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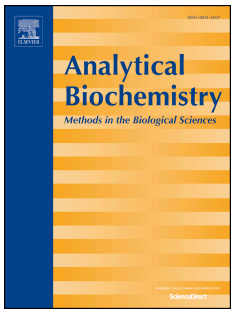


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# Carboxylic group riched Graphene Oxide based Disposable Electrochemical Immunosensor for Cancer Biomarker Detection

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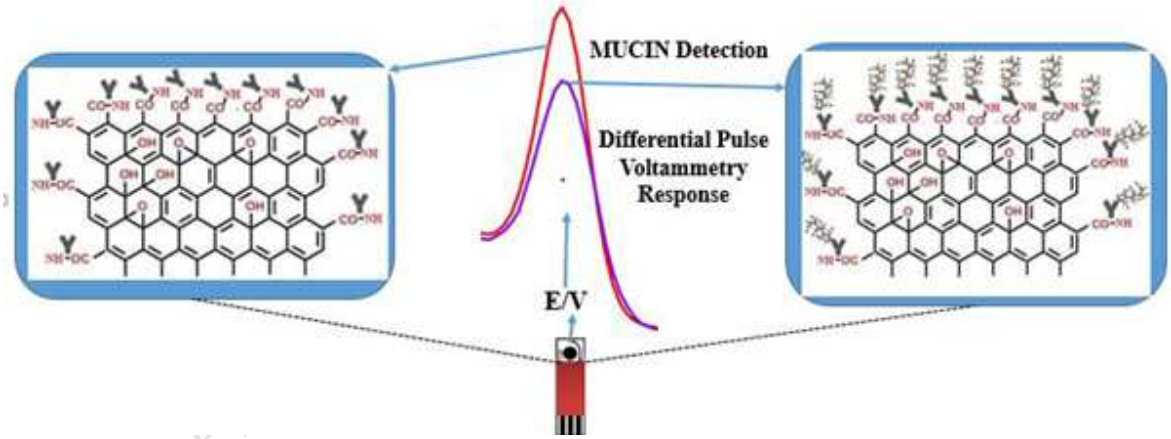
## Graphical abstract




**Abstract:**

In this work, we have developed for the first time a carboxylic group riched graphene oxide based disposable electrochemical immunosensor for cancer biomarker detection using methylene blue (MB). The developed immunosensor is highly sensitive for detection of biomarker Mucin1

(MUC1) in human serum samples. Development of this disposable electrochemical immunosensor was premeditated by applying specific monoclonal antibodies against MUC1. In this method, we explored highly conductive surface of carboxylic group (-COOH-) rich graphene oxide (GO) on screen-printed carbon electrodes (SPCE). This modified GO-COOH-SPCE was employed for the detection of MUC1 protein based on the reaction with methylene blue (MB) redox probe using differential pulse voltammetry (DPV) technique. Developed immunosensor exhibited good detection range for MUC1 with excellent linearity (0.1 U/mL- 2 U/mL), with a limit of detection of 0.04 U/mL. Upon potential application of developed biosensor, good recoveries were recorded in the range of 96-96.67 % with % R.S.D 4.2. Analytical performance of the developed immunosensor assures the applicability in clinical diagnostic applications.

## 1. Introduction

Protein biomarkers are one of the important classes of biomarkers, which can be indicative of disease state according to their high or low expression in serum [1]. Mucins are a family of high molecular weight, heavily glycosylated proteins that are bound to cells by an integral transmembrane domain via the formation of a gel matrix [2]. Mucin 1 (MUC1) protein is a membrane-associated glycoprotein, which contains a hydrophobic domain of 31 amino acids, cytoplasmic domain of 69 amino acids and an extracellular domain of 20 identical amino acids per repeat. MUC1 is also a well-known tumor marker, existing in a variety of malignant tumors. It is commonly over expressed on a broad range of different human epithelia, including breast, gastric, colorectal, lung, prostate, ovarian, pancreatic, and bladder carcinomas [2, 3]. Several tumor markers found in biological fluids, such as MUC1, are important for early stage screening of cancers because they are usually asymptomatic until advanced stages when the prognosis of survival is poor. Although, cutoff points for MUC1 were used between 25-40 KU/L and the generally accepted value for cutoff is 35 KU/L in serum. Diagnostic accuracy for detection of this cancer marker is very limited therefore; current research needs to focus on developing disposable immuno-sensing devices for the reliable and sensitive detection of these cancer biomarkers.

In recent years, biosensors have become increasingly prevalent in clinical diagnosis and point-of-care testing. This development is motivated in part by the aspiration to decrease the cost of health care tools and shift some of the crucial analytical tests from centralized facilities to

frontline users. Moreover, it also helped to obtain more precise information regarding the patient's health status more quickly [4]. Among all the reported biosensors, electrochemical biosensors have achieved specific importance due to its advantages of sensitivity, portability and miniaturization [5]. Electrochemical biosensors have conventionally established the major share of the consideration in biosensor development [6]. Such electrochemical devices produce a simple, inexpensive and an accurate platform for diagnosis purpose when a molecular sensing device closely couples a biological recognition element to an electrode transducer. The function of the electrochemical transducer is to convert the biological recognition event into a useful electrical signal [7].

Ample of immunological and biochemical methods have been advanced for the detection of biomarker proteins connected to cancer and cardiovascular diseases in biological fluids [8, 9]. However, very limited studies have been performed for the detection of MUC1 biomarker. Various biosensing techniques have been exploited to develop detection methods for MUC1 in recent years. Some of the methods are based on the fluorescence intensity of DNA hybridization on quantum dots by MUC1peptide [10]. Aptamer based fluorescence quenching assay utilizing graphene oxide (GO) was developed by some researchers where GO quench the fluorescence of single-stranded dye-labelled MUC1 specific aptamer which helps in the quantification of MUC1 protein [2]. In another study an aptamer based strategy was proposed for the sensitive detection of MUC1 based on electro-chemiluminescence resonance energy transfer (ERET) from Bis (2,2′-bipyridine)-(5-amino phenanthroline) ruthenium(II) to GO [11]. Moreover, several methods were developed using gold nanoparticles (AuNPs) conjugated with hairpin oligonucleotide and horseradish peroxidase (HRP) for ultrasensitive electrochemical detection of MUC1 protein [3]. In another approach MUC1 protein was detected using fluorescence resonance energy transfer (FRET) between carbon dots (CDs) GO in nano-molar (nM) range [12].

