

RESEARCH ARTICLE

Electrochemical Detection of Insulin in Blood serum using Ppy/GF Nanocomposite Modified Pencil Graphite Electrode

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ABSTRACT

In this study, pencil graphite electrode (PGE) was modified using conductive polypyrrole (Ppy) and graphene (GF) nanocomposite for electrochemical determination of insulin. Electrochemical behavior of insulin on PGE was evaluated using differential pulse voltammetric (DPV), cyclic voltammetric (CV) and chronoamperometry (CA) methods. Several effective variables including pH, concentration, and scan rate for electrochemical modification of electrode were investigated and optimal conditions were proposed. Kinetics of the oxidation reaction and diffusion coefficient of the sensor was studied. The performed steps allow the measurement of insulin with a linear repeatability curve and appropriate accuracy at a range of 0.225 to 1.235 μM . The limit of detection was obtained at 8.65nM for insulin. The amount of electron transfer coefficient between modified electrode and insulin was obtained to 0.5 with 0.84~1 number of electrons exchanged during oxidation of insulin. The proposed nano biosensor was chosen for insulin measurements in human blood serum analysis.

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INTRODUCTION

Insulin is a polypeptide hormone acts as a control center for the metabolism of the body and is used for the therapy of insulin-dependent type I diabetes. In the presence of insulin, the liver cells take blood sugar and store it as glycogen and stopped using fat as a fuel source. If the insulin does not exist or is lacking in the blood, the body uses fat as a fuel source [1-2]. Given the crucial role of insulin in living organisms, measuring insulin in diagnostic laboratories and diabetic clinics is very important [3-4].

For this reason, several methods including immune tests, capillary electrophoresis and high performance liquid chromatography have been investigated for quantitative analysis of insulin [5-8]. But, in general these methods are time-consuming,

cost-effective, laborious and lower detectors sensitivity toward samples with nano-molar or lower concentrations [9-10]. Electrochemical method compared to the above methods is suitable, sensitive and inexpensive with short testing time and availability [11]. Several studies have been reported in the area of electrochemical detection of biomaterials over the years. For instance an electrochemical immunosensor for the measurement of carcinoembryonic antigen in saliva and serum was developed using multiwall carbon nanotubes (MWCNT) and screen-printed electrode by Viswanathan et al. A detection limit of 1×10^{-12} g/mL (Signal to noise = 3) was obtained using this process for determination of carcinoembryonic in human blood serum [12]. Also, MWCNT bearing terminal monomeric unit was reported

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for the construction of epinephrine imprinted polymer-based electrochemical sensor. This system, responded a highly sensitive and selective response for epinephrine, with linear range 0.09–5.90 ng mL⁻¹ and limit of detection of 0.02 ng mL⁻¹ [13]. Serafin et al was developed an amperometric immuno sensor for the discovery of hormone insulin-like growth factor using modified glassy carbon electrode. Excellent reproducibility and selectivity were evidenced with a detection limit of 0.25 pg mL⁻¹, [14]. In the following, Ppy, reduced graphene oxide, and Au nanocomposite was developed on a glassy carbon electrode. High electrocatalytic activity toward hydrogen peroxide, 32 μM–2mM linear range of detection and 2.7 μM limit of detection was obtained for proposed sensor. As well as acceptable reproducibility and selectivity and a good stability was observed for this sensor [15].

Pencil graphite electrode application as working electrode with a large working potentials window, low cost, stability, simplicity and is attracted more attention in recent studies [16-17]. In addition, a renewal surface for pretreatment, fast and simple polishing procedures and good reproducibility are more advantage of pencil graphite electrode [18]. Higher performance of PGE in compare to different electrodes also were reported for ozone in acidic media and dopamine amperometric determination [19-20]. This provides a potential electrochemical approach for the fabrication of different electrochemical biosensors such as cholesterol, glucose, lactic acid, and choline.

