

# Fabrication of Functionalize Reduce Graphene Oxide and Its Application in Ampicillin Detection

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**Abstract** Graphene oxide (GO) was prepared from graphite (GT) with Hammer method, the GO was reduced with hydrazine hydrate to produce a reduced graphene oxide (RGO). The RGO was reacted with thiocarbohydrazide (TCH) to functionalize the RGO with 4-amino-3-symbl-1h-1, 2, 4-triazol-5 (4H) –thion group and to obtain (RGOT). All the prepared nanomaterial and the product of the functionalization RGOT were characterized with Fourier transformer infrared (FT-IR) spectroscopy, X-ray diffraction (XRD) analysis. RGOT mixed with ultrasonic device at different pH values of phosphate buffer solution (PBS), the mixture used to modifying a screen printed carbon electrodes SPCE and with cyclic voltammetry the sensitivity of selectivity of the new modifying electrode to examine. The results show good selectivity at pH<sub>2</sub> to the ampicillin and a redox reaction of the  $\beta$ - lactam group of the antibiotic.

**Keywords** Graphene oxide (GO), Reduced graphene oxide (rGO), Reduced graphene oxide (rGO) functionalization with thiocarbohydrazide, Screen printed carbon electrode (SPCE), Cyclic voltammetry, Effect pH on the redox of AMP, Scan rate and limit of detection LOD

## 1. Introduction

Graphene (g) is a mono layer of graphite. It is the ideal thinnest 2dimentional-substance sheet structure with a good conduction of charge carriers' and bipolar field effect, doing it proper for electronics applications [1]. Graphene has been choose for the applications of various nanodevices e.g. optical sensors, capacitors, electrical and field-effect transistors, etc. [2-5]. In the sensing field graphene is very required because it has the high ratio of surface area to volume. Make plans exactly and the layer of graphene is 2630 m<sup>2</sup>/g [6]. For optical bio-devices graphene oxide (GO) is much desired because of its optical properties and existence of functional groups in biomolecules. The graphene and graphene composites or derivatives have been applied in modification of electrodes of sensors. Various compounds have been detected with electrochemical graphene-modified electrodes [7-11]. Many results were mentioned for sensing on the employment of graphene- field effect transistor (G-FET). The first G-FET sensors used for the gases detection [12, 13] and pH measurement [14] also G- FETs used for the proteins and cells identification [15-17]. G-FET sensors were in prospect to take a good quality of reproducibility better than CNT-FETs [18].

**Functionalization of carbon nanostructures.** Many

carbon nanomaterials applications (such as molecular filters, optoelectronics, microelectronics, sensors and others) need the modification of the nanomaterial surface to get better stability, optical properties or electrical properties, selectivity or solubility and biocompatibility of nanostructures [19, 20]. In case biosensing uses is the binding and functionalization of graphene and CNTs important for their service options with analyte molecules with their receptor molecules. The binding of receptor may be happened as non-covalently or covalently attachment [21].

**Non-covalent functionalization.** The hydrophobic surfaces property of CNTs and graphene adsorb the aromatic molecules by  $\pi$ - $\pi$  bonding. Certain receptor materials, such as hydrophobic proteins or nucleic acids can be attached the carbon nanostructures [22-24]. A molecular interaction occurs when e.g. pyrene-like molecules and or other aromatic molecules maybe attached with an anchor for receptor [25, 26].

**Covalent functionalization.** A variety of reactions like thermic activated electrochemical and photochemical can be made for the covalent modified carbon nanostructures. The oxidation of CNT and Graphene had been created with the formation of epoxide or hydroxyl and reactive carboxyl groups [27]. Different reactions of oxygen groups can represent a location for the dependent addition of the receptor, e.g. the molecules can connected to carboxyl groups through esterification or amidation reactions [25, 27, 28].

**Electrochemical functionalization.** Electrochemical

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functionalization supply's the binding of biomolecules with the carbon atoms surfaces in a multilateral way of a non-covalent or covalent direction [29, 30]. An electrochemical cell or screen printed carbon electrode SPCE made of a counter electrode (CE), a reference electrode (RE) and working electrode (WE). In case a positive potential was applied the oxidation of electricity can be achieved the active component solution, negative potential can lead to reduction process. The reference potential is controlled by (RE) and the observation of the electrical current of the electrochemical process observed with (CE). The non-covalent and covalent changes can be recognized for example; by using receptor molecules have amino phenyl or heterocyclic amino groups. In these cases antibiotics have reached special attention according to some earlier reports [32, 33], the antibiotics and pharmaceutical remnants and endocrine disruptors that have been completely discarded in the environment. An ampicillin antibiotic is useful for the treatment of a number of bacterial infections. In this work was investigated coupling the WE with the functionalized graphene oxide systems. The modified screen printed carbon electrode SPCE was examined for the electrochemical oxidation and reduction of ampicillin antibiotic using cyclic voltammetry.

## 2. Experimental

### 2.1. Chemicals and Reagents

All drugs were analytical grad, double distilled water was used for all the preparations, and the antibiotics AMP

purchased from Samarra drug factory. All used without further clean. The phosphate buffer solution prepared according to [34].

