

Supporting information

Electrochemical redox signaling of hemoglobin in human whole blood and its clinical relevance to hemoglobin analysis and *thalassemia* diagnosis

*Khairunnisa Amreen and Annamalai Senthil Kumar**

Environmental and Analytical Chemistry Division, School of Advanced Sciences, Vellore Institute of Technology University, Vellore-632 014, India.

1.Experimental Section

1.1 Materials and Reagents

Human blood sample was taken from an adult 22 year old female from the health care center of VIT University, Vellore, Tamil Nadu. This was a normal blood sample used as an optimal. 15 more blood samples were collected from various VIT students, VIT health center's laboratory and Nalam medical center – Vellore. Diseased blood samples were taken from a government hospital in Chennai. Lyophilized Hemoglobin was purchased from Sigma Aldrich, graphitized mesoporous carbon (GMC, purity assay= >99.95%, <500nm pore size), multi-walled carbon nano tube (MWCNT, with ~90% purity assay), activated charcoal, graphene, carbon mesoporous hydrophilic, carbon mesoporous hydrophobic were purchased from Sigma Aldrich (USA). Graphite nanopowder (400nm, ~98% purity) was received from SRL(India), Nafion (5%) was purchased from Sigma Aldrich (USA). The supporting electrolyte used here is 20 min N₂ purged pH 7 phosphate buffer solution (PBS). Other basic chemicals of analytical grade were also used.

1.2 Apparatus.

Cyclic voltammetry (CV) experiment was performed on CHI model 660C work-station, USA with 10 mL working volume. The three working electrode system consists of Ag/AgCl with 3 M KCl as reference electrode, platinum wire as a counter electrode and modified glassy carbon electrode (GCE) as a working electrode with the surface area of 0.0707 cm^2 . Raman spectroscopy analyses were carried out using AZILTRON, PRO 532(USA) with 532 nm Laser excitation source. FTIR analysis was done by using JASCO 4100 Spectrophotometer by KBr method. ATR/FTIR spectroscopy measurements for SPE modified with the proposed hybrid materials were performed by using JASCO FTIR-460 PLUS spectrometer equipped with an ATR cell. UV-Vis analysis was carried out by using UV-Vis NIR spectrophotometer, JASCO V-670, Germany.

1.3 Procedure

The surface of GCE was cleaned mechanically by polishing with $0.5\text{ }\mu\text{m}$ alumina powder, washing with DD water followed by sonication of electrode in acetone for 1 min. Then cyclic voltammetry was used for electrochemical treatment of electrode in a potential window of -0.2 to 1 V vs Ag/AgCl for 10 cycles at a potential scan rate $\nu = 50\text{ mV s}^{-1}$ in pH 7 PBS. GCE/GMC was prepared by coating of $5\text{ }\mu\text{L}$ of GMC-ethanol suspension (2 mg mL^{-1}) on a clean GCE surface and air dried at room temperature for 5 ± 1 minutes. Then, a mixture of $8\text{ }\mu\text{L}$ of human whole blood (anticoagulant EDTA (electro-inactive compound) added and stored in refrigerator at $5\pm 2^\circ\text{C}$) + $2\text{ }\mu\text{L}$ of pH 7 PBS was drop casted and air dried for 20 ± 2 minutes. $5\text{ }\mu\text{L}$ of 1% Nf-ethanolic solution were successively drop casted on the GCE/GMC@Blood and air-dried for 5 ± 1 minutes. All other carbon nanomaterial-blood modified electrodes were prepared same as above.

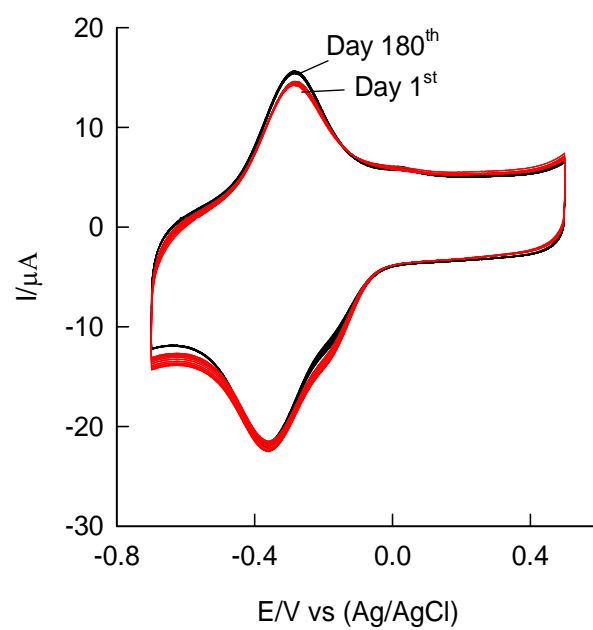
Figure S1

Figure S1. GCE/GMC@Blood : Continuous CV responses of GCE/GMC@Blood/Nf in N₂ purged pH 7 PBS at scan rate of 50 mV s⁻¹ stored in refrigerator for six months.

