# Mediatorless Electrocatalysis at a Conducting Polymer Electrode: Application to Ascorbate and NADH Measurement

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Voltammetric studies of a poly(indole-5-carboxylic acid)-modified glassy carbon electrode showed a catalytic effect to ascorbate and NADH, without the use of electron-transfer mediators.

Ascorbic acid analysis is of interest to the food industry owing to its role as a chemical marker for assessing food deterioration.[1] The interest in NADH measurement lies in the commercial availability of many $NAD^+$-linked dehydrogenases applicable to biosensor research. The oxidation of both compounds shows a single peak and involves the removal of two electrons. Ascorbate oxidation also requires the cleavage of two oxygen–hydrogen bonds, while NADH oxidation proceeds *via* the cleavage of a carbon–hydrogen bond. At clean electrodes both reactions require a high overpotential,[2,3] with NADH oxidation also often poisoning the electrode material. This applies to platinum, glassy carbon, pyrolytic graphite and gold electrodes.[3]

Much research has been directed at the avoidance of these problems by modification of the working electrode with an electron-transfer mediator. Methods have involved the adsorption onto the electrode of either a mediator monolayer,[4] or a polymer which enables three-dimensional mediator coverage.[5] The latter approach is more favourable as it provides higher catalytic currents.[6]

One of the most promising methods of electrode modification is that of electropolymerisation to produce an adherent and conducting polymer film, and electrocatalytic compounds have been entrapped successfully in conducting polymers during deposition.[7,8] However, this method can suffer from lack of long-term stability, owing to exchange of anions with the external electrolyte. A preferable, but as yet little examined technique, would be to use a conducting polymer which is inherently electrocatalytic for the analyte. Thus far, only one such polymer, poly(3-methylthiophene), has been

reported.[9] In this paper we decribe the catalytic properties of a second such material, poly(indole-5-carboxylic acid).

Poly(indole-5-carboxylic acid) films were grown on a glassy carbon disc from solutions of 5 mmol $dm^{-3}$ monomer in 50 mmol $dm^{-3}$ Tris-HCl, pH 8.8 containing 0.1 mol $dm^{-3}$ KCl. Polymerisation was initiated by cycling the electrode potential between −0.8 and 2.5 V *vs.* saturated calomel electrode (SCE) at 0.2 V $s^{-1}$. Film growth was followed by noting the increase in redox peak heights with subsequent scans. Maximum coverage was achieved by six to seven cycles, which corresponded to accumulating a charge density of approximately 0.2 C $cm^{-2}$.

Figs. 1 and 2 show voltammograms of ascorbate and NADH at bare and modified electrodes, illustrating the electrocatalytic effect of the polymer towards both compounds. In both cases polymer deposition also caused an increase in the effective surface area, as is evident in the voltammograms by increased non-faradaic currents. The shift in oxidation potential is greater for ascorbate than for NADH. At this stage the reason for this is unclear and is perhaps related to the particular mechanism for the NADH oxidation.

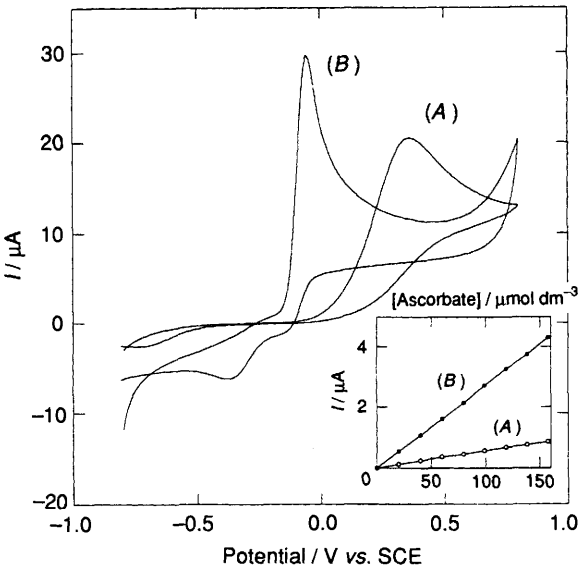


**Fig. 1** Cyclic voltammogram of 2 mmol $dm^{-3}$ ascorbate in Tris-HCl, pH 8.8 at bare (*A*) and polymer-modified electrode (*B*). Scan rate: 20 mV $s^{-1}$. *Inset*: Calibration of ascorbate at 0 mV at bare (*A*) and modified (*B*) electrode.

It would appear that, as in the case of poly(3-methylthiophene),[9] the polymer is apparently electrocatalytic without acting as an electron-transfer mediator. Our reasons for suggesting this are that in buffer solution the polymer film shows only an irreversible reduction peak(−400 mV *vs.* SCE), whereas to mediate oxidation of the analyte, the polymer would need to be reoxidised at the electrode. Hence, this catalytic effect can perhaps be viewed as analogous to electrode modifications such as platinization,[10] in which the oxidation potential of an analyte is lowered without the action of a redox-mediating species.

For both analytes, electron transfer occurs at potentials considerably negative of the polymers' conducting state (*ca.* 2.3 V). This was also found by Atta *et al.* using poly(3-methylthiophene).[9] Atta *et al.* have speculated that this form of electrocatalysis might occur *via* charge tunnelling across the polymer film. The initial NADH adsorption required for this was observed from spectroelectrochemical measurements on an $SnO_x$-coated glass electrode. Our own studies have determined that the oxidative peak current at a modified electrode incubated in a 2 mmol $dm^{-3}$ NADH solution increases with time (from 13.85 to 16.93 μA in 1 h), again suggesting uptake into the polymer film. Also, cyclic voltammograms of a modified electrode in a blank buffer solution, following longer incubation times (1.5–3 h), showed the presence of adsorbed NADH on the electrode.