Although being selective and sensitive, these reported biosensors have the disadvantages of high cost, long analysis, requirement of qualified personnel and sophisticated instrumentation; also their sensitivity is not high enough to detect tumor biomarkers at an early stage. Moreover, currently used fluorescent probes, including organic dyes and inorganic semiconductor quantum dots, have their own drawbacks including poor photostability, easy photobleaching, short lifetimes and significant toxicity even at relatively low concentrations.

Owing to their extraordinary physicochemical properties, integrating GO nanostructures on screen-printed electrodes has proved to be advantageous in enhancing sensitivities of electrochemical sensors and develop new generation of biosensors. Although pristine graphene is chemically inert and electrochemically inactive, tunable functionalization and reduction of GO is very promising for development of a wide range of biosensors [13]. Modification of GO with functional groups to improve their solubility or binding with modified electrode provide new insight into the application of nanomaterials medical biosensing and clinical diagnostics. Increased carboxyl group modified graphene oxide (GO-COOH) shown to possess intrinsic peroxidase-like activity that can catalyze the methylene blue (MB) probe and provides highly conductive surface for the detection of MUC1 protein [14].

The present work is first report on the integration of carboxy rich graphene oxide towards the construction of MUC1 immunosensor while integrating MB as redox probe. The role of functionalized graphene oxide was not only to serve as a covalent immobilization support for antibody, but its catalytic signal amplification behavior towards methylene blue was demonstrated for the first time in the construction of label free immunosensor. DPV was used for this study since it offers high sensitivity, low limit of determination, easy operation and the use of simple instrumentation [15]. It is known that MB is reduced to a leucomethylene blue (LB) at an electrode surface by accepting two electrons. This indicator was therefore used for detection of antibody-protein interactions [16]. Presented immunosensor showed excellent linearity (0.1 U/mL- 2 U/mL) in the spiked serum samples and good recoveries were recorded in the range of 96-96.67 % with % R.S.D 4.2.

## 2. Experimental Details

### 2.1. Materials

Sulfuric acid ($H_2SO_4$, 98%), potassium ferrocyanide (K4[Fe(CN)6]), potassium ferricyanide (K3[Fe(CN)6]), fetal bovine serum, human serum, Prestige Antibodies (75-100 U), Methylene Blue (MB) and bovine serum albumin (BSA) were purchased from Sigma (Taufkirchen, Germany). Lysozyme was from Carbosynth (Berkshire, UK). N-(3-dimethylaminopropyle)-N-ethyle-carbodiimide hydrochloride (EDC) was obtained from Alfa Aesar (Heysham, UK), while cancer antigen mucin (25 kU) was purchased from Lee bio

(Maryland Heights, MO, USA). Antibody solutions and Mucin solutions were prepared in phosphate buffer saline (PBS), pH 7.4. All solutions were prepared in deionized water from ELGA PURELAB® Ultra water deionizer (High Wycombe, UK).

### 2.2. Apparatus

Scanning electron microscope (SEM) studies were performed at VEGA 3 TESCAN variable pressure mode (LMU) version (Brno, Czech Republic). Images were taken in different magnification ranges at an accelerated voltage of 20 kV, Atomic Force Microscopy was performed by a Park xe-7 Atomic Force Microscope (Suwon, Korea) and electrochemical measurements were performed on a Gamry Reference 3000 potentiostat/galvanostat (Warminster, PA, USA).

### 2.3. Functionalization of Graphene:

Modified Hummers method [17, 18] was used to synthesize GO from natural graphite nano powder. In brief, graphite powder (2 g), $NaNO_3$ (2 g) and 80 mL of $H_2SO_4$ (98%) were mixed together in an ice bath. Later, $KMnO_4$ (12 g) was slowly added as portion under ice bath condition for 2 h. Subsequently, the solution was stirred at 40˚C for 2 h, 80 mL of water was added, and the mixture was stirred for 1 h at 90˚C. Finally, 180 mL of water and 20 mL of $H_2O_2$ (30%) solution were added and stirred for 3 h. The final mixture was thoroughly washed with 5% HCl and deionized water by using repetition of centrifugation and filtration process. The obtained suspension was kept in an oven at 60˚C for 24 h for the drying process.

We followed the earlier reported methods [19, 20] to obtain the carboxyl functionalized graphene oxide from GO. In brief, GO was ultra-sonicated for 2 h to achieve a homogeneous suspension solution (1 mg/mL). Later, 1.5 g of NaOH and 1 g of chloro-acetic acid were added to the 5 ml of GO suspension and ultra-sonicated for 12 h. The final mixture can directly use for the electrochemical assay.

