Biogenic preparation of doughnut shaped manganese nanograins embellished on graphene for superior interfacial binding of biomarkers

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ABSTRACT

The astounding characteristics of the nanomaterials can be crafted to prepare tailor-made interfaces, especially for the development of biomedical applications. In the present study, we illustrate the preparation of a new class of doughnut-shaped manganese nanograins embellished on the graphene sheets to develop hybrid interfaces using a gentle and nontoxic approach. We investigate its potential to detect clinically important biomarkers and glutathione is chosen as a case study. The unprecedented structural morphology and chemical characteristics are described. Two-dimensional sheets of graphene are uniformly occupied by manganese nanograins. Each individual grain is doughnut-shaped with the external diameter of about 5–10 nm, the thickness of 2 nm with a small central cavity of about 1 nm. We show the unique morphology of hybrid offers conducting features of graphene, metallic nature of nanograins, and amelioration of greater surface area to promote enhanced binding capability. Resultantly, the hybrid yields remarkable sensitivity of 7 nM. A binding mechanism has been proposed along with extensive kinetics assessment. The developed hybrid can selectively bind glutathione in comparison of protein and non-protein molecules. The results validate that this approach can be used to design green hybrid materials for accurate diagnosis of biomarkers and metabolites.

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1. Introduction

Two-dimensional (2D) materials provide structural skeletons that enable the fabrication of heterostructures with novel morphologies, which can be potentially harnessed to develop outstanding applications including electronic devices, catalysts, sensors, drug delivery, energy conversion and storage devices [1]. A plethora of opportunities arises, when we combine 2D nanostructures with other compositions of nanomaterials to fabricate hybrids. These materials constitute a large family and show extraordinary characteristics owing to the synergistic effects of nanomaterials, in addition to inherent quantum properties. As the family of 2D materials is expanding day by day, it opens new platforms for diverse utility, especially in biomedical analysis [2,3].

Carbon based nanomaterials, such as carbon nanotubes and carbon nanofibers are most commonly used as the frame to fabricate hybrids. Among this material family, most popular remains graphene with its unique structural, chemical, mechanical, optical, and electrical properties [4–6]. The higher electron mobility as compared to monocrystalline silicon and carbon nanotubes, makes graphene one of the lowest resistive materials at room temperatures [7]. Due to its two-dimensional structure, large surface-to-volume ratio, and excellent interfacial properties, it is considered as a favorable candidate to synthesize hybrids. This fusion of graphene with other nanomaterials leads to enhanced electrochemical properties that can be utilized to detect various biological molecules [8].

Metal nanomaterials with exceptional inherent features are the best choice to incorporate with 2D materials. When combined with suitable transducers, it allows the fabrication of advanced biosensors with remarkable capabilities for the detection of specific bioanalytes [9]. Among metals, manganese is the twelfth most significant transition element of the earth, and the third most common element after iron and titanium [10]. MnO2 nanoparticles potentially hold great promise to be used in molecular sieves, batteries, solar cells, catalysts, optoelectronics, drug delivery systems, and diagnostics due to their outstanding physicochemical properties such as surface-to-volume ratio, low melting point, mechanical strengths, optical, and magnetic properties [11–15]. Moreover, they are less toxic, cost-efficient, and highly active due to its oxidation state [16].

Now-a-days, scientific interest is more focused towards mild synthesis approaches. Despite, the fact that chemical methods are usually preferred in synthesizing nanomaterials, biogenic synthesis methods have received considerable attention as it offers eco-friendly, sustainable, and inexpensive routes [17,18]. Here, the reducing agents in the chemical synthesis of nanomaterials are replaced by natural products, such as plant leaf extracts (biogenic reduction). The resulting materials benefit from the biomolecules available in the extract e.g. enzymes, amino acids, proteins, polysaccharides, vitamins, and organic acids [5].

