

Fabrication of GQD-Electrodeposited Screen-Printed Carbon Electrodes for the Detection of the CRP Biomarker

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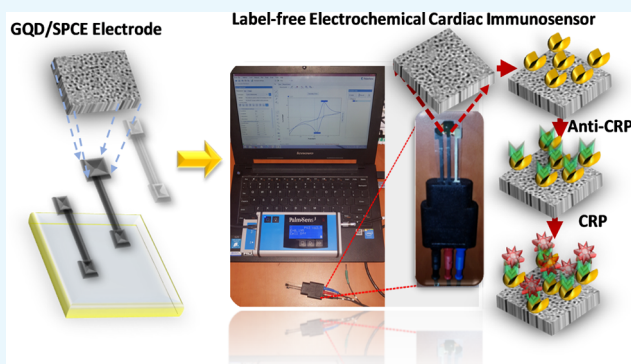
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Supporting Information

ABSTRACT: The traditional three-electrode electrochemical system used in the development of biosensors for detecting various biomarkers of interest necessitates the use of bulk electrodes, which precludes the deployment of handheld electrochemical devices in clinical applications. Affordable screen-printed carbon electrodes (SPCEs) modified with functional interfaces are being developed to enhance the sensitivity of a compact sensing system as a whole. In this work, SPCEs were fabricated on an overhead projection (OHP) sheet in three different active areas of 2×2 , 3×3 , and 4×4 mm² using a screen printing technique, and then ~ 2 nm sized graphene quantum dots (GQDs) were electrodeposited over the SPCE surface to add functionality for the detection of ultralow levels of one of the cardiac biomarkers, C-reactive protein (CRP).

The proposed mediator-dependent voltammetric biosensor exhibited good sensitivity, a low detection limit, and a linear range of $2.45 \mu\text{A ng}^{-1} \text{mL}^{-1} \text{cm}^{-2}$, 0.036 ng mL^{-1} , and $0.5\text{--}10 \text{ ng mL}^{-1}$, respectively. The fabricated SPCE/GQDs/anti-CRP biosensor could rapidly detect CRP in less than 25 s. The intra- and interassays were performed with five sensor strips, which showed a minimum standard deviation of 1.85 and 2.8%, respectively. The SPCE/GQDs/anti-CRP electrode was used to detect CRP concentrations in a ringer lactate solution. Thus, the developed biosensor has all of the characteristics such as rapidity, inexpensive disposable electrodes, miniaturization, and a lower detection limit needed to evolve as a point-of-care (PoC) application.



1. INTRODUCTION

Over the past few decades, much attention has been paid to the risk assessment of cardiovascular diseases (CVDs) using C-reactive protein (CRP) as a standard inflammation biomarker. CRP has been linked to stroke, incident myocardial infarction, peripheral arterial diseases, atherosclerosis, and sudden cardiac death in several studies.¹ CRP concentration levels in the range of <1 , $1\text{--}3$, and $3\text{--}10 \text{ mg L}^{-1}$ indicate a low, moderate, and high risk for developing CVDs, respectively.^{2,3} So, monitoring CRP at ultralow levels is critical for assessing the risk level and saving lives. Conventionally, the levels of CRP are measured using a microplate reader,⁴ enzyme-linked immunosorbent assay (ELISA), turbidimetric method,⁵ and nephelometric technology.⁶ However, the aforementioned techniques have some drawbacks, such as the requirement of trained individuals, well-established laboratories, and higher costs. In an effort to overcome these limitations, piezoelectric microcantilevers,⁷ microfluidics,⁸ quartz crystal microbalance,⁹ surface plasmon resonance (SPR),^{10,11} and electrochemical methods¹² have been widely used. Although these techniques achieve high sensitivity, the fabrication steps and analysis of CRP samples have different levels of complexity.

Among them, the electrochemical technique has sparked a lot of interest due to its numerous advantages, such as sensitivity, rapidity, small sample volume, repeatability, and so on.^{13,14} At the same time, large background signals and nonspecificity are considered as its limitations. In this background, it is critical to construct a label-free electrochemical immunosensor to meet the most appropriate sensing technique for CRP detection. Also, it is highly desirable to use a redox mediator to determine the difference in the current response of an anti-CRP-modified electrode with and without CRP. The formation of an immunocomplex on the electrode can be tracked with the help of an external redox indicator, and one such widely used molecule is potassium ferrocyanide.¹⁵ Further, it eliminates the need for secondary antibodies and labeling, reduces fabrication complexity, is highly immune to conjugated biomarkers, and has the most efficient antibody–

