# An Organic Conductor Electrode for the Oxidation of NADH

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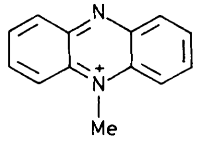
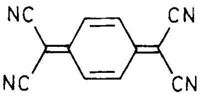
The conducting organic salt *N*-methylphenazinium tetracyanoquinodimethanide can be used as an electrode for the oxidation of NADH at a sufficiently rapid rate to be a good detector in an enzyme coupled sensor.

Many enzymes use the coenzyme β-nicotinamide adenine dinucleotide ($NAD^+$) producing the reduced form NADH. The rate or amount of NADH production is used for a wide range of systems to assay the enzyme activity or concentration of the enzyme's substrate, respectively.[1] Such measurements at present are done by bench top procedures using spectrophotometry. The use of *in situ* electrochemical regeneration of NADH to $NAD^+$, where the current is proportional to the rate of reaction, allows the construction of more convenient, cheap, direct reading sensors which can be used on line and *in vivo*. Unfortunately the direct oxidation of NADH on a metal electrode, such as platinum, is complicated and requires a large overvoltage.[2,3] The reaction takes place through radical intermediates and the large overvoltage invites interference from other reactions on the electrode. To avoid these problems several groups have investigated the uses of media-



NMP$^+$



TCNQ

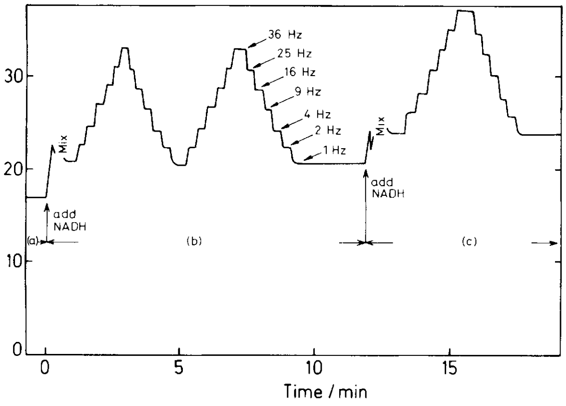


**Figure 1.** Typical results for the oxidation of NADH on a rotating dip coated NMP$^+$TCNQ$\cdot^-$ electrode (0.39 cm$^2$; $E = 0.15$ V in 0.5 M KCl, 0.1 M Tris, pH 7.0), showing the effects of adding aliquots of NADH and of changing the rotation speed, as shown in the diagram. The concentrations of NADH (in mM) were: (a) 0.84; (b) 1.05; (c) 1.25.

tors, which, when coated on the electrode surface, catalyse the oxidation of NADH.[4] Such catalysis has indeed been observed, but hitherto there have been problems with the stability of the modified electrode.[5]

We report the development of a novel electrode using the conducting organic salt NMP$^+$TCNQ$\cdot^-$. This material was first prepared by Melby[6] who found that its conductivity was comparable to that of copper. Kulys[7,8] has investigated enzyme electrochemistry on electrodes made of this material. In our work we have found that NMP$^+$TCNQ$\cdot^-$ electrodes readily oxidise NADH and furthermore they suffer no deterioration over a matter of days.

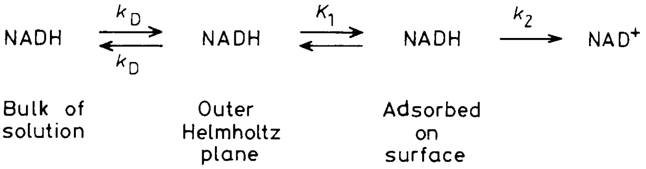
The conducting salt was prepared by the method of Melby.[6] Rotating disc electrodes were fabricated from the crystalline solid by three methods: by cold pressing a pellet,[7] by successive dip coatings of a glassy carbon disc electrode, and by packing a small cavity with a paste made of crystallites and polyvinyl chloride in tetrahydrofuran. All potentials are reported with respect to the saturated calomel electrode (S.C.E.).

The electrochemical stability of a NMP$^+$TCNQ$\cdot^-$ electrode has been investigated by Jaeger and Bard.[9] In agreement with them, we find that the electrode is stable between $-0.1$ and $+0.3$ V. Throughout this potential region NADH is oxidized; the rate of oxidation is virtually independent of the electrode potential. Typical results for the current from the oxidation of NADH on a rotating dip coated glassy carbon electrode are shown in Figure 1 for successive aliquots of NADH and for different rotation speeds.

The results are analysed according to the kinetic scheme shown in Scheme 1. In this scheme $k_D$ [equation (1)] is a mass

$$k_D = 1.554\, D^{2/3}\, \nu^{-1/6}\, W^{1/2} \qquad (1)$$

transfer rate constant[10] determined from the Levich equation for a rotating disc electrode,[11] where $D$ is the diffusion coefficient of NADH, $\nu$ is the kinematic viscosity, and $W$ is the rotation speed in Hz. On the electrode surface we find that NADH obeys Michaelis Menten kinetics[12] or in other words it is adsorbed according to a Langmuir isotherm.