Glutathione (GSH), an abundant non-protein biologic mercaptan, plays an important role in maintaining redox homeostasis in humans by scavenging free radicals and toxins. It is involved in gene regulation [16,19], but also in many cellular processes, including cell differentiation, proliferation and apoptosis [18]. The clinical range of glutathione in solid tissues and blood is 10–15 μM and 2–5 μM, respectively. Abnormal levels of glutathione are associated with increased oxidative stress [20]. Disturbances in GSH homeostasis are implicated in the progression of different human diseases such as cancer, cystic fibrosis, aging, cardiovascular, immune disorders, inflammatory, metabolic, and neurodegenerative diseases [21,22]. Therefore, tracing the level of GSH can generate very important clinical and pharmacokinetics data which can help in early diagnosis of high-risk diseases. In this respect, some techniques are available, including high-performance liquid chromatography, capillary electrophoresis, and enzymatic based assays [23]. These conventional methods perform well, but the development of less technically challenging techniques would greatly help clinicians and health care providers. Electrochemical methods can be a promising choice being sensitive, less time consuming, and relatively easy to handle [24]. Therefore, these methods can be integrated with suitable nanomaterials for sensitive recognition of disease biomarkers.

In the present study, we report a benign (nontoxic), single step, and cost-efficient approach to synthesize graphene oxide hybrid, which is augmented with ultra-small doughnut shaped manganese nanograins. We used Caryota mitis extract as it contains a large number of important biomolecules which can act as potential reducing agents due to their outstanding antioxidant properties. The purpose of doughnut shaped morphological work was to achieve an interesting three-dimensional hybrid structure with the prospect of large surface area and improved sensor response. An efficient hybrid interface was developed that has a novel morphology and binding affinity to detect glutathione. The morphology of the hybrid was investigated by different microscopic techniques including field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). To analyze the functional groups and surface potential of the hybrid, Fourier transform infrared (FTIR) spectroscopy and dynamic light scattering (DLS) experiments were performed. The electrochemical activity of the hybrid is investigated in detail by using cyclic voltammetry and differential pulse techniques. This study opens a new platform of green synthesis to design efficient hybrids that can be used in clinical applications.

2. Materials and methods

All chemicals/reagents used in this study were either of highest procured or analytical grade. They were purchased from either Sigma–Aldrich or Merck and used without further modifications. Sodium phosphate buffer (monobasic monohydrate) was prepared using deionized water (50 mM; pH 7.0) as a background medium to conduct all experiments. Graphene oxide was purchased from the Shenzhen Nanotech Port. Co. Ltd (China). Flowers (Caryota mitis) were collected from the Botanical Gardens of Agriculture University Faisalabad, Pakistan.
2.1. Preparation of hybrid (Mn NGs@GO)

2.1.1. Preparation of Caryota mitis extract
To prepare the extract, typically, Caryota mitis flowers were collected from the Botanical Gardens of Agriculture University Faisalabad, Pakistan. They were dried under sunlight for 3–5 days. Afterwards, the parched flowers were ground mechanically with a pestle and mortar, till a very fine powder is obtained. A suspension was prepared by mixing 10 g powder in 100 mL distilled water with constant stirring for 2 h at 80 °C. After that the resulting mixture was cool down to room temperature. To obtain the pure extract, it was centrifuged at 15,000 RPM for 15 min, followed by filtration to remove residues, through Whatman filter paper (grade 1; with a pore size of 11 µm). Finally, in pursuance of further work, the as-prepared extract was stored at 2 °C to circumvent the microbe culturing and growth.

2.1.2. Surface modification of 2D sheets of graphene oxide
Graphene oxide (GO) was purchased from the Shenzhen Nanotech Port. Co. Ltd (China). To modify the surface, 0.08 g of GO was dissolved in 334 µL of 1% w/w polyethyleneimine (PEI) in methanol solution. The GO suspension was sonicated for 60 min and then centrifuged (8000 RPM) at 25 °C to isolate the pallet from suspension. The supernatant was discarded and a pallet (2 mg) of PEI wrapped graphene oxide was obtained and sonicated until it formed a stable dispersion, after reconstituted in 50 mL methanol.

