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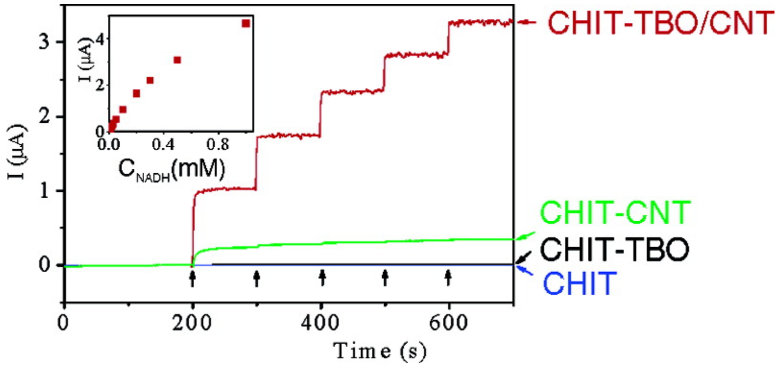

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Electrochemical Sensing Platform Based on the Carbon Nanotubes/Redox Mediators-Biopolymer System

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This report describes a new electrode system that utilizes synergy between carbon nanotubes (CNT) and redox mediators (RM) to facilitate electron-transfer processes. The integration of CNT and RM for such purposes has not been explored thus far. At first, such integration may even appear to be counterintuitive because both CNT and RM provide similar electrochemical benefits in terms of lowering the overpotential (η) of electrode processes.[1−12] Nevertheless, the present communication demonstrates that integration of CNT and RM can provide a remarkable synergistic augmentation of the current because of the oxidation of redox-active species. To demonstrate the concept, CNT and a redox mediator Toluidine Blue O (TBO) were co-immobilized in a matrix of the biopolymer chitosan (CHIT) and used for the oxidation of the enzyme cofactor β-nicotinamide adenine dinucleotide (NADH).

The attachment of redox mediators to a polymeric matrix that can be cast on the electrode surface is an attractive route to improve efficiency of the heterogeneous mediation. However, while polymers modified with one-electron mediators have shown great progress for enzyme electrodes,[13] the corresponding progress for mediator-containing polymers for NADH oxidation has been less successful. The latter has been ascribed to the limited affinity of a polymeric matrix for NADH, slow diffusion of NADH within a polymer film, or redox self-exchange restrictions within the film.[14−16] As presented below, these complications can be overcome by integrating the hydrophilic ion-conducting matrix of CHIT with a two-electron mediator TBO and an electronic conductor CNT.

CHIT is a polysaccharide polymer that exists as a cationic polyelectrolyte in acidic aqueous solutions, precipitates at pH > 6, and can be chemically modified by covalently attaching molecules to its amino and/or hydroxyl groups.[17,18] In the present work, the amino groups of CHIT were used to covalently link amino groups of TBO via Schiff base chemistry using glutaric dialdehyde as a tether (Figure 1). Solutions of 0.02 wt % CHIT-TBO were used to solubilize multiwalled CNT (Nanolab, 20−50 nm diam, 1−5 μm long). The 10-μL aliquots of CHIT-TBO/CNT solutions (0.25 mg of CNT mL^{-1}) were mixed with 20 μL of water and cast on the surface of glassy carbon electrodes (0.07 cm^2). The evaporation of water resulted in thin CHIT-TBO/CNT surface films that contained 2.5 μg of CNT. Other films such as CHIT, CHIT-TBO, and CHIT/CNT were prepared in the analogous manner by casting relevant solutions on the surface of glassy carbon electrodes.

To discern the role of individual components and possible synergy between them, films of CHIT, CHIT-TBO, CHIT/CNT, and CHIT-TBO/CNT were studied in NADH solutions. Figure 2 shows current traces recorded at film electrodes that were polarized at −0.10 V in a stirred solution, which was spiked with NADH stock solution. The CHIT film (trace a) yielded no current response to NADH, which indicated that CHIT played the role of an electrochemically silent structural matrix. At CHIT-TBO films (trace b), a detectable but very small current response to NADH on the order of ~10^{-8} A was observed. The presence of the small current was due to the mediated redox process, which involved the oxidation of NADH by TBO and reoxidation of the reduced TBO molecules at the glassy carbon surface.[19] The small current was indicative of an inefficient redox self-exchange in CHIT-TBO films, which probably related to slow electron hopping between the TBO molecules attached to CHIT chains. Indeed, slower charge transfer in such films was indicated by the increase in separation of voltammetric peaks of TBO from 35 mV for free TBO to 110 mV for TBO bound in CHIT-TBO films (Supporting Information). The direct oxidation of NADH at CNT of CHIT/CNT films (trace c) was also ineffective at −0.10 V because the current saturated quickly after the first addition of NADH to a solution. However, the incorporation of CNT into CHIT-TBO films yielded a high and dynamic current output due to TBO-mediated oxidation of NADH (trace d).

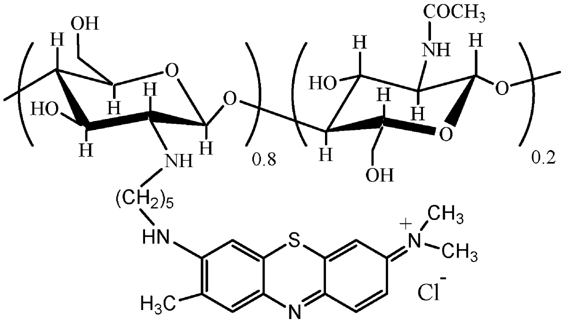

Figure 1. Polysaccharide CHIT with covalently attached redox mediator TBO. The Schiff bases in the CHIT-TBO material were reduced using sodium cyanoborohydride.

