

Full Paper

Amperometric Sensor for Detection of the Reduced Form of Nicotinamide Adenine Dinucleotide Using a Poly(pyronin B) Film Modified Electrode

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Abstract

An electrochemical method for the preparation of poly(pyronin B) film was proposed in this paper. A poly(pyronin B) (poly(PyB)) film modified glassy carbon electrode (GCE) has been fabricated via an electrochemical oxidation procedure and applied to the electrocatalytic oxidation of reduced form of nicotinamide adenine dinucleotide (NADH). The poly(PyB) film modified electrode surface has been characterized by atomic force microscope (AFM), scanning electron microscope (SEM), electrochemical impedance spectroscopy (EIS), UV-visible absorption spectrophotometry (UV-vis) and cyclic voltammetry (CV). These studies have been used to investigate the poly(PyB) film, which demonstrates the formation of the polymer film and the excellent electroactivity of poly(PyB) in neutral and even in alkaline media. Due to its potent catalytic effects towards the electrooxidation of NADH at lower potential (0.0 V), poly(PyB) film modified electrode can be used for the selective determination of NADH in real samples because of dopamine, ascorbic acid and uric acid oxidation can be avoided at this potential. The catalytic peak currents are linearly dependent on the concentrations of NADH in the range of 1.0×10^{-6} to 5.0×10^{-4} mol/L with correlation coefficients of 0.999. The detection limits for NADH is 0.5×10^{-6} mol/L. Poly(PyB) modified electrode also shows good stability and reproducibility due to the irreversible attachment of polymer film at GCE surface.

Keywords: Detection of NADH, Electroanalysis, Modified electrode, Sensor, Electropolymerization, Redox polymer

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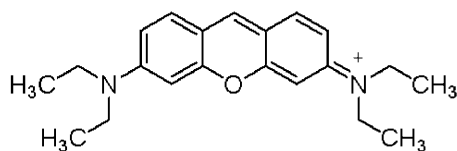
1. Introduction

Amperometric sensors preparation and their use in the detection of nicotinamide adenine dinucleotide reduced form (NADH) are of considerable interest [1–4]. Since NADH is one of the most important coenzymes in the human brain and body, this coenzyme is a common cofactor of about 500 dehydrogenases and its reversible regeneration is a key step in the development of amperometric sensors [5]. However, direct oxidation of NADH at unmodified electrodes shows high irreversibility and requires high over potentials (> 1.0 V) [6]. In addition, electrochemical oxidation of NADH and other biomolecules takes place at nearly same potential on bare electrode and consequently, results surface contamination (fouling) by their oxidized products which cause unstable analytical response. To overcome these problems, considerable efforts devoted to the determination of NADH at lower potential using variety of chemically modified electrodes [7–9].

Electron transfer redox mediators incorporated electrodes have been used for determination of NADH at lower potentials. These are *ortho*- and *para*-quinones, phenylenediamines, phenoxazines, alkylphenazines and pheno-

thiazines [1, 10–12], and electropolymerized quinones [13, 14], electrodeposited TCNQ [15, 16], *N*-methylphenazine [17], metal complexes [18], the phenothiazine and phenoxazine dyes [12], and nitrofluorenes [19–22].

The electrochemically polymerized phenothiazine dye film modified electrodes were used for NADH sensors [11, 23]. The phenothiazine dyes easily also adsorb directly onto a wide range of substrates including graphite [24, 25], titanium phosphate [26], carbon nanotubes [27], zinc oxide [28, 29] and these dyes have been covalently attached on silica gel [30] and gold electrodes through a cysteamine linkage [31]. All these modified electrodes have been used for detection of NADH at lower potential. However, the stability of electrodes is major concern in the development of electrochemical sensors. Recently, visible light based on thionine bridged carbon nanotubes and gold nanoparticles multilayer [32], poly(*p*-aminobenzene sulfonic acid) films doped with flavins [33], carbon electrode modified with a redox-active $(\text{NC})_2\text{C}_6\text{H}_5\text{-NO}/(\text{NC})_2\text{C}_6\text{H}_5\text{-NHOH}(\text{NOPH}/\text{NHOHPH})$ film [34], electropolymerized mediator-based SPEs and mediator-modified polysulfone composite film-based SPEs, [35], dispersing gold nanoparticles onto the matrix of poly(3,4-ethylenedioxythiophene)–poly(styrene



Scheme 1. Structural formula of pyronin B.

sulfonic acid) towards the oxidation of NADH is investigated [36]. The oxidation products of flavin adenine dinucleotide, coenzyme A and 5-hydroxytryptophan have a common structure of electroactive quinone-imine that acted as efficient catalyst of the oxidation of NADH at low potentials [37–39]. A comparative study of six different redox mediators for the electrocatalysis of NADH for the construction of dehydrogenase-based biosensor have been reported [40] and they have suggested that electrogenerated polymers are promising tools for the development of dehydrogenase-based sensors.