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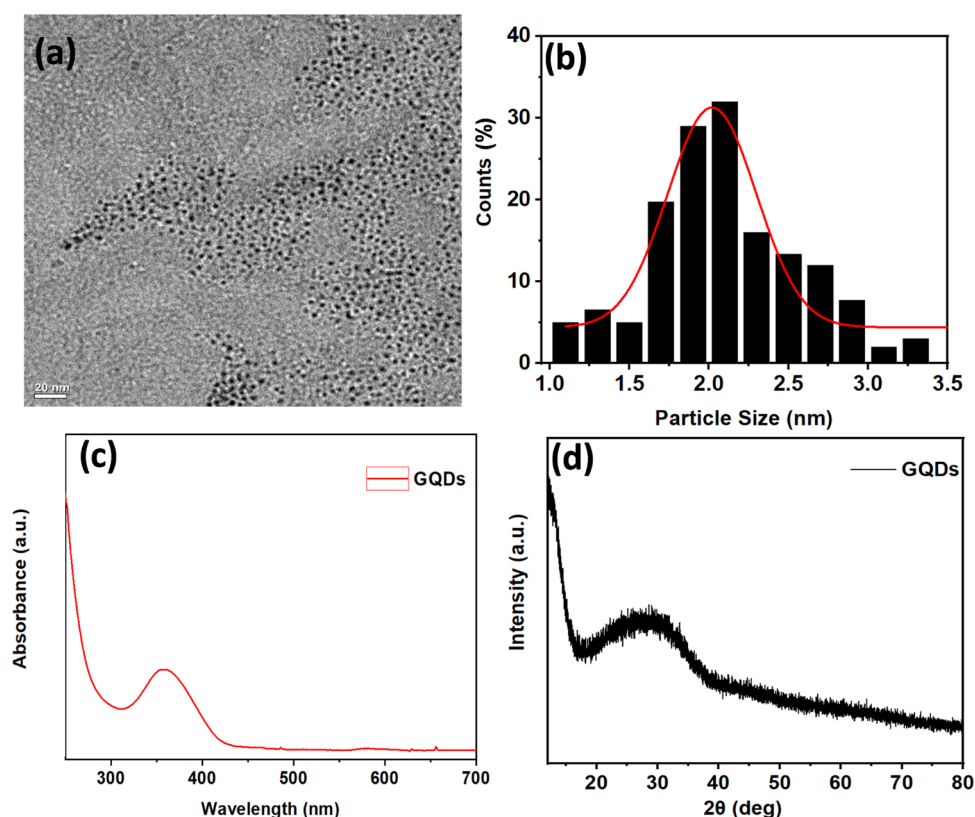


Figure 1. (a) HR-TEM image of GQDs; (b) particle size distribution of GQDs; (c) UV-vis absorption spectrum of GQDs; and (d) XRD pattern of GQDs.

antigen-binding ability. Electrochemical sensors based on the interaction between CRP and CRP-antibody using gold nanoparticles (AuNPs) and carbon nanotubes have been reported for label-free CRP analysis that lack sensitivity and are insufficient to be within the clinically acceptable range.^{12,16,17} To enhance the sensitivity and stability of the sensors, nanomaterials such as gold nanoparticles (AuNPs), carbon nanotubes, metal-oxide nanoparticles, and quantum dots have been used as the functional interfaces on the working electrode. In this context, Zhu et al. developed a CRP electrochemical immunosensor using a AuNP-amplified label-free approach,¹⁸ whereas Bryan et al. employed polycrystalline gold to increase anti-CRP immobilization as an electrochemical impedimetric sensor.¹⁹ Recently, Gupta et al. used carbon nanofibers in the development of the CRP immunosensor.²⁰ Even though the abovementioned immunosensors showed higher sensitivities, complexity in fabrication and analysis with small sample volumes were considered as limitations.

In recent years, screen-printed electrodes (SPEs) have been popular in the design of biosensors due to their low sample volume requirement and affordability.²¹ Although the use of nanointerfaces for detecting trace amounts of CRP in human blood serum has gained popularity, its sensitivity can be further enhanced by engineering the electrode surface with quantum dots.²² Graphene quantum dots (GQDs) have gained a lot of attention owing to their specific edge effects and quantum confinement phenomenon, which reflect well on electrochemical sensing properties. It has also been reported that GQDs have low toxicity, excellent solubility, and high electrical conductivity.^{23,24} As a result, GQDs are widely used as a matrix to provide effective bioreceptor immobilization and to rapidly

shuttle electrons to the electrode surface. Given these benefits, GQDs are employed in the fabrication of low-cost SPCEs for the detection and quantification of CRP.^{25–28} In this present work, SPCEs were fabricated on an overhead projection (OHP) sheet in three different active areas of 2×2 , 3×3 , and 4×4 mm² using the screen printing technique, followed by preparation and electrodeposition of ~ 2 nm sized graphene quantum dots (GQDs) over the SPCE surface to introduce nanomaterials having a high electroactive area for the detection of ultralow levels of CRP using amperometry and differential pulse voltammetry (DPV). And we have made an attempt to detect CRP using the artificial blood solution (ringer lactate solution). Also, a label-free electrochemical CRP immunoassay-based SPCE/GQDs/anti-CRP was used to measure the current response using the amperometry technique for the known concentrations of CRP in a ringer lactate solution, a commonly used substituent for blood serum.²⁷

2. RESULTS AND DISCUSSION

2.1. GQD Characterization. The surface morphology of GQDs was observed with high-resolution transmission electron microscopy (HR-TEM) and is shown in Figure 1a, which clearly shows the well-dispersed and homogeneous distribution of GQDs. To further confirm the homogeneous size of the as-prepared GQDs, the particle size distribution of the same is shown in Figure 1b. The particle size distribution curve suggests that the average size of GQDs is around 2 nm, with a size range of 1–3.4 nm. Figure 1c displays the UV-vis absorption spectrum of GQDs with a characteristic absorption peak at 365 nm, which is in good agreement with the UV absorption results of GQDs.²⁹ The X-ray diffraction (XRD) pattern of GQDs exhibited a broad peak of (002) associated