### 2.2. Apparatus

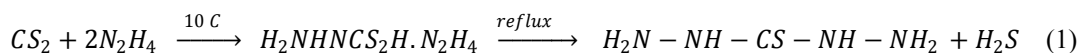
Electrochemical measurement recorded with a portable potentiostat 200  $\mu$  stat (drop Sens S.L. Oviedo, Spain). Screen printed carbon electrode (SPCE) existing carbon CE, RE and a carbon working electrode (4 mm diameter) printed on a ceramic. All pH values were measured with a PH meter BP3001 and an ultrasonic device type (soniprep 150).

### 2.3. Preparation of Graphene Oxide (GO) Reduces Graphene Oxide (RGO)

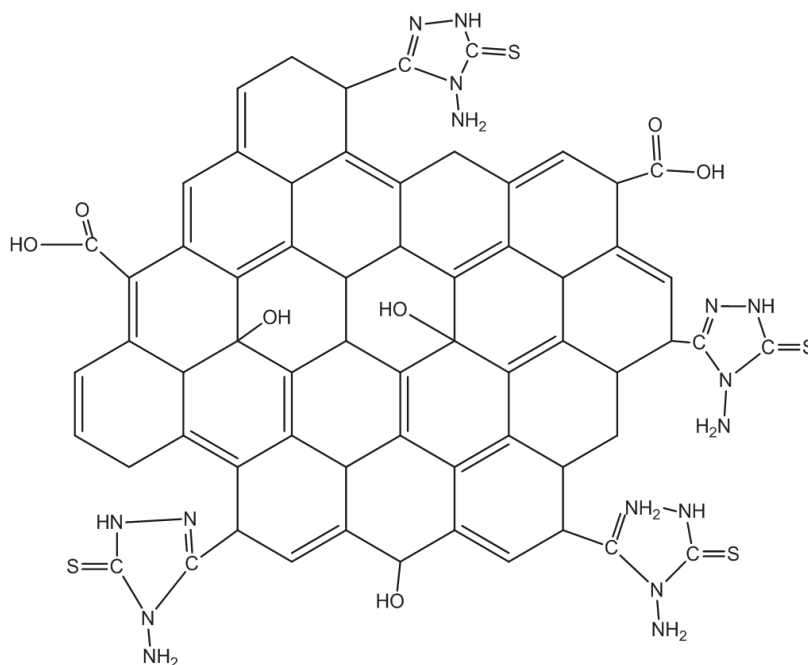
Graphene oxide (GO) are synthesized by the hummers' method [35]. The Reductions of GO was according to be like [36]. Briefly as follows, 1g was sonicated and 100 ml double distilled water in closed round bottle flask until the suspension was without visible particulate matter, hydrazine monohydrate was added and reflux and 100°C for 24h. The black precipitate rinsed with methanol to stop the reaction and then washed with double distilled water. The product was dried at 60°C under the vacuum for 12h.

### 2.4. Preparation of Thiocarbohydrazide TCH

TCH prepared by the reaction of hydrazine  $N_2H_4$  and carbon disulfide  $CS_2$  to produce hydrazinium dithiocarbazinate HDTC.