Conducting polymers have potential applications in various fields such as fuel cells, biosensors, electroanalysis, electrocatalysis, electrochromic displays and others [41–43]. In recent years, electrochemically synthesized polymer films on electrode surface considered for potential applications in electroanalysis of biological molecules [9, 44]. Electropolymerization is a good approach to prepare polymer modified electrodes (PMEs) as adjusting electrochemical parameters can control film thickness, permeation and charge transport characteristics. PME's have many advantages in the detection of analytes because of its selectivity, sensitivity and homogeneity in electrochemical deposition, strong adherence to electrode surface and chemical stability of the film [45, 46].

Pyronin B (PyB) (Scheme 1, a derivative of xanthene dye) is a water soluble dye and has found applications in biology as a differential stain for nucleic acids and synthetic polyelectrolyte systems [47–50]. In this paper, for the first time, electrochemical polymerization of PyB is carried out in acidic solution using glassy carbon electrode. The electrochemically generated poly-pyronin B film modified electrode has been characterized using atomic force microscope (AFM), scanning electron microscope (SEM), UV-visible spectrophotometry (UV-vis), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). Poly-pyronin B film modified electrode shows excellent electrocatalytic effect towards NADH at lower potential. Consequently, an amperometric sensor has been developed for the detection of NADH using poly(PyB) modified electrode.

2. Experimental

2.1. Reagents

All chemicals were of analytical reagent grade unless otherwise specified. Pyronin B was purchased from Aldrich Chemical Company. β -NADH, dopamine hydrochloride,

myoglobin, catalase, tyrosine and cysteine and uric acid received from Sigma-Aldrich (St. Louis, MO, USA). *p*-acetaminophen, sulfuric acid and sodium hydroxide were purchased from Wako pure chemicals (Osaka, Japan). Ascorbic acid, sodium acetate and sodium dihydrogen phosphate were received from E-Merck (Darmstadt, Germany). Double distilled water was obtained from a Millipore Alpha-Q Lotun ultrapure water system. Solutions and buffers were prepared employing standard laboratory procedures. Before each experiment the solutions were deoxygenated by purging with pre-purified nitrogen gas.

2.2. Apparatus

Electrochemical measurements were performed with CH Instruments (TX, USA) Model-400 potentiostat with conventional three-electrode cell. A BAS glassy carbon electrode (GCE) and platinum wire are used as the working electrode and auxiliary electrode, respectively. All the cell potentials were measured with respect to an Ag/AgCl [KCl (sat)] reference electrode. Amperometric studies were performed on a Bi-potentiostat Model CHI750A having an analytical rotator model AFMSRK with MSRX speed control (PINE Instruments, USA). Hitachi scientific instruments (London, UK) Model S-3000H SEM was used for surface image measurements. The AFM image was recorded with a multimode scanning probe microscope system operated in tapping mode using model CSPM4000 Instruments, Ben Yuan Ltd (Beijing, China). EIS were performed using impedance measurement unit, IM6ex ZAHNER, Messsysteme (Kroanach, Germany). The UV-visible absorption spectra were checked by using a U3300 Spectrophotometer (HITACHI).

2.3. Modification of Electrode Surface

Prior to the use, the GCE was mechanically polished with alumina powder (Al_2O_3 , 0.05 micron) up to a mirror finish and ultrasonicated in distilled water for 5 min. Then the electrodes were cycled in 0.1 M sulfuric acid in a potential range -0.5 to 1 V at a sweep rate of 100 mVs^{-1} until a stable voltammogram obtained. The electrochemical deposition of poly(PyB) film on well cleaned GCE was carried out by cyclic voltammetry (between -0.8 and 2.0 V at 50 mVs^{-1}) for 20 cycles. The electrolyte consisted of 1×10^{-3} M PyB monomer in aqueous solution of 0.1 M H_2SO_4 . After this step, a dark purple green color polymer film was observed on electrode surface. The resulting polymer film modified electrode washed with doubly distilled deionized water before proceed electrochemical measurements. To remove unbounded molecules or physically adsorbed monomers from the electrode surface, the poly(PyB)/GCE was electroactivated by scanning in the potential range between -0.6 and 0.8 V in pH 7.0 buffer solutions using cyclic voltammetry at a scan rate of 50 mVs^{-1} for five cycles. We observed that, after the polymerization process, the modified elec-