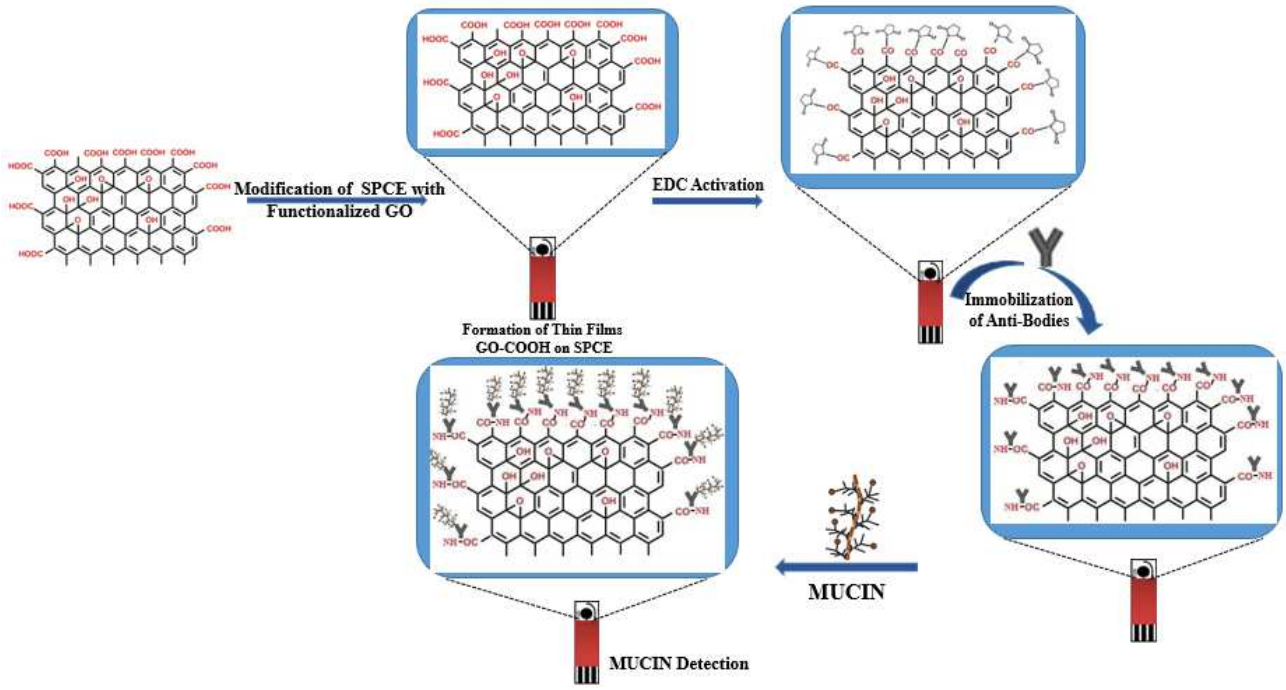
### 2.4. Assembly of GO-COOH on SPCE

Assembly of GO-COOH was performed with slight modification in our previously reported work [21]. In brief, screen-printed carbon electrodes (SPCE) were subjected to electrochemical pretreatment before depositing GO-COOH on SPCE. Several potential cycles

between 1 and -1.5 V/pseudo Ag reference electrode were applied using 0.5 M $H_2SO_4$ solution with 100 $mVs^{-1}$ scan rate until the characteristic cyclic voltammetry (CV) graph for a clean SPCE surface was obtained. Aqueous solution of functionalized GO-COOH was introduced on electrochemically cleaned SPCE surface for 1 h followed by thoroughly rinsing with large amounts of water to remove non-specifically bound GO-COOH. The GO-COOH assembled SPCE was dried at room temperature and used directly to perform the experiments or were stored at room temperature for use in further experiments.

### 2.5. Immobilization of MUC1 Antibodies on GO-COOH Modified SPCE

The terminal benzoic acid groups on SPCE surface were activated by immersing the SPCE into a solution of 100 mM N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) in PBS (pH 7.4) for 60 min. After washing the electrode surface with water, 20 µL of antibodies solution at an optimized concentration of 0.35 U/mL at the dilution of (1/200) were incubated in water environment onto the activated SPCE surface for 1 h. After incubation, the electrode was rinsed with distilled water to remove unbound antibodies, and, subsequently, the modified SPCE was incubated with 100 µL of 1% Bovine serum albumin (BSA) solution for 1 h to deactivate the remaining terminal groups and block unreacted sites. Illustration of the experimental procedures for immobilization of MUC1 antibody on SPCE is presented as Scheme 1.




**Scheme 1.** Schematic illustration of the immobilization of MUC1 antibody on GO-COOH modified SPCE for electrochemical detection.

### 2.6. Electrochemical Differential Pulse Voltammetry (DPV) Measurement

Electrochemical measurements were performed on Gamry Reference 3000 potentiostat/Galvano stat. SPCEs were fabricated using a DEK 248 screen-printing system. The SPCE consists of a conventional three-electrode configuration with graphite as working (4 mm diameter disk) and counter (16 mm × 1.5 mm curved line) electrodes, and Ag/AgCl (16 mm × 1.5 mm straight line) as pseudo reference electrode. DPV experiments were carried out under optimized conditions, obtained from the redox potential of 0.5 µM MB with a potential range of 0.0 to -0.5 V and a current peak located at -0.35 V. The DPV spectra were plotted in the form of Current (I/µA) verses Potential (E/A). For reproducibility of results from different electrodes, Δ ratio was calculated for each electrode using the following equations where, Δs represents the current before addition of MUC1 protein, Δp represents current after addition of MUC1 protein, and A represents the current.

$$\Delta ratio = \Delta s/\Delta p,$$

$$\Delta s = (A_{\text{before MUC1}} - A_{\text{bare electrode}})$$

$$\Delta p = (A_{\text{after MUC1}} - A_{\text{bare electrode}})$$

Where,

$\Delta s$ = current before addition of MUC1 protein,

$\Delta p$ =current after addition of MUC1 protein,

A = current

### 2.7. Interference Study and analyte detection

Detection of the target protein was carried out using 50 µL of varying concentrations of MUC1 solutions, incubated on to MUC1 antibody modified SPCE for 60 min. Bovine Serum Albumin (BSA), Lysozyme (Lys) and Fetal Bovine Serum (FBS) were selected as possible interfering analytes due to their structural and physiochemical similarity. Electrochemical experiments were performed in the similar way as described for analysis of MUC1 detection. Potential application of the developed electrochemical immunosensor in clinical analysis was demonstrated by analyzing the spiked human serum samples with MUC1 protein.

## 3. Result and Discussion

### 3.1. Characterization of modified GO-COOH SPCE

#### 3.1.1. Surface characterization of GO-COOH SPCE using SEM

The SEM images of bare SPCE and modified GO-COOH SPCE are represented as Fig. 1. It can be observed from the figure that the surface of modified GO-COOH SPCE has different morphology (Fig 1a) as compared to the bare SPCE with equally distributed spherical grains and granular thread like structure of GO-COOH (Fig 1b). These thread like structures showed good density with some porosity and making the uniform modified SPCE surface for immobilization of MUC1 antibody as compared to the bare SPCE.

