Fabrication of a Mesoporous CoFe₂O₄/rGO Nanohybrid and Laccase Interface Biosensor for Rapid Detection of Adrenaline for Neurodegenerative Disease Diagnosis

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1. Synthesis of CF/rGO nanohybrid

Scheme S1: *The general presentation of hydrothermal synthesis of cobalt ferrite (CF)/rGO nanohybrid.*

2. Result and Discussion
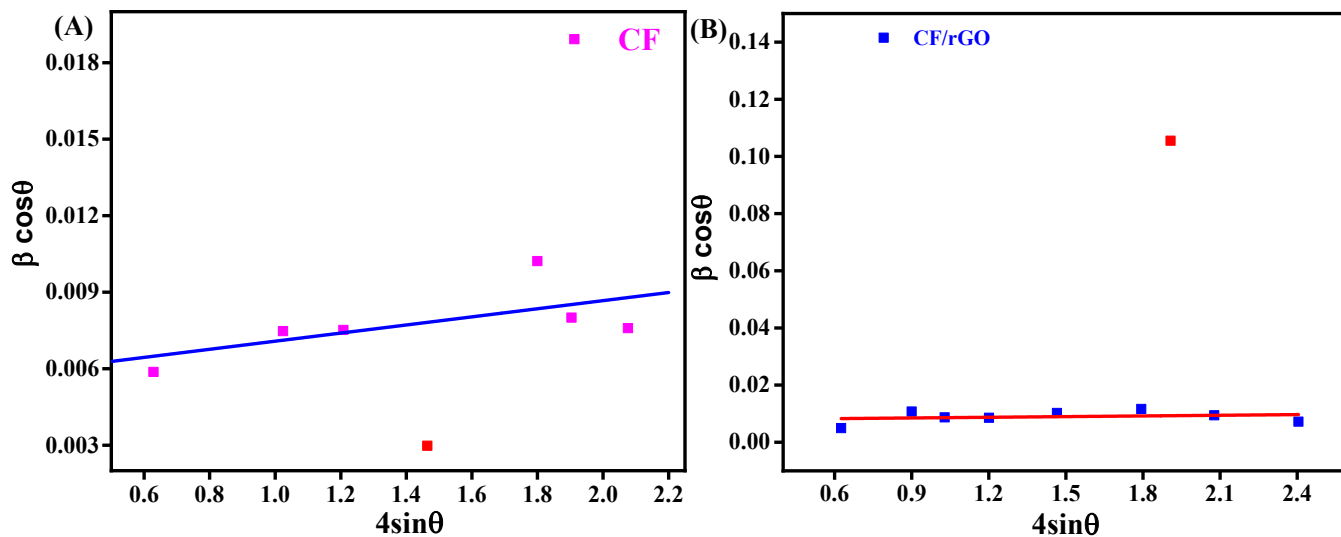
XRD Analysis, and Williamson-Hall plot

Figure S1 (A&B): *Shows the Williamson-Hall plot to calculate the average crystalline size and strain in the CF and CF/rGO nanohybrid.*

The strain had been calculated based on the Hall- Williamson relationship mentioned below.

$$\beta cos\theta = \frac{k\lambda}{D} + 4\varepsilon*sin\theta\ldots\ldots\textbf{Equation S1}$$

Here β is the Bragg peak, k shape factor (0.9), X-ray wavelength (λ=1.5418 Å), ε is the lattice strain.