2.1.3. Preparation of Mn NGs@GO hybrid
The PEI functionalized GO dispersion was added to 20 mL of manganese chloride precursor methanol solution, with rigorous and continuous stirring. It was kept at 45 °C for further 30 min. Then a 10 mL of freshly prepared extract was added into the above reaction solution with added stirring of 5 min. Afterwards, the solution was centrifuged at 8000 RPM for 10 min and a pallet of approximately 2 mg was collected. It was washed with methanol (70%) three times, to ensure purity. This cleaning procedure was repeated many times under the same reaction conditions. To conduct sensor experiments, the final product was vacuum dried at 60 °C overnight. This is termed as Mn NGs@GO hybrid.

2.2. Characterization methods
To investigate the morphology of the designed hybrid extensive studies were carried out employing field emission scanning electron microscopy (JEOL, JSM-7500F, Tokyo, Japan). The projection images of the materials were obtained by transmission electron microscopy (JEOL, JSM-1010 Tokyo, Japan). The surface properties were examined by atomic force microscopy (SHIMADZU WET-SPM 9600, Tokyo, Japan).

To investigate the optical properties of Mn NGs@GO hybrid, UV–vis spectroscopy was used, and experiments were carried out in triplicate for every sample (1 mg mL⁻¹). The surface potential of Mn NGs@GO hybrid was studied using a Zeta Sizer Nano Series instrument (Malvern). To do this, homogeneous dispersions (1 mL) of GO-PEI and hybrid were obtained by sonication and respective potentials were measured.

To get information about the functional groups and their transformation occurred during the preparation of Mn NGs@GO hybrid, Fourier transform infrared (FTIR) scans were conducted using attenuated total reflection mode of Perkin Elmer system 2000. To make the measurements, a 5 mg pallet of hybrid material was collected at different experimental stages, i.e. suspension of PEI wrapped graphene oxide and Mn NGs@GO hybrid. Additionally, each sample was prepared in triplicates and 10 consecutive scans of each sample were calculated to obtain the spectra.

2.3. Preparation of sensor interfaces
To prepare sensor interfaces, working electrodes were coated using different sizes of alumina slurries, 0.1, 0.2, and 0.3 µm, respectively. Afterwards it was followed by washing with deionized water and methanol, to eradicate adsorbed organic or inorganic contaminants. In order to further clean the surfaces, GCE electrodes were exposed to ten cycles in the same supporting media (NaH₂PO₄, H₂O; 50 mM) between −0.2 V and 1 V potential window, at a scan rate of 0.1 V s⁻¹.

To fabricate the sensor, 1 mg of Mn NGs@GO hybrid was suspended in 1 mL of methanol. 20 µL of this suspension was drop-cast on the clean surface, and dried at room temperature for 30 min. The resulting layers of hybrid suspension were strengthened with 2 µL of nafion (2%). Control experiments were performed by following the same procedure, but without the hybrid. Here, 10 µL of manganese nanograins (Mn NGs) and graphene oxide dispersions (0.5 mg mL⁻¹), each were coated to get the separate responses for Mn NGs and graphene oxide modified electrodes, respectively.

The electrochemical capability of the hybrid to bind glutathione was assessed by cyclic voltammery (CV) and differential pulse voltammetry (DPV) methods. All experiments were carried out with a Galvanostat/Potentiostat (PGSTAT; Autolab, AUTF2284, Netherlands). A three- electrode setup was constituted: consisting GCE with Mn NGs@GO interfaces as working electrode, a carbon rod counter electrode, whereas an Ag/AgCl was adjusted as the reference electrode (3 M KCl). The electrochemical experiments were conducted in the background media of 25 mL of NaH₂PO₄, H₂O (50 mM). Prior to start sensor experiments, the reaction medium was purged with nitrogen gas (99%) for approximately 5 min to remove trapped oxygen. CV responses were measured using the potential range −0.2 to 1 V, with a scan rate of 100 mVs⁻¹. For DPV experiments, the reaction conditions were adjusted at a pulse amplitude of 50 mV and a period of 0.3 s. The equilibration time was set at 60 s and scans were obtained with a potential range of −0.5 V and 1.2 V. All electrochemical scans were recorded using the General Purpose Electrochemical System software (GPES version 4.9), whereas data was handled using Microsoft Excel.
3. Results and discussion

