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*Short Communication*

# Electrochemical Studies on the Response to Glucose in the Presence of Bilirubin, Creatinine and Uric Acid at Nafion/Pd-GOx Modified Screen Printed Carbon Electrode

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In the present work, a glucose sensor was developed based on the glucose oxidase immobilized on Pd (Pd-GOx) and nafion modified electrode. The modified electrode was characterized by atomic force microscopy and field emission scanning electron microcopy. The electrochemical analysis for electrochemical activity was accessed by cyclic voltammetry. The modified electrode displayed an excellent electrooxidation behavior to glucose and was detected by cyclic voltammetry and amperometry. In optimized conditions, the fabricated Nafion/Pd-GOx modified electrode exhibited a sharp amperometric response to Human blood and glucose. The modified electrode also holds its high selectivity in the presence of bilirubin, creatinine, and uric acid, indicating it can be an ideal electrode material for detection of glucose in diabetes.

**Keywords:** Palladium; glucose oxidase; glucose biosensor; amperometry, selectivity

## 1. INTRODUCTION

Over the past decade, the research on glucose monitoring have been attractive in various fields, especially in medical diagnostics, pharmaceuticals, and food processing [1-2]. Owing to the importance of glucose, the sensitive, selective and stable determination of glucose is mandatory and highly appreciable. Glucose oxidase (GOx) is an ideal model enzyme; has been widely used in amperometric biosensors for the selective detection of glucose [3-5]. There are numerous reports were available for glucose biosensor based on various materials modified electrodes, such as carbon nanotubes (CNT) [6], graphene [7], metal nanoparticles [8] and conducting polymers [9]. Some of the

recently reported palladium (Pd) based glucose biosensors are GOx immobilized PtPd nanoparticles on carbon nanotubes-ionic liquid composite film [9], Au@Pd corshell nanoparticles-ionic liquids composite film modified glassy carbon electrodes[10], Pd-Ni/SiNW electrode[11], electrochemical deposition of palladium and glucose oxidase on a glassy carbon electrode[12].

In the electrochemical glucose biosensors the selectivity is always play a vital role especially in the presence of creatinine, bilirubin and uric acid [13, 14]. Creatinine is the 2-amino-1-methyl-5H-imidazol-4-one and presents certain level in human blood and urine [15]. It also naturally produced by the body and is filtered from the bloodstream by the kidneys in relatively constant amounts every day. The normal physiological concentration of creatinine in blood is 40–150 □M. On the other hand, bilirubin exists in human serum as two major forms: unconjugated bilirubin (Bu) and conjugated bilirubin, where bilirubin can be bilirubin monoglucuronide or bilirubin diglucuronide [16]. The uric acid (UA) is an important end product in the human metabolism [17]. Hence the detection of glucose in the presence of these interfering compounds is still challenging.

In the present work, a selective and sensitive glucose sensor was developed based on Nafion/Pd-GOx modified electrode. The modified electrode displayed an excellent electrooxidation behavior to glucose and process good selectivity in the presence of creatinine, bilirubin and uric acid. The further optimization studies for glucose detection have also been discussed in detail.

## 2. EXPERIMENTAL

### *2.1 Materials*

SPCEs were purchased from Zensor Research & Development Co., Taiwan. D-(+)-Glucose, uric acid, bilirubin, creatinine, 5% Nafion-117 were purchased from Sigma-Aldrich(USA). The Human blood obtained from K-Jump© Health Co. Taiwan. The Pd-GOx composite material was received from K-Jump© Health Co. Taiwan. All other chemicals used were of analytical trade and used without any further purification. All the stock solutions were prepared using Double distilled deionized water. The 0.05 M phosphate buffer solution (PBS) of pH 7.0 was prepared using $Na_2HPO_4$ and $NaH_2PO_4$.

### *2.2 Apparatus*

All electrochemical experiments were performed using CHI 1205a Potentiostats (CH Instruments, USA). Field emission scanning electron microscope (FE-SEM) images were recorded using a HITACHI S-4700 (Japan). The AFM images were recorded with a multimode scanning probe microscope system operated in tapping mode using Being Nano-Instruments CSPM-4000, Ben Yuan Ltd. (Beijing, China). The three electrode setup consisting of modified SPCE (geometric area 0.3 $cm^2$) as a working electrode, Pt wire as a counter electrode and a saturated Ag|AgCl as a reference electrode. All the experiments were carried out at a room temperature.