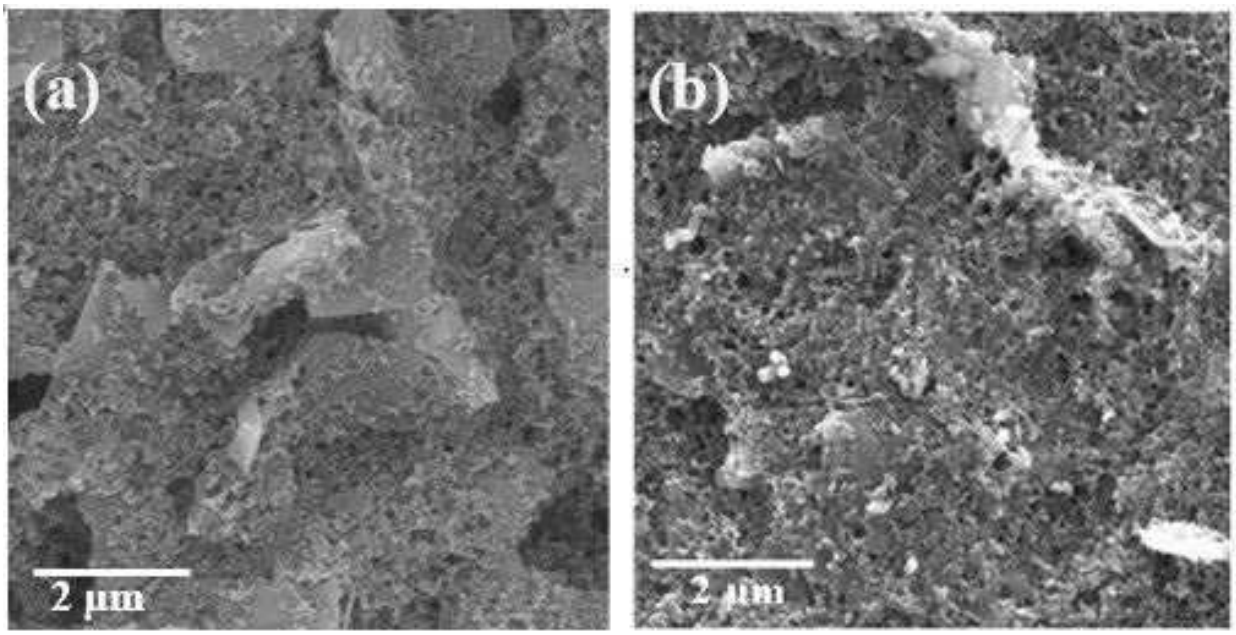



**Figure 1.** Scanning electron microscope (SEM) images of bare-screen printed carbon electrode (SPCE) (a), GO-COOH-SPCE (b).

### 3.1.2. Surface characterization of GO-COOH SPCE using AFM

Figure 2 represents the AFM topographic surface analysis of bare SPCE (Fig 2a) and GO-COOH modified SPCE (Fig 2b). Phase differences between both the samples can be explained from surface roughness and texture of each step. As it can be seen from figure 1, the GO-COOH SPCE surface was found to have regular surface pattern (forest) due to the GO thin layer formation, which is making it smoother as compared to surface of the bare SPCE surface. Moreover, calculated values of surface roughness for bare SPCE (0.480 µm) GO-COOH modifies SPCE (0.076 µm) demonstrates the difference between both the samples and confirms the modification of SPCE surface.

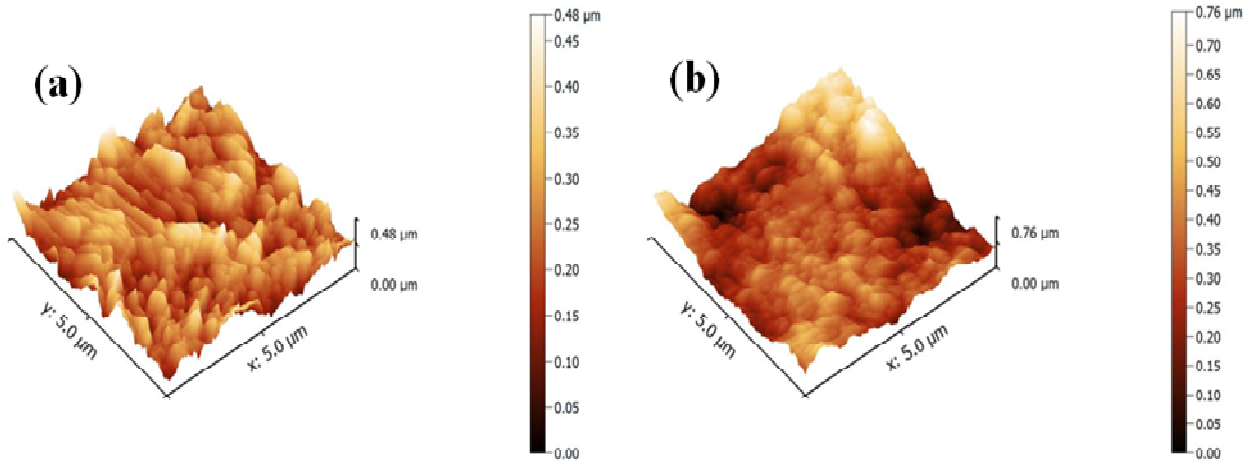



**Figure 2**. Atomic Force Microscopy (AFM) images of bare-screen printed carbon electrode (SPCE) **(a)**, GO-COOH-SPCE **(b).**

### 3.1.3 Electrochemical characterization of modified GO-COOH SPCE

Cyclic voltammetry (CV) was selected for the characterization of the modified electrode surface modification after each assembly step. As reported in the literature, CV in ferri/ferrocyanide solution is a useful and an important tool to monitor the behavior of modified electrode because electron transfer could take place either through the barrier or through the defects in the barrier. All electrochemical measurements were performed in a relatively low concentration (2 mM) of the redox probe [Fe(CN)6]. The presence of $[Fe(CN)6]^{4-/3-}$ allows the detection of a higher current response against the response obtained from an electrochemically inert solution. Cyclic voltammograms for each modification steps were shown in Fig. 3. The characteristic anodic and cathodic peak potentials were observed in case of (a) bare SPCE, (b) SPCE/GO-COOH modified electrode, (c) SPCE/GO-COOH/ EDC activated modified electrode, (d) SPCE/GO-COOH/EDC activated/ 0.35 U/mL concentration of Anti-body modified electrode, and (e) SPCE/GO-COOH/EDC activated/ 0.35 U/mL concentration of Anti-body/ 1 U/mL concentration of Mucin modified electrode. The bare SPCE showed the characteristic quasi reversible redox peak with the anodic and cathodic current peak ratio of ~1 and the peak-to-peak separation of ~1.12 V. After formation of SPCE/GO-COOH modified electrode, the electron transfer resistance ($R_{et}$) between the redox probe and electrode surface was increased due to the

presence of an organic layer with negatively charged carboxyl groups (-COO-), resulting in an increased peak to peak distance. The negative end group activation using EDC introduced a succinimide moiety, cause increase in response due to devoid of negative charge groups. Immobilization of MUC1 antibody on the electrode surface repels a negatively charged redox probe, resulting in an increased $R_{et}$ value. Similarly, binding of MUC1 protein to the modified SPCE immobilized with MUC1 antibody was confirmed with the increased $R_{et}$ value.

