Disposable Redox Polymer Coated Screen-Printed Carbon Electrode for NADH Sensing

Ashok K. Sundramoorthy1,*, Szu-Ling Chen and Shen-Ming Chen#

School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637459, Singapore
Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No. 1, Section 3, Chung-Hsiao East Road, Taipei, 106, Taiwan (ROC)

Abstract: Electrochemical behavior of electro-generated poly-pyronin B (PyB) film was reported at a screen-printed carbon electrode (SPCE). The poly(PyB) modified SPCE showed excellent redox activity in neutral and alkaline media. Surface topography of poly(PyB) film modified electrode was analyzed by using atomic force microscopy (AFM) and scanning electron microscopy (SEM). Poly(PyB) film coated electrode was characterized by electrochemical impedance spectroscopy (EIS), UV–visible absorption spectroscopy (UV–vis) and cyclic voltammetry (CV). The poly(PyB) modified electrode showed electrocatalytic response to the reduced form of nicotinamide adenine dinucleotide (NADH) in physiological condition, and was used for the detection of NADH with high selectivity. The anodic peak current was linearly related to concentrations of NADH over the range from 1×10^-5 M to 5×10^-4 M, and the detection limit was 5×10^-7 M (S/N = 3).

Keywords: Electroactive polymer, Modified Electrode, Chemical sensors, Electrocatalytic oxidation, NADH.

1. INTRODUCTION

Reduced form of nicotinamide adenine dinucleotide (NADH) is an important coenzyme and a most common cofactor of dehydrogenases. NADH can be reversibly oxidized into an enzymatically-active form (NAD+) via electrochemical oxidation [1-8]. Generally, oxidation of NADH requires high positive voltage (>0.7 V) at bare electrodes (glassy carbon, Au, Pt etc.) [9]. Also, adsorption of oxidation products of NADH on the electrode surface greatly affects the performance of the working electrode. To reduce the overpotential required for the oxidation of NADH, electrochemically or chemically modified electrodes (with redox active functional groups) have been developed for electrochemical oxidation of NADH in neutral buffer solution at lower potential [1-8].

Redox chemicals and dyes [5, 10-26], electroactive polymers [27-30], metal complexes [31], thionine bridged carbon nanotubes/gold nanoparticles multilayer [32], poly(β-aminobenzene sulfonic acid) film [33], redox-active (NC)2C6H3–NO/(NC)2C6H3–NHOH(NOPH/NHOHPH) film [34], polysulfone composite film [35], gold nanoparticles/poly(3,4-ethylenedioxythiophene)–poly (styrene sulfonic acid) films were reported for oxidation of NADH [36].

Electrochemically generated polymers are more promising in the development of chemical and biosensors [37-40] and also have found applications in fuel cells, electrochromic displays [41, 42]. One of the most promising methods to modify an electrode surface is electropolymerization which can produce adherent and conducting polymer thin-films. Polymer film thickness, permeation and charge transport characteristics can be easily controlled.

Electrochemical polymerization will also help to achieve a thin uniform polymer film with strong adherence to the electrode surface [37, 40]. Polymer modified electrodes can be easily employed for the electrocatalytic oxidation of NADH because they offer more reactive sites than monolayer modified electrodes [8, 37, 39, 40].

Pyronin B (PyB) (Scheme 1A) is a water soluble organic dye and used as a staining agent in biology [43-46]. We reported electrochemical polymerization of PyB on a glassy carbon electrode for the detection of NADH [47]. In this paper, we report the electrochemical polymerization of PyB and its electrochemical properties on a screen printed carbon electrode (SPCE). Electro-polymerization of PyB is carried out in acidic solution using SPCE or indium tin oxide coated glass electrode as working electrode. Surface characterization of poly-Pyronin B film was investigated by atomic force microscopy (AFM) and scanning electron microscopy (SEM). We studied oxidation of NADH at poly-Pyronin B/SPCE in 0.1 M phosphate buffer solution (pH 7.0).