trode was thoroughly washed and transferred into phosphate buffer solution, the redox peak current of poly(PyB) was increased for few cycles and became stable, this is because of some of physically adsorbed monomers blocked the electroactive centers of polymerized film. To overcome this, we have electroactivated the modified electrode by potential sweeping in 0.1 M phosphate buffer solution. After this step, the poly(PyB) film modified electrode was used for further studies.

For electrocatalytic experiments, prior to start experiments, the stock solution of 5 mM NADH was prepared and then, the required quantity of NADH solution was injected into the 10 mL of deoxygenated phosphate buffer solution (PBS) (pH 7.0) using micro syringe. The concentrations of added NADH solutions were calculated.

3. Results and Discussion

3.1. Electropolymerization of PyB

Figure 1A shows the successive cyclic voltammograms (CVs) of GCE in 0.1 M H₂SO₄ solution containing 1×10^{-3} M PyB in a potential range from -0.80 to $+2.0$ V at a scan rate of 50 mV s^{-1} . During the first anodic scan, an irreversible oxidation wave was observed at $+1.0$ V (Pa1) which can be assigned to the oxidation of tertiary amino group of PyB monomers [10, 51]. In the first cathodic scan a cathodic peak at -0.2 V (Pc1) was observed. In the second and subsequent cycles, a broad shoulder was observed at $+1.57$ V (Pa2) and a set of redox peak (Pc2 and Pa3) was started to appear at $E^{\circ'} = +0.3$ V. As can be seen, that gradual increases in the amplitude of the peaks were observed as a consequence of the repeated potential scans, indicative of the electropolymerization of PyB and subsequent deposition of the polymeric film at the GCE surface. The polymer film hardly grew after the 20th cycle which

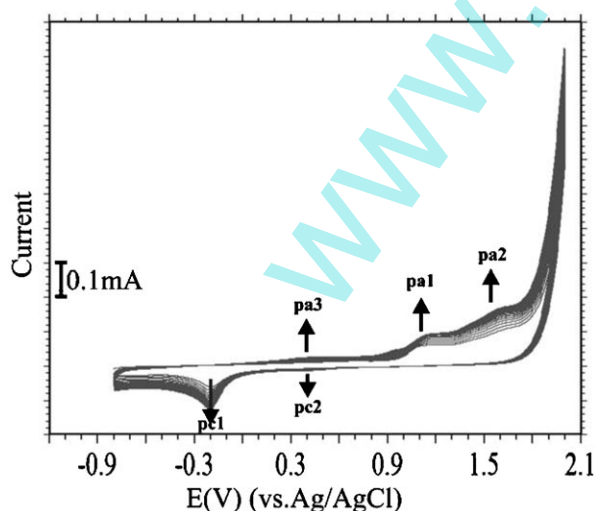


Fig. 1. Cyclic voltammograms (CVs) of the poly(PyB) film growth on GCE from the electrolyte 0.1 M H₂SO₄ solution + 1×10^{-3} M PyB monomers. Scan rate = 0.05 V/s .

indicated the saturation of polymer growth. After electropolymerization, an adherent polymer observed on the GCE. The similar results were obtained in the electrochemical polymerization of methylene blue and methylene green [52]. Karyakin et al. [53] reported that the polymerization of monomer containing one or two tertiary amine groups was taking place through tertiary amine. Moreover, as polymerization occurs, more tertiary amine groups become nitrogen bridges and these bridges become electroactive similar to those in polyaniline and poly(thionine) films [54, 55]. PyB monomers were able to form an electroactive polymer by anodic oxidation from acidic aqueous solutions similar to poly(methylene blue) nor poly(methylene green) since its chemical structure resembles with methylene blue and methylene green [10, 52, 53]. The structure of poly(PyB) film might similar to the hypothetical structure proposed for poly(methylene blue). After the modification, the electrode thoroughly washed with water and dried in air for 30 min.

