Highly sensitive and selective detection of glutathione using ultrasonic aided synthesis of graphene quantum dots embedded over amine-functionalized silica nanoparticles

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ABSTRACT

Glutathione (GSH) is the most abundant antioxidant in the majority of cells and tissues; and its use as a biomarker has been known for decades. In this study, a facile electrochemical method was developed for glutathione sensing using voltammetry and amperometry analyses. In this study, a novel glassy carbon electrode composed of graphene quantum dots (GQDs) embedded on amine-functionalized silica nanoparticles (SiNPs) was synthesized. GQDs embedded on amine-functionalized SiNPs were physical-chemically characterized by different techniques that included high resolution-transmission electron microscopy (HR-TEM), X-ray diffraction spectroscopy (XRD), UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and Raman spectroscopy. The newly developed electrode exhibits a good response to glutathione with a wide linear range (0.5–7 µM) and a low detection limit (0.5 µM) with high sensitivity (2.64 µA µM⁻¹). The fabricated GQDs-SiNPs/GC electrode shows highly attractive electrocatalytic activity towards glutathione detection in the neutral media at low potential due to a synergistic surface effect caused by the incorporation of GQDs over SiNPs. It leads to higher surface area and conductivity, improving electron transfer and promoting redox reactions. Besides, it provides outstanding selectivity, reproducibility, long-term stability, and can be used in the presence of interferences typically found in real sample analysis.

1. Introduction

In living organisms, the thiol group is part of critical components present in many redox-mediated or regulated processes in the cells. It participates in different metabolic processes, protein synthesis, cofactors for thiol-dependent enzymes, and also it plays a part in antioxidants [1]. The unique chemistry of organothiol and thiolate groups provides functional bases with specific properties (e.g., high-affinity metal binding, ability to form disulfide bonds, and high nucleophilic strength). Biological organisms produce millimolar concentrations of distinct low-molecular-weight (LMW) thiols [2,3]. Among them, the most abundant is the tripeptide γ-L-glutamyl-L-cysteinyl-glycine, glutathione (GSH) found in human tissues, particularly, in liver tissues in the range of 0.5 to 10 mM, which exists in both reduced (GSH) and oxidized (GSSG) states. Changes in their ratio are often associated with a variety of diseases, such as stress, aging, cancer, cystic fibrosis, diabetes, neurodegenerative diseases, and HIV. Thus, glutathione becomes a vital molecule in maintaining proper biological functions such as protecting cells from oxidative damage, protein, and DNA synthesis detoxification metabolism, among other components responsible for staying healthy [4–6].

Based on this biomedical relevance, the accurate, selective detection and quantification of GSH has been an area of interest in the past decades. Regarding this, some analytical methods such as high-performance liquid chromatography, capillary electrophoresis, and...
titrimetry have been investigated together with other detection systems based on photometric and fluorimetric [7]. However, such methods result to be time-consuming, being expensive, less selective, and less sensitive [8]. Electrochemical methods appear as an alternative technique for in vitro analysis of GSH, due to its simplicity, cost-effectiveness, minimal sample pretreatment, rapidity, highly sensitive, and simple experimental approach [9]. The improvement in electrochemical detection of GSH has become effective by using chemically modified electrodes possessing a high electron transfer rate from the substrate to the electrode [10].

Among various materials used for electrode modifications, silica nanoparticles are a promising material in electrochemical biosensing. They occupy an important place in scientific research due to their facile synthesis procedure and extensive applications in various fields including medical, pharmaceutical, catalysis, and sensor applications [11,12]. Moreover, the presence of ammonia as part of a catalyst has been reported as a precursor in the synthesis of spherical and monodisperse silica nanoparticles from an aqueous alcohol solution of silicon alkoxides. Additionally, various methods have been employed in the preparation of silica nanoparticles, including sonoochemical, microemulsion, and Stober methods [13–15] with different sizes and morphology [16–18]. In recent years, the sonochemical method has become an important way to synthesize novel nano-sized materials under ambient conditions. However, nano-sized silica materials are known to be toxic, environmental hazard, and their chemical instability may limit their use for sensing biomolecules.

