

Optimizing the Electrocatalytic Oxidation of NADH at Nitrogen-Doped Carbon Nanotubes

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The cofactor nicotinamide adenine dinucleotide (NAD⁺) is essential for the enzymatic turnover of dehydrogenase enzymes, commonly used in the formation of electrochemical biosensors^{1,2} and biofuel cells^{3,4}. The available array of dehydrogenase enzymes (over 300) provide substantial opportunity to expand the range of fuels that can be used to create energy in biofuel cells, and the types of metabolites that can be monitored in biosensing applications. Fundamental principals of biosensors and biofuel cells overlap, and it can be envisioned that a sufficiently small biofuel cell⁵ could provide power for an implantable biosensor, an attractive method for painless blood glucose monitoring in the management of diabetes.⁶ In many dehydrogenase-based systems, dihydronicotinamide adenine dinucleotide (NADH), the reduced form NAD⁺, provides the flow of electrons by being oxidized at the electrode surface, subsequently converting NADH back to the enzymatically active form. Oxidation of NADH, however, requires a large overpotential, lowering the power of a biofuel cell and causing the appearance of oxidizable interferents in biosensing. Mediators are often introduced to mitigate these effects, of which many options have been investigated.⁷

Nanotechnology has opened the door for alternative oxidation materials, with carbon nanotubes (CNTs) being an attractive option for bioanalytical applications. CNTs were shown to display electrocatalytic activity towards NADH oxidation, significantly lowering the necessary overpotential compared to traditional carbon electrodes such as glassy carbon.⁸ Further enhancements have been made by heteroatom doping CNTs, which can alter both the composition of the electroactive site, and the electronic properties of the CNTs. Boron-doped CNTs, a p-type dopant in carbon, significantly lowered the overpotential for NADH oxidation compared to the nondoped variety.⁹ We present the use of nitrogen-doped CNTs (N-CNTs), a n-type dopant in carbon, as an efficient NADH electrocatalyst. In addition, we demonstrate their use in a biosensing application, as N-CNTs have been noted as being more biocompatible than nondoped CNTs¹⁰ and display spontaneous adsorption of biologically active enzyme on the N-CNT surface.¹¹ Figure 1 displays the electrochemical detection of NADH by oxidation at a 7.4 atom % N-CNT rotating disk electrode set at a potential of -0.32 V (vs Hg/Hg₂SO₄). Figure 2 displays the response of a 7.4 atom % N-CNT rotating disk electrode to aliquots of glucose, after glucose dehydrogenase was allowed to spontaneously adsorb onto the surface of the N-CNTs.

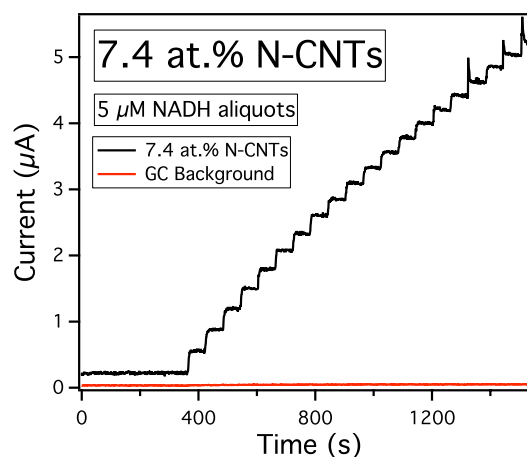


Figure 1. Chronoamperograms of a 7.4 atom % N-CNT and a glassy carbon rotating disk electrode held at -0.32 V (vs Hg/Hg₂SO₄) in an argon purged 0.1 M phosphate buffer (SPB, pH 7.00) solution as aliquots of 5 µM NADH are introduced (rotation rate 1000 rpm)

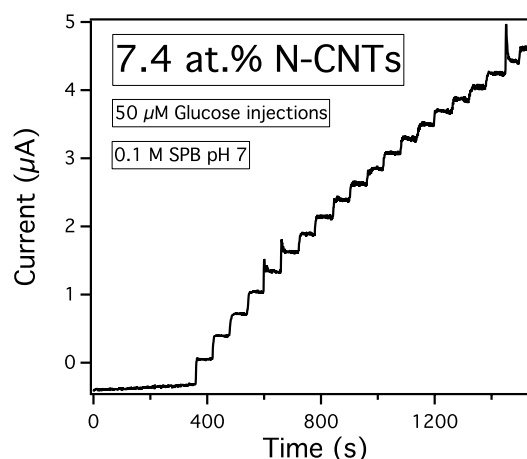


Figure 2. Chronoamperogram of a 7.4 atom % N-CNT rotating disk electrode after glucose dehydrogenase was allowed to spontaneously adsorb on the surface, held at -0.32 V (vs Hg/Hg₂SO₄) as aliquots of 50 µM glucose are introduced (0.1 SPB, pH 7, rotation rate 1000 rpm)

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