

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

Ashok K. Sundramoorthy^{1,*}, Szu-Ling Chen and Shen-Ming Chen[#]

¹School 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 5.2×10^{-4} M, and the detection limit was 5×10^{-7} M (S/N = 3).

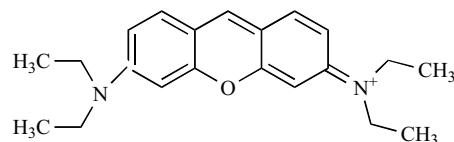
Keywords: Electroactive polymer, Modified Electrode, Chemical sensors, Electrocatalytic oxidation, NADH.

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(*p*-aminobenzene sulfonic acid)/flavin films [33], redox-active $(\text{NC})_2\text{C}_6\text{H}_3\text{-NO}/(\text{NC})_2\text{C}_6\text{H}_3\text{-NHOH}(\text{NOPH}/\text{NHOHPH})$ 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 electropoly-



Scheme 1A. Chemical structure of PyB.

merization 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,

*Address correspondence to this author at the Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No. 1, Section 3, Chung-Hsiao East Road, Taipei, 106, Taiwan (ROC); Tel: +886- 2- 27017147; Fax: +886- 2- 27025238; E-mail: sakumar80@gmail.com; smchen78@ms15.hinet.net

[#]both authors contribute equally

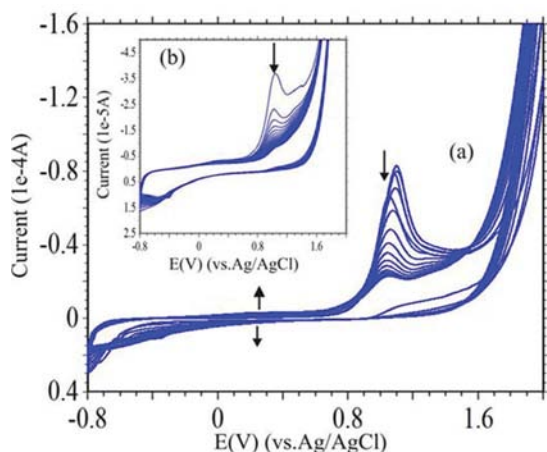


Fig. (1a). CVs of the poly(PyB) film growth on SPCE from the electrolyte 0.1 M H_2SO_4 solution containing 1×10^{-3} M PyB monomers. **(b)** CVs of poly(PyB)/SPCE in 0.1 M phosphate buffer solution. Scan rate = 0.05 V/s.

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.

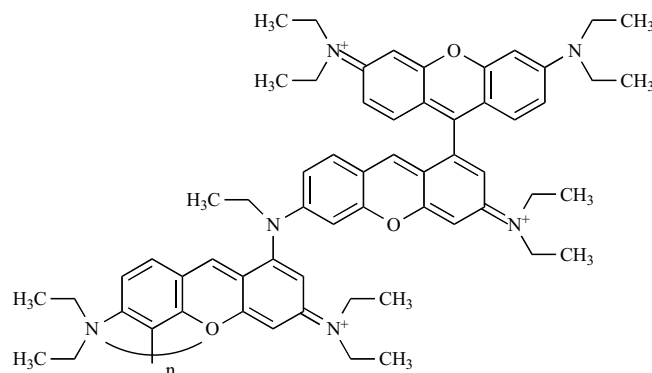
2.2. Apparatus

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 unit, IM6ex ZAHNER, Messsysteme (Kroach, 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 1.0×10^{-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



Scheme 1B. Hypothetical structure of poly(PyB).