In order to overcome such limitations, different kinds of nano-materials, having good stability, large surface area, and remarkable electrical conductivity, have been embedded over nano-sized silica materials, enhancing its catalytic performance. Among those materials, carbon-based nanomaterials (carbon nanotubes (CNTs) [19], graphene (sheets and quantum dots (QDs)) [20,21], carbon nanofibers (CNF) [22], and carbon aerosols [23] have been used because of their large surface area, distinctive structure, exceptional mechanical properties, high electrical conductivity, and uniform particle size distribution [24,25]. In particular, zero-dimensional (0D) GQDs (having lateral dimensions of 3 to less than 10 nm) with edge effects, electron and quantum confinement behavior have become an attractive material used in biosensors, bioimaging, photovoltaic and energy-saving devices [26–32]. Furthermore, GQDs embedded over amine-functionalized silica nanoparticles have been proved to enhance electrocatalytic performance in comparison to bare GQDs due to an increase in the amount of active sites with better chemical interaction.

In this research work, an electrochemical sensor based on graphene quantum dots embedded over silica nanoparticles (GQDs-SiNPs/GC) is developed via an eco-friendly ultrasound-assisted process in view to detect GSH in real samples. Synthesized GQDs-SiNPs nanocomposite is characterized using high resolution-transmission electron microscopy, X-ray diffraction spectroscopy, UV–visible spectroscopy, Fourier transform infrared spectroscopy and Raman spectroscopy. Moreover, the electrochemical performance of the glassy carbon electrode modified with GQDs-SiNPs is assessed with voltammetry and amperometry analyses. Finally, the response of the newly developed electrode is evaluated in the presence of biological interferents, such as glucose, urea, dopamine, and ascorbic acid. As a result stability, selectivity, sensitivity, and detection limit are identified.

2. Experimental procedures

2.1. Materials

Tetraethyl orthosilicate (TEOS), (3-Aminopropyl) triethoxysilane (APTS), citric acid (CA), L-ascorbic acid (AA), dopamine hydrochloride (DA), urea, glucose, potassium ferricyanide (K₃[Fe(CN)₆]), ammonium hydroxide (NH₄OH), ethanol, and Nanion (5 wt%, Fluka) were purchased from Sigma-Aldrich. L-glutathione (GSH) and sodium hydroxide (NaOH) were purchased from Alfa Aesar. Mono and disodium phosphate (99%) were obtained from Merck. All the chemicals utilized were of analytical grade and were used without any further purification. All solutions were prepared with deionized water of resistivity not less than 18.2 MΩ cm⁻¹.

2.2. Synthesis methods

In this study, an effortless and eco-friendly process was used to synthesis GQDs-SiNPs through sonochemical technique. Fig. 1 shows a schematic illustration of the different preparation steps used during the synthesis of GQDs-SiNPs nanocomposite. In the first step, citric acid was reduced by an incomplete pyrolysis method to prepare GQDs. Then, silica nanoparticles were obtained in an ethanol medium with tetraethyl orthosilicate (TEOS) by hydrolysis. Subsequently, silica nanoparticles were functionalized with (3-Aminopropyl) triethoxysilane (APTS), generating a catalyst with a high surface area. Finally, a composite material was prepared by mixing GQDs with amine-functionalized SiNPs using ultrasonication. It is well known that chemical synthesis assisted by ultrasonication reduces the reaction time and the amount of chemicals used. Moreover, ultrasonication has been proved that contribute to the formation of nanoparticles with different morphology. Furthermore, ultrasound disruption is more energy-efficient and can achieve a higher degree of powder fragmentation, at constant specific energy than other conventional dispersion techniques [33].

2.2.1. Synthesis of graphene quantum dots

About 2 g of CA were taken in a small beaker and kept in an oil bath at 160 °C, melting of CA in 5 min. Subsequently, the color of the liquid changed from colorless to pale yellow, followed by pale orange in 30 min. The obtained orange-colored solution was immediately added dropwise into 120 mL of 10 mg mL⁻¹ NaOH under vigorous stirring, resulting in an aqueous solution of GQDs [34].

2.2.2. Synthesis of graphene quantum dots embedded on SiNPs (GQDs-SiNPs) nanocomposites

After 10 min of sonication, TEOS was introduced into a mixture of ethanol/water (1:1 v/v). Then after 20 min, a solution of aqueous ammonium hydroxide (14.53 M) was dropped as a catalyst to stimulate the condensation, and sonication was continued for another 60 min at room temperature to obtain a white suspension [35,36]. After that, the prepared solution was sonicated with 0.05 M of APTS (1:0.5 v/v), leading to the formation of SiNPs. The formed SiNPs were washed and separated with ethanol by centrifugation for 20 min at 6000 rpm.

