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## Synthesis of modified Graphene Oxide and its application as Electrochemical Sensor

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Bу

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## Abstract

This study involved preparation of Graphene oxide (GO) and reduced graphene oxide (RGO) using Hummer method and chemical method respectively.

These carbon nanomaterials were used as starting material to make novel functionalize with thiocarbohydrazide (TCH) which was prepared by reacting  $CS_2$  with hydrazine to form GO or RGO- 4-amino,5substituted 1H,1,2,4 Triazole 5(4H) thion (ASTT) ,(GOT) and( RGOT) respectively via cyclocondensation reaction.

Also  $MnO_2$  nanorod was prepared to form hybridized with GOT and RGOT. A commercial multiwall carbon nanotube (MWCNT) and functionalization with carboxylic groups' (f-MWCNT) and its nanocomposite with GOT were also prepared.

All carbon nanomaterials were characterized with different techniques such as Fourier transform infrared (FT-IR), X-ray diffraction (XRD), atomic force microscope (AFM) scanning electron microscope (SEM) and elemental analysis. XRD showed presence diffraction peak at 11.95 for GO and this diffraction disappeared for RGO. Diffraction peak of crystal planes for  $MnO_2$  matched well with standard data. The diameter of  $MnO_2$  nanotubes was determined using Debye scherrer equation and found to be 11.6nm corresponding with AFM image. The AFM images proves the growth of  $MnO_2$  nanotubes from the  $MnO_2$  nano spherical shape these images are very rare in the scientific literature.

The real permittivity ( $\epsilon$ '), imaginary permittivity ( $\epsilon$ ") and a.c conductivity (S.m<sup>-1</sup>) of all nanomaterials were measured by LCR meter at frequencies ranging from 100Hz to 100 KHz.

The result showed the values of the real permittivity for RGO higher than GO at all frequencies while RGOTM have lower values of real permittivity at low frequency due to presence of MnO<sub>2</sub> nanorods which affected the accumulation of charges. The imaginary permittivity of f-MWCNT-GOT and RGO were at low frequency higher than the real values due to their high conductivity. Also imaginary permittivity of f-MWCNT-GOT nanocomposites at all frequencies higher than real which have negative values at frequencies in range 400 to 4KHz .a.c conductivity for RGO and f-MWCNT-GOT nanocomposite have higher values compared with all prepared nanomaterial, at the same time the modified WE with f-MWCNT-GOT nanocomposite show the best detection limits in comparison with other prepared modified WE.

Also the prepared nanomaterials were used to study novel sensing system and develop electrochemical sensor capable of detecting some of antibiotics such as Ampicillin (AMP), Amoxilline (AMOX) which have  $\beta$ -lactam ring and Tetracycline (TET) which contains four hydrocarbon rings using cyclic voltammetry (CV) technique via modification of the working electrode of the SPCE with the prepared nanomaterial by deposition process. f-MWCNT-GOT/SPCE nanocomposite showed higher electrochemical reaction response and lower limit of detection. The working electrodes surfaces were studied with AFM and SEM techniques. The value of apparent heterogeneous electron transfer rate constant (k<sub>s</sub>) was determined using the value of electron transfer coefficient ( $\alpha$ ) and the result showed that f-MWCNT-GOT/SPCE showed higher (k<sub>s</sub>).

## Introduction

#### **General Introduction**

Carbon (coal in Latin language) can be found in every living organism. It is a non-metal element, the basic building block in millions of various compounds. Carbon have allotropes such as amorphous carbon, diamond (very hard) and graphite (very soft) depending on ways of their bonding. Carbon nanomaterials have attracted enormous attention since their arising and have been intensively studied because of their unique structure, chemical electronic, mechanical, and optical characteristics. Low-dimensional carbon nanomaterials can be distributed to classes of different dimensionality depending on their nanoscale range (<100 nm) in different spatial directions, one-dimensional (1-D) like carbonencapsulated, onion-like carbon, nanodiamonds, carbon nanofibers, carbon nanotubes, metal nanoparticles and fullerene, and twodimensional (2-D)like graphene G, and graphene oxide GO. All the above carbon nanomaterials structure were depended on the graphite sheets structure [1].

## 1. Carbone nanomaterials

#### **1.1 Graphite**

Graphite (GT), has two dimensional layers (planar structure), and the distance between planes is 0.335 nm [2]. Carbon atoms are arranged in a <u>honeycomb lattice</u> in covalently sp<sup>2</sup> hybridization, with separation 0.246 nm. Van der Waals force held together in the stacks [Fig. (1-1)].



Graphite

Figure (1-1) Structure of graphite, consisting 8 stacked graphene layers

GT is a soft material with Mohs hardness of 1-2 and a density of 2.1-2.3 g.cm<sup>-3</sup>.It is present in two forms *alpha* (hexagonal) which is thermodynamically stable form and *beta* (rhombohedral), which has very similar physical properties. Only weak interactions between the  $\pi$ orbitals, these weak interactions explain the using of graphite as lubricants. The sp<sup>2</sup> hybridization and associated delocalized  $\pi$ -electrons also explain the electrical conductivity of the graphite.

#### 1.2 Graphene

An individual graphite layer is called pristine graphene. The name G comes from graphite + ene. It is the thinnest imaginable and realizable sheet of carbon atoms. Graphene is a planar single sheet of carbon atoms with thickness of one atom. Tightly packed into a two - dimensional lattice (honeycomb) [3] [Fig. (1-2)].



Figure (1-2) Atomistic structure of a graphene monolayer.

Graphene has metallic character and consists solely of carbon and hydrogen. G can be used as a basis not only of graphite, but also the carbon nanotubes and fullerenes.

The carbon nanotubes CNT discovered in1991 in graphite <u>electrodes</u> during an arc discharge [4]. While fullerenes, new allotrope of carbon formed by the three-dimensional folding of graphene in a spherical shape which is discovered in the 20th century. Fullerene molecules consist of 60, 70, or more carbon atoms. C60 has the form of football and is also called Bucky ball made up from 20 hexagons and 12 pentagons. Thus graphene was taken an intermediate position between the three-

dimensional graphite and the one-dimensional carbon nanotubes, as shown in the Fig. (1-3).



Figure (1-3) Schematic of graphene, and graphitic forms (fullerene, carbon nanotube, and graphite) [3]

## **1.3 Properties of graphene**

Graphene was a semimetal with a very small electron density or a semiconductor [5 and 6] with a vanishing band gap. The  $p_z$  electron completely fill the lower band (valence), the upper (conduction) band has no electron as a result valence band and the conduction band touch at the Dirac points (six corners of the Brillouin zone) as shown in Fig.(1-4).The charge carriers has a zero effective mass at the K and K point [7]. By virtue of its hexagonal lattice structure, the charges carriers in the two-dimensional electron system of graphene was protected against backscatter and therefore have huge mobility [8].



## Figure (1-4) The band structure of graphene, the conductance bands touch the valence band at $K_x$ and $K_y$ points [7].

The energy bands start to overlap as the number of layers in graphene samples increases, (the overlap is 1.6 meV for two layers) and it becomes semi-metallic. Many of the interesting physical properties of graphene were measured experimentally in recent years which has theoretical investigations in advance forecast [9]. Graphene is a single layer which has a theoretical specific surface area of 2630  $\text{m}^2.\text{g}^{-1}$  surpassing that of graphite 10  $\text{m}^2.\text{g}^{-1}$  and two times larger than of single walled carbon nanotube ~1315  $\text{m}^2.\text{g}^{-1}$  [10] surface area of graphene sample (few layer) was found to be in range 270-1550 m<sup>2</sup>.g<sup>-1</sup> using Brunauer-Emmett –Teller (BET) method [11] Large surface is an essential characteristic of an electrode material, particularly in sensing devices. This can give rise to fast electron transfer and high densities of attached analyte molecules. Electrical and thermal conductivity is essential properties for material that used in electronic and photonic industry, graphene has a very high electrical conductivity 64 mS cm<sup>-1</sup> at room temperature which is about 60 times larger than of single wall carbon nanotube(SWCNT) and has the highest known thermal conductivity 5000W.m<sup>-1</sup>.K<sup>-1</sup> [12] 25 times than that of silicon [13]. Optically, graphene is quite transparent with 70% transmittance over the visible and infrared regions of the spectrum [12] and exhibit high optical absorptivity 2.3% [14]. Nano indentation test in AFM microscope of monolayer graphene membrane showed that the graphene is the strongest material with intrinsic strength equals to130 GPa [15 and 16] the breaking force of 42 N m<sup>-1</sup> and Young's modulus of 1 TPa.

## 1.4 Graphene oxide (GO)

## 1.4.1 Brief history of graphene oxide and graphite oxide

Graphite oxide (GTO) history can be traced back to some of the earliest studies about intercalation and exfoliation. intercalation of graphite by oxidizing and reducing agent was discovered in 1840 by German scientist Schafhautt [17] who observed "blue graphite" upon reaction with sulfuric acid and nitric acid then, in 1859 the oxford chemist Benjamin Brodie was the first who prepared graphite oxide by treating graphite with an oxidation mixture which consists of fuming nitric acid and potassium chlorate [18].

Improved method was developed by L. Staudenmaier in 1898[19]. He reduced the reaction time of the Brodie method by adding sulfuric acid into the mixture of  $KClO_3$  and  $HNO_3$ this change made the production of highly oxidized GO significantly more practical.

The oxidative treatment of bulk graphite transforms it into graphite oxide (GTO) which consists of multilayers structure of graphene oxide (GO). Graphene oxide is one layer of a polycyclic hydrocarbon network and is partially aromatic, has various oxygen functional groups (CO, OH, and COOH) as shown in Fig. (1-5) .From the chemical oxidation process which introduces high density electronegative oxygen atoms on the GO basal plane and cause a rise in the energy gap making GO non-conductive [20]. The epoxide and the hydroxyl groups lay on the surface sheet while carboxyl and carbonyl groups attached at the edges. A strong sonication of GTO aqueous suspension can produce individual layers of GO [21 and 22].



Figure (1-5) The chemical structure of GO contains different oxygenate groups, including carboxyl, hydroxyl, and epoxide.

GO was electrically insulating due to the  $sp^3$  of carbon atoms on the basal plane. The presence of negatively charged (carboxylate surface groups) results in an electrostatic repulsion between the GO layers, therefore obtained stable GO aqueous dispersions which consist of a large part of monolayers over weeks.

Besides GO shows excellent hydrophilicity and relatively easily dispersed in water and in polar organic solvents make it biocompatible. The hydrophilicity of GO allows it to be uniformly deposited in various substrate by methods such as spraying ,spinning ,coating ,drop-casting by forming Langmuir –Blodgett films[22]which is used for application in electronics and as excellent electrode materials[23].

## 1.4.2 Structure of graphene oxide (GO)

The exact structure of GO isn't known because a sample to sample variability. It's highly amorphous even though it is composed from(C, O and H) and overlapping of broad bands and the multiple closely related vibrations. Theoretical calculations, spectroscopic data and all forms of analytical techniques were used to disclose the accurate structure of GO, such as X-Ray diffraction (XRD), X-Ray photoelectron spectroscopy (XPS), elemental analysis technique and vibrational spectroscopy, including IR, Raman, UV-visible and photoluminescence spectroscopy.

The first structure of GO was proposed by Hoffman & Holst in1939, in this proposed structure, the atomic surface of GO was embellished with only epoxy were covalently bonded to carbon atom and the molecular formula C/O ratio was 2 [24]. Later, in 1947, Ruess proposed that the backbone of GO consist of a Trans –cyclohexane instead of flat sp<sup>2</sup>. He also included axial hydroxyl in the structure and replaced epoxy group with 1, 3 ether. This model was the first to account for the presence of hydrogen in the GO which later modified with double bond [25]. Alternating rows of 1,4 benzoquinones and cyclohexane with additional OH group was the model proposed by the Schulz and Boehm model.

The Nakajima-Matsuo model include only hydroxyl groups attached to carbon atoms with a fully sp<sup>3</sup>-hybridised structure [26 and 27], also the more acceptable model was the Lerf-Klinowski model consisted of in addition to randomly distributed 1,2-epoxides, it included also hydroxyl groups[28 and 29]. The most recent models have focused on amorphous and on stoichiometric instead of lattice based model was Szabo, Berkesi–model involved quinone –cyclohexane structure with OH groups and 1-3 ethers [30].

The Lerf-Klinowski model was the first to utilize 1D & 2D C<sup>13</sup> MAS Solid-state NMR to reveal structural insights into GO and this showed present 1, 2 ether (epoxy), tertiary alcohol and alkenes on the graphitic plane[31]. By the harsh oxidation reaction conditions of Hummer's method the isolated double bonds would break down, and an aromatic graphitic domain would be strong withstand and display in NMR spectra. lastly by combination IR spectra with C<sup>13</sup> NMR deduced present carboxylic acid and carbonyl groups attached on the edges of GO. Gao and coworkers in2009 published another paper using C<sup>13</sup> NMR their paper reports the presence of 5- and 6-emberered-ring lactols along the edges of GO[32]. This structure of GO is widely accepted today, although a few reports have been contradictory [33 and 34]. Fig. (1-6) showed the most of GO proposal structure over the years.