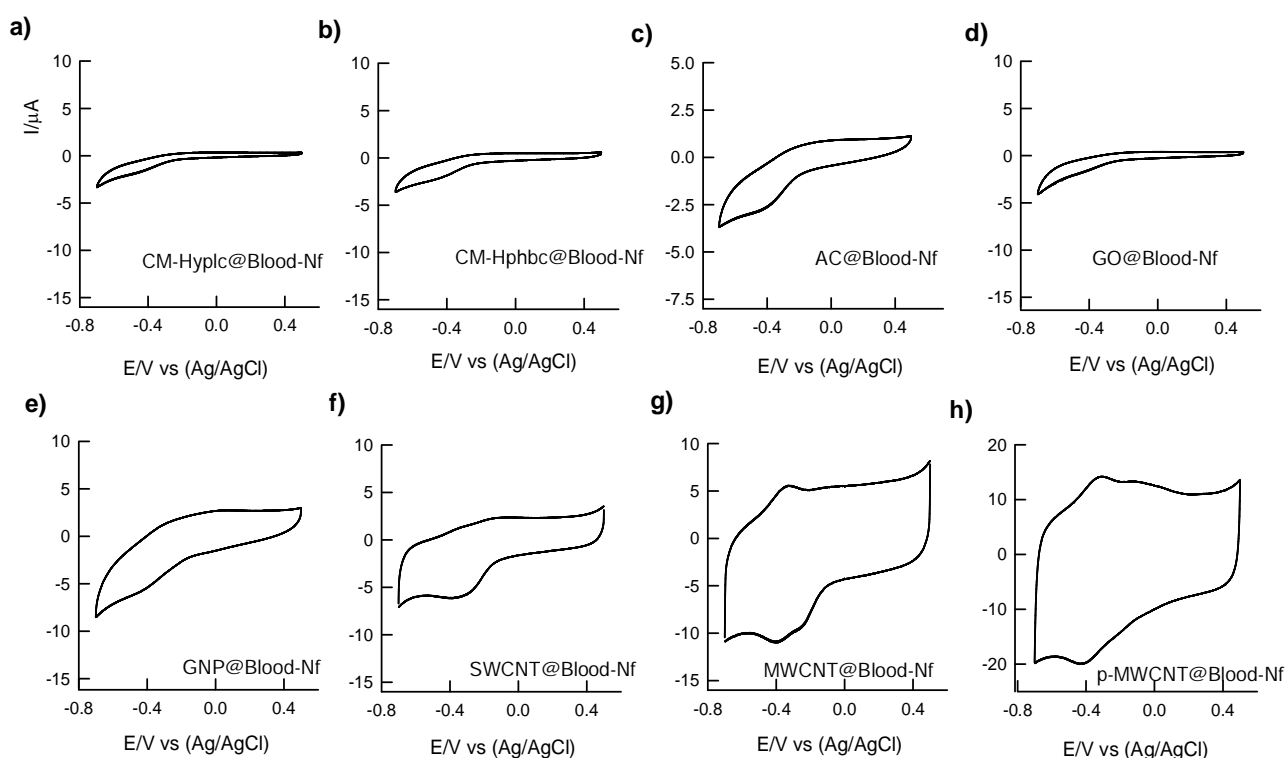
Figure S2

Figure S2. CV responses of various GCE/carbon nanomaterial@ Blood-Nf systems: a) Carbon mesoporous hydrophilic (CM-HypIc), b) Carbon mesoporous hydrophobic (CM-Hphbc), c) Activated charcoal (AC), d) Graphene oxide (GO), e) Graphite nano powder (GNP), f) single walled carbon nanotube (SWCNT), g) multiwalled carbon nanotube (MWCNT) and (h) purified MWCNT (p-MWCNT) as a matrix for the blood chemically modified electrode. Electrolyte= pH 7 nitrogen purged phosphate buffer solution. Scan rate=50 mV s⁻¹.

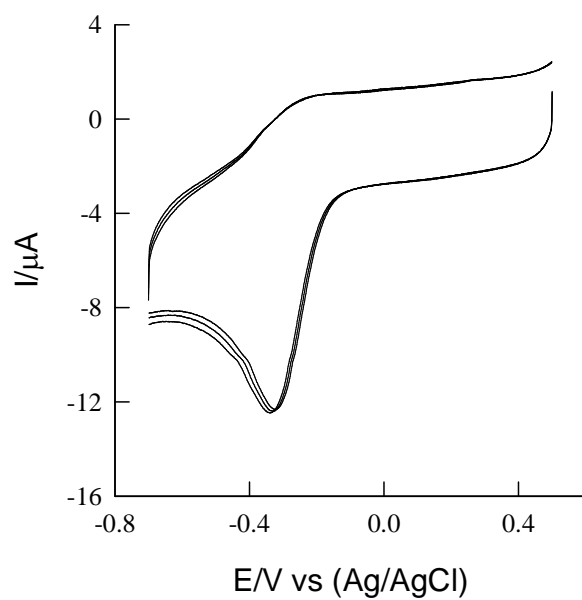
Figure S3

Figure S3. GCE/GMC@Blood : (Control) Continuous CV responses of GCE/GMC@Blood (without Nafion) in N_2 purged pH 7 PBS at scan rate of 50 mV s^{-1} .