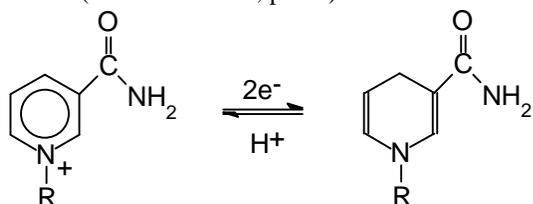


Equilibrium (NAD⁺/NADH) Potential on Poly(Neutral Red) Modified Electrode.

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The largest group of known oxidoreductases enzymes (>500) uses the turnover of nicotinamide adenine dinucleotide (NAD⁺/NADH) as a coupled redox reaction. Electrochemical regeneration of this cofactor allows the development of the biosensors for a great number of analytes, the biofuel cells with different cheap fuels and provide the specific electrosynthesis of organic compounds. However, (NAD⁺/NADH) is the mediator with the lowest potential as can be found in aerobic organisms (-0.56 V vs SCE, pH 7):



It is not surprised that *nature* discovered how to prevent oxidation of its reduced form in aerobic media. As a result, regeneration of (NAD⁺/NADH) on carbon and platinum electrodes requires extremely high overvoltages (>1.0 V). Due to continuous efforts of bioelectrochemists during last three decades the mediators for (NAD⁺/NADH) regeneration were proposed: quinones, azines, transition metal complexes. However, the known systems still require overvoltages and are not able to catalyze both NAD⁺ reduction and NADH oxidation.

To mimic biological catalysis we used the electropolymerization technique. When the structure of the monomer is similar to the chemical structure in biological system of interest, the desired affinity or catalytic center can appear in the corresponding polymer due to various ring and heteroatom substitutions. In particular, phenazine Neutral Red reminds the molecular structure of flavins.

Due to optimization of Neutral Red electropolymerization, the electrocatalyst for both NAD⁺ reduction and NADH oxidation was synthesized. As seen in Fig. 1a the addition of pure NADH provides the anodic (oxidation) current on poly(Neutral Red) modified electrode, where as the addition of pure NAD⁺ causes the cathodic (reduction) current. Thus, poly(Neutral Red) is active in both NAD⁺ reduction and NADH oxidation. Indeed, in the presence of equimolar mixture of NAD⁺ and NADH the current-potential curve intersects the abscissa axis (Fig. 1b). The solid lines in Fig. 1 are the corresponding polarographic waves fit to the typical exponential equations.

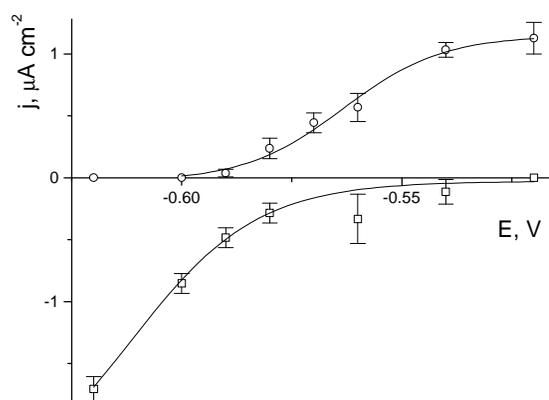
Let us consider the current-potential curve in the presence of equimolar mixture of NAD⁺ and NADH (Fig. 1b). The observed zero-current potential of -0.59 V (SCE) was rather far from both determined poly(Neutral Red) redox potential (-0.53 V) and the reported in (1) standard potential for NAD⁺/NADH (-0.53 V). It is however seen, that the current-voltage curve in Fig. 1b is an overlap of the corresponding curves for NAD⁺ reduction and NADH oxidation (Fig. 1a). The algebraic sum of the voltammograms in Fig. 1a is not able to reach a zero current around -0.53 V. Thus, the zero-current potential observed in fig. 1b is due to NAD⁺/NADH conversion.

The current-voltage dependence for equimolar mixture of NAD⁺ and NADH was fit to the above exponential equation (Fig. 1b, solid line). The computer treatment indicated the transfer of two electrons per

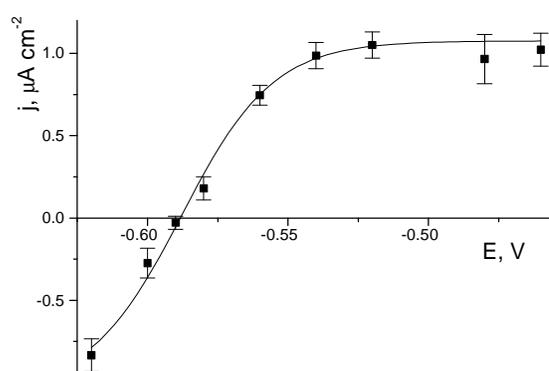
reaction turnover, what exactly in the frame of NAD⁺/NADH reaction scheme (see above). Thus, the steady-state current-voltage dependence in Fig. 1b is the reversible polarographic wave of nicotinamide adenine dinucleotide reduction-oxidation.

We supposed that the reported (rather long ago by the way) standard potential for NAD⁺/NADH [1] had not been precisely determined in particular at pH 6.0; its real value is equal to the zero-current potential (-0.59 V) observed in Fig. 1b. Since the standard potential of NAD⁺/NADH is lower than the redox potential of poly(Neutral Red) (-0.53 V), the activity of the electrocatalyst in NAD⁺ reduction should be higher compared to its activity in NADH oxidation. Indeed, the slightly improved activity towards NAD⁺ reduction is observed in Fig. 1. This confirms, that the observed zero-current potential in Fig. 1b is the equilibrium potential for NAD⁺/NADH reduction-oxidation.

We conclude, that using poly(Neutral Red) modified electrodes, the reversible polarographic wave of nicotinamide adenine dinucleotide reduction-oxidation and the equilibrium (NAD⁺/NADH) potential were observed. This was impossible using all known catalytic and mediator systems. The unique poly(Neutral Red) based electrocatalyst allowed us to determine the standard (NAD⁺/NADH) potential more precisely ($E' \cong 0.59$ V SCE, pH 6.0).



a



b

Figure 1. Current responses of poly(Neutral Red) modified electrodes to nicotinamide adenine dinucleotide at different potentials: (a) 0.1 mM NADH (o) or 0.1 mM NAD⁺ (□), (b): mixture of 0.1 mM NADH and 0.1 mM

NAD⁺; 0.02 M phosphate pH 6.0 with 0.1 M KNO₃.