3.2. Electrochemical Properties by CV

CVs of the poly(PyB) modified GCE in phosphate buffer solution (PBS) (pH 7.00) at different scan rates were recorded (data not shown). A couple of redox peak appears at a formal potential of -0.047 V versus Ag/AgCl at a scan rate of 20 mV/s . The cathodic peak current (I_{pc}) and anodic peak current (I_{pa}) were proportional to the scan rate which is an indication of a surface confined redox process [56, 57]. Therefore, the peak currents could be correlative with scan rate by Equation 1.

$$I_p = n^2 F^2 \Gamma A \nu / 4RT \quad (1)$$

$$Q_f = nFA\Gamma \quad (2)$$

Where Γ represents the surface coverage concentration (mol/cm^2), ν is the scan rate, A (0.0707 cm^2) is the electrode surface area, I_p is the peak current, n ($=2$) is the number of electrons per reactant molecule, Q the charge obtained by integrating the anodic peak at low voltage scan rate (10 mV s^{-1}), F is the Faraday constant and other parameters have their usual meanings. We assume that all of the immobilized redox centers are electroactive on the voltammetry time scale. The slope of anodic peak current against scan rate was 0.2 V . Thus, the surface coverage was calculated to be $3.552 \times 10^{-9} \text{ mol/cm}^2$, further confirming the immobilized state of the poly(PyB) on the electrode surface.

To ascertain the effect of pH, the voltammetric response of poly(PyB) film modified GCE was obtained in solutions of different pH from 1 to 11. As we observed (data not shown), the $E^{\circ'}$ ($E^{\circ'} = (E_{pc} + E_{pa})/2$) of polymer redox couple was pH dependent with a slope of -60.5 mV per pH for polymer redox couple which is very close to the anticipated Nernstian value of -59 mV for electrochemical processes involving the same number of protons and electrons. In addition there was no significant variation in

the slope suggesting that the polymer film remain protonated over the entire pH range studied. This clearly indicates that polymer redox processes is stable over the entire range of pH. This kind of observations were observed for poly(methylene blue) and poly(methylene green) [53].

3.3. Surface Characterizations by SEM, AFM, EIS, and UV-vis

Figure 2a shows the SEM image of poly(PyB) coated electrode which indicated that a polymer film layer was formed on the surface. As we seen, highly porous film morphology was observed in SEM image which indicated that several beads like polymer particles were attached on electrode surface. Figure 2b depicts AFM 3-dimensional image of poly(PyB) film modified electrode, from this image the hills-valley like polymer particles were seen. Using AFM image analyzer software, the approximate poly(PyB) film thickness was found to be 70 nm.

EIS can give information on the impedance changes of the electrode surface with the surface modification [58]. The EIS experiments were carried out in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ + 0.1 M KCl solution. Figure 3 shows the type complex plane diagram (Z' versus Z'' Nyquist plot) of the EIS obtained at the pretreated bare GCE (curve a) and poly (PyB) modified electrode (curve b). EIS spectra of pretreated GCE included