Finally, the GQDs-SiNPs nanocomposites were synthesized under ultrasonication at room temperature for 1 h by mixing volumes of SiNPs and GQDs previously prepared by ultrasonication as described above, using a 1:0.5 ratio. Ultrasonic irradiation was accomplished at 50 W and 38 kHz (Kaijo 30,110 (QR-003) type lot. No. SM146009, Oscillator 30,301 type). After the synthesis procedure, the obtained sample was directly used for characterization assays and electrochemical applications. In this case, neither separation nor a drying process were applied.

2.3. Characterization of synthesized materials

High-resolution transmission electron microscopy (HR-TEM) was carried out in FEI Tecnai 20 G2. The crystalline phases of the samples were recognized by X-ray diffraction (XRD) analysis in a Bruker AXS D4 Endeavor using Cu-Kα radiation. Raman spectra were obtained on a Lab RAM HR Evolution, Horiba Raman Spectrometer. A spectrod 600S diode array UV–Visible spectrophotometer (Jena, Germany) was used to record ultraviolet–visible (UV–Vis) absorption spectra. Infrared spectra were obtained in a Thermo Scientific Nicolet iS5 FT-IR spectrometer (Crawley, Perth).
2.4. Electrochemical procedure for glutathione detection using modified GC electrode with GQDs, SiNPs, and GQDs-SiNPs

Electrochemical detection of glutathione was made by a conventional three-electrode system with the assistance of the CHI 650C electrochemical workstation (Austin, TX, USA). The system is comprised of glassy carbon (GC) as the working electrode, a platinum wire as the counter electrode, and Ag/AgCl saturated with KCl, as the reference electrode. Measurements were carried out in a phosphate buffer solution (PBS), at pH 7.0 and room temperature. Before analysis, the GC working electrode (3 mm diameter) was polished with an alumina suspension (α-Al₂O₃), with particle sizes of 1, 0.3, and 0.05 μm. Then, it was washed with double distilled water. After that, three different modified GC electrodes were prepared. For that, volumes of 10 μL of each synthesized GQDs, SiNPs, GQDs-SiNPs were drop-cast over bare GC electrodes and then dried overnight at room temperature. Finally, 2 μL of a Nafion suspension was used as a binder that was dropped over each modified GC and permitted to dry before electrochemical measurements.

2.5. Real sample preparation

Human blood samples were used for glutathione measurements. In blood samples, erythrocytes were separated from the serum by centrifugation at 6000 rpm for 10 min. Then, the erythrocytes were gathered and diluting with distilled water (1:2) for haemolyzed. In this regard, 50 μL of the blood sample was diluted to 5 mL by adding PBS (pH 7.0). Subsequently, evaluations of blood samples were carried out using the standard addition method using the modified GC electrode with GQDs-SiNPs.

In addition, commercially available GSH tablets (having a labeled value of 500 mg GSH/tablet) were used for real GSH detection. GSH tablets were supplied by a permitted medical center in India. The main
components of the tablet besides GSH are ethylcellulose, diethyl phthalate, lactose monohydrate, croscarmellose sodium, polyethylene glycol, colloidal silicon dioxide, magnesium stearate, triacetin, hydroxypropyl methylcellulose, and sodium starch glycolate. Tablets were dissolving in water by ultrasonication. Thus, 10 mL of a 0.1 M aqueous solution of GSH was prepared as stock solution. Then, a required amount of GSH solution was added to an electrochemical container consisting of 0.1 M of PBS (pH 7.0) for GSH detection.

Spiked sample solutions were analyzed according to the proposed analytical procedure and the recovery percentage was calculated, as follow:

\[
Recovery(\%) = \left( \frac{S_{\text{Spiked}} - R_{\text{Real}}}{S_{\text{Spiked}}} \right) \times 100
\]

where \( S_{\text{Spiked}} \) represents the calculated analyte concentration in the spiked sample (\( \mu g/mL \)) and \( R_{\text{Real}} \) stands for the concentration of the analyte in the real sample solution (\( \mu g/mL \)).