Figure (1-6) Proposal structures of GO [35]

## 1.5 Preparation of graphene oxide and graphene

Graphite is the original source for the preparation and manufacturing of the different carbone nanomaterial. Reduced graphene oxide (RGO) was prepared using chemical methods from the reduction process of the graphene oxide. Hummer and Offeman in 1958 developed an efficient process to prepare GTO called Hummer method using mixture of strong acid and oxidant H<sub>2</sub>SO<sub>4</sub>, NaNO<sub>3</sub> and KMnO<sub>4</sub> [36]. This method is currently the most commonly used process.

The unprecedented surge in interest in graphene was promoted the development a number of different manufacturing methods. First, topdown approach which is commonly employed in literature, i.e. started with graphite, either in the form of millimeter-sized crystal substrates (highly oriented pyrolytic graphite, HOPG) or graphite oxide. Second, bottom-up approach started with carbon precursors. In both cases; the aim was the exfoliation.

#### **1.5.1 Reduction of graphene oxide.**

Researcher developing some strategies for reduction graphene oxide to remove the oxygen groups and to rehybridize  $sp^3$  to  $sp^2$  carbon atoms. The result in, the resistance decreased by several orders of magnitude, which transforms the GO into a semiconductor or a graphene-like semimetal. Experimentally after the reduction process number of oxygen groups and abundant structural defect remain [37]. These defects (such as rings with 5 or 7 carbon atoms instead of 6 and holes) disrupt the classical carrier transportation among  $sp^2$  carbon clusters [38]. For this reason G produced through reduction of GO often called reduced graphene oxide (RGO) and should discriminate it from pristine graphene.

RGO is few layers about 2-10 of a polycyclic hydrocarbon network, it has oxygen fraction of around or below 10% .It is mostly aromatic and resemble pristine graphene in the term of mechanical and thermal properties, but RGO lacks the excellent electrical conductivity of G due to broken conjugated structure. It's conductivity at values below of pristine G. The reduction process of the graphene oxide may be occurred with many different ways e.g. chemical, thermal methods, photo catalyst etc.

#### **1.5.1.1 Chemical treatment**

GO can be reduced by using reducing agents such as hydrazine [39], and many other compounds as shown in Fig 1-7(a). Hydrazine has activity to eliminate most functional groups of GO. Hydrazine reacted with epoxide groups by opening ring reaction to form hydrazine alcohol as shown in Fig. 1-7(b) [40]. This derivative in turn reacts to form amino aziridine. A double bond consists through thermal elimination of di imide. The hydrazine didn't reduce carboxylic groups [41]. Hydrazine reductant was found the best one to produce fine, thin and stable RGO aqueous dispersion [42]. When dried, a black powder, electrically conductive was obtained [43].



Figure (1-7) (a) Reduction GO to RGO. (b) A proposed reaction pathway for epoxy reduction by hydrazine. [41]

The advantage from this fabricated methodology is high yield, low cost, practicability of sample handling (liquid suspension), offers the greatest ease for functionalization and to a wide variety of application such as conductive inks and paints, polymer fillers, supercapacitors, sensors and battery electrodes.

#### **1.5.1.2 Photocatalyst reduction**

Photochemical reaction is another method to reduce colloidal GO by using photocatalyst such as  $TiO_2$  particles (semiconductor) under UV irradiation [44]. When UV irradiate , charge separation occurs on the surface of  $TiO_2$  particles, ethoxy radicals (from ethanol) will accumulated electrons within  $TiO_2$  particles and reduced the functional group of GO.As shown in the equations below

$$\begin{split} TiO_2 + h\nu &\rightarrow TiO_2(h+e) \rightarrow TiO_2(e) + C_2^{\circ}H_4OH + H^+ \qquad (1-1) \\ TiO_2(e) + GO \rightarrow RGO \qquad (1-2) \end{split}$$

#### **1.5.1.3 Electrochemical reduction**

This method needs no special reduction chemical agent, but using electrochemical cell with aqueous of buffer solution or in various organic solvents, too. GO reduced by applying potential range from 0 to 1.0 V (in respect with a saturated calomel electrode) using cyclic voltammetry. The thin film of GO deposited on a substrate such as indium tin oxide (ITO), glass, plastic will reduce through the electron exchange between GO and electrodes.

Reduction of GO began at 0.6 V and reached a maximum at 0.87 V[45]. Zhou *et al* [46] reported that, the pH value of the buffer solution affected the potential needed to reduction also a low pH value was favorable in the reduction of GO proposing that  $H^+$  ions participate in the reaction.

#### **1.5.1.4 Thermal treatment**

Graphite oxide (GTO) can be exfoliated and reduced by exposing it to extremely high temperature (1050 C° in the argon atmosphere) [47]. This result in formation CO or CO<sub>2</sub> gases from oxygen functionalities of graphene oxide which creates pressure between graphene oxides (GO) in graphite oxide and thus helps to exfoliate [48]. RGO according to another report has electrical conductance which is four times of magnitude higher than of GO.

#### 1.5.2 Chemical vapor deposition (CVD)

Chemical vapor deposition is the one of the important methods (The bottom-up methods) used carbon precursors like (methane, ethylene) on various transition metal substrate [Fig. 1-8(a)] [49 and 50]. Few layers G, using Champher as precursor, was fist successfully prepared in 2006 in Ni foil[ 51] to prevent formation of multilayers of G(deposit monolayer), thin film of Ni (30nm) on silicon dioxide –silicon substrate was deposited. Recently, workers [52] product thin film of G (30inch) by rolled –to rolled process graphene in this process have transmittance about 97.4% and resistance125 ohm. Square [Fig. 1-8(b)].

The principle of CVD is decomposed precursor molecules from the gas phase or from a liquid phase at elevated temperature. There are many main variables in every CVD processes must optimized to obtain large area from graphene (monolayers) such as quality of the metal substrates, rate flow of precursors, quality of the metal substrates and the thickness , temperature of the reaction chamber ,heating and cooling rates. Study has shown that faster cooling rates didn't gave large area of graphene films while very slow cooling rates of less than 4  $C^{0}$ . min<sup>-1</sup> gave the 87% maximum for less than double-layer graphene. Also cover temperature affected the bonding between the carbon atoms to make  $sp^2$ hybridization. CVD can be used to fabricate a great area of graphene and has a small number of defects. Graphene from this method can be used in optical polarizers [53] a touch screen [52], smart windows, flexible liquid crystal display (LCD), organic light-emitting diodes (OLEDS) [54] highfrequency transistors [55 and 56] and photodetectors [57]



Figure (1-8). (a)The scheme of CVD, (b) few layers graphene **1.5.3 Epitaxial growth on silicon carbide SiC surface** 

It is one of the very important ways for the synthesis graphene film in SiC. Berger *et al* were first reported the controlled growth of graphene films on SiC [58]. Electrical transports of G is similar to those obtained by mechanical exfoliated on HOPG. Single crystal 6H and 4H-SiC (0001) substrate is heated to approximately 1573K and pressure below 1.3Kpa excess carbon is left behind on the surface to form graphene due to the sublimation rate of silicon which is higher than that of carbon [59 and 60]. It has an advantage of being very clean because the epitaxial matching support crystal provides the carbon itself and no metal is involved. Graphene fabricated in this method is used in a transistor circuit and interconnect memory.

## 1.5.4 Micromechanical exfoliation of graphite

The mechanical exfoliation was deducted by means of an adhesive film of the top layer of a HOPG. This layer was transferred mostly from graphene multilayers by means of repeated folding back the foil further separated and then by pressing on a suitable substrate (a conventional manner, a silicon wafer with a silica coating) [61]. Thus obtained graphene layers reach a size of several 10 microns as shown in Fig. (1-9).The mechanical exfoliation can be considered as a common and successful method to obtain one or a few layers of G.



Figure (1-9) Light microscopy (a) and Atomic Force Microscopy (b)

This method is direct, low cost, simple, and high structural and electronic quality. Possible contamination of a sample from adhesive tape utilized low yield, poor reproducibility were the most important disadvantages of this method [62].

# 1.6 Functionalization of graphene oxide and reduced graphene oxide

Pristine G and reduced graphene oxide RGO have hydrophobic properties towards water and organic solvent, tend to form irreversible agglomerate via van der Waals interaction which limits its application. This drives researchers to manipulate properties of G and RGO by introducing organic and inorganic molecules on their surface (surface modification). The surface functionalized has taken by two approaches covalent attachment and non-covalent attachment.

#### 1.6.1 Covalent attachment

The edge and/or the basal plane of GO and RGO can be used as sites to covalent functionalize .The existence of functional groups on these sites can provide chemical reactivity and utilizes to change their surface functionality. In addition, simple reduction of GO removes partially oxygen groups and rehybridize the sp<sup>3</sup> carbon atoms to sp<sup>2</sup> leaving it aromatic. The advantage from using residual oxygen groups for chemical functionalize was that it doesn't create additional defects on the graphene surface compared with the reaction with double bonds. The strategies of covalent functionalization of GO and RGO including acylation, amidation, nucleophilic and electrophilic substitution and an esterification. The most important functionalized methods were explain below.

### 1.6.1.1 Covalent linkage to polymer

A polymeric chain such as polyethylene glycol (PEG), poly L-Lysine (PLL) [63], poly vinyl alcohol (PVA) and poly allyl amine (PAA) can be grafted onto GO nanosheet. Amine terminated PEG can be grafted onto nano plates through an amide bond [64]. (PEG) is biocompatible, super hydrophilic polymer. Thus (PEG-GO) has these characteristics of PEG which makes it highly disperse in biological solutions such as cell medium or serum and water this makes it plays an important role in field of drug delivery. So comptochecin named SN38 (hydrophobic drug) [Fig.(1-10)] immobilized on the surface of (PEG-GO) by Vander Waals interaction can be easily dispersed .Also (PAA) can react with GO through cross –linking between amine groups and epoxy group of GO[65] forming thin paper –like membrane making stiff GO membrane work successfully. PVA can be grafted onto GO sheet [66] through formation of ester bond between carboxylic group of GO and the hydroxyl group of (PVA), either by direct formation or by convert, the carboxylic group into acyl chloride by using thionyl group (SOCl<sub>2</sub>). The PVA-GO composite

dispersed in DMSO and hot water. Also PVA-GO can be partially reduced by hydrazine to form PVA-RGO.



Figure (1-10) Comptochecin drug on a PEG- GO system [64]

## 1.6.1.2 Addition chromophores

Conjugated polymeric material such as 3-hexcyl amine poly thiophene poly (3-hexylthiophene) (P3HT) can be grafted on GO nano platelets through amide and ester covalent bonds [Fig. (1-11)] respectively.  $\pi$  electrons delocalized (high charge mobility) along conjugated chains of polymeric material make it used in various optoelectronic application, such as, solar cell [67 and 68].



Figure (1-11) Reaction between GO and CH<sub>2</sub>OH terminated (P3HT) [68]

Simple organic chromophores that have interesting optoelectronic properties such as azobenzene porphyrins and phthalocyanines can functionalize with GO through covalently attachment. Porphyrins

functionalized amine formed amide bonding with COOH groups of GO as shown in Fig. (1-12). [69 and 70]



Figure (1-12) Reaction between GO and amine functionalized Porphyrins

#### 1.6.1.3 Addition of organic molecules

Linking molecular moieties onto oxygenated groups at the edges of GO are most common approaches via amidation [Fig.1-13(a)] [71] and esterification [Fig.1-13(b)][72].

Carboxylic groups of GO can be converted to acyl chloride by using of thionyl chloride in DMF to activate carboxylic groups [73] or by carbodiimide (R-N = C = N-R). Acyl chloride is an excellent leaving group. Another coupling type used the epoxide groups ring opening with nucleophilic reagents, especially amines or azide ions [Fig. 1-13(c and d)] respectively.

The azide groups can then, be used for further functionalization with alkyne (click reaction) [Fig. 1-13(e)] or reduction with Lithium aluminum hydride LiAlH<sub>4</sub> [Fig. 1-13(f)] resulting amino functionalized GO. [Fig. (1-13)] shows covalent functionalize strategies.



Figure (1-13) Important reaction types for covalent functionalization of graphene oxide by utilizing the carboxyl, hydroxyl and epoxide groups.

## 1.6.2 Non covalent functionalization

The non-covalent functionalize of graphene and their derivatives based on forces such as  $\pi$ - $\pi$  interaction, Van der Waals and hydrophobic force in this type of functionalize the  $sp^2$  structure wasn't disturbed (no defect formed), this was provided certain application's advantage. Noncovalent interaction can be achieved by first adsorption polymers such as sulfonated poly aniline (SPANI) [73], poly aniline PANI [74 and 75] poly pyrrole (PPY)[76-78], amine terminate polystyrene and ploy methyl methacrylate (PMMA)<sup>[79]</sup>. The functionalization with polymer can happen either by adsorption the monomer [80 and 81] or adsorption a polymer chain on the surface of nano sheet [77 and 82]. Second by adsorption of small aromatic molecules such as pyrene butanoic acid succidymidyl ester (PBASE) [83] and perylene derivatives [84]. Graphite exfoliates into single- and bilayer graphene flakes in presence pyrene in aqueous solution [85] as shown in Fig. (1-14). The  $\pi$ - $\pi$  interaction is proved by fluorescence spectroscopy and UV-Vis absorption. From AFM measurements graphene flakes were composed of single-layer sheets with a thickness of 2nm. Scanning Electron Microscope (SEM) and Scanning Tunneling Microscope (STM) images showed that the sizes of the graphene flakes are 2 - 4 micrometer in diameter. Raman spectra demonstrated that the graphene dispersions predominantly consisted of single- and bi-layer graphene flakes. The prepared graphene films showed high conductivity and transparency.