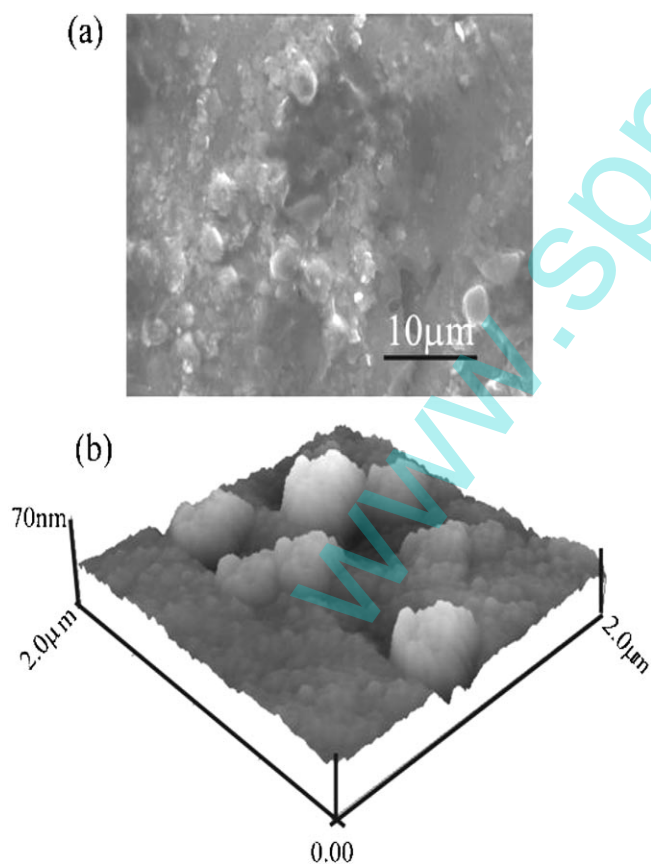


Fig. 2. Surface images of poly(PyB) modified electrode: a) SEM and b) AFM (three dimensional image).

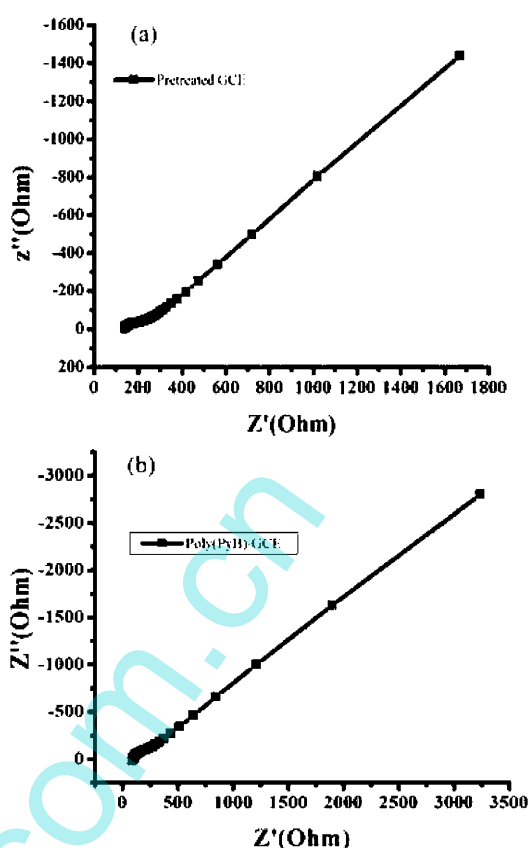


Fig. 3. EIS obtained for bare GCE (a) and poly(PyB) modified electrode (b) in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ + 0.1 M KCl solutions.

a semicircle part and a linear part, the semicircle part at higher frequencies corresponds to the electron-transfer limited process, and the linear part at lower frequencies corresponds to the diffusion process. The electron-transfer resistance, R_{et} , equals to 200 Ω . However, the diameter of the high frequency semicircle was obviously reduced to 50 Ω by the surface modification of the poly(PyB) film, suggesting that a significant acceleration of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox reaction occurred due to the presence of positively charged polymer film. The decreasing of charge transfer resistance value for poly(PyB)/GCE is because that cationic polymer film can act as a electron transfer medium and enhanced electron transfer and also indicated that poly(PyB) had been attached to the GCE surface.

Exciton theory [59] predicts that monomer dimerization have an absorption band at the shorter wavelengths compared to the monomer band. In addition, the oblique dimers yield a band splitting on absorption spectra of the monomer into two allowed structures as a function of increasing dye concentration [59]. Most of the dyes are planar and cationic in nature. They exhibit blue shift on dimerization [59, 60]. The monomer absorption spectra of PyB and poly(PyB) coated electrode is given in Figures 4a and b, respectively. The main peak that belongs to monomer occurs at 553 nm with the dilute concentration of 1.0×10^{-3} M PyB (curve a) [60]. After the polymerization process on electrode surface the intensity of monomer band decreases,

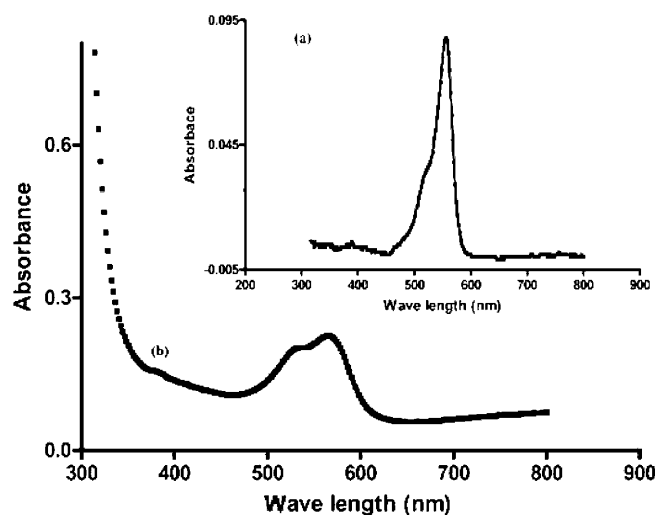


Fig. 4. UV-vis spectra of monomer PyB solution (curve a) and poly(PyB) film modified electrode (curve b).