3. Results and discussion

3.1. Characterization of synthesized materials

The size distribution and morphology of as-prepared amino-functionalized GQDs, SiNPs, and GQDs-SiNPs were primarily studied using TEM, selected area electron diffraction (SAED) energy dispersive X-Ray (EDX) analyses, and the results are shown in Fig. S1 & S2 (in supporting information). Fig. S1 (A-B) shows TEM images of GQDs, with diameters of 3–7 nm, which are densely and consistently dispersed. HR-TEM images reveal localized graphene lattice fringes with an in-plane lattice parameter of ~ 0.24 nm (Fig. S1-C) that could be attributed to the (1120) plane [37]. SAED pattern for GQDs exhibited in Fig. S1(D), shows a bright circular ring, confirming the crystallinity of the prepared GQDs. TEM images of the amino-functionalized silica nanoparticles depict spherical structures with uniform particle sizes in the range of 30–40 nm (Fig. S2 A-B). Preliminary experiments indicated the need for the pre-modification of silica with APTS to contribute to the immobilization of GQDs over the SiNPs surface [38,39]. EDX analysis (Fig. S2C) shows the presence of Si (silicon) and O (oxygen), where the presence of oxygen suggests that silicon is in the form of amorphous silica (SiOx). In Fig. S2D, the SAED pattern of SiNPs shows only diffraction rings without any diffraction dots, confirming that the structures of SiNPs have an amorphous nature.

TEM images of GQDs-SiNPs are shown in Fig. 2 (A and B). As expected, there is a subtle increase in particle size due to the presence of GQDs with an average particle size of 140–150 nm. Results of energy-dispersive X-ray (EDX) spectroscopy analysis are displayed in Fig. 2 (C). Elements such as Si, C, and O are detected in the prepared GQDs-SiNPs (Fig. 2(C)). Moreover, the SAED pattern of GQDs on silica crystal structure is confirmed as shown in Fig. 2(D). Peptide bonds (–CO-NH–) could be formed by the interaction of –NH2 from SiNPs with –COOH groups of GQDs, being responsible for the observed embedding of GQDs over SiNPs.

The optical properties of pristine and composite materials are displayed in Fig. 3, where (a), (b), and (c) stand for GQDs, SiNPs, and GQDs-SiNPs, respectively. UV–Vis absorption spectrum in Fig. 3a shows strong absorption peaks at around 230 and 280 nm that could be due to \( \pi-\pi^* \) and \( n-\pi^* \) transition of C=C and C=O bond, respectively, evidencing the formation of GQDs [40]. In Fig. 3b, a characteristic absorption peak at 288 nm is observed for the colloidal solution, proving the successful formation of SiNPs (Eg ~ 11 eV) with a uniform size [41]. In the case of the GQDs-SiNPs, an absorption peak at 230 nm is registered (Fig. 3c). Such absorption peak could be assigned to \( \pi-\pi^* \) transition of aromatic rings. Moreover, the weaker peak observed at 288 nm corresponds to the embedding of GQDs molecules over the surface of SiNPs.

Fig. 2. Different scale magnification for TEM images of (A & B) GQDs-SiNPs. (C) EDX spectra and (D) patterns of selected area electron diffraction (SAED) for GQDs-SiNPs.
Fig. 4. FT-IR spectra of (a) pristine GQDs, (b) pristine SiNPs, and (c) GQDs-SiNPs.

[42]. Additionally, the disappearance of the shoulder peak seen at 280 nm indicates that the functional groups were reduced by reaction with silane moieties [43]. Such results are in agreement with those obtained by TEM analysis, confirming the formation of nanoparticles through applied sonochemical synthesis method.

Fourier transform infrared (FT-IR) spectroscopy analyses contribute to reveal the chemical interactions that take place between GQDs and SiNPs in the formation of GQDs-SiNPs (Fig. 4). Fig. 4(a) depicts the IR spectrum of GQDs, IR bands observed at 1727 and 1565 cm\(^{-1}\) could be related to the stretching vibrations of \(\text{C} = \text{O}\) and \(\text{C} = \text{C}\) groups, respectively, being the core part of GQDs. The IR bands found at 3445, 1378 and 1093 cm\(^{-1}\) for GQDs could emerge from the high concentration of \(\text{OH}\), \(\text{C-H}\) and \(\text{C-O}\) groups, respectively [44]. In Fig. 4b, the observed broadband at \(\sim 3455\) cm\(^{-1}\) could correspond to the \(\text{N-H}\) stretching vibration in the amine-functionalized silica nanoparticles [40]. The registered bands at 1112, 1084 cm\(^{-1}\) could be attributed to the siloxane groups (\(-\text{Si-O-Si}\)). Moreover, the characteristic band that appears at 940 cm\(^{-1}\) could be responsible for silanol groups (\(-\text{Si-OH}\)). In Fig. 4c, broad IR bands are noticed in the range of 3400–3500 cm\(^{-1}\) that could be ascribed to secondary amine bonds coming from the formation of GQDs-SiNPs. The observed band reduction and broadening at 940 cm\(^{-1}\) could be due to the intramolecular hydrogen vibration within the silanol groups [45]. As it can be seen, the intensities of the IR band related to \(-\text{Si-O-Si}\) and \(-\text{Si-OH}\) are reduced significantly, after the interaction of amine-functionalized silica nanoparticles with GQDs in the GQDs-SiNPs [46]. Furthermore, the observed band shifts at 1634, 1384, and 561 cm\(^{-1}\) could be attributed to the formation of an amide functional group \(\text{C} = \text{ONH}\) (identified here as \(\text{C} = \text{O}\), \(\text{C-N}\) stretching, and \(-\text{N-H}\) wagging vibrations) coming due to the reaction between the aminosilane of SiNPs and carboxylic acids of GQDs in the GQDs-SiNPs [47].

