

Supporting Information

Hemozoin Anchored MWCNT for Mediated Reduction of Hydrogen Peroxide and Real-Time Intracellular Oxidative Stress Monitoring in Colon Cancer Cells

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1.1. Preparation of chitosan solution

At first, 5 mg of chitosan was weighed and mixed with 3 mL of distilled water and the pH of this solution was adjusted using glacial acetic acid. Further the mixture was allowed for stirring at 100 rpm for 3 hr. Then the uniform homogenous solution was collected and neutralized to pH 7 using 0.1M NaOH solution. The as-prepared solution was used for electrode modification purpose.

Initially, a control cyclic voltammetry was performed on a GCE/MWCNT-Chit electrode in a nitrogen-purged 0.1 M PBS solution at pH 7 (Fig. S1A). A weak, irreversible faradaic response was observed at approximately -0.2 V vs. Ag/AgCl, likely due to the reduction of protonated amino groups on the MWCNT surface. This response was accompanied by a significant background current. Notably, no specific redox peak appeared at around -0.4 V vs. Ag/AgCl, indicating that chitosan does not directly participate in the HZ redox reaction. Next, we studied the effect of varying chitosan loading (1-5 μ L of chitosan stock solution) on the formation of the GCE/MWCNT@HZ-Redox electrode. The quantitative peak current values obtained from the redox peaks were plotted against the loading volume, as shown in Fig S1 B. A sharp increase in the anodic peak current (i_{pa}) was observed from 1 to 3 μ L, followed by a plateau. Based on these results, 3 μ L of chitosan solution was selected as the optimal volume for modified electrode preparation.

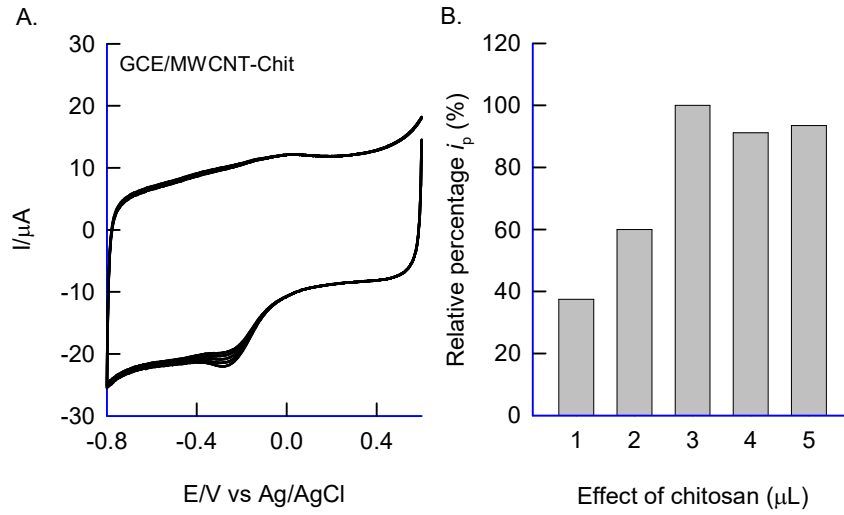


Fig S1. (A) Continuous CV response of GCE/MWCNT-Chit and (B) Bar chart representing effect of chitosan solution on the GCE/MWCNT@HZ-Redox modified electrode in presence of N_2 purged 0.1 M PBS solution at pH 7 at $v=50$ mV/s.

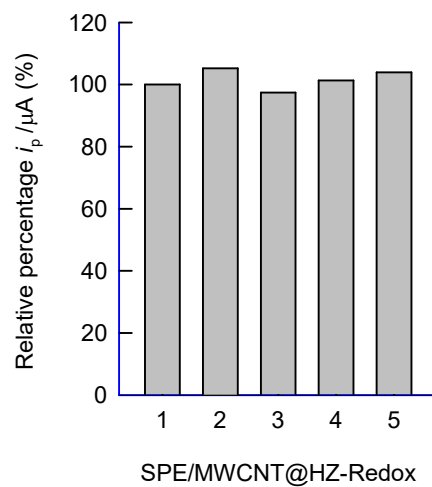


Fig S2. A bar chart illustrating the reproducibility of peak currents (i_p) obtained from CVs of five different SPE/MWCNT@HZ-Redox modified electrode recorded in a N_2 purged 0.1 M PBS solution at pH 7 at $v=50$ mV/s.

