Supporting Information

Biocompatibility Assessment of Chemically Modified GONR with Hemoglobin and Histopathological Studies for its Toxicity Evaluation

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Experimental Section

Materials:

Carbon nanotubes (MWCNTs; type 5; min. 95%) and $NiCl_2.6H_2O$ was purchased from Sisco Research Laboratories Pvt. Ltd., India. Hemoglobin porcine, lyophilized powder (molecular weight: 66.7 kDa), Pyridine-2-Carbaldehyde (99%) was obtained from Sigma Aldrich. Hydrochloric acid (HCl, LR) and sulfuric acid (H_2SO_4, LR) were prurchased from Thomas Baker (Chemicals) Pvt. Ltd., India. Orthophosphoric acid (H_3PO_4, GR) was purchased from Molychem, India. (3-Aminopropyl)triethoxysilane (APTES, 98%) was purchased from Alfa Aesar. Hydrogen peroxide (H_2O_2, 30%) was procured from Merck, India. Sodium phosphate buffer was prepared using sodium phosphate dibasic dehydrate and sodium phosphate monobasic anhydrous procured from Sisco Research Laboratories Pvt. Ltd., India. All other materials were of AR grade that has been used in the study. Double distilled water was used for washing and preparation of solutions.

Synthesis of GONR:

The method available in the literature with slight modification was used for the synthesis of GONR from carbon nanotubes (CNT).[1] In a 500 mL round bottom flask, 1.5 g of CNT was taken to which a 200 mL mixture of 9:1 by volume of concentrate H_2SO_4 and H_3PO_4 was added. The resultant mixture was stirred initially for 15 mins and followed by slow addition of 9.0 g $KMnO_4$ to the mixture while stirring. Then the mixture was kept stirring for 12 h at 60°C. After that mixture was poured into a 1L beaker containing 400 mL crushed ice, to which 5 mL of 30% H_2O_2 was added and mixed well. Further to obtain solid, the mixture was centrifuged. Obtained solid was washed with water several times by centrifugation. Followed by three concentrated HCl washing and then five washing with ethanol by centrifugation. The solid so obtained was dried in a vacuum desiccator at room temperature. The dried solid was labeled as GONR.

Synthesis of H_2N-GNR:

In a 250 mL round bottom flask, 400 mg of GONR was taken and 100 mL of ethanol was added. After this mixture was sonicated well for the dispersion of GONR in ethanol. Then 1.5 mL of APTES was added to the ethanolic solution of GONR and mixture was sonicated for 30 mins. Later, the mixture was stirred under reflux condition for 6 h. Then, G-4 sintered glass crucible was taken and mixture was filtered through it. Solid so obtained was washed several times with ethanol and was dried at 60°C. This obtained solid was labeled as H_2N-GNR.

Synthesis of S-GNR:

In a 250 mL round bottom flask, 500 mg of H_2N-GNR and 2 mmol of Pyridine-2-carbaldehyde was taken and 100 mL of ethanol was added. The mixture was stirred under reflux condition for 5 h. Then, G-4 sintered glass crucible was taken and mixture was filtered through it. Solid so obtained was washed repeatedly with ethanol and was dried at 50 °C. This obtained solid was labeled as S-GNR.

Synthesis of Ni-S-GNR:

In a 250 mL round bottom flask, 400 mg of S-GNR and 3 mmol of $NiCl_2.6H_2O$ was taken and 50 mL of ethanol was added. The mixture was stirred under reflux condition for 6 h. Then, G-4 sintered glass crucible was taken and mixture was filtered through it. Solid so obtained was

washed repeatedly with ethanol and was dried at 50 ℃. This obtained solid was labeled as Ni-S-GNR.

Sample preparation:

10 mM phosphate buffer of pH 7 was prepared and its pH was adjusted to pH 7.4 by using NaOH solution. The samples were prepared using this phosphate buffer of pH 7.4. Ni-S-GNR stock solution (1 mg/mL) was prepared in the buffer by ultrasonication for biophysical studies. Hemoglobin (Hb) stock solution (1 mg/mL) was prepared in the buffer by mixing. For all biophysical studies, 0.25 mg/mL Hb concentration was used with varying volumes of Ni-S-GNR. The equilibrium time was 30 mins at 25 ℃ for all the samples.