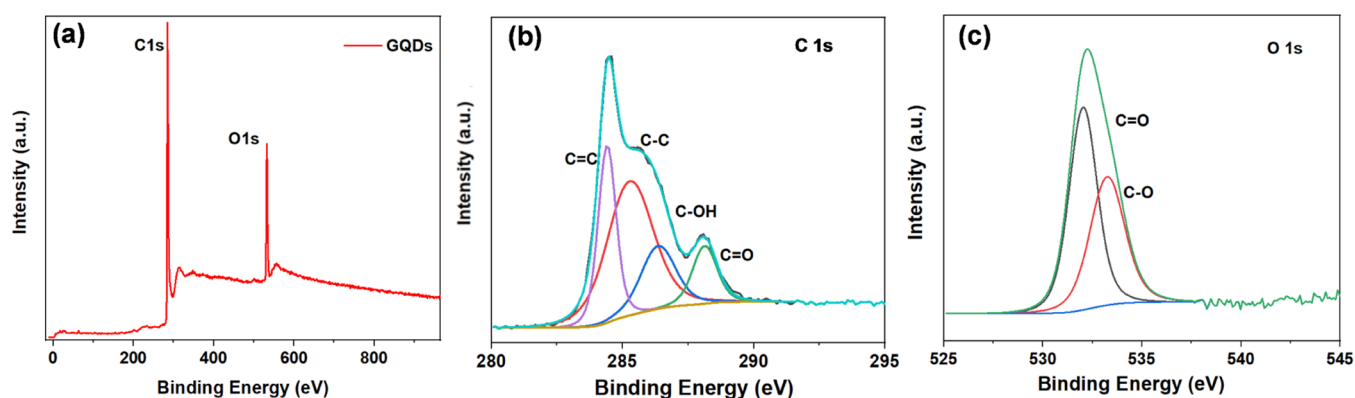


Figure 2. (a) XPS survey, (b) C 1s, and (c) O 1s spectra of GQDs.

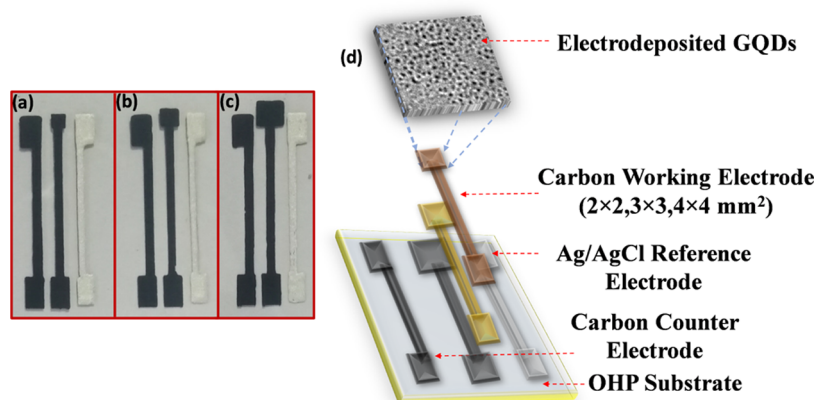


Figure 3. Fabricated SPCEs of (a) 2×2 , (b) 3×3 , and (c) 4×4 mm². (d) Schematic of electrodeposited SPCEs.

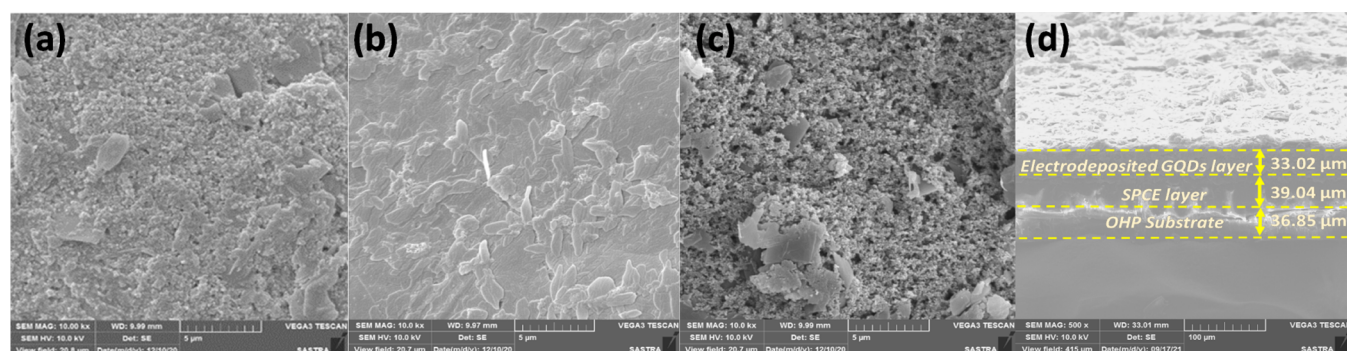


Figure 4. (a) SEM images of 2×2 mm² optimized SPCE, (b) carbon working electrode after oxygen plasma treatment, (c) electrodeposited GQDs on SPCE, and (d) cross-sectional image of SPCE/GQDs.

with the interlayer spacing of 0.37 nm formed due to the disordered stacking of GQDs, as shown in Figure 1d.^{29,32} Further, X-ray photoelectron spectrometer (XPS) spectra were observed to examine the functional groups and composition states found in GQDs.