a new absorption band at 518 nm appears on the vibronic shoulder of the monomer band, which is an indication of poly(PyB) film formation (curve b).

3.4. Electrocatalysis of NADH

Since the poly(PyB) redox processes is stable over the entire range of pH, the optimistic working pH value of 7.0 was selected for electrocatalysis of NADH at poly(PyB) modified electrode. To investigate electrocatalytic activity of poly(PyB) film modified electrode, electrochemical catalytic oxidation of NADH in 0.1 M PBS + 7.5 mM NADH at poly(PyB)/GCE was investigated by CV (Fig. 5). There was oxidation peak observed at pretreated GCE (GCE was electrochemically pretreated by using 20 times cycling potential sweeps in the range of -0.8 to 2.0 V in 0.1 M H_2SO_4 solution at a scan rate of 50 mV/s) in the presence of NADH at about $+0.62$ V, suggesting that bare GCE electrode was failed to reduce over potential required for NADH oxidation (curves c). However, at poly(PyB)/GCE, the oxidation peak current at about 0.0 V was greatly enhanced in the presence of 7.5 mM NADH corresponding with the decrease of the reduction peak current, suggesting a typical electrocatalytic oxidation process of NADH (curve b). Compared to pretreated GCE, poly(PyB) modified electrode reduced the overpotential about 620 mV. Based on these observations, it is concluded that electrocatalytic oxidation of NADH takes place by poly(PyB) film modified electrode. Not due to the electrogenerated surface quinone groups during the strong anodization of the electrode in acidic medium [38]. PyB monomers also showed electrocatalytic effect towards NADH in 0.1 M phosphate buffer solution, however, the physically adsorbed PyB monomers on the GCE easily leached out of the surface so it is not suitable for preparation of stable sensors.

A new group of electroactive polymers known as polyazines have been extensively studied and reported for

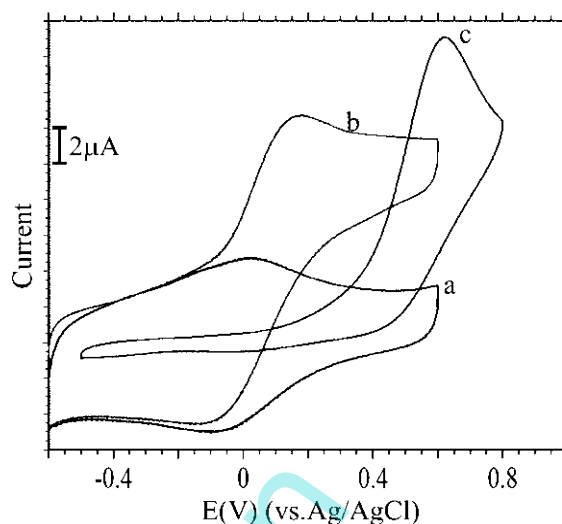
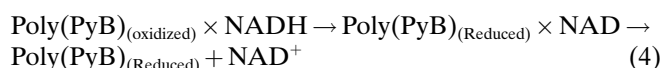
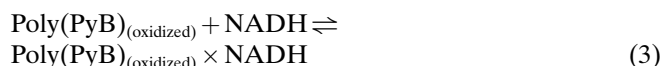


Fig. 5. CVs of poly(PyB) modified GCE in 0.1 M PBS (pH 7.0): NADH = a) 0.0 mM, b) 7.5 mM, and c) pretreated GCE with 7.5 mM NADH; scan rate = 20 mV/s.