Instrumentation:

Instruments used for the study are as follow: Benchtop powder X-ray diffractometer (Rigaku XRD MiniFlex 600), FTIR spectrometer (Thermo Fisher, Nicolet iS50 FTIR), Renishaw Laser Raman Spectrometer (Invia II) with a laser source of 514 nm, X-ray photoelectron spectroscope (Physical Electronics, PHI 5000 VersaProbe III), Transmission electron microscope (Thermo Scientific, Cryo-TEM (TALOS S)), Scanning electron microscope (JEOL Japan Mode: JSM 6610LV) with EDS, UV-VIS Spectrophotometer (Labindia Analytical, UV 3092), Fluorescence Spectrophotometer (Hitachi High-Tech Science Corporation, F-4700), Circular Dichroism spectrometer (JASCO-CD, J-815(150-S)).

Methods for Biophysical Studies:

For pure Hb and Hb in the presence of different concentration of Ni-S-GNR, spectroscopic measurements such as UV-visible, Fluorescence, far- and near-UV CD, FTIR and Raman were performed. The UV-visible absorption spectra were recorded on Labindia Analytical UV 3092 at room temperature (25℃). The steady-state fluorescence spectra were recorded on Hitachi High-Tech Science Corporation F-4700 at room temperature (25℃) using a Peltier device. 295 nm was the excitation wavelength, PMT voltage was 400 V with a slit width of 5 nm for both excitation and emission. The CD spectra were recorded on JASCO-CD, J-815(150-S) equipped with a Peltier system, and an accuracy of ±0.1 °C. Far- and near-UV CD spectra were observed in the range of 190−250 and 250−350 nm, respectively. All spectra were averaged over three scans. All spectra shown are obtained after subtracting respective blank solutions. The FTIR spectra were recorded on Thermo Fisher, Nicolet iS50 at room temperature (25℃). Samples for FTIR were prepared specially in D_2O and spectra shown are obtained after subtracting respective blank solutions. The Raman spectra were recorded on Renishaw Laser Raman Spectrometer (Invia II) at room temperature (25℃). The samples prepared in buffer were freeze dried and spectra was obtained for the obtained powder.

Animals and Treatment Groups

The male Wistar rats were used for this study. The whole protocol for the animal study was approved by Institutional Animal Ethics Committee of the Department of Pharmaceutical Sciences, RTMNU, Nagpur, India. Male Wistar rats, weighing 180 – 200 g, were housed in a temperature- and humidity-controlled facility. The rats were randomly divided into 2 groups with 6 animals in each group. One of the groups served as the control and received the normal saline solution only. The animals in the treatment group were treated with a single

intraperitoneal injection of Ni-S-GNR (40 mg/kg). The control and treatment group animals were sacrificed on the next day (after 24 h).

Histopathology

The isolated liver and kidney kept in 10 % formaldehyde solution were processed overnight for dehydration, clearing, and impregnation using an automatic tissue processor (Sakura, Japan). The specimens were embedded in paraffin blocks using an embedding station (Sakura, Japan) and serial sections of 4 μm thickness were cut using a microtome (ModelRM2245, Leica Biosystems, Wetzlar, Germany). An autostainer (Model 5020, Leica Biosystems, Wetzlar, Germany) was used for Hematoxylin & Eosin staining of the sections. The mounted specimens were observed and were scored under light microscopy.