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 1×10^{-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 $E^{0'} = +0.3$ 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

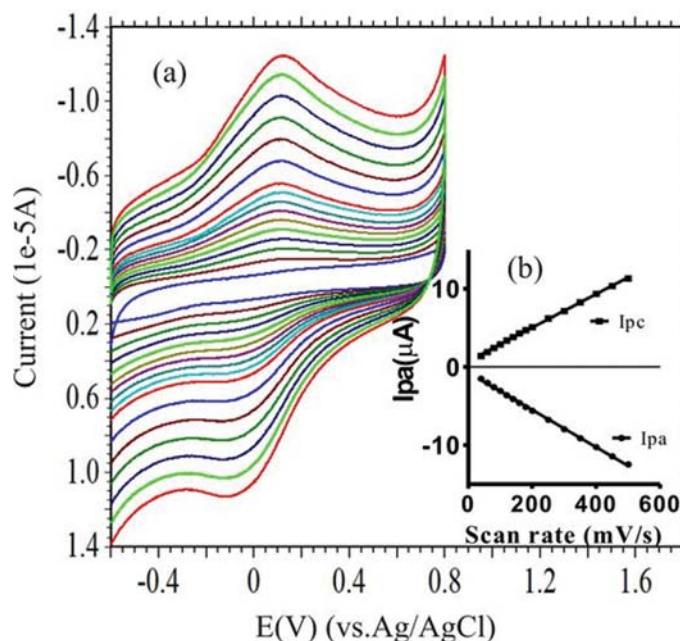


Fig. (2a). CVs of poly(PyB)/SPCE in 0.1M PBS (pH 7.0) at different scan rates. The scan rates from inner to outer are 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50 V/s, respectively. (b) Inset plot shows I_{pa} and I_{pc} vs. scan rate.

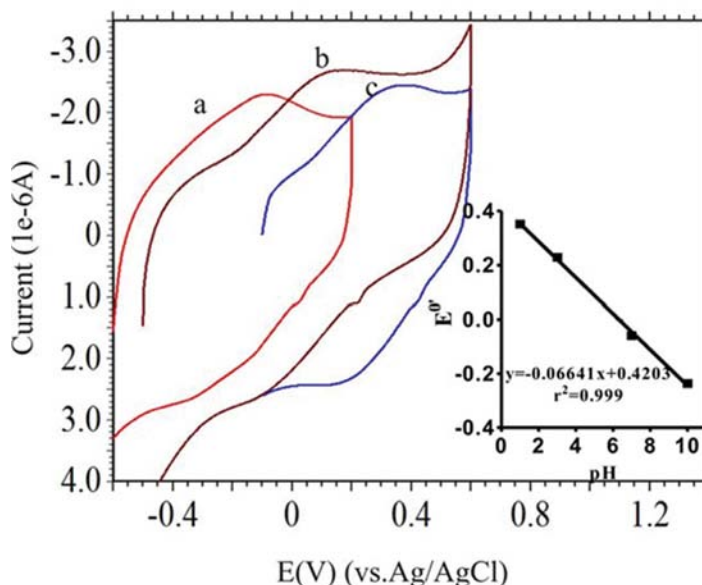


Fig. (3). CVs of poly(PyB)/SPCE in various pH solutions (a) 10.0, (b) 7.0 and (c) 1.0. Scan rate 0.05 V/s.

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, $I_p = n^2 F^2 \Gamma A \nu / 4RT$ and $Q_r = nFA\Gamma$, the electron transfer number was two for poly(PyB) redox couple and the surface coverage was 8.672×10^{-11} mol/cm².

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, $E^{o'}$, ($E^{o'} = (E_{pc} + E_{pa})/2$) (Inset of Fig. 3). The shift in $E^{o'}$ 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].

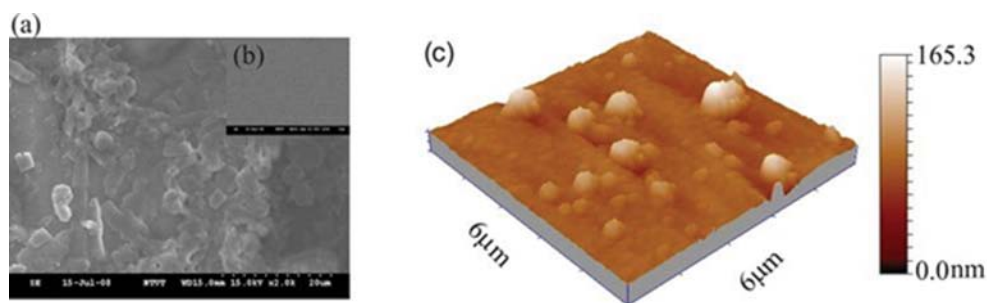


Fig. (4). SEM (a, b) and AFM (c) (three-dimensional image) images of poly(PyB) modified electrode.

