Electronic Supplementary Information

In Situ Stabilization of Hydroxylamine via Electrochemical Immobilization of 4-Nitrophenol on GCE/MWCNT Electrodes: NADH Electrocatalysis at Zero Potential†

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† Electronic Supplementary Information (ESI) available.

1. Materials, Instrumentation and Methods

Pristine MWCNTs (outer diameter: 10-15 nm; inner diameter: 2-6 nm; length 0.1-10 μm and 90% purity) and single-walled carbon nanotubes (SWCNT: 1-1.5 nm diameter, 1-15 μm length) were purchased from Sigma-Aldrich, India. 4-nitrophenol, 3-nitrophenol and 4-aminophenol were purchased from SD Fine Chemicals, India. Other chemicals used in this work were all of ACS-certified reagent grade and used without further purification. Screen-printed gold electrodes (AuSPE) were obtained from Zensor R&D, Taiwan. Aqueous solutions were prepared using deionized and alkaline $KMnO_4$ distilled water (designated as DD water). Unless otherwise stated, pH 7 phosphate buffer solution (PBS) of ionic strength $I = 0.1$ M was used as a supporting electrolyte in this work.

Voltammetric measurements were all carried out with a CHI Model 660C electrochemical workstation (USA). A three-electrode system consisting of glassy carbon or a screen-printed substrate and its chemically modified electrodes of geometric area 0.0707 cm^2 were used as a working electrode, Ag/AgCl was used as a reference electrode, and platinum wire was used as an auxiliary electrode. The surface of the GCE was cleaned (i) mechanically by polishing with 0.05-micron alumina powder, washing with DD water and sonicating for 5 minutes and (ii) electrochemically by performing cyclic voltammetry (CV) for 10 cycles (n=10, n = no. of cycles) in the potential window of -1 to 1 V vs Ag/AgCl at a potential scan rate (v) of 50 $mV.s^{-1}$ in pH 7 PBS. For the nanoparticle characterization and surface morphology examination, a high-resolution transmission electron microscope instrument (HRTEM, Zeiss EM902A, Germany) and an X-ray diffraction (XRD) Bruker D8 Advanced system were used.

For practical convenience, the ØNHOH@p-MWCNT sample was prepared for the physicochemical characterization (by TEM) on a screen-printed gold electrode (AuSPE/ØNHOH@p-MWCNT) in the optimal condition. The AuSPE-modified samples also showed qualitatively similar electrochemical patterns in comparison with the GCE modified electrodes (figures not shown). For TEM characterization, the film particles from the above AuSPE/ØNHOH@p-MWCNT electrode were scratched out with a needle of dimension 0.5 mm x 3.5 cm, suspended in 500 µL ethanol solutions and analysed. For XRD, a simulated ØNHOH@p-MWCNT powder sample was prepared by the solution phase procedure as follows: 10 mg p-MWCNT and 5 mg 4-NP were dissolved in pH 7 PBS and continuously stirred for 30 minutes in room temperature, washed more than five times to completely remove the loosely adsorbed 4-NP and dried in vacuum overnight.

2. Preparation of different types of CNT modified electrodes and their electrochemical behaviour

Functionalized and purified MWCNT (f-MWCNT and p-MWCNT, where f= functionalized and p=purified) were prepared by treating the pristine MWCNTs with concentrated (13 N HNO_3) and diluted (2 N HNO_3) acid solutions as per the literature procedure.[S1,S2] The MWCNT, f-MWCNT, p-MWCNT and SWCNT modified GCEs are designated as GCE/MWCNT, GCE/f-MWCNT, GCE/p-MWCNT and GCE/SWCNT, respectively, and were prepared by a following common procedure: 3 μL of respective CNT dispersed in ethanol (2 mg/500 μL) was drop coated on the pretreated GCE and was dried in air for 15 minutes in room temperature. Because dissolved oxygen did not influence the electrochemical response, all of the experiments were carried out without any degasification, similar to physiological systems.

