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Modified Glass Carbon Electrode (GCE) Electropolymerized Polypyrrole Nanofibers with Hemoglobin (Hb) Film as a Unique Biosensor for Nitrite Determination

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Abstract: In this study, we were investigated behavior the electrochemical reduction of nitrite at a hemoglobin (Hb) immobilized on glass carbon electrode (GCE) containing polypyrol nanofiber (ppy) films. Polypyrrole (PPy) nanofibers have been constructed on GCE applying electrochemical technique, and can to deposit diverse polymers on miniaturized electrodes with this common method. The structure of the acquired electrode and the electrochemical behavior were characterized using Scanning Electron Microscopy (SEM), Cyclic Voltammetry (CV), and Linear Sweep Voltammetry (LSV). The immobilized Hb showed an irreversible reduction peak with a formal potential of –0.72 versus Ag/AgCl, in 0.1M pH 4.5 acetate buffer. The immobilized Hb exhibited remarkable electrocatalytic activity for the reduction of nitrite. Under the optimal condition the nitrite concentrations range 2.0×10^{-4} to 46.0×10^{-4} M with a detection limit of 6.2×10^{-5} M (at S/N=3). The resulting biosensor has been successfully applied to the determination of nitrite in water samples. The produced modified electrode for nitrite determination has been explored within the presence of the interfering substances including a number of common ions and cations, demonstrating great selectivity.

Keywords: Hemoglobin, Polypyrrole Nanofibers, Nitrite, electrochemical sensor, biosensor.

1. INTRODUCTION

Nitrite ion one of important ions that has been reported as a human wellbeing risk that broadly exists in the environment. Also, Nitrate can convert to nitrite by nitrate-reducing bacteria, so the excess nitrite can produce N-nitrosamines,

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which would cause carcinogens. Forming met hemoglobin, when nitrites react with the iron (III) of the hemoglobin, which has no oxygen transfer, this phenomenon occurs, when the concentration of nitrites in the blood are high. In addition, nitrite create nitrosamines with ceratain amine that these found in food which recognized to be powerful cancer-causing chemicals. Antioxidants like Vitamin C and Vitamin E that extant in food may be inhibit Nitrosamine formation. Some research facility contemplates when rodents were given significant levels of nitrites alongside amine-containing chemicals, cancer of the liver, lung, and throat were watched [1, 2].

However, nitrite extant ubiquitous naturally in waters, physiological systems, soil, and foods such as vegetables owing to the use of nitrogen-based manure, or may be added to foods to the preservation of meat product as regularly as it was hundreds of years back. In addition, nitrite is additionally utilized widely in prepared nourishment for a specific aim, especially to reestablish shading or to improve flavor to a nourishment, and participating in many physiological processes including neurotransmission, apoptosis, inflammation, vasodilatation, and, cell proliferation [1, 3, 4]. Accordingly, long haul utilization of nourishments containing nitrite will cause a potential danger of malignancy or death [5]. Because of these realities Committee of the Food and Agriculture Organization of the United Nations and World Health Association have been built up greatest admission level for nitrate in the scope of 0.1–3.7 mg/kg body weight [6, 7] .

Consequently, the quantitatively recognize nitrite in food, environmental, and biological samples is of incredible significance [4]. To date, several analitycal method such as electrothermal-atomic absorption spectrometry (ET-AAS) [8] high-performance liquid chromatography (HPLC) [9] capillary electrophoresis [10, 11] spectrophotometery [12] have been utilized in the analysis of nitrate in various examples. In any case, there are numerous downsides related with these technologies, and the most significant of these are high detection limits, poor repeatability, tedious pretreatment prerequisites, extreme interference from other ionic species, and complex, and low separation efficiency. Subsequently, it stays a gigantic test to acquire, specific, quick, simple and sensitive detection of nitrite [4].