electrocatalytic oxidation of NADH [8, 10]. Redox mediators with tertiary nitrogen atoms such as methylene blue, methylene green, oxazine 170, azure A etc. served as efficient electrocatalyst towards oxidation of NADH [10, 53]. In the same way, PyB monomer structure resembles with azine monomers (Scheme 1). As shown in Figure 5 curve b, polymerized PyB film on GCE was performed as an excellent redox mediator for electrocatalytic oxidation of NADH in 0.1 M PBS. PyB monomers were able to form an electroactive polymer by anodic oxidation from acidic aqueous solutions similar to poly(methylene blue) nor poly(methylene green) [10, 53]. The structure of poly(PyB) film might similar to the hypothetical structure proposed for poly(methylene blue). Like other polyazines [10, 11, 18, 51, 53], the electrocatalytic reactions of PyB with NADH might follow the formation of the substrate-catalyst complex. The oxidation peak of current of NADH on poly(PyB) modified electrode was directly proportional to the square root of scan rate from 10 to 100 mV/s which is confirmed that NADH diffuses from its solution to polymer film where it reduces the oxidized poly(PyB) film to form NAD^+ and reduced form of poly(PyB). The electrooxidation of NADH at poly(PyB) film modified electrode may proceed as follows [8, 10, 53], however, further studies are required to evaluate such a kinetic process.



3.5. Amperometric Detection of NADH

The amperometric response of poly(PyB) modified electrode to NADH was investigated in the stirred 0.1 PBS

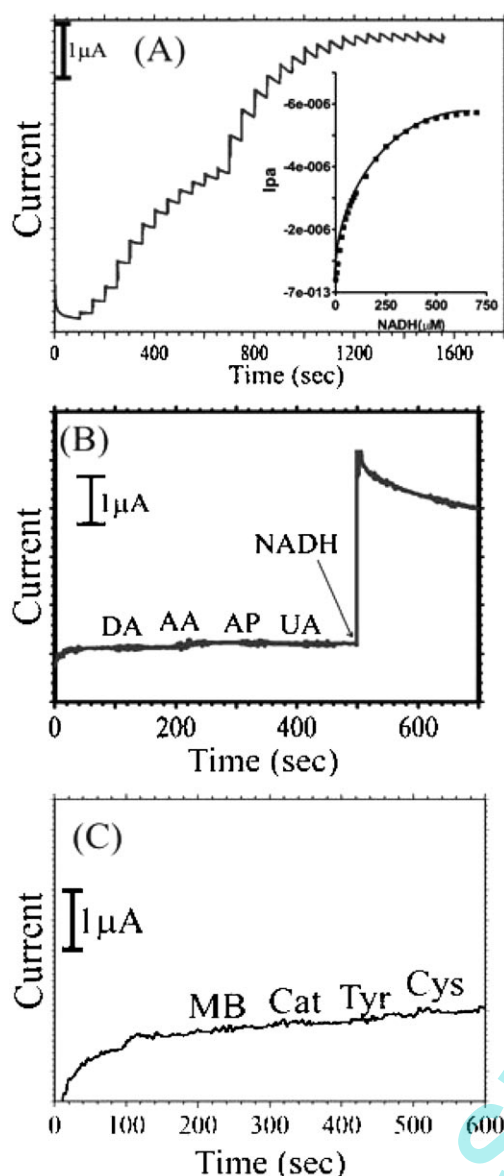


Fig. 6. A) Typical amperometric curve obtained with a poly(PyB)/GCE in 0.1 M PBS (pH 7.0) at 0.0 V, stirring rate = 1000 rpm. Successive additions of NADH in the range from 1.0×10^{-6} M to 5.0×10^{-4} mol/L and inset figure shows the corresponding calibration plot. B) Typical amperometric curve obtained with a poly(PyB)/GCE in 0.1 M PBS (pH 7.0) at 0.0 V. Successive additions of a) 5 mM dopamine, b) 5 mM AA, c) 5 mM acetaminophen, d) 5 mM UA, and e) 5 mM NADH. C) Typical amperometric curve obtained using a poly(PyB)/GCE in 0.1 M PBS at 0.0 V. Successive additions of 5 mM myoglobin (MB), 5 mM catalase (Cat), 5 mM tyrosine (Tyr), and 5 mM cysteine (Cys).