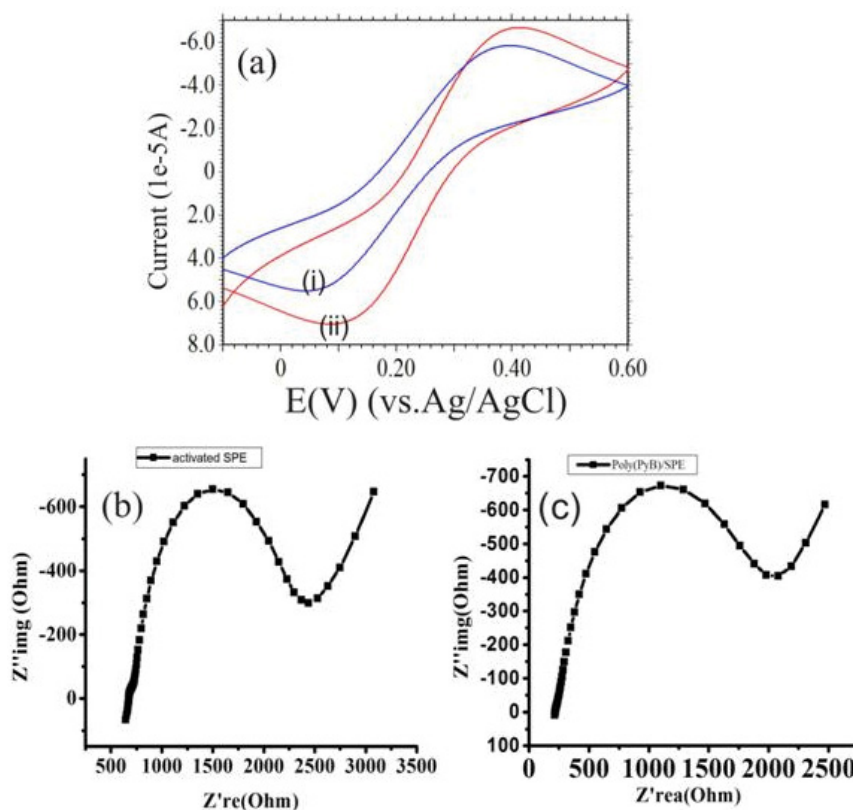


Fig. (5a). CVs of pretreated SPCE (curve i) and poly(PyB)/SPCE (curve ii) in 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$ + 0.1 M KCl. EIS obtained for (b) pretreated SPCE and (c) poly(PyB)/SPCE in 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$ + 0.1M KCl solutions.

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 \sim 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 $[\text{Fe}(\text{CN})_6]^{4-/3-}$ + 0.1M KCl solution. As shown in From Fig. (5a), 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 $[\text{Fe}(\text{CN})_6]^{4-/3-}$ 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

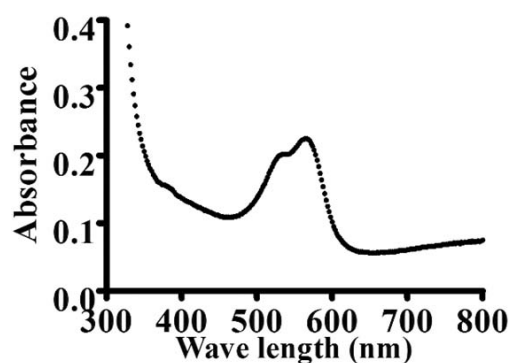


Fig. (6). Uv-vis absorption spectra of poly(PyB) film modified electrode.

(c) shows EIS results (Z' versus Z'' Nyquist plot) of pretreated SPCE (curve b) and poly(PyB) film modified SPCE in the presence of redox probe $[\text{Fe}(\text{CN})_6]^{4-/3-}$ + 0.1 M KCl solutions. A semicircle and a linear part were observed for unmodified SPCE. It is reported that semicircle at higher frequencies and the linear part at lower frequencies are related to the electron-transfer limited process and to the diffusion process, respectively.