3.1. Characterization of as-prepared materials

3.1.1. Surface morphology and structural analysis of Mn NGs@GO hybrid

The Mn NGs@GO interface was designed to impart both conducting and metallic characteristics which can support catalysis of GSH. The morphology of the as-prepared hybrid was investigated by FESEM (Fig. 1A–C). A clear difference can be seen between Mn NGs@GO hybrid and GO. Fig. 1A shows the large inherent wrinkled sheets of GO with interconnected network and folds. In the case of the hybrid (Fig. 1B), doughnut shaped manganese nanograins with uniform shape and size, inherited on the layers of graphene can be observed. The grains were homogeneously populated on the surface of graphene sheets (ca 100 grains/500 nm² ± 10). The structure of doughnut consists of a central pit of ca 1 nm with a surrounding grain ring of less than 1 nm (Fig. 1C). The external diameter of individual grains was found to be ca 5–10 nm with the thickness of ca 2 nm and a small cavity can be seen in the center of every single grain.

A facile, biologically induced reaction, using a simple and in-situ approach was followed. The detailed synthesis mechanism can be quite complex, however in this strategy two major steps can play very important role in the formation of hybrid morphology. Firstly, the functionalization of GO, and secondly the nucleation and growth of nanograins. We assume that the size and distribution of the NGs are determined by these two steps. GO acts as a substrate which provides an active platform for the nucleation and growth of NGs. Moreover, well-formed sheet morphology of GO ensures the presence of its conductive characteristics in the hybrid. Undoubtedly, the doughnut shape morphology and a large population of Mn nanograins can impact metallic and catalytic characteristics during the binding process [19].

In most cases, polydispersity of the active sites on the surface of GO results in the uneven attachment of nanomaterials [25]. However, the synthesis of metallic structures by bioextract produces monodispersed and homogenous discrete manganese nanostructures. The morphology depends on the biological extract which is used to grow NGs. This structure exhibits discrete features like ultra-high conductivity and a unique network of unified sheets of graphene oxide augmented with manganese nanograins. The hybrid is electrically conducting owing to the presence of free electrons between graphene sheets and metallic nanograins. This continuous network along with surface area is expected to play decisive role in the sensitive electrocatalysis and suggests that the hybrid can be used in electrochemical diagnostic applications. Further, TEM images (Fig. 1D,F) validate the ability of the above synthesis protocol to attain a uniform distribution of 5–10 nm diameter manganese nanograins anchored to the graphene sheets. Moreover, the fluted and groovy surface of graphene oxide is also apparent from this analysis. Both microscopy analysis of SEM and TEM, support the novelty of our synthesized hybrid.

The topography of GO and hybrid material was examined by AFM and representative images are shown in Fig. 1G,H. The layer thickness of GO was analyzed to be 100–200 nm (Fig. 1G). On the other hand, Mn NGs@GO hybrid showed dispersion of 5–10 nm Mn nanograins, embossing the sheets of GO (Fig. 1H). Comparing these AFM topographs revealed the height of structures decreased from 400 nm to 25 nm which indicated that the flattening of GO sheets has occurred during the formation of hybrid probably due to its functionalization and the growth of nanograins.