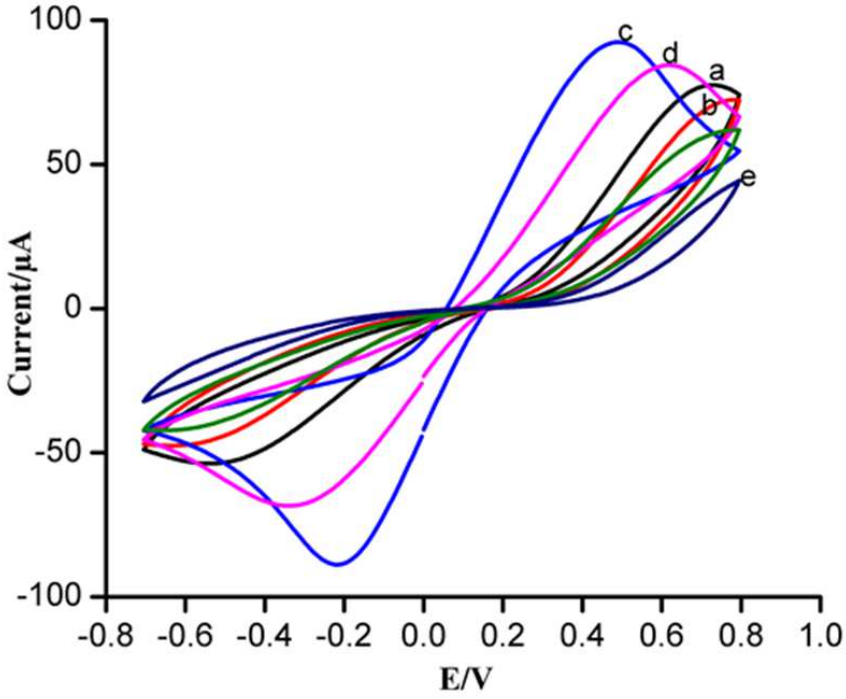


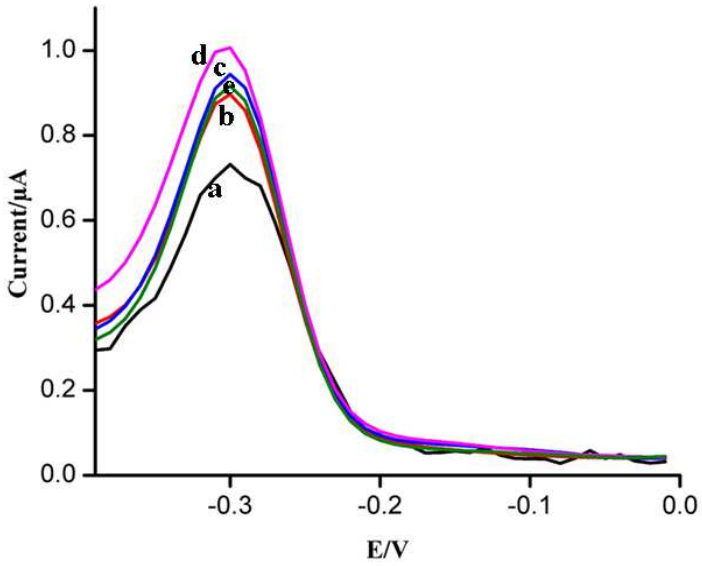

**Figure 3.** Cyclic voltammograms of 2.0 mM $[Fe(CN)6]^{4-/3-}$probe at scan rate of 100 mV/s **(a)** bare SPCE, **(b)** SPCE/GO-COOH modified electrode, **(c)** SPCE/GO-COOH/N-(3-dimethylaminopropyle)-N-ethyle-carbodiimide (EDC) activated modified electrode, **(d)** SPCE/GO-COOH/EDC activated/ 0.35 U/mL concentration of Anti-body modified electrode, and **(e)** SPCE/GO-COOH/EDC activated/ 0.35 U/mL concentration of Anti-body/ 1 U/mL concentration of Mucin modified electrode.

A similar trend to CV was observed in case of electrochemical impedance spectroscopy characterization. In brief, the Rct value for SPCE was found to be 70 KΩ. For the electrode modified with FGO, the Rct was increased to 280 KΩ. Activation of the terminal carboxylic group by EDC resulted in a decrease in Rct to 10 KΩ. Subsequently, anti-MUC1-antibody was

covalently immobilized onto the modified electrode via ester intermediate and an increase in Rct (50 KΩ) was observed demonstrating the successful immobilization of antibody. After binding of MUC1 to surface-bound anti-MUC1-antibody, a higher increase in Rct was observed (350 KΩ).

### 3.2. Optimization of experimental variables for MUC1 detection

Optimization of various parameters in construction of an immunosensor is crucial to achieve a better response against a target analyte. Experimental variables such as antibody dilutions and incubation time could significantly affect the performance of an immunosensor thus prior to MUC1 detection these parameters were optimized. Higher concentration of immobilized antibody may decrease the detection value of the analyte and lower concentration may reduce the signal so different concentrations of antibody (U/mL) (0.35, 0.25, 0.15, 0.5, 0.1) were evaluated to find the optimum response against MUC1 protein. Based on the observation from Fig. 4a, 0.35 U/mL at 1/200 dilution of antibody was adapted for further analysis of MUC1 since a remarkable difference of the signal was observed in case of concentration of 0.35 U/mL as compared to those obtained with other concentrations. Incubation time of analyte protein on antibody-immobilized electrode is another important parameter for immunosensor development. Electrochemical responses were evaluated for varying incubation time (10, 20, 30, 45, 60, 80 min) of MUC1 and immobilized MUC1 antibody in presence of MB probe. As shown in Fig 4b, optimal incubation time was found to be at 60 min for the concentration of 0.1 U/mL of MUC1 since no prominent difference was observed afterwards.

