Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Hierarchical structure of molybdenum disulfide-reduced graphene oxide nanocomposite for development of highly efficient serotonin biosensing platform

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1. Instrumentation

The crystalline structure and phase purity of the synthesized MoS_2-rGO nanocomposite is analyzed using Rigaku miniflex 600 X-ray diffractometer (XRD) with Cu Kα radiation of wavelength, λ = 1.5406 Å. Surface functionalization studies of the MoS_2-rGO nanocomposite with APTES molecule and formation of amide bond after immobilization of antibodies are examined using Fourier transform infrared spectrometer (FT-IR, Shimadzu IR Affinity 1S). Structural properties and surface morphology of the nanocomposite and fabricated electrodes are observed by Transmission electron microscopy (TEM, FEI Tecnai $G^2$20 S-Twin) and scanning electron microscope (SEM, JEOL JSM 6610LV; Japan). All the electrochemical studies are recorded using an Autolab Potentiostat Galvanostat (AUT204, Netherlands) and utilizing a conventional three-electrode system. An ITO coated glass electrode, a Pt wire and Ag/AgCl were used as working electrode, a counter electrode and reference electrode respectively. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) are scrutinized for characterizing the electron transfer kinetics of the fabricated electrode. Freshly prepared PBS (pH 7.0) solution containing 5 mM of $[Fe(CN)_6]^{3-/4-}$ as redox probe is used as a mediator. For CV measurements the potential was scanned from -0.7 to 0.7 V (vs. Ag/AgCl) at a fixed scan rate of 50 mV s^{-1} except for the different scan rate experiments, whereas for DPV measurements the potential was executed in the range from -0.4 V to 0.7 V with an amplitude of 0.025 V, pulse width of 0.005 s, pulse period of 0.5 s and increments of 0.005 V.

2. Chemicals and reagents

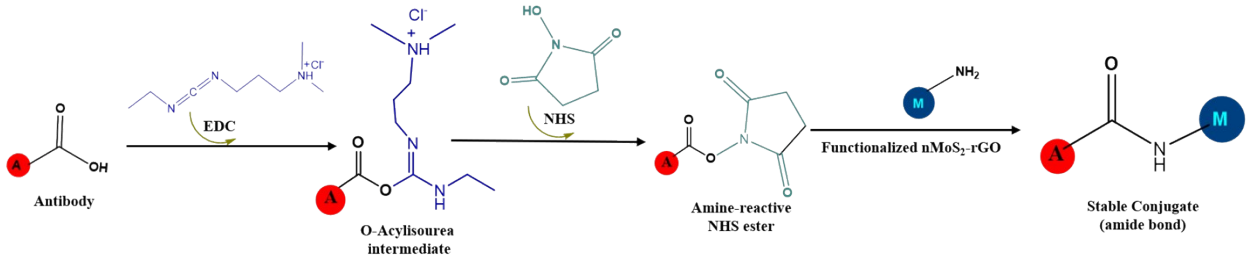
All chemicals of analytical grade were used without any further modification. Graphene oxide for the synthesis of rGO, 1-(3-(dimethylamino)-propyl)-3-ethyl carbodiimide hydrochloride (EDC) and bovine serum albumin (BSA) were supplied by Sigma-Aldrich. N-hydroxysuccinimide (NHS)

was purchased from Fisher Scientific. Sodium diphosphate dihydrate [$Na_2HPO_4.2H_2O$], sodium monophosphate [$NaH_2PO_4.2H_2O$], potassium ferrocyanide [$K_4(Fe(CN)_6).3H_2O$)], potassium ferricyanide [$K_3(Fe(CN)_6)$] and sodium chloride (NaCl) were procured from Merck Life Science Pvt. Ltd. Magnesium nitrate hexahydrate [$Mg(NO_3)_2.6H_2O$] and sodium molybdate dihydrate [$Na_2Mo_4.2H_2O$] were supplied by Thermo Fischer Scientific India Pvt. Ltd. Thiourea was supplied by Loba Chemie Pvt. Ltd. 3- aminopropyl triethoxy silane (APTES, 98%) and Serotonin were purchased from Alfa-aesar. Phosphate buffer saline (PBS) solution of pH 7.0 was freshly prepared using $NaH_2PO_4.2H_2O$ (0.2 mol L^{-1}), $Na_2HPO_4.2H_2O$ (0.2 mol L^{-1}) and 0.9% NaCl as supporting electrolyte. The mouse monoclonal serotonin antibody (anti-serotonin) was procured from Merck Life Science Pvt. Ltd. (California, USA). The biomolecule was diluted using PBS of pH 7.0 and stored at -20°C until further use.