*2.3. Preparation of Nafion/Pd-GOx/SPCE modified electrodes*

The obtained Pd-GOx composite solution was drop casted onto the SPCE and dried in a room temperature. The nafion about 3 µL was covered on the Pd-GOx composite modified SPCE and dried at a room temperature. The fabricated electrode was further used for all electrochemical studies.

## 3. RESULTS AND DISCUSSION

*3.1 Electrochemical characterization of Nafion/Pd-GOx modified electrode*

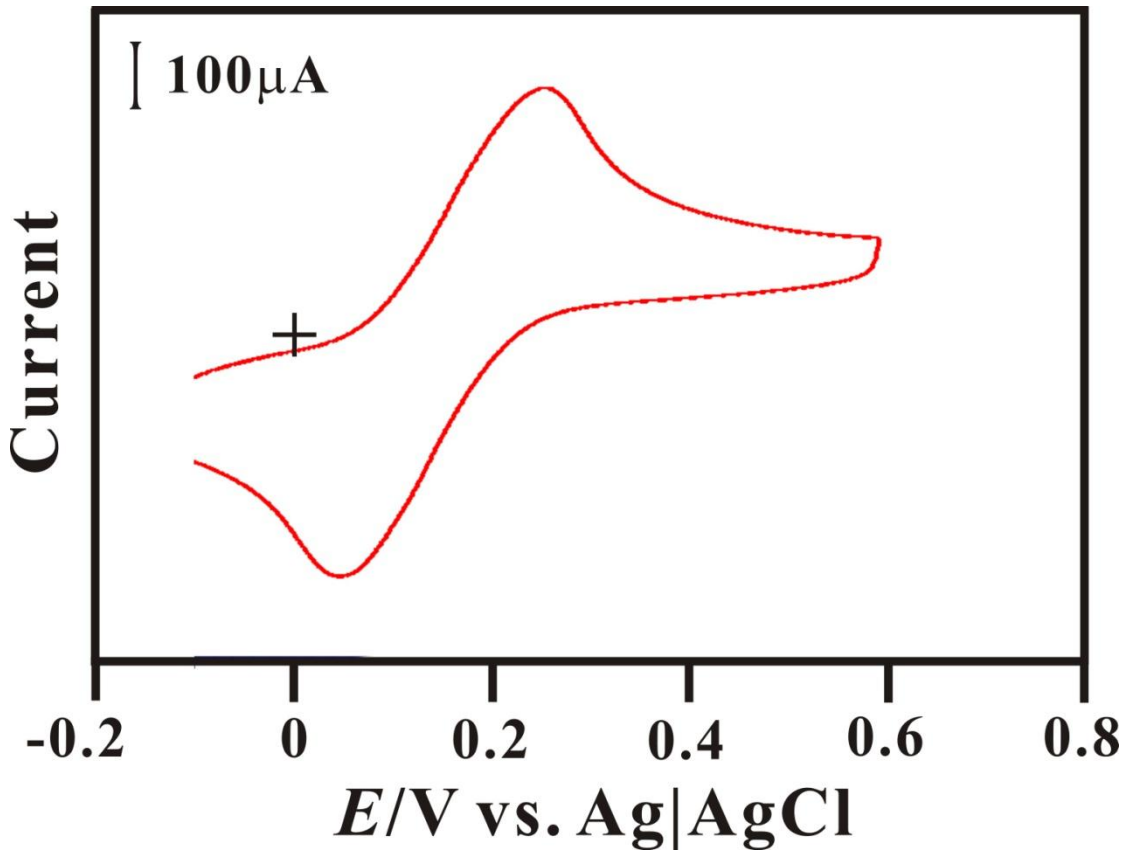


**Figure 1.** Cyclic voltammetry response of a Nafion/Pd-GOx modified SPCE in pH 7 containing 1 mM $K_4Fe(CN)_6$ at a scan rate of 0.1 V/s.

