (PAN) loaded with 5 wt.% of multiwall carbon nanotubes (MWCNTs) was electrospun onto a carbon layer. The CNT-embedded PAN electrospun nanofibers (diameter ~ 200 nm) were subsequently coated with a polypyrrole layer via vapor-phase polymerization using *p*-toluenesulfonate (FeTos) as an oxidizing agent in a vacuum system. The electrochemical behavior of both the unmodified and the modified SPCEs were compared using cyclic voltammetry (CV) with common electroactive analytes, such as ferri/ferrocyanide ($\text{Fe}(\text{CN})_6^{3-/4-}$) redox couples and hydrogen peroxide (H_2O_2), to optimize the electrospinning and the vapor-phase parameters. The voltammograms of the $\text{Fe}(\text{CN})_6^{3-/4-}$ and H_2O_2 solutions demonstrated that the modified electrodes produced a decreased separation between the peak potential, ΔE_p , of +1.4 V compared with that of the unmodified electrode (BARE). The amperometric study indicated that steady-state currents were obtained with the modified electrode at a fixed potential of +1.2 V for a variety of H_2O_2 concentrations, with a linear relationship in the concentration range of 0.125 to 10 mM and with a limit of detection (LOD) of 1.25 μM . By incorporating glucose oxidase (GOX) as an enzymatic model into the modified electrode, we conducted a calibration study that showed that glucose could be detected by amperometry over a linear range of 0.25-6 mM with a LOD of 15.51 μM and a sensitivity of 7.09

mA/Mcm^2 . This study introduces a new SCPE with a conductive nanostructure for monitoring H_2O_2 produced from the catalytic reaction of oxidase enzymes without the use of a mediator. Therefore, the novel modified electrode is a promising new device for biosensor applications.

Keywords: Biosensor, Hydrogen peroxide detection, Electrospun electrode, Vapor-phase polymerization, Conducting polymer

3.2 Introduction

In recent years, the vast number of reports on screen-printing carbon electrode (SPCE) technology have been used to develop biosensors that detect biological molecules in applications, such as environmental (Soo, 2010), biomedical (Rawson, 2009), microbiological (Thanyani, 2008), healthcare (Zheng, 2013), and chemical/biochemical analyses (Yang, 2010). Because an SPCE is inexpensive and can be used as a disposable electrode with large-scale production capability, a number of methods have been devised to increase the surface area of SPCEs to enhance their sensitivity for electrochemical detection. For example, this enhanced sensitivity can be achieved by coating an SPCE with a conductive polymer, such as polypyrrole (PPy) (Oliveira, 2012, Wu, 2011), polyaniline (PANI) (Chang, 2007), or poly(3,4-ethylenedioxythiophene):poly(styrene-sulfonate) acid (PEDOT:PSS) (Donavan, 2011), via an electropolymerization processes using a potentiodynamic, potentiostatic, or galvanostatic mode. These processes can be used in combination with chemical/biological components that are embedded in the SPCE structures. In amperometric biosensors, enzyme electrodes that contain oxidase enzymes can catalyze substrates, such as glucose (Ahmad, 2010), uric acid (Kiran, 2012) or lactate (Rawson, 2009), by reducing oxygen to form hydrogen peroxide (H_2O_2), and the oxidation current can be evaluated (Dungchai, 2009).

Among the different techniques that are used to obtain nanostructured surfaces, electrospinning techniques produce nanofibers with a large surface area that is one to two orders of magnitude larger than those found in continuous films; electrospun fibers also exhibit a high porosity and good features for the incorporation

of active composites, such as metal nanoparticles (Liu, 2008), graphene (Karuwan, 2012), or carbon nanotubes (CNTs) (Ju, 2008, Rujitanaroj, 2008), to improve the electron-transfer activities. CNTs are extensively used in biosensors because of their unique tubular structure with a nanoscale diameter, high conductivity, and outstanding mechanical properties (Choi, 2010).

Vapor-phase polymerization (VPP) has been used to synthesize and to optimize intrinsically conducting polymers (ICPs). The substrates were coated with oxidizing agents, such as FeCl_3 (Jang, 2009), Fe(III) *p*-toluenesulfonate (FeTos) (Laforge, 2010), benzenesulfonic acid, *p*-dodecylbenzenesulfonic acid (DBSA), and *p*-ethylbenzenesulfonic acid (Subramanian, 2008), using simple coating methods. Then, the oxidant-coated substrates were exposed to ICP monomers. When the monomer vapors evaporated from the oxidant-coated locations, they were rapidly polymerized to form a conductive thin film on the substrate surface. The VPP method has been reported by numerous scientists to produce excellent electronic conductivity properties on various material surfaces. In the present work, VPP was used to coat a PPy layer onto electrospun nanofibers.

The aim of this study is to modify an SCPE surface through the preparation of a BARE–CNT–PPy electrode with a PPy coating on CNT-embedded PAN electrospun nanofibers, which are a key factor in the enhancement of the electrode electrochemical performance. First, the redox behaviors of the unmodified/modified electrode were studied in a ferri/ferrocyanide solution to optimize the electrode surface modification. Second, the H_2O_2 detection of the modified electrode and any interference were studied. Glucose detection was also studied; glucose oxidase was used as a representative enzyme without the addition of a mediator. The performance of BARE–CNT–PPy electrodes with respect to their sensitivity, detection limits, and calibration curves are described and discussed.

3.3 Experimental

3.3.1 Screen-printed Electrode Fabrications

SPCEs were fabricated on plastic sheet made from PVC. Firstly, the silver ink was twice spread over on the PVC surface by a spin-coater. Next, the

carbon layer was also coated 2 times on the top of silver layer; for each spreading, it is baked in a hot oven at 60 °C for 60 minutes to evaporate the solvent. Further surface modifications on SPCE electrode surfaces were carried out by electrospinning and vapor-phase polymerization techniques as shown in **Fig. 3.1** Unmodified SPCE was labeled as BARE.

3.3.2 Electrospinning Process

The SPCE surfaces were modified with conductive PAN-based nanofibers by electrospinning labeled as SPCE using a 10 wt.% PAN solution in DMF which 2.5 wt.% MWCNT was dispersed to PAN solution and sonicated for 30 minutes with a homogenizer. In this research, the amount of MWCNT was fixed at 2.5 wt.%. **Fig 3.1** shows the experiment apparatus used for the electrospinning process. A 20-ml syringe with capillary tip ($D=0.5$ mm) was placed and clamped with anode of high voltage power supply. The cathode was connected to a silver layer with 15 kV applied voltage. The distance between electrode and nozzle was 15 cm with various electrospinning times (1-30 mins) at room temperature (25 ± 1 °C).

3.3.3 Vapor-phase Polymerization of PPy Layer

After electrospinning, vapor-phase polymerization of PPy layer was carried out in a vacuum chamber setup as shown in **Fig 3.1** The PAN-MWCNT/SPCE electrodes to be covered with PPy layer are initially coated with FeTos at various concentrations (20-60 %w/v) in n-butanol and pyridine. Then, the oxidant-coated electrodes were heated on hot plate at 60 °C for 3 mins until the solvent evaporated. The electrodes were then exposed to pyrrole vapor in a sealed vacuum chamber. After polymerization, the electrodes were heated at 60 °C for 1 hour to ensure complete evaporation of the pyrrole monomer. They were washed with absolute ethanol for 5 mins followed by rinsing with deionized water for 5 mins. Finally, they were dried under vacuum at room temperature for 2 hours. The completely PPy coated electrodes were labeled as PPy/PAN-MWCNT/SPCE.

3.3.4 Electrochemical Study of PPy/PAN-MWCNT/SPCE in Ferri/Ferro Solution

Electrochemical behavior of modified/unmodified electrodes was studied by cyclic voltammetry (CV). Cyclic voltammograms were obtained by scanning rate from 10 to 500 mV/s using 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ or $(\text{Fe}^{2+/3+})$ in 0.1 M PBS of pH 7.4. For the performance of electrodes for anodic current response, the scanning rate was fixed at 50 mV/s in 0.1 M PBS of pH 7.4. The anodic current values from CVs of studied conditions were obtained from amperometric current subtracted by background current.

3.3.5 Cyclic voltammetric measurement of hydrogen peroxide at electrodes

Cyclic voltammograms of electrodes were evaluated by scanning from 0 to +1.6 V at a scan rate of 50 mV/s using a 2 ml aliquot of 10 mM H_2O_2 in 0.1 M PBS of pH 7.4. The CVs of background of each electrode were illustrated in **Fig 3.10(a)**.

3.3.6 Amperometric Response for Hydrogen Peroxide Using Modified Electrodes

The potential response to anodic current peak of SPCE and PPy/PAN-MWCNT/SPCE is +1.4 and +1.2 V with respect to Ag/AgCl electrode to detect oxidation current of H_2O_2 . These potential responses were used to obtain anodic current versus time plots as shown in **Fig 3.10(b)**. Initially, the background current was measured until the current became constant. Then, the concentration series of H_2O_2 were added to the solution which immediately resulted in the increase of the anodic current and it reaches a steady state when approaching the 50 second mark. Finally, the differences between these anodic current values were observed. The effect of pH, concentrations of supporting electrolyte and calibration for H_2O_2 detection were also studied.

3.3.7 Enzyme Electrode Preparation

The dropping method was selected for the enzyme electrode preparation. The procedures were as follows; the various concentrations of GOx

solutions (0.1-1.0 g/ml) were prepared in 0.1 M PBS, pH 7.4 in a vial. Afterwards, 20 μ l of prepared enzyme solutions were dropped onto PPy/PAN-MWCNT/SPCE electrode surface followed by 1 hour of air drying. The sample was kept at 4 °C before use.