The phase composition and crystallinity of the synthesized materials were determined by XRD analysis. XRD patterns of GQDs (a), SiNPs (b), and GQDs-SiNPs (c) are shown in Fig. 5. The XRD pattern of the pristine GQD (a) reveals a broad diffraction peak at 28.4° (JCPDS: 00-001-0640), corresponding to the (002) plane [48,49]. The diffraction peaks of the pristine SiNPs (b) and GQDs-SiNPs (c) appear at 28.4°, 47.3°, 56.1°, 69.1°, and 76.4°, corresponding to (111), (220), (311), (400) and (331) planes, respectively; which are clearly indexed with the silica phase and well-matched with JCPDS 27–1402 [50,51]. In addition, a characteristic peak is observed at 26.5°, which confirms the existence of GQDs embedded on the surface of SiNPs in the formed GQDs-SiNPs (c). Moreover, there are no other peaks found in the XRD spectrum of GQDs-SiNPs that could be related to any impurity, which suggests that the synthesized material is highly pure.

Raman spectra of GQDs (a) and GQDs-SiNPs (b) are presented in Fig. 6. As it can be seen, there are two major peaks observed at 1363 and 1597 cm\(^{-1}\), corresponding to the D and G bands, respectively. The D-band is known as a disordered band emerging from the disorder in sp\(^2\) hybridized carbon, whereas the G band is correlated with sp\(^2\) carbon that domains the first-order scattering of the E\(_{2g}\) stretching vibration mode. The intensity ratio (I\(_D\)/I\(_G\)) is a factor for assessing the degree of graphitization of carbon-based materials and the density of defects in graphene-based materials [52]. Comparing the I\(_D\)/I\(_G\) intensity ratios of pristine GQDs (1.07) with GQDs-SiNPs (0.70), a decrease is observed after embedding GQDs on the surface of SiNPs. Such changes could be due to the variation in the size of the in-plane sp\(^2\) region that is increased notably, while the intensity of the D-band decreases as the degree of carbonization increases [53]. In addition, contrasting the FT-IR spectra (Fig. 4) of GQDs and GQDs-SiNPs, it can be seen that there are some oxygen-containing groups from the GQDs within the GQDs-SiNPs, including carbonyl, epoxy, and alkoxy groups; however, the content of
these groups are much less than in the pristine GQDs, as expected [54].

3.2. Electrocatalytic oxidation of glutathione

Fig. 7 displays electrochemical activities of the bare GC (a), GQDs/GC (b), SiNPs/GC (c), and GQDs-SiNPs/GC (d) electrodes, obtained by cyclic voltammetry (CV) studies, using 5.0 mM [Fe(CN)$_6$]$^{3-/4-}$ in a 0.1 M KCl electrolytic solution at a scan rate of 50 mVs$^{-1}$. As shown in Fig. 7a, the separation of bare GC peak potential ($\Delta$Ep) is as large as 112 mV and the shapes of redox peaks are moderately broad. After modifying the surface of GC with GQDs and SiNPs, the current peaks increase compared to the bare electrode; whereas the separations of the peaks ($\Delta$Ep) have not significant changes (Fig. 7 b-c). In the case of GQDs-SiNPs modified GC (Fig. 7d), the anodic and cathodic peaks’ currents increase highly, accompanying a considerable reduction in peak-to-peak separation. Such results indicate that the GQDs-SiNPs can significantly accelerate the electron and mass transfer processes, due to the incorporation of GQDs over SiNPs in the synthesized GQDs-SiNPs material, which provides an additional conduction path due to the increase in the surface area.