Figure (1-14) Adsorption of pyrene on graphene flake.

## 1.7 Carbon nanotubes

Carbon nanotube (CNTs) are a novel nanomaterial, with diameter on the order of few nanometers (approximately 50,000 times smaller than the width of a human hair) and micrometer-sized length. A CNT discovered in 1991 by Japanese scientist Sumio Iijima [86].

CNT lying between fullerene and graphite sheet. CNT includes cylindrical buckyballs or enrolled cylindrical graphitic sheets [87]. Each atom in CNT honeycomb lattices joined to three neighbors in sp2 hybridize. According to the number of rolled up graphene sheet, CNTs can be either single walled (SWNTs) or multiwall (MWNTs). Thus SWCNT [Fig. (1-15)] is obtained by rolling a graphene layer along a surface direction with diameter of several nanometers SWNTs are cylindrical graphite sheets of 0.5 - 1 nm diameter while MWNTs comprise several concentric cylinders of these graphitic shells with a layer spacing of 0.3 - 0.4 nm MWNTs tend to have diameters in the range 2 - 100 nm [88].

CNTs can be produced by chemical vapor deposition (CVD) [89] arc - discharge methods [90], and laser ablation [91]



Figure (1-15) Synthesis of SWCNT by rolling G layer.

SWCNT have two separated regions, side walls and end cup of tube with different chemical and physical properties. Diameter is close to 1 nm The type of SWCNT (metallic or semiconducting) depends on how the graphene sheet is oriented on rolling .The basic building block of this system is the electric wire. SWCNT has excellent electrical properties. Multi-walled carbon nanotubes (MWCNTs) consist of multiple layers of graphite rolled in on themselves to form a tube shape [Fig. (1-16)].



Figure (1-16) Multiwall carbon nanotube MWCNT

Length, diameter and properties of MWCNT are different from SWCNT [86]. The interlayer distance in MWCNTs is approximately 3.3A the growth mechanisms of CNT is not exactly known. But the most accepted growth mechanisms are tip-growth [92] and base growth [93].

In the two mechanisms, catalyst – substrate interaction (an obtuse or acute) play important role.

In tip-growth mechanism metal has an acute contact angle with substrate, carbon atoms were added (decomposing) on the top surface of the metal, diffused through metal bottom and added freshly to its circumference (due to existence of concentration gradient in the metal) pushing the whole metal particle off the substrate .Once the metal is fully covered with surplus carbon, CNT catalytic activity discontinues and the growth is stopped [Fig. (1-17)].



Figure (1-17) Tip growth mechanism of CNT

While in base growth mechanism, the catalyst–substrate has an obtuse contact angle with the substrate, CNT precipitation fails to push the metal particle up so the precipitation is forced to emerge out from the metal top. Carbon crystallizes out as a hemispherical cupola which then extends up in the form of seamless graphitic cylinder. Subsequent hydrocarbon deposition takes place on the lower circumferential surface of the metal, and as-dissolved carbon diffuses upward [Fig. (1-18)]



Figure (1-18) Base growth mechanism of CNT

## **1.8 Carbon nanotubes functionalization**

Great efforts made recently to functionalize carbon nanotubes with specific analyte molecules. Covalent and non-covalent functionalization are two main routes to functionalize surface of CNTs. Covalent functionalization of MWCNT is based on the approach to modify the outer shell only without modified inner core. This approach doesn't change the electronic properties of MWCNT. Polymer can be functionalized with CNT non –covalently. Degrees of swelling of the polymers through  $\pi$ - $\pi$  stacking and van der Waals interaction which is very important in field sensing.

## **1.9 Metal oxides nanostructures**

Productions of metal and metal oxides nanostructures were found thresholds of scale in the field of Nanotechnology and nanoscience. Nanoparticles include, Nobel metal (Ag, Au, Pt and Pd), magnetics magnetite (Fe<sub>3</sub>O<sub>4</sub>) and maghemits (Fe<sub>2</sub>O<sub>3</sub>), semiconductor (TiO<sub>2</sub> and ZnO), and transition metal oxide (CuO and MnO<sub>2</sub>). Manganese oxide nanoparticles were one of the most attractive inorganic materials having a wide applications due to its chemical and physical properties, in energy storage, catalysis [94], molecular adsorption [95], lithium battery [96], sensor and biosensor [97] and particularly electrochemical capacitor [98]. MnO<sub>2</sub> crystals have been synthesized with different morphologies. Onedimensional manganese dioxide (MnO<sub>2</sub>) nanostructures like fibers, wires and nanorod have wide research interests due to their excellent electrochemical, optical magnetic and electrical properties [99-101]. Manganese has various oxidation state like  $Mn_3O_4$ ,  $Mn_2O_3$  and  $MnO_2$ . There are many polymorphs (crystallographic form) for  $MnO_2$ : ( $\beta$ ,  $\delta$ ,  $\alpha$ ,  $\gamma$ )  $MnO_2$  offering distinctive properties and wide application.  $MnO_6$  octahedron is a basic structural unit for all types of  $MnO_2$ . The combination of  $MnO_6$  octahedral plays role in synthesize, design, and optimize specific  $MnO_2$ . Therefore, give a brief review on crystalline structures of  $MnO_2$  was necessary. As shown in Fig. 1-19(a),  $\alpha$ -MnO<sub>2</sub> consists of a series of [2\*2] and [1\*1] tunnels, these tunnels are formed by a double chain of edge –sharing [MnO<sub>6</sub>] octahedral cross is linked by sharing a corner [102].  $\beta$  MnO<sub>2</sub> (pyrolusite) is the simple one within the family compound with tunnel structure ,consists of mono chain of the octahedral (MnO<sub>6</sub>) [103] [Fig. 1-19(b)].



Figure (1-19) The structure of double chains  $\alpha$ -MnO<sub>2</sub> (a) and single chains  $\beta$ -MnO<sub>2</sub> (b) both consist of (MnO<sub>6</sub>) octahedron.

 $\gamma$ - MnO<sub>2</sub> [104] intergrowth (irregular) of two or more tunnel phases [Fig. 1-20(a)] [105]. (1×1) tunnels (pyrolusite) and (1×2) tunnels (ramsdellite). Ramsdellite According the De Wolff model is composed of double MnO<sub>6</sub> chains [Fig. 1-20(b)] (only observed in nature). While  $\gamma$ -MnO2 contains pyrolusite (1×1) tunnels which were synthesized.



Figure (1-20) (a) The structure of  $\gamma$ -MnO<sub>2</sub> with irregular intergrowth of (1×1) tunnels (pyrolusite) and (1×2) tunnels (ramsdellite) (b) aramsdellite-MnO2.

 $\delta$ - MnO<sub>2</sub> Fig. [1-21] two dimension (2D) layered structure with an interlayer separation of ~7 A. It has a significant amount of water and stabilizing cations such as sodium or potassium between the sheets of MnO<sub>6</sub> octahedral [106]



Figure (1-21) 2D layered structure of  $\delta$  - MnO<sub>2</sub>

## 1.10 Electrochemical sensor.

The device that measures or detects a physical property and records it is called sensor consists of an analyte-selective interface, which is connected to a device that converts the binding event into a quantifiable output signal called transducer [107]. Sensor could be classified depending on its transducer, into an optical, mass, magnetic and electrochemical sensor. The most widespread kinds of optical method are fluorescence and colorimetric. The transduction system was based on using a light source and optical receiver. Mass transducer transforms mass change in the surface due to analyte presence by means of piezoelectric material which is sensitive to mass change. The magnetic measurement in sensor was depending on the changing that occurs in paramagnetic properties of material after interacting with analyte. All this describes sensor which has complexity and the high cost fabrication. Electrochemical sensor measured the electron transfer between the analyte and electrode directly, whereby the electrical resistance changes depend on the analyte concentration and related material are based on their exceptional properties have been brought to the forefront in field of electrochemical sensor [108 and 109]. The presence of defect and oxygen functional groups on the surface of RGO and GO made them a goal template to obtain nanocomposite through covalent and noncovalent bonding. G and related material nanoparticles nanocomposite are widely used for electrochemical sensing application through decreasing over potential of electrochemical reaction. In this work GO, RGO and their functionalize were used to detect some of antibiotics such as Ampicillin (AMP), Amoxicillin (AMOX) and Tetracycline (TET).

## 1.11 Electrochemical technique

The electrochemical sensor can be classified based on technique that is used in measurement. There are two general types of electrochemical technique that can be used in electroanalysis: potentiometric and voltammetric techniques.

## 1.11.1 Potentiometric technique

The first quantitative potentiometric applications appeared in 1889, which describe the relationship between activity of electroactive species potential and electrochemical cell without drawing current. Potentiometric measurements consist of two electrodes; measuring working electrode (WE) which gives response to the target analyte and reference electrode (RE), has constant potential independence on the properties of the solution. In potentiometric measurement, no current flow occurs, when the electrodes are placed in solution, the potential difference ( $\Delta E$ ) is measured from the difference in free Gibbs energy  $(\Delta G)$ 

$$\Delta G = -zF\Delta E \tag{1-3}$$

Where F, Faraday constant and *z* electrons number.

:[Comment [S1

The potential between measuring and reference electrodes gives an idea in the concentration of certain ions in the solution. Current flowing through the electrode is equal to or near zero [110 and 111].

## **1.11.2** Voltammetric technique.

In voltammetric technique, the potential of WE is controlled and the resulting current flow is measured relative to reference electrode (RE). Controlled potential techniques have many advantages including high sensitivity and selectivity towards electroactive species. The relationship and between current electrode potential was determined bv electrochemical reaction at one electrode only WE. The voltammetric experiment was performed in chemical cell using three electrodes, WE, RE and counter electrode CE. The DropSens system was used which the three electrodes printed in ceramic surface. To determine the current at the WE only, WE must have a surface area much smaller than the surface area of CE .In addition CE should be non-reactive and have high surface area such as platinum or carbon. WE can be a multiple of configurations and compositions such as

1. Inert (metallic)electrode, which doesn't chemically or electrochemically react over a wide range of potentials like Hg drop, Au, Pt with variety of geometric configurations(wires, rods, and flat sheet), and carbon electrodes such as glassy carbon electrodes, screen printed electrodes and pyrolytic graphite

2. Reactive electrode (metallic electrode) like Fe, Ni and Cd at some sufficiently anodic potential.

3. Semiconductor electrode has a narrow band gap like (Si and  $MoS_2$ ) or high band gap like (ZnO and TiO<sub>2</sub>)

4. Synthesized or chemically modified electrodes (CMEs).

Modification of electrodes can be accomplished using many methods such as applying irreversible adsorbed material with various functionalities, coating the electrode with films like polymers or other material and covalent attachment of component.

RE must have stable potential that not varied when the external potential is applied in the WE, insensitive to the composition of the target analyte and well known potential such as the standard hydrogen electrode SHE, calomel electrode and Ag, AgCl electrode.

Unstirred solution of the voltammetric measurements usually contains electroactive species and supporting electrolyte with a high degree of ionization like, ionic salts KCl.KNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>, HCl, KOH. Supporting electrolyte improved the conductivity of the solution and

minimized ohmic (IR) drop or potential drop. All electrodes are connected to potentiostat (electronic hardware) [112] or a bipotentiostat (capable of controlling two working electrodes). This equipment was fundamental to modern electrochemical studies. Fig. (1-22) shows potentiostat an arrangement of the three-electrode technique allowed the potential difference between RE and WE to be controlled, and at the same time the current flowed between the CE and WE was measured. The current flowing through the RE is minimized which keeps the applied potential between RE and WE stable and the RE is non polarizable.



Figure (1-22) Voltammetric measurement including three-electrodes

## 1.12 Cyclic voltammetry (CV)

An electroanalytical method (CV) was used to investigate the electrochemical behavior of a system, providing qualitative information about the electrochemical reaction, obtaining information about the redox potential and study of electroactive species therefore, cyclic voltammetry is often used to characterize the performance of electrochemical biosensors. It was first reported in 1938 and described theoretically by Randle [113]. It has been used widely in the field of electrochemistry, biochemistry, organic and inorganic chemistry

CV and Linear sweep voltammetry (LSV) are an example of several potential sweep techniques. LSV involves scanning the potential of WE linearly from initial potential  $E_i$  to final potential  $E_f$  at the fixed scan rate. The scan rate (v) was the change of potential as a function of time and can be deduced from the gradient of the curve

$$(\nu) = \frac{dE}{dt} \tag{1-4}$$

Typically scan rate measured in V.s<sup>-1</sup>.

In CV, the scan swept back at the same rate to initial potential, so-called switching potential ( $E_{sp}$ ). Potential of the working electrode (considered as an excitation signal) was varied linearly with time (with a triangular waveform) as shown in Fig. (1-23). The potential varied between three values initial, switching and final potential .The variation in potentials of WE causes redox the electroactive chemical species at the electrode. The faradic current was proportional to the concentration of the electroactive species present in the solution [112] .One or more potential cycles can be performed.



Figure (1-23) triangle waveform for cyclic voltammetry signal

Cyclic voltammogram was recorded using recording device such as a computer or plotter. The resulting voltammogram is considered as graph between the current on the vertical axis versus the applied potential on a horizontal axis. According to an International Union of Pure and Applied Chemistry (IUPAC), anodic currents were plotted positive, and positive potentials increasing to the right. The important parameter in cyclic voltammogram are $E_{pc}$ ,  $E_{pa}$ ,  $I_{pc}$ ,  $I_{pa}$  potential cathodic, anodic peak and current cathodic, anodic peak, respectively [Fig. (1-24)].