Fig. 1 shows the cycle voltammogram of Nafion/Pd-GOx modified electrode in pH 7.0 containing 1 mM $K_4Fe(CN)_6$ at a scan rate of 0.1 $Vs^{-1}$.The redox couple with the anodic and cathodic peak at 0.26 and 0.046 V with a formal potential of ($E^{0'}$) 0.153 V observed at Nafion/Pd-GOx modified electrode. The peak to peak separation ($\Delta_{Ep}$) was found as 0.214 V. This is the characteristic redox processes of $Fe(CN)_6^{4-/3-}$ which involving of one electron transfer on the electrode surface. Compared to GOx immobilized SPCE/Nafion (160 mV) the ($\Delta_{Ep}$) of the modified electrode shows more peak separation (figure not shown), indicates that the GOx firmly immobilized onto the Nafion/Pd surface.

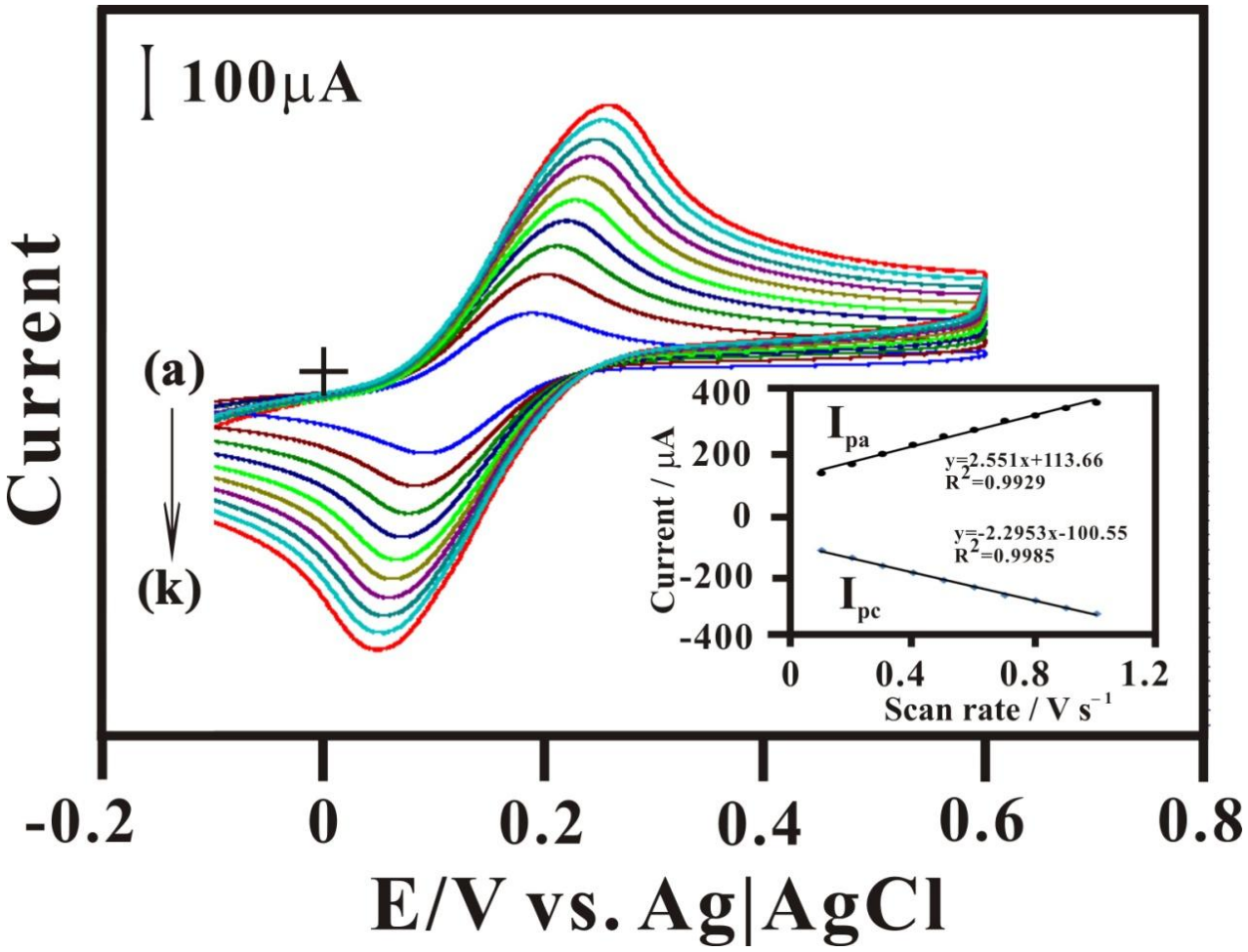


**Figure 2.** The cyclic voltammetry response of Nafion/Pd-GOx modified SPCE in pH 7 at sweeping of different scans rate from 0.01 to 0.1 V/s. Inset shows the calibration plot for scan rate vs. current response.

Fig. 2 shows the effect of scan rate on the response of Nafion/Pd-GOx modified electrode in pH 7.0 containing 1 mM $K_4Fe(CN)_6$. It can be seen that the anodic and cathodic peak currents increased upon increase the scan rate from 0.01 to 0.1 V/s. The inset (a) in Fia. 2 shows the plot of the Nafion/Pd-GOx/SPCE modified electrodes signal of the anode peak and cathode peak current vs. scan rate. The corresponding linear regression equations were Ipa (μA) = 2.551($Vs^{-1}$) +113.66, $R^2$ = 0.9929, and Ipc(μA) = -2.2953($Vs^{-1}$) -100.55, $R^2$ = 0.9985 for anod and catohd peak current, respectively.

*3.2 Surface morphology of Pd-GOx composite*

The surface morphology of the Pd-GOx composite was investigated using AFM and FESEM and shown in Fig. 3a and b. The AFM image clearly reveals that the GOx particles are firmly attached on the Pd nanorods. From Fig. 3(a) and Fig.3(b) we could seen the Pd columnar structure aggregate, and the GOx attached on the palladium cylindrical surface. The average particle size of the Nafion/Pd-GOx were 99.9 nm and the average roughness (Sa) were 24.3 nm.