In Fig. 8 a-d, CV curves of the bare/GC (a), GQDs/GC (b), SiNPs/GC (c), GQDs-SiNPs/GC (d) electrodes were recorded over a potential range between –1.4 V to 0.6 V at a scan rate of 50 mVs$^{-1}$ in 0.1 M phosphate-buffered saline (PBS) solution containing 0.5 mM GSH. In the case of the bare GC electrode, the oxidation of the GSH does not take place across the studied potential window, maybe due to the slow kinetics of the electron transfer. Voltammograms results of GQDs/GC and SiNPs/GC electrodes shown in Fig. 8 (b and c) exhibit a small broad anodic peak potential and peak current at about +0.15 V. Moreover, reduction peaks are observed at about –0.75 and –0.63 V, respectively. These results reveal that GQDs/GC and SiNPs/GC electrodes offer several active sites to enhance effectively the transfer rate of electrons between the electrodes and analytes compared to the bare/GC electrode. This experimental evidence indicates that GQDs and SiNPs display a crucial factor in the oxidation of GSH using the modified electrodes. Likewise, the GQDs-SiNPs/GC (d) shows the appearance of the oxidation peak at 0.25 V that is related to the arrival of the reduction peak at –0.8 V (vs. Ag/AgCl) in 0.1 M PBS (pH = 7), containing 0.5 mM GSH, during the reverse scan. In the case of the reduction peak observed at around –0.8 V, it is associated with a successive reduction of disulfide (GSSG) [55]. Compared to other electrodes the values of CV peak currents at the GQDs-SiNPs/GC electrode are 3.98, 4.84, and 8.10 folds higher than that at the SiNPs/GC, GQDs/GC, and bare GC electrodes, respectively. This indicates that the electrocatalytic performance of SiNPs has been enhanced due to the incorporation of GQDs, increasing not only the specific surface area but also the electric conductivity, resulting in better detection of GSH. Therefore, the observed improvement in the electrode performance comes as a result of the distinctive and integrated properties of SiNPs and GQDs in the synthesized GQDs-SiNPs.

Fig. 9 compares the current response of bare GC (A), GQDs/GC (B), SiNPs/GC (C), and GQDs-SiNPs/GC (D) electrodes in the presence and the absence of 0.5 mM GSH in a PBS solution (0.1 M) at a scan rate of 50 mVs$^{-1}$. For the bare GC electrode (Fig. 9 A), in the presence and the absence of GSH, a similar response is noticed and there are no peaks found for electro-oxidation of GSH. This indicates that the bare GC electrode has insufficient electrocatalytic activity towards GSH oxidation. In the absence of GSH, a negligible amount of redox peaks are observed when all modified GC electrodes GQDs/GC (Fig. 9B), SiNPs/GC (Fig. 9C), and GQDs-SiNPs/GC (Fig. 9D) are used. In the case when GSH is present, GQDs/GC (Fig. 9B) and SiNPs/GC (Fig. 9C) do not show any proper oxidation peaks but reduction peaks are observed at around

![Fig. 6. Raman spectra of pristine GQDs (a), and GQDs-SiNPs (b).](image)

![Fig. 7. Evaluation of modified electrode performances using CV studies in 5.0 mM [Fe(CN)$_6$]$^{3-/4-}$ containing 0.1 M KCl with a scan rate of 50 mVs$^{-1}$: (a) Bare/GC, (b) GQDs/GC, (c) SiNPs/GC, and (d) GQDs-SiNPs/GC.](image)

![Fig. 8. Evaluation of GSH oxidation with different modified electrodes using CV studies in 0.1 M PBS (pH 7.0) containing 0.5 mM GSH with a scan rate of 50 mVs$^{-1}$: (a) Bare/GC, (b) GQDs/GC, (c) SiNPs/GC, and (d) GQDs-SiNPs/GC.](image)
As for fabricated GQDs-SiNPs/GC (Fig. 9D), a sharp oxidation peak (0.25 V) is found in the presence of GSH and it is related to the appearance of the reduction peak at 0.75 V. Such reduction peak is associated with a successive reduction of disulfide. The GQDs-SiNPs/GC electrode exhibits a typical electrooxidation response to GSH, suggesting that this nanocomposite material has good electrocatalytic ability towards GSH sensing. The performance of GQDs-SiNPs electrode is enhanced by the synergistic surface effect caused by the incorporation of GQDs over SiNPs, being this a key factor in accelerating the electron transfer and promoting redox reactions.