3. Processing and collection of serum sample

The blood sample was collected and processed at the National Liver Disease Biobank, Institute of Liver and Biliary Sciences, New Delhi, India after ethical approval by the Institutional Ethical Committee (Ref. No. Chem/2020/1953), Department of Chemistry, University of Delhi. Written consent from the subject was taken before the sample collection. Briefly, the sample collected in a sterilized tube was centrifuged to remove cell debris and other unwanted materials. The resultant supernatant obtained after centrifugation of the blood sample was collected in a sterilized tube and stored at -20°C until further use.

Scheme 1



Scheme 1: Reaction scheme for EDC-NHS based covalent bioconjugation of anti-serotonin biomolecule with APTES/$nMoS_2$-rGO/ITO electrode.

Fig S1.

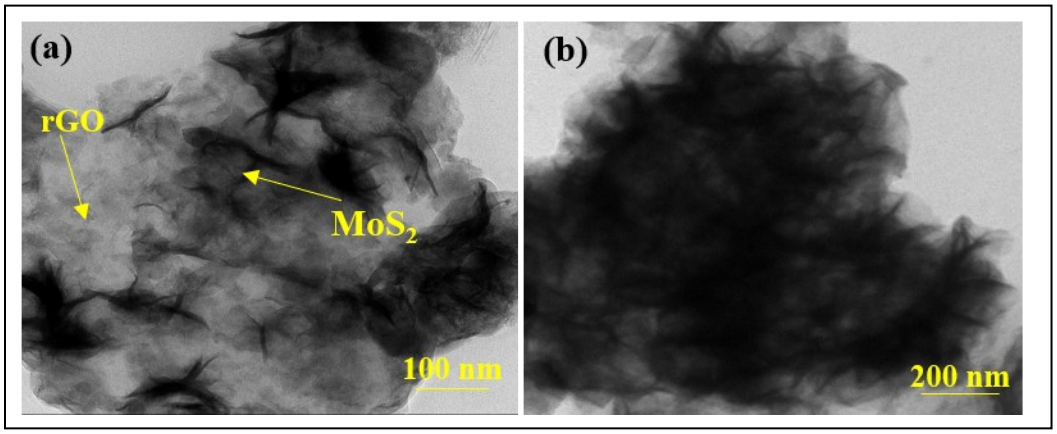


Fig S1: TEM image of MoS_2-rGO nanocomposite at different scale.

