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

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RECEIVED DATE (to be automatically inserted after your manuscript is accepted if required according to the journal that you are submitting your paper to)

EXPERIMENTAL SECTION

Reagents. Multi-walled carbon nanotubes (CNT, 20-50 nm dia, ~1-5 μm length, ~95 % nominal purity) were purchased from Nanolab (Brighton, MA). Chitosan (CHIT, MW ~ 1 x 10^6 Da; ~80 % deacetylation), Toluidine Blue O, NADH, $NaBH_3CN$, and $OHC(CH_2)_3CHO$ were purchased from Sigma-Aldrich. Other chemicals, $NaH_2PO_4 \cdot H_2O$, Na_2HPO_4, HCl, and NaOH were from Fisher. All solutions were prepared using deionized water that was purified with a Barnstead NANOpure cartridge system. The pH 7.40 phosphate buffer solution (0.05 M) served as a background electrolyte in all experiments.

Electrochemical Measurements. A CHI 832B workstation (CH Instruments, Inc.) was used to collect electrochemical data. Experiments were performed at room temperature (20 ± 1 ^oC) in a conventional three-electrode system with 3.0-mm-diameter glassy carbon (GC) disk working electrode (Bioanalytical Systems, Inc.), a platinum wire as the auxiliary electrode, and a Ag/AgCl/3MNaCl (BAS) reference electrode. All of the potentials are reported vs. this reference. Prior to use, the glassy carbon electrodes were wet polished on an Alpha A polishing cloth (Mark V Lab) with successively smaller particles (0.3 and 0.05 μm diameter) of alumina. The slurry that accumulated on the electrode surface was removed by ultrasonication for 30 s in deionized water and methanol.

Determination of Active Surface Areas. The electrode areas were determined by analyzing current-time (I-t) traces for the reduction of a model analyte $K_3Fe(CN)_6$ (C = 1.0 mM, D = 7.7 x10^{-6} cm^2 s^{-1}) at the electrodes (E = 0.0 V). The electrode surface area was calculated from a slope of the I vs. $t^{-1/2}$ plot at short times (80-200 ms, R^2 = 0.999) using the Cottrell equation. In control experiments with bare GC electrodes, such a procedure yielded an active surface area equal to 0.0712 cm^2 (±1.7 %, N=3), which was close to the geometrical area of a 3.0-mm diameter GC disk electrodes (0.0707 cm^2) used in

these studies. The experiments with GC/CHIT-TBO film electrodes yielded surface area equal to 0.144 cm^2 (±1.1 %, N=3), which was twice that of a bare GC electrode. Such a difference in surface areas probably reflected a difference in $CD^{1/2}$ products for the $Fe(CN)_6^{3-}$ ions in CHIT-TBO film and solution. The active surface area of the GC/CHIT-TBO/CNT film electrodes was determined to be equal to 0.231 cm^2 (±3.3 %, N=3), which was ~2 times larger than that of the GC/CHIT-TBO film electrodes. This corresponded to the increase in the geometrical surface area of the CHIT-TBO/CNT film, which had a diameter of ~4-5 mm in order to cover the 3.0-mm GC disk. Apparently, in the time window of the experiment, the diffusion lengths exceeded the distances between CNT in the film, and the Cottrell plot reflected the cross-sectional area of the overall film.

Synthesis of CHIT-TBO structures. A 0.10 wt % CHIT stock solution was prepared by dissolving chitosan flakes in hot (80-90 ^oC) aqueous solution of 0.10 M HCl. The solution was cooled to room temperature and its pH was adjusted to 5.0 using the NaOH solutions. The chitosan solutions were filtered using a 0.45-μm Millex-HA syringe filter unit (Millipore) and stored in a refrigerator (4 ^oC) when not in use. All chitosan solutions were colorless.