(pH 7.0) at a working potential of 0.0 V. Figure 6A shows typical current–time curve for successive addition of NADH for reaching different concentrations between 1.0×10^{-6} to 5.0×10^{-4} mol/L. The current immediately changed after the addition of NADH and reached another steady-state current within 5 s. The slower decrease of the

current intensity, as observed after the current achieved the maximum value, is due to the slow diffusion of NADH at the electrode–solution interface. Hence, one min of elapsed time, between one addition to other of the NADH solution was maintained. However, which is a common characteristic of amperometric experiments. The inset of Figure 6A shows the calibration curve of NADH (the correlation coefficient was 0.999) and detection limit was found to be 0.5×10^{-6} M at a signal to noise ratio of three. To evaluate the advantage of the present of method, we have compared the pH, applied potential, linear range and detection limit of the present method with the recently reported methods and some of other polyazine modified electrodes in Table 1. As shown in Table 1, present method showed wide linear range [33, 38, 64, 65] and lower limit of detection [33, 61, 62, 64] than earlier reports. In addition, compared to other methods [33, 38, 62–65], NADH determination can be studied at considerably lower applied potential using the present method.

The selectivity and potential interference on poly(PyB) modified electrode were tested using amperometric method. Amperometric responses were obtained by successive injection of 5 mM dopamine, 5 mM ascorbic acid, 5 mM acetaminophen, 5 mM uric acid and 5 mM NADH in stirred 0.1 M PBS solution of pH 7.0 at the same working potential used for NADH (0.0 V). From the amperogram shown in Figure 6B, we can see that no response was obtained for interfering species at this working potential. In addition, we also tested the response of other biological matrix such as myoglobin (MB), catalase (Cat), tyrosine (Tyr) and cysteine (Cys) using poly(PyB) modified electrode as same condition used for detection of NADH. As shown in Figure 6C, poly(PyB) modified electrode do not responded to these biomolecules. These observations show that the poly(PyB) modified electrode can be useful for selective detection of NADH in real samples.

3.6. Reproducibility and Stability

The reproducibility was estimated by eight times repetitive injections of 500 μ M NADH under the same conditions every 20 min. The relative standard deviations of the peak currents were found to be 2.3% for NADH. The turnover frequency of NADH on poly(PyB) modified electrode was calculated to be 56 s^{-1} with no loss of activity which is considerably higher value and comparable with other polyazine modified electrode [11, 18, 23]. In addition, the long-term stability of poly(PyB) modified electrode stored at 4 °C 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 five-week periods. The results showed that the poly(PyB) modified electrode had a good reproducibility and stability when it was used as the sensor to determine NADH in physiological condition.

Table 1. Comparison of the performance characteristics of modified electrodes proposed for the detection of NADH. GCE: glassy carbon electrode, SPE: screen printed carbon electrode, SWNT: single walled carbon nanotubes, MWNTs: multiwalled carbon nanotubes, PABS: poly(*p*-amino benzenesulfonic acid).

Electrode	Matrix	Mediator used	pH	E_{app} (V)	Linear range	Detection limit	Reference
GCE	PABS	Poly(ABSA)	6.4	0.2	10 to 300 μ M	1 μ M	[33]
Pyrolytic graphite	–	Mediator arising from the oxidized adenine moiety of FAD	7.4	0.125	25 nM to 10 μ M	40 nM	[38]
Graphite	SiO ₂ /TiO ₂	Meldola blue	7.5	–0.12	0.018 to 7.29 mM	0.008 mM	[61]
GCE	SWNT	Thionine	8.3	0.05	10 μ M to 1.5 mM	2 μ M	[62]
GCE	MWNTs	6-Ferrocenyl-hexanethiol	7.4	0.65	5.0 μ M–1.5 mM	0.54 μ M	[63]
GCE	–	Poly(methylene green)	6.2	0.05	5.6–420 μ M	3.8 μ M	[64]
SPE	–	Poly(azure B)	6.9	0.2	0.5 to 100 μ M	0.2 μ M	[65]
GCE	–	Poly(Pyronin B)	7.0	0.0	1 to 500 μ M	0.5 μ M	This work

4. Conclusions

In summary, we reported a new redox polymer, poly(PyB) modified electrode and its electrochemical properties. In this work, poly(PyB) modified electrode has been employed for the determination of NADH at lower potential. Furthermore, amperometric determination of NADH has been demonstrated using poly(PyB) modified electrode as an electrochemical sensor for the first time. This described new method is very simple and the modified electrode can be prepared easily within 30 min. However, the poly(PyB) modified electrode have the good stability in neutral buffer solution. These features advocate the potential application of poly(PyB) modified electrode as an amperometric sensor for detection of NADH.

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6. References

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