**Scheme 1.** The reaction of hydrazine with to produce HDTC



**Figure 1.** Functionalized RGO with 5-substituted 4-amino, 3-mercapto 1, 2, 4 Triazole –thion

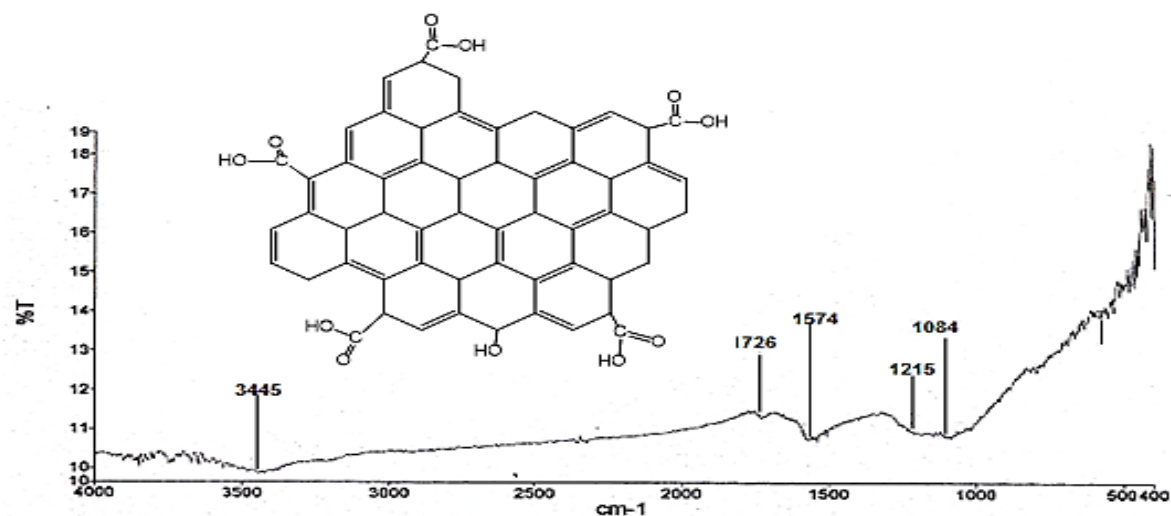


Figure 2. FT-IR spectrum of RGO

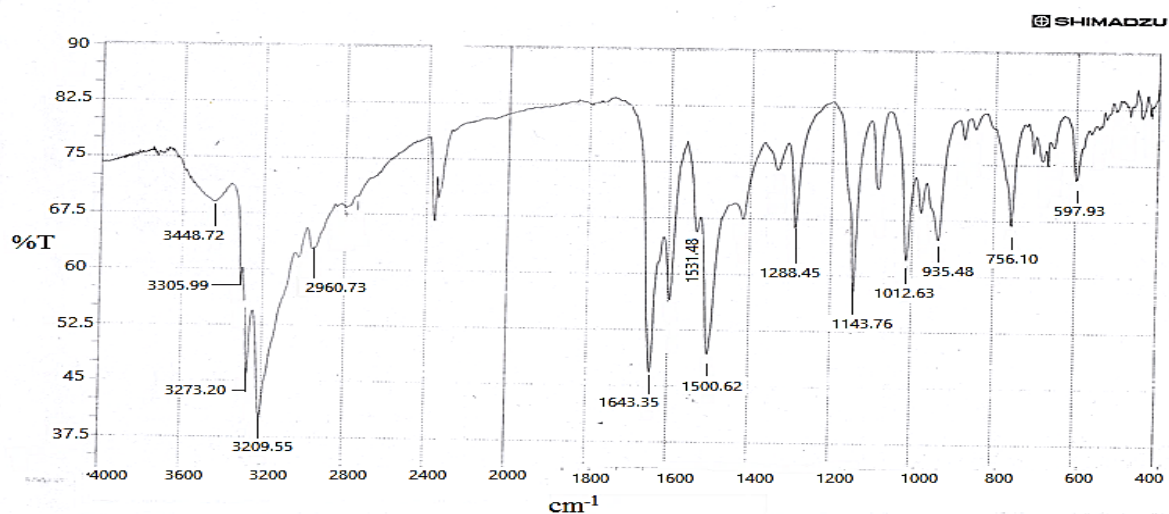


Figure 3. FT-IR spectrum of thiocarbohydrazide TCH

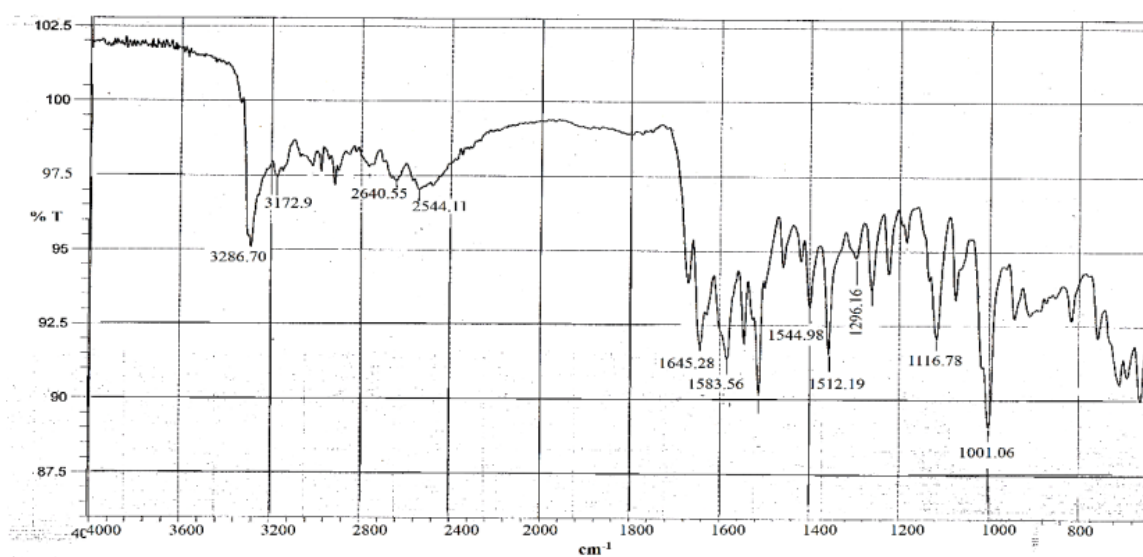


Figure 4. FT-IR spectrum of RGO functionalized with (5-substituted 4-amino, 3-mercapto 1, 2, 4 Triazole -thion) ASTT

About 20 ml of carbon disulfide was placed in a flask in water bath to maintain the temperature at 10°C. Then 80 ml of hydrazine was added with dropping and stirring until the formation of yellow precipitate then resultant mixture refluxed for half an hour to remove H<sub>2</sub>S (equation 1) [37].