The synthesis of CHIT-TBO product involved two steps. In step I, an aqueous solution of 0.10 wt. % CHIT was reacted with an excess of glutaric dialdehyde. In order to react only one aldehyde group of dialdehyde with amino groups of CHIT, a high molar ratio of glutaric dialdehyde to CHIT glucosamine units (200:1) was used. The unreacted dialdehyde was removed from the solution by multiple extractions with ethyl ether. In step II, the solution after extractions was reacted with an excess of TBO. The Schiff bases in the CHIT-TBO product were reduced to more stable secondary amines using sodium cyanoborohydride. The results of the synthesis were corroborated by infrared spectroscopy (IR) of films I and II that were prepared by evaporating water from reaction mixtures after steps I and II, respectively. Prior to IR measurement, the films were extensively soaked in pH 7.40 phosphate buffer solutions and washed with deionized water to remove loosely bound material. Figure S1 shows IR spectra of films I and II as well as a reference spectrum of CHIT film (curve a). Film I (curve b) displayed the absorption band at 1720 cm^{-1}, which is characteristic for an aldehyde group. This demonstrated that molecules of glutaric dialdehyde were attached to CHIT with one end, leaving a free aldehyde group on the other end. Film II (curve c) displayed no absorption at 1720 cm^{-1}, which indicated that the free aldehyde groups were used up in the reaction with TBO. Instead, it displayed a new band at 1600 cm^{-1}, which was due to absorption by the aromatic rings of TBO molecules that were bonded to CHIT chains. In addition, the UV-visible spectra of the CHIT-TBO films displayed the absorption band at ~663 nm, which was close to absorption band of free TBO (~633 nm).

Analytical performance. The CHIT-TBO/CNT films displayed a sensitive (8 mA M^{-1}) and fast (~5 s) response to NADH in a wide linear range of concentrations from 0.5 μM to 0.30 mM (Figure 2, trace d and inset) with a dynamic range up to 10 mM NADH (Figure S3). The films provided a low detection limit (0.5 μM NADH at the signal-to-noise ratio 3) and retained a stable signal for several hours under continuous polarization and continuous exposure to elevated levels (0.50 mM) of NADH (Figure S4). In addition, their low operating potential (–0.10 V) allowed eliminating interferences from other redox active species such as ascorbic acid, uric acid, and acetaminophen, which are typically present in physiological fluids (Figure S5). Such a combination of good sensitivity, response time, detection limit, stability, and selectivity is an exception rather than a rule among current electrochemical sensors for NADH.[1]

1. (a) Dominguez, E.; Lan, H. L.; Okamoto, Y.; Hale, P. D.; Skotheim, T. A.; Gorton, L. *Biosens. Bioelectron.* **1993**, *8*, 167; (b) Munteanu, F.-D.; Okamoto, Y.; Gorton, L. *Anal. Chem. Acta*, **2003**, *476*, 43; (c) Karyakin, A. A.; Ivanova, Y.; Revunova, K. V.; Karyakina, E. E. *Anal. Chem.* **2004**, *76*, 2004; (d) Wu, Q.; Maskus, M.; Pariente, F.; Tobalina, F.; Fernandez, V. M.; Lorenzo, E.; Abruna, H. D. *Anal. Chem.* **1996**, *68*, 3688; (e) Prieto-Simon, B.; Fabregas, E. *Biosens. Bioelectron.* **2004**, *19*, 1131.

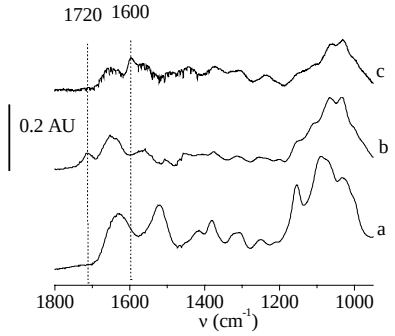

Figure S1. Infrared spectra of (a) CHIT, (b) CHIT-glutaric dialdehyde, and (c) CHIT- glutaric dialdehyde -TBO films.

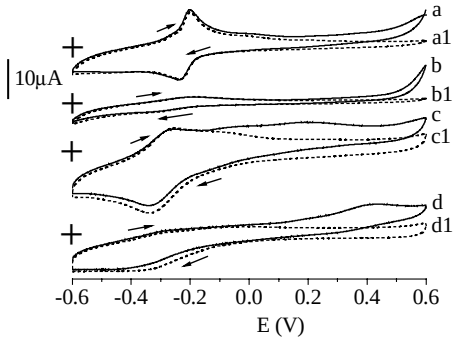

Figure S2. Cyclic voltammograms recorded in 1.0 mM NADH solutions at (a) bare glassy carbon electrode, and (b) CHIT-TBO, (c) CHIT-TBO/CNT, and (d) CHIT/CNT films that were cast on the surface of glassy carbon electrodes. Voltammograms a and a1 were recorded in NADH + 0.25 mM TBO and 0.25 mM TBO solutions, respectively. Current peaks in the potential window from –0.2 to –0.4 V are due to the redox of TBO. Background voltammograms for the film electrodes b1, c1, and d1 were recorded in pH 7.40 phosphate buffer. Scan rate, 50 mV s^{-1}.

