

**Measurement of the Kinetic Isotope Effect for the Oxidation of NADH at a Poly(aniline)-poly(vinylsulfonate) Modified Electrode**

P. N. Bartlett and E. Simon

University of Southampton

Department of Chemistry, University of Southampton, Southampton, SO17 1BJ, UK.

The electrochemical oxidation of NADH at modified electrodes has been widely studied because of its potential applications in biosensors and biofuel cells based on dehydrogenase dependent enzymes.

In a series of papers [1-3] we have shown that poly(aniline)-poly(anion) modified electrodes can be used at neutral pH to carry out oxidation of NADH at potentials around 0.1 V vs. SCE. In these studies we have established that the reaction can be described by the following mechanism



where  $\text{PANi}_{\text{ox}}$  represents an oxidised site on the polymer chain,  $\{\text{NADH.PANi}\}$  the complex formed between the NADH and the polymer, and  $\text{PANi}_{\text{red}}$  the reduced site on the polymer chain.

In the present work we have studied the kinetics of the reaction in greater detail by examining the relative rates of oxidation of NADH and doubly deuterated NADH at the poly(aniline) modified electrode surface. Doubly deuterated NADH was prepared in three steps. First,  $\text{NAD}^+$  is reduced by YADH in presence of  $\text{C}_2\text{D}_5\text{OD}$  to form the monodeuterated [4,4-D,H]-NADH. In a second step deuterated  $\text{NAD}^+$  is synthesized by oxidation of the mono-deuterated NADH by L-glutamate dehydrogenase in presence of  $\alpha$ -ketoglutaric acid. Finally, the dideuterated NADH, [4,4-D,D]-NADH, is obtained by oxidation of the deuterated  $\text{NAD}^+$  by YADH in presence of  $\text{C}_2\text{D}_5\text{OD}$ . The poly(aniline)-poly(vinylsulfonate) composite films were deposited on a rotating glassy carbon disc ( $0.38\text{cm}^2$ ) by cyclic voltammetry in a 0.5 M aniline solution in 1.0 M  $\text{H}_2\text{SO}_4$  in presence of 22% PVS. The poly(aniline)-poly(vinylsulfonate) modified electrode was placed in a thermostated cell at 25 °C, containing 0.1 M citrate-phosphate buffer pH 7.0, under argon. The electrode was rotated at 9 Hz and the potential held at 0.10 V vs SCE. Aliquots of a concentrated solution of NADH or [4,4-D,D]-NADH in 0.1M citrate-phosphate were added in the electrochemical cell and the steady state catalytic currents were recorded.

Figure 2 shows typical results for the oxidation of NADH at a poly(aniline) coated electrode under conditions where the rate of reaction of the NADH in the bound state becomes rate limiting at high NADH concentration. It is clear from the results that the currents for the doubly deuterated compound are significantly smaller. Based on our analysis of the data using our model from the reaction we find that deuteration of the NADH has no effect on the partition and diffusion of the NADH into the film and no effect on the binding of the NADH to the active sites within the film. However the rate of reaction of the bound NADH shows a significant kinetic isotope effect of  $k_{\text{H}}/k_{\text{D}}$  of 5.4. This is consistent with typical values in the literature for hydride transfer reactions and confirms that the C-H bond is broken in the rate limiting step for the reaction of the bound NADH.

To the best of our knowledge this is the first recorded example of the measurement of a kinetic isotope effect for a reaction at a modified electrode.

**ACKNOWLEDGEMENT**

This work was supported by the Office of Naval Research

**REFERENCES**

1. P. N. Bartlett, P. R. Birkin and E. N. K. Wallace, *J. C. S., Faraday Trans.*, **93**, 1951 (1997).
2. P. N. Bartlett and E. N. K. Wallace, *J. Electroanal. Chem.*, **486**, 23 (2000).
3. P. N. Bartlett and E. Simon, *PCCP*, **2**, 2599 (2000).

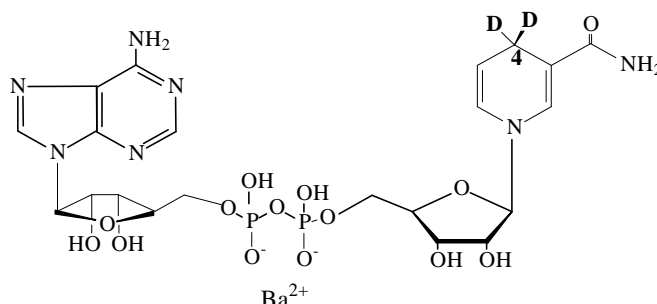


Figure 1 Structure of doubly deuterated NADH

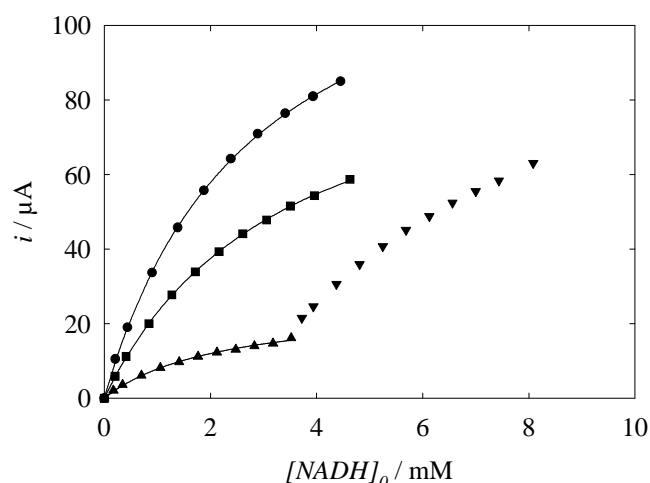


Figure 2 Plot of catalytic currents for oxidation of NADH or [4,4-D,D]-NADH, recorded at 0.10V vs SCE at PANi-PVS modified electrode (electropolymerisation by cyclic voltammetry, deposition charge ~ 70mC, geometric area =  $0.38\text{cm}^2$ ) rotated at 9 Hz in 0.1M citrate/phosphate buffer pH 7.0 at 25°C. First Run: (●) NADH; second run: (□), [4,4-D,D]-NADH, (→), NADH; third run: (▲), NADH.