

Nitrogen-doped Carbon Nanotube Electrodes for Enzyme Based Electrochemical Biosensing

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Enzyme based electrochemical biosensors use two main types of enzymes, oxidases and dehydrogenases. Although these enzymes perform essentially the same chemical reaction to the substrate, they differ in the reaction mechanism, and more importantly, the cofactors employed to catalyze the enzymatic reaction. In general, oxidases use oxygen as a natural cofactor (subsequently generating H_2O_2), while dehydrogenases utilize the oxidized form of nicotinamide adenine dinucleotide (NAD^+ , subsequently generating NADH). One of the main problems of enzymatic biosensing is the potential necessary to detect an enzymatic event at the electrode surface, such as the oxidation of H_2O_2 or NADH . Carbon is more biocompatible than traditional noble metal electrodes, and is overwhelmingly the choice of electrode material for both commercial and academic electrochemical biosensing. Carbon nanotubes (CNTs), have been shown to be superior to traditional carbon electrodes, such as glassy carbon, for detection of biogenic analytes such as H_2O_2 and NADH .¹⁻³ We present an extension of these findings by showing the advanced chemical properties of CNTs when a foreign atom, nitrogen, is introduced into the carbon lattice, creating nitrogen-doped CNTs (N-CNTs). N-CNTs provide enhanced and unique electrochemical properties for both oxidase and dehydrogenase based electrochemical biosensing.

In the case of oxidases, which use oxygen as a cofactor, the oxygen reduction reaction (ORR) at N-CNTs displays an important difference versus CNTs. Figure 1 shows cyclic voltammograms (CVs) of the ORR at CNTs and N-CNTs with increasing amounts of incorporated nitrogen. It is clear that the potential necessary to reduce oxygen is greatly reduced when nitrogen is introduced into the CNTs. More importantly, the second two-electron reduction of H_2O_2 is not observed with N-CNTs, while clearly visible at CNTs as a second peak more negative than the initial two-electron reduction of O_2 to H_2O_2 .

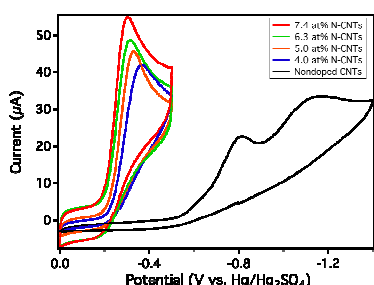


Figure 1. ORR at CNTs and N-CNTs

We believe this unique ORR at N-CNTs

indicates a spontaneous chemical reaction, whereby the H_2O_2 created in the first two-electron reduction of O_2 disproportionates at the N-CNT surface back into O_2 and H_2O .^{4,5} Figure 2 displays this idea by showing cyclic voltammograms of a 7.4 at% N-CNT electrode in an

oxygen saturated solution, and the same solution when H_2O_2 is introduced. After adding H_2O_2 , an increase in the reduction current from O_2 is observed; clearly indicating that introduction of H_2O_2 creates a local increase in O_2 .

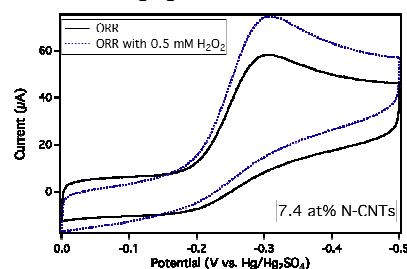


Figure 2. CVs of 7.4 at% N-CNTs in oxygen saturated 0.1M phosphate buffer (pH 7.00)

This unique property provides a different method of detecting enzymatic turnover by measuring the increase, rather than the decrease like most “Clark” type oxygen electrodes, of oxygen as H_2O_2 is enzymatically created on the N-CNT surface. Figure 3 shows this type of biosensing method applied to detect L-lactate with 7.4 at% N-CNTs modified with lactate oxidase.⁶

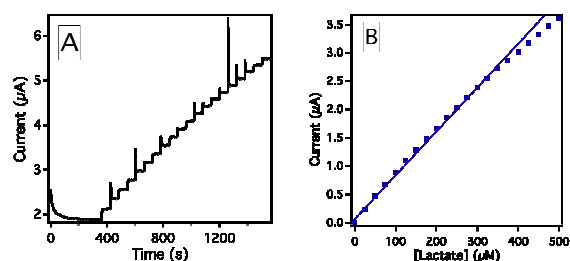


Figure 3. (A) Current increase as L-lactate is added to solution (B) The resulting current response and linear range

In the case of dehydrogenases, which use NADH as a cofactor, N-CNTs further decrease the potential necessary to oxidize NADH versus nondoped CNTs. Figure 4 displays CVs of glassy carbon (GC), edge-plane pyrolytic graphite (EPPG), nondoped CNT, 4.0 at% N-CNT, and 7.4 at% N-CNT electrodes in 0.1 M phosphate buffer (pH 7.00) with 2 mM NADH . Often described as an increase in edge plane character,⁷ N-CNTs display a marked decrease in the NADH oxidation potential from nondoped CNTs, and an over 500 mV decrease from glassy carbon.

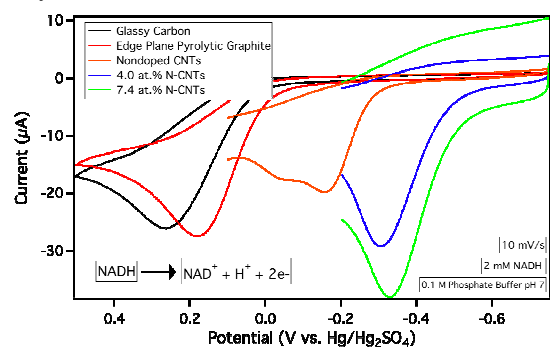


Figure 4. Oxidation of 2 mM NADH by GC, EPPG, CNT, and N-CNT electrodes

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