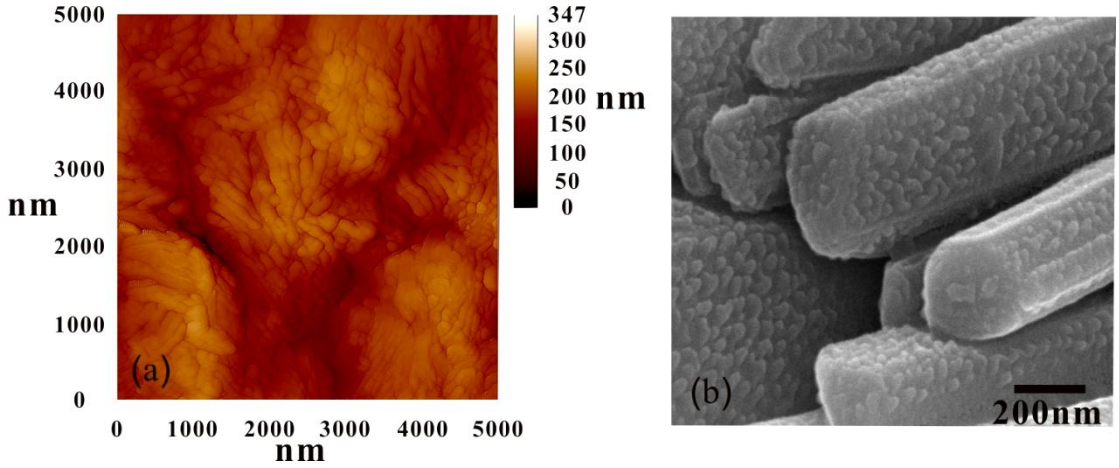


**Figure 3.** a) AFM image of Nafion/Pd-GOx and b) FE-SEM image of Nafion/Pd-GOx.

*3.3 Electrocatalytic detection of glucose*

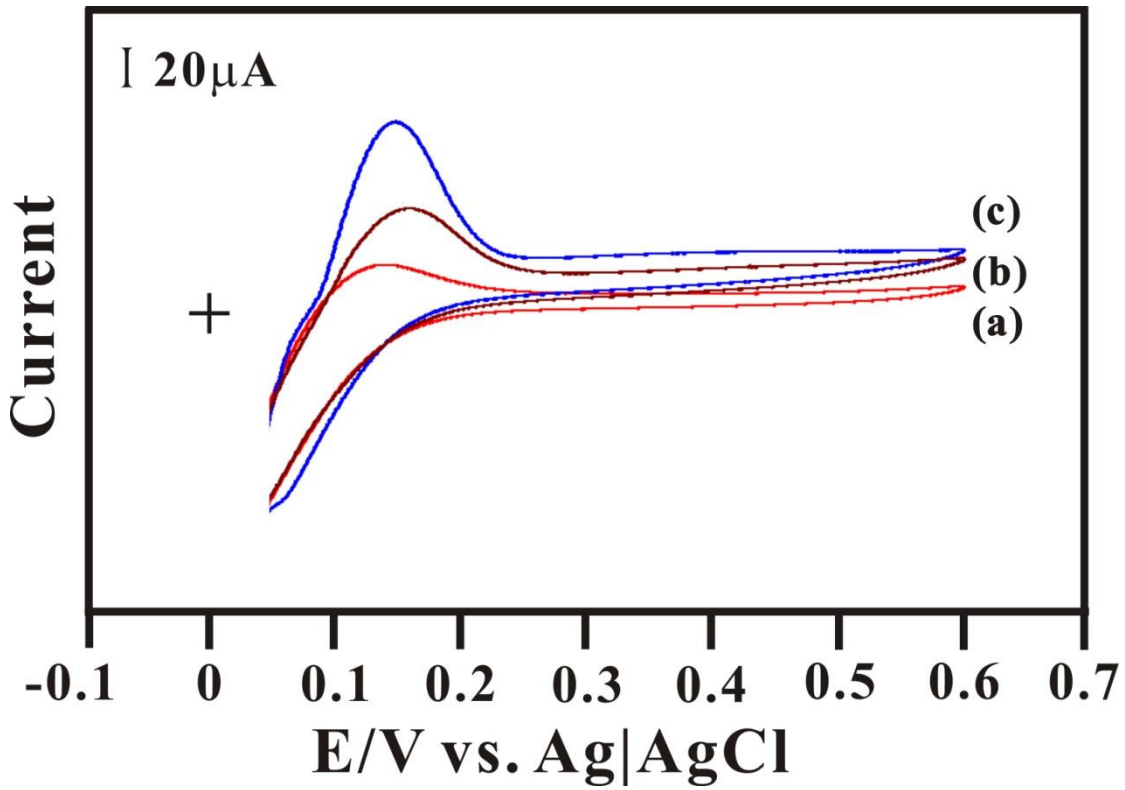


**Figure 4.** The cyclic voltammetry response of Nafion/Pd-GOx modified electrodes in pH 7.0 in the absence (a) and presence of 0.005 M (b) and 0.01M (c) glucose at a scan rate of 0.1 V/s.

The electrochemical detection of glucose was investigated by using CV and amperometric method. Fig. 4 displays the cyclic voltammogram response of Nafion/Pd-GOx modified electrode in pH 7.0 in the absence (a) and presence of 0.005 M (b) and 0.01M (c) glucose at a scan rate of 0.1 $Vs^{-1}$. It can be seen that in the presence of 0.005 M glucose, a sharp oxidation peak was observed at 0.15 V; which is due to the electrocatalytic oxidation of glucose to gluconolactone by the modified electrode.

The oxidation peak current was increased further increased the glucose concentration (005 to 0.01 M). Whereas, in the absence of glucose the modified electrode did not show any signal on this potential window, clearly suggesting that the modified electrode is electrochemically inactive.