The survey spectrum of GQDs in Figure 2a shows the intense graphitic O 1s peak at 532 eV and the C 1s peak at 285 eV. Figure 2b displays the high-resolution XPS spectra of the C 1s peak. The peaks observed at binding energies of 284.6 and 285.3 eV are due to C=C and C-C bonds, respectively. The binding energy peaks of 288.1 and 286.4 eV are attributed to the oxygen-containing functional groups of C=O and C-OH, respectively. Figure 2c shows the XPS spectra of O 1s. The binding energy peaks observed at 531.6 and 533.2 eV

correspond to the carbonyl-containing groups of C=O and C-O, respectively. The surface quantification revealed the presence of carbonyl and hydroxyl groups in GQDs.^{33,34}

2.2. Fabrication and Optimization of SPCE/GQDs. The microband SPCEs were fabricated in three different active areas of 2×2 , 3×3 , and 4×4 mm² with a full electrode dimension of 3.5×1.2 cm², as shown in Figure 3a–c. Then, the synthesized GQDs were electrodeposited onto the optimized SPCEs, as shown in Figure 3d. Surface morphologies of 2×2 , 3×3 , and 4×4 mm² carbon working electrodes on the OHP sheet revealed a uniform coating of carbon ink with dense microstructures (Figures 4a, S1a, and S1b). As shown in Figure 4b, scanning electron microscope (SEM) images clearly show that the binders and organic

solvents adsorbed on the surface of the electrode were effectively removed after oxygen plasma treatment.³⁰

Using the cyclic voltammograms (CV) technique, the peak-to-peak separation between the cathodic peak potential (E_{pc}) and anodic peak potential (E_{pa}) evaluated by $\Delta E_p = (E_{pa} - E_{pc})$ (mV) of three different fabricated SPCE sizes (2×2 , 3×3 , and 4×4 mm²) was analyzed. The ΔE_p values were observed for various plasma-activated electrodes and compared to select the optimized pretreated electrode.³⁰ The SPCE with dimensions of 2×2 mm² showed a lower ΔE_p of about 60 mV after being subjected to 100 W oxygen plasma treatment for 10 min. The surface morphology of 2×2 mm² SPCE revealed the activation of edge planes of the working electrode, as shown in Figure 4b. Then, the optimized 2×2 mm² electrode was electrodeposited with GQDs and exhibited a mesoporous form of GQDs (see Figure 4c). Each layer was characterized using a scanning electron microscope (SEM). The cross-sectional SEM images show the uniform layer thickness of the electrodeposited GQDs (33.02 μ m), the screen-printed carbon electrode (39.04 μ m), and the thickness of the OHP substrate (36.85 μ m) (see Figure 4d). The fabricated SPCE/GQDs was connected to Palmsens S3 for electrochemical experiments, as shown in Figure 5. Hence, the optimized 2×2 mm² SPCE/

GQDs electrode was employed for further electrochemical analysis. Other plasma-treated SPCEs with dimensions of 3×3 and 4×4 mm² showed higher ΔE_p (>60 mV), as shown in Figure 6a.

2.3. Electrochemical Characteristics of the SPCE/GQDs Electrode. The control experiments were performed with various unmodified and modified SPCE/GQDs. When the surface of bare SPCE was modified with GQDs, an increased current response was observed in the presence of redox probe species $\text{Fe}(\text{CN})_6^{3-/4-}$ due to the higher surface-to-volume ratio of GQDs. Further, the SPCE/GQDs was modified with a thin layer of anti-CRP and its voltammetric response was observed, as shown in Figure 6b. The anti-CRP layer-modified surface acted as a barrier to electron transport, resulting in a decrease in the current response with a wide separation of anodic and cathodic peaks. However, the current response of SPCE/GQDs/anti-CRP was decreased after incubation with 1 nM CRP due to the CRP-anti-CRP association. When the CRP interacts with anti-CRP, it forms an anti-CRP and CRP complex that serves as an interkinetic obstacle, which blocks the charge transfer process between the redox couple and the SPCE.¹² The significance of the stepwise modification of electrode can be understood by estimating the electroactive surface area. The surface area was calculated using eq 1 for pre- and postmodification of the electrode.²⁹

$$I_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} \nu^{1/2} C^* \quad (1)$$

where I_p is the peak current of the forward scan, n is the number of electrons ($n = 1$), ν is the scan rate (0.01 V s^{-1}), C is the concentration of $\text{K}_3[\text{Fe}(\text{CN})_6]$ ($5 \times 10^{-7} \text{ mol cm}^{-3}$), D is the diffusion coefficient of $\text{Fe}(\text{CN})_6^{3-/4-}$ ($7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), and A represents the active area of the electrode (cm^2). The calculated surface area for the stepwise modification of each electrode is as follows: SPCE = 4.13 cm^2 , SPCE/GQDs = 4.88 cm^2 , and SPCE/GQDs/anti-CRP = 3.52 cm^2 .