Graphene (GF) is a potent conductor of thermal and electrical energy. It's extremely light, pure, amazing thin, chemically inert, and flexible with a low thermal interface resistance. Due to these superb features, it has found many applications such as random access memory, flexible OLED displays, superconductors, supercapacitors, textile electrodes and solar cells as well as super conductors. The main advantage of graphen is low cost in comparison with carbon nanotubes. These advantages make graphene a successful filler to manufacture polymer-based composite materials with improved thermal conductivity, surface to volume ratio and porosity in the film texture [21-23].

Polypyrrole (Ppy) is a conjugated polymer with potential electrical and sensing properties for organic, inorganic and enzyme substrates [24-25]. It has attracted more attention because of its unique physical, electroconductive, antioxidant and biocompatibility effect [26-28].

In this study, a facile eco-friendly one-step

electrochemical method for the fabrication of a Ppy-GF nanocomposite was developed as an electroactive surface modifying material on PGE electrode for insulin detection in human blood serum. Electrochemical behavior of the proposed nanosensor and kinetic of the reaction and diffusion coefficient of the reaction was investigated.

EXPERIMENTAL

Chemicals

Pyrrole (Sigma Aldrich, 99%), HCl (Sigma Aldrich), NaOH (SigmaAldrich, 98-100.5%), phosphoric acid (Sigma Aldrich, 85%), boric acid (Merck), acetic acid glacial (Sigma Aldrich, ≥99.85%), sodium dodecyl sulfate (Sigma Aldrich, 99%), Insulin (Sigma Aldrich) and NaClO₄ and KCl as carrier electrolytes (Sigma Aldrich, 70.0-70.2%), were purchased and used without any purification. Graphene nanoplates, (powder, thickness 4-20 nm, purity >99.5wt%, platelet planar size 0.3-5μm) was purchased from Sigma Aldrich. Pencil lead (RotringGermany, R505210N type H) with diameter of 2.0 mm was used as working electrode in all electrochemical measurements. Deionized water was used to prepare all the solutions. The blood serum sample was prepared from a personal lab.

Instrument

The AUTOLAB (model: PGSTAT302N) with the Nova 1.8 software package (Eco Chemie, the Netherlands) was used to perform electrochemical tests. Measurements conducted in a three-electrode system using PGE, platinum wire and saturated calomel electrode (SCE) as working, auxiliary and reference electrode consequently. All experiments were carried out at room temperature without oxygen dehydration of solutions.

PGE modification

PG electrode was chosen as a working electrode. About 2 mm of the electrode was polished on 2000 mm soft sandpaper before starting each test. So that, the preserved effects of the previous test were completely cleaned up. GF nanoparticles were coated on the tip of the electrode according to our previous method [29]. The GF dispersions were first dropped on PGE exterior and then pyrrole was electrochemically deposited in aqueous solution using CV method. Pyrrole was electro-polymerized on the top of GF electrode using cyclic sweep for 8 cycles in potential range of 0.1V to 1V and at scan rate of 50mV/s to get of uniform thickness film. The

grapheme modified PGE was washed thoroughly and immersed into Ppy 0.1M solution.

The fabrication process of the Ppy-GF nanocomposite modified PG electrode is schematically illustrated in Fig. 1. GF has become the next generation of photonic and electronic components due to its superb properties in electrical conductivity as the replacement of silicon [30]. During the polymerization of pyrrole on GF/PGE positive charges are created in its structure [31]. Thus, electrostatic interactions assisted the immobilization of GF on the electrode surface as an efficient linker. Appropriate conjugation to the nanocomposite and mechanical stability was obtained likewise [32-34]. Furthermore the carboxylic acid group of insulin strongly conjugated to amin group of pyrrole in electrochemical reaction. The resulting Ppy-GF composite supposed to exhibit excellent

immobilization ability, charge transport properties, and sensing responses due to the synergistic effect [15]. Modified electrode was submerged in a solution of sodium hydroxide 0.5 M and applies a constant voltage of 1.2 volts increased to 8 volt and then applied for insulin detection.