3.3.8 Calibration Study Using Amperometry with Modified Electrode for Glucose Detection

Calibration studies were carried out through PPy/PAN-MWCNT/SPCE electrode with 0.1 g/ml of glucose oxidase (GOx) as the enzyme model. The glucose used as substrate was prepared in various concentrations of 0.125 – 20 mM in 0.1 M PBS of pH 7.4. In amperometric measurements, the potential was fixed at +1.2 V which was a characteristic response of PPy/PAN-MWCNT/SPCE to H₂O₂. The times were monitored in a range of 0-50 secs. The glucose additions were made without stirring the solution in the cell. Limit of detection (LOD); (3S/N) was estimated PPy/PAN-MWCNT/SPCE.

3.3.9 Surface Morphology Studies

Morphologies of both modified and unmodified electrodes were observed by Hitachi S-4800 Field Emission electron microscope (FE-SEM) at 10 kV and SemAphore 4.0 software. Each electrode was coated with a thin layer of platinum using platinum sputtering device prior to SEM observation.

3.3.10 Incorporation with Mediator

In this work, various concentrations (1-100 mM) of Fe(CN)₆³⁻ were used as mediator in 0.1 g/ml of GOX system. The linear range and sensitivity were evaluated as the same approach of the calibration in condition without mediator in **section 3.3.8**.

3.4 Results and Discussion

3.4.1 Electrospinning Process for PAN-MWCNT Nanofiber

The most common method for preparing electrospun loaded MWCNT has been to mix both components into solution. The low portions of MWCNT were dispersed into PAN solution using DMF as the solvent. In this study, we set our experiment conditions followed by P. Kampalanon (Kampalanonwat *et al.*, 2010) that they fabricated PAN metal removable nanofiber by using conditions (15 kV and PAN 10% w/v in DMF). In this work, various concentrations of MWCNT (0-7.5 w/v) were dissolved in PAN solution and electrospun onto SPCE surface as shown in **Fig 3.1**. The SEM images illustrated that at the high concentration of MWCNT loading resulting to the increase of PAN-MWCNT fiber diameter increase from 198 to 622 nm as shown in **Fig 3.2**. Because, MWCNT portions can enhance the conductivity of polymer solution. However, we cannot carry out at high concentration (7.5% w/v) of MWCNT due to the high viscosity and agglomeration of MWCNT particles. In order to obtain the smooth nanofiber, the concentrations of 2.5 %w/v of MWCNT was chosen to modify electrode for this work that The average conductive fiber diameter of PAN-MWCNT/SPCE was approximately 308±48 nm with a large surface area(Ju, 2008).

3.4.2 The Effect of Electrospinning Time on Current Response of Modified SPCEs

In the electrospinning process, the PAN solution containing 2.5% w/v of MWCNT was applied with positive charges to produce a large amount of nanofibers which were coated on the black SPCE surface. The white layer appeared immediately on the electrode surface within the first electrospinning time and the nanofibers were continuously coated until 60 minutes as shown in **Fig 3.3**.

To study of current response behavior of electrodes, the redox couple solution (5 mM $\text{Fe}^{2+}/\text{Fe}^{3+}$ in PBS) were used for observation of electrode characteristic. The black bar in **Fig 3.4**, the results indicated that the anodic current increased with the electrospinning time until to 5 minutes. Afterwards, the current response exhibited to decrease. Because, too thick CP layer can cause the charge barrier between redox

species and electrode surface (Shiu *et al.*, 1999). This phenomenon was also observed by Jang *et al.* Therefore, the best condition for electrospinning of PAN-MWCNT nanofiber with an excellent oxidation current on SPCE surface was 5 minutes which was used for further study. This electrode was labeled as PAN-MWCNT/SPCE.

3.4.3 Vapor-phase Polymerization of Polypyrrole Coated on Modified SPCEs

For the final modification step, the conductive electrospun layer coat was coated by PPy via vapor-phase polymerization in a vacuum chamber. Pyrrole monomer evaporated rapidly and then polymerized to be polypyrrole on the fiber surface resulting in a drastic increase in fiber diameter in the range of 420-590 nm within the first time exposure at 1 min, color change from uncoated white fiber to black. **Fig. 3.5(a)** shows the thickness of the PPy film covering the electrospun fiber which increased in proportion to the polymerization time. However, the thickness and morphology of the coated fiber depends on the oxidizing agent's concentration. By increasing the oxidizing agent's concentration, the PPy film formation was also increased. Moreover, in high FeTos concentration (60%w/v), the viscosity of coating was also high and PPy film formation polymerized as a thick film; covering electrode causing film-like polymer coating on electrode surface (Laforgue *et al.*, 2010) as shown in **Fig 3.6**. Therefore, from the results in **Fig 3.5(b)** displayed that the best condition to modify electrode by vapor-phase polymerization to obtain the highest anodic current response (230 μA) was the use of FeTos 40% w/v and 5 minutes of exposure time. Eventually, the three kinds of modified electrode; SPCE, PAN-MWCNT/SPCE and PPy/PAN-MWCNT/SPCE were study for use as biosensor application. The SEM images in **Fig 3.7** show the different of each electrode surface morphology.

The FTIR spectra of PAN-MWCNT/SPCE and PPy/PAN-MWCNT/SPCE are shown in **Fig 3.8**. The spectrum of the uncoated PPy SPCE exhibited adsorption peaks at 2242 cm^{-1} , corresponding to the stretching vibrations of the nitrile group of the polyacrylonitrile (Kampalanonwat, 2010). The spectra of coated PPy SPCE showed new adsorption bands at 3346 and 1650 cm^{-1} . These can be assigned to the stretching vibration of secondary amine and amidine group of polypyrrole molecules. After the electrode achieved vapor-phase polymerization, the

nanofiber were completely coated with PPy layer. Therefore the band at 2242 cm^{-1} (nitrile group) of PPy/PAN-MWCNT/SPCE was disappeared. From the results can confirm the completed vapor-phase polymerization of conducting polymer on the PAN-MWCNT and the electrochemical behavior were also studied in next part.

3.4.4 Electrochemical Behavior of Modified SPCEs

The electrochemical behavior of the fabricated electrodes was studied by cyclic voltammetry using $5\text{ mM Fe}^{2+/3+}$ as a reversible redox couple model (Njagi *et al.*, 2007). The redox peaks (oxidation/reduction) and voltage windows were recorded in range -0.6 to $+1.0\text{ V}$. Cathodic and anodic current responses of each electrode was observed; SPCE ($+0.55\text{ V}/-0.2\text{ V}$), PAN-MWCNT/SPCE ($+0.4\text{ V}/-0.1\text{ V}$), and PPy/PAN-MWCNT/SPCE ($+0.3\text{ V}/+0.15\text{ V}$). The observation was quite interesting. The electrochemical efficacy of electrodes were evaluated from CV responses and compared to electrode modified and unmodified surface. **Fig. 3.9 (a)** illustrated that the CV of each electrode exhibited reversible redox peaks of $\text{Fe}^{2+/3+}$ with an increased oxidation/reduction current (233 ± 6 and $231\pm 2\text{ }\mu\text{A}$ for PPy/PAN-MWCNT/SPCE as compared to 70.9 ± 3 and $69.3\pm 2\text{ }\mu\text{A}$ for PAN-MWCNT/SPCE and 25.1 ± 1 and $23.2\pm 2\text{ }\mu\text{A}$ for SPCE). Moreover, the presence of conductive nano-fiber structure and the PPy layer on electrode surfaces exhibited a decrease in separation between E_{pa} and E_{pc} ; ΔE_p , and the increase of electrochemical sensitivity (Palomera *et al.*, 2011).

Through electrospinning process of conductive nanofiber, the electron transfer on electrode surface can be improved. From the CV, SPCE covered with conductive nanofiber coated with PPy layer (PPy/PAN-MWCNT/SPCE) showed the highest of oxidation peak amplitudes which are approximately 4.5 and 2.5 times higher than SPCE and PPy/PAN-MWCNT/SPCE, respectively. The effect of conductive nanofiber coating on BARE surface and vapor-phase polymerization of PPy were also studied in terms of the anodic current for each condition. **Fig. 3.4** shows the anodic current responses of PAN-MWCNT/SPCE before (black bar) and after (grey bar) coating with PPy layer. This is evident at the 5-min mark of coating time, which is the best condition to fabricate PPy/PAN-MWCNT/SPCE, as longer coating time can create a current barrier on the electrode surface (Lakard *et al.*, 2007).

Nevertheless, only PAN electrospun nanofiber without loading MWCNT covered on SPCE surface exhibited drastic increase in ΔE_p ; +0.6 V for oxidation (E_{pa}) and -0.3 V for reduction (E_{pc}). Enhancing the anodic current by coating with PPy layer on nanofiber exhibited the same trend. In the case of vapor-phase polymerization, **Fig. 3.5(b)** illustrated that using high concentrations of oxidizing agent to obtain high thickness of PPy layer is not the suitable method in obtaining excellent electrochemical activity of electrode surface (Atta *et al.*, 2007, Jang *et al.*, 2007). In this experiment, the best condition to obtain the highest anodic current is using the 40 (%w/v) FeTos and 5 mins of polymerization time. Therefore, those conditions were used to fabricate PPy/PAN-MWCNT/SPCE.

In addition, the effect of CV scan rate on the performance of electrodes was also studied and the results are shown in **Fig. 3.9(b)** shows characteristic of CV spectra of PPy/PAN-MWCNT/SPCE electrode in 0.1 M PBS of pH 7.4 at different scan rates. With the increase in scan rate from 10 to 500 mVs^{-1} , the oxidation peak potential gradually shifts to the positive potential. The oxidation peak current, I_p , and reduction peak, I_c , are governed by Randle-Sevcik relationship as shown in Eq(9)

$$I_p = kn^{3/2}AD^{1/2}C^b\nu^{1/2} \quad \text{Eq (9)}$$

Where the constant $k = 2.72 \times 10^5$; n is the number of moles electrons transferred per mole of electroactive species (ferricyanide); A is the area electrode in cm^2 ; D is the diffusion coefficient in cm^2/s ; C^b is the solution concentration in mole/L; and ν is the scan rate of potential in volt/s. The I_p is linearly proportional to the bulk concentration, C_b , of the electroactive species and the square root of the scan rate, $\nu^{1/2}$. Therefore, an interesting diagnostic is a plot of I_p vs. $\nu^{1/2}$ as shown in **Fig. 3.9(b)**; both the oxidation peak current (I_a) and reduction peak current (I_c) of PPy/PAN-MWCNT/SPCE electrode exhibited linear responses. It is reasonably safe to imply that the electrode reaction is controlled by radial diffusion process (Rawson, 2009), which is the mass transport rate of the electroactive species to the surface of electrode (Frasconi *et al.*, 2009, Kadara *et al.*, 2009). Moreover, incorporation of conductive nanofiber and coated PPy layer on electrode surface makes the PPy/PAN-MWCNT/SPCE system quasi-reversible ($I_c/I_a \neq 1$) (Palomera, 2011).