2.4. Binding Property of Anti-CRP onto GQDs. From the control experiment (Figure 6b), the amount of the antibody surface coverage onto the SPCE was estimated using eq 2.³³

$$\Gamma = \frac{Q}{nFA} \quad (2)$$

where Γ is the surface coverage of the CRP-antibody, Q is the amount of charge accumulated on the surface of SPCE/

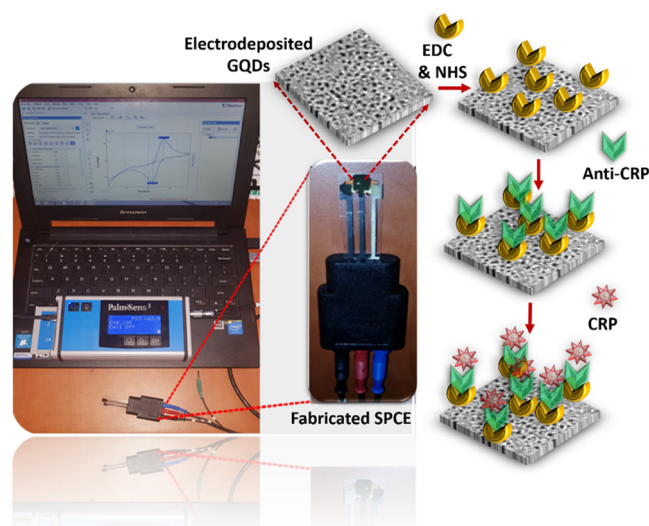


Figure 5. GQDs/SPCE immunosensor connected with a Palmsens S3 device.

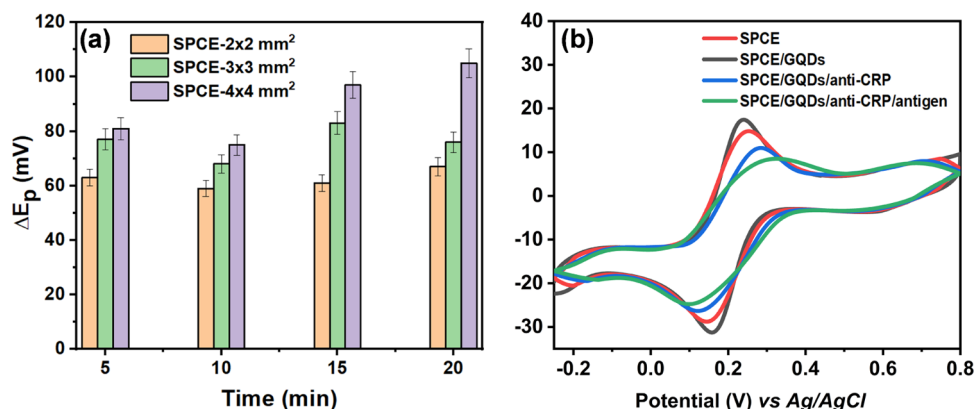


Figure 6. (a) ΔE_p values of three different (2×2 , 3×3 , and 4×4 mm²) sizes of fabricated SPCEs at 100 W oxygen plasma treatment from 5 to 20 min. (b) Cyclic voltammograms of bare SPCE, SPCE/GQDs, SPCE/GQDs/anti-CRP, and SPCE/GQDs/anti-CRP/antigen.

GQDs/anti-CRP ($2.35 \times 10^{-6} \text{ cm}^2$), n is the number of electrons ($n = 1$), A is the area of the working electrode (3.52 cm^2), and F represents Faraday's constant ($96,485 \text{ C} \cdot \text{mol}^{-1}$). The amount of CRP-antibody being attached onto the surface was $6.93 \times 10^{-12} \text{ M} \cdot \text{cm}^{-2}$. Figure 7 displays the Fourier

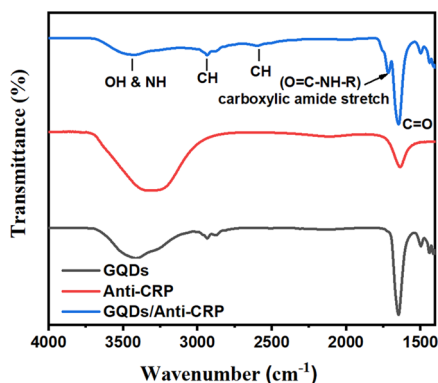


Figure 7. FT-IR spectra of GQDs, anti-CRP, and GQDs/anti-CRP.

transform infrared (FT-IR) spectra of GQDs, anti-CRP, and GQDs/anti-CRP. The wide band between 3410 and 3323 cm^{-1} is assigned to the O–H stretching vibrations of GQDs and anti-CRP, respectively. The band observed at 3429 cm^{-1} corresponds to N–H and O–H stretching vibrations from immobilized anti-CRP GQDs, where the broadness is caused due to the extensive hydrogen bonding. The band observed between 1645 and 1631 cm^{-1} is attributed to the carbonyl stretch ($\text{C}=\text{O}$) of GQDs and anti-CRP, respectively. The

binding between GQDs and anti-CRP is introduced using a carbodiimide cross-linker, wherein in the presence of 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (EDC), the functional group in GQDs gets activated *via* an intermediate ester. The role of *N*-hydroxysuccinimide (NHS) is to convert unstable NHS-ester on reaction with protein molecules leads to the formation of an amide bond. The immobilization of anti-CRP onto GQDs is confirmed from the occurrence of the carboxylic amide band ($\text{O}=\text{C}-\text{NH}-\text{R}$) at 1715 cm^{-1} .^{35,36} The other two stretches at 2939 and 2585 cm^{-1} are caused by the CH bonding between the GQDs and anti-CRP protein.