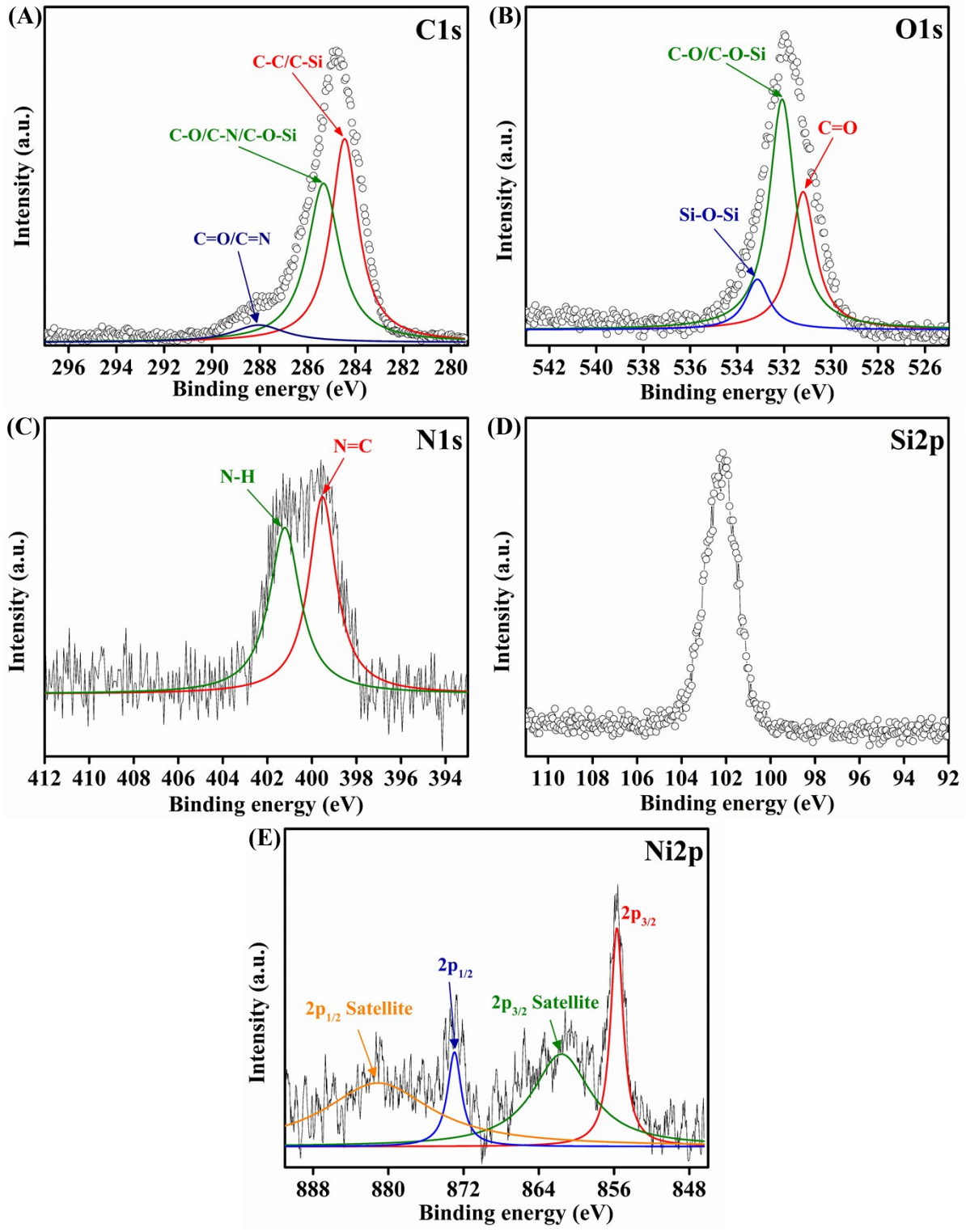
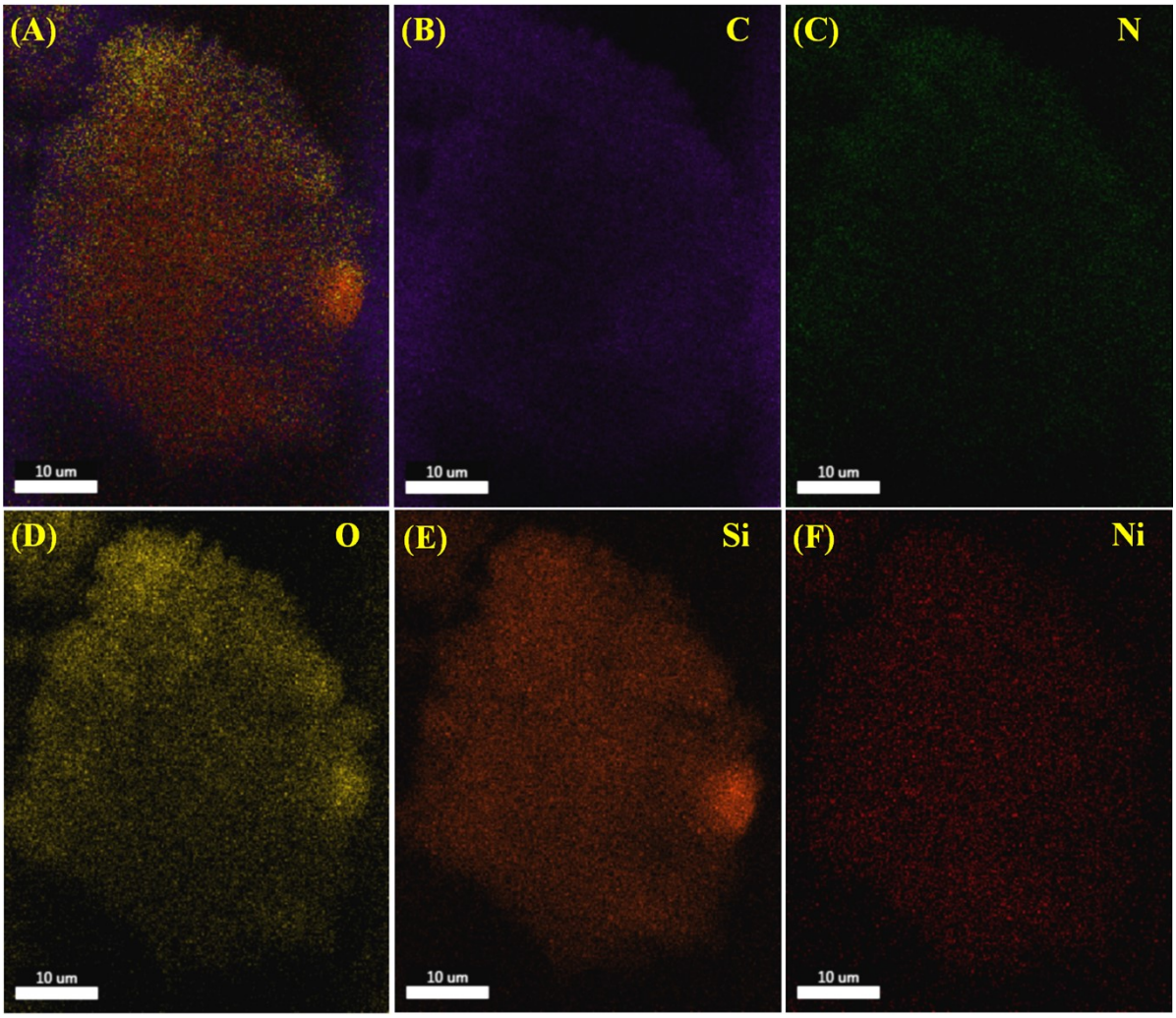