3.4.5 The Study of Modified SPCEs on Hydrogen Peroxide (H₂O₂) Detection

The CV study was evaluated to establish whether H₂O₂ could be measured using the proposed electrode as shown in **Fig. 3.10(a)**. SPCE electrode exhibits an electrolytic oxidation signal of 10 mM H₂O₂ at +1.4 V with current response at approximately 110 μ A. No oxidation peak was observed in a blank PBS buffer under identical experiment conditions. It is interesting to note that PPy/PAN-MWCNT/SPCE electrode exhibits a higher anodic current response (420 μ A) at a lower potential (+1.2 V). This is due to more efficient mass transport and electron transfer properties of the PPy/PAN-MWCNT/SPCE electrode; conductive nanofiber covered on electrode surface can improve the oxidation reaction area between electrode surface and H₂O₂ (Ju, 2008, Ren *et al.*, 2006). Similarly, enhancing PPy layer on conductive nanofiber promotes electron transfer. Such high current values have been previously observed when screen-printed electrode incorporated with PPy layer was used.

The anodic current of PPy/PAN-MWCNT/SPCE electrode for H₂O₂ was to determine the basis for purposed H₂O₂ biosensor and applied potential of +1.2 V. **Fig. 3.10(b)** illustrates the drastically different current response between SPCE and PAN-MWCNT/SPCE fixed at +1.4 V and PPy/PAN-MWCNT/SPCE fixed at +1.2 V, which is the lowest potential to detect H₂O₂. However, during the amperometric measurement, it is observed that bubbles covered the electrode surface when the potential applied was more than +1.4 V. In addition, using too high poised potential can increase the background current and possible direct oxidations of electroactive species at the underlying electrode (Gao *et al.*, 2005).

The intensity of the signal was shown to be proportional to the concentration of H₂O₂ over the range studied as illustrated in the inset in **Fig. 3.11**, which was clear that the oxidation peak current varied linearly with concentration in the range from 0.125 to 10 mM. The regression equation is given by $y=2.4089x + 48.029$ ($r^2=0.997$), where y and x are the magnitude of peak current (μ A) and H₂O₂ concentration (mM), respectively. The slope of the equation corresponds to a linear sensitivity of 4.77 mA/ M cm^2 and LOD of 1.25 μ M

3.4.6 The Effect of pH and Buffer Strength on the Anodic Current

Response on Hydrogen Peroxide Detection

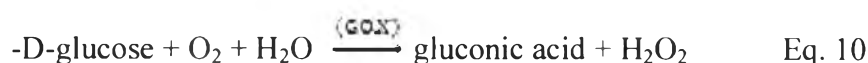
The effect of pH on anodic current response of 10 mM H₂O₂ detection at various system conditions was also studied **Fig 3.12** obviously shows the maximum current response at pH 8. But at pH 9, the experiment could not be conducted due to bubble formation occurring on the electrode surface (over oxidation). The low current response in pH 3 may cause from the low ionic strength in PBS system. For this application, H₂O₂ detection in buffer solution commonly used in biological research is the focus. Therefore, we have measured pH 7.4 for further studies.

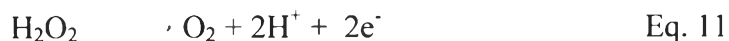
The salt ion in PBS solution in **Fig 3.13**, balances the amount of salt ions inside the cell. The osmolality and ion concentrations of PBS solution match of human body fluid. The effect of salt concentration, according to its anodic current for detection of 10 mM H₂O₂, we found that there is not much change in anodic current response. It can be said that the range of KCl concentration for H₂O₂ detection should be 0.1-1 M KCl. Normally, 0.1 M KCl has been used for blood simulation in medical research. Therefore, we used this value for our further studies.

The effect of supporting electrolyte concentration on the anodic current response **Fig 3.14**, the graph illustrated that the anodic current declined when the supporting electrolyte concentrations were decreased. This will definitely make it clear that the overall ionic strength in system was disturbed. So, we can inferred that the ionic strength of solution was proportional to the anodic current response.

3.4.7 Incorporation with Glucose Oxidase for Glucose Detection

In order to study the performance of H₂O₂ biosensor which can be used to detect many oxidase-substrate compounds from the catalytic reaction of various enzymes, in this work, GOx was chosen as a model enzyme to detect glucose without using a mediator. The amperometric current detection during oxidation at the enzyme electrode is as shown in **Eq. 10** and anodic current response at working electrode followed by **Eq. 11**.

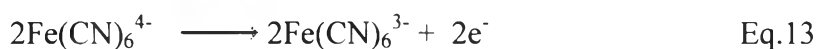
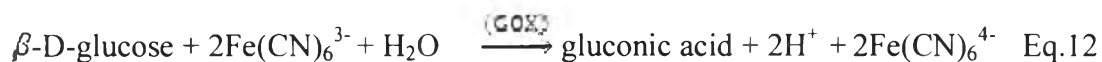




In the catalytic reaction between GOx and glucose molecule, GOx must be high enough to have a good sensitivity. **Fig. 3.15** illustrated that the anodic current was directly proportional to GOx concentration up to 1.0 g/ml. Anodic current for the GOx concentration over 1.0 g/ml could not be measured due to the low solubility of GOx in the PBS solution. The GOx concentrations of 0.1 g/ml was taken into both sensitivity and calibration curve as shown in **Fig. 3.16**. The amperometric response of PPy/PAN-MWCNT/SPCE electrode shows an obvious increase in oxidation current upon addition of glucose occurred which were monitored in range of 50 seconds. The amperometric responses show a linear relation with glucose concentration from 0.25 mM to 6.00 mM with correlation coefficient of 0.996 (**Fig. 3.16**). The LOD was evaluated to be 15.51 μM and sensitivity of 10.81 mA/ M cm². The modified electrode shows high sensitivity and low detection limit because the high conductivity of PPy layer and surface-to-volume ratio of conductive electrospun fiber were covered on electrode surface. Compared to another report on amperometric glucose biosensor without using mediator, LOD in this work was lower than conventional blood glucometers; the detected level was as low as 1.7 mM (Dungchai, 2009)

3.4.8 Mediator-based Glucose Biosensor

The problems of using GOX alone in reaction system are the interfere substances such as ascorbic acid and urea which can be oxidized at the >0.6 V and the variable oxygen concentration. So, the anodic current of these compound could lead to electrochemical interference. The way to eliminate the interference signal is using mediator. In this study, we used $\text{Fe}(\text{CN})_6^{3-}$ as mediator which can react with GOX instead of oxygen thus reduced mediator is formed instead of hydrogen peroxide. As shown in **Eq. 12** and **Eq. 13**, the electron transfer between the mediator and the GOX. The characteristic of CV of SPCE and PPy/PAN-MWCNT/SPCE were shown in **Fig 3.17**. The contrasts greatly with ΔE_p of both types of electrode, in this system, the oxidation peak of PPy/PAN-MWCNT/SPCE occurred at low potential with the high anodic current compared to SPCE.



Cyclic voltammetry of PPy/PAN-MWCNT/SPCE containing various mediator concentrations and constant 0.1 g/ml of GOX as shown in **Fig 3.18**, at scan rate of 50 mV/s. The results showed that the oxidation peak of PPy/PAN-MWCNT/SPCE was slightly shifted to the higher voltage (0.4-0.6 V) compared to the system without mediator and GOX. The addition of mediator from 1 mM to 100 mM, the current and potential responses were different; the current and potential response were proportional to the mediator concentration. In **Fig 3.19** showed the amperometric response, the detection of 1 mM glucose in systems containing constant GOX and various concentrations of mediators, at the highest concentration of mediator (100 mM), exhibited the high current response. But, there is no increase of anodic current after adding 1 mM of glucose due to signal disturbed by background current. Therefore, in this work, the 20 mM of mediator was chosen for incorporation with GOX. In addition, at this concentration, can detect glucose in more wide range of 0.25-7 mM.

The calibration curve of Mediator+GOX/PPy/PAN-MWCNT/SPCE was evaluated by amperometry technique shown in **Fig 3.20(bottom)**. Further with this method, glucose can be estimated up to 7 mM. Linear current response with concentrations reveal that the modified SPCE can be used to estimate glucose concentration in 0.25-7 mM range with LOD (3S/N) of 0.98 mM and the sensitivity of this electrode is 116.8 mA/M cm²

3.4.9 Michaelis-Menten Constant (K_m) Calculation

To study enzyme-substrate kinetics process, Michaelis-Menten constant, K_m was estimated from **Fig 3.18**, graph between glucose concentration and glucose concentration/ amperometric response current. The K_m values of GOX/PPy/PAN-MWCNT/SPCE and GOX+Mediator/PPy/PAN-MWCNT/SPCE were 2.602 mM and 0.469 mM, respectively. For GOX+Mediator/PPy/PAN-MWCNT/SPCE, the K_m value was lower than mediatorless system and smaller than

reported value of 23 mM for plain PPy/GOx . Smaller value of K_m reveal the higher affinity of enzyme for its substrate and can be attributes to the better conformation of adsorbed enzyme for enzyme reaction. The result suggest that the presence of mediator in the reaction system helps to promote the redox reaction with biological component and thus help in the rapid electron transfer

3.5 Conclusions

We have shown that a PPy/PAN–MWCNT/SPCE can be fabricated using electrospinning and vapor-phase polymerization techniques. The presence of a conductive nanofiber structure coated with a PPy layer on the SPCE surface enhances the electrochemical redox activity, where the resulting current is produced by radial diffusion and is quasi-reversible with an excellent anodic/cathodic current response and a low ΔE_p in a $\text{Fe}(\text{CN})_6^{3-/4-}$ redox-couple system. In the case of standard H_2O_2 detection, the PPy/PAN–MWCNT/SPCE gave a well-defined electrocatalytic response, which indicated that the modified electrodes behave as a disposable device with an anodic current greater than that of the conventional planar SPCE and could be used to measure H_2O_2 over a wide concentration range from 0.125 to 10 mM. The incorporation of GOX as an enzymatic model with the PPy/PAN–MWCNT/SPCE led to glucose detection over a linear range of 0.25-6 mM. In addition, The experimental results showed that mediator retained its catalytic activity with low K_m value and increase sensitivity and linear range of detection. This work represents our first report on SPCE surface modification. In future works, enzymatic immobilization on a PPy layer will be investigated.