In this exploration, direct electrochemistry of hemoglobin as a model protein has been examined on polished carbon electrode altered by electropolymerized polypyrrole (PPy) nanofibers. At that point, the electrocatalytic conduct of joined hemoglobin has been researched toward nitrite.

2. EXPERIMENTAL

2.1. APPARATUS AND REAGENTS

Electrochemical measurements were performed with 302N electrochemical station (Autolab Instrument, Swiss). The electrochemical cell consisted of a three-electrode system with a modified glass carbon electrode as the working electrode. An Ag/AgCl (3.0 M KCl) electrode and platinum wire electrode were used as the reference and the auxiliary electrode, respectively. All potential in the paper are referenced to this Ag/AgCl (3.0 M KCl) electrode. All experiments were performed at ambient temperature (20±2 ºC). All solutions were purged with purified nitrogen for at least 5.0 min prior to each set of experiments. A nitrogen environment was then maintained over the solutions used in the cell. A Metrohm pH meter 691 was employed for pH measurements. All the reagents were of analytical reagent grade and were used without further purification. The standard stock solution of nitrite (0.1M) was prepared by dissolving 0.690 g of $NaNO_2$ (Merck) in water in 100 cm^3 volumetric flask.

Working solutions were prepared by appropriated dilution of the standard solution used in the calibration curve was obtained by subsequent dilution of the stock solutions. A 1.55×10^{-5} M solution of Hb was prepared by dissolving 0.010 g of Hb in 10 cm^3 of water. Buffer solutions of pH 4.5 were prepared by mixing soda (1M), acetic acid solution (0.05M). The stock solutions of sodium carbonate (Merck, 1.0 M), sodium perchlorate (Merck, 1.0 M), and pyrrole (Merck, 0.5 M) were prepared by dissolving appropriate amounts of their solids, and liquid in water, and diluting to the mark with water. A pyrrole (py, Merck) was vacuum distilled, and stored at lower than 5 ºC at dark.

2.2. GENERAL PROCEDURE

All glassware used in the following procedures was cleaned in the bath of freshly prepared concentrated sulfuric, and nitric acid (1:1), rinsed thoroughly in doubly- distilled water, and dried in air. Briefly, 0.013g of Hb was added to 10 cm^3 of double distilled water.

Glass carbon electrode (GCE, 3.0 mm in diameter) was polished with 0.3 μm alumina slurry, and rinsed thoroughly with double- distilled water. Them the electrodes were successively sonicated in 1:1 nitric acid, acetone, and doubly-distilled water, and allowed to dry at room temperature. For the modified electrode, GCE was immersed in solution containing 0.1M sodium carbonate, 0.2M sodium perchlorate, and 0.015M py. Then, a fixed potential of about 0.95V vs. A/AgCl (3.0 M KCl) reference electrode was applied to the electrode for 400s. Then, the modified electrode surface was washed three time with

perchloric acid (0.1M), and distilled water for removing physically absorbed ppy on the electrode surface. 0.015g Hb was dissolved in 10 cm^3 of distilled water. The GCE/ppy was immersed in solution Hb (1.55×10^{-5} M), and then was left for a least 2h at 5ºC. Thus, GCE/ppy/Hb electrode were obtained. The modified electrode was stored in buffer solution pH 4.5 at 5ºC in a refrigerator when not in use.

3. RESULTS, AND DISCUSSION

3.1. CHARACTERIZATION OF GCE/PPY/HB ELECTRODE

3.1.1. SCAN ELECTRON MICROSCOPY (SEM)

The SEM image (Fig. 1) exhibit the morphology of (a) ppy nanofibers electro synthesized on GCE (b) cross-linked GCE/PPy/Hb layer. Obviously, the SEM image of Hb cross-linked PPy layer (Fig. 1b) was different from that of GCE/PPy (Fig. 1a). Some small flower link accumulations with different shapes were observed in Hb cross- linked PPy fiber, which was formed by aggregates of Hb molecules with PPy fiber during adsorption process.

