

Voltammetric Studies of the Oxidation of Reduced Nicotinamide Adenine Dinucleotide at a Conducting Polymer Electrode

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Voltammetric studies of the redox behaviour of NADH at a poly(3-methylthiophene) conducting polymer electrode showed a large electrocatalytic effect without the use of electron-transfer mediators.

The electrochemical oxidation of dihydronicotinamide adenine dinucleotide (NADH) in aqueous solution is of increasing interest in order to develop amperometric biosensor electrodes¹ for the determination of substrates which react under enzymatic conditions with NAD⁺ to produce NADH. Problems associated with the electro-oxidation of NADH, namely, the considerable overpotentials, 1.1 V at carbon^{2a} and 1.3 V at platinum^{2b} electrodes, and the adsorbed molecules of NAD⁺^{2c} resulted in the interference from more easily oxidizable species for the amperometric NADH detection in serum samples and electrode fouling at NADH concentrations above 0.1 mM, respectively. The electrocatalytic oxidation of NADH at modified electrode surfaces has been extensively investigated.³ Transfer of electrons has been shown to be effectively catalysed by the introduction of mediators. However, these electrodes suffered from the lack of long-term stability.

We describe here voltammetric studies of NADH in aqueous media at a poly(3-methylthiophene) modified electrode (PMTME) with no additionally immobilized mediators. We observed a noticeable reduction in the activation energy for NADH oxidation and a remarkable stable voltammetric response at the conducting polymer electrode surface. The electron transfer necessary for the conversion in reaction (1) is realized perhaps by charge tunnelling across the polymer film to the adsorbed NADH molecule. Evidence for the prerequisite adsorption of NADH at the PMTME was confirmed by the necessary cycling of the electrode in NADH-electrolyte solution between 0 and -500 mV (*vs.* Ag/AgCl), prior to the analysis step, and from the UV-VIS spectra obtained for the polymer film on an optically transparent SnO_x coated glass

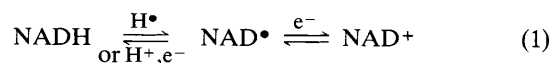


Table 1. Effect of the electrolyte type on the peak potentials (E_{Pa}) and anodic peak currents (I_{Pa}) observed on the PMTME electrode. Scan rate: 50 mV s^{-1} .

Electrolyte ^a	E_{Pa}/mV	$\Delta E_{Pa}/\text{mV}^b$	I_{Pa}/A
Na_2SO_4	455	545–845	1.28×10^{-5}
H_2SO_4	450	550–850	5.90×10^{-6}
NaCl	472	528–828	9.07×10^{-6}
NaNO_3	758	242–542	7.20×10^{-5}
Buffer I ^c	448	552–852	1.57×10^{-4}

^a All electrolytes were 0.1 M in concentration in nano-pure water.

^b Peak differences from those measured in glassy carbon and Pt electrodes, respectively. ^c Buffer I: 5 mM Na_2HPO_4 , 5 mM NaH_2PO_4 , and 0.1 M NaCl.

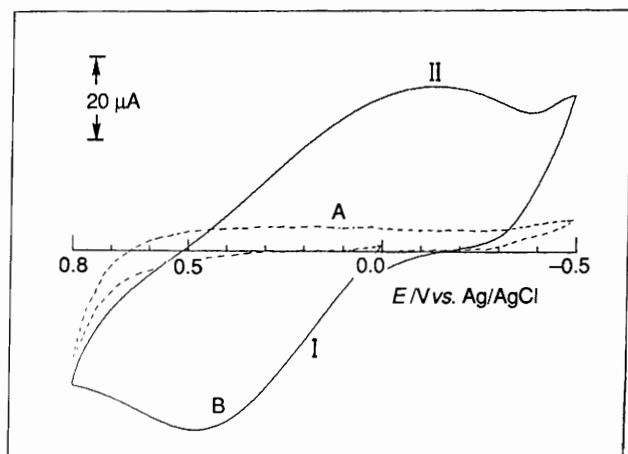


Figure 1. Cyclic voltammetric response of PMTME grown on Pt in absence (A) and in presence (B) of 1 mM NADH (in 0.01 M H_2SO_4). Scan rate: 50 mV s^{-1} . (I) Anodic, (II) cathodic.