Figure (1-24) Cyclic voltammogram according to IUPAC

Cyclic voltammetry (CV) was used for the illustration of redox processes. In reversible electrochemically active material the reduction and an oxidation peak have the same height ( $I_{pa}/I_{pc}$  equal 1) and the same peak distance  $\Delta E_P$  (V), regardless the scanning speed [113]

$$\Delta E_{p} = E_{p_{a}} - E_{p_{c}} = \frac{2.303RT}{nF}$$
(1-5)

Where R, T and F have the usual meaning. n the number of electrons exchanged. Because of factors such as cell resistance, this value in practice was difficult to attain. The potential in the middle of the peaks (the half-wave potential  $E_{1/2}$ ), is an important characteristic as it is related to the formal potential  $E^{\circ}$  of the reaction

$$E_{1/2} = E^{\circ} + \frac{RT}{nF} \ln \left[ \frac{D_R}{D_o} \right]$$
(1-6)

 $D_R$  and  $D_o$  diffusion coefficient for reduced and oxidized form of electroactive species other symbol has the usual meanings. The half-wave potential  $E_{1/2}$  for CV can be calculated from anodic and cathodic peaks potential as described below[114]

$$E_{1/2} = \frac{E_{pa} + E_{pc}}{2} \tag{1-7}$$

## 1.13 Differential pulse voltammetry (DPV)

Pulse method was presented in 1952 by Baker and Jenkin. The potential applied to the WE with a series of pulses. Each potential pulse is fixed, of a small amplitude (10 to 100 mV), and is superimposed on a slowly changing base potential. Current is measured at two points for each pulse, before and at the end of a pulse [Fig. 1-25(a)] these sampling points are selected to allow for the decay of the non-faradaic (charging) current. Typical parameters for DPV measurements of simple faradic reaction are pulse heights  $\Delta_p E$  and pulse width  $t_p$ . The difference between current  $\Delta_i$  measurements at these points for each pulse is plotted against the sweeping potential yields to voltammogram distinct with a peak-shaped response of the electrode reaction [Fig. 1-25(b)].



Figure (1-25) Differential pulse voltammetry. (a) Linear voltage rises in a black, Voltage pulses are shown in red (b) differential voltammogram

The DPV peak potential  $E_P$  corresponds to or lies close to the approximately half wave potential for small  $\Delta_p E$  [114]

$$E_p = E_{1/2} - \Delta_p E/2$$

Where  $\Delta_p E$  represented pulse amplitude. The peak current (Ip) for reversible faradic reaction related as described below.

(1 - 9)

$$I_p = nFAc \sqrt{\left(D/\pi t_p \left[\frac{1-\sigma}{1+\sigma}\right]\right)} \tag{1-10}$$

$$\sigma = \exp\left[\frac{\mathrm{nF}\Delta_p \mathrm{E}}{RT2}\right] \tag{1-11}$$

Where n, number of electrons, F faraday, C concentration of analyte (mole.cm<sup>-3</sup>) A electrode surface area (cm<sup>2</sup>) The sensitivity of the DPV was to some powers of 10 greater than that of the linear sweep voltammetry. They were considered most powerful and most commonly used direct method.

## 1.14 Dielectric measurement

Dielectric properties measurement such as real and imaginary permittivity, permeability and conductivity of material (liquid or solid) provided the engineers and scientists information about the materials to use in different applications [115]. Dielectric properties were varied with frequency of the external electric field, density, temperature and composition and structure of materials. Several techniques were used to measure dielectric properties ranging from direct current to microwave, the used techniques will be determined according to the nature of material (liquid, solid, powder and sheet) the response of material to an applied electric field causes displacement (called polarization) of charge but no flow of charge, permittivity was used to quantify this response [116].

## 1.15 General description of polarization

All materials as composed of atoms affected by an applied electric field, the force result in this field exerted on each charge particle where positive electric charge pushed in the direction of the field and negative charged particles of each atom are displaced from their equilibrium positions in opposite directions. This displacement is called polarization (p) the polarization is the electric dipole moment density which depends on the total electric field (E) in the material

 $p = \propto E$  (1-12)

Polarizability of the molecule is  $\propto$ . Polarizability is the ability of a molecule to the redistribution of charge in response to the electric field. Degree of polarization depends on the electric field and the properties of material atoms. Polarization (*p*), electric displacement (D) and electric field (E) are related to each other [117 and 118]. As shown below

$$D = p + \varepsilon E \tag{1-13}$$

Where  $\varepsilon_{\circ}$  is permittivity of space  $(\frac{1}{36\pi} \times 10^{-9})$  F m<sup>-1</sup>

And
$$D = \varepsilon_{\circ} \epsilon_{r} E \tag{1-14}$$

From eqns (1-13) and (1-14) we obtained relation between polarization and relative dielectric constant

$$p = \varepsilon \cdot E(\epsilon_r - 1) \tag{1-15}$$

In equation (1-15) magnitude of relative permittivity  $\varepsilon_r$  must be greater than the unity

#### 1.17 Dielectric theory

Interaction of material with electric field can be expressed in term of permittivity  $\varepsilon$  where the dimensionless relative permittivity ( $\varepsilon$ <sub>r</sub>) equals to

$$\varepsilon_r = \frac{\varepsilon}{\varepsilon_\circ} \tag{1-16}$$

The relative permittivity was usually known as dielectric constant, this term was no longer used in chemistry and engineering [119] as well as in physics [120]. Also permittivity is known as real part of permittivity( $\epsilon$ ) which is determined as the ratio of the measured capacitance (C) of capacitor in which the space between the electrode filled with insulating material to the capacitance of the same configuration of electrode in a vacuum ( $C_{\circ}$ ) where

$$\dot{\varepsilon} = \frac{C}{C_{\circ}} \tag{1-17}$$

c. Can be obtained from the same specimen geometry

$$c_{\circ=\varepsilon} \frac{A}{d} \tag{1-18}$$

A is the surface area of the parallel electrodes  $(m^2)$ , d a thickness of the sample (m).Substitution eq (1-18) in eq (1-17) give eq (1-19) [121]

$$\dot{\varepsilon} = \frac{C d}{\varepsilon \cdot A} \tag{1-19}$$

Where  $(\epsilon)$  was the permittivity of free space  $(8.854 \times 10^{-12})$  F.m<sup>-1</sup>, This equation related the ability of substances to store electrical energy with their capacitance when an external electric field was applied [122]. Permittivity values vary and change with T, humidity, frequency of applied field and crystal structure to different extend according to material type [123].Relative Permittivity is normally written in a complex number under alternate an electric field which can be defined as

$$\varepsilon_r^* = \varepsilon' - j \varepsilon''$$

$$(1 - 20)$$

$$\varepsilon_r^* = \varepsilon_* \varepsilon_r - j \varepsilon^{"} \tag{1-21}$$

Where  $\varepsilon$  is the real value of permittivity and means the electrical energy stored within the material,  $\varepsilon$  is the imaginary value of permittivity also called (relative dielectric loss factor or energy disspated) is a measure of the amount of energy that material dissipated when subjected to an alternating electric field ,J is  $\sqrt{-1}$  value. Complex permittivity is represented as a simple vector diagram [Fig. (1-26)] the real and imaginary component 90° out of phase. The vector sum forms an angle  $\delta$  with the real axis ( $\varepsilon$ ). The relative "lossiness" of a material is the ratio of the energy lost to the energy stored which is termed as tangent loss.



Figure (1-26) diagram of loss tangent vector

$$\tan \delta = \varepsilon'' / \varepsilon' = D = \frac{1}{Q} = \frac{1}{\omega CR}$$
(1-22)

R is the resistance in ohm, D is dissipation factor, Q is quality factor and  $\omega$  is angular frequency in radian.s<sup>-1</sup>. Angular frequency refers to change the phase of sinusoidal wave form, a complete rotation is a unit of angle measurement equals to 360° or  $2\pi$  radians hence [124 and 125]

$$\omega = \frac{2\pi}{T} = 2\pi f \tag{1-23}$$

T is the period in second, f is the frequency in Hertz.

Or

#### **1.18 Literature survey**

Literatures report a wide variety of works on functionalized graphene and graphene oxide and their potential applications.

Zhou *et al* [126] studied electrodes modified with graphite, RGO and carbon nanotubes and compared their activity. Cyclic voltammetry showed that the modified electrode gave a higher sensitivity towards hydrogen peroxide H<sub>2</sub>O<sub>2</sub> at a low over potential of -0.20 V due to formation of strong hydrogen bonds between hydrogen peroxide and RGO. A limit of detection (LOD) of 0.05 µM was found. No interfere for uric acid, dopamine, acetaminophen and ascorbic acid was found.

Chang *et al.* [127] used SPCE modified with RGO to detect of uric acid (UA) and ascorbic acid (AA). Peak separation and sensitivity of the plain carbon and RGO modified electrodes were compared. It illustrates that hydrogen bonds formed between of functional groups on the surface of the electrode and the analyte [Fig (1-27)] [127]. The peak separation (oxidation step) of UA at 0.3 V instead of 0.5 V and ascorbic acid is 19 mV instead of 0.5 V with increasing (double) of the peak current (simultaneous). It demonstrated that the peak separation affected the strength of the bond and the distance of interaction site to the reaction center.



← Carbon ← Oxygen ← Hydrogen III Reaction center ----- Hydrogen bonding Figure (1-27) A scheme illustrates using RGO sensor for UA and AA

Zhang *et al.* [128] studied sensing (AA) and dopamine using blending of G and MWCNT in a cross linked cyclodextrin matrix. The potential oxidation and LOD for sense AA was -0.06V and 1.65  $\mu$ M versus saturated calomel electrode SCE respectively. The LOD and the potential oxidation for dopamine was 0.05  $\mu$ M and 0.17V by voltammetry. The sensitivity and facilitate the electron transfer (oxidation) for dopamine was observed higher than AA about 23-fold due to, first presence of  $\pi$ - $\pi$  interaction between the phenyl ring of dopamine and the graphene/carbon nanotubes made easy contact with the dispersed G/MWCNT in contrast to AA, second diffusion of dopamine through cavities of cyclodextrin due to its host-guest chemical reaction.

P. Umek *et al* [129] synthesized  $\alpha$ - MnO<sub>2</sub> nanorod in acidic condition by hydrothermal method. The reaction based on the redox reaction of potassium permanganate in different reaction parameters such as number of moles of potassium permanganate, temperature and reaction time. The result showed that the diameter and the length of  $\alpha$ - MnO<sub>2</sub> are affected by concentration of KMnO<sub>4</sub> in the reaction mixture where the average lengths and diameters of the isolated MnO<sub>2</sub> nanorod are reduced with an increasing concentration of KMnO<sub>4</sub>. It was found that the reaction time has a strong influence on the length of the obtained nanorod.

L. Zhang *et al*[130] used simple synthetic method for preparation nanoscale graphene oxide (NGO) functionalized by sulfonic acid groups ,followed by covalent attaching to folic acid(FA) molecules as receptors to MCF-7 cells, breast cancer cells .NGO-FA was investigated by loading anticancer drugs comptochecin (CPT) and doxorubicin (DOX) via hydrophobic interactions and  $\pi - \pi$  stacking, which shows specific targeting to MCF-7 cells, and remarkably high cytotoxicity compared to NGO loaded with either DOX or CPT only.

J.A. Yan and M. Y. Chou [131] studied the oxidation epoxide and hydroxyl functional groups on graphene based on density-functional calculations and investigated effects of various combinations of single functional groups on the structural and electronic properties. The result showed that hydroxyl and epoxy groups tend to aggregate on the graphene sheet using energetics analysis. By forming hydrogen bonding between hydroxyl groups stabilize chain like structures which can be formed, nearness of the epoxy groups. Calculations showed functionalization of graphene by oxidation will significantly alter the electronic properties of graphene where energy gap of graphene oxide increases in range of 0–4.0 eV.

N. Zhou *et al* [132] studied graphene oxide functionalized ionic liquid and gold nanoparticles (AuNPs) as modifier for glassy carbon electrode (GCE) as sensor AuNPs–GO-IL–GCE to detect mercury ions (Hg<sup>2+</sup>) presence in environmental and drinking water. Using anodic stripping voltammetry as sensitive electrochemical technique. The modifier (nanostructured membrane) is examined by cyclic voltammetry CV and scanning electron microscopy SEM. Also it showed good electron conductive and large electroactive surface area. The relation between the concentration of the Hg<sup>2+</sup> standard and peak current under the optimal conditions, showed linear behavior (R = 0.9808) in range of concentration 0.1–100 nM. Using a signal-to-noise ratio of  $3\sigma$  the limit of detection was found to be 0.03 nM.

K. Sablok *et al* [133]proposed sensor to detect trinitrotoluene (TNT) from amine functionalized reduced graphene oxide/carbon nanotubes (a-rGO-CNT through charge-transfer Jackson–Meisenheimer (JM) complex The formation of amine functionalization on modified electrodes was confirmed using confocal microscopy ,X- ray photoelectron spectroscopy (XPS) and energy dispersive X-ray (EDX). The formation of JM complex between amine functionalized electrodes and TNT was confirmed using Raman and FT-IR spectra from altering the broadening of peak and shifted the peak intensity. Ultra-trace of TNT was detected up to 0.01 ppb using a-rGO-CNT nanocomposite as modifier.