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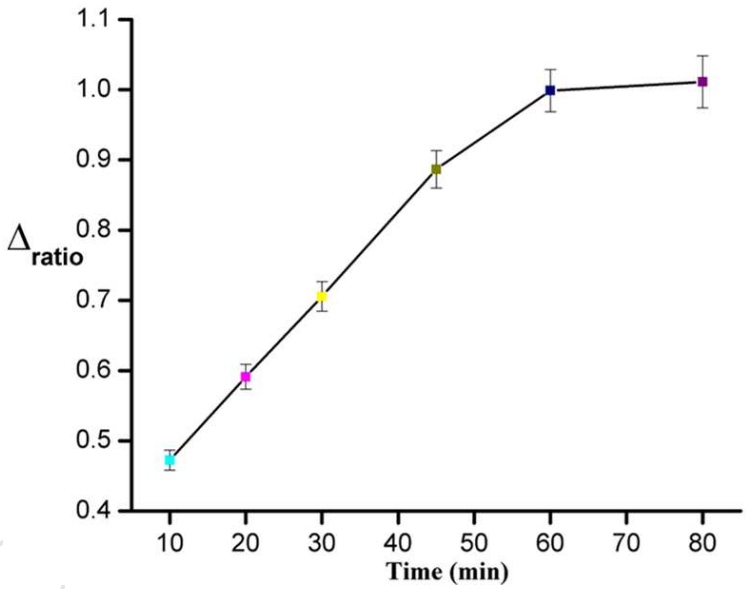
278 **Figure 4a.** Differential Pulse Voltammetry of different Concentrations of Anti-body **(U/mL);**
279 0.35 (a), 0.25 (b), 0.15 (c), 0.5 (d), 0.1 (e).

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281 **Figure 4b**. Optimization of incubation time for concentration of 0.1 U/mL MUC1 detection
282 using developed immunosensor.

283 **3.3. Electrocatalytic study of methylene blue probe**

284 DPV is an important electro-analytical technique to study the redox behavior of a
285 compound. The electrochemical behavior of MB was investigated after interaction with different

MUC1 protein concentrations. After incubation with antibody solution, the electrode surface was washed with buffer. MB was accumulated on the antibody by immersing for 60 min in a 5 µM MB solution prepared in 0.1 M PBS buffer (pH 7.4). Unbound MB was removed by washing the electrode multiple times with rinsing buffer. DPV of 5 µM MB probe at scan rate of 100 mV/s was carried out for comparison of bare and SPCE/GO-COOH modified electrode. Fig 5 represents the DPV voltamograms for bare SPCE (a) and SPCE/GO-COOH modified electrode (b). It is evident from the figure that modified SPCE gives the higher response as compared to bare SPCE when methylene blue was reduces on the electrode surface. The modified SPCE electrode were found to have 0.23 fold better electrochemical response with a difference of 0.4 µM as compared to that of bare SPCE. This response can be attributed to the introduction of carboxylic groups on the modified SPCE, while retaining the improved catalytic behavior towards the reduction of MB probe.

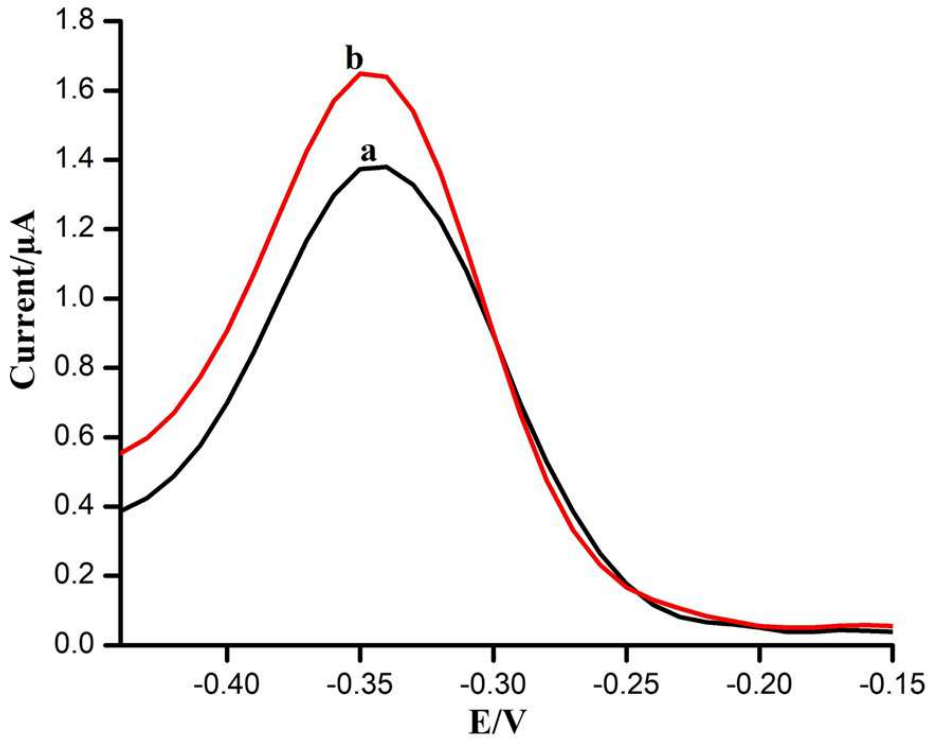


**Figure 5.** Differential Pulse Voltammetry of 5 µM Methylene Blue probe at scan rate of 100 mV/s, bare SPCE **(a)**, SPCE/GO-COOH modified electrode **(b).**