The zeta potential of the Mn NGs@GO hybrid was investigated to determine the surface potential and the stability of the hybrid. The high packing of 2D materials by different functional groups decreases the agglomeration and aggregation of nanostructures on the scaffolds and increases its potential to assemble more ions leading the nucleation of nanostructures. When GO sheets are functionalized with amine entities, the sheets hold a negative potential of −28 V (Fig. 2A). The negative potential indicates the suitability of the surface of graphene sheets to attract the positive manganese ions of the precursor. The hybrid exhibited 32 mV positive surface potential validating the growth of nanograins (Mn NGs) on the skeleton of GO, and its ability to catalyze the negatively charged amine. The stability of the formed hybrid suspension and surface potential makes it a very favorable support for the fast oxidation of glutathione.

UV–vis spectroscopic analysis was done to investigate the surface plasmon resonance effects of the hybrid, in the wavelength range of 200–800 nm (Fig. 2B). It revealed a broad absorbance band at 320 nm of Mn NGs@GO hybrid due to the uniform attachment of Mn NGs to the 2D sheets of graphene oxide (Fig. 2B). GO shows two distinct absorption bands, one at 230 nm and the other small band at 300 nm, due to the π−π aromatic transition of C−C bonds and the n−π transition of carbonyl functional groups, respectively [26]. The UV–vis spectra of as-synthesized manganese nanograins following the same method, shows an absorption peak at 280 nm. However, in the case of hybrid, a board absorption peak can be correlated to the presence of manganese nanograins attached to the sheets of graphene oxide.

To investigate the functional groups, present on GO, FTIR analysis was done for GO and Mn NGs@GO hybrid. Fig. 2C shows the absorption bands in the FTIR spectra of GO at about 3400 cm⁻¹ and 1020 cm⁻¹ for stretching vibrations of N−H and C−N groups, respectively, which indicates the functionalization of GO with amine groups. These absorption bands of amine groups are also reported in the literature [27]. The FTIR spectra of GO also illustrate the absorption band at 2435 cm⁻¹ ensuring the presence of the C=O stretching in GO. Conversely, all these absorption bands are diminished in the FTIR spectra Mn NGs@GO hybrid, which indicates that these functional groups act as affixing sites for the nucleation of manganese nanograins on GO.

3.2. Sensor signals

To investigate the binding capability of Mn NGs@GO hybrid, cyclic voltammetry was performed. Fig. 3A–C shows the CV signals of bare glassy carbon electrode and Mn NGs (A), graphene oxide (B) and Mn NGs@GO hybrid (C) in the presence of 250 μM glutathione. The electrochemical signal of bare glassy carbon electrode was found insufficient due to lower
facilitation of electron flow. Compared to it, a more defined CV response was observed for NGs due to the conducting and metallic nature of manganese (Fig. 3A). The oxidation peak current was recorded ($8.0 \pm 0.3 \, \mu A$) at 300 mV, when the electrode modified with Mn NGs was challenged. In the case of GO, the CV shows a blunt curve, which indicates its trivial activity towards glutathione binding. The broad shape of voltammogram can be attributed to the flow of higher amount of non-Faradaic current because of greater electron mobility on GO interfaces (Fig. 3B). In contrast, the CV response of Mn NGs@GO hybrid demonstrates an unprecedented catalytic activity (Fig. 3C), with high peak current intensity of oxidation ($55.1 \pm 0.1 \, \mu A$) and reduction ($48.3 \pm 0.2 \, \mu A$). The high surface area and remarkable catalytic activity of the designed hybrid increase its efficiency to interact with an analyte. The oxidation and reduction peaks were found completely reversible suggesting all the intermediates are fully converted back into its reactants. Another feature of the Mn NGs@GO hybrid that makes it a suitable candidate for electrochemical sensing is the separation of both peaks, which was found to be 160 mV, with an anodic peak at 210 mV and cathodic at 50 mV. Both peaks of redox reaction of the glutathione are sharply separated due to excellent kinetics and higher conductance of materials. The enhanced capabilities of the Mn NGs@GO hybrid make it highly suitable for the electrochemical interactions. The outstanding electrochemical signals of the prepared hybrid towards glutathione can be conferred due to the coherence of the metallic nature of manganese, conducting feature of graphene oxide and quantum effect of their nano-regimen [28]. Further, it provides a continuous flow of the electrons in the Mn NGs@GO hybrid that increases the flow of current. These results show the higher efficiency of hybrid for the electrochemical sensing of clinically important biomolecules.
3.3. Electrochemical mechanism of GSH by Mn NGs@GO hybrid