P. Wongkaew and S. Poosittisak [134] studied modification of the screen printed carbon electrode (SPCE) with chitosan and ratio 1:1 from activated carbon blending chitosan. The surface topographic characteristics and investigated by atomic force microscopy and a complementary electrochemical techniques. The working electrode surface area was modified via self-assembled deposition. Each modified surface was obviously differentiated by all roughness parameters such as height different, root mean square, an average roughness, grain and fractal. The highest roughness value with the least grain was from chitosan modified SPCE. A good change in current response was appeared with electrochemical cyclic voltammetry, slope value 0.5 followed by their regression coefficient of 0.99. Thus these modifications were affordable and efficient platforms for a fabrication of desirable pesticide biosensor.

L. Feng, *et al* [135] prepared  $\alpha$ -MnO<sub>2</sub> crystal with two morphologies like urchin in acidic solution and caddice-clew in neutral condition via hydrothermal method. X-ray diffraction (XRD), electrochemical impedance spectroscopy (EIS), cyclic voltammetry, scanning electron microscope (SEM) and galvanostatic cell cycling was used to characterize the electrochemical performance and structure of  $\alpha$ - MnO<sub>2</sub> crystal. The result was showed presence of relationship between morphology of MnO<sub>2</sub> and its electrochemical performance. Urchin-like MnO<sub>2</sub> material can be used for lithium-ion battery application due to better electrochemical performance.

#### 1.19 Aims of this study

1. Preparation of graphene oxide and reduced graphene oxide with a chemical method, and functionalization of these carbon nanomaterials with 4-amino-3-substituted-1H-1, 2, 4-triozol-5(4H)-thion, doping of the above new functionalized carbone nanomaterials with metal oxide nanoparticles. Moreover: A commercial MWCNTs functionalization with carboxylic groups, synthesis of nanocomposites between the functionalized MWCNT and one or more of the prepared new carbone nanomaterials.

2. Study the structural and morphological properties of prepared nanomaterial above using AFM and SEM as characterization tools as well as XRD and FT-IR.

3. Study the electrical and some of the physical properties of all the above synthesized carbone nanomaterials.

4. Modification of the surface of working electrode WE of screen printed carbon electrode SPCE with all the above prepared carbone nanomaterials.

5. Compare the electrochemical properties of nanomaterial above using cyclic voltammetry technique.

6. Fabricate electrodes for applications in the field of electrochemical sensing to sense some antibiotics using a cyclic voltammetry technique.

### 2. Experimental work

### 2.1 Instrument

Apparatus used in this thesis are tabulated in table (2-1).

Table (2-1) Instrument used, suppliers and origins.

No.	Instrument and model	location	
1	X-ray diffraction 6000 shimadzu (Japan)		
2	FT-IR spectrophotometer, Shimadzu, 8400s ,(Japan)		
3	Ultrasonic instrument type soniprep 150, (United Kingdom)		
4	Potentiostat µ Stat 200, Drop Sens S.L. Oviedo, (Spain)		
5	pH-meter BP3001(Singapore)	College of Education for Pure Science Baghdad	
6	Electric balance ,Sartorius Lab. BL 210 S, (Germany), $\pm 0.0001$ g.		
7	Vacuum drying oven ( Han Yang Scientific Equipment LTD ), Seoul, Korea		
8	LCR-meter (4274A) MULTI-FREQUENCY HEWLETT.PACKARD(Japan)		
9	Atomic Force Microscopy compact AFM PHYWE (Germany)		
10	Micrometer(JIANGXI) China		
11	Elemental analysis Euro EA3000 (Italy)		
12	Scanning Electron Microscopy SEM (Zeiss) (Germany)	Sharif university of technology/ Iran	

### 2.2 Chemicals

The chemical compounds with molecular weight and company that are used in this research were shown in Table (2-2)

× ,	1	
Chemicals	Company	% Purity
) Potassium permanganate( KMnO <sub>4</sub>	BHD	99
Sulphuric acid (H <sub>2</sub> SO <sub>4</sub> )	DAGENHAM	(96-98% acid)
	England	
Sodium nitrate (NaNO <sub>3</sub> )	Fluka	99.93
graphite	Fluka	95
Hydrogen Peroxide(H <sub>2</sub> O <sub>2</sub> )	DAGENHAM	30
	England	
Carbon disulfide ( $CS_2$ )	ASPAIN	anhydrous <mark>≥ 99</mark>
)Hydrazine (N <sub>2</sub> H <sub>4</sub>	ASPAIN	50-60
) Manganes sulfate (MnSO <sub>4</sub>	E.Merck	98
Nitric acid (HNO <sub>3</sub> )	GCC	69
Sodium phosphate monobasic dihydrate	E.Merck	98
(NaH <sub>2</sub> PO <sub>4</sub> .2H <sub>2</sub> O)		
Sodium phosphate dibasic	BDH	Analar
$(Na_2 HPO_4)$		
Phosphoric acid (H <sub>3</sub> PO <sub>4</sub> )	Aldrich	85
Sodium hydroxide(NaOH)	GCC	99
(Sulfonated tetrafluoroethylene)	Aldrich	5
(Nafion)		
Potassium ferricyanide( $K_3$ [Fe (CN) <sub>6</sub> ] <sup>-3</sup> )	BDH	99.96
Tetracycline (TET)	Samarra drug factory	99.98
Amoxicillin (AMOX)	Samarra drug factory	99.995
Ampicillin (AMP)	Samarra drug factory	99.995
Multi wall carbon nanotube (MWCNT)	Zhengzhou Dongyao	98.77
	Nano material	
	Co.,Ltd /china	
Potassium chloride (KCl)	Riedel-DE-HAEN	99.0
	AG	
Hydrochloric acid( HCl)	Aldrich	37

Table (2-2) Chemical compounds used in this work

#### 2.3 Preparation of graphene oxide (GO)

Graphite oxide (GTO) was synthesized by hummers' method [36] In ice bath at 0°C, 69 ml concentrated H<sub>2</sub>SO<sub>4</sub> 98% was mixed with 15g sodium nitrate NaNO<sub>3</sub> using magnetic stirrer for 30 minute, followed by adding 3g graphite (150  $\mu$ m) with stirring for 30 minute afterwards, 9g KMnO<sub>4</sub> was carefully added to the mixture preserving its temperature below 20 °C. The color of suspension was dark red from the reaction of potassium permanganate with sulfuric acid according to following reactions:

$$KMnO_4 + 3H_2SO_4 \to K^+ + MnO_3^+ + H_3O^+ + 3HSO_4^- \qquad (2-1)$$
  
$$MnO_3^+ + MnO_4^- \to Mn_2O_7 \qquad (2-2)$$

The double bonds oxidize by  $MnO_4^-$  and formed diols. The dehydration of diols produced epoxides. The reactivity of bimetallic heptoxide (manganite dimer) Mn<sub>2</sub>O<sub>7</sub> was more than monometallic tertraoxide counterpart. Manganite dimer exploded when placed in contact with organic compound or exposed to temperature greater than 55 <sup>o</sup>C [136 and 137]. Mn<sub>2</sub>O<sub>7</sub> acts as effective oxidizing agent [138] oxidize the unsaturated double bond. At intermediate temperature stage the mixture was heated to  $35 \pm 3$ °Cin water bath for 30 minutes .138ml distilled water was added carefully to increase the temperature to 98 <sup>o</sup>C for 15min. Also large amount of distilled water was added to dilute the solution at room temperature. Finally 35ml (30%) of hydrogen peroxide  $H_2O_2$  was added to terminate oxidation process by reducing (residual) permanganate and manganes dioxide to colorless soluble manganese sulfate [139]. The produced GTO mixture was filtered and washed with 5% aqueous solution of HCL and distilled water to make the pH of rinsing water neutral pH (6-7). In oven vacuum at 55 °C the product was dried for 48h. The GTO aqueous solution mixture was exfoliated by sonication and stirring for long time [20].

#### 2.4 Preparation of reduced graphene oxide RGO

The chemically reduction of aqueous dispersed GO was an important reaction stage because the similarity of reduced graphene oxide RGO with pristine graphene G. The reaction depended on the sonication of 1g GTO in 100ml distilled water until the suspension became without visible particulate matter and converted to GO.

Then 5ml of hydrazine monohydrate was added in closed round bottom flask with reflux at 100 °C for 24h. To terminate the reaction

firstly, the mixture rinsed with methanol and secondly, washed with double distilled water. The precipitate was dried in vacuum at 60 °C for 12h [140 and 141].

#### 2.5 Preparation of thiocarbohydrazide (TCH)

There are several ways to prepare the thiocarbohydrazide, reaction of hydrazine  $N_2H_4$  with carbon disulfide  $CS_2$  to produce hydrazinium dithiocarbazinate HDTC was the most important way [142] as shown below

$$CS_2 + 2N_2H_4 \rightarrow H_2NHNCS_2H.N_2H_4$$
 (2-3)

80ml of hydrazine were added dropwise with stirring to flask containing about 20ml of carbon disulfide kept in water bath which its temperature is about 10 °C until the formation of yellow precipitate. The color of precipitate changed to brown after refluxed for half hour. As shown in equation below,  $H_2S$  was removed as gas

$$H_2NHNCS_2H.N_2H_4 \rightarrow H_2NNH - CS - NHNH_2 + H_2S \uparrow$$
 (2-4)

The reaction mixture was filtrated to separate crystalline precipitate of TCH then washed with ethanol and water. The TCH crystals were dried at oven vacuum for 6 hours at 40-50 °C.

#### 2.6 Preparation of $MnO_2$

 $MnO_2$  nanoparticles was synthesized according to method reported by Xaio and Deguzman [143 and144] where 0.3 M KMnO<sub>4</sub> was added with spray technique to 1.9M MnSO<sub>4</sub>. HNO<sub>3</sub> was added to adjust pH to 1, the reaction was refluxed at 60-100 °C for 8h (this condition developed distinctive  $MnO_2$  nano rod) aging temperature plays crucial rule in accelerate rate of evolution of  $MnO_2$  nanostructure from spherical agglomeration  $MnO_2$  to aggregate of well-defined nano rod [145]. The product is ultrasonicated for a half hour, filtered, washed and dried in vacuum at 100°C the reaction pathway to yield  $MnO_2$  in acidic media may be occurred according to equa.5 and 6 [146]

$$2H_2O \rightarrow 4H^+(aq) + O_{2(q)} + 4e^-$$
 (2-5)

$$MnO_{4}^{-}(aq) + 4H^{+}(aq) + 3e^{-} \rightarrow MnO_{2}(s) + 2H_{2}O$$
 (2-6)

#### 2.7 Functionalization of GO and RGO with TCH

0.3g of GO or RGO with 1.2g TCH were put in round bottom flask with thermometer. The mixture was heated in sand bath on hot plate till the mixture melt. At fusion temperature of TCH the mixture was maintained at 168 °C for 10min with stirring with glass rod. To dissolve the TCH none reacting the product was cooled and washed with distilled water then dried at 60°C. Carboxylic groups in GO and RGO can be used as starting point to react with TCH at the melting temperature in cyclocondensation reaction. This could be allowed to prepare RGO and GO-4-amino,3-substituted 1H,1,2,4 Triazole 5(4H) thion(ASTT),(RGOT and GOT) respectively as shown in Fig. (2-1)



Figure (2-1) Functionalize graphene oxide GO with TCH

# 2.8 Preparation of nanocomposite of GOT, RGOT with $MnO_2$

In this work, simple mixing method and efficient approach were used to fabricate GOTM and RGOTM nanocomposites. Saturated solution of  $MnSO_4$  was prepared then 0.5g from GOT or RGOT was added to the filtrated  $MnSO_4$  and sonication for 1h to bond Mn(II) ions to the surface of GOT and RGOT by electrostatic force[147]. The mixture GOT, RGOT/  $Mn^{+2}$  was filtered and 0.3 M KMnO<sub>4</sub> was added with 1-2 drops of HNO<sub>3</sub> the reaction mixture cap with Para film were put in an oven for 8 to 10 h to transform  $Mn^{+2}$  to  $MnO_2$  as shown in the following equation [148].

$$2Mn^{+2} + MnO_4^- + 2H_2O \rightarrow 3MnO_2 + 4H^+$$
 (2-7)

Thus, MnO<sub>2</sub> nanoparticle were deposit and anchor on the surface of GOT, RGOT.

#### 2.9 Instrumentation and characterization procedure

#### 2.9.1 XRD analysis

One of the most important and useful techniques that is used to determine the internal structure of crystals and the arrangement of atoms within crystals was X-ray diffraction (XRD). The peak intensities in the diffraction pattern from a regular crystal lattice and interplaner distance are determined by Bragg's equation [149]:  $n\lambda = 2d \sin \theta$  (2-8)

Here, n-integer referring to the order of reflection,  $\lambda$  was wavelength of characteristic X-rays radiation, d was a lattice interplaner spacing of the crystal, and  $\theta$  is the X-ray incident angle. From x-ray diffraction scherrer equation that was published in 1918[150] was used to find out the mean size of nanoparticles [151] Based on the basic principles the crystal structure and composition of the material were related to the angle of reflection of X-rays.

The XRD technique was used to differentiate between crystalline and amorphous compound. Crystalline compounds produce many diffraction bands, while amorphous materials display a more or less regular baseline. Moreover, the Miller index, diffraction peak position and the relative intensity of unknown materials can be identified by comparing the diffraction data with diffraction pattern from Joint Committee on Powder Diffraction Standard (JCPDS). Powder XRD analysis was carried out by using powder diffractometer (Japan) XRD Shimadzu 6000 with an incident Cu-K $\alpha$  radiation of 1.54 $A^{\circ}$  40.0Kv and 30mA; scan range (2 $\theta$ = 5-80°) and; scan speed: 10 (deg/min).