3. Control Electrochemical Experiments

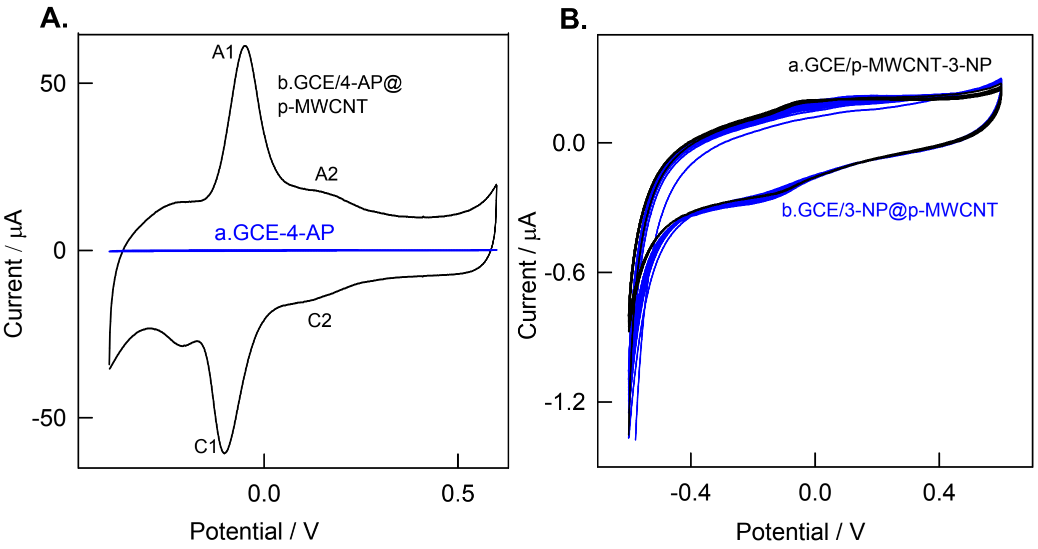


Fig. S1 The 20 continuous CV responses of the (A) GCE/hydroxylamine(4-AP)@p-MWCNT (b) in comparison with the bare GCE (a) and (B) GCE/p-MWCNT in 1 mM 3-NP solution (a) in comparison with the GCE/3-NP@p-MWCNT in the blank pH 7 PBS (b). All experiments were conducted at the scan rate of 50 mV.s^{-1}.

4. Electrochemical behavior of GCE/ØNHOH@different CNTs

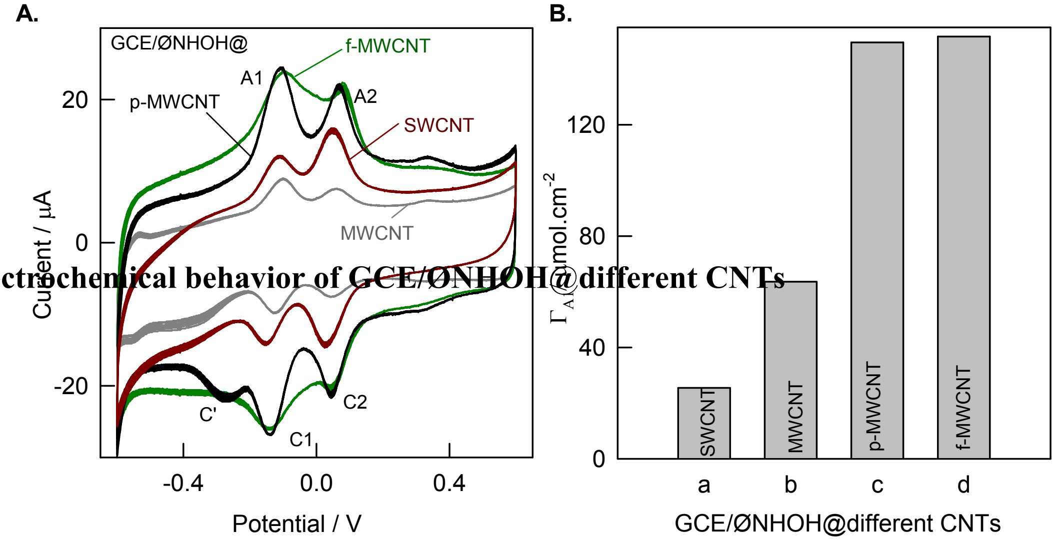
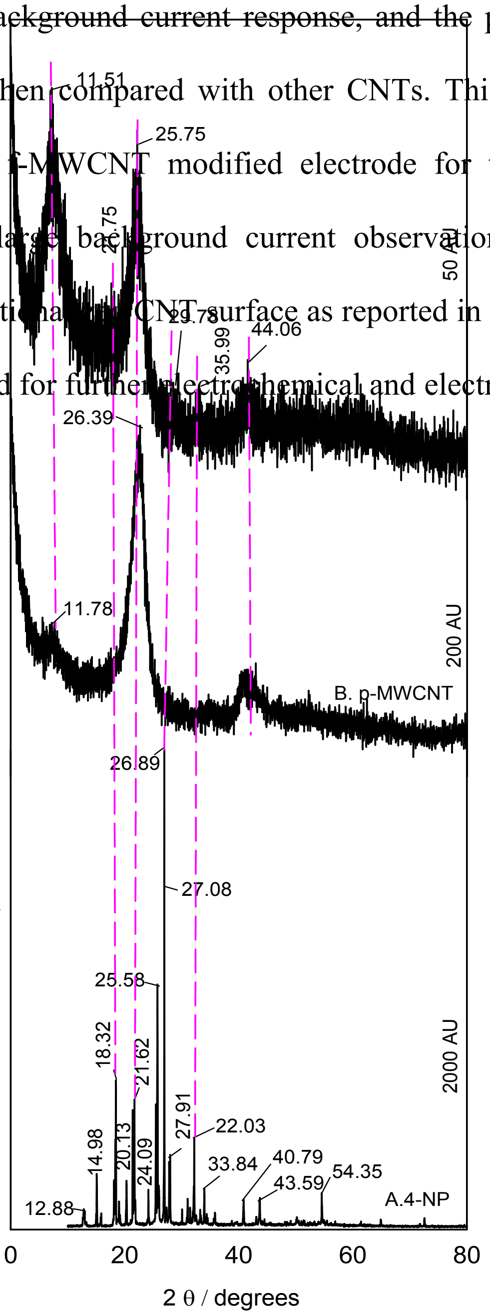