We have tried to account for a plausible mechanism to explain the high amount of current generated as the result of the oxidation of glutathione by Mn NGs@GO hybrid. Overall mechanism has been illustrated in Fig. 3D. This may involve the following steps: firstly, enhancement of the current peak due to the nucleophilic reaction of GSH, being an effective electron donor [29,30] (1, 2). The unstable oxidized forms of glutathione interact with each other to make a stable reduced species (3). These electrons react with Mn NGs@GO hybrid and this reversible reaction goes on generating both anodic and cathodic peaks in the CV response (4).

The kinetic parameters such as diffusion coefficient and number of electrons involved in the oxidation and reduction reaction are calculated theoretically using the Randles-Sevcik Eq. (6) and the Tafel plot [16].

\[
i_p = 2.65 \times 10^{3/2} AD^{1/2} C \times v^{1/2}
\]

Where, \(i_p\) is the maximum peak current (A), \(n\) is the number of electrons in the rate determining step, calculated by the Tafel plot Eq. (7), \(A\) is the electrode surface area (0.03 cm²), \(D\) is the diffusion coefficient of the analyte, \(C\) is the bulk concentration of analyte (mol L⁻¹), and \(v\) is the scan rate (Vs⁻¹). From Eq. (6), the diffusion coefficient for the oxidation of GSH is estimated as \(1.4 \times 10^{-5}\) cm² s⁻¹.

\[
b = \alpha n F / 2.303RT
\]

The calculated total number of electrons for GSH are found to be 2.1. It indicates that 2 electrons participated in the redox reaction of GSH. This data shows the fast electrochemical kinetics of Mn NGs@GO hybrid to bind GSH.

\[
\begin{align*}
\text{GSH} & \leftrightarrow \text{GS}^- + \text{H}^+ \\
\text{GS}^- & \rightarrow \text{GS} + e^- \\
2\text{GS} & \rightarrow \text{GSSH} + 2e^- + 2\text{H}^+ \\
\text{Mn} + 2e^- + 2\text{H}^+ & \leftrightarrow \text{Mn}^0
\end{align*}
\]
3.4. **Analytical performance**

The cyclic voltammograms of the Mn NGs@GO hybrid were also recorded by increasing the concentration of glutathione, under optimized conditions. A wide range of analyte concentration from 0.01 to 400 μM was selected and the respective responses are presented in Fig. 4A. It can be observed that by increasing the concentration of glutathione, the oxidation current increases gradually from 12.8 ± 0.2 μA to 182.5 ± 1.7 μA (linear regression r = 0.985). The limit of detection (LoD) was calculated as low as 7 nM at (S/N = 3) and limit of quantification (LoQ) was found to be 23 nM, which qualifies the applied utility of fabricated sensor. This remarkable sensitivity can be credited to the outstanding morphology of Mn NGs@GO hybrid and the synergistic effect of graphene oxide and manganese nanograins. These characteristics provide a greater surface area with huge electron cloud that promoted rapid electron transfer. To support our extraordinary data, we compared these values with other reports in the literature (Table 1). It manifests more satisfactory analytical performance of our designed sensor towards glutathione as compared to other nanomaterials.