Ascorbate and NADH were determined by amperometry at 0 and +450 mV *vs.* SCE respectively. In each case calibrations were performed before and after electrode modification. As illustrated in Fig. 3 and the inset to Fig. 1, the increase in sensitivity to both analytes was similar. In addition to this, polymer-modification prevented electrode poisoning during NADH oxidation, producing steady-state rather than peaked responses and increasing the linear range, as can be seen by comparison with the inset to Fig. 3.

As an illustratory application of NADH determination, alcohol dehydrogeanse (ADH) was immobilised onto a polymer-modified electrode by cross-linking with glutaraldehyde. Calibrations were performed at +450 mV in Tris-HCl, pH 8.8. As has been previously demonstrated,[11] an

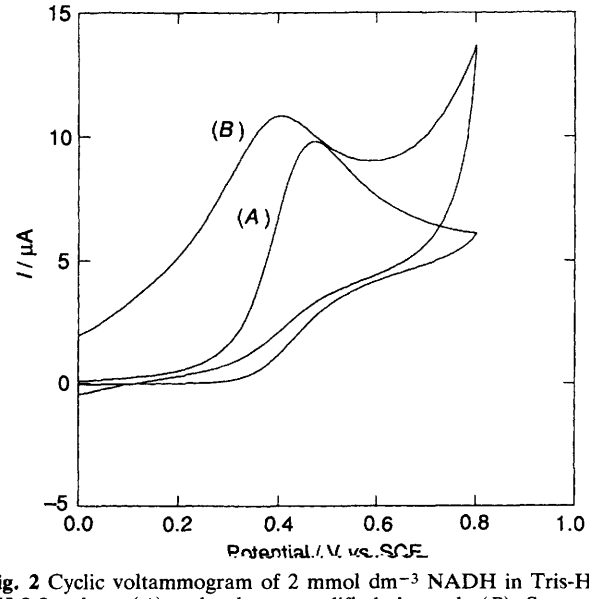


**Fig. 2** Cyclic voltammogram of 2 mmol $dm^{-3}$ NADH in Tris-HCl, pH 8.8 at bare (*A*) and polymer-modified electrode (*B*). Scan rate: 20 mV $s^{-1}$.

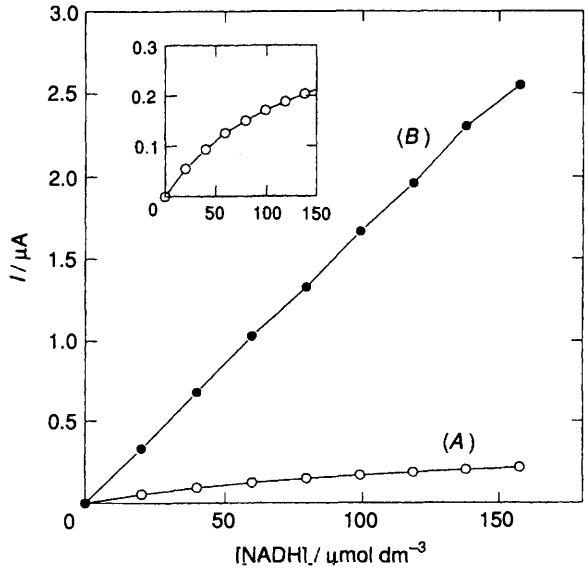


**Fig. 3** Calibration of NADH at +450 mV *vs.* SCE at bare (*A*) and modified (*B*) electrode. *Inset*: Bare electrode calibration shown with expanded *y* axis.

apparent Michaelis–Menten constant ($K'_m$) can be calculated for an immobilised enzyme by amperometric methods, taking a double reciprocal plot of steady-state current against substrate concentration. In such a case, we would expect a higher $K'_m$ value than for the enzyme in solution, owing to the presence of the unstirred Nernst diffusion layer adjacent to the electrode and the resulting concentration gradient across it.[12] Further diffusional restriction could also be caused by the presence of inactive immobilised enzyme. These factors are reflected in our results, which show $K'_m$ values for ethanol and $NAD^+$ (9.2 and 3.8 mmol $dm^{-3}$ respectively) notably higher than previously reported data taken at this pH, using dissolved ADH[13] (3.2 mmol $dm^{-3}$ for ethanol, 0.08 mmol $dm^{-3}$ for $NAD^+$). An additional cause of $K'_m$ elevation could also be the presence of adsorbed NADH lowering the rate of formation of the enzyme–substrate complex.

Many of the applications for NADH determination involve clinical analytes.[14] In cases requiring measurement in blood or serum, the possible interference of an ascorbate signal should be considered. The presence of ADH on the polymer-modified electrode lowered the sensitivity of the ascorbate response at +450 mV from 0.60 to 0.07 μA μmol $dm^{-3}$ $cm^{-2}$. This is likely to be due to the diffusional barrier created by the cross-linked enzyme, and also to the fact that the ADH used was reported by the manufacturer (Calbiochem, Nottingham, UK) as having an isoelectric point of pH 5.4 and at pH 8.8 would therefore present an overall negative charge to the ascorbate anions. Further diminution of the ascorbate response could possibly be achieved by charge- or size-selective membranes.

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