Acknowledgements

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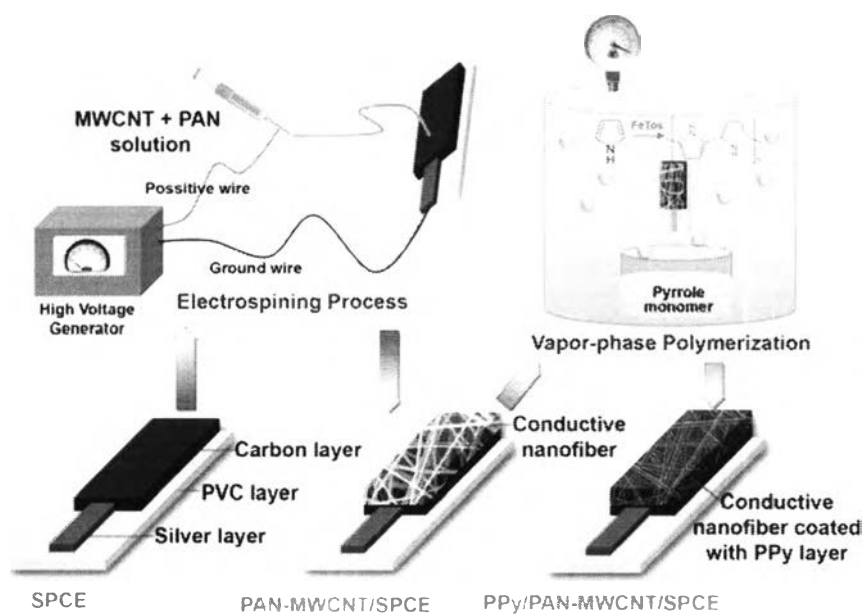


Figure 3.1 Schematics of electrode fabrications

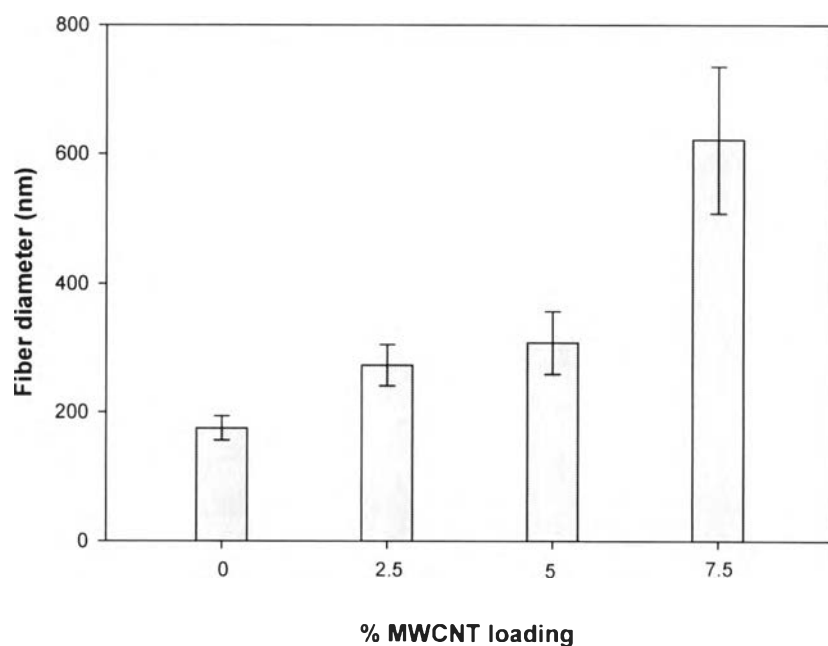


Figure 3.2 Effect of % MWCNT loading on fiber diameter

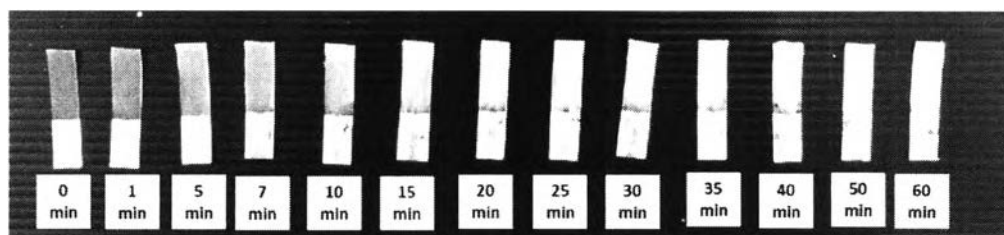


Figure 3.3 The samples of SPCE coated with PAN-MWCNT nanofiber at various times

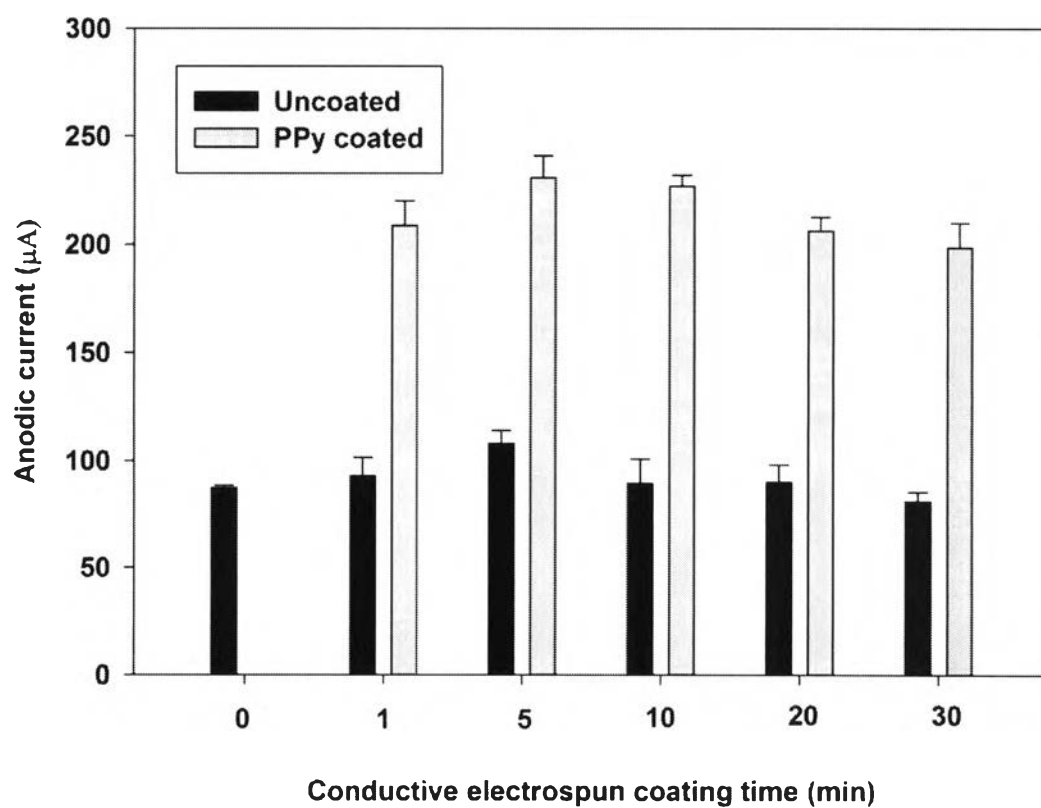


Figure 3.4 Anodic current response of modified electrodes before and after coating with PPy layer of 5 mM $\text{Fe}^{2+/3+}$ in 0.1 M PBS of pH 7.4 as a function of electrospinning times