The electron-transfer resistance of unmodified SPCE was 2300 Ω . After the deposition of poly(PyB) on the electrode surface, the high frequency semicircle was decreased ($\sim 2000 \Omega$). It may due to the acceleration of the $[\text{Fe}(\text{CN})_6]^{4-/3-}$ redox reaction at the electrode surface in the presence of poly(PyB) film.

PyB dimers have an absorption band at the shorter wavelengths compared to the monomer band [56,57]. The absorption spectra of poly(PyB) coated electrode is given in Fig. (6). The PyB monomer has an absorption at 553 nm [57]. As shown in Fig. (6), for Poly(PyB) film, the intensity of monomer band decreased and a new absorption band at 518 nm appeared on the vibronic shoulder of the monomer band.

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

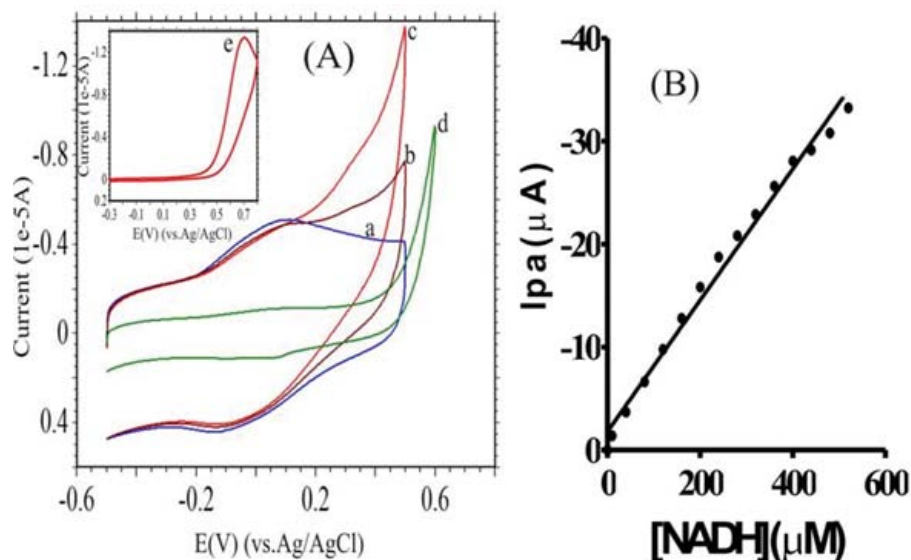
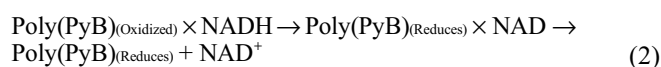
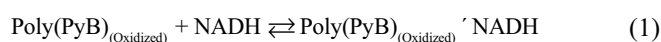


Fig. 7(A). CVs of poly(PyB)/SPCE in 0.1 M PBS (pH 7.0): NADH= (a) 0.0 mM, (b) 1.0 mM, (c) 3.0 mM and (d and e) pretreated SPCE with 3.0 mM NADH; scan rate 100 mV/s. **(B)** Calibration plot of $[\text{NADH}]$ vs. I_{pa} .

Table 1. Comparison of Electro-Analytical Data of Modified Electrodes Proposed for the Detection of NADH

Modified Electrode	pH Used for Catalysis	Potential (V)	Linear Range	Detection Limit	References
GCE/Poly(ABSA)	6.4	0.2	10 to 300 μM	1 μM	[31]
GCE/ MWNTs/6-ferrocenyl-hexanethiol	7.4	0.65	5.0 μM –1.5 mM	0.54 μM	[55]
Au/(PANI-PSS) ₆	Phosphate buffer	0.3	1 to 320 μM	0.31 μM	[56]
Poly-(3-methylthiophene)-multi-walled carbon nanotubes hybrid composite electrode	7.0	0.3	5.0×10^{-7} and 2.0×10^{-5} M	0.17 μM	[57]
o-aminophenol film/GCE	7.0	0.15	7.5×10^{-7} to 2.5×10^{-6} M	0.15 μM	[58]
Poly(Pyronin B)/SPCE	7.0	0.2	1×10^{-5} M to 5.2×10^{-4} M	0.5 μM	This method

NADH at poly(PyB) film modified electrode may proceed as follows [11, 50].