To investigate enhanced discrimination of Faradaic currents of Mn NGs@GO and sensitive quantitative chemical analysis of glutathione, differential pulse voltammetry was also used. The electrocatalysis of glutathione using Mn...
NGs@GO was evaluated with the successive increase in the concentration of glutathione and the resultant DPVs are shown in Fig. 4B. There was an obvious increase in the oxidative peak current from 10.8 ± 0.2 μA to 182.5 ± 0.4 μA at 0.175 V. A calibration curve was obtained by plotting the concentration of glutathione versus peak current response and evaluated by its correlation coefficients (R² = 0.981) as shown in Fig. 4C. The calibration curve for values between 0.01 and 7 μM (R² = 0.998) is shown in the inset of Fig. 4C. It depicts that the value of current is changing gradually with the increase of analyte concentrations and is strongly co-related to the current response.

3.5. Selectivity

The electrochemical response of designed sensor was further investigated by studying its selectivity. There are many molecules such as lysine, glycine, tyrosine, ascorbic acid, leucine, glucose, histidine, cysteine, arginine and some metabolites FeCl₂, ZnSO₄, and MgCl₂ that can affect the sensor responses during the analysis of glutathione [20]. They have similar chemical structure and in biological system they co-exist with glutathione. To monitor the performance of our designed Mn NGs@GO hybrid, it was challenged with glutathione and its structural and functional analogs. The peak current intensity was recorded in the presence of glutathione and interfering agents (250 μM, each). The oxidation peak current of interfering molecules is less than 50 μA on average as compared to glutathione (Fig. 4D). The result shows that Mn NGs@GO hybrid is an efficient material for electrochemical sensing of desired analyte (glutathione) with the increased peak current at a specific potential. The hybrid shows the sensor response to other competing molecules with low current intensity at different potential that indicates it has excellent specificity to differentiate glutathione with high accuracy from its competing structural analogs (SI. Table 1). This outstanding specificity of fabricated hybrid material makes it extremely suitable to diagnose disease specific biomarkers in clinical applications.

3.6. Reproducibility

In order to test for reproducibility, 10 sensors were prepared and subjected to 350 μM glutathione under optimum conditions. The results of each individual sensor response were investigated and compiled in Fig. 5A. The results indicate that the responses are quite reproducible for the 10 sensors. Different biomolecules present in green extract play an important role to maintain the stability of Mn NGs@GO hybrid [19]. This outstanding result is attributed to the special morphology, polydispersity of manganese nanograins, and increased surface area of hybrid. These features ensure a greater number of the sites that can catalyze the analyte, providing higher

Fig. 4 – Sensor Characteristics: (A) CVs of Mn NGs@GO hybrid towards different concentrations of glutathione (0.01–400 μM) with background electrolyte NaH₂PO₄, H₂O (50 mM), scan rate of 100 mV s⁻¹; (B) Differential pulse voltammogram obtained with Mn NGs@GO hybrid towards 0.01–400 μM of glutathione at scan rate 100 mV s⁻¹; (C) The respective regression plot showing all the sensing response values are highly correlated with each other; inset shows the linear relation of 0.01–7 μM of glutathione at scan rate 100 mV s⁻¹; (D) Selectivity profile of Mn NGs@GO interface towards 250 μM concentration each, of glutathione and interfering agents.
### Table 1 - Sensor performance of already reported different nanomaterials and techniques towards recognition of glutathione.