AFM analysis

The tapping mode of analysis by NT-MDT, Model-solver Next instrument, was used to analyze the surface topography of both CF and CF/rGO thin film on ITO acquired by atomic force microscopy (AFM). **Figure S2(A&B)** shows that the CF average roughness (Sa) is 6.3 nm, root mean square roughness (Sq) is 8.1 nm, and maximum area peak height (Sp) is 33.2 nm. **Figure S2(C&D)** shows the roughness parameters of CF/rGO, such as Sa is 4.3 nm, Sq is 5.6 nm, and Sp is 25.9 nm, respectively. The roughness parameter decreases due to the addition of rGO on the CF surface as intercalated and electrostatically bound by a metal of CF with the oxygen of rGO to form a stable nanohybrid material. Thus, these results were used in loading analytes to small amounts and made more specific towards the analyte. The active site of the nanohybrid surface is a suitable platform for analyte detection.
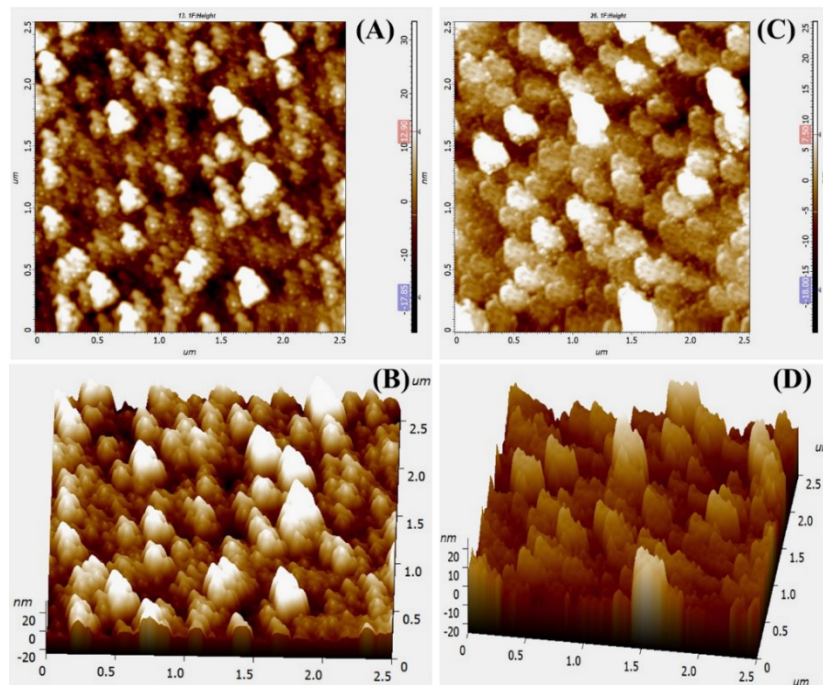
Figure S2 *(A and B); AFM micrograph of CF 2D and 3D image, and (C and D); AFM micrograph of CF/rGO nanohybrid 2D and 3D image on ITO surface.*
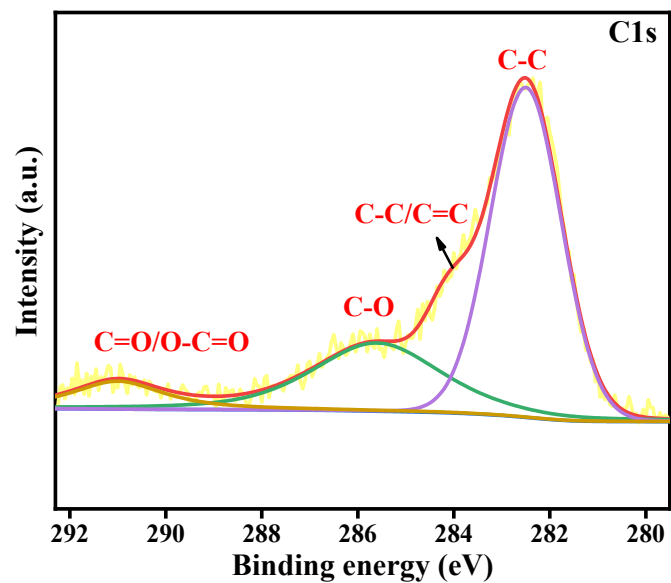
XPS analysis

2.1. Electrode Functionalization, Fabrication, Laccase Immobilization, Optimized Electrochemical Parameters (OEP), and Analyte Optimization

The electrode functionalization of glass sheets coated with Indium Tin Oxide (ITO) was cut into small pieces (1 cm × 2 cm) and dipped in a mixture of H_2O_2: NH_3: H_2O with a volume ratio of 1:1:5, respectively. After that, the pieces were baked in an oven for one hour at 80 °C for adherence of the OH groups uniformly on the ITO surfaces. Thus, the functionalized ITO pieces were washed with milli-Q water and acetone, dried at room temperature, and stored in a fresh atmosphere (4 °C) for forthcoming use in various applications as working electrodes.