### 3.4 Detection of MUC1 protein using DPV

Antibody immobilized SPCE/GO-COOH modified electrode and MB probe was incubated with the target protein MUC1 for 1 h to allow sufficient interaction between antibody and target analyte. The DPV experiments were performed under optimized conditions for quantitative analysis of MUC1. MUC1 concentration incubated on the modified electrode surface were varied in a range of (U/mL) (a) 0.1, (b) 0.25, (c) 0.5, (d) 1.0, (e) 1.25, (f) 1.5. (g) 2. The obtained results were showed in Fig 6. A linear decrease pattern was observed in peak current values with increasing MUC1 concentrations. The binding interaction between MUC1 and antibody layer was followed by an increase in the electron transfer resistance. The decrease of current could be due to the fact that the formation of anti-MUC1 antibody-MUC1 complex offers a physical barrier, which causes the decrease in the electron-transfer on SPCE surface. The specific antibody-MUC1 recognition interaction induces a decrease in the electron transfer corresponds to the different concentration of MUC1 The corresponding decrease in current was calculated and presented as Δ ratio against various concentrations of MUC1. The original voltammograms of MUC1 detection and calibration curve (linear range) were presented in Fig 6A and 6B. A very low limit of detection (0.04 U/mL) with a linear range from (U/mL) (a) 0.1, (b) 0.25, (c) 0.5, (d) 1.0, (e) 1.25, (f) 1.5. (g) 2 was achieved with good linearity ($R^2$ = 0.97). The reproducibility of immunosensor was verified by the analysis of each concentration of MUC1 using three equally prepared GO-COOH-SPCE modified electrodes. A relative standard deviation of 3-4 % showed a good reproducibility of the developed immunosensor. Obtained results with a linear response of calibration curve and reproducibility of the immunosensor indicates the successful application of GO-COOH modified SPCE electrodes for quantitative analysis of MUC1 protein. The immunosensor showed better analytical performance in terms of sensitivity, linear range and LOD (0.04 U/mL). In Table 1, the results of the proposed immunosensor were displayed in comparable with other biomarkers. This confirms that the presented immunosensor shows better analytical performances to than other technique.

331 Table 2. A comparison of current work with reported literature regarding the performance (e.g.
332 linear range and LOD) of the breast cancer biomarkers.

| **Materials** | **Detection methods** | **Limit of detection (U/mL)** | **Linear range (U/mL)** | **Ref #** |
|---|---|---|---|---|
| ZnO coated nanorods | Quartz crystal microbalance | – | 0.5 – 26 | [22] |
| Gold/Zinc Oxide Thin Film Surface | Plasmon Resonance-Based | 0.025 | 1 – 40 | [23] |
| L-Lys-Ru(dcbpy)$_3^{2+}$ functionalized porous six arrises column nanorods | Electrochemiluminescence Immunosensor | 0.014 | 0.05 – 120 | [24] |
| Coated Polymethylmethacrylate (PMMA) beads | Kinetic-exclusion analytical technology | 0.21 | 0.3 – 20 | [25] |
| Pt nanoclusters | Enzyme-linked Immunosensor | 0.04 | 0.1 – 160 | [26] |
| Catalytic activity of cobalt sulfide/graphene nanocomposite | Enzyme-free electrochemical Immunosensor | 0.03 | 0.1 – 150 | [27] |
| Carboxylic group riched Graphene Oxide | Disposable electrochemical Immunosensor | 0.04 | 0.1 – 2 | **This work** |

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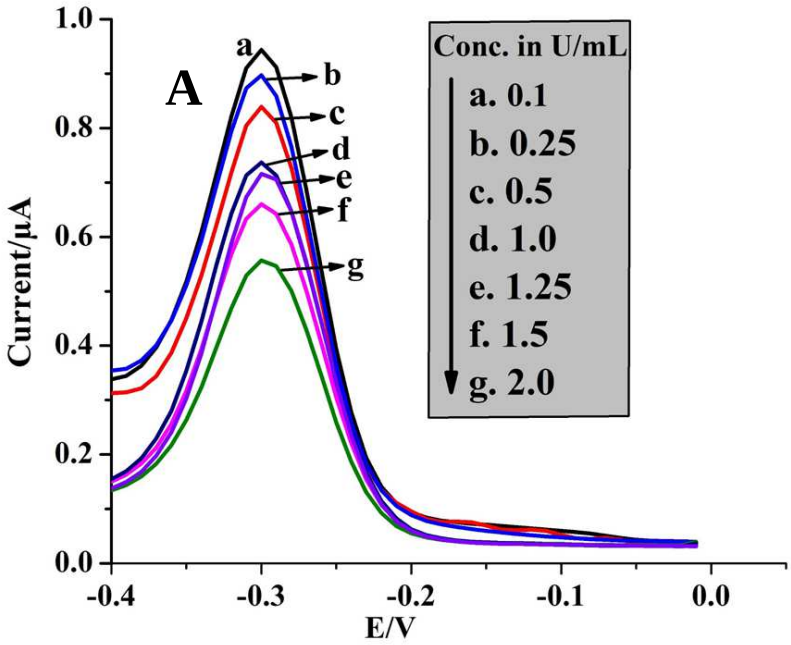


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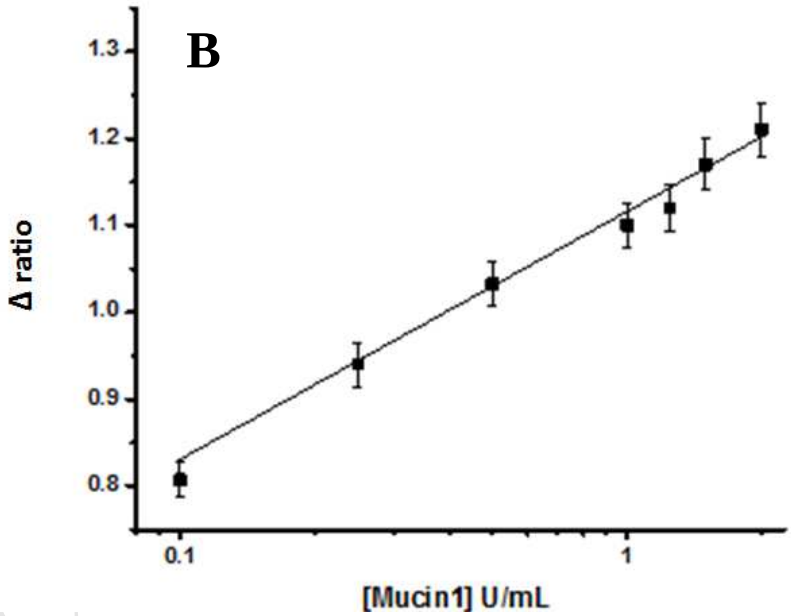


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336 Figure 6 (A) Differential pilse voltamograms of antibody immobilized GO-COOH-SPCE
337 modified electrode with varying concentration of MUC1 protein (U/mL) (a) 0.1, (b) 0.25, (c) 0.5,
338 (d) 1.0, (e) 1.25, (f) 1.5. (g) 2, (B) The calibration curve corresponding to the detection of MUC1
339 protein based on changes of electron transfer rersistance ($R_{et}$), presented as Δratio.