The precipitate changed to brown, filtrated, and washed with double distilled water and ethanol. The TCH crystals are dried at 40-50°C under the vacuum for 6h.

### 2.5. Functionalization of RGO with (5-substituted 4-amino, 3-mercapto 1, 2, 4 Triazole –thion)

0.3g RGO was mixed with 1.2g TCH in around bottle flask and heated in the sand bath until TCH melted. The mixture was kept on the 168° C (Fusion temperature of TCH) for 10min and stirring with glass rod. The product of RGO functionalized with 5-substituted 4-amino, 3-mercapto 1, 2, 4 Triazole –thion (RGOT) Figure 1, and washed with distilled water to dissolve the non-reacted TCH and dried under vacuum 60°C.

### 2.6. Modification and Pretreatment of (RGOT- SPCE)

A mixture from 1 mg of RGOT 3μL Nafion was dispersed in 10 ml of double distilled water for 1 h with ultrasonic devices. The WE has been coated with 4 μL of suspended RGOT leaved 2 hours evaporate the solvent in the air. The prepared SPCE was initially pre-treated to increase the sensitivity and to achieve a stable base line for a numerous experiments by sweeping the potential between -0.1 and +1.9 V in 0,1M H<sub>2</sub>SO<sub>4</sub> for 15 times to achieve a repeatable voltammogram [38]. After the activation of the SPCEs were washed and dried, then to the modified and pre-treated SPCEs were added 50μL freshly prepared solution of AMP with PBS as a supporting electrolyte to cover all the reservoir area of the modified WE, CE and RE.

## 3. Results and Discussion

### 3.1. FTIR Characterization

Reduced Graphene oxide FT-IR showed O-H stretching

vibration peak 3445 cm<sup>-1</sup> was significantly small due to the deoxygenation. The stretching at 1726 cm<sup>-1</sup> assigned the C = O (carbonyl / carboxy), (C = C) 1574 cm<sup>-1</sup> (aromatics) and 1215 cm<sup>-1</sup> to 1084 cm<sup>-1</sup> for C-O stretching, some residual oxygen groups were present at 3454 cm<sup>-1</sup> [39, 40] Figure (2).

Figure (3) show FT-IR spectrum of prepared TCH, and peaks at 3209.5, 3273.2, 3305.9 cm<sup>-1</sup> assigned to NH and NH<sub>2</sub> stretching vibrations, The NH<sub>2</sub> 1643.35cm<sup>-1</sup> in 1143, 79 cm<sup>-1</sup> respectively, NH in 1531 and 1500.6 cm<sup>-1</sup> assigned to the N-H and C-N stretching vibrations C = S stretching of the two peaks at 1288.45 and 935.48 cm<sup>-1</sup> [41].

The FT-IR spectrum of RGO functionalized with 5-substituted 4-amino, 3-mercapto 1, 2, 4 Triazole –thion (ASTT) figure (4) was to show a band in 3286, 7 cm<sup>-1</sup> and 3172.9 cm<sup>-1</sup> attributed to NH<sub>2</sub>, N-H at 3154 cm<sup>-1</sup> and 2430cm<sup>-1</sup>, C = S at 1325-1288 cm<sup>-1</sup>.

### XRD characterization of the prepared RGO

Fig (5) shows XRD patterns of RGO peak at 2θ = 24.5° was appeared like the peak of graphite and agreed with (JCPDS Card no.75 -1621) with plane (002) the d- spacing d<sub>002</sub> = 3.3 Å, the constant lattice was a = 2.78Å [35]. The broad peak indicated the amorphous nature of the RGO.

### 3.2. Surface Characterization

SEM imaging was necessary to reveal the morphology of Graphene-based working electrodes. Fig. 6 (A and B) show typical SEM images of two electrodes utilized throughout this work. There is a clear similarity between bare-SPCE (Figs. 6A) and RGOT-SPCE (Figs. 6B) electrode, where (Figs. 6A) can be seen that the surface is slightly rough and disordered in bare-SPCE, while RGO functionalized with (ASTT) RGOT-SPCE (Figs. 6B) seems to be an overall slightly smoother than the bare-SPCE, which is likely due to the RGOT with binder polymer nafion utilizing the electrode ink of the latter to fabricate a more compacted surface. However, the granule's sizes appear smaller than for the bare electrode on the existence of accumulated RGOT sheets. Fig. 6B appears similar to that previously reported SEM image in [42].