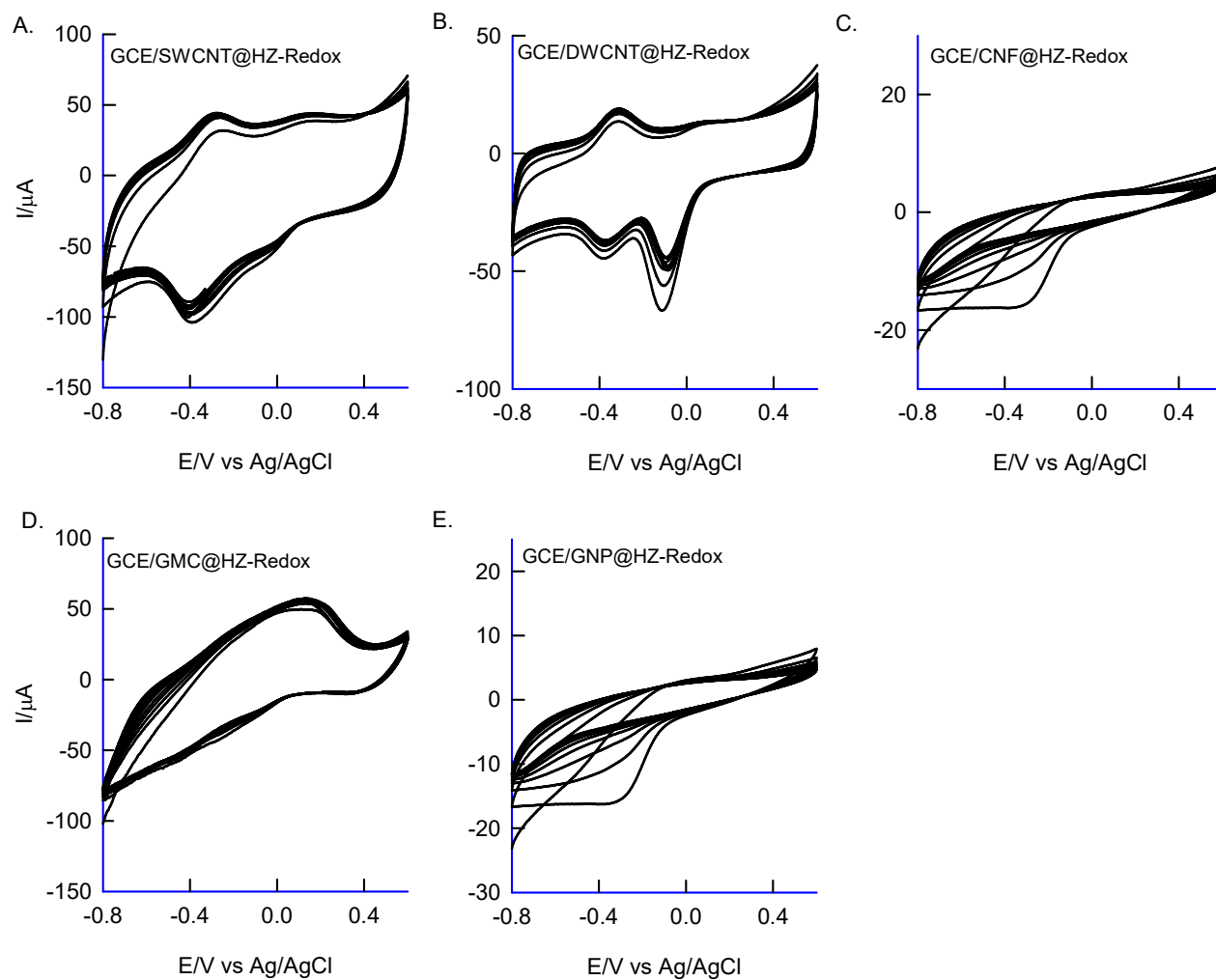


Fig. S3. Continuous CV responses of HZ-Redox immobilized on various GCE modified carbon materials, (A) SWCNT, (B) DWCNT, (C) CNF, (D) GMC and (E) GNP in N_2 purged 0.1M PBS of pH 7 at the scan rate $\nu= 50 \text{ mVs}^{-1}$.