2. EXPERIMENTAL

2.1. Reagents

Pyronin B, β-NADH, dopamine hydrochloride and uric acid were received from Sigma–Aldrich. P-acetaminophen,
H_2SO_4 and NaOH were purchased from Wako pure chemicals (Osaka, Japan). Ascorbic acid, CH_3COONa and NaH_2PO_4 were received from E-Merck (Darmstadt, Germany). Nitrogen gas was purged to remove oxygen from the electrochemical cell.

2.2. Apparatus

Electrochemical measurements were performed with CH Instruments (TX, USA) Model-400 potentiostat with conventional three-electrode cell. SPCE (Purchased from Zensor R&D, Taichung, Taiwan) or indium tin oxide coated electrode (ITO from Merck display technologies, ltd, Darmstadt, Germany) and platinum wire were used as the working electrode and auxiliary electrode, respectively. ITO substrates were cleaned by using a detergent and followed by diluted hydrochloric acid and distilled water.

Ag/AgCl [KCl (sat)] electrode was used as a reference electrode. Surface analysis was performed by using Hitachi scientific instruments (London, UK) Model S-3000H SEM and a multimode scanning probe microscope system (CSPM4000 Instruments, Ben Yuan Ltd, Beijing, China). Impedance measurement units IM6ex ZAHNER, Messsysteme (Kroanch, Germany) was used to measure the resistance of modified electrodes. Absorption spectra were recorded with a U3300 Spectrophotometer (HITACHI).

2.3. Preparation of Poly(PyB) Modified SPCE

The SPCE with an area of 0.196 cm^2 was used as the working electrode. Before use, it was washed with doubly distilled deionized water. Then, the electrode was pretreated by continuous cyclic sweeps from -0.8 to +2.0 V (vs. Ag/AgCl) at 50 mVs^{-1} in 0.1 M H_2SO_4 solution until a constant background was obtained. Poly(PyB) modified electrodes were prepared by cyclic sweeping at a pretreated SPCE from -0.8 to +2.0 V at 50 mVs^{-1} in aqueous solution of 0.1 M H_2SO_4 containing 1x10^{-3} M PyB monomers for 20 cycles. Subsequently, the resulting polymer film modified electrode was washed thoroughly with doubly distilled deionized water. The poly(PyB)/SPCE was electro-activated by scanning in the potential range between -0.8 and 2.0 V in pH 7.0 buffer solutions using cyclic voltammetry at a scan rate of 50 mVs^{-1} for 10 cycles (This treatment was performed to achieve complete polymerization of physically adsorbed monomers on the electrode surface). After this step, the poly(PyB) film modified electrode was used for further studies.

3. RESULTS AND DISCUSSION

3.1. Electrochemical Polymerization

The cyclic voltammograms (CVs) recorded in PyB aqueous acidic solution indicate the deposition of the poly(PyB) film on the electrode surface (Fig. 1) [47]. The potential sweep range, especially the upper limit, is the most important factor for preparing the poly(PyB) modified electrode. If the potential sweep was confined within the region of -0.8 to 0.7 V, no polymerization was observed. However, electropolymerization was observed when the value of anodic upper potential reached beyond 1.2 V. Fig. (1a) shows the successive CVs of pretreated SPCE in 0.1 M H_2SO_4 solution containing 1x10^{-3} M PyB in a potential range from -0.80 to +2.0 V at a scan rate of 50 mVs^{-1}. During the first anodic scan, an irreversible oxidation wave was observed at +1.1 V which can be assigned to the oxidation of tertiary amino group of PyB monomers [9, 11, 48]. In the first cathodic scan, a cathodic peak was observed at -0.73 V. With increasing potential cycles, polymer film deposition on the electrode surface was confirmed by a redox peak with E' = +0.3 V. The monomer oxidation peak gradually decreased at 1.1 V. The polymer film deposition was almost stopped after the 20th cycle which indicated the saturation of polymer growth. Similar observation is already reported for growth of poly-methylene blue and methylene green [49, 50].