2.5. CRP Detection Using Differential Pulse Voltammetry. The observed current response of SPCE/GQDs/anti-CRP was recorded in the applied potential frame of 0 – 0.6 V (vs Ag/AgCl) against varying concentrations of CRP (0.5 – 10 ng mL^{-1}) with an electrolyte containing 0.1 M phosphate buffer solution (PBS) and 5 mM of $\text{K}_3[\text{Fe}(\text{CN})_6]$. The current response was gradually decreased with the concentration of CRP due to the binding between CRP and anti-CRP and immune complex formation, which blocked the charge transport from the probe. The calibration plot showed a linear response ($y = -0.07x + 5.83$, $R^2 = 0.99$) to CRP (Figure 8a,b). The sensor showed a limit of detection (LOD) of 0.024 ng mL^{-1} ($S/N = 3$) and limit of quantification (LOQ) of 0.072 ng mL^{-1} . The SPCE/GQDs/anti-CRP sensor exhibited a sensitivity of $0.075 \mu\text{A ng}^{-1} \text{ mL}^{-1} \text{ cm}^{-2}$ over a linear range of 0.5 – 10 ng mL^{-1} .

2.6. CRP Detection Using Amperometry Studies. Amperometry study was also performed at 0.3 V potential between SPCE/GQDs and the reference electrode to

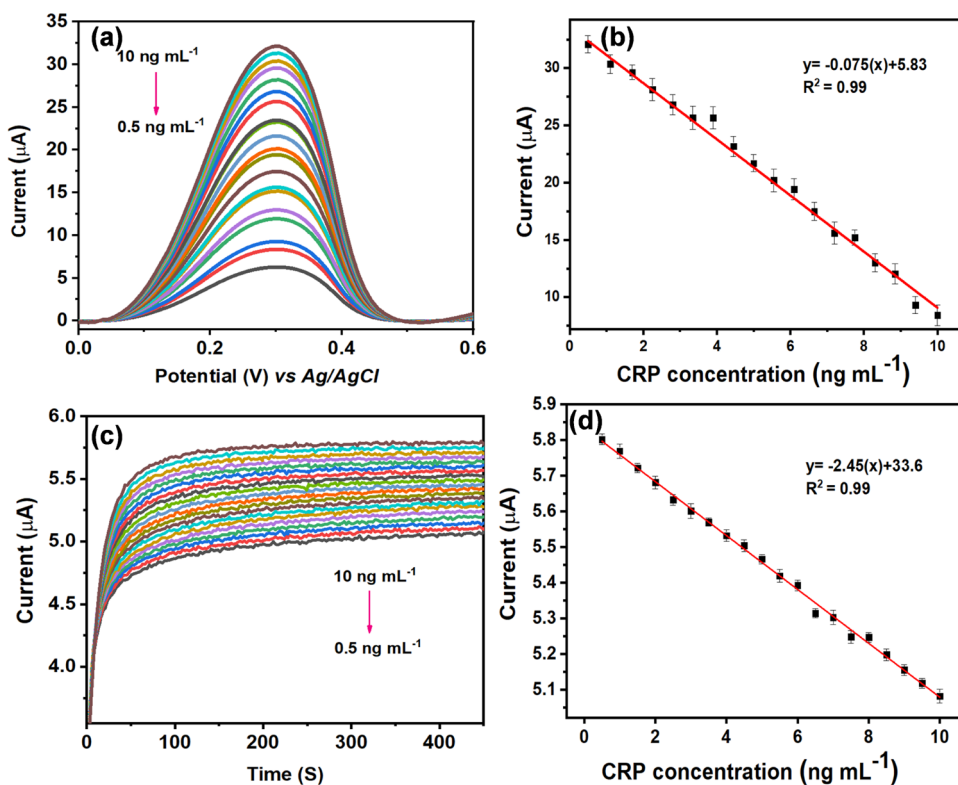


Figure 8. (a) Differential pulse voltammograms and (b) amperograms of the SPCE/GQDs/anti-CRP electrode for varying CRP concentrations. (c) Calibration plot of the SPCE/GQDs/anti-CRP electrode for varying CRP concentrations detected using the CV technique. (d) Calibration plot of the SPCE/GQDs/anti-CRP electrode for varying concentrations of CRP detected using the DPV technique.

Table 1. Comparison of Electrochemical Detection of CRP Using Label-Free Methods

s. no.	immunosensor	techniques	linearity (ng mL ⁻¹)	detection limit (ng mL ⁻¹)	reference
1	Au–SAM–anti–CRP	EIS	0.15–50	0.055	19
2	SiO ₂ –VACNFs–anti–CRP	EIS	0.13–13	0.028	20
3	Au–SAM–anti–CRP	DPV	0.030–3.06	0.015	12
4	SPE–AuNPs–SAM–anti–CRP	Amp	0.125–62.89	0.047	31
5	SPCE/GQDs/anti–CRP	Amp	0.5–10	0.036	this work