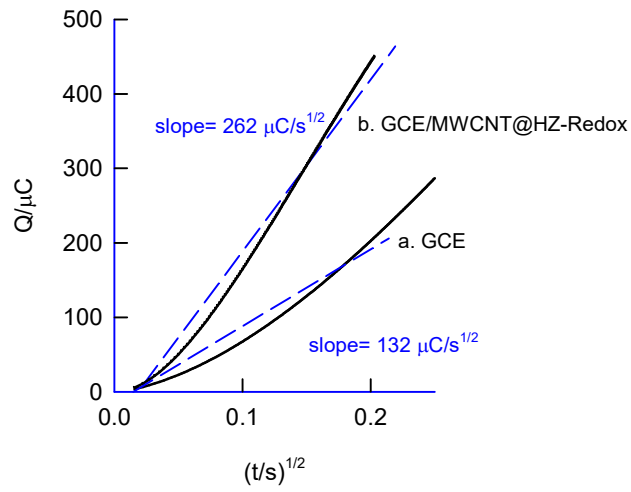


Fig S4. (A) Anson's plot (Q vs $t^{1/2}$) of chronocoulometric response recorded for (a) GCE and (b) GCE/MWCNT@HZ-Redox modified electrode at the potential window of -0.2 to 0.6V vs Ag/AgCl in presence of 5mM of $[\text{Fe}(\text{CN})_6]^{3-}$ containing 0.1M PBS solution at pH 7.

1.2. Real sample analysis using tannery waste water

In the context of potential industrial applications, tannery wastewater sample was collected from nearby industries in Vellore, Tamil Nadu. This sample was filtered using Whatman and polypropylene Buchner funnels. An independent experiment demonstrated the analysis of real samples using an SPE/MWCNT@HZ-Redox modified electrode coupled BIA (Fig. S5). The developed electrode system achieved a 98.25% recovery rate, as detailed Table S1.

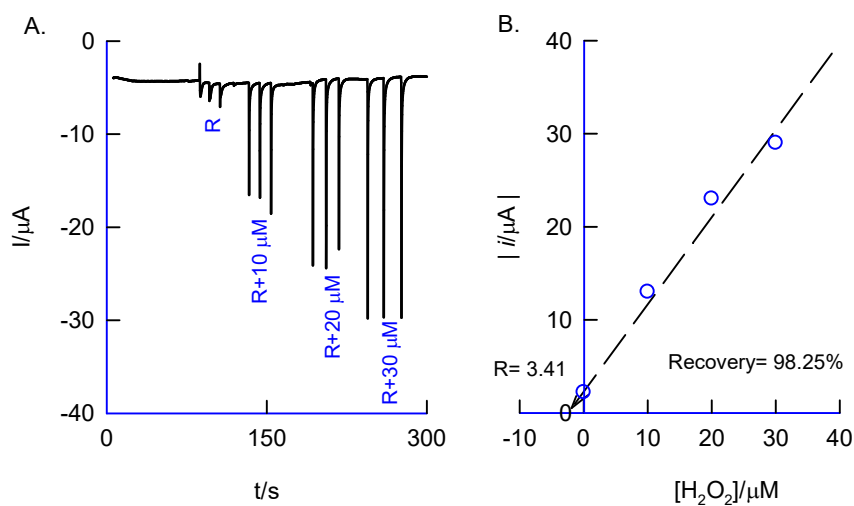


Fig. S5. (A) BIA responses of SPE/MWCNT@HZ-Redox for the analysis of H_2O_2 in tannery waste sample using standard addition approach at an applied potential, $E_{\text{app}} = -0.25$ V vs Ag/AgCl in presence of PBS of pH 7. (B) Typical standard addition approach calibration plot. R= Real sample and S1-S3= Standard H_2O_2 samples of 10,20 and 30 μM respectively.

Table S1. Electrochemical H₂O₂ detection in tannery waste by SPE/MWCNT@HZ-Redox modified electrode using batch injection technique through standard addition method.

S.No	Parameter	Tanney waste
1.	Original detected / μ M	3.41
2.	Spiked/ μ M	10
3.	Detection after the spike/ μ M	9.8
4.	Recovery /%	98.25