The standard analyte can be prepared using the AD hydrochloride powder to prepare a solution in milli-Q water (resistivity 15 Ω/sq) with 0.2 M concentrated solution. Additionally, this standard AD solution was further diluted to 2 mM solution and again continued with serial dilution to prepare the different micromolar concentrations in one ml; these are important for the physiological range of detection.
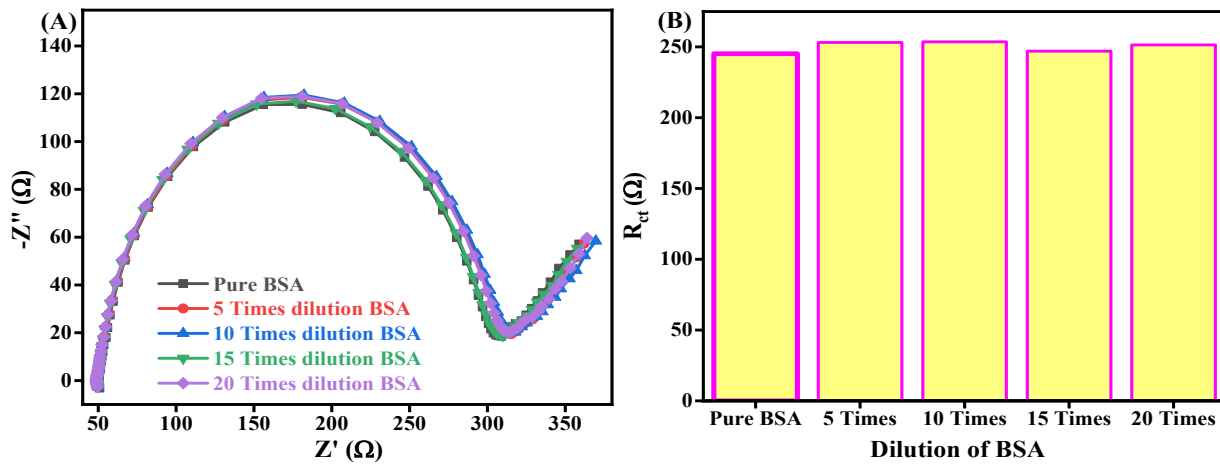
Figure S4: *(A) Shows the Nyquist plot of optimization of BSA dilution, and (B) R_{ct} with different times dilution on fabricated La/CF/rGO/ITO bioelectrodes.*

2.2. Electrochemical behavior of nanofabricated electrode

Cyclic-Voltammetry (CV)

Figure S5 (A) illustrates four distinct electrodes: ITO, CF/ITO, CF/rGO/ITO, and La/CF/rGO/ITO. These were developed to record current responses in a potential range from -0.7 to +1 V at OEP. The CF and CF/rGO nanohybrids enhanced the current response to 0.60 mA and 0.73 mA, respectively, compared to the base ITO electrode, which had a response of 0.47 mA. This improvement is attributed to the electrostatic interactions between CF and rGO and the increased surface area provided by the nanohybrid structure. Adding the Laccase (La) enzyme onto the nanohybrid electrode's surface further elevated the current response to 0.80 mA. This increase is due to the effective enzyme analyte interface and enzyme filling the electrode's surface pores, enhancing charge mobility through the increased surface available for charge transport and reducing biomolecule diffusion, thus favoring selective analyte adsorption.
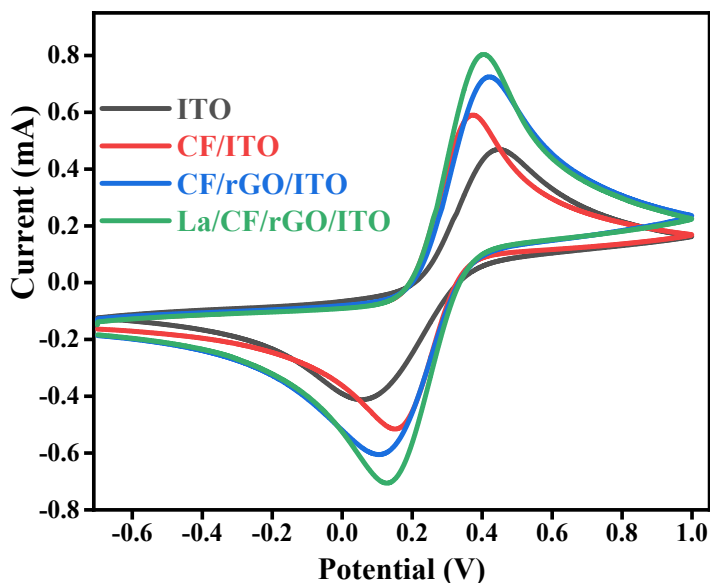
Figure S5: *Cyclic voltammetry of the ITO, CF/ITO, CF/rGO/ITO, and La/CF/rGO/ITO electrode plotted between current versus potential.*

Scan rate and kinetic analysis

CV analysis is being accomplished at various scan rates from 10 to 100 mVs^{-1} to estimate the interfacial kinetics of the La/CF/rGO/ITO bioelectrode with regular intervals of scan rates (10 mVs^{-1}), shown in **Figure S6(A)**. The anodic/oxidation current peak (Ipa) and cathodic/reduction current peak (Ipc) extents rise linearly as a function of scan rate, which is represented in the calibration graph between the square root of scan rate ($v^{1/2}$) versus current (mA). Additionally, the scan rate increased with the increase of Ipa and Ipc due to the decrease of diffusion layer with increasing scan rate. Moreover, the potential shifting is also recorded in scan rate variations, resulting in a higher scan rate causing further shifting of peak potential from a more positive side towards oxidation peak potential (Epa) and a more negative side towards reduction peak potential (Epc) represented in **Figure S6(B)**. The difference of peak potential (ΔE) versus $v^{1/2}$ gives a linear relationship to confirm the electron transfer administered by diffusion, i.e., diffusion-controlled process. Throughout the whole region in which these chemical systems transit from the reversible limit E_{pa} - E_{pc} (ΔE) = 57 mV to beyond 300 mV, the range of peak separations is between 167 and 349 mV, during which the electrode process is advancing from reversible to irreversible. Which also demonstrates the quasi-reversible nature of the electrode reactions[1].