As reported earlier, in the polymerization processes, tertiary amine groups become electroactive nitrogen bridges such as in polyaniline and poly(thionine) films [51, 52]. The hypothetical structure of poly(PyB) is presented in Scheme 1B. After the modification, the modified poly(PyB)/SPCE was electro-activated by scanning in the potential range between -0.8 and 2.0 V in pH 7.0 buffer solutions using cyclic voltammetry at a scan rate of 50 mVs^{-1} for 10 cycles. As it can be seen in Fig. (1b), physically adsorbed monomers with polymeric film on the electrode surface get polymerized. By this treatment, a complete polymerization was obtained on the electrode surface. Consequently, the...
modified electrode was thoroughly washed with water and dried in air for 30 min.

3.2. Electrochemical Properties of poly(PyB)/SPCE

Fig. (2a) shows the CVs for the poly(PyB) film in pH 7.0 with different scan rates. A redox wave appeared and the peak potentials were +0.11 V for $E_{pa}$ and -0.15 V for $E_{pc}$. The peak current was proportional to the scan rates which indicated a surface confined redox process [53,54] (Fig. 2b). According to the equation, $I_p = n^2F^2A^2/4RT$ and $Q_p = nF\alpha$, the electron transfer number was two for poly(PyB) redox couple and the surface coverage was $8.672 \times 10^{-11}$ mol/cm$^2$.

Nearly reversible CVs with stable and well-defined redox peak were obtained for poly(PyB)/SPCE in the pH range of 1.0 to 10.0 (Fig. 3). The pH increase led to a negative shift in formal potential, $E^{\circ'} = (E_{pc} + E_{pa})/2$ (Inset of Fig. 3). The shift in $E^{\circ'}$ was dependent on pH, suggesting that the redox reaction was accompanied by the transfer of proton. The value of the slope (-66 mV/pH) was little higher than the theoretically expected value, -59 mV/pH for same number of electron and proton transfer reaction. This clearly indicates that polymer redox processes are stable over the entire range of pH. Similar observations were also reported for poly(methylene blue) and poly(methylene green) [50].
3.3. Surface Characterizations

Fig. (4a) shows the SEM image of poly(PyB) coated ITO which indicated that a polymer film layer was formed on the surface. Fig. (4b) shows the surface morphology of unmodified electrode. The difference between Fig. (4a) and (b) confirms polymer particles (sizes ~ 200 nm to 2 µm) on the electrode surface. Fig. (4c) represents AFM 3-dimensional image of poly(PyB) film modified electrode and the hills-valley like polymer particles were seen on the modified electrode. Observations from AFM image were almost similar to the SEM image. The approximate polymer film thickness and average surface roughness were found to be 165.3 nm and 6.97 nm, respectively.

Fig. (5a) shows the CVs of pretreated SPCE (curve i) and poly(PyB)/SPCE (curve ii) in 5 mM [Fe(CN)₆]₃⁻/⁴⁻ + 0.1 M KCl. EIS obtained for (b) pretreated SPCE and (c) poly(PyB)/SPCE in 5 mM [Fe(CN)₆]₃⁻/⁴⁻ + 0.1 M KCl solutions.

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The redox currents of the poly poly(PyB)/SPCE considerably increased and the anodic and cathodic potentials significantly shifted to less positive and less negative values, respectively as compared to pretreated SPCE (unmodified). The increased peak currents could be due to the attached polymer which acted as a promoter to the interfacial electron transfer. In addition, the peak potential separation (ΔE_p) decreases for poly(PyB) modified electrode. This can be attributed to the polymer film on the modified electrode surface which is improving the conductive property of the electrode. The permeable characteristic of the porous polymer film should be highly useful for such [Fe(CN)₆]₃⁻/⁴⁻ probe diffusion, and therefore enhances reaction efficiency. This may be due to possible interaction between positively charged polymer film and negatively charged redox probe.