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 in 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.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Education and the National Science Council of Taiwan (ROC).

REFERENCES

- Arechederra, M. N.; Jenkins, C.; Rincon, R. A.; Artyushkova, K.; Atanassov, P.; Minter, S. D.: Chemical polymerization and electrochemical characterization of thiazines for NADH electrocatalysis applications. *Electrochim. Acta* **2010**, *55*, 6659-6664.
- Guo, K.; Qian, K.; Zhang, S.; Kong, J. L.; Yu, C. Z.; Liu, B. H.: Bio-electrocatalysis of NADH and ethanol based on graphene sheets modified electrodes. *Talanta* **2011**, *85*, 1174-1179.
- Saleh, F. S.; Okajima, T.; Kitamura, F.; Mao, L. Q.; Ohsaka, T.: Poly(phenosafranin)-functionalized single-walled carbon nanotube as nanocomposite electrocatalysts: Fabrication and electrocatalysis for NADH oxidation. *Electrochim. Acta* **2011**, *56*, 4916-4923.
- Blaedel, W. J.; Jenkins, R. A.: Electrochemical oxidation of reduced nicotinamide adenine dinucleotide. *Anal. Chem.* **1975**, *47*, 1337-1343.
- Gorton, L.: Chemically modified electrodes for the electrocatalytic oxidation of nicotinamide coenzymes. *J. Chem. Soc. Farad. T 1*: **1986**, *82*, 1245-1258.
- Gorton, L.; Dominguez, E.: *Bioelectrochemistry*; Wiley-VCH: Weinheim, 2002; Vol. 9.
- Silber, A.; Bräuchle, C.; Hampp, N.: Electrocatalytic oxidation of reduced nicotinamide adenine dinucleotide (NADH) at thick-film gold electrodes. *J. Electroanal. Chem.* **1995**, *390*, 83-89.
- Kumar, S. A.; Chen, S. M.: Electroanalysis of NADH using conducting and redox active polymer/carbon nanotubes modified electrodes - A review. *Sensors* **2008**, *8*, 739-766.
- Cai, C. X.; Xue, K. H.: Electrocatalysis of NADH oxidation with electropolymerized films of Nile blue A. *Anal. Chim. Acta* **1997**, *343*, 69-77.
- Gao, Q.; Wang, W.; Ma, Y.; Yang, X.: Electrooxidative polymerization of phenothiazine derivatives on screen-printed carbon electrode and its application to determine NADH in flow injection analysis system. *Talanta* **2004**, *62*, 477-482.