## **2.9.2** Fourier transform infrared (FTIR) analysis for chemical bonding studies

The vibration characteristics of chemical functional groups in a sample was detected using Fourier transform infrared spectroscopy (FTIR). FTIR spectra were obtained on Shimadzu IR affinity 8400s, Japan. Scan range 4000–400 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup>. Infrared spectrophotometer using potassium bromide disc.

#### 2.9.3 Scanning electron microscope (SEM) analysis

SEM was a very powerful imaging technique commonly used to visualize nano- and microstructures [152–154] by scanning a sample surface with high electrons energy (about 50 Kev) high-resolution image was produced through [155] interact the bombarded electron beams with the surface atoms that make up the sample producing signals which provide information about the sample surface topography. The examined specimens must be electrically conductive, at least at the surface, Nonconductive specimens should be coated with an ultra-thin layer of conductive material either by low vacuum sputter coating or high vacuum evaporation. Chromium, gold, Au/Pd alloy, platinum, osmium, graphite and tungsten materials were currently used for coating. Fig. (2-2) shows diagram of SEM microscope [155]. SEM image obtained by Oxford instrument (Zeiss) in Sharif University of Technology, Tehran, Iran.



Figure (2-2) Diagram of SEM microscope

#### 2.9.4 Atomic force microscope (AFM)

To investigate the exfoliation rate and thickness of nanosheet, AFM microscope was used. AFM was a scanning probe technique (high resolution) that offers a capability of three-dimensional visualization, in which the end of a sharp tip positioned on deflective cantilever was scanned along a specimen surface (back and forth) the magnitude of the deflection is measured by the change in direction of laser beam that is reflected off the surface of cantilever into sensitive detector [Fig.(2-3)] [156]. It is possible to record the deflection as a function of time and thereby form an image of the surface topography. AFM promises

scanning in the two or three axis to rebuild the surface image from different viewpoints. Moreover, AFM measurement didn't require surface treatment such as coating with metal or carbon. Measurements carried out using Compact Atomic Force Microscope made by PHYWE (Germany).



Figure (2-3) Diagram of an atomic force microscope using the beam deflection detector.

#### 2.9.5 LCR meter

The permittivity and conductivity of the nanomaterial in this research was measured by LCR Meter (HP4274A) Multi-Frequency (Japan). The measurement was conducted at frequency range from 100Hz to 100 KHz. The real ( $\epsilon$ ') and imaginary part ( $\epsilon$ ") of the complex permittivity of the nanomaterial were calculated from capacitance(C) measurements.

The sample cell has two squared silver electrodes each having an area of  $1.5 \times 2 \text{ cm}^2$ . The nanomaterial fabricated as laminate (its thickness measured using micrometer (0-25mm) was indirect contact with electrode so the surface of the sample should be smooth, flat, to prevent an air gap between the sample and electrode and the area of material as large as enough to completely cover the electrode, the test fixture was connected to the measuring instrument (LCR) meter and dielectric properties were evaluated.

#### 2.9.6 Cyclic voltammetry

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used in this work using a portable potentiostat  $\mu$  Stat 200 (Drop Sens S.L. Oviedo, Spain) connected via a USB connection to a laptop computer with software Drop View (DropSens). The dimensions of potentiostat (Length 8.5, Width5.4, Height2.3) cm [Fig. (2-4)]. the

principle function of a potentiostat is to control potential and measure current.



Figure (2-4) Bipotentiostat

SPCE shown in Fig. (2-5) having, three electrodes printed on a ceramic surface [157] a carbon working electrode WE (4 mm diameter) a carbon counter electrode CE, and silver/silver chloride reference electrode RE. A small area enables a very low quantity of sample to perform the measurement. The measured volumes were smaller than 10  $\mu$ l deposited directly on the electrode surface [158] are adequate for an analysis.

An electrical contact area is placed at the end, which is connected with the active part of each electrode via internally conducting carbon parts. The WE is the principal electrode on which electrochemical reactions are performed, while the RE and CE are used to complete the electronic circuit. The advantage for SPCEs are considered as disposable devices that can be discarded carrying out the only analysis. The disposability of the electrodes provides a solution for problems like the electrode surface fouling by products of redox processes and an unintentional adsorption that can arise by using solid electrode materials (e.g. metal, amalgam and composite electrodes) [159].



Figure (2-5) SPCE used in voltammetric measurement

SPCE was connected to a box with dimensions (Length 8-Width4-3.5Height) cm [Fig. (2-6)].This box is operated as interface between DropSens SPCEs and potentiostat.



Figure (2-6) Boxed connector for SPCE

### 2.10 Experimental set-up and instrumentation

Experimentally, for the present study screen printed methodology was used to design new electrochemical (voltammetric) sensors. which consist of three electrodes include reference electrode silver-silver chloride( at fixed potential),counter electrode (carbon electrode) and working electrode could consist of chemically modified electrode CMEs, this expression was first used by P.Moses and co-workers to classify an electrode with a chemically active species immobilized onto the surface of the electrode [160]. Modification includes coating the WE (carbon electrode) with RGO, GO functionalities with TCH and their composites with MnO<sub>2</sub>. The voltammetric measurement was performed in quiescent solution with large excess of supporting electrolyte (10-100) fold excess over concentration of the species being studied).

One of the most important for using CMEs is their ability to catalyze the redox reaction of solute. Three electrodes are connected to potentiostat to control the potential applied to the WE [112]. Control and acquisition of response can be conveniently done by computer through adequate interface in based potentiostat. Following that, CV scan and the current is plotted versus the voltage to obtain a voltammogram, which is used to study the electrochemical properties of analyte in solution. Before measuring, the quality of the working electrode was tested by using ferroferricyanide redox couple in a 0.1 M KCL solvent [161]. Ferricyanide is a very popular compound to test the redox reaction quality of the electrodes in sensing and bio sensing experiments which showed well, defined redox peaks [162-164].

**2.11 Pretreatment of SPCEs** At scan rate 0.1 V s<sup>-1</sup> the potential was swept between -0.5 and +1.0V in 0.1 M H<sub>2</sub>SO<sub>4</sub> in order to pretreat the SPCE and to get a reproducible voltammogram increase sensitivity of sensors as well as to obtain a stable baseline for long term experiments [165]. Then, the SPCE was washed with double distilled water and dried at room temperature 24-25°C.

#### 2.12 Fabrication of GO, GOT, RGO, RGOT, GOTM and **RGOTM** with nafion modified SPCE.

To modify the working electrode the SPCE was first washed with distilled water and dried by N<sub>2</sub> stream. 10mg from each nano material were dispersed in 10 ml of water with  $5\mu L$  Nation (sulfonated tetrafluoroethylene) [Fig. (2-7)]. The suspension was completely dispersed via sonicated at room temperature for 1 h. A drop-casting method was used to immobilize the given material onto the working electrode. The modified electrodes (labeled as GO, GOT, RGO, RGOT, GOTM and RGOTM) -SPCE were rinsed with deionized water to remove unbounded material and dried completely at room temperature to get uniform thin film from the above solution on SPCEs, then kept for 24 h at 25°C

Appropriate volume about 50µL from each analyte solution was dropped carefully onto the reservoir area surface of modified SPCE using a micro-pipette to cover the three electrodes. The modified SPCE was

activated by sweeping an extreme anodic potentials +1.9 V in blank solution 0.1M PBS (pH 7.4) to augment the electron transfer kinetics of the working electrode surface [166]. This advancement increases the hydrophilicity amount of carbon-oxygen functional groups and further eliminate possible surface [167-169].



Figure (2-7) structure of Nafion

#### 2.13 Preparation of antibiotics solution

Appropriate concentration from antibiotics tetracycline TET( $1 \times 10^{-4}$ M), amoxicillin AMOX( $5 \times 10^{-4}$ M) or ampicillin AMP( $1 \times 10^{-4}$ M) was daily fresh prepared with phosphate buffer solution PBS as supporting electrolyte. Because TET is decomposing in existence of light. Therefore TET solution was covered with brown paper to provide the dark environment. Phosphate Buffer has some advantages: such as non-toxic, little pH changes with temperature and stable for several weeks at  $4^{\circ}$ C. PBS was prepared by mixing solutions of sodium phosphate monobasic dihydrate NaH<sub>2</sub>PO<sub>4</sub>.2H<sub>2</sub>O and sodium phosphate dibasic Na<sub>2</sub>HPO<sub>4</sub>, adjusted by 1M H<sub>3</sub>PO<sub>4</sub> or 1M NaOH as shown in the table (2-3) below.

pН	$Na_2HPO_4(g)$	$NaH_2PO_4.2H_2O(g)$
2	0.0001	1.7926
3	0.0003	1.7926
4	0.0036	1.7913
5	0.036	1.7700
6	0.3218	1.5785
7	1.5466	0.7586
8	2.4970	0.1221
9	2.6605	0.0129

Table (2-3).Preparation of 0.05M PBS at different pH

 $50\mu$ L from the solution of antibiotics was dropped on reservoir area to cover modified working electrode (with nanomaterial and nanocomposites), counter and reference electrodes for at least one minute to allow the electrode to equilibrate with the analyte solution. The electrochemical behavior of the antibiotics was analyzed with CV and DPV. CV measurement was carried out at a scan rate 0.1V.s<sup>-1</sup> between +1to -1V with potential step 0.01V with blown stream of nitrogen into solution for 3 minutes to avoid interference caused by oxygen and maintain an aerobic condition. To remove dissolved oxygen and water vapors from commercial nitrogen gas was passed through a series of traps. DPV carried out from -1 to 0.9 V, potential step 0.01V, time of pulse 10 (ms).

# 2.14 Synthesis of functionalized MWCNT (f-MWCNT) and its nanocomposite with GOT

Two types of MWCNT were prepare nanocomposites. CNT was used as received second, MWCNT was chemically treated. The dispersion of MWCNT with mixture of sulphuric acid 96 wt % and nitric acid was refluxed for 5 h. The resulting dispersion was diluted in water, washed until the pH would be neutral and dried in vacuum oven at 55 °C for 12 h as shown in Fig (2-8) [170 and 171]. To prepare suspension (1:1) GOT/f-MWCNT nanocomposite 0.1 mg/10ml from both GOT and CNT was mixed by sonication for 1h with  $3\mu$ L Nafion  $.2\mu$ L GOT/f-MWCNT nanocomposite was deposit on the working area of SPCE dried at 25°C and washed with double distilled water.



Figure (2-8) Chemically functionalized MWCNT with COOH-group

#### 3. Results and Discussion

#### 3.1. Identification and characterization of nanomaterial

#### 3.1.1 FT-IR analysis

### **3.1.1.1 FT-IR** spectra of graphene oxide (GO) and reduced graphene oxide (RGO).

The chemical composition of GO and RGO has been characterized with FT-IR measurement. Fig. (3-1) shows the FT-IR spectrum of GO. The stretching vibration of (C-OH, COOH, and residue of  $H_2O$ ) appeared at 3401 cm<sup>-1</sup> with broad and strong band. The peak at 1587cm<sup>-1</sup> was assigned to unoxidized graphitic domain.

The two bands at 1221 cm<sup>-1</sup> and 1400 cm<sup>-1</sup> were corresponded to (C-O) stretching vibration of epoxide groups (C-O-C) and (C-O-H) bending vibration of COOH group or C-OH group respectively. The OH bending vibration of COOH groups can be used to evaluate the amount of COOH groups [172]. Vibration at 1060 cm<sup>-1</sup> is assigned to alkoxy (C-OH) groups. Besides, the band at 1721 cm<sup>-1</sup> might referred to not only the carbonyl group stretching vibration of COOH situated at the edges but also to ketones or quinone [173].



Reduced graphene oxide spectrum (RGO) [Fig. (3-2)] shows that, the O-H stretching vibration peak at 3445 cm<sup>-1</sup> is significantly

reduced in intensity indicated that C-OH was still existed but in lower proportion. The peak at 1726 cm<sup>-1</sup> is assigned to carbonyl groups of carboxylic groups. Hydrazine cannot remove it [174]. The bands remained at 1215 cm<sup>-1</sup> and 1084 cm<sup>-1</sup> shows that even after reduction process, some residual oxygen groups were present may be due to the carboxylic groups. A peak at 1574cm<sup>-1</sup> was assigned to conjugate double bonds(c=c).



3.1.1.2 FT-IR of thiocarbohydrazide (TCH)

Fig. (3-3) shows FT-IR spectrum of prepared TCH .The peaks at 3273.2, 3209.5, 3305.9 cm<sup>-1</sup>corsspondes to N-H and NH<sub>2</sub> stretching vibrations respectively. The NH<sub>2</sub> bending and wagging vibrations contributed to the two peaks at 1643.35 and 1143.79 cm<sup>-1</sup> respectively [175]. The characteristic peaks 1531 and1500.6 cm<sup>-1</sup> assigns to the coupled modes N-H wagging and C-N stretching vibrations [175].The C=S stretching contributes to two peaks at 1288 cm<sup>-1</sup> and 935 cm<sup>-1</sup> also these peaks contain contributions of other vibration such as C-N stretching and C-N-N bending vibration .