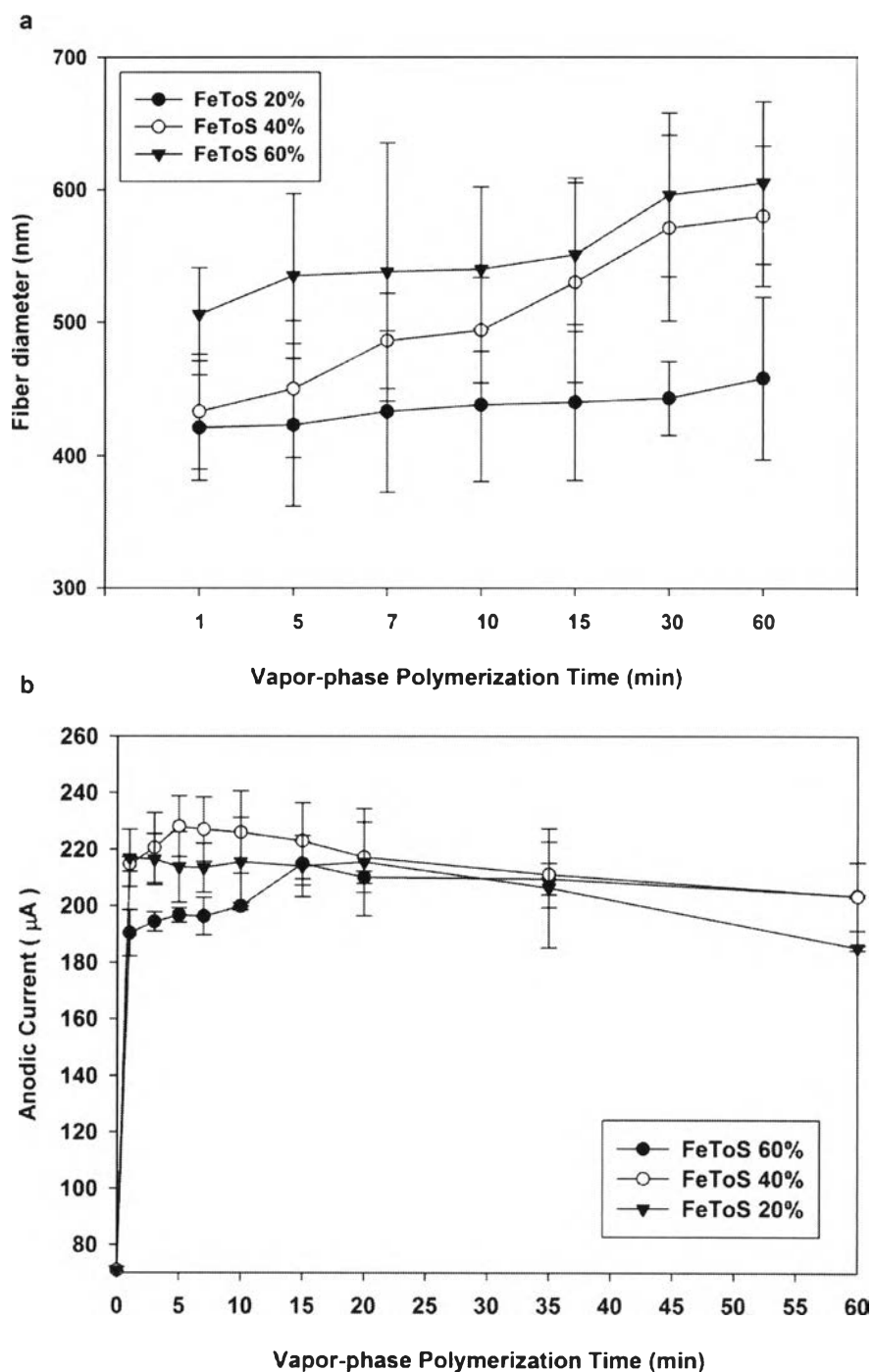


Figure 3.5 (a) Effect of concentration of FeTos oxidant on fiber diameter and (b) Effect of concentration of FeTos oxidant on anodic current response of 5 mM $\text{Fe}^{2+/3+}$ in 0.1 M PBS of pH 7.4

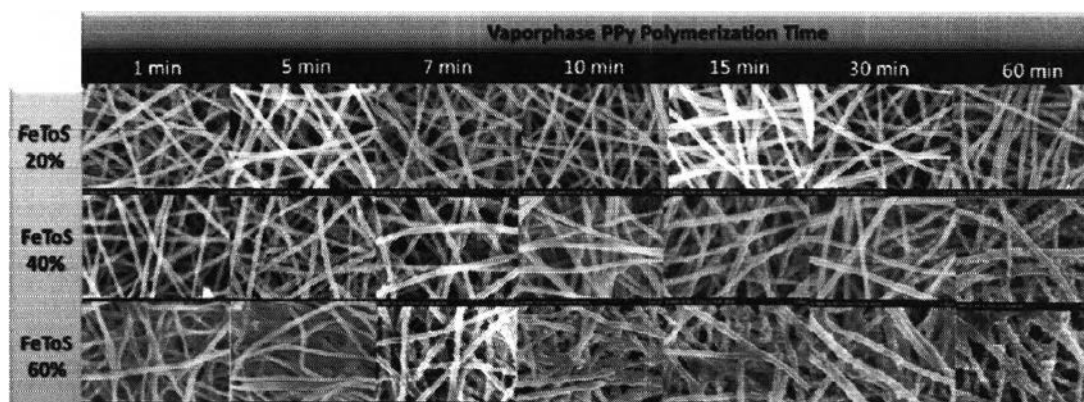


Figure 3.6 SEM images of modified SPCE coated with PPy layer by vapor-phase polymerization at various times

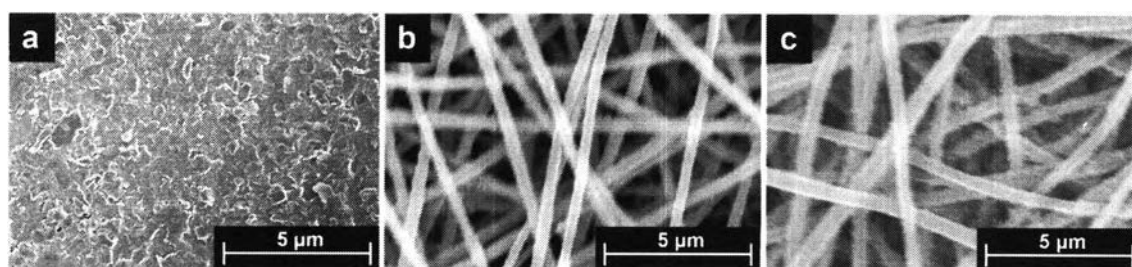


Figure 3.7 SEM images of the surface morphologies of (a) SPCE, (b) PAN-MWCNT/SPCE, and (c) PPy/PAN-MWCNT/SPCE electrodes.

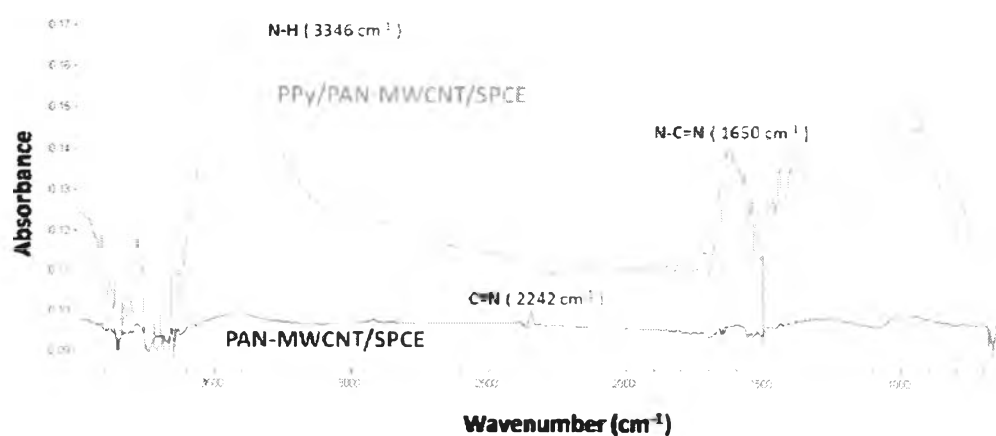


Fig. 3.8 FT-IR spectra of PAN-MWCNT/SPCE (bottom line) and PPy/PAN-MWCNT/SPCE (top line)

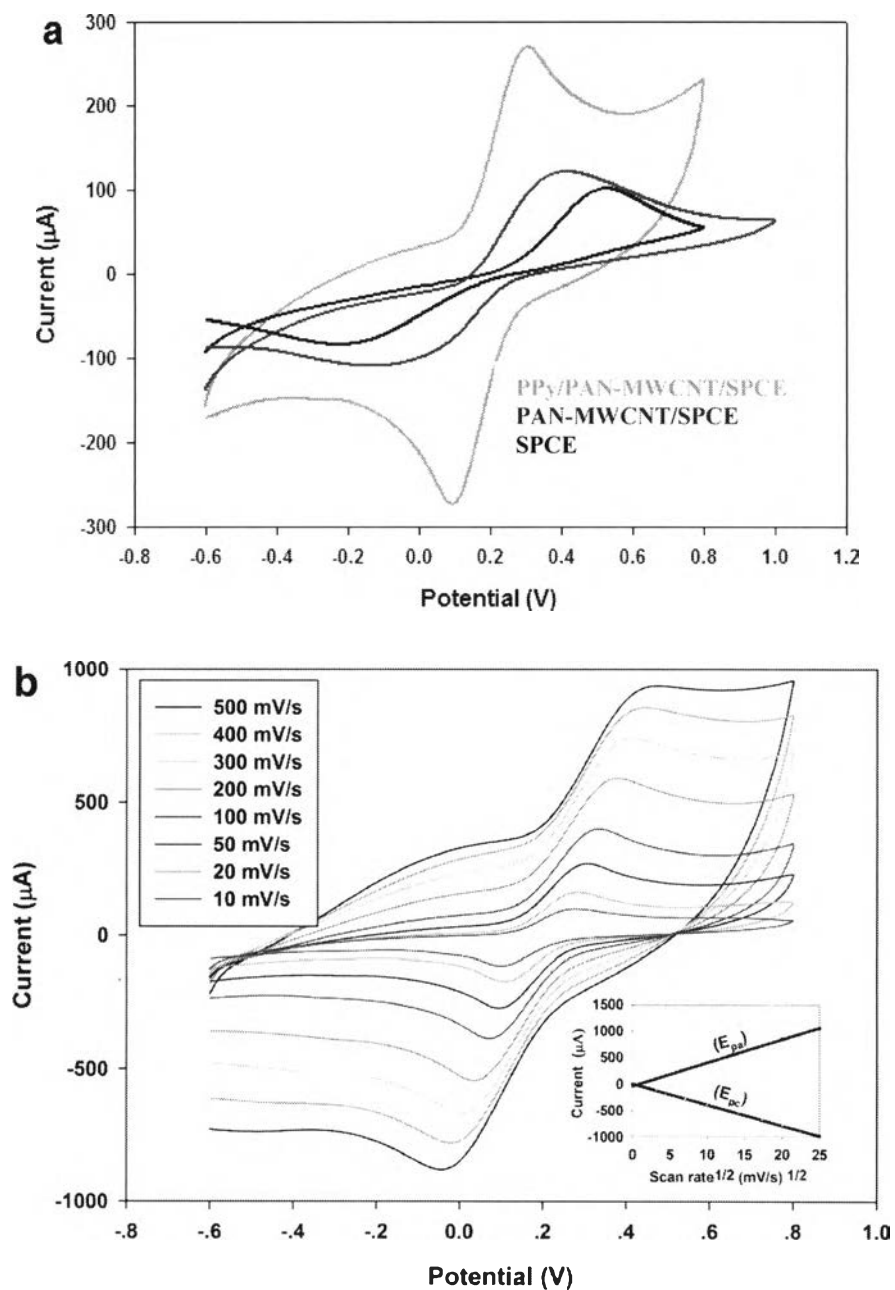


Figure 3.9 (a) Cyclic voltammograms of 5 mM Fe^{2+/3+} in 0.1 M PBS of pH 7.4 at different modified electrodes measured with scan rate of 50 mV/s (b) Cyclic voltammograms of BARE-CNT-PPy electrode at different scan rate. (inset) plot linear relation current vs. scan rate^{1/2}