electrode revealing two distinguishable peaks for the adsorbed NADH at ≈ 340 and 260 nm , in addition to the polymer absorption peak around 510 nm .⁴

Poly(3-methylthiophene) films were electrochemically grown on a Pt disc substrate from deaerated 50 mM 3-methylthiophene and 100 mM tetrabutylammonium tetrafluoroborate-dry acetonitrile solutions at an applied constant potential of 1.6 V (vs. Ag/AgCl). Film thicknesses of 300–2400 Å were estimated from coulometric measurements during the film formation. Figure 1 shows a typical CV behaviour of PMT in 0.01 M H_2SO_4 in the absence (A) and presence (B) of 1 mM NADH, respectively. As may be seen from the results obtained on PMTME, the anodic peak potential values, E_{Pa} , for NADH oxidation is ca. 450–760 mV, which is 250–850 mV less positive at the modified electrode than at the non-treated platinum and carbon electrodes. However, the increase in the effective surface area is manifested by the increase in the background charging current in the case of PMTME. Summaries of the voltammetric results of NADH at PMTME in different electrolytes are given in Table 1.

The position of the E_{Pa} was a function of both the nature of electrolyte and the pH employed. This might be attributed to the different kinetic effects for different electrolytes used. The 'doping' level by the anions and the conductivity of the polymer film electrode are a function of the charge/size ratio as reported by Garnier *et al.*⁵ This will also affect the electrochemical response of these films towards electroactive species upon varying the supporting electrolyte. However, the

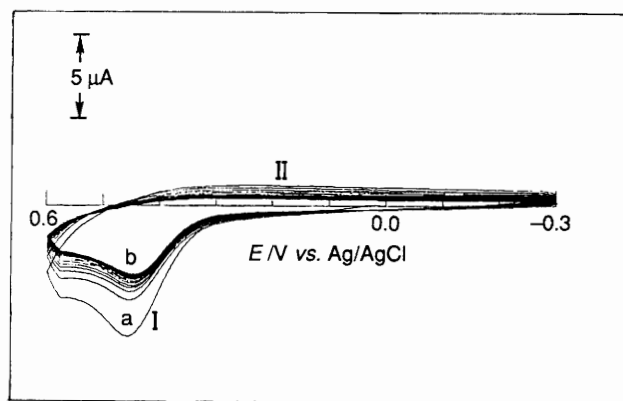


Figure 2. Effect of repeated cycles on the stability of PMTME grown on Pt on the response towards 1 mM NADH (in 0.01 M Na_2SO_4) solution. First cycle (a) and 100th cycle (b). Anodic, (II) cathodic.

reversibility vs. irreversibility shown in Figures 1 and 2 cannot be explained at this stage of the present work. The highest catalytic effect was observed with the use of Buffer I. The stability of the polymer film was examined by repeatedly cycling the electrode within a narrow potential window as is shown in Figure 2. The usual adverse adsorption effect onto the electrode which resulted in the fouling of its surface and the significant attenuation of the voltammetric current signal was not observed. The anodic peak current values (I_{Pa}) correlate linearly with the square root of the scan rate ($v^{1/2}$) for all the electrolytes used, indicating that the charge transfer process is controlled mainly by diffusion as illustrated in Figure 3a. Moreover, the peak potential, E_{Pa} , exhibited a positive linear shift with the logarithm of the scan rate, indicating the irreversible nature of the electrode reaction. It could also be observed from the cyclic voltammogram shown in Figure 1 that the electron transfer occurs through the polymer film at potentials well negative to its doped (oxidized) state, where the film becomes highly conducting.⁶