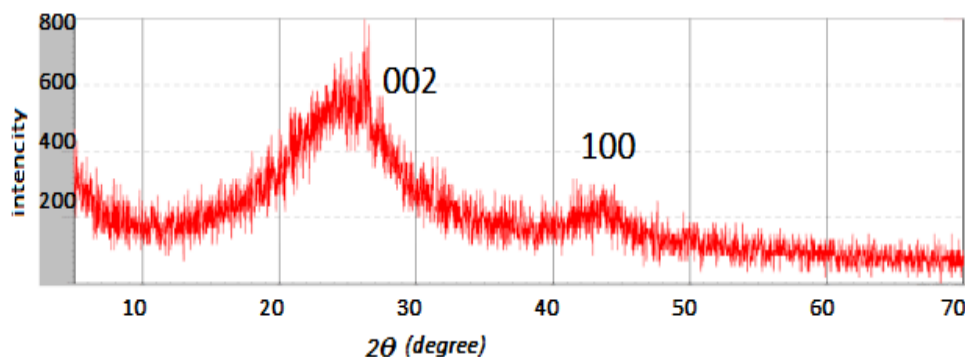
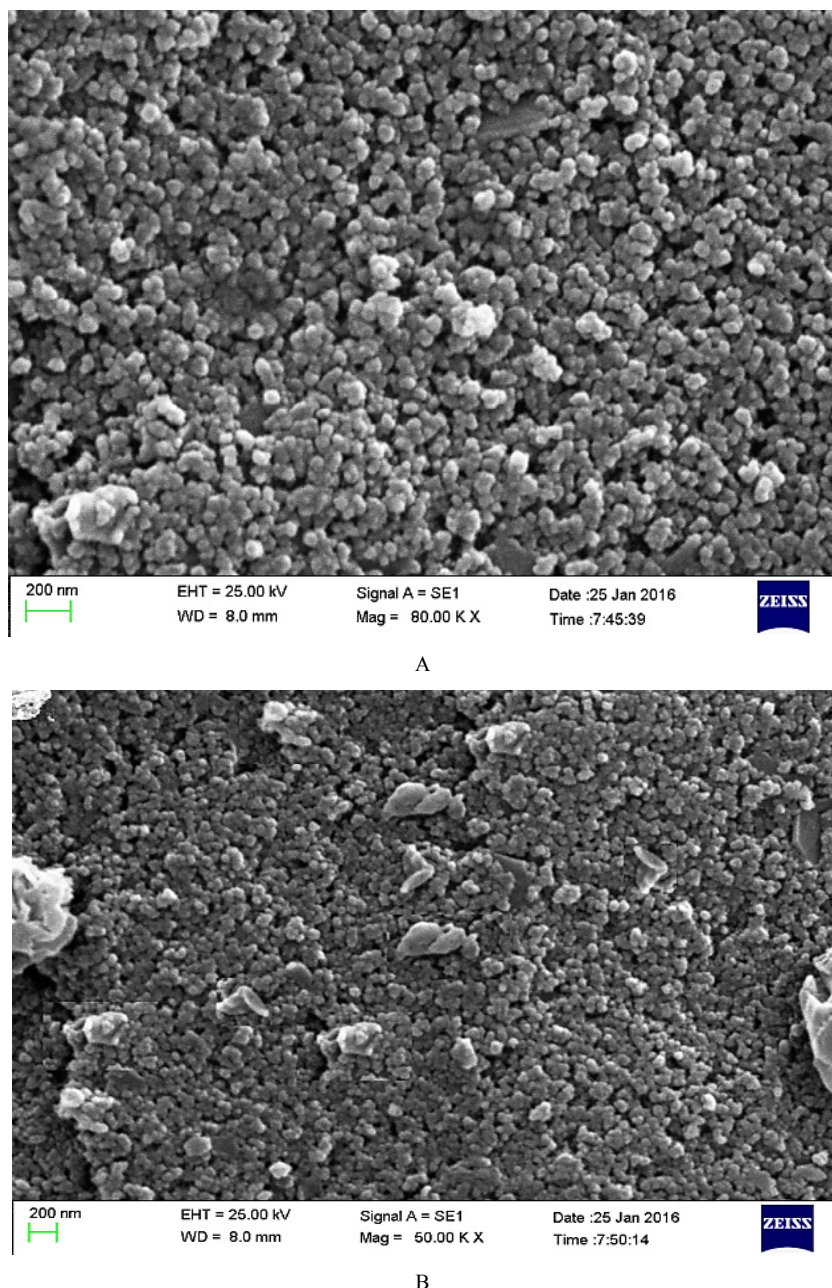


Figure 5. XRD pattern of RGO



**Figure 6.** SEM images of the bare-SPCE (A) and RGOT-SPCE

### 3.3. Voltammetric of Ampicillin and RGOT-SPCE

The  $\text{Fe}(\text{CN})_6^{3-}$  compound is sensitive to the carbon-based electrodes; the  $\text{Fe}(\text{CN})_6^{3-}$  was chosen to study RGOT-SPCE modified electrodes. Bare and reduces Graphene oxide-SPCE modified (WE) electrodes was coated with 0, 5 m M ferrocyanide solution and the cyclic voltammograms (CVs) shows. Fig.7. the CV cycles display of oxidation and reduction process for the redox of  $\text{Fe}_3^+ / \text{Fe}_2^+$ . It was evident that the peak current intensity of bare-SPCE was lower than that of modified electrode with the reduced Graphene oxide. It was because the oxygen functional groups reinforce the electric resistance and decreases the charge transfer between the modifying layer and substrate but the functionalized reduced graphene oxide with (ASTT) increased the