RESULTS AND DISCUSSION

Morphological structure

Morphological structure of cathode was examined after modification process of PGE by scanning electron microscope (SEM). The irregular granular pattern of particles with minor ripples for graphite and two-dimensional sheet for GF have been reported in previous studies [35]. Surface feature of the GF/PGE is presented at different magnification in Fig. 2 (A and B). A lot of GF plates were homogeneously covered the electrode surface

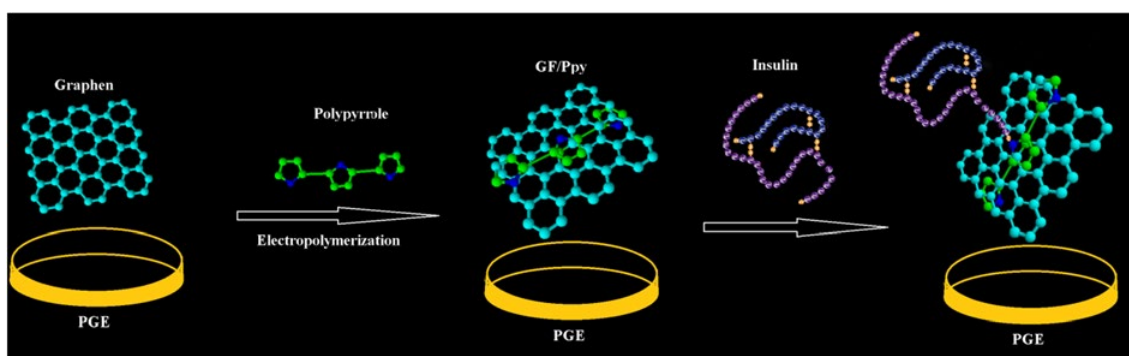


Fig. 1. Schematic illustration of polypyrrole/graphene naocomposite (PPy-GF) modified pencil graphite electrode (PGE) for insulin detection

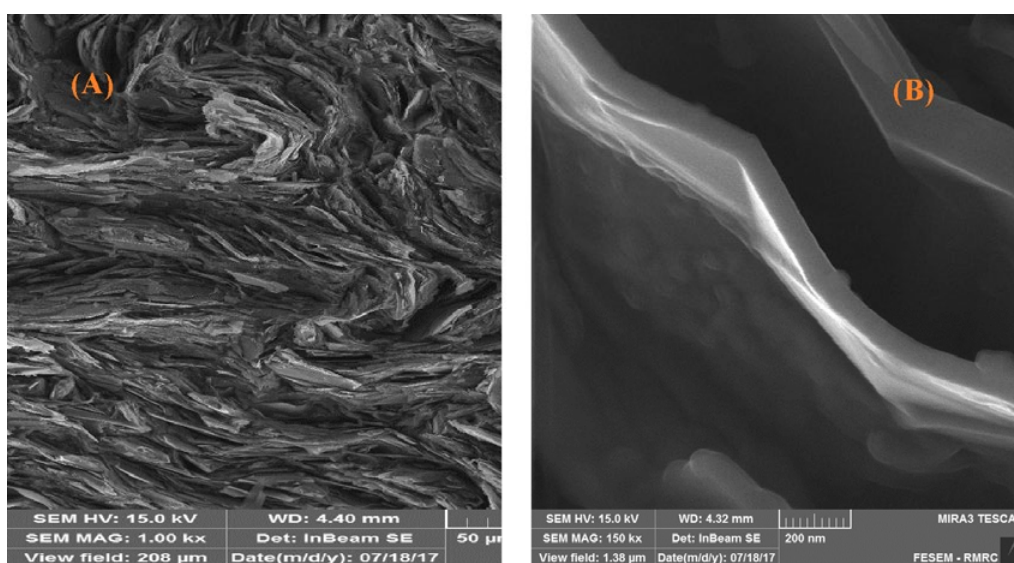


Fig. 2. SEM of GF modified PGEat different magnification (A) at 1.0 Kx and (B) at 150 Kx

and confirmed effective immobilization of GF on the electrode. The average thickness of plate was approximately 20–50 nm (Fig. 1B). Highly porous matrix was obtained for interconnected network of Ppy which provides large surface area for immobilization of insulin.