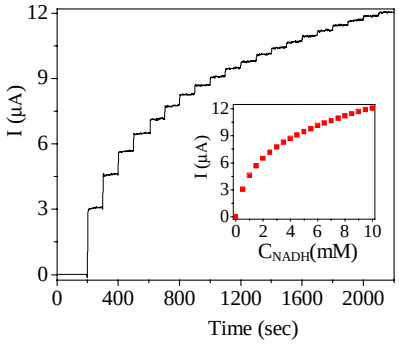

Figure S3. Response of the CHIT-TBO/CNT film to additions of 0.50 mM NADH at E = –0.10 V). Inset: calibration plot in the wide concentration range from 0.50 mM to 10 mM NADH. Background electrolyte, pH 7.40 stirred phosphate buffer.

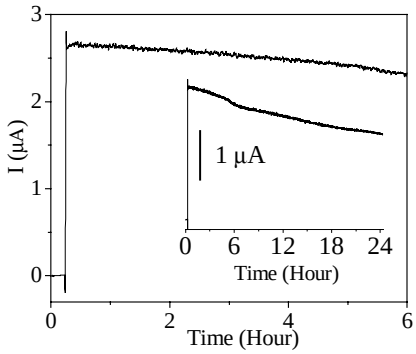

Figure S4. Operational stability of a CHIT-TBO/CNT film under continuous polarization (E = –0.10 V) and continuous exposure to a stirred solution of 0.50 mM NADH. Inset: 24-hour stability study, which shows a slow decay in current probably because of the decomposition of NADH in the solution. Background electrolyte, pH 7.40 phosphate buffer.

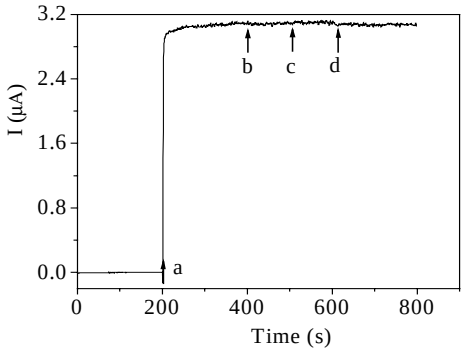

Figure S5. Interference-free behavior of a CHIT-TBO/CNT film illustrated by the amperometric trace (E = –0.10 V) that was recorded at the film in a stirred solution, which was spiked with (a) 0.50 mM NADH, (b) 0.10 mM uric acid, (c) 0.10 mM ascorbic acid, (d) 0.10 mM acetaminophen. Background electrolyte, pH 7.40 phosphate buffer.

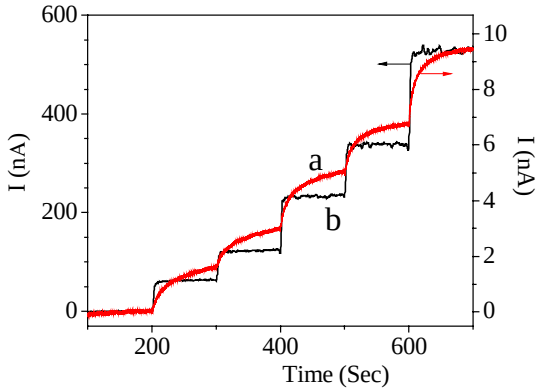

Figure S6. A comparison of the response times of CHIT-TBO (a, red trace, right scale) and CHIT-TBO/CNT (b, black trace, left scale) films at E = –0.10 V. Current steps represent the response of the

film to additions of 5, 5, 10, 10 and 20 μM NADH. Background electrolyte, pH 7.40 stirred phosphate buffer.

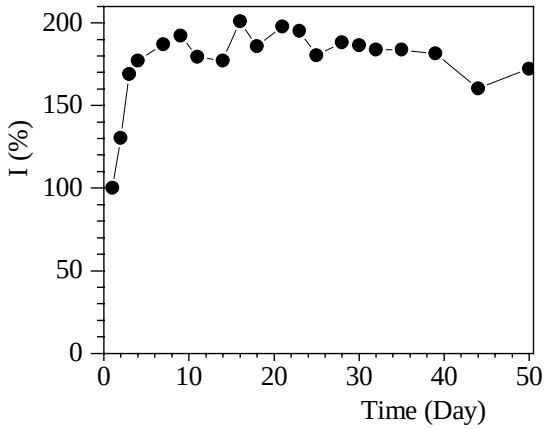

Figure S7. Long-term stability study of a CHIT-TBO/CNT film electrode that was kept in deionized water when not in use. The data points represent film's current response to additions of 0.50 mM NADH (E = −0.10 V). The response was normalized with respect to that on day 1. Background electrolyte, pH 7.40 stirred phosphate buffer.