The surface concentration (I*) of the fabricated La/CF/rGO/ITO bioelectrode is conceivably estimated by applying the Brown-Anson model[2], and the diffusion coefficient (D) of the La/CF/rGO/ITO bioelectrode has been estimated from the Randels-Sevcik **equation S2 and S3,** respectively. The calculated value of I* is 1.095×10^{-7} mol cm^{-2}, and D is 5.25×10^{-2} cm^2 s^{-1},

respectively. The effective surface area of the electrode is 0.974 cm², also calculated by the Randels-Sevcik equation by using the standard diffusion coefficient of $[Fe(CN)_6]^{3-/4-}$ (7.6×10^{-6} cm² s⁻¹)[3,4].

$$Ip = (2.69 \times 10^5)\, n^{3/2}\, AD^{1/2}\, v^{1/2}\, C \qquad ..... (S2)$$

$$Ip = (n^2\, F^2\, I^*\, Av) / 4RT \qquad .....(S3)$$

Here, Ip (peak current in volt), n (number of electrons involved in this reaction), A (the effective surface area is 0.974 cm²), v (scan rate, 0.05 Vs⁻¹), C (electrolyte concentration, ($[Fe(CN)6]^{-3/-4}$ concentration in 5×10^{-6} mol cm³), F (Faraday constant 96500 C/mol), R (gas constant, 8.314 J mol⁻¹ K⁻¹), T (absolute temperature, 300 K).
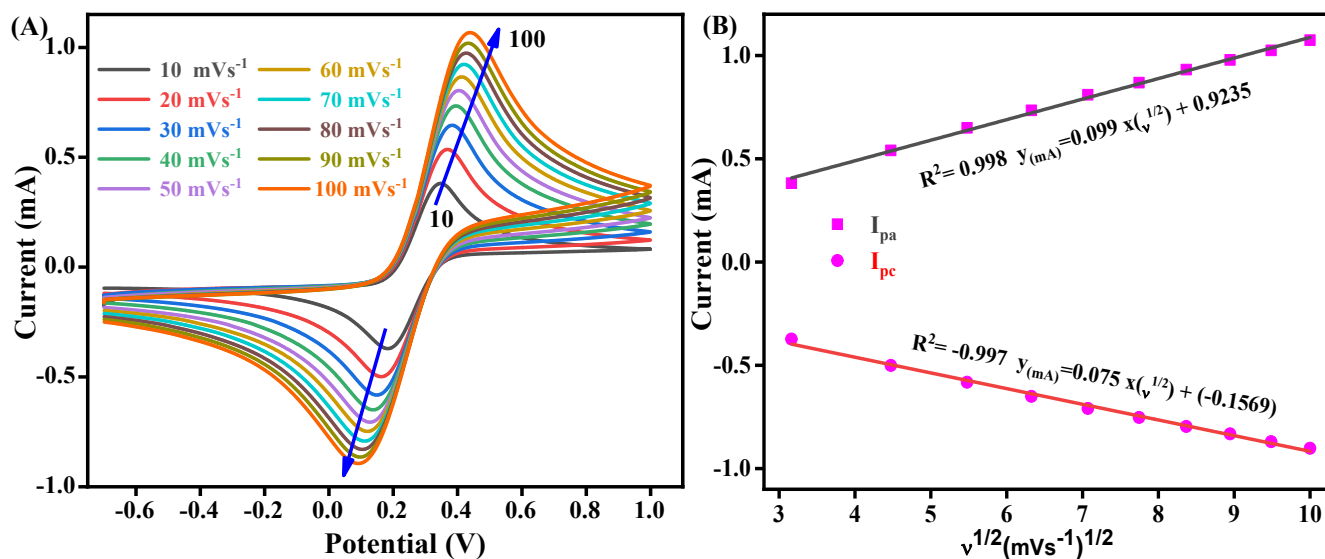
Figure S6: *(A&B); Scan rate analysis from 10-100 mV/s and a Linear calibration curve of oxidation and reduction peak current versus $v^{1/2}$ utilized in kinetic analysis.*

References:

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