<table>
<thead>
<tr>
<th>Nanomaterial</th>
<th>Technique</th>
<th>Linear range μM</th>
<th>LoD nM</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrocene dicarboxylic acid modified carbon nanotubes paste electrode (FDCCNTE)</td>
<td>Differential pulse voltammetry</td>
<td>0.5–24</td>
<td>200</td>
<td>[31]</td>
</tr>
<tr>
<td>AgNP(TMSPE)-rGO/GC</td>
<td>Amperometry</td>
<td>0.1–2.75</td>
<td>100</td>
<td>[32]</td>
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<tr>
<td>Multi-wall carbon nanotubes/platinum</td>
<td>Hydrodynamic voltammetry with liquid chromatography</td>
<td>0.2–100</td>
<td>29</td>
<td>[33]</td>
</tr>
<tr>
<td>Ag–ZnO nanoplates</td>
<td>Square wave voltammetry</td>
<td>500–20000</td>
<td>20</td>
<td>[34]</td>
</tr>
<tr>
<td>Pyrogallol red modified carbon nanotube paste (PGRMWCNTE)</td>
<td>Square wave voltammetry</td>
<td>0.3–500</td>
<td>190</td>
<td>[35]</td>
</tr>
<tr>
<td>Copper hydroxide composite carbon ionic liquid</td>
<td>Cyclic voltammetry</td>
<td>1–50</td>
<td>30</td>
<td>[36]</td>
</tr>
<tr>
<td>Cobalt tetrasulfonated phthalocyanine/poly(l-lysine)</td>
<td>Amperometry</td>
<td>0.5–3.5</td>
<td>80</td>
<td>[37]</td>
</tr>
<tr>
<td>Multi-wall carbon nanotubes/isoprenalinene</td>
<td>Sweep voltammetry</td>
<td>0.5–300</td>
<td>90</td>
<td>[30]</td>
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<tr>
<td>Cobalt phthalocyanine on a thioctic acid modified gold</td>
<td>Differential pulse voltammetry</td>
<td>10–100</td>
<td>5500</td>
<td>[38]</td>
</tr>
<tr>
<td>Mn NGs@GO hybrid</td>
<td>Cyclic voltammetry and differential pulse voltammetry</td>
<td>0.01–400</td>
<td>7</td>
<td>This work</td>
</tr>
</tbody>
</table>

Fig. 5 – Reproducibility and Stability Parameters: (A) The reproducibility pattern of the Mn NGs@GO sensor towards 400 μM glutathione, (B) The stability profile of Mn NGs@GO sensor towards 350 μM glutathione; reaction conditions are 50 mM NaH2PO4, H2O for all experiments as the background media; scan rate 100 mVs⁻¹.

currents and good stability of the sensor. The sensor signals with higher repeatability (98%) indicates the analytical accuracy of the sensor, strongly suggesting it can be utilized for diagnostic purposes. To investigate the stability of designed sensor, the responses were recorded towards 350 μM strength of glutathione for 10 consecutive days on the same modified interface at a 100 mVs⁻¹ scan rate. The results are shown in Fig. 5B. The current values of each response were found statistically the same, indicating good stability of Mn NGs@GO owing of the strong coherence of graphene oxide sheet with Mn NGs.

### 4. Conclusion

Our study describes in-situ formation of doughnut shaped manganese nanograins on the graphene sheets to develop interfaces to recognize biological markers. The hybrid possesses unique morphology where, 5–10 nm doughnut shaped nanograins are uniformly spread all over graphene surfaces. The addition of these nanoscale structures resulted in the greater surface area, conducting, and metallic character and thus more availability of binding sites for the binding of biomolecules. The sensor responses against glutathione are
found remarkably sensitive, as low as 7 nM. Further, the hybrid interfaces can differentiate among a range of bioanalytes, including protein and non-protein molecules. This study paves the way to designing exciting morphologies of nanomaterials which can efficiently bind clinically significant biomarkers and metabolites for disease diagnosis.

**Novelty statement**

In this study, we presented a simple and in-situ formation of doughnut shaped manganese nanograins inhabited on the layers of graphene. The grains were homogeneously populated on the surface of graphene sheets (ca. 100 grains/500 nm² ± 10). The structure of doughnut consists of a central pit of ca. 1 nm with a surrounding grain ring of less than 1 nm. The external diameter of individual grains was found to be ca. 5–10 nm with the thickness of ca. 2 nm and a small cavity can be seen in the center of every single grain. The large conducting surface area of graphene oxide sheets and catalytic activity of metal nanograins provide excellent platform for the electrochemical interaction with glutathione leading to greater sensitivity. The selective binding was assessed choosing a range of protein and non-protein molecules. This is the first report where, such interfaces are proposed for clinical diagnostics.

**Conflict of interest**

The authors declare no conflicts of interest.

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**Appendix A. Supplementary data**

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jmrt.2020.06.054.

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