Differential Pulse voltammetry

Differential pulse voltammetry belongs to one of the most selective and sensitive electrochemical methods for determination of materials. Electrochemical behavior of insulin on the bare and modified electrode was investigated by differential pulse voltammetric method. In this method PGE and Ppy-GF modified PGE were immersed in the Britton-Robinson buffer solution (BRBS) (0.04M) containing 0.695 μM insulin and the corresponding voltammogram was presented in Fig. 3. At a constant concentration a weak oxidation peak was observed in voltammogram of insulin. The intensity

of the oxidation peak significantly increases in GF modified PGE and maximum intensity was observed for Ppy-GF modified PGE. It seems that electrode modification create active sites on the surface of the electrode. Thus, the modified Ppy-GF/PGE has been used in further studies.

Effect of pH

The effects of pH on the electrochemical response of insulin at PGE were recorded in 0.659 μM insulin in BRBS at pH values between 6.0 and 12 and are presented in Fig. 4A. As shown in Fig. 4(A, B and C) anodic peak potential of insulin moved to the positive potentials and a decrease of pH according to the equation, $E_p (mv) = -0.3pH + 0.740$ ($R^2 = 0.994$) (Fig. 4B) in the range of pH 6.0 to 12. It reveals that, the peak potential of insulin was irreversible and pH-dependent between pH 6.0 and 12.0 with a slope of -0.3. There was no significant peak for insulin at pH less than 6.

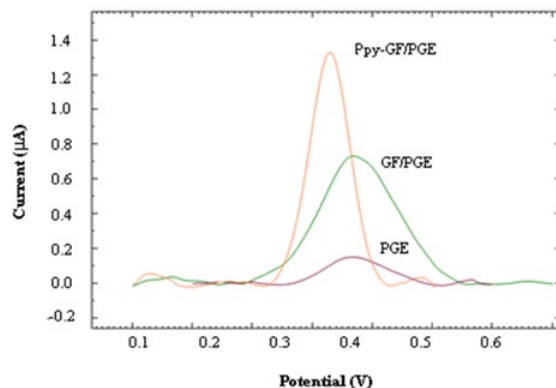


Fig. 3. Differential pulse voltammogram of 0.695 μM of insulin in BRBS (pH=11) and KCl (0.2 M) at PGE, modified GF/PGE and Ppy-GF/PGE (pulse amplitude: 0.1-1V; scan rate: 50 mV s⁻¹; pulse time: 25 s)

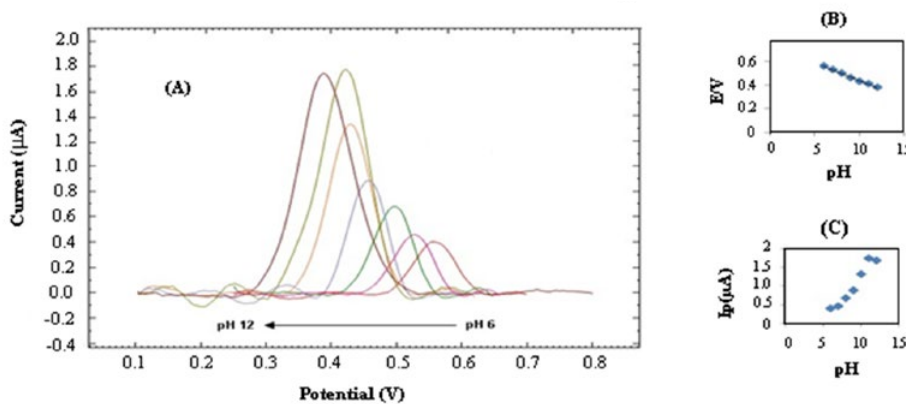


Fig. 4. (A) Differential pulse voltammogram of 0.695 μM insulin in BRBS and KCl (0.2 M) in different pH (B) potential versus pH, (C) current versus pH.