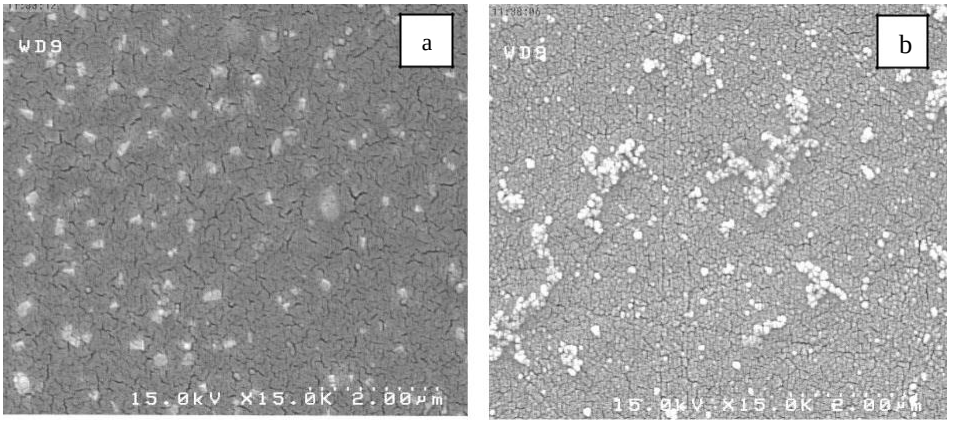

Fig. 1. SEM image of a) PPy nanofibers formed on GCE by electrochemical oxidation of Py, b) adsorbed Hb on GCE/PPy.

3.1.2. ELECTROCHEMICAL BEHAVIOR OF GCE/PPY/HB

The electrochemical behavior of the Hb immobilized in adsorbed PPy fiber modified electrodes in 0.1 M acetate buffer pH 4.5 at 100 mV/s was studied by linear sweep voltammetry (LSV). Fig 2 shows the LSV obtained with GCE (a) GCE/PPy (b), and GCE/PPy/Hb (c) in presence nitrite (0.012 M). No peak was observed at GCE, GCE/PPy (curve a and b in Fig. 2), which indicated that GCE and GCE/PPy were not electroactive in the potential window. However, when Hb was adsorbed on the surface GCE/PPy electrode, and fabrication modified electrode, the LSV displayed reduction peak between Hb, and nitrite (curve c in Fig. 2).

The formal potential $E^{0/}$ (reduction peak potentials in the linear sweep voltagram) is about -0.72 V versus. A/AgCl (3.0 M KCl). The results present strong evidence that direct electron transfer between Hb, and nitrite. Thus, the heme groups in Hb molecules still remained their structure, and electrochemical activity.

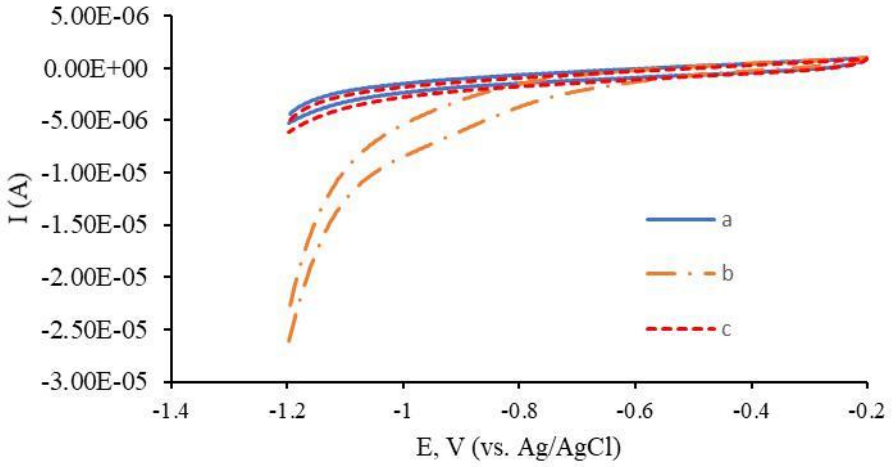

Fig. 2. Linear sweep voltammograms of (a) GCE, (b) GCE/PPy, and (c) GCE/PPy/Hb in buffer acetate pH 4.5 (0.1M), and in the presence nitrite 0.012 M at a scan rate of 100 mV/s.