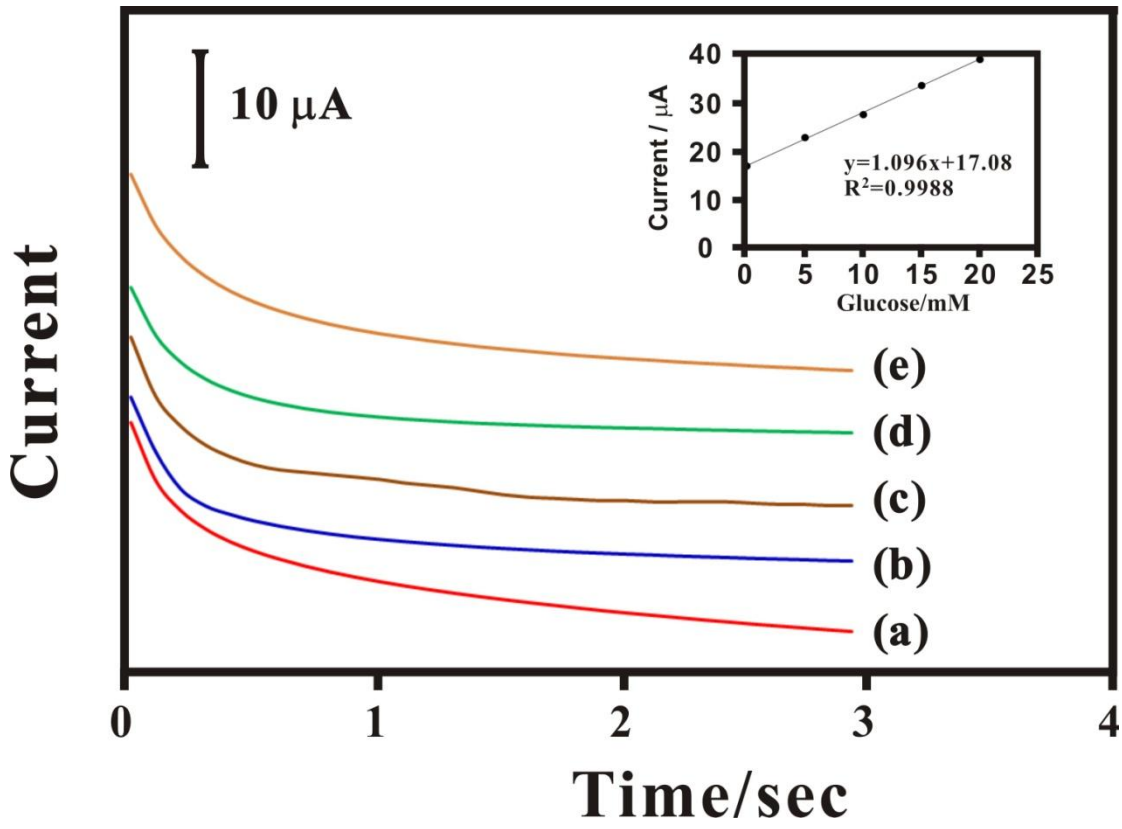


**Figure 5.** Amperometric i-t response obtained at Nafion/Pd-GOx modified SPCE in the in the absence (a) and presence of Human blood (b) and each addition of 0.005 M glucose (c–e). Working potential = 0.4 V.

Fig. 5 displays the amperometric response of Nafion/Pd-GOx modified SPCE in the in the absence (a) and presence of Human blood (b) and each addition of 0.005 M glucose (c–e). Working potential = 0.4 V. In the absence of Human blood and glucose, the modified electrode does not show any amperometric response. Whereas, the current response increased in the presence of Human blood (b) and further increased increasing the glucose concentration (c–e). The linear regression equation was found from the fitted linear regression equation as $I_{pa}(\mu A) = 1.096 + 17.08$ C (mM) , $R^2 = 0.9988$. Fig 6 A-a to A-c shows the amperometric i-t responses obtained at Nafion/Pd-GOx modified SPCE in the Human blood in the absence (a) and presence (b) of bilirubin (A-a), creatinine and (A-b) and uric acid (A-c). Fig 6 B-a to B-c shows amperometric i-t responses of Nafion/Pd-GOx modified SPCE in the Human blood containing 0.005 M glucose in the absence (a) and presence (b) of bilirubin (B-a), creatinine and (B-b) and uric acid (B-c). The working potential was held at 0.4 V. It can be seen that a sharp response observed for Human blood and 0.005 M glucose at the composite modified electrode. Whereas, in the presence of the interfering electroactive compounds such bilirubin, creatinine uric acid

the response current became stable in pH 7.0 containing glucose. The concentrations were used for bilirubin, creatinine uric acid as 1 mM, 2 mM and 5 mM, respectively.