Fig S2

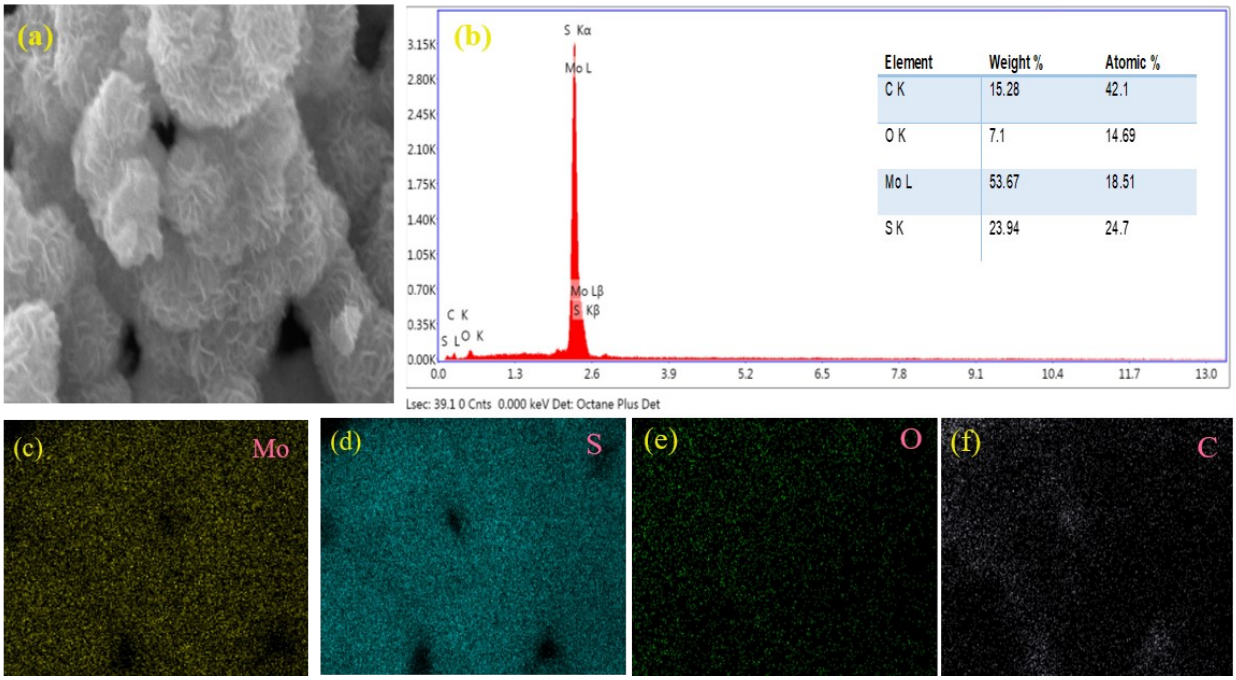


Fig S2: (a) SEM image (b) EDX spectrum of the nMoS_2-rGO and Elemental mapping of (c) Mo, (d) S, (e) O and (f) C at same place.

