Supporting Information

Cu(0) nanoparticles decorated functionalized reduced graphene oxide sheets as artificial peroxidase enzymes: Application for colorimetric detection of Cr(VI) ion

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**Characterization techniques**

Diffuse reflectance infrared Fourier transform (DRIFT) spectra were recorded in transmittance mode at 4 cm$^{-1}$ spectral resolution using an IR Affinity-1 FTIR spectrophotometer (Shimadzu, Japan) equipped with a DRS-8000 DRIFT accessory in the 400–4000 cm$^{-1}$ range.

The morphology and the particle size of the synthesized nanocomposite were determined by high resolution transmission electron microscopy (HRTEM) using JEOL JEM 2100 PLUS, transmission electron microscope, Japan, operated at an accelerating voltage of 200 kV. The surface morphology of the synthesized nanocomposite was analysed by field emission scanning electron microscopy (FESEM) using ZEISS Gemini scanning electron microscope (Germany) operated at an accelerating voltage of 9–7 kV.

The crystallographic structure of the nanocomposite was investigated by X-ray diffraction (XRD) using a Rigaku X-ray diffractometer (model: ULTIMA IV, Rigaku, Japan) with a scanning rate of 3$^\circ$ min$^{-1}$ at 2$\theta$ values 5–100$^\circ$ with a Cu K$\alpha$ X-ray radiation ($\lambda = 1.54056$ Å) at a generator voltage of 40 kV and a current of 40 mA.

The chemical composition of the synthesized fGO sheets and nanocomposite Cu-fGS was examined by X-ray photoelectron spectroscopy (XPS) measurements using a Thermo-Scientific ESCALAB Xi+ spectrometer with a monochromatic Al K$\alpha$ X-ray source (1486.6 eV) and a spherical energy analyser that operates in the CAE (constant analyser energy) mode using the electromagnetic lens mode. The CAE is 100 eV for survey spectra and that for high-resolution spectra is 50 eV. All the XPS data were deconvoluted in the Casa XPS processing software. After the peroxidase mimic activity, the nanocomposite materials were analysed by XRD and XPS.
Comparison of morphology and functionalities of rGO and fGO sheets in Cu(0) nanoparticles based composite materials

The TEM images of GO and fGO sheets (Fig. S2a and S2b) show that after functionalization, graphene oxide sheets were further exfoliated and thus a single layer of graphene oxide possesses high surface area to accommodate higher amounts of Cu(0) nanoparticles on its surface. From FTIR spectra (Fig. S3), it is seen that after decoration of Cu(0) nanoparticles, the intensity of the characteristic –C=O (1732 cm\(^{-1}\)) and –OH (3130 cm\(^{-1}\)) groups decreased, illustrating the reduction of GO to rGO. Similarly, in case of Cu-f-rGO nanocomposite,
reduction of the intensity of both –C=O (1797 cm$^{-1}$) and –OH (3274 cm$^{-1}$) groups occurred. Thus, the reduction of fGO to f-rGO took place during the synthesis of Cu-frGO.

Fig. S2 TEM images of (a) GO and (b) fGO sheets.

Fig. S3 FTIR of GO, Cu-rGO, fGO and Cu-frGO
Fig. S4 X-ray photoelectron survey spectrum of Cu-rGO nanocomposite.

Kinetic analysis of peroxidase nanoenzyme

For kinetic analysis, TMB oxidation was carried out by varying the concentration of the substrates (TMB and H₂O₂), at constant concentration of other reagents. The absorbance value for a particular concentration of the substrate was evaluated using the Beer Lambert’s law:

\[ A = \varepsilon_{\text{TMBDI}} \times C \times L \]

where \( A \) is the absorbance and \( \varepsilon_{\text{TMBDI}} \) is the molar extinction coefficient of 3,3′,5,5′-tetramethylbenzidinediimmine (TMBDI); its value is 39,000 M⁻¹cm⁻¹ at 652 nm [1]. \( C \) is the concentration of the substrate and \( L \) is the path length. The catalytic parameters \( K_m \) (Michaelis constant) and \( V_m \) (maximal velocity) of the enzyme mimic were evaluated from Lineweaver-Burk double reciprocal plots shown in Fig. S4 (insets). Fig. S4 depicts the typical
Michaelis-Menten curves for H$_2$O$_2$ and TMB, respectively, as substrates in a certain concentration range in presence of Cu-rGO nanoenzyme.

**Fig. S5** Steady-state kinetic assay of Cu-rGO: (a) variation of H$_2$O$_2$ concentration (0.06-5.0 mM) at constant concentration of TMB (0.5 mM), (b) variation of TMB concentration (0.02-10.0 mM) at constant concentration of H$_2$O$_2$ (50 mM). The corresponding Lineweaver-Burk double reciprocal plots of Michaelis-Menton equation are shown inset.
Fig. S6 Reusability study of Cu-frGO toward TMB oxidation in presence of 50 nM Cr(VI) ions: acetate buffer (0.2 M, pH 4), 0.5 mM TMB, 6 mg/L catalyst and 50 µL H$_2$O$_2$.

References