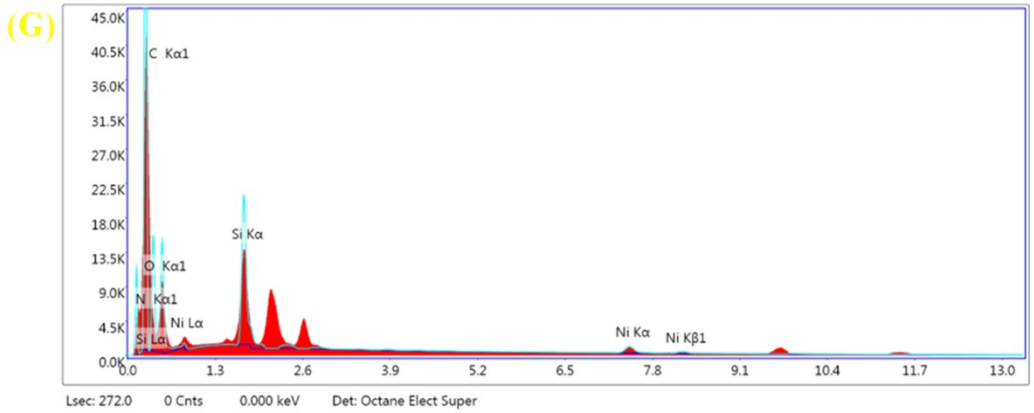