3.1.3 OPTIMIZATION OF PH

The redox demeanor of protein is often crucially dependent on the solution pH. The pH-dependence of the formal potential $E^{0/}$ for the redox couple indicates the contribution of proton in the electrochemical process[13]. The

effect of pH, cyclic voltammetry (CV) study of nitrite reduction at GCE/PPy/Hb was carry out in 0.1 M acetate buffer solution (from 3.5 to 5.5), and 0.1 M phosphate buffer solution (from 6.5-8.5). Figure 3 shows peak current for nitrite reduction on the GCE/PPy/Hb against different pHs of the buffer solution. As seen in figure 3, the maximal peak current for nitrite reduction was attained at pH 4.5. Thus pH 4.5 was chosen as the optimal pH for the determination of nitrite. Also in plenty study nitrite reduction was studied about pH 4.5 [1], [3], [14]–[17].

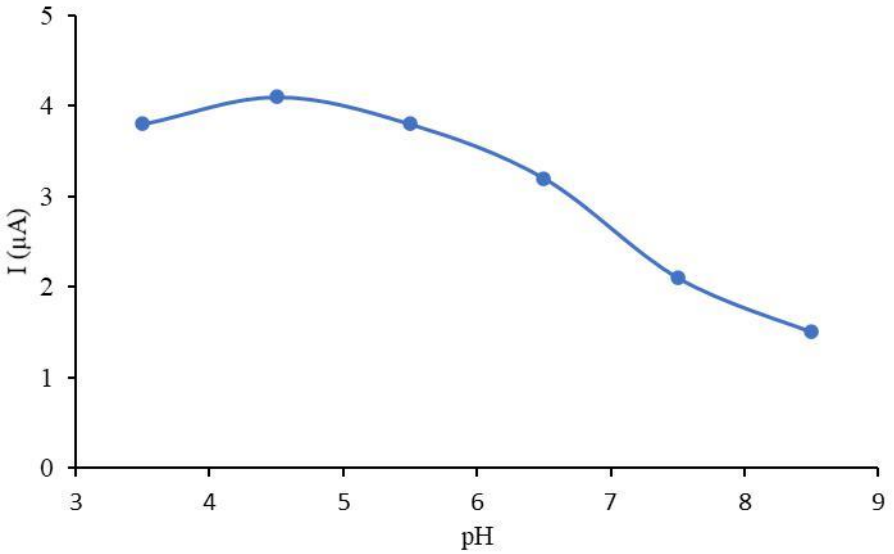

Fig. 3. Plot of values of NO^{2-} reduction peak currents versus pHs. Scan rate: 0.1V s^{-1}.

Furthermore, there is a linear relationship with the pH value (Fig. 4) with the slope value of −49.1 mV pH^{-1}. This slope value was close to the theoretical value of 59.0 mV pH^{-1} at 25℃, indicating an one proton and one electron transfer reaction [18]. The reaction conspire for the electron transfer procedure of Hb can be depicted as reference [19]:

$$\text{Hb heme Fe (III)} + H^{+} + e^{-} \rightarrow \text{Hb heme Fe (II)}$$

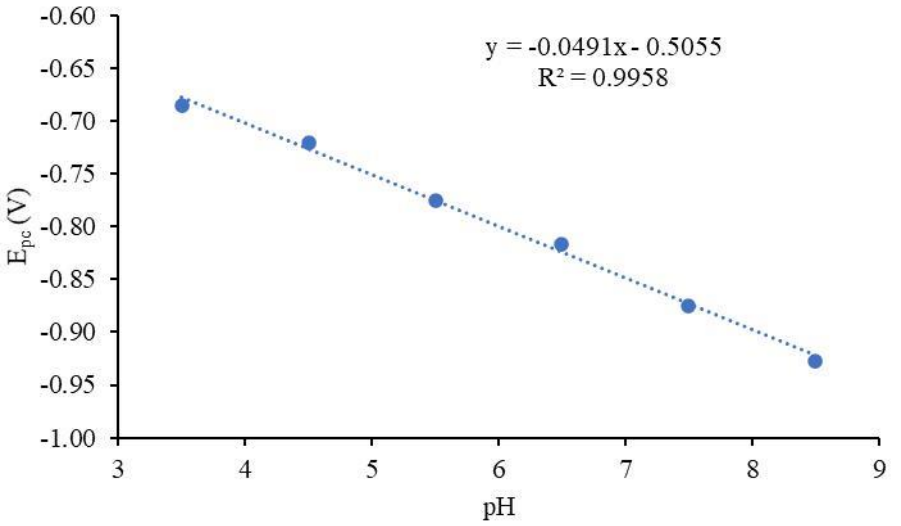

Fig. 4. Plot of Ep vs. pH.

3.1. 4. INTERFERENCE STUDIES

Generally, at negative potential, less interference affects the detection of NO_2^- except the dissolved oxygen. All solutions in this work were deoxygenated by bubbling pure nitrogen for at least 15 min and maintained under nitrogen atmosphere during the measurements so that it avoided the interference from the dissolved oxygen.

3.2. ANALYTICAL FIGURES OF MERIT

The calibration curve for the determination of nitrite was prepared according to the general batch procedure under the optimum conditions developed above (see Fig. 5). The detection limit (S/N=3) was 6.24×10^{-5} M for nitrite under the optimum conditions. Calibration linearity was obtained in the range of 2.0×10^{-4} - 46.0×10^{-4} M of nitrite with a correlation coefficient (R^2) of 0.998. The relative standard deviation was found to be 0.42% for 30.0×10^{-4} M of nitrite for ten repetitive determinations. The results demonstrated that a 200-fold of, like NO_3^-, Cl^-, Al^{3+}, CO_3^{2-}, PO_4^{3-} , Ac^-, Cd^{2+}, Na^+, K^+, Ca^{2+} and Mg^{2+}, and 50-fold of Mn^{2+}, Pb^{2+}, Zn^{2+} and SO_4^{2-} doesn't interfere in the determination of nitrite at 10.0×10^{-4}M.