- [11] Karyakin, A. A.; Karyakina, E. E.; Schuhmann, W.; Schmidt, H.-L.: Electropolymerized Azines: Part II. In a Search of the Best Electrocatalyst of NADH Oxidation. *Electroanal. Chem.* **1999**, *11*, 553-557.
- [12] Malinauskas, A.; Ruzgas, T.; Gorton, L.: Electrochemical study of the redox dyes Nile Blue and Toluidine Blue adsorbed on graphite and zirconium phosphate modified graphite. *J. Electroanal. Chem.* **2000**, *484*, 55-63.
- [13] Gründig, B.; Wittstock, G.; Rüdell, U.; Strehlitz, B.: Mediator-modified electrodes for electrocatalytic oxidation of NADH. *J. Electroanal. Chem.* **1995**, *395*, 143-157.
- [14] Mano, N.; Kuhn, A.: Cation induced amplification of the electrocatalytic oxidation of NADH by immobilized nitrofluorenone derivatives. *J. Electroanal. Chem.* **2001**, *498*, 58-66.
- [15] Mano, N.; Kuhn, A.: Electrodes modified with nitrofluorenone derivatives as a basis for new biosensors. *Biosens. Bioelectron.* **2001**, *16*, 653-660.
- [16] Mano, N.; Thienpont, A.; Kuhn, A.: Adsorption and catalytic activity of trinitro-fluorenone derivatives towards NADH oxidation on different electrode materials. *Electrochem. Commun.* **2001**, *3*, 585-589.
- [17] Munteanu, F. D.; Mano, N.; Kuhn, A.; Gorton, L.: NADH electrooxidation using carbon paste electrodes modified with nitrofluorenone derivatives immobilized on zirconium phosphate. *J. Electroanal. Chem.* **2004**, *564*, 167-178.
- [18] de-los-Santos-Álvarez, N.; de-los-Santos-Álvarez, P.; Lobo-Castañón, M. J.; Miranda-Ordieres, A. J.; Tuñón-Blanco, P.: Flavin Adenine Dinucleotide As Precursor for NADH Electrocatalyst. *Anal. Chem.* **2005**, *77*, 4286-4289.
- [19] de-los-Santos-Álvarez, N.; Lobo-Castañón, M. J.; Miranda-Ordieres, A. J.; Tuñón-Blanco, P.: Electrochemical and Catalytic Properties of the Adenine Coenzymes FAD and Coenzyme A on Pyrolytic Graphite Electrodes. *Electroanal. Chem.* **2005**, *17*, 445-451.
- [20] de-los-Santos-Álvarez, N.; Lobo-Castañón, M. J.; Miranda-Ordieres, A. J.; Tuñón-Blanco, P.; Abruña, H. D.: 5-Hydroxytryptophan as a Precursor of a Catalyst for the Oxidation of NADH. *Anal. Chem.* **2005**, *77*, 2624-2631.
- [21] Prieto-Simón, B.; Fàbregas, E.: Comparative study of electron mediators used in the electrochemical oxidation of NADH. *Biosens. Bioelectron.* **2004**, *19*, 1131-1138.
- [22] Dicu, D.; Muresan, L.; Popescu, I. C.; Cristea, C.; Silberg, I. A.; Brouant, P.: Modified electrodes with new phenothiazine derivatives for electrocatalytic oxidation of NADH. *Electrochim. Acta* **2000**, *45*, 3951-3957.
- [23] Kumar, S. A.; Chen, S.-M.: Fabrication and characterization of Meldola's blue/zinc oxide hybrid electrodes for efficient detection of the reduced form of nicotinamide adenine dinucleotide at low potential. *Anal. Chim. Acta* **2007**, *592*, 36-44.
- [24] Kumar, S. A.; Chen, S.-M.: Electrocatalysis and Amperometric Detection of the Reduced Form of Nicotinamide Adenine Dinucleotide at Toluidine Blue/Zinc Oxide Coated Electrodes. *Electroanal. Chem.* **2007**, *19*, 1952-1958.
- [25] Lawrence, N. S.; Wang, J.: Chemical adsorption of phenothiazine dyes onto carbon nanotubes: Toward the low potential detection of NADH. *Electrochem. Commun.* **2006**, *8*, 71-76.
- [26] Lobo, M. J.; Miranda, A. J.; Tuñón, P.: A comparative study of some phenoxazine and phenothiazine modified carbon paste electrodes for ethanol determination. *Electroanal. Chem.* **1996**, *8*, 591-596.
- [27] Lorenzo, E.; Sánchez, L.; Pariente, F.; Tirado, J.; Abruña, H. D.: Thermodynamics and kinetics of adsorption and electrocatalysis of NADH oxidation with a self-assembling quinone derivative. *Anal. Chim. Acta* **1995**, *309*, 79-88.
- [28] Schuhmann, W.; Huber, J.; Wohlschläger, H.; Strehlitz, B.; Gründig, B.: Electrocatalytic oxidation of NADH at mediator-modified electrode surfaces. *J. Biotechnol.* **1993**, *27*, 129-142.
- [29] Murthy, A. S. N.; Gupta, A. R. L.: NADH sensor with electrochemically modified TCNQ electrode. *Anal. Chim. Acta* **1994**, *289*, 43-46.
- [30] Surya, A.; Murthy, N.; Anita: Tetracyanoquinodimethane (TCNQ) modified electrode for NADH oxidation. *Bioelectrochem. Bioener.* **1994**, *33*, 71-73.
- [31] Cai, C.-X.; Ju, H.-X.; Chen, H.-Y.: Cobalt hexacyanoferrate modified microband gold electrode and its electrocatalytic activity for oxidation of NADH. *J. Electroanal. Chem.* **1995**, *397*, 185-190.