Figure S1. Deconvoluted XPS spectra for (A) C1s, (B) O1s, (C) N1s, (D) Si2p, and (E) Ni2p of Ni-S-GNR.





eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	49.68	56.30	2736.90	6.03	0.2536	1.0223	0.4995	1.0000
N K	26.73	25.98	465.00	11.34	0.0288	0.9980	0.1078	1.0000
O K	18.10	15.40	534.30	11.24	0.0201	0.9769	0.1136	1.0000
SiK	4.17	2.02	1174.20	2.78	0.0317	0.8860	0.8542	1.0040
NiK	1.32	0.31	102.50	5.33	0.0124	0.7504	1.0208	1.2219

Figure S2. Elemental mapping images of (A) area mapped, (B) carbon, (C) Nitrogen, (D) Oxygen, (E) Silicon, and (F) Nickel of Ni-S-GNR. (G) EDAX elemental analysis of Ni-S-GNR.

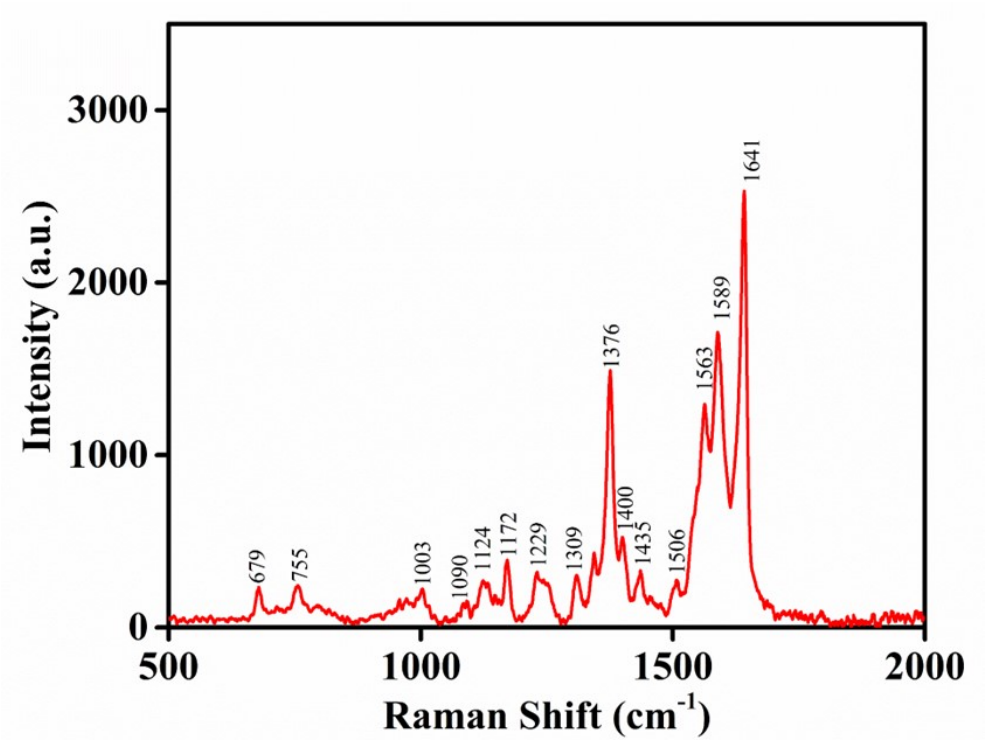


Figure S3. Raman spectra for pure Hemoglobin (Hb).

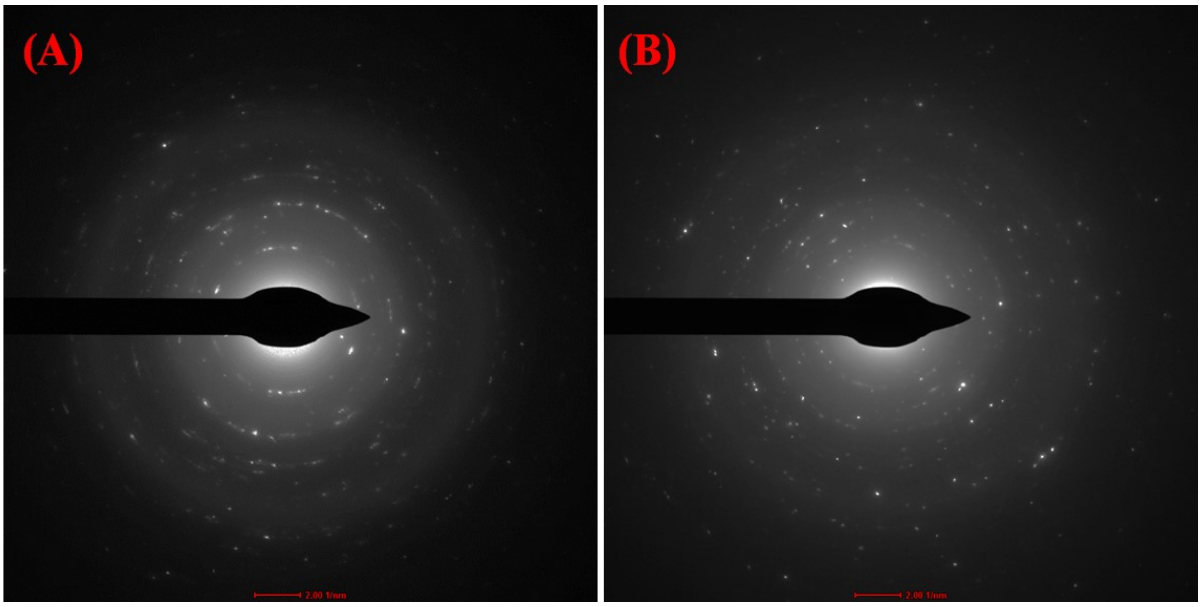


Figure S4. The SAED patterns for the complex formed of (A) Hb with 0.05 mg/mL of Ni-S-GNR, and (B) Hb with 0.07 mg/mL of Ni-S-GNR.

Reference

1 A. L. Higginbotham, D. V. Kosynkin, A. Sinitskii, Z. Sun and J. M. Tour, *ACS Nano*, 2010, **4**, 2059–2069.