Calibration curve of nanosensor

The concentration dependence of insulin in BRBS(pH=11) and KCl 0.3 M was measured in the range of 0.252 to 1.235 $\mu\text{mol.L}^{-1}$ and presented in Fig. 5A. Fig. 5B is the calibration curve obtained from the information of previous concentration voltammogram. Linear response was observed in the range between 0.252 and 1.235 μM based on equation of $I_p(\mu\text{A})=2.711C(\mu\text{M})-0.002$, $R^2=0.999$. The LOD was computed using calibration curve and obtained about 7.82nM. The result shows that the Ppy-GF/PGE electrode has a high sensitivity and LOD compared to the other reported electrodes [1, 36-37]. Accordingly, Ppy-GF/PGE has good electrocatalytic activities versus insulin. Therefore, the Ppy-GF/PGE electrode can be recommended as an alternative method for insulin characterization.

Electrochemical behavior of insulin

The voltammetric behavior of insulin (0.695 μM) at PGE in the BRBS (pH=11) and different scan rate was studied (Fig. 6A). It is revealed that the peak current increase with increasing scan rate and peak positions are shifted positively in higher scan rates. The change in the intensity of cranial oxidation peak of insulin in terms of scan rate was linear with r square of 0.9719 (Fig. 6B).

Electrochemical behavior of insulin on the electrode surface was obtained from the relationship between peak current and square root of scan rate. A linear correlation in the potential range of 0.1-0.8V and scan rate of 10–400 mV/s was observed for insulin (Fig. 6C), which is of a typical diffusion controlled reaction [38-39]. The equation can be exhibited as

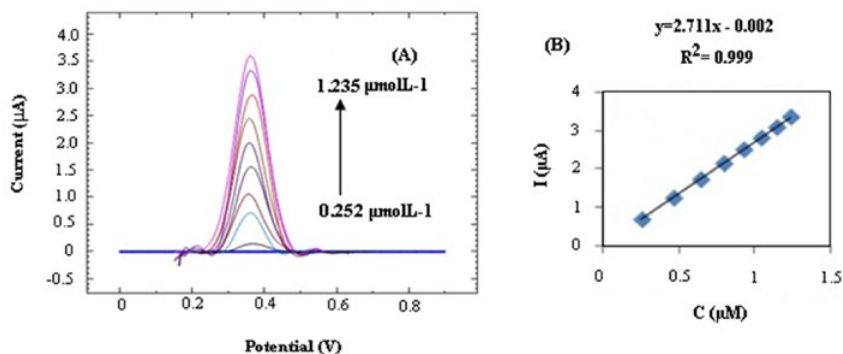


Fig. 5. Differential pulse anodic voltammogram of insulin at GCE in the BRBS(pH=11) in the concentration range of 0.252 to 1.235 μmolL^{-1} , (B) concentration dependence of insulin

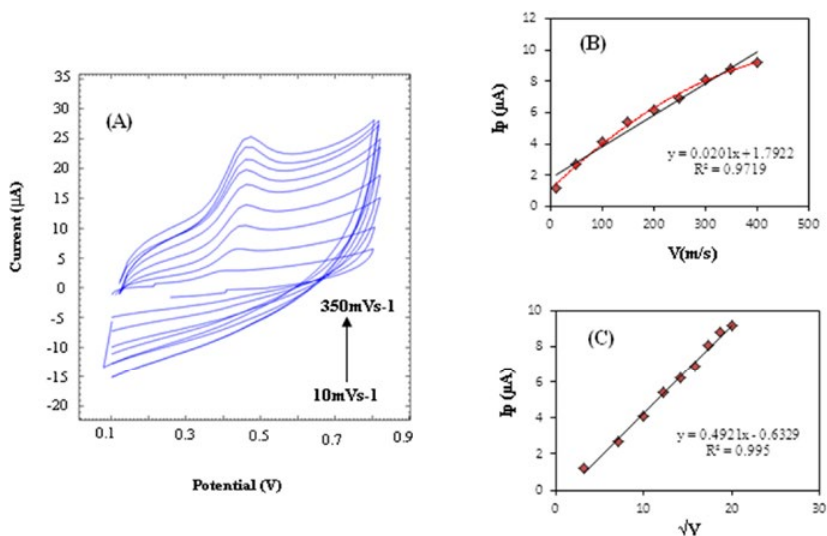


Fig. 6. Cyclic voltammograms for the oxidation of insulin at several scan rates (1) 10 (2) 50 (3) 100 (4) 150 (5) 200 (6) 250 (7) 300 (8) 350 mVs^{-1} (B) Changes in the intensity of cranial oxidation peak of insulin in terms of scan rate (C) square root of scan rate versus oxidation peak current

$$I_{pa} (\mu A) = 3.8178v^{1/2} (V^{1/2}S^{-1/2}) + 0.3857, (r = 0.9874) \quad (1)$$