340

### 3.5 Spiked sample analysis and recovery studies

To prove the clinical applicability of the developed immunosensor for detection of MUC1 proteins, artificially spiked human serum samples were used. Standard addition method was used to detect MUC1 spiked in human serum. Several samples were prepared by adding different concentrations of MUC1 (0.25, 0.5, 1.5 U/mL) to healthy human serum samples obtained from Shaukat Khanum Memorial Cancer Hospital & Research Centre, Islamabad, Pakistan. Antibody immobilized GO-COOH-SPCE modified electrodes were incubated with different concentrations of MUC1 as describes earlier protocol. DPV measurements were performed in triplicate and average MUC1 concentrations were determined from the obtained calibration curve. Good recoveries were obtained (96-96.67 %) with % R.S.D. in range of 3.5-4.2%. Recovery results were summarized and presented as Table 2. Recovery results for the real sample analysis and relative standard deviation values indicate the applicability of the developed immunosensor in clinical diagnosis. The developed method can be used to detect MUC1 protein in real serum samples with good reproducibility and sensitivity. The immunosensor can be a promising tool in clinical applications for complex biological matrix.

**Table 2.** Recovery percentages obtained for real serum samples against different concentration of MUC1 using developed electrochemical immunosensor

| **Mucin 1 added (U/mL)** | **Mucin 1found (U/mL)** | **R.S.D %** | **R.E %** | **R%** |
|---|---|---|---|---|
| 0.25 | 0.24 | 3.5 | 4 | 96 |
| 0.5 | 0.48 | 3.7 | 4 | 96 |
| 1.5 | 1.45 | 4.2 | 3.33 | 96.67 |

*R.S.D % = relative standard deviation percentage; R.E % = relative error percentage; R% = recovery percentage.*

### 3.6. Specificity of the developed Immunosensor

Selectivity and specificity of the immunosensor towards the target analyte are some important parameters to prove the practical applicability. An immunosensor is considered reliable only when it does not generate a signal for nonspecific molecules. Therefore, specificity

of the developed immunosensor was evaluated by performing control experiment using non-specific binding proteins, including BSA, FBS and lysozymes. GO-COOH-SPCE modified electrodes were incubated for 1 h with these proteins. Figure 7 shows the Δratio response of the antibody immobilized GO-COOH-SPCE modified electrode after incubating with non-specific proteins, BSA, FBS and lysozymes. Δratio was calculated following the equation described in section 2.6. It is evident from the figure that Δratio values for nonspecific proteins are much smaller than those MUC1. These results demonstrate that the effect of nonspecific proteins is insignificant on MUC1 detection and the developed immunosensor is has sufficient specificity towards MUC1 protein.

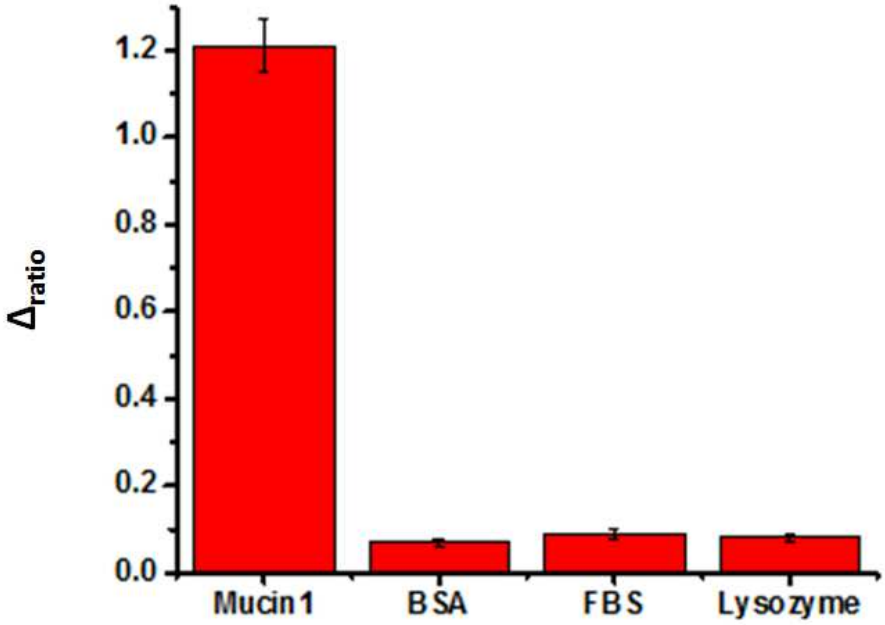


Figure 7. Specificity of the developed immunosensor for MUC1 detection during the competition between non-specific proteins BSA, FBS, and lysozyme.

## 4. Conclusions

In conclusion, we have presented a very simple and inexpensive way for modification of SPCE electrode with GO-COOH and its first time application for the detection of MUC1 protein in

serum samples using a MB probe. Modification of SPCE electrode with –COOH- functionalized GO provided a highly conductive and ideal platform for surface chemistry to immobilize specific antibodies against MUC1, and thus has been demonstrated in the construction of a sensitive and disposable electrochemical immunosensor. Proposed immunosensor offers sensitive and reproducible detection of MUC1 protein for screening of cancer in serum samples. Developed immunosensor can find widespread application in the field of clinical diagnostic and can easily extended to the designing of other type of bio receptor surface based on enzyme, aptamer, and antibodies for various protein biomarkers.

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## Highlights

- Construction of a label free electrochemical immunosensor for cancer biomarker.
- Catalytic behavior of carboxyl rich graphene oxide towards MB in immunosensor
- Excellent performance of the immunosensor for MUC1 detection
- Monitoring of MUC1 in spiked human serum samples