The effect of polymer film thickness on the I_{Pa} and E_{Pa} values was also examined. Figure 3b shows the effect of time of deposition of PMT films on the peak potential values, E_{Pa} . A limiting value was generally obtained which varied with the nature of the electrolyte. The highest current signals were observed for films formed with 50 mM methylthiophene and tetrabutylammonium hexafluorophosphate in acetonitrile on platinum for 4 minutes. Details of film preparation are mentioned elsewhere.⁴ This shift in I_{Pa} values may be a result of the increase in the effective surface area or the number of active sites within the polymer film. Successive standard additions of NADH (10^{-4} – 10^{-3} M) showed a linear increase in the I_{Pa} values of the cyclic voltammogram.

Flow injection analysis of NADH with a constant potential amperometric detector using a PMTME working electrode proved to be a sensitive detection means with stable responses.⁷ Moreover, other redox systems such as ascorbic acid, dopamine, phenols, aminophenols, catechols, hydroquinones, *etc.* have also been studied with results indicating the applicability of PMTME for the selective and sensitive determination of these compounds.⁸

In conclusion, the present investigation shows the possibility of using a conducting polymer modified electrode for the determination of NADH. The PMTME showed excellent stable response and high resistance against surface fouling. The charge-transfer process was proved to be diffusion-controlled as illustrated from the film thickness and effect of scan rate studies. The possible application of the proposed electrode for the determination of other organic and biological

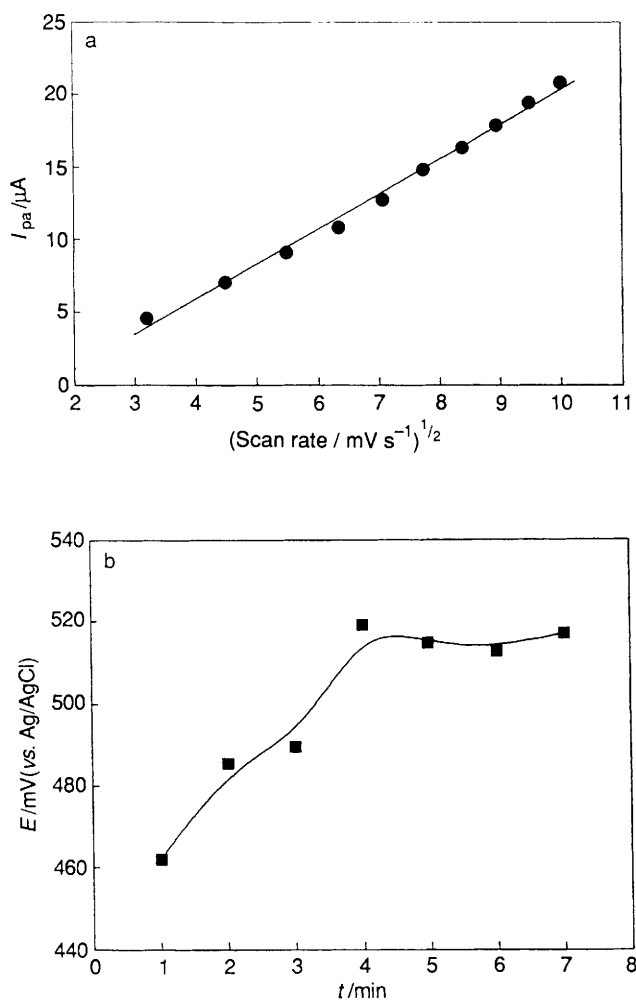


Figure 3. (a) Effects of increasing scan rate on anodic peak current, I_{pa} . (b) Effect of increasing deposition time of PMT film grown on Pt on anodic peak potential, E_{pa} . 1 mM NADH (in 0.01 M Na_2SO_4).

systems of clinical, environmental, and industrial importance has also been verified.⁷

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