These results can be related to the adsorption of the analyte at PGE, indicating that the oxidation of insulin was a surface-controlled red-ox process. Logarithm of scan rate versus logarithm of anodic peak current is plotted in Fig. 7. In this analysis a linear relation with a slope value of 7.06 was observed for modified electrode. This indicated diffusion controlled process in which electroactive insulin was diffused to a planar electrode surface from bulk solution.

Kinetics of the oxidation reaction

An successful electrode kinetic theory must explain Tafel behavior. It was experimentally observed that at low currents, the current was

exponentially related to the overpotential according to Tafel equation. To study the kinetics of insulin oxidation reaction a cyclic voltammogram of a modified PG electrode in a 0.04 M BRBS (pH = 11) and KCl (0.2 M) in insulin solution $0.46 \mu\text{mol.L}^{-1}$ was recorded with scan rate of 10 mVs^{-1} (Fig. 8A). The Tafel curve drawn for this voltammogram is presented in Fig. 8B. Electron transfer coefficient was calculated using Tafel equation. In this chart, $\log I$ is plotted versus potential (in the upturn of the voltammogram). The amount of electron transfer coefficient between modified electrode and insulin was obtained to 0.5. Also the number of electrons exchanged during oxidation of insulin was calculated $0.84 \sim 1$.

Diffusion coefficient of nanosensor

Fig. 9A shows chronoamperometric measurements of insulin with modified Ppy-GF/PGE to

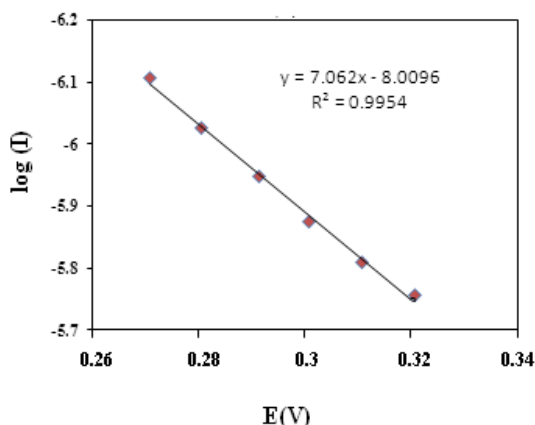


Fig. 7. Dependence of oxidation peak potential on the logarithm of scan rate for the oxidation of insulin at various scan rates

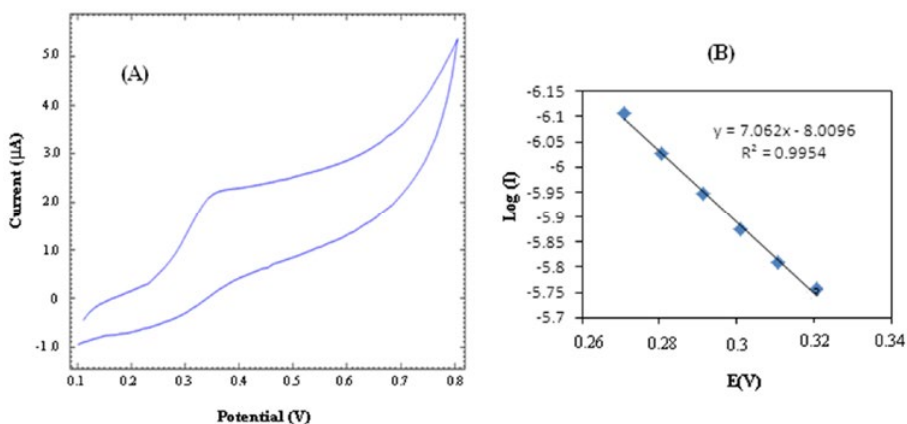


Fig. 8.(A)Cyclic voltammogram of a modified PG electrode in a 0.04 M BRBS(pH = 11) and KCl (0.2 M) in insulin solution $0.46 \mu\text{mol.L}^{-1}$ with scan rate of 10 mVs^{-1} , (B) Tafel curve of related CV on modified PGE.