Fig. S3

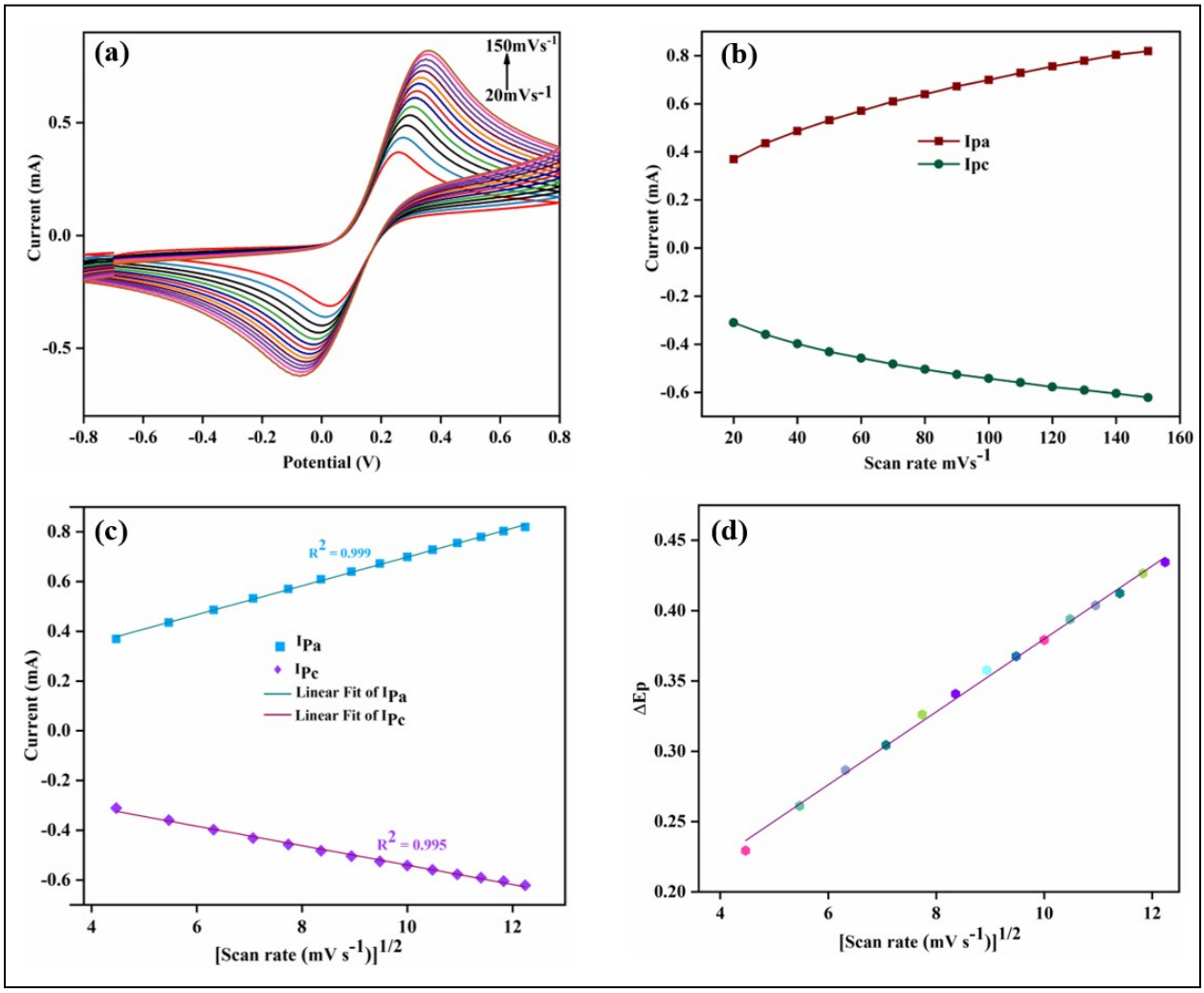


Fig S3: CV response of APTES/nMoS$_2$-rGO/ITO immunoelectrode at different scan rate (20-150 mV s-1), (b) Magnitude of anodic (I_{pa}) and cathodic (I_{pc}) peak current of as a function of scan rate (υ) and (c) Relationship between oxidation and reduction peak current vs square root of scan rate (mV s^{-1}) and (d) Difference of peak potential ($\Delta E_p = E_{pa} - E_{pc}$) as a function of the square root of scan rate.