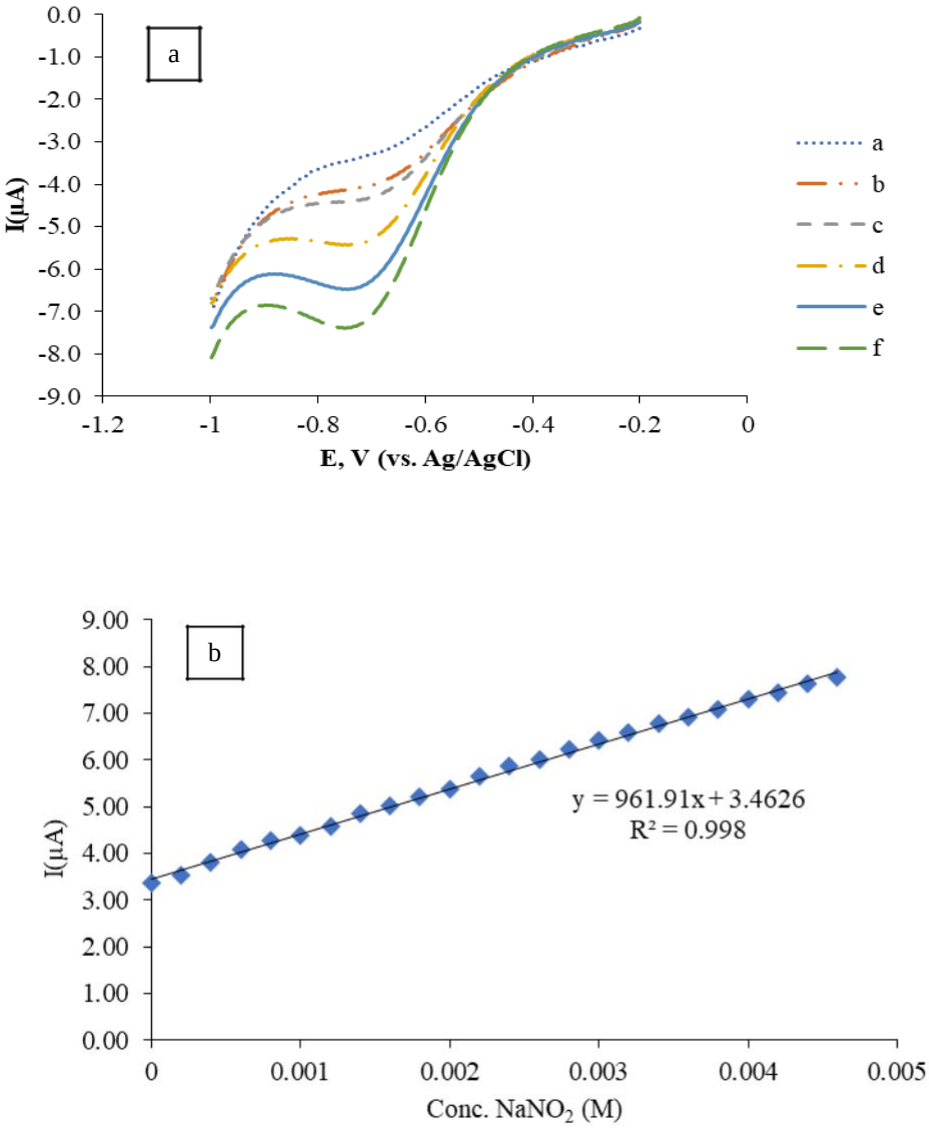

Fig. 5. (a) Typical LSV curves of different concentrations of nitrite on GCE/PPy/Hb in 0.1 M acetate buffer (pH 4.5), Concentration of nitrite (a-f): 0.000, 0.0006, 0.001, 0.002, 0.003, and 0.004 M. Scan rate 0.100 V/s. (b) Calibration curve for different concentrations of nitrite at the optimum condition.

4. CONCLUSION

We have successfully developed a novel, and simple biosensor. In this paper, we have demonstrated the use of modified electrode (GCE/PPy/Hb) for a greatly enhanced linear sweep voltammetry biosensor of nitrite in pH 4.5 acetate buffer. The cross-linked polypyrrol nano fiber substrate, can form networks, and provide a suitable biomembrane-like microenvironment for Hb, which greatly facilitated the electron exchange between Hb and electrodes. Hemoglobin can be effectively immobilized on GCE surface by incorporated with polypyrrol nano fiber, this (ppy/Hb) film proposed high electrochemical activity and shows a fast-direct electron transfer of Hb. A comparative study of electrodes, bare GCE, GCE/ppy, and GCE/ppy/Hb demonstrates that the adhesion of GCE/ppy into Hb can fundamentally enhance the direct electron transfer between Hb and electrode, and therefore improve the electrocatalytic ability towards nitrite reduction. Subsequently, the GCE/ppy/Hb demonstrate good selectively and sensitivity for the LSV determination of nitrite. This method offers an economical, simple, and convenient way to obtain high quality detectors for the detection of nitrite.

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