better understand the processes occurring at the electrode-electrolyte interface. The useful net current signals were obtained for various concentrations of insulin at 100 seconds. It can be observed that the current intensity linearly is dependent on insulin concentration in the explored concentration range between 4.63 μM and 10.41 μM (Fig. 9B).

Since the transfer of materials in these conditions is entirely through diffusion, the current-time plots reflect the concentration gradient near the electrode surface. By gradually expanding the diffusion layer, the reagent concentration decreases and the slope of the concentration profile versus time decreases consequently. Therefore, the current is reduced according to the Catterle equation in proportion of time:

$$I = \frac{nFACD^{1/2}}{\pi^{1/2}t^{1/2}} = Kt^{-1/2} \quad (3)$$

Where D is diffusion coefficient, C bulk concentration, n number of electrons, F Faraday's constant, and A electrode area.

The slope changes of the current-reverse square time plot were presented in Fig. 8C for different concentrations of insulin. The slope of the plot

demonstrated to diffusion coefficient which was obtained as $5.014 \times 10^{-2} \text{ cm}^2\text{s}^{-1}$ for insulin with voltage step of 0.5 V.

Analysis in blood serum

In order to evaluate the effectiveness of the proposed sensor for determining insulin in biological samples, the sensor was used to insulin analysis in human blood serum by standard addition method (Fig. 10A). To explore relative recovery of the method, blood serum samples were diluted 10 times with 0.04 M BRBS (pH =11) and KCl (0.2 M) and spiked with concentrations 0.245 μM, 0.45 μM, 0.775 μM, 0.906 μM of insulin (Fig. 10B). Linear equation of regression, $I_p \text{ (nA)} = 0.254 C \text{ (}\mu\text{M)} + 0.222$, $R^2 = 0.993$, in Fig. 10B was used for measurement of the insulin in a blood serum sample.

Recovery and reliability

The result of the recovery for the spiked samples was 97.8% which indicated that the detection procedures were free from interrupting by the blood serum sample matrix. The results demonstrated a comparable detection limit with a wide linearity range of insulin determination in compare to other established sensors [40-41].