Fig. S4

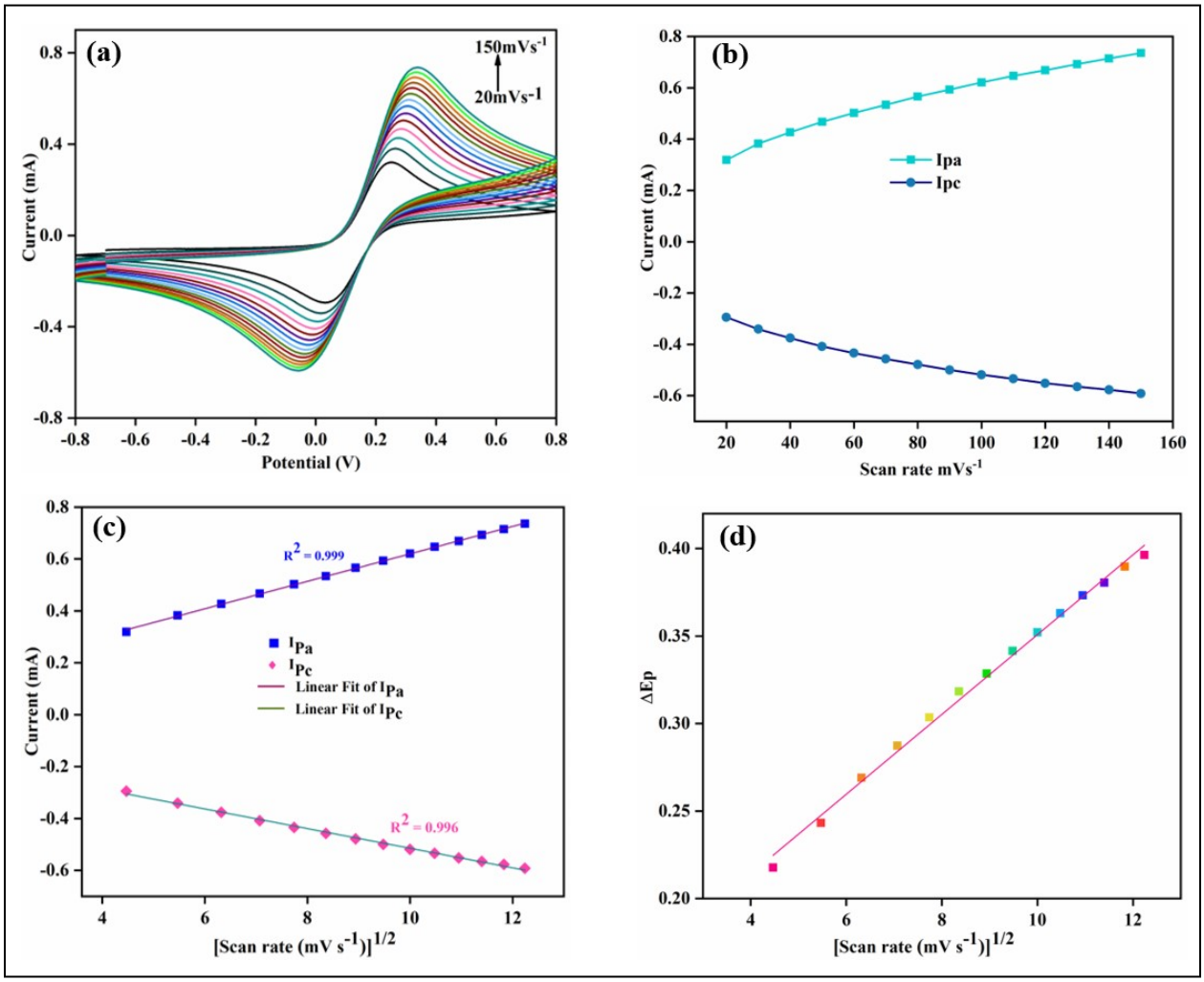


Fig S4: CV response of BSA/anti-serotonin/APTES/nMoS$_2$-rGO/ITO immunoelectrode at different scan rate (20-150 mV s^{-1}), (b) Magnitude of anodic (I_{pa}) and cathodic (I_{pc}) peak current of as a function of scan rate (υ), (c) Relationship between oxidation and reduction peak current vs square root of scan rate (mV s^{-1}) and (d) Difference of peak potential ($\Delta E_p = E_{pa} - E_{pc}$) as a function of the square root of scan rate.

Quantification of serotonin using DPV analysis

The limit of detection (LOD) of the fabricated immunosensor is evaluated using following equation:

$$\text{Limit of Detection} = \frac{k \times \sigma}{m}$$

……..Eq. S1

Where, k is the confidence level of parameters (k = 3), σ is the standard deviation of the BSA/anti-Serotonin/APTES/nMoS_2-rGO/ITO immunoelectrode and m is the sensitivity.

Fig. S5

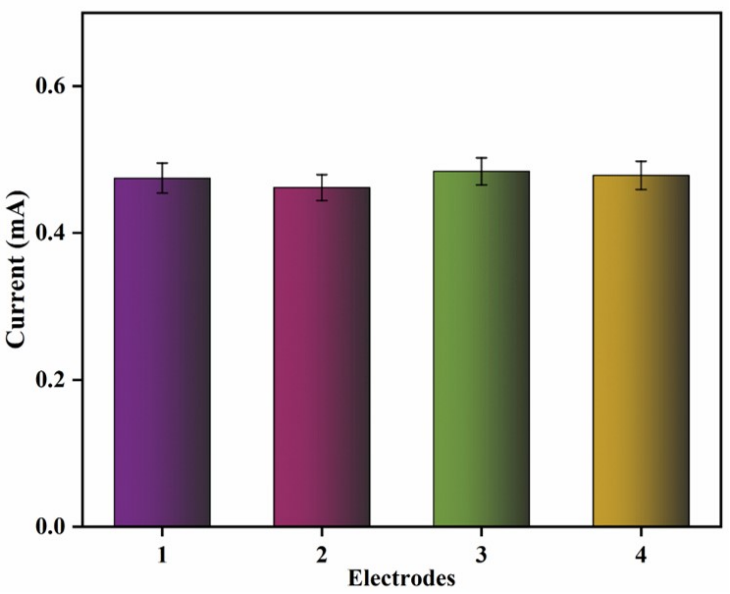


Fig S5: Reproducibility studies of four different BSA/anti-Serotonin/APTES/nMoS_2-rGO/ITO immunoelectrodes.