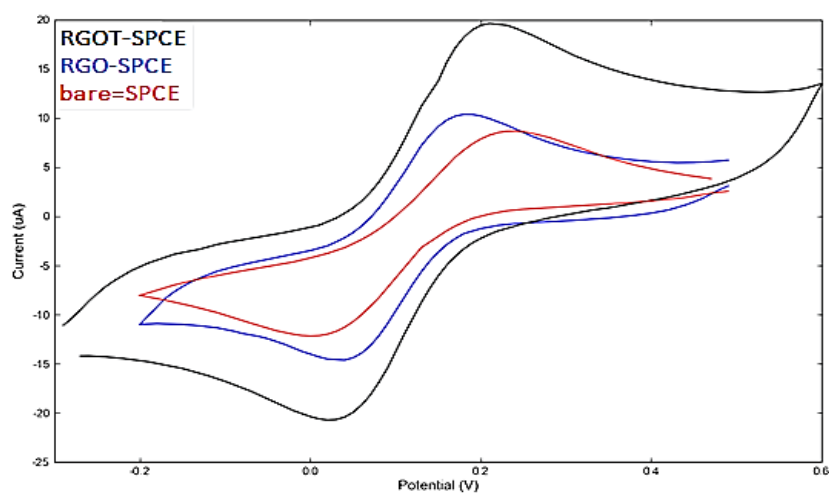
electroactive surface area and provided the conductive bridges for the electron-transfer of ferrocyanide. Therefore, electro catalytic activity of reduced Graphene oxide was further enhanced by the electrode by introducing various ASTT group in the structure of the RGO. However, RGOT-SPCE modified electrodes show better results than that bare-SPCE.

Moreover, the electrochemical stability of the RGOT-SPCE tested. The results show that the highest number of definitions of modified electrode was almost the same as the second CV cycle on the 50<sup>th</sup> repeated CV cycling fig.8.

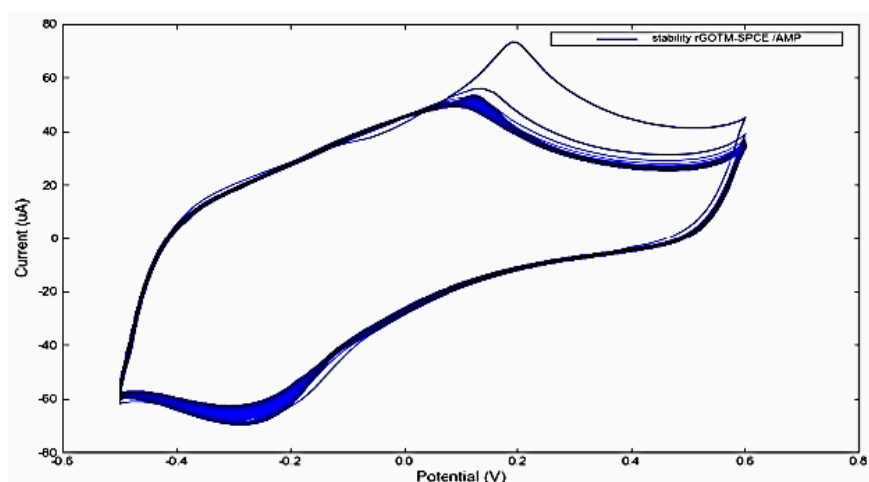
Figure 9. A very well difference appeared between the cyclic voltammograms of RGOT-SPCE composites and bare-SPCE in the presence of ampicillin AMP in PBS at the



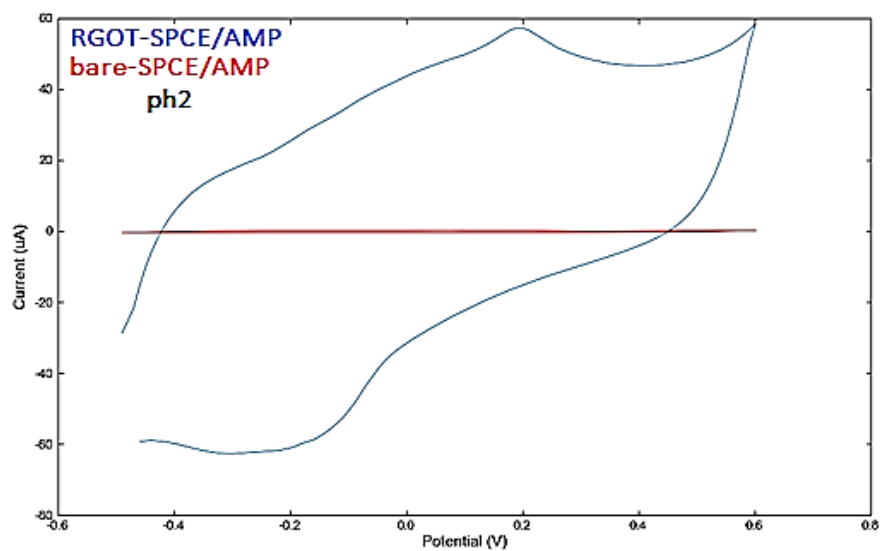
pH<sub>2</sub> value the scan was with rates  $0.1\text{V}\cdot\text{s}^{-1}$ . The obvious -0.19V on the modified RGOT-SPCE. oxidation peak was found ca. +0.19V and the reduction of ca.