Additionally, GSH sensing using bare GC (a), GQDs/GC (b), SiNPs/GC (c) and GQDs-SiNPs/GC (d) electrodes, were assessed by differential pulse voltammograms (DPV) and results are depicted in Fig. 10. Detection was conducted in 0.1 M of PBS solution (pH 7.0), containing 0.5 mM of GSH at a scan rate of 50 mVs⁻¹. On the bare GC (a) electrode, a small background current is observed, indicating poor electrocatalytic activity towards GSH. After the modification of GQDs (b) and SiNPs (c) on the GC electrode surface, broad peaks are obtained at potential values of 0.10 and 0.06 V, respectively, and peak currents increase in comparison to the bare GC electrode (a), due to an increase in the promotion of electron transfer by GQDs (b) and SiNPs (c). In the case of the use of GQDs-SiNPs/GC electrode, it shows a further negative shift (-0.12 V) in the value of anodic peak potential as well as in the value of the peak current that increases greatly towards oxidation of GSH compared to the other three electrodes. The observed increase in the value of peak current towards negative shift can be taken as evidence of the increase in
the electron transfer rate during GSH sensing, which signifies good detection activity towards GSH.

The effect of the scan rate value, ranging from 10 to 200 mV s\(^{-1}\), on GSH detection using GQDs-SiNPs/GC electrode was followed by CV analyses and results are displayed in Fig. 11. Detection was done using a solution of GSH (0.5 mM) dissolved in PBS (0.1 M pH 7.0). As it can be seen, the resulting redox peak currents increase linearly with the increase of the scan rate. Redox peak currents are linearly proportional to the square root of scan rate (\(v^{1/2}\)) in the studied range, as indicated by the inserted graph in Fig. 11. Linear regression equations for the anodic and cathodic regions were obtained: \(y = 3.3699x - 7.4795\), \(R^2 = 0.9912\) and \(y = -3.844x - 9.7326\) (\(R^2 = 0.9903\)), respectively. Such linear relationships confirm that electro-oxidation/reduction of GSH at the GQDs-SiNPs/GC is a surface-controlled process, as it is represented by equations (2)–(4).

\[
\text{GSH} \rightarrow \text{GS}^- + \text{H}^+ \quad (2)
\]

\[
\text{GS}^- \rightarrow \text{GS} + e^- \quad (3)
\]

\[
2\text{GS} \rightarrow \text{GS}_2^- \quad (4)
\]

Electrode performances (bare/GC (a), GQDs/GC (b), SiNPs/GC (c), GQDs-SiNPs/GC (d)) were also evaluated using amperometric analyses during successive addition of GSH in a solution of PBS (0.1 M, pH 7.0) (see Fig. 12). In Fig. 12A, the amperometric current–time (i-t) response in 0.1 M PBS solution was examined by consecutively adding the GSH with continuous stirring, whereas the applied potential of modified electrodes was kept at 0.25 V vs. Ag/AgCl. When successive amounts of GSH are added to the PBS solution, small current responses are observed upon the addition of GSH at the bare GC (a), GQDs/GC (b) and SiNPs/GC (c) electrodes. On the contrary, in the case of the use of GQDs-SiNPs/GC, it is noticed a prompt reaction with the successive addition of the analyte. As a result, abruptly increased responses in the current values are detected. Results shown in Fig. 12B represent the resultant linear plots of the corresponding current vs concentration of the analyte. Such experimental evidence demonstrate that the SiNPs-GQD/GC has an increased sensitivity of around 16, 12 and 10 fold compared to bare/GC, GQDs/GC, SiNPs/GC electrodes, respectively. The amperometric results of SiNPs-GQD/GC electrode show a linear range between 0.5 and 7 \(\mu\)M, having a linear regression equation defined by \(I_{\text{GSH}} = 2.6495C_{\text{GSH}} - 0.7595\), with a correlation coefficient of \(R^2 = 0.9916\), sensitivity of 2.64 \(\mu\)A \(\mu\)M\(^{-1}\), and a detection limit of 0.5 \(\mu\)M. Such results indicate that the modified GQDs-SiNPs/GC electrode is appropriate for the electrochemical determination of glutathione. A comparison of detection limit using GQDs-SiNPs/GC electrode to various modified electrodes using the amperometric method previously reported in the literature is summarized in Table S1.