Table S1: Determination of %RSD in presence of different analytes in PBS solution.

S.No.	Analyte in PBS solution	Magnitude of peak currents (µA)	RSD (%)
1	Ser + Dp	67.60	0.62
2	Ser + Dp + UA	67.80	0.42
3	Ser + Dp + UA + Glu	66.90	1.36
4	Ser + Dp + UA + Glu+ AA	66.40	1.89
5	Ser + Dp+ UA + Glu +AA + Urea	67.50	0.73
6	Ser + Dp + UA +Glu + AA + Urea + NaCl	67.15	1.10
7	Ser + Dp + UA +Glu + AA + Urea + NaCl+ LA	67.40	0.83

Fig. S6

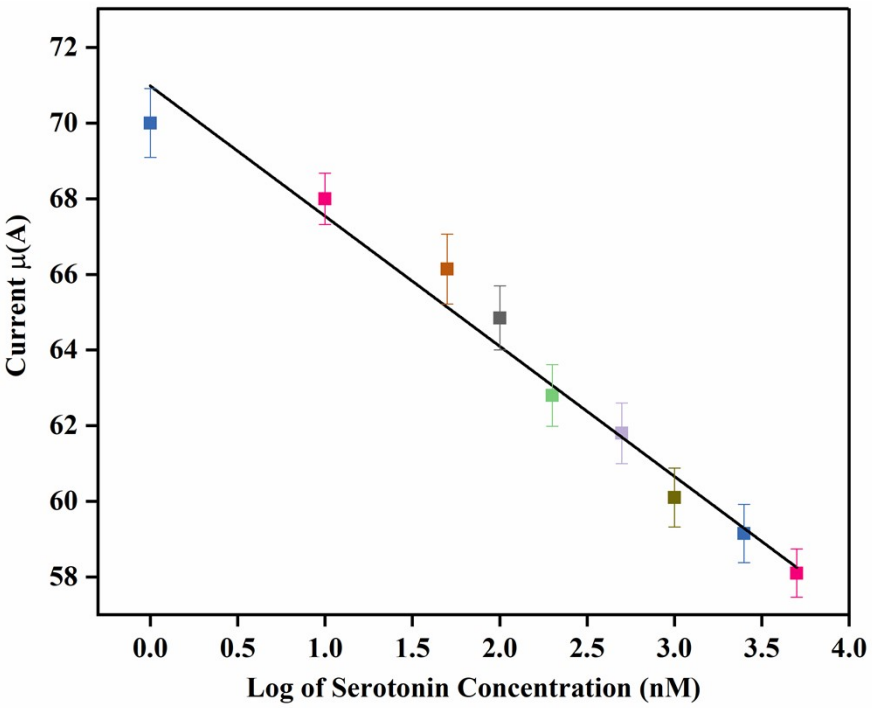


Fig S6: Calibration curve of current response (μA) vs Log of serotonin concentration (nM) in spiked sera samples.

Table S2: Determination of % RSD (relative standard deviation) of serotonin concentration in serum using BSA/anti-Serotonin/APTES/nMoS_2-rGO/ITO immunoelectrode.

Concentration (nM)	Value of current (μA) obtained with standard serotonin solutions	Value of current (μA) obtained with serotonin spiked sera samples	% RSD
1	71.5	70	1.50
10	68.25	68	0.26
50	66.75	66.14	0.65
100	65.30	64.85	0.49
200	64.40	62.80	1.78
500	63.85	61.80	2.31
1000	63.35	60.10	3.72
2500	62.75	59.15	4.18
5000	62.40	58.10	5.05

Average % RSD	**2.21**