Figure (3-3) FT-IR spectrum of TCH

#### 3.1.1.3 FT-IR of functionalize GO and RGO with TCH

The infrared spectrum of GO functionalized with thiocarbohydrazide (GOT) shown in Fig. (3-4) the peaks in the region 3000-3500 referred to stretching vibration of  $NH_2$  groups. These peaks were not appeared in the FT-IR spectra of GO and they were suggested to the formation of functionalization of GO with TCH.

A band in the 1590-1650 cm<sup>-1</sup> region is characteristic of the NH<sub>2</sub> scissoring vibration; additionally, absorption band at 750-850cm<sup>-1</sup> assigns to NH<sub>2</sub> twisting and wagging deformations. Also , absorption bands at 1333.32, 1501.4, 1175cm<sup>-1</sup> that appeared in spectrum of (GOT)are correspond to stretching vibration of (C=S) , C=N, and N-N respectively which denotes to stretching vibration that had been introduced to GO by functionalized with TCH.



Figure (3-4) FT-IR spectrum of GO functionalized with TCH.

The FT-IR spectrum of RGO functionalized with TCH (RGOT) Fig. (3-5) exhibit a band at 3286 cm<sup>-1</sup> and 3172 cm<sup>-1</sup> attributed to the NH<sub>2</sub> asymmetric and symmetric stretching vibration. The presence of C=S absorption band at about 1296 cm<sup>-1</sup> for thion and characteristic SH absorption band at about 2600-2550 cm<sup>-1</sup> for thiol forms confirm that the thion-thiol tautomeric form was exist.

The band at 1583.5 was assignable to stretching vibration of azomethane linkage. The new peaks appeared in the spectra of RGOT confirm that cyclocondensation of TCH with carboxylic groups attached at the edge of RGO sheet was happened.





The hydrogen atom may be bonded either to the sulfur atoms or to nitrogen atom so triazole functionalized with GO or RGO can exist in two forms thion (a) and thiol (b) tautomeric as shown in Fig.(3-6) where x is (RGO or GO)





#### **3.1.1.4 FT-IR of MnO<sub>2</sub> nanoparticles**

Generally, oxide and hydroxide of metal nanoparticles give absorption peaks in the finger print region below wavelength of 1000 cm<sup>-1</sup> arising from inter-atomic vibration. The peaks appear in Fig. (3-7) at 526 cm<sup>-1</sup> and 480 cm<sup>-1</sup> were assigned to the Mn-O bond [176]. Beside, a peak at 716 cm<sup>-1</sup> referred to stretching mode of MnO<sub>6</sub> octahedral along the double chain [177 and 178].

These peaks suggest that the  $\alpha$ -MnO<sub>2</sub> was obtained. The absorption peak observed in 3383 cm<sup>-1</sup> can be attributed to expose to the ambient water vapor. The bands at 1635 cm<sup>-1</sup> and 1074 cm<sup>-1</sup> are attributed to the O-H bending vibration.



Figure (3-7) FT-IR spectrum of MnO<sub>2</sub>

# 3.1.1.5 FT-IR spectra of nanocomposites GOT and RGOT with $MnO_2nanopatricles$

Fig. (3-8) and Fig. (3-9) show FT-IR spectra for functionalized GOT and RGOT with MnO<sub>2</sub> respectively. Both spectra show all stretching vibration of GOT and RGOT in lower intensity and appearance of the stretching of Mn-O bonds in MnO<sub>6</sub> octahedra in the region (450–750 cm<sup>-1</sup>) [179,180].







gure (3-9) FT-IR spectrum of RGOTM nanocomposite.

#### 3.1.1.6 FT-IR of MWCNT and f-MWCNT

A comparison between MWCNT without treatment [Fig. (3-10)] and acidic functionalized of MWCNT (f-MWCNT) Fig. (3-11) shows significant differences between the two spectra. The presence of the strong peak at 1739 cm<sup>-1</sup>[181] that corresponded to stretching

Fi

vibration of carboxylic group indicated clearly that carboxylic group present on the surface of MWCNT is due to the success of the carbon atoms oxidation [182].The peak at 3741 cm<sup>-1</sup> is assigned to free OH groups. The peak at 3448 cm<sup>-1</sup>can be attributed to the O-H vibration of carboxyl groups (O=C-OH) and C-OH. Appearance of peak at 1360 cm<sup>-1</sup> may be related with O-H bending deformation in COOH groups' also increased strength of the signal at 1219 cm<sup>-1</sup> may be related with C-O stretching vibration in the carboxylic groups [183]. The peaks at 1547 cm<sup>-1</sup> in the Fig. (3-10) and 1512 cm<sup>-1</sup> in the Fig. (3-11) associated with the stretching vibration of the CNT backbone [183]. Which shifted to lower wave number after chemical treatment with the acid mixture.



Figure (3-10) FT-IR spectrum of MWCNT without treatment



Figure (3-11) FT-IR spectrum of acidic f-MWCNT.

3.1.1.7 FT-IR of f-MWCNT-GOT nanocomposite

FT-IR spectrum of f-MWCNT-GOT nanocomposite [Fig. (3-12)] shows a broad peak at 3440 cm<sup>-1</sup> which confirms formation of hydrogen bonding between carboxylic groups of f-MWCNT and functional groups of GOT. The absorption of carbonyl group, OH bending, C–O stretching vibration in the carboxylic groups and aromatic C=C bonds are still presence but at slightly lower wave number, due to formation the nanocomposites f-MWCNT-GOT.





#### 3.2 Elemental analysis

The elemental analysis was carried out in type Euro EA3000 for GO, RGO, RGOT and GOT using 1 mg of sample. The results indicated the high purity of GO when compared with reference [22] and shows the hydrogen percentage in the RGO less than in the GO this referred to the formation of the double bond between the C-C atoms and the removing of OH groups in the middle of the sheets, also the nitrogen was present in a very small percentage (a residue from the preparation process). The presence of sulfur and the increase of percentage of nitrogen in RGOT and GOT indicated that the functionalization happened as shown in table (3-1).

Table (3-1) elemental analysis of GO, RGO, GOT and RGOT

sample	C%	H%	N%	S%
RGO	$75.3 \pm 0.41$	$1.38 \pm 0.05$	$3.70 \pm 0.04$	-
RGOT	69.30	1.005	17.53	5.288

GO	55.09 ± 0.14	$2.55 \pm 0.05$	$0.12 \pm 0.006$	-
GOT	55.914	1.783	22.04	8.260

### 3.3 XRD analysis

#### 3.3.1 XRD of graphite GT, GO and RGO

XRD patterns of graphite flake GT [Fig. (3-13)] show distinct diffraction peaks at 26.4° (d= 0.336 nm) [184], 43.36° and 54.54° correspond to planes (002), (101) and (004) respectively. The XRD for GT was compared with reference card [No.00-056-0159c] and with literature data (JCPDS 75-2078, 3.347 Å) and we found the position and intensity of the reference matched with data with very small amount of mismatch due to experimental errors[184].



Figure (3-13) XRD pattern of Graphite

A powder sample of GO was examined with x-rays diffractometer. [Fig. (3-14)] shows the XRD pattern of GO. It is found that GO exhibits a strong diffraction at 11.95° corresponding to d-spacing of 0.73 nm [184,185] which is higher than that of graphite (0.336 nm) indicates introduce functional groups (carboxylic acid, carbonyl, hydroxyl and epoxy) on the basal and edges planes of GO sheets [186] Moreover, the increase in d-spacing proved the oxidation of graphite flakes (GT) Since the degree of oxidation is proportional to the interlayer spacing (d) of GO [187 and 188].

The thickness of GO sheets (t) was calculated using the classical Debye–Scherrer.

$$t = \lambda \, k / \beta \cos\theta \tag{3-1}$$

Where, k is the Scherrer constant depends on the shape of the crystal, and the size distribution (shape factor).  $\beta$  is the angular full width at half maximum peak (FWHM) intensity in radian, ( $\lambda = 0.1541$  nm) for Cu K $\alpha$  radiation source and  $\theta$  is the Bragg angle (deg). The diffractograms were recorded in range of 10-80°. The thickness was found to be 6.6 nm. The numbers of layers stacked (n) can be estimated from the following relation

 $n = t/d \tag{3-2}$ 

Where, d is the interlayer spacing .the number of perpendicular stacked GO sheets was found to be 9 as shown in height profile obtained from AFM measurement Fig (3-24). The value of thickness and the number of layers were lower than values obtained in the literature [189].The peaks at 26.5° and 44.3° and it's d-spacing, resemble the one of pristine graphite which indicated that trace of starting material was present in the sample [184]



Figure (3-14) XRD patterns of GO

The XRD patterns of RGO [Fig. (3-15)] shows a broad diffraction peak appeared at  $2\theta 23.7^{\circ}$  [190] indicates poor ordering of the sheets along the stacking direction, which implies the sample was comprised mainly from a few layers of RGO and 43.6<sup>°</sup> corresponding to (002) and (100) planes of RGO respectively, with an interlayer d-spacing of 0.4805 nm which is smaller than

GO due to the removal of most oxygen functional groups [191] as indicated in the FT-IR results. The interlayer d-spacing of RGO was little higher than graphite GT may be attributed to remaining some of oxygen functional group, referring to partial reduction of GTO nanoplates by hydrazine, the appearance of this peak and the disappearance of the diffraction of GO (2 theta 11.95°) implying that the RGO nanosheets were formed, and result a new lattice structure. The thickness or height and the numbers of layers stacked (n) were also estimated and found to be 3.699 nm and 7 respectively. This number of stacked layers referred to graphene nanoplatlets which have distinct properties [192] Reduced graphene oxide (graphene nanoplatlets) is considered as intermediate phase between graphite and pristin graphene known as *quasi*-graphene [193]



Figure (3-15) XRD pattern of RGO

#### 3.3.2 XRD of thiocarbohydrazide (TCH).

Fig. (3-16) shows the XRD of TCH. Comparing the diffraction peak of TCH with known standards in the JCPDS file [35-1800] showed the presence of diffraction peaks in the same position with very small variation in intensity.



Figure (3-16) XRD of TCH

#### 3.3.3 XRD of functionalize GO and RGO with TCH

The XRD pattern of the prepared GOT showed the appearing of many other miller indices due to the TCH crystallinity structure [Fig.(3-17)] and also a decrease in the intensity of GO at  $2\theta = 44.3^{\circ}$ . On other hand a decrease in the d-spacing of GO from 0.739 nm to 0.688 nm confirmed functionalize of GO with TCH and indicated the formation of a new lattice structure. Also, there was an emergence of the new well-defined Bragg reflections at  $2\theta$  angles in the diffraction patterns which suggests that the GOT was formed.



Figure (3-17) XRD of functionalize GO with TCH (GOT)

The XRD pattern for RGOT have a different diffraction intensity from RGO as shown in Fig. (3-18) and the chemicals

functionalization of TCH molecules onto RGO gives rise to decrease the d-spacing from 0.364 nm to 0.354 nm.



Figure (3-18) XRD of functionalize RGO with TCH (RGOT)

#### 3.3.4 XRD of MnO<sub>2</sub> nanoparticles

The XRD pattern of MnO<sub>2</sub> is shown in Fig. (3-19).The diffraction that appeared at  $2\theta = 12.69$ , 18.1, 28.75, 37.5, 42.02, 49.8, 56.4 and 60.09 were matched well with the diffraction peak of crystal planes of  $\alpha$  –MnO<sub>2</sub> standard data (JCPDS card No.44-0141) [135]. No peaks of other phases were detected, indicating the high purity of the synthesized product. Crystalline size for the powder sample obtained at 25 <sup>o</sup>C was estimated according to the Scherrer equation using the diffraction of maximum intensity.

The diameter of the MnO<sub>2</sub> nanorod was calculated to be (11.8 nm) using the strongest diffraction peak of (211) and by substituting the diffraction angle  $\theta$  (18.7766°) and  $\beta$  (0.0124 rad) in the Debye –scherrer equation. This value was corresponding with the value observed by AFM. Increasing number of mole of KMnO<sub>4</sub> in the reaction mixture plays important role in decreasing average diameter of nanorods. According to the literature [194] using 6mmole of KMnO<sub>4</sub> made the diameter of nanorods 20nm.



Figure (3-19) XDR of MnO<sub>2</sub> nanoparticles

#### 3.3.5 XRD of nanocomposites GOT and RGOT with MnO<sub>2</sub>

The XRD pattern [Fig. (3-20)] represented the diffractograms of RGOTM. Its diffraction shows a good similarity to the GOTM [Fig. (3-21)] except its peaks more intensity. The peaks at  $2\theta$ = 37 (211), 66 (002) and 54.7(600) indexed to  $\alpha$ -MnO2 (JCPDS No. 44-0141)



Figure (3-20) XRD of nanocomposite RGOT with MnO<sub>2</sub> (RGOTM)



Figure (3-21) XRD of GOTM nanocomposite

## **3.3.6 XRD** of functionalized multiwall carbon nanotube (f-MWCNT).

X-ray diffractions could provide useful information on the structural properties of MWCNT based material [195].Fig (3-22) shows the XRD pattern of f- MWCNT. XRD shows strong diffraction peaks at  $2\theta = 26.2^{\circ}$  in addition to two diffractions at  $43^{\circ}$  and  $54^{\circ}$  coresponding to the (002) , (100) and (004) planes respectively of graphitic structure of MWCNT [196 and 197]. The peaks of the functionalized MWCNT are very sharped and intense, indicating the graphitic structure of MWCNT have excellent crystallinity (without damaged). The XRD of CNT and graphite GT is very similar due to XRD method can disclose the graphitic structure of carbon and these results were consistent with the reference [198].