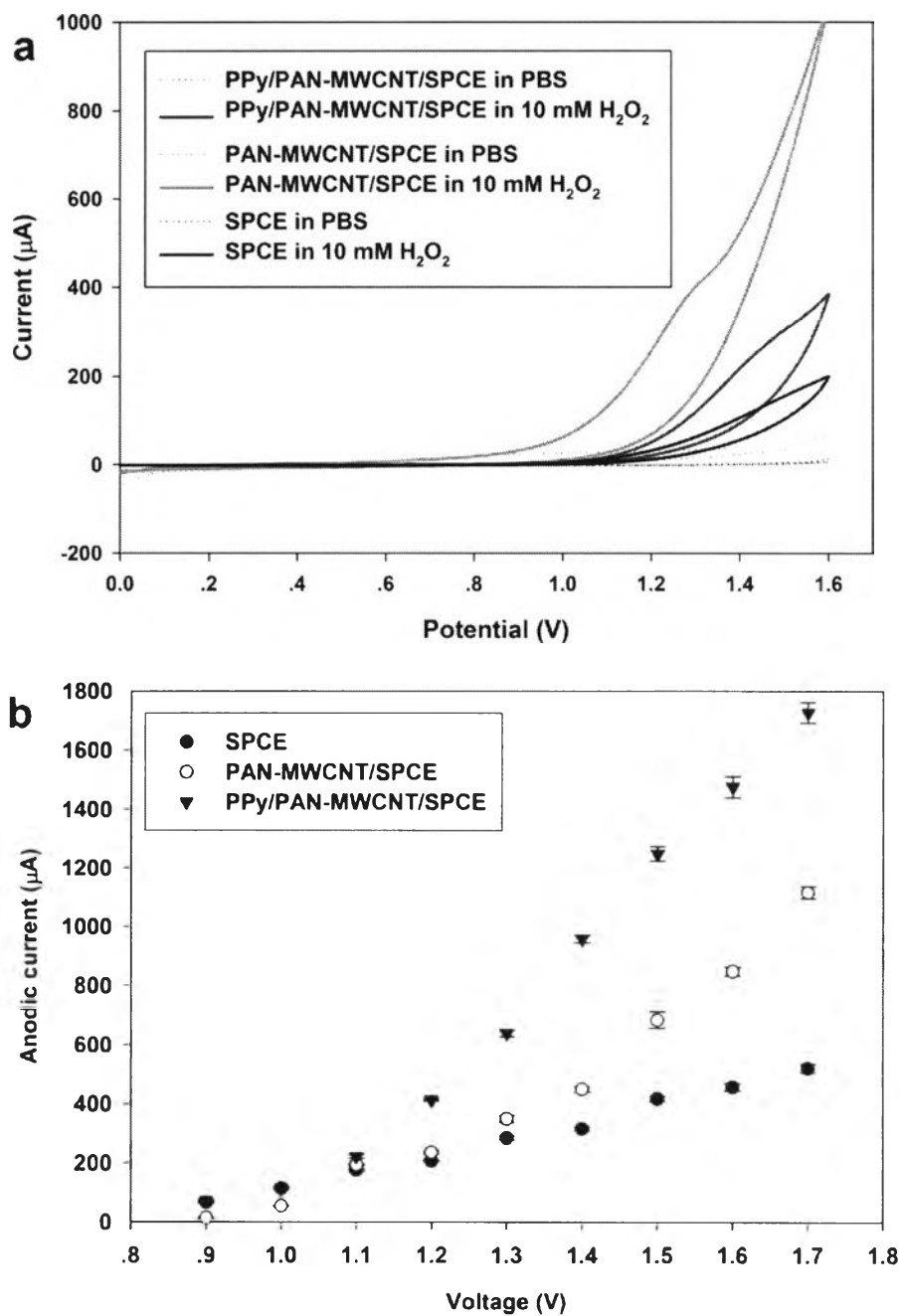


Figure 3.10 (a) Cyclic voltammograms of 10 mM H_2O_2 , 0.1 M PBS in of pH 7.4 at modified electrodes (b) Amperometric response of electrodes of 10 mM H_2O_2 in 0.1 M PBS of pH 7.4 potential fixed at +1.4 V for SPCE and PAN-MWCNT/SPCE and +1.2 V for PPy/PAN-MWCNT/SPCE, respectively

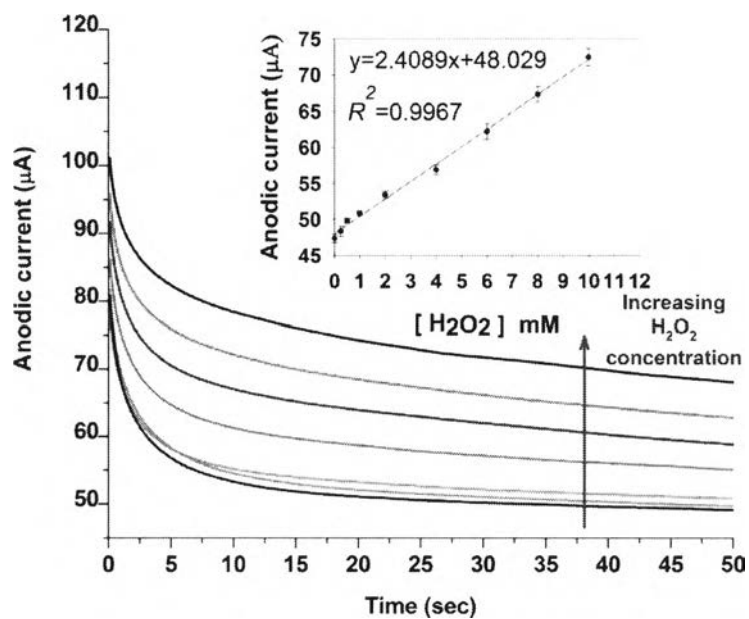


Fig. 3.11 Amperometric responses of glucose in 0.1 M PBS of pH 7.4. at PPy/PAN-MWCNT/SPCE electrode. (inset) Linear current response with H_2O_2 concentrations from 0.125 to 10 mM poised potential at +1.2

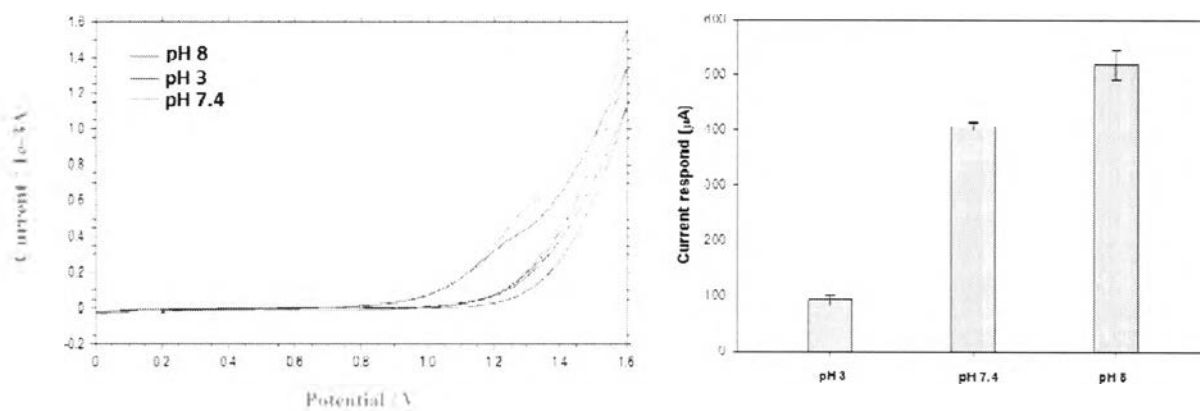


Fig. 3.12 Cyclic voltammogram and anodic current plots of 10 mM H_2O_2 detection at PPy/PAN/MWCNT/SPCE with various pHs.