**Scheme 1**

**Table 1.** Kinetic parameters for the oxidation of NADH.

| Electrode | $k_2$/s$^{-1}$ [a] | $K_1^{-1}$/mM [b] | $k'$/cm ks$^{-1}$ [c] |
|---|---|---|---|
| Dip coat | 13 | 3 | 0.56 |
| Polymer paste | 3 | 1.3 | 0.30 |
| Pellet | 2 | 0.15 | 1.70 |
| Pellet | 1.5 | 1.0 | 0.20 |
| Pellet[d] | 3.3 | 0.45 | 0.95 |

[a] $k_2$ obtained from intercept of equation (2). [b] $K_1$ obtained from gradient of equation (2). [c] $k'$ calculated from equation (3). [d] In this experiment the electrolyte was 0.1 M LiCl, 0.1 M Tris, pH 8.0 instead of 0.5 M KCl.

In the steady state we then obtain a form of the Koutecky Levich equation[13] modified for the more complicated kinetics on the electrode surface [equation (2)], where $i$ is the current, $A$ is the area of the electrode, $k_D(W{=}1)$ is the value of $k_D$ for $W{=}1$ Hz, and $N$ is the number of sites on the electrode surface. From the crystal geometry[14] we estimate that $N$ is 1.3 $\times$ 10$^{-10}$ mol cm$^{-2}$.

$$2FA/i = 1/\{k_D(W{=}1)\,[\mathrm{NADH}]W^{1/2}\} + 1/(K_1k_2N[\mathrm{NADH}]) + 1/(k_2N) \qquad (2)$$

Figure 2(a) shows typical results plotted according to equation (2) where the rotation speed ($W$) has been varied at constant bulk concentration of NADH ([NADH]). Good straight lines are obtained. From the gradients and equation (1) we find that $D = 4.4 \times 10^{-6}$ cm$^2$ s$^{-1}$ which is the same as the value found by Elving.[2] The intercepts which correspond to infinite rotation speed, where there could be no concentration polarization, are given by the last two kinetic terms in equation (2). Figure 2(b) is the equivalent of a Lineweaver Burk plot and shows the intercepts plotted against [NADH]$^{-1}$. From the gradients and intercepts of plots such as these for different electrodes, we obtain the values for $K_1$ and $k_2$ given in Table 1. We also report values of the effective electrochemical rate constant $k'$ (cm s$^{-1}$) [given by equation (3)] which is found at low concentrations of NADH before the surface becomes saturated. From the results in Table 1 it can be seen that $k'$ is about 10$^{-3}$ cm s$^{-1}$. This corresponds to a relatively fast electrochemical rate constant; a redox system with this standard rate constant would be quasi-reversible. Hence we conclude that the kinetics of the NADH oxidation on the NMP$^+$TCNQ$\cdot^-$ electrode are sufficiently rapid for the electrode to be a good detector in an enzyme coupled sensor.

$$k' = K_1k_2N \qquad (3)$$

Another advantage of this new type of electrode material is that by sweeping the potential outside the region of stability, one can dissolve the outer layers in a controlled fashion and so present a fresh surface to the electrolyte. This type of regeneration is impossible for electrodes which are modified with a covalent monolayer or with a polymer layer containing

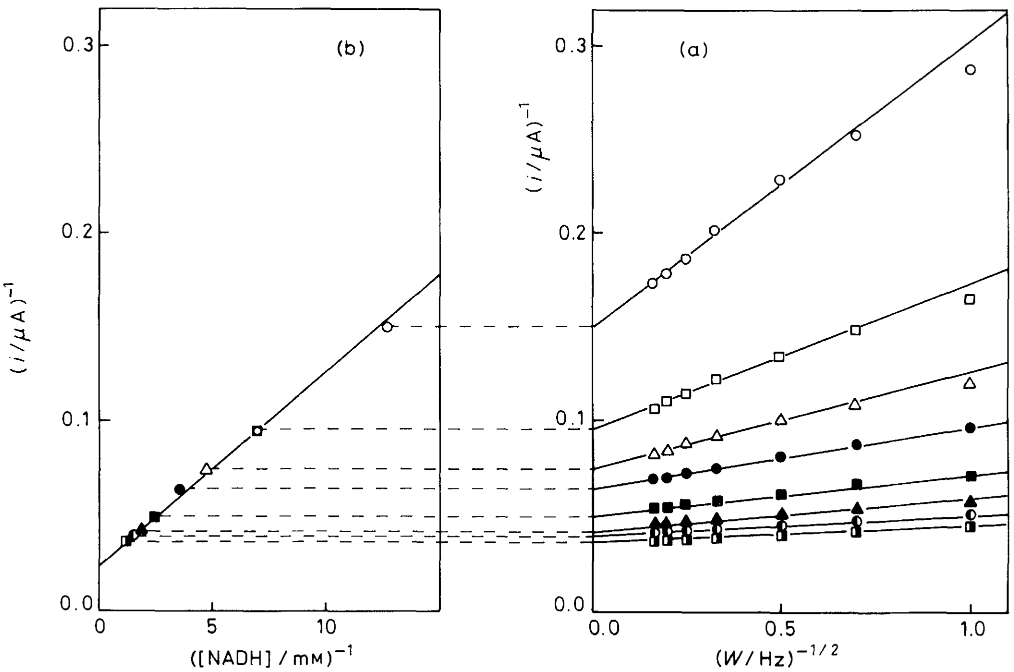


**Figure 2.** Koutecky Levich plots (ref. 14) of the current, *i*, against the rotation speed *W* according to equation (2), obtained on a rotating pellet electrode (0.52 cm$^2$; $E$ = 0.25 V in 0.1 M LiCl, 0.1 M Tris, pH 8.0). The intercepts of the plots of (a) are plotted against [NADH]$^{-1}$ in (b).

redox groups. We conclude that molecular conductors which have specific catalytic properties promise to be an exciting new class of electrode materials.

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