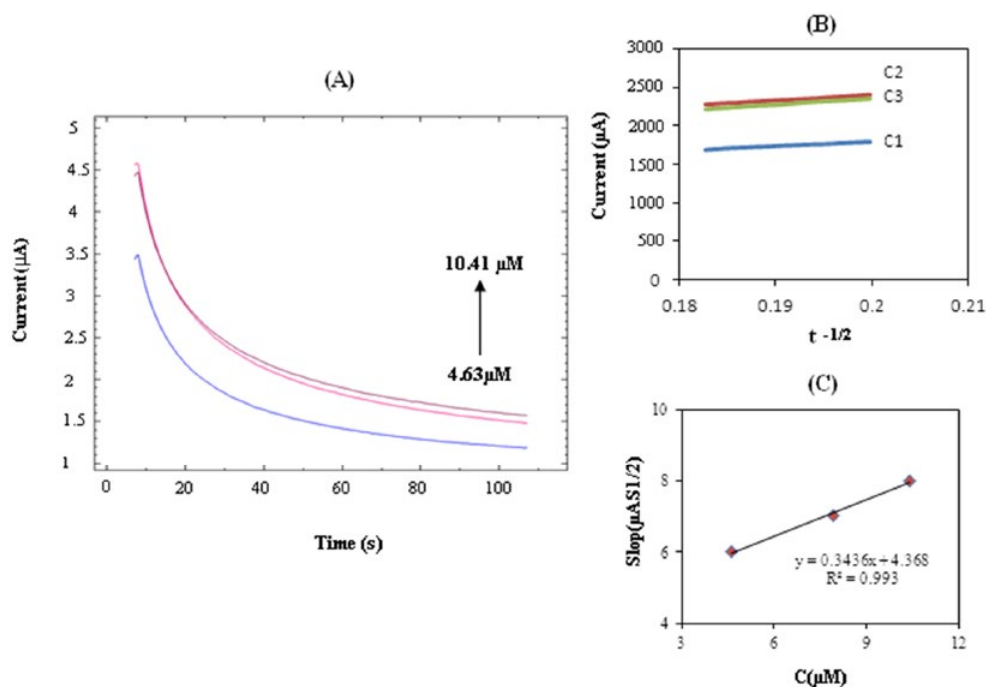


Fig. 9. (A) Chronoamperograms obtained at Ppy-GF/PGE in the presence of (C1) 4.63 μM, (C2) 7.94 μM, (C3) 10.41 μM, insulin; 0.1 M Na₂SO₄ supporting electrolyte; (B and C) the calibration plots of current

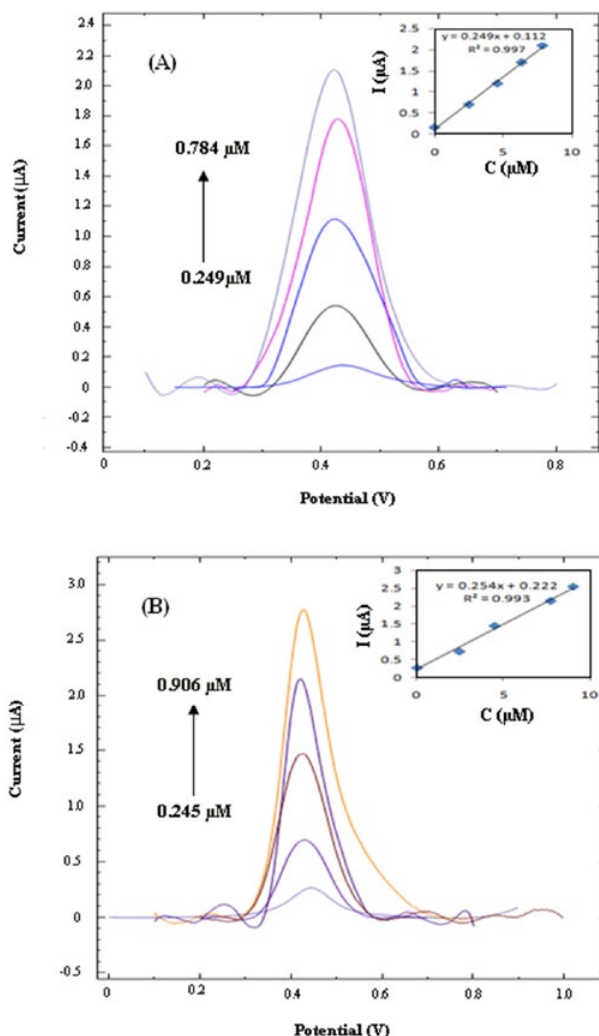


Fig. 10. (A) Chronoamperogram of different concentrations of insulin at the PGE: (a) 0.249 μM , (b) 0.457 μM , (c) 0.633 μM , and (d) 0.784 μM . standard addition method. Inset: related calibration graph. (B) Chronoamperogram of insulin spiked with concentrations (a) 0.245 μM , (b) 0.45 μM , (c) 0.775 μM , and (d) 0.906 μM added to a blood serum sample. Inset: related calibration graph.

CONCLUSION

In this paper, Ppy-GF nanocomposite with high electroactive material was synthesized and used for accurate and rapid electrochemical detection of insulin. Different voltammetric techniques (CV, DPV and CA) were used for the optimization and analysis. The effect of various kinetic parameters for electrochemical oxidation of insulin on modified electrode was investigated. The calibration curve was obtained using the CA method in a vast linear and low detection limit. The standard addition method was used to determine insulin in blood serum samples. The reliability and applicability of the Ppy-GF/PGE were studied by spiking insulin into blood serum samples and good recovery values demonstrated this issue.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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