EIS is an effective method to probe the interfacial properties of modified electrode and often used for understanding chemical transformations and processes associated with the conductive supports [55]. Fig. (5b) and
3.4. Electrocatalytic Properties of Poly(PyB)/SPCE

The poly(PyB) film modified SPCE shows good electrocatalytic activity towards the electrochemical oxidation of NADH in 0.1 M phosphate buffer solution. As shown in Fig. (7A), in the absence of NADH, a reversible redox peak was obtained (curve a) at (E\(^{0}\)) = -0.03 V, however, in the presence of NADH, the oxidation of NADH at the modified electrode starts at around 0.2 V (curve b and c). Obviously, the oxidation of NADH at the poly(PyB) film modified electrode requires much lower over potential as compared to the bare electrode (curve d and e). In addition, the catalytic currents for the oxidation of NADH at the polymer film modified electrode increase significantly (curves b and c). However, pretreated SPCE did not reveal oxidation current in the potential range between -0.5 and +0.6 V (curve d). As shown in Fig. (7A) curve e, NADH oxidation takes place on pretreated SPCE (unmodified) at 0.65 V. Compared to unmodified SPCE, poly(PyB)/SPCE reduced the overpotential about 450 mV and considerably higher catalytic peak current was observed. These increased catalytic currents and reduced overpotential may due to the unique properties of the attached poly(PyB) film. Furthermore, the peak current for the oxidation of NADH at the poly(PyB)/SPCE increases with the NADH concentration (Calibration plot is shown in Fig. 7B), the oxidation current at +0.2 V shows a linear dependence on the NADH concentration in a range of 1×10^{-5} M to 5.2×10^{-4} M with a detection limit of 5×10^{-7} M (S/N = 3). This interesting behavior could be potentially useful for selective detection of NADH which is an important coenzyme in biological reactions.

Karyakin et al. found that ring substitution with only tertiary nitrogen atoms as ligands provides higher catalytic activity towards NADH and they also reported that polyazines are good electrocatalysts for oxidation of NADH [11]. Since poly(PyB) belongs to the group of polyazines, the catalytic reactions might include the formation of the substrate-catalyst complex, the electrocatalytic oxidation of...
The linear range, pH, potential and lower limit of detection of the proposed method were compared with the earlier reports in Table 1. From the data presented in Table 1, a wide linear range of NADH can be achieved using the proposed method [33, 58-60]. NADH can be detected at lower oxidation potential using poly(PyB)/SPCE other than reported methods [33, 58, 60, 61].

3.5. Selectivity, Reproducibility and Stability of Poly(PyB)/SPCE

To ascertain the role of other biological molecules on the poly(PyB) modified SPCE, we have recorded CVs separately in the presence of 5 mM dopamine, 5 mM ascorbic acid, 5 mM acetaminophen and 5 mM uric acid to 0.1 M PBS solution (pH 7.0) in the potential range of 0.5 to +0.5 V. We observed that no significant response was obtained for interfering molecules at this potential range. It indicates that the poly(PyB) modified electrode may be useful for selective detection of NADH.

The reproducibility was estimated by repetitive measurements of 300 μM NADH under the same conditions at every 30 min. The relative standard deviations of the peak currents were found to be 3.4% for NADH. In addition, the long-term stability of poly(PyB)/SPCE stored at room temperature in phosphate buffered solution was examined by checking its relative activity periodically. No apparent change in the current responses on this analyte was observed over three-weeks period.

4. CONCLUSIONS

Electrochemical polymerization and electrochemical properties of poly(PyB) were reported. Poly(PyB) film showed excellent electrochemical stability in the pH range between 1 and 10. In addition, poly(PyB) modified SPCE showed excellent electrocatalytic properties towards NADH in physiological solution (pH 7.0, 0.1 M PBS). Poly(PyB) modified SPCE has been employed for the determination of NADH from 1×10⁻⁵ M to 5.2×10⁻⁴ M with a detection limit of 5×10⁻⁷ M (S/N = 3). This new method is very simple and the modified electrode can be prepared easily within 30 min. The poly(PyB) modified electrode had good stability in neutral buffer solution. We also studied the effect of other biological molecules such as dopamine, ascorbic acid, acetaminophen, and uric acid using poly(PyB) modified electrode. From our observations, we found that selective detection of NADH can be performed using poly(PyB)/SPCE in 0.1 M phosphate buffer solution.

CONFLICT OF INTEREST

The author confirms that this article content has no conflicts of interest.

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