**Figure 7.** Cyclicvoltagram in red the bar-SPCE, in blue RGO-SPCE and in black RGOT-SPCE



**Figure 8.** The electrochemical stability modified electrode for more than 50 time repeated cyclic voltammetry cycle



**Figure 9.** The behavior of RGOT-SPCE and bare-SPCE in the presence of AMP at pH<sub>2</sub>

The results show that on the application of RGOT-SPCE, the electrochemical redox of AMP is much stronger than in the bare-SPCE, so that the composites electrodes attractive for applications in the field of electrochemical Sensing. The bare-SPCE indicates low conductivity because of the carbon material in the SPCE poor with the covalent double bonds cause an increase in the band gap. However, the conductance of the prepared RGOT-SPCE displayed higher conductivity than the bare-SPCE, In addition, AMP interaction with ASTT and the deprotonation of ASTT induced the changes in the electronic structure of the ASTT and during the deprotonation was added the hydrogen ion to the  $\beta$ -lactam group of AMP in aqueous solution to yield the carboxylic acid group instead of ketone group scheme 2.

Therefore, we consider that both the deprotonation of ASTT and high conductivity of RGOT-SPCE facilitate better sensing performance for AMP. To show the dependence of the electrocatalytic current concentration of APM was measured cyclic voltammograms of RGOT-SPCE and various PBS solution pH values. The figure10 appeared at pH<sub>2</sub> the highest current value, and at this pH value was the best deprotonation of ASTT and at pH<sub>2</sub> examined a series concentrations of AMP have been obtained. Fig. 11

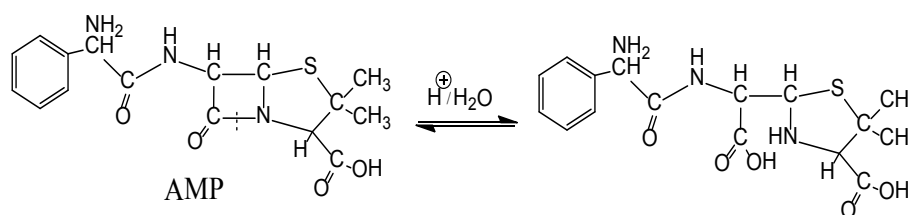
depending on the application of AMP the oxidation peak current was increased with the increasing of AMP concentration. At present, there was a slight shift in the peak position of concentration, it may be said that the adsorption of the side-product of the electrochemical oxidation products on the electrode surface.

Figure 12 show linear relationships of the oxidation peak at different concentration of AMP ( $1 \times 10^{-6}$  -  $5 \times 10^{-5}$  mol.). The linear regression equation was  $I(\mu A) = 1.4648 (\mu M) + 26.264$ , with the correlation coefficients of 0.8139. The limit of detection was calculated according to equa.1 and obtained the result 0.12  $\mu\text{mol/L}$

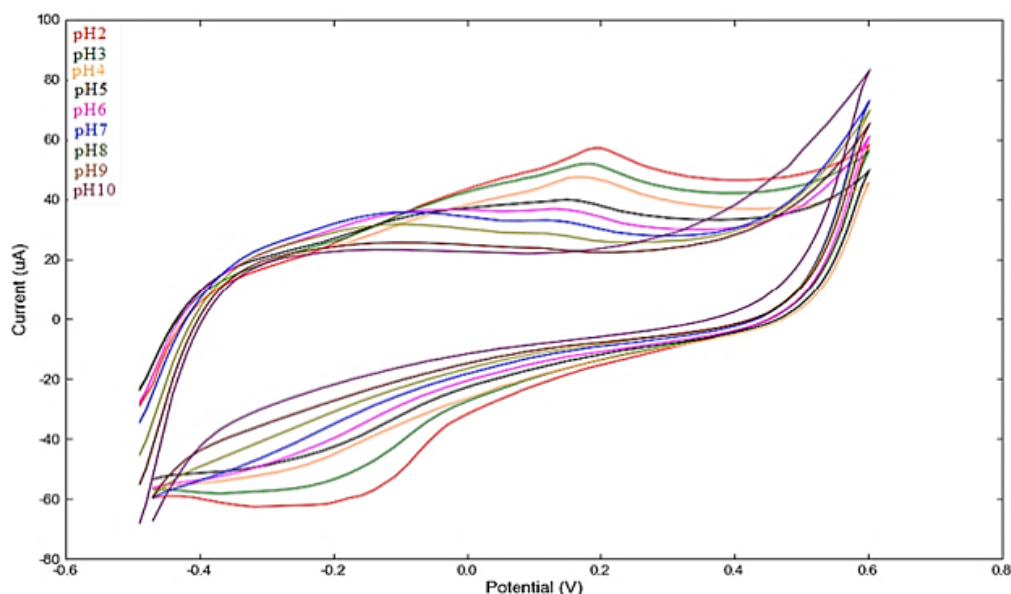
$$LOD = \frac{3S_y}{b} \quad (1)$$

Where  $S_{y/x}$  was the standard deviation of peak current and  $b$  is the slope of the related calibration curve.