- [32] Deng, L.; Wang, Y.; Shang, L.; Wen, D.; Wang, F.; Dong, S.: A sensitive NADH and glucose biosensor tuned by visible light based on thionine bridged carbon nanotubes and gold nanoparticles multilayer. *Biosens. Bioelectron.* **2008**, *24*, 951-957.
- [33] Kumar, S. A.; Chen, S.-M.: Electrochemically polymerized composites of conducting poly(p-ABSA) and flavins (FAD, FMN, RF) films and their use as electrochemical sensors: A new potent electroanalysis of NADH and NAD⁺. *Sensor. Actuat. B-Chem.* **2007**, *123*, 964-977.
- [34] Lima, P. R.; Santos, W. d. J. R.; de Oliveira, A. B.; Goulart, M. O. F.; Kubota, L. T.: Electrochemical investigations of the reaction mechanism and kinetics between NADH and redox-active (NC)2C6H3-NHOH/(NC)2C6H3-NO from 4-nitrophthalonitrile-(NC)2C6H3-NO₂-modified electrode. *Biosens. Bioelectron.* **2008**, *24*, 448-454.
- [35] Prieto-Simón, B.; Macanás, J.; Muñoz, M.; Fàbregas, E.: Evaluation of different mediator-modified screen-printed electrodes used in a flow system as amperometric sensors for NADH. *Talanta* **2007**, *71*, 2102-2107.
- [36] Manesh, K. M.; Santhosh, P.; Gopalan, A.; Lee, K. P.: Electrocatalytic oxidation of NADH at gold nanoparticles loaded poly(3,4-ethylenedioxythiophene)-poly(styrene sulfonic acid) film modified electrode and integration of alcohol dehydrogenase for alcohol sensing. *Talanta* **2008**, *75*, 1307-1314.
- [37] Cosnier, S.: Biosensors based on electropolymerized films: new trends. *Anal. Bioanal. Chem.* **2003**, *377*, 507-520.
- [38] Kumar, S. A.; Tang, C.-F.; Chen, S.-M.: Poly(4-amino-1-1'-azobenzene-3, 4'-disulfonic acid) coated electrode for selective detection of dopamine from its interferences. *Talanta* **2008**, *74*, 860-866.
- [39] Malinauskas, A.; Garjonytė, R.; Mažeikienė, R.; Jurevičiūtė, I.: Electrochemical response of ascorbic acid at conducting and electrogenerated polymer modified electrodes for electroanalytical applications: a review. *Talanta* **2004**, *64*, 121-129.
- [40] Malinauskas, A.; Malinauskienė, J.; Ramanavicius, A.: Conducting polymer-based nanostructured materials: electrochemical aspects. *Nanotechnology* **2005**, *16*, R51-R62.
- [41] *Handbook of Oligo and Polythiophenes*; Kossmehl, G.; Engelmann, G., Eds.; Wiley-VCH: New York, 1999.
- [42] *Handbook of Organic Conductive Molecules and Polymers*; Lamy, C.; Leger, J. M.; Garnier, F., Eds.; John Wiley & Sons: New York, 1997; Vol. 3.
- [43] Acemioglu, B.; Arık, M.; Onganer, Y.: Solvent effect on nonradiative process of pyronin B in protic and aprotic solvent systems. *J. Lumin.* **2002**, *97*, 153-160.
- [44] Çelebi, N.; Arık, M.; Onganer, Y.: Analysis of fluorescence quenching of pyronin B and pyronin Y by molecular oxygen in aqueous solution. *J. Lumin.* **2007**, *126*, 103-108.
- [45] Li, B.; Wu, Y.; Gao, X.-M.: Pyronin Y as a Fluorescent Stain for Paraffin Sections. *Histochem. J.* **2002**, *34*, 299-303.
- [46] Snare, M. J.; Tan, K. L.; Treloar, F. E.: Fluorescence Studies Of Solubilization And Dye Binding In Hypercoiled Poly(Methacrylic Acid) - A Connected Cluster Model. *J. Macromol. Sci. A* **1982**, *A17*, 189-201.
- [47] Ashok Kumar, S.; Chen, S.-L.; Chen, S.-M.: Amperometric Sensor for Detection of the Reduced Form of Nicotinamide Adenine Dinucleotide Using a Poly(pyronin B) Film Modified Electrode. *Electroanal. Chem.* **2009**, *21*, 1379-1386.
- [48] Cai, C.-X.; Xue, K.-H.: Electrocatalysis of NADH oxidation with electropolymerized films of azure I. *J. Electroanal. Chem.* **1997**, *427*, 147-153.
- [49] Barsan, M. M.; Pinto, E. M.; Brett, C. M. A.: Electrosynthesis and electrochemical characterisation of phenazine polymers for application in biosensors. *Electrochim. Acta* **2008**, *53*, 3973-3982.
- [50] Karyakin, A. A.; Karyakina, E. E.; Schmidt, H.-L.: Electropolymerized Azines: A New Group of Electroactive Polymers. *Electroanal. Chem.* **1999**, *11*, 149-155.
- [51] Bauldreay, J. M.; Archer, M. D.: Dye-modified electrodes for photogalvanic cells. *Electrochim. Acta* **1983**, *28*, 1515-1522.
- [52] Ohsaka, T.; Tanaka, K.; Tokuda, K.: Electrocatalysis Of Poly(Thionine)-Modified Electrodes For Oxidation Of Reduced Nicotinamide Adenine-Dinucleotide. *J. Chem. Soc. Chem. Comm.* **1993**, 222-224.
- [53] Turner, A. P. F.; Karube, I.; Wilson, G. S.: *Biosensors-Fundamentals and Applications*, 1987.