For sensor applications, selectivity is one of the desirable essential characteristics. Fig. 13 exhibits the amperometric responses of the newly developed GQDs-SiNPs/GC electrode during GSH detection in the presence of typical interferent molecules. Ascorbic acid (AA), dopamine (DA), glucose and urea were used here as the main interferents analytes. Due to the very low oxidation potential (applied potential: \(+0.25\) V), there is not any distinguished current response while adding the common blood interferents molecules as AA, DA, glucose and urea in 0.1 M PBS. In contrast, after the addition of GSH in the presence of some common interferent species, the current response is rapidly increased, indicating an outstanding selectivity of GQDs-SiNPs/GC electrodes for GSH sensing. Consequently, the anti-interference performance suggests that the newly developed GQDs-SiNPs/GC electrode could be used as a promising tool for the detection of GSH in biological fluids.

3.3. Stability, reproducibility and repeatability

The long-term stability of the GQDs-SiNPs/GC electrode was determined using CV measurements by following the current response of the electrode during the detection of GSH. Detections were conducted using a solution of GSH (0.5 mM) dissolved in PBS (0.1 M pH 7.0). After one month of daily sensing of GSH, the peak current response for the GQDs-SiNPs/GC electrode remains 90.8% of its original value. Such results may be due to the chemical stability of SiNPs and GQDs in the modified GQDs-SiNPs/GC electrode. In the reproducibility tests, the variation coefficient of the peak currents of CV curves was performed in the PBS solution (pH 7.0) containing 0.1 mM of GSH. For five repeated measurements, the calculated RSD result to be 1.3% (n = 5). The repeatability of the GQDs-SiNPs/GC electrode was examined by measuring the response to 0.1 mM GSH in 0.1 M PBS (pH 7.0); a value of 3.5% was obtained for the RSD with 5 successive measurements.

3.4. Real sample analysis

Results of real detection of glutathione in blood samples and commercial tablets using the newly developed GQDs-SiNPs/GC electrode are listed in Tables S2 and S3, respectively. Good recovery values were obtained for all the tested concentrations. Recovery values ranged from 97 to 99.2% for blood samples, and from 98.5 to 100.5% for the pharmaceutical tablet with a relative standard deviation (RSD (n = 3)) less than 5%. Such experimental evidence confirm the ability of the GQDs-SiNPs/GC electrode as a suitable electrochemical sensor to determine GSH with high accuracy, and good selectivity in real samples even in the presence of interferents.

4. Conclusions

A novel modified glassy carbon electrode comprised of graphene quantum dots embedded over amine-functionalized silica nanoparticles (GQDs-SiNPs/GC) was successfully prepared and used in the detection of glutathione. The fabricated GQDs-SiNPs/GC electrode exhibits very attractive electrocatalytic activity towards glutathione sensing in neutral media at low potential due to a synergistic surface effect caused by the incorporation of GQDs over SiNPs that leads to the generation of a GQDs-SiNPs/GC electrode with a higher conductivity and surface area, enhancing electron transfer and promoting redox reactions. Experimental results show that the GQDs-SiNPs/GC electrode has high electrocatalytic activity and excellent analytical properties such as wide linearity, high sensitivity, and great selectivity towards glutathione.
sensing. The GQDs-SiNPs/GC electrode provides better electrocatalytic performance for GSH detection than mono-counter modified GC electrodes made of GQDs/GC and SiNPs/GC. Furthermore, the prepared electrochemical sensor displays remarkable stability, superior reproducibility, good repeatability even in the presence of common interferents coexisting biomolecules, such as: glucose, urea, dopamine, and ascorbic acid. The newly developed GQDs-SiNPs/GC electrode proves its practical compatibility and high recovery yield in the detection of glutathione in real sample analysis.

CRediT authorship contribution statement

Reshma Kaimal: Investigation, Data curation, Writing – original draft. Victor Vinoth: Investigation, Validation, Writing – original draft. Amol Shrikrishna Salunke: Formal analysis, Data curation. Hector Valdés: Supervision, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2021.105868.

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Fig. 12. (A) Comparison of the different electrode performances during successive addition of GSH in 0.1 M PBS (pH 7.0) at different concentrations using amperometric analyses (i-t): (a) Bare/GC, (b) GQDs/GC, (c) SiNPs/GC, and (d) GQDs-SiNPs/GC; applied potential of + 0.25 V. (B) Calibration curve of i-t current response (I_p) as a function of the concentration of GSH.

Fig. 13. Evaluation of the effect of different interfering substances during GSH sensing in 0.1 M PBS at pH 7.0 by amperometric analysis, using the new developed GQDs-SiNPs/GC electrode: applied potential (+0.25 V).