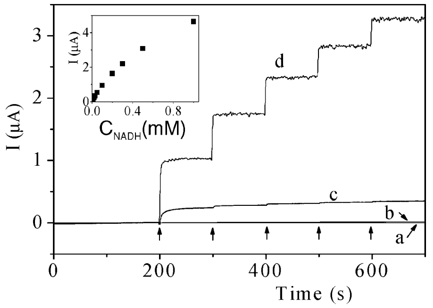

Figure 2. Current traces ($E = -0.10$ V) recorded at (a) CHIT, (b) CHIT-TBO, (c) CHIT/CNT, and (d) CHIT-TBO/CNT film electrodes. Steps represent the response of the films to additions of 0.10 mM NADH (arrows) to a phosphate buffer solution, pH 7.40. Inset: calibration plot at a CHIT-TBO/CNT film electrode.

The comparison of current traces in Figure 2 revealed the presence of synergistic effects in CHIT-TBO/CNT films. The

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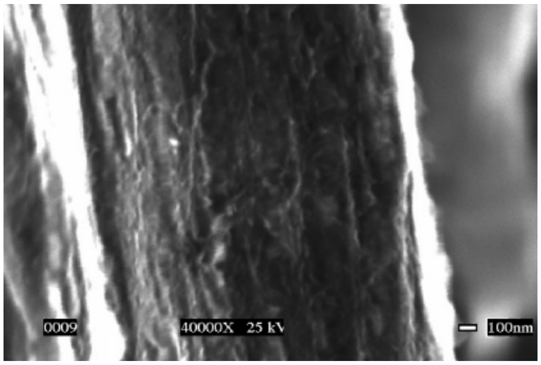

Figure 3. Scanning electron micrograph of a cross section of a CHIT-TBO/CNT film.

synergy was indicated by the condition $I_{(TBO+CNT)} \gg I_{TBO} + I_{CNT}$, where $I_{(TBO+CNT)}$ is the current due to oxidation of NADH at the integrated CHIT-TBO/CNT film (trace d), and I_{TBO} and I_{CNT} are the NADH currents recorded at individual CHIT-TBO and CHIT/CNT films, respectively.

To explain the enhancement of the $I_{(TBO+CNT)}$ current, the following hypothesis was formulated. The introduction of CNT into a CHIT-TBO film improved its electronic and ionic transport capacity because the CNT provided a three-dimensional electron-conductive network, which extended throughout the ion-conductive matrix of CHIT.

The hypothesis was supported and tested by the following observations and experiments. Scanning electron micrographs (Figure 3) demonstrated the homogeneity of the cross sections of composite CHIT-TBO/CNT and CHIT/CNT films. This indicated that CNTs were distributed throughout the CHIT matrix rather than clustered in a distinct zone. One can relate this to the ability of CHIT solutions to form stable colloidal suspensions of CNT.[11] Clearly, the CNT/CHIT interactions promoted a dispersion of CNT in the CHIT matrix upon evaporation of water from such suspensions.

Electrochemical testing (Supporting Information) demonstrated that the introduction of CNT into CHIT-TBO films increased voltammetric peak currents of TBO by ∼25 times, while the active electrode area increased only ∼2 times. This indicated that the increase in TBO peaks involved more than just the extra surface area provided by CNT for the redox of TBO. Additional effects of CNT on the voltammetry of CHIT-TBO films included the lowering of TBO overpotential (by ∼60 mV) and the decrease in the separation of TBO peaks (by ∼40 mV). Such changes were indicative of more efficient electron transfer and ion transport in CHIT-TBO/CNT than that in CHIT-TBO films. The improvement in charge transport can be ascribed to shortened pathways for the electron hopping and accompanying ion movement because of a dispersion of electron-conductive CNT in the CHIT-TBO matrix. The enhanced charge-transport capacity of the film facilitated the redox of TBO and, thus, contributed to augmentation of TBO peak currents.

The incorporation of CNT into CHIT-TBO films also facilitated the electrooxidation of NADH. In particular, it amplified the NADH current by ∼60 times (Figure 2, traces d and b) while reducing the response time from ∼50 s for CHIT-TBO to ∼5 s for CHIT-TBO/CNT films. The latter was another indicator of enhanced charge propagation in the films with CNT. The large amplification of the NADH current could not be explained in terms of the increased active electrode area alone. Rather, it could be viewed as a result of the TBO/CNT synergy leading to improved redox of TBO, which, in turn, facilitated the TBO-mediated oxidation of NADH.

In the control experiments, the CNTs were substituted with a comparable amount of graphite particles (Gp, 1−2 μm diam). However, such CHIT-TBO/Gp film electrodes yielded NADH currents (∼10^{-8} A) that were comparable to those generated by particle-free CHIT-TBO films. This correlated with the inability of CHIT solutions to form colloidal suspensions of graphite particles. Apparently, weak interactions between graphite particles and CHIT precluded their intimate mixing to improve charge transport in such films.

The CHIT-TBO/CNT system represents a new electrochemical platform that provides operational access to a large group of NAD^+-dependent dehydrogenase enzymes for designing bioelectrochemical devices such as biosensors, biological fuel cells, and bioreactors. This concept has a potential to be of general use because the synergistic effects reported here were also observed when redox mediators other than TBO were employed.

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Supporting Information Available: Synthesis of the CHIT-TBO material and electrochemical testing of surface films. This material is available free of charge via the Internet at http://pubs.acs.org.

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