- [54] Gosser, J. D. K.: *Cyclic voltammetry -Simulation and analysis of reaction mechanisms*; VCH Inc., 1993.
- [55] Suni, I. I.: Impedance methods for electrochemical sensors using nanomaterials. *TrAC- Trends Anal. Chem.* **2008**, *27*, 604-611.
- [56] Valdes-Aguilera, O.; Neckers, D. C.: Rose bengal ethyl ester aggregation in aqueous solution. *J. Phys. Chem.* **1988**, *92*, 4286-4289.
- [57] Arik, M.; Onganer, Y.: Molecular excitons of Pyronin B and Pyronin Y in colloidal silica suspension. *Chem. Phys. Lett.* **2003**, *375*, 126-133.
- [58] Agüí, L.; Peña-Farfal, C.; Yáñez-Sedeño, P.; Pingarrón, J. M.: Poly-(3-methylthiophene)/carbon nanotubes hybrid composite-modified electrodes. *Electrochim. Acta* **2007**, *52*, 7946-7952.
- [59] Nassef, H. M.; Radi, A.-E.; O'Sullivan, C. K.: Electrocatalytic sensing of NADH on a glassy carbon electrode modified with electrografted o-aminophenol film. *Electrochem. Commun.* **2006**, *8*, 1719-1725.
- [60] Zhang, M.; Yamaguchi, A.; Morita, K.; Teramae, N.: Electrochemical synthesis of Au/polyaniline-poly(4-styrenesulfonate) hybrid nanoarray for sensitive biosensor design. *Electrochem. Commun.* **2008**, *10*, 1090-1093.
- [61] Yao, X.; Wang, Y.; Wen, L.: Sensitive Detection of NADH by Ferrocenylalkanethiol Functionalized Multiwall Carbon Nanotubes Electrodes. *Anal. Lett.* **2008**, *41*, 1236-1247.

Received: March 03, 2012

Revised: April 02, 2012

Accepted: May 14, 2012