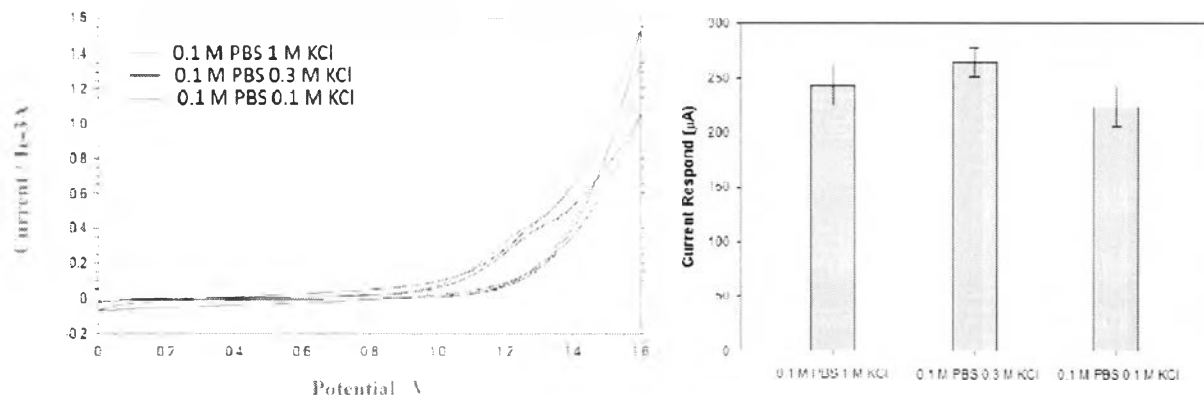


Fig. 3.13 Cyclic voltammogram and anodic current plots of with various KCl concentrations for 10 mM H_2O_2 detection at PPy/PAN-MWCNT/SPCE

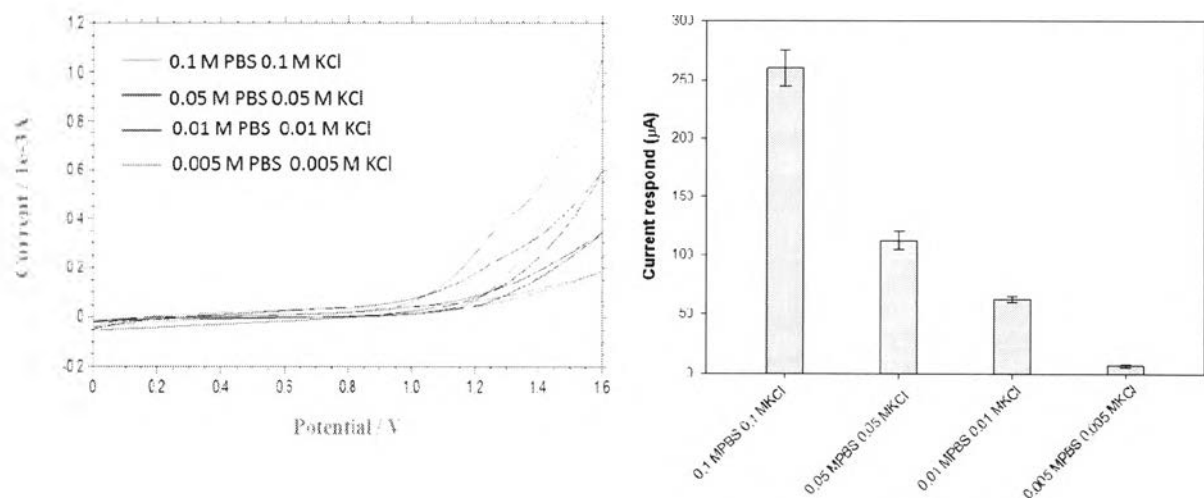


Fig. 3.14 Cyclic voltammogram and anodic current of 10 mM H_2O_2 detection at PPy/PAN-MWCNT/SPCE.

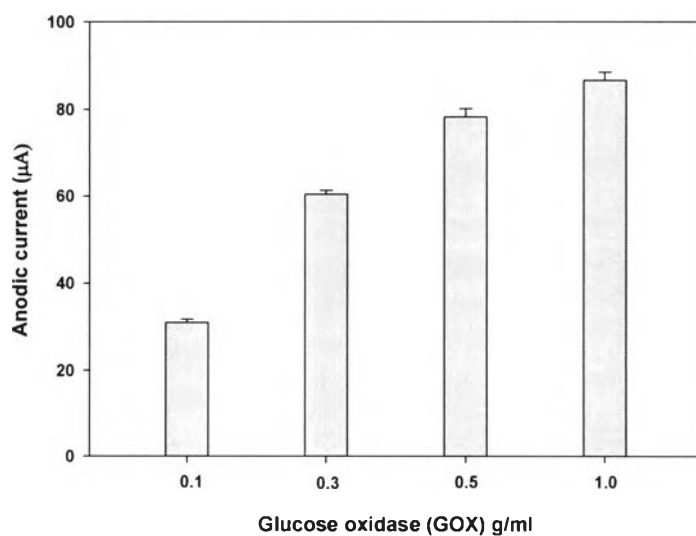


Figure 3.15 Amperometric peak current of 1 mM glucose in various GOX concentrations

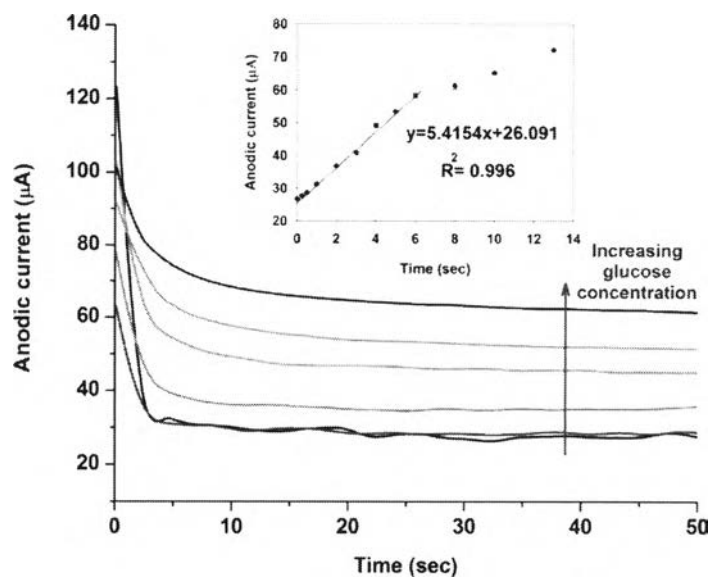


Fig. 3.16 Amperometric measurements obtained for standard glucose additions using an applied potential of +1.25 V with BARE-CNT-PPy electrode. Inset is a calibration curve of BARE-CNT-PPy electrode

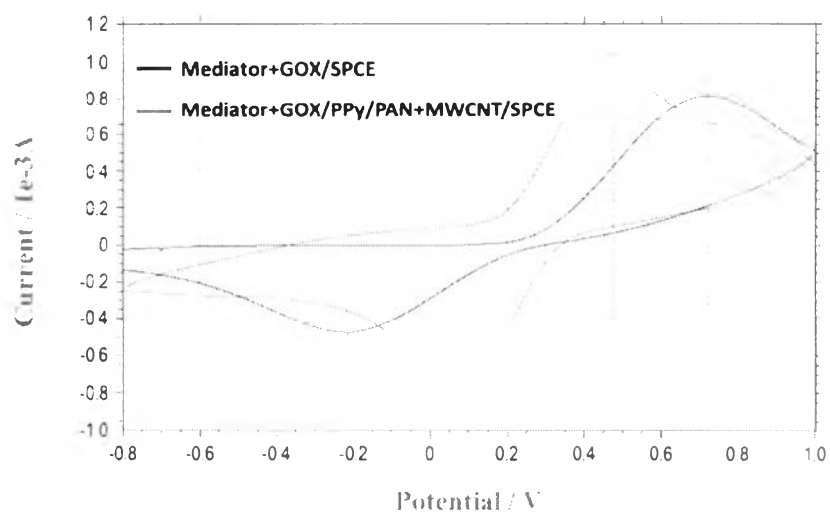


Fig. 3.17 Cyclic voltammogram of SPCE and PPy/PAN-MWCNT/SPCE in 50 mM mediator system

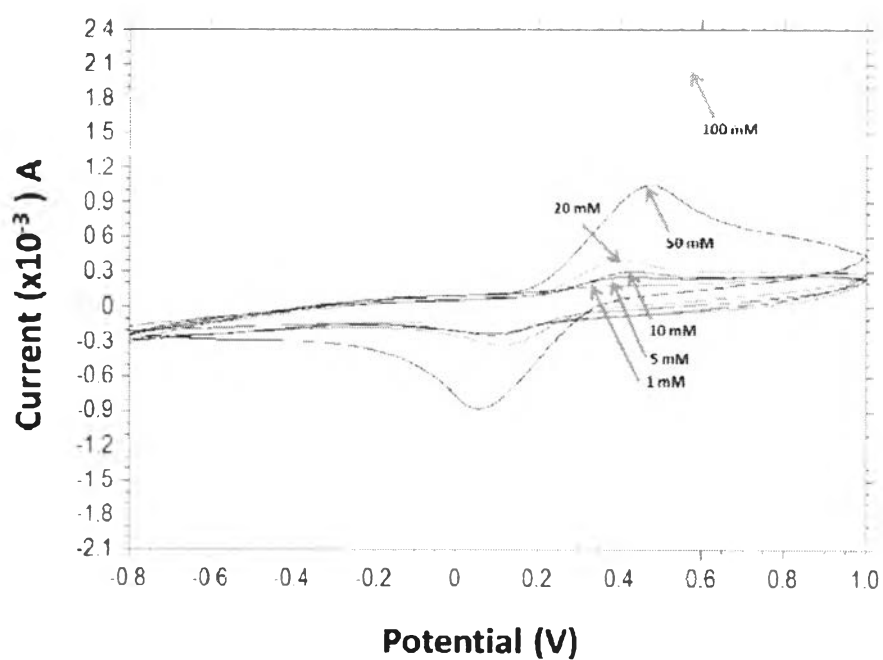


Fig. 3.18 Cyclic voltammogram of 0.1 g/ml of GOX and various mediator concentrations at PPy/PAN-MWCNT/SPCE.