Fig. S2 (A) 10 CV cycles of the GCE/ØNHOH at different CNTs and (B) Bar chart for the comparative surface coverage values for the GCE/ ØNHOH at different CNTs for the A1 peak. All experiments were conducted at a scan rate of 50 mVs^{-1} in pH 7 PBS.

The comparative CV responses taken for the last 10 out of 20 continuous potential cycles of various 4-NP immobilized types of CNT-modified GCEs are given in the ESI† Fig. S2A. It was interesting to notice that all of the CNTs showed qualitatively similar ØNHOH formation with A1/C1 and A2/C2 redox peaks at 0.117±0.020 V and 0.086±0.027 V vs Ag/AgCl. However, the quantitative calculated surface coverage, $\Gamma_{\text{ØNHOH}}$, values were different for various types of CNTs. The values were in the order of, 37.78 ±0.02 < 45.68 ±0.03 < 117.62 ±0.05 < 122.42 ±0.02 $\mu mol.cm^{-2}$ for the A2/C2 redox couple and 22.54 ±0.05 < 63.62 ±0.02 < 149.60 ±0.06 < 151.66 ±0.01 $\mu mol.cm^{-2}$ for the A1/C1 redox couple for the SWCNT, MWCNT, p-MWCNT and f-MWCNT, respectively. A comparison bar chart for the $\Gamma_{\text{ØNHOH}}$ values against different types of CNTs for the GCE/ØNHOH@CNTs are given in the ESI† Fig. S2B. The lowest $\Gamma_{ØNHOH}$ values obtained for the SWCNT may be due to the single wall arrangement, and larger amounts of impurities could have hindered the immobilization and stabilization of the electrogenerated ØNHOH species through π-π interactions within the inter-walls of the CNT.[10,33] However, because the "as commercially received" MWCNT contained metallic and carbonaceous impurities within the multiwalls, which might have hindered the immobilization of ØNHOH species.[10] Therefore, there was a decrease in the $\Gamma_{ØNHOH}$ value for the MWCNT in comparison with the p-MWCNT and f-MWCNT. Although the f-MWCNT modified electrode showed higher peak current and $\Gamma_{ØHOH}$ values for the immobilization of ØNHOH species, there was a relatively larger background current response, and the peak resolution between the two redox peaks was poor when compared with other CNTs. This CV response behaviour led to lower sensitivity of the f-MWCNT modified electrode for the immobilization of ØNHOH species. Moreover, the large background current observation may be due to the charging characteristics of the functionalized CNT surface as reported in our earlier work.[33] Therefore, the p-MWCNT was optimized for further electrochemical and electrocatalysis studies in this work.