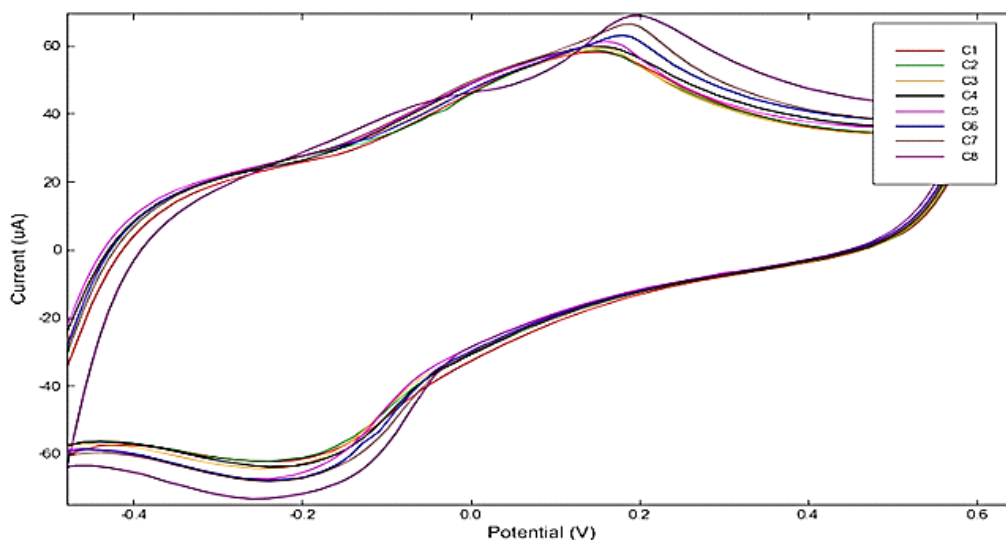
It was noted that the research's focusing on identifying and determination the LOD of the ampicillin with the CV technique very few. For comparison, kloth *et al.* were mention to 1.1  $\mu\text{g/L}$  for different antibiotic in raw milk, Aderian *et al.* were mention to LOD value of 58-190nmol/L and Leyden MR *et al.* were mention to 0.67  $\mu\text{mol/L}$ .



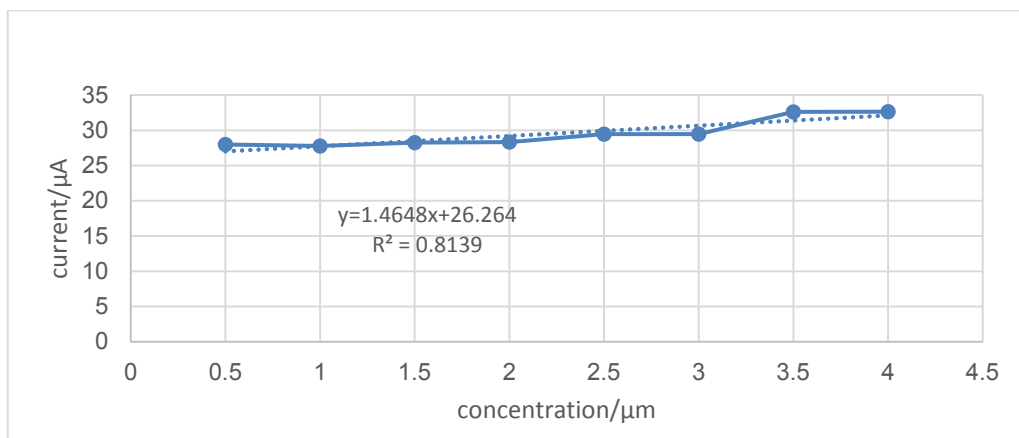
**Scheme 2.** The formation of carboxylic acid group instead of ketone group during the protonation process on the  $\beta$ -lactam group



**Figure 10.** Cyclic voltammogram of RGOT-SPCE/AMP in different pH



**Figure 11.** Cyclic voltammogram of AMP (PBS pH<sub>2</sub>) at different con. ( $1 \times 10^{-6}$  to  $5 \times 10^{-5}$  mol.) with scan rate  $0.1 \text{ V.s}^{-1}$



**Figure 12.** Oxidation peak at the different concentration of AMP

## 4. Conclusions

In summary, we have prepared RGOT SPCEs composites with covalence bonding of ASTT with RGO. The characterization process shows a successful formation of composites. The detection of ferrocyanide was with electrochemical reaction and shows that the signal enhancement of composite, lead to suggest that the application of RGOT in electrochemical sensors. Furthermore, it is clear that the modified RGOT- SPCE display the electro catalytic activity to the oxidation and reduction of AMP.

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