determine the selectivity/specificity of the developed sensor toward CRP. The amperometric current response was recorded for various concentrations of CRP ranging from 0.5 to 10 ng mL⁻¹. The steady-state current response decreased significantly as the CRP level increased from 0.5 to 10 ng mL⁻¹. The calibration plot over the different concentration ranges exhibited a linear response ($y = -2.45x + 33.6$, $R^2 = 0.99$) to CRP, as shown in Figure 8c,d. The rapid response time of the sensor was estimated to be less than 25 s. The sensor showed a LOD of 0.036 ng mL⁻¹ (S/N = 3) and LOQ of 0.108 ng mL⁻¹, suggesting that the proposed sensor can detect ultralow concentrations of CRP. Moreover, the SPCE/GQDs/anti–CRP sensor exhibited a sensitivity of 2.45 $\mu\text{A ng}^{-1}\text{ mL}^{-1}\text{ cm}^{-2}$ over a linear range from 0.5 to 10 ng mL⁻¹. However, after 10 ng mL⁻¹ anti–CRP, the sensor reached a saturation point and maintained the same current response, suggesting the formation of an immunocomplex on the fabricated SPCE/GQDs. The sensor performance was compared to the other reported immunosensors shown in Table 1, and it was observed that the SPCE/GQDs/anti–CRP sensor has better electrochemical characteristics for CRP detection.

2.7. Selectivity, Stability, and Reproducibility. Additionally, the selectivity of the sensor was tested against other commonly found biomarkers in human serum. The SPCE/GQDs was incubated in 0.5 ng mL⁻¹ interfering species like ascorbic acid (AA), bovine serum albumin (BSA), and glucose with 0.5 ng mL⁻¹ CRP. There was no significant change in the current response in the presence of interfering biomolecules, indicating that the developed sensor can overcome possible interfering biomolecules (Figure S2a). The sensor stability was investigated by storing it at 4 °C. Thereafter, the current response was tested for 4 weeks and is shown in Figure S2b. At the end of the fourth week, the developed sensor showed a 5.21% decrease in amperometric response, confirming stability. The intra- and interassays were performed with five sensor strips, and the corresponding relative standard deviations (RSD) were 1.85 and 2.8%, respectively. These results demonstrated that the developed electrode could rapidly detect CRP with enhanced sensitivity and specificity. As a result, this label-free method is more useful in point-of-care applications, and the proposed sensor is suitable for CRP detection.

2.8. Recovery Studies. A label-free electrochemical CRP immunoassay-based SPCE/GQDs/anti–CRP was used to measure the current response of the various spiked CRP concentrations in a ringer lactate solution. The CRP concentration was quantified for each addition, as given in Table 2. The practical applicability of the present immunoassay was tested by determining the recovery of the known concentrations of CRP that were added to the ringer lactate solution. The recovered values between 94.6 and 101% indicated the capability of the sensor to detect CRP in artificial serum.

Table 2. Measurements of CRP Antigen Concentrations in the Ringer Lactate Solution

sample no.	CRP added (ng mL ⁻¹)	CRP measured (ng mL ⁻¹)	recovery (%)	RSD (%) ($n = 3$)
1	0.5	0.49	98	1.4
2	2.5	2.37	94.8	3.7
3	5.0	5.05	101	0.7
4	7.5	7.35	98	1.4
5	10	9.46	94.6	3.9

3. CONCLUSIONS

In summary, a label-free SPCE electrochemical immunosensor has been successfully fabricated and tested to detect one of the cardiac biomarkers, CRP. The GQD-modified SPCE interface played a decisive role in anti–CRP immobilization. The current response obtained from the probe species decreased linearly with an increased binding between CRP and anti–CRP, confirming the effective immunocomplex formation. The fabricated SPCE/GQDs/anti–CRP biosensor was used for the quantitative analysis of the CRP biomarker. The developed immunosensor displayed good sensitivity and stability over a linear range and a low LOD of 0.5–10 and 0.036 ng mL⁻¹, respectively. Moreover, a recovery study was also performed in the ringer lactate solution by spiking known levels of CRP, and its recovery range was estimated as 94.6–101%, affirming the practical applicability of the developed electrode. The developed label-free SPCE-based electrochemical immunosensor can be used for the assessment of cardiac-related healthcare applications.

4. EXPERIMENTAL SECTION

4.1. Materials and Methods. CRP polyclonal and monoclonal antibodies were procured from Sigma-Aldrich. *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), bovine serum albumin (BSA), and glucose were obtained from Sigma-Aldrich. Potassium hexacyanoferrate (III) [$\text{K}_3\text{Fe}(\text{CN})_6$], sodium hydroxide (NaOH), ascorbic acid (AA), and citric acid (CA) were obtained from Merck, India. Conductive inks like silver/silver chloride (Ag/AgCl) (Product No: 113-09, 102-03) were obtained from Creative Materials, Inc. (Massachusetts). Ringer lactate solution and phosphate buffer solution (PBS) with a pH of 7.4 were purchased from Merck, India. The additional chemicals utilized were of scientific grade and used without further purification.