3. XRD characterization

Fig. S3 Comparative XRD graphs of 4-NP (A), p-MWCNT(B) and ØNHOH@p-MWCNT (C).

The CV response obtained for the ØNHOH@p-MWCNT powder sample prepared for XRD on modified GCE is given in the ESI† Fig. S4.

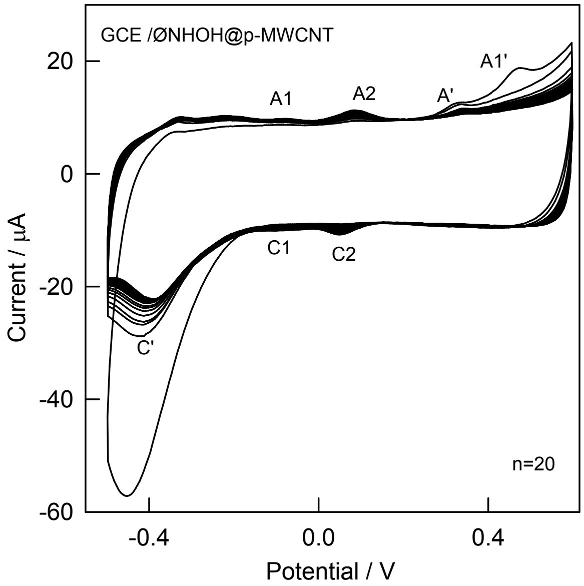


Fig. S4 The 20 continuous CV responses of the (A) GCE/ØNHOH@p-MWCNT for the powder sample at the scan rate of 50 mV.s^{-1} in pH 7 PBS.

Table S1. Comparative results of the electrocatalysis of NADH on various carbon electrodes

S. No.	Electrode	pH	Detection Potential (V)	Sensitivity (nA/µM)	LOD (µM)	Linear range (µM-mM)	Ref.
1	GCE/np-quinones@MWCNT	7.0	-0.01	-	8.00	20-0.200	[12]
2	MWCNT/PEI-3,5-DNBA[$]	7.0 (SCE)	-0.03	71	1.2	4-0.042	[30]
3	[#] DHPB/CNTPE[@]	7.0	0.7	48	1.00	5-0.600	[34]
4	GCE/[&]OCNT	6.8	0.650	-	0.50	20-1.00	[35]
5	Edge Plane Pyrolitic Graphite-CNT	7.4 (SCE)	0.55	0.036	0.03	8.2-0.108	[36]
6	GCE/MWCNT	7.4	0.3	-	-	0.1-0.8	[37]
7	GCE/*PolyXa/FAD/MWCNT	7.0	0.15	0.155	64	5-0.17	[38]
8	GCE/[@]PTBO/MWCNT	4.0 (SCE)	0.0	-	0.50	2-4.5	[39]
9	GCE/Boron-doped CNTs	7.0	0.3	0.022	0.05	-	[40]
10	Screen Printed Shallow Recessed Electrodes	7.0	0.45	0.00047	5.2	10-0.09	[41]
11	Paper based Graphite Screen Printed Electrode	7.0 (SCE)	0.6	0.015	1.8	10-0.11	[42]
12	GCE/ØNHOH@p-MWCNT	7.0	0.02	0.0029	0.043	100-1.00	This work

PEI-3,5-DNBA=Polyethyleneimine-3,5-dinitrobenzoic acid; [#]DHPB=*N*-(3,4-dihydroxyphenethyl)-3,5-dinitrobenzamide; [@]CNTPE=carbon nanotube paste electrode; [&]OCNT=Ordered CNT; *PolyXa=Polyxanthurenic acid; FAD=Flavin Adenine Dinucleotide; [@]PTBO=polytoluidene blue.

References

S1. P.D. Tam, *J. Immunol. Methods.*, 2009, **350**, 118-124.

S2. E.B. Craig, C. Alison, S. Christopher, J.W. Shelley and R.G. Compton, *Angew. Chem., Int. Ed.*, 2006, **45**, 2533-2537.