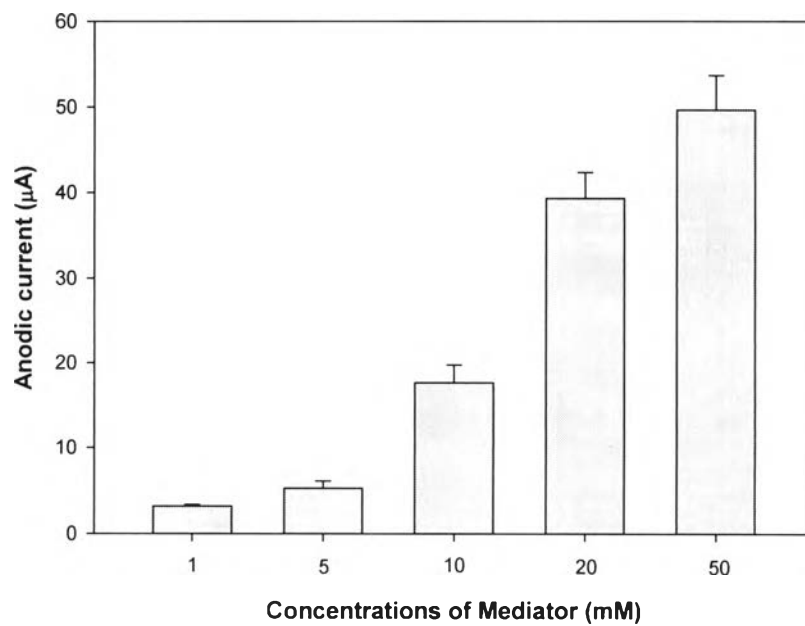


Fig. 3.19 Amperometric responses of 1 mM of glucose at PPy/PAN-MWCNT/SPCE electrode incorporate with various mediator concentrations.

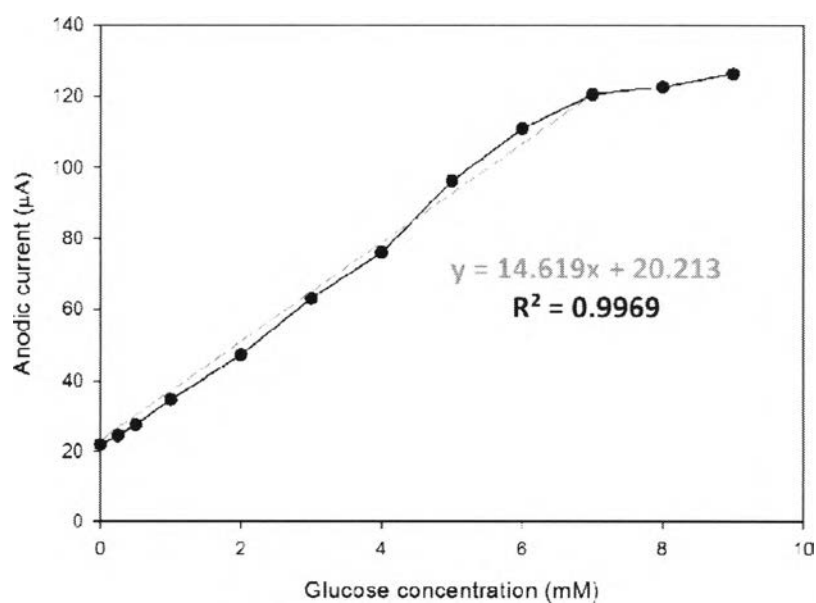
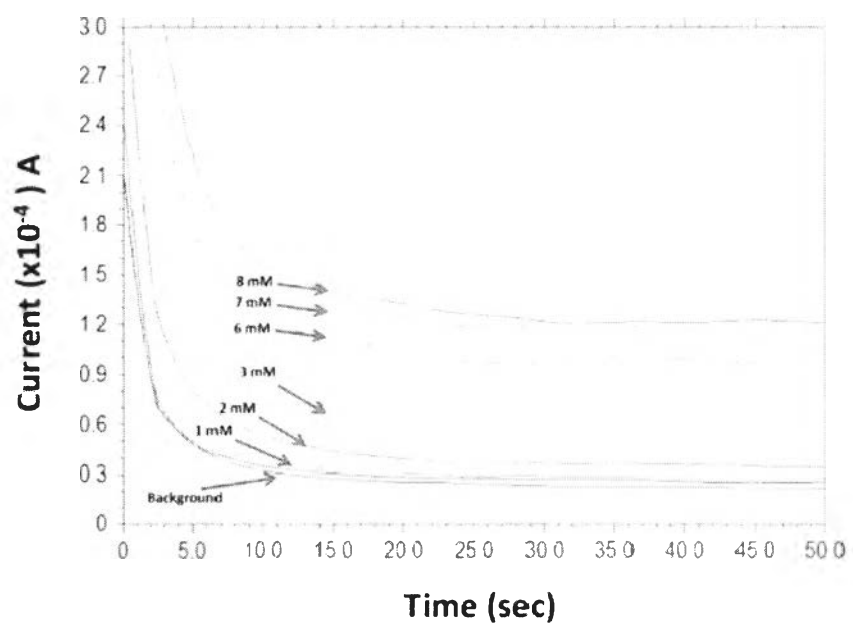


Fig. 3.20 Amperometric measurements obtained for standard glucose additions using an applied potential of +0.45 V of PPy/PAN-MWCNT/SPCE incorporation with mediator (top) and calibration curve (bottom)

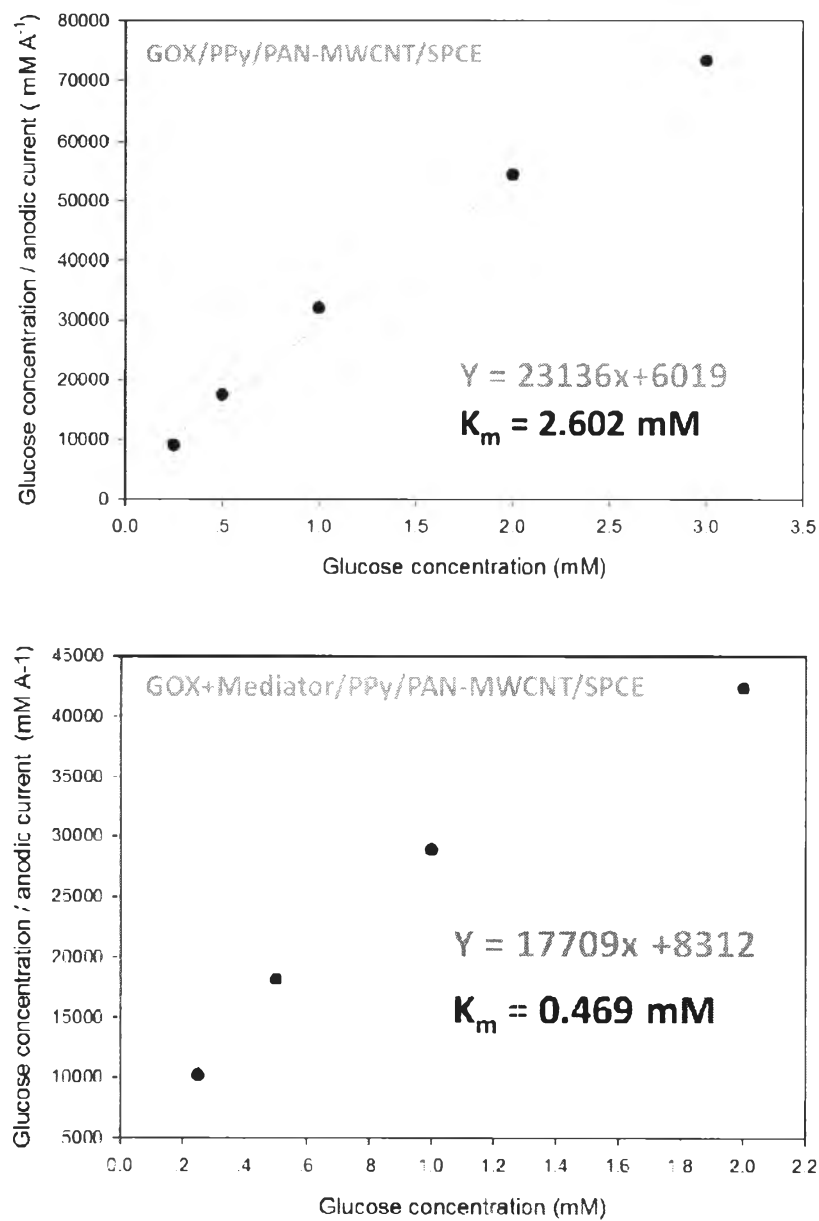


Fig. 3.21 Hanes plot for comparison of GOX /PPy/PAN-MWCNT/SPCE(top) and GOX+Mediator/PPy/PAN-MWCNT/SPCE(bottom)

Table 3.1 Comparison of modified electrodes with others GOX based amperometric glucose biosensors reported in literature.

Electrode modified with	Linear range	K _m (mM)	Sensitivity	Ref
PS/PANI/Au/ITO	0.04-2.04 mM	0.76	-	(Liu <i>et al.</i> , 2008)
Au-PPy/ITO	2.5 μM-5 mM	43.5	1.089 μAmM ⁻¹	(Njagi, 2007)
PPy/Fc/ITO	2-10 mM	-	0.23 μAmM ⁻¹	(Fiorito <i>et al.</i> , 2001)
PPy/ITO	5 μM- 20 mM	23.3	-	(Chen <i>et al.</i> , 2006)
PPy/MWCNT/ITO	1-4 mM	-	95 nA mM ⁻¹	(Tsai <i>et al.</i> , 2006)
P(PPy-FcPy)/ITO	1-16.8 mM	1.6	19.21 μAmM ⁻¹	(Palomera, 2011)
PPy/PAN-MWCNT/SPCE	0.25-6 mM	2.602	5.415 μAmM ⁻¹	[This work]
GOX+Mediator /PPy/PAN-MWCNT/SPCE	0.25-7 mM	0.469	14.62 μAmM ⁻¹	[This work]
GOD/CoPC/SPCE	0.2-5 mM	-	1.12 μAmM ⁻¹	(Crouch <i>et al.</i> , 2005)
GOD/CNT/Pt	0.1-13.5 Mm	-	91 mAM ⁻¹ cm ⁻²	(Tang <i>et al.</i> , 2004)
GOD/Pd-Ni/SiNW	2-20 mM	-	190 μAmM ⁻¹	(Hui <i>et al.</i> , 2011)