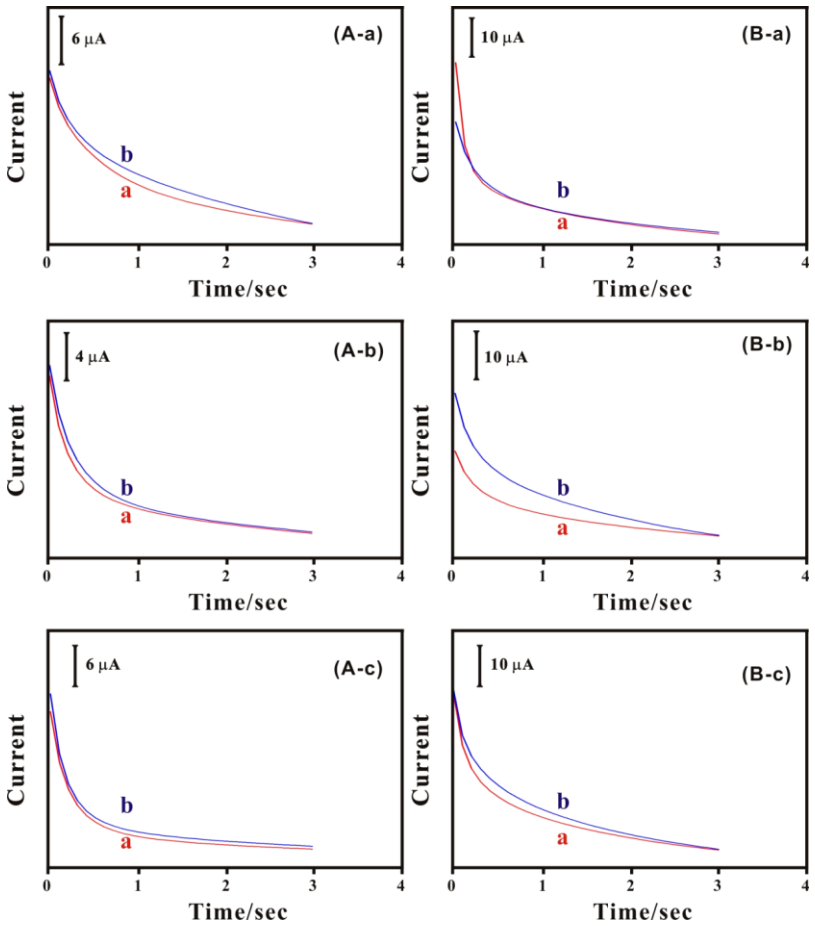


**Figure 6.** Amperometric i-t responses obtained at Nafion/Pd-GOx modified SPCE in the Human blood in the absence (a) and presence (b) of 1 mM bilirubin (A-a), 2 mM creatinine and (A-b) and 5 mM uric acid (A-c). At same conditions, amperometric i-t responses of Nafion/Pd-GOx modified SPCE in the Human blood containing 0.005 M glucose in the absence (a) and presence (b) of 1 mM bilirubin (B-a), 2 mM creatinine and (B-b) and 5 mM uric acid (B-c). Working potential = 0.4 V.

The amperometric response was slighted affect in the presence of bilirubin, creatinine uric acid at only in Human blood. However, compared to the glucose response in pure glucose and Human blood samples the responses of electroactive compounds are negligible. The obtained results further indicate the high selectivity of the fabricated electrode in the presence of electroactive interfering species.

## 4. CONCLUSIONS

The Nafion/Pd-GOx modified electrode has been successfully applied for the detection of glucose in Human blood sample. The modified electrode was characterized by AFM and FESEM and

those confirmed the presence of GOx on Pd. Cyclic voltammetry and amperometry was used for the detection of glucose in pH 7 and it further applied for detection of glucose in Human blood. Amperometric results further confirmed that the fabricated Nafion/Pd-GOx modified electrode is interference free for the detection glucose in the presence of bilirubin, creatinine, and uric acid. In addition, the fabricated electrode could be a more sensitive and selective electrode material for the detection of glucose in Human blood in near future.

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