An automatic DEK Horizon printer (ASM Assembly System Singapore Pte. Ltd, Singapore) was used for screen printing SPCE on an overhead projection (OHP) sheet. The desired stencil pattern was procured from Satchitanand Stencils Pvt. Ltd., India. Curing was performed using the Tarsons HOTOP 5040 digital hot plate procured from Tarsons, India. TESCAN Vega-3 scanning electron microscope (SEM) was used to examine the surface morphologies of SPCEs and SPCE/

GQDs. Electrochemical measurements were performed using Palmsens3 from Dropsens (the Netherlands, Europe). X-ray diffraction (XRD) investigation was conducted with the D8 Focus X-ray diffractometer (Bruker, Germany) with a diffraction angle from 10 to 80° with Cu K α radiation of wavelength 1.5418 Å. The absorption spectra of GQDs were obtained at a wavelength range of 200–600 nm with a UV–vis spectrophotometer (PerkinElmer Lambda 25 system). The functional groups and amounts of carbon and oxygen elements present in GQDs were investigated with the use of the ESCA model VG 3000 X-ray photoelectron spectrometer (XPS). The oxygen plasma treatment was performed with the help of a Zepto plasma cleaner system purchased from Diener Electronic, Germany.

4.2. Synthesis of GQDs. GQDs were synthesized by placing 2 g of citric acid (CA) in a precleaned 5 mL beaker and kept for heating on a heating mantle at 200 °C. The formation of GQDs was confirmed by the change in color from a colorless liquid to pale yellow, which finally transformed into orange after 30 min. To obtain GQDs, the orange-colored liquid procured after 2 h was slowly added to a 100 mL solution containing 10 mg mL⁻¹ NaOH solution while being continuously stirred at 1000 rpm. The aqueous GQDs solution was derived after it was neutralized to pH 8.0 with NaOH.²⁹

4.3. Fabrication of Electrodeposited GQDs on the SPCE. A commercial carbon ink (Product Code: 124-50; Creative Materials Inc.) was used for the fabrication of counter and working electrodes. At first, carbon ink was screen-printed onto the OHP sheet, and the as-printed carbon layer was kept at 100 °C for 5 min. Further, Ag/AgCl ink (Product Code: 120-07, Creative Materials Inc.) was screen-printed as a reference electrode and then cured at 100 °C for 5 min. Finally, an oxygen plasma technique was used to pretreat the fabricated electrodes of varying dimensions.³⁰ A plasma system operating at a frequency of 40 kHz was used to perform oxygen plasma treatment of the fabricated SPCEs for a period of 5–20 min. First, 100 μ L of a 1.0 mM GQDs solution was drop-casted onto the fabricated SPCE, and cyclic voltammetry (CV) was performed by applying potentials in the range of 0.0 to -1.4 V (vs Ag/AgCl) operating at 50 mV s⁻¹ scan rate.²⁵

4.4. Immobilization of Anti-CRP. An equimolar mixture of 10 mM EDC and NHS in a 1:1 ratio was prepared by stirring the mixture continuously in PBS at an rpm of 500 for 45 min, and the resultant solution was mixed with 10 μ L of anti-CRP at 300 K for 3 h. Later, 3 μ L of the obtained mixture was drop-casted onto the SPCE/GQDs electrode. As a result, the carboxylic terminal groups of the electrodeposited GQDs on the SPCE surface reacted with the amine groups of the anti-CRP to form a stable amide bond.³¹ Subsequently, the unreacted loosely bound antibodies were removed by washing the surface of SPCE/GQDs/anti-CRP with PBS four times. Finally, the fabricated SPCE/GQDs/anti-CRP was stored in a deep freezer at 4 °C prior to electrochemical analysis.

4.5. Electrochemical Detection of the Target CRP. The performance of the fabricated SPCE was investigated using CV with 5 mM of K₃[Fe(CN)₆] and 0.1 M PBS solution at pH 7.4 at the applied potential range of -0.2 to +0.8 V with a scan rate of 0.01 V s⁻¹ (optimized). Prior to the electrochemical measurement, the SPCE/GQDs/anti-CRP electrode was incubated with CRP for 10 min to enhance the interaction between GQDs and the CRP biomarker. After incubation, the fabricated electrode was rinsed with 0.1 M PBS (pH 7.4), and CV studies were carried out using the fabricated electrode in

20 μ L of K₃[Fe(CN)₆] and 0.1 M PBS solution. In addition, DPV and amperometry measurements were performed with various concentrations of CRP. Moreover, the specificity of the sensor was determined in the presence of nonspecific antigens such as ascorbic acid, glucose, and bovine serum albumin (BSA) produced in human blood under various physiologic conditions. For determination of CRP using an artificial serum solution, namely, ringer lactate solution, known levels of CRP were spiked at varying concentrations from 0.5 to 10 ng mL⁻¹ into 20 μ L of the ringer lactate solution and K₃[Fe(CN)₆]. The change in the current behavior for varying concentrations of CRP was observed in the amperometry studies, and subsequently, the recovery percentage and RSD values were evaluated.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.1c04043>.

Surface morphologies of 3 $\times$ 3 and 4 $\times$ 4 mm² carbon working electrodes on an OHP sheet (Figure S1a,b); selectivity of the SPCE/GQDs sensor tested against other commonly found biomarkers in human serum; the SPCE/GQDs electrode incubated in 0.5 ng mL⁻¹ interfering species like ascorbic acid, BSA, and glucose with 0.5 ng mL⁻¹ CRP (Figure S2a); and stability of the sensor investigated for 4 weeks (Figure S2b) (PDF)

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Notes

The authors declare no competing financial interest.

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