Figure (3-22) XRD of f-MWCNT

#### 3.3.7 XRD of f-MWCNT-GOT composite

Fig. (3-23) shows the XRD pattern of f-MWCNT-GOT nanocomposite, the peak at  $2\theta = 26.4^{\circ}$  was relevant to the characteristic of MWCNT. It was also observed that GOT didn't shift the plane (002) of graphite, the distance between planes is little increased (0.337 nm).Presence of GOT nanosheets affected the peak intensity of the f-MWCNT and caused the growth of new diffraction peaks (low intensity) indicated the f-MWCNT-GOT nanocomposites were synthesized.



Figure (3-23) XRD of f-MWCNT-GOT nanocomposites

#### 3.4 AFM measurement

#### **3.4.1 AFM of GO**

AFM is a good technique to give image for the surface morphology. Two dimensional (2D) image for GO sheets observed many sheets, the area A in the cross section at the black arrow. [Fig. 3-24 (A)] represented a double layers sheets with thickness about 5.94 nm, also [Fig. 3-24(B)] show the B area which represented the area of mono layer sheet with thickness 3.71nm.

Three dimensional (3D) image was shown in Fig. 3-249(C) and demonstrated the morphology of the surface and showed the difference in the high between the single and double layers. On other hand these images indicated good chemical preparation of



GO material, which consisted one and two layers sheet. The average lateral size of overall GO flakes is  $1.25\mu m$ 

Figure (3-24) AFM imaged for GO sheets (A) 2D image (B) A cross section (C) 3D image.

#### 3.4.2 AFM of RGO

AFM was also used to examine the morphology and the thickness of the RGO sheets. As shown in Fig. 3-25(A, B and C). Fig. A shows the 2D image and appeared the RGO sheets with total accumulation reached to 33.4nm, the cross-section at the black arrow [Fig. A] shows the lowest thickness of the accumulated sheets about 12.68 nm [Fig. B] which indicated about 3 sheets one on the others[199]. The three dimension image figure C shows almost sheets appear equal in value rate of them clumping above the other.


Figure (3-25) (A) An AFM image of RGO sheets, (B) thickness profile (cross-section) and (C) 3D view.

#### 3.4.3 AFM of MnO<sub>2</sub> nanoparticles

 $MnO_2$  nanotubes showed good images in two and three dimensions [Fig 3-26(A)] image showed the growth of nanorods from the body of the spherical  $MnO_2$  nanoparticles and this case it came in accordance with the literature [200].The nanorods appeared very clear with the length ca. 127.1 nm. A cross-section in [Fig. 3-26(B)] of this nanorod was with a diameter of ca. 5.48 -12.48 nm appeared, the cross-section was made at the black arrow in the two dimensions (A). This value was a good agreement with value obtained from XRD. Many  $MnO_2$  nanorods were shown in the Fig. 3-26 (C).



Figure (3-26) AFM image of MnO<sub>2</sub> Nanoparticles (A) 2 D (B) crosssection (C) 3D MnO<sub>2</sub> nanorod.

### 3.5 SEM analysis

Fig. (3-27) Shows SEM images of  $\alpha$ - MnO<sub>2</sub> nanoparticles in micro (A) and nanoscale (B). It is clearly shown that the product is made up of distinctive  $\alpha$ -MnO<sub>2</sub> nanorod (almost equal in length) was observed to initiated from the surface of MnO<sub>2</sub> formed from spherical MnO<sub>2</sub> nanoparticles.



Figure (3-27) SEM image of MnO<sub>2</sub> nanorods (A) microscale (B) nanoscale

### 3.6 Electrical properties

The permittivity can change widely between solids. Furthermore, the response of permittivity with frequency of applied field and temperature can be either linear or nonlinear [201]. Electric properties such as real permittivity ( $\epsilon$ '), imaginary permittivity ( $\epsilon$ ") and Alternating electrical conductivity ( $\sigma_{a,c}$ ) were measured for all prepared nanomaterials.

### **3.6.1 Real permittivity** $(\epsilon')$

Real permittivity ( $\epsilon$ ') characterizes materials to store charge (energy) when the material exposed to an electric field. The capacitance (c) in F was determined by the LCR meter measurements so the permittivity can be calculated using equation (1-19) .Table (3-2) shows the values of real permittivity of GO and their nanocomposites.

Table (3-2) The real permittivity of GO and its nanocomposites

Frequency(Hz)Log fReal Permittivity(ε')	
---	--

		GO	GOT	GOTM
100	2	32090	51086252	28166855
120	2.1	26636	46467043	23696422
200	2.3	14463	25031714	13436309
400	2.6	8376	27187345	5970998
1000	3	3397	3013484	3266855
2000	3.3	1729	1.6	1254847
4000	3.6	900	1.1	1250158
10000	4	386	2.9	321371
20000	4.3	213	0.6	114543
40000	4.6	125	708	126704
100000	5	69	717	61085

The variation of real permittivity ( $\epsilon$ ') of GO, GOT and GOTM with logarithm frequency shows a sharp decrease with increase of the frequency till 2 KHz and nearly continued the permittivity in very low range as presented in Fig. (3-28).



Figure (3-28) Real permittivity ( $\epsilon$ ') of GO, GOT, and GOTM versus logarithm frequency

Table (3-2) shows the values of real permittivity of RGO and their nanocomposites.

Table (3-3) The real permittivity of RGO and their nanocomposites

Frequency(Hz)	Log f	Re	Real Permittivity (ɛ')		
		RGO	RGOT	RGOTM	

100	2	45657250	33111864	0.5
120	2.1	31658606	27593220	0.6
200	2.3	20779510	16564293	0.5
400	2.6	8598418	8290508	0.4
1000	3	2796233	3143954	0.4
2000	3.3	1512150	1.3	99662
4000	3.6	720903	0.9	59053
10000	4	270448	0.8	15049416
20000	4.3	45049	0.5	13742674
40000	4.6	50201	29934	23915254
100000	5	69032	1199	29448587

Fig. (3-29) shows the relation between real permittivity ( $\epsilon$ ') of RGO, RGOT and RGOTM with logarithm frequency. The real permittivity of RGO and RGOT shows extremely high positive permittivity values at low frequency and decrease with the increasing of the frequency per contra the RGOTM shows very low values at the low frequency and at 2 KHz the real permittivity began to increase with frequency increasing.

This difference in the behavior between the RGO and RGOT and on the other hand the RGOTM, the presence of manganese nanoparticles which may affect the accumulation of the charges and caused the dispersion of the electric charges and reduced the real permittivity values at the low frequency, but at high frequency, the haste of frequency relay was faster than the dispersion of the electrical charge process and whenever the frequency increasing the amount of charge accumulated increases inside the material leaded to an increase in real permittivity.



# Figure (3-29) Real permittivity ( $\varepsilon$ ') of RGO, RGOT, and RGOTM versus logarithm frequency.

### **3.6.2 Imaginary permittivity (ε")**

The imaginary permittivity was quantity of electric energy that transforms into heat energy. The ratio of the imaginary part of the permittivity to the real part of the permittivity [202] was known as loss tangent (tan  $\delta$ ) or the dissipation factor (D) which is shown in the following equation [121].

$$\tan \delta = \varepsilon'' / \varepsilon'$$
 (3-3)

Rearrangement of equ. (3-3) the imaginary permittivity ( $\epsilon$ ") can calculate from equation:

$$\varepsilon'' = \varepsilon' \tan \delta$$
 (3-4)

The values of imaginary part of permittivity ( $\varepsilon$ ") for GO, GOT and GOTM tabulated in table (3-4) and plotted with logarithm frequency [Fig. (3-30)], these data shows lower but almost high values in comparison with real permittivity values. The GOT nanomaterials showed the highest imaginary permittivity which indicated that this carbon nanomaterial have a high conductivity values at least at the low frequency range.

Table (3-4) The imaginary component of permittivity of GO and their nanocomposite

Frequency(Hz)	Log f	Imaginary permittivity (ε")		
		GO	GOT	GOTM
100	2	28467	48991716	21437793
120	2.1	27869	46480983	17672791
200	2.3	16712	29191984	9867625
400	2.6	9418	30398170	6842763
1000	3	3464	3537830	3569692
2000	3.3	605	0.52	1005132
4000	3.6	315	0.38	1259159
10000	4	18	0.05	341681

20000	4.3	25	0.12	98071
40000	4.6	148	70	132684
100000	5	103	92	119929





GO, GOT, GOTM with logarithm frequency.

Table (3-5) shows the imaginary component of permittivity of RGO and their nanocomposites.

Table (3-5) The in	maginary com	ponent of	permittivity	of RGO	and
their nanocomposition	ites.				

Frequency(Hz)	Log f	<b>Imaginary permittivity</b> (ε")		
		RGO	RGOT	RGOTM
100	2	61751431	32101952	0.4
120	2.1	56035733	28175437	0.4
200	2.3	23707343	18000418	0.2
400	2.6	9514149	8087391	0.05
1000	3	2782811	3047435	0.03
2000	3.3	1449850	0.4	81593
4000	3.6	657320	0.3	227769
10000	4	208461	0.04	16038162
20000	4.3	24295	0.08	9791655
40000	4.6	29608	17628	15482735
100000	5	148405	87	18119715

At low frequency the result showed that imaginary permittivity values of RGO have high positive values when compared with RGOT and RGOTM. RGOTM have very low positive values. Also imaginary permittivity of RGO higher than real permittivity may be due to RGO which has high a.c. conductivity. At higher frequencies the real permittivity of RGO and RGOTM is higher than imaginary while real and imaginary values of RGOTM have fluctuated behavior as shown in Fig. (3-31).



Figure (3-31) The variation of imaginary permittivity  $\hat{\epsilon}$  of RGO, RGOT, and RGOTM with logarithm frequency.

### 3.6.3 Alternating electrical conductivity ( $\sigma_{a.c}$ )

The a.c conductivity  $(S.m^{-1})$  of GO, RGO and their nanocomposites films was calculated with varying frequency (f) in Hz ranging from 100 Hz to 100KHz. using the relation [203].

 $σ_{a,c} = ω \varepsilon_{\circ} \dot{\epsilon} tan \delta$ 

where  $\omega$  is the angular frequency which equals to (2 $\pi$ f). Table (3-6) represent the correlation between the a.c conductivity versus frequency of the GO their nanocomposites respectively.

(3 - 5)

Table (3-6) The alternative conductivity ( $\sigma_{a,c}$ ) of GO and their composites

Frequency(Hz)	Log f	$(\sigma) (S.m^{-1})$		
		GO	GOT	GOTM
100	2	0.00015	0.27	0.12
120	2.1	0.00018	0.30	0.12

200	2.3	0.000185	0.32	0.10
400	2.6	0.0002	0.67	0.15
1000	3	0.00019	0.19	0.19
2000	3.3	6.72E-05	5.86E-08	0.11
4000	3.6	7.01E-05	8.64E-08	0.27
10000	4	1.03E-05	3.30E-08	0.18
20000	4.3	2.85E-05	1.37E-07	0.10
40000	4.6	0.0003	0.00015	0.29
100000	5	0.0005	0.0005	0.66

The ac conductivity of GOT at low frequencies as shown in Fig(3-32)was higher than the GO and GOTM and decreased after 400Hz and stayed with very low values after 2KHz, the GOTM showed an increase in the ac conductivity at frequency values more than 20KHz but the GO showed very low values at all frequencies. Functionalize GO with TCH made a.c conductivity for GOT almost equal or greater than a.c. conductivity for RGO at low frequencies.



Figure (3-32) Variation of a.c. conductivity ( $\sigma_{ac}$ ) with logarithm frequency of GO, GOT and GOTM.

Table (3-7) shows the a.c conductivity for RGO and their composites.

Table (3-7) The alternative conductivity ( $\sigma_{a.c}$ ) of RGO and their composites

Frequency(Hz)	Log f	$(\sigma) (S.m^{-1})$		
		RGO	RGOT	RGOTM

100	2	0.34	0.17	2.08E-09
120	2.1	0.37	0.18	3.06E-09
200	2.3	0.26	0.20	2.55E-09
400	2.6	0.21	0.17	1.22E-09
1000	3	0.15	0.16	1.67E-09
2000	3.3	0.16	4.75E-08	0.01
4000	3.6	0.14	6.16E-08	0.05
10000	4	0.11	2.53E-08	8.91
20000	4.3	0.02	9.81E-08	10.88
40000	4.6	0.06	0.03	34.41
100000	5	0.82	0.0004	100.70

The reduced carbone nanomaterials shows a different conductivity behavior than the unreduced materials, the RGO showed relatively higher conductivity at the low frequencies and these conductivity values decrease with increasing of the frequencies, on the other hand the conductivity of RGOT nanomaterials decreased with increasing of the frequencies and stayed at the minimum value in 2KHz. The RGOTM doesn't show any mentioned differences in its conductivity with change of the frequencies in low range of frequencies while its conductivity began to increase from frequency 2 KHz and reached maximum at 100 KHz as shown in Fig. (3-33)



Figure (3-33) Variation of a.c conductivity ( $\sigma_{ac}$ ) with logarithm frequency of RGO, RGOT, and RGOTM.

Also the real and imaginary permittivity of the f-MWCNT-GOT nanocomposites material were studied and electrical conductivity for MWCNT and f-MWCNT-GOT nanocomposite were studied within the frequency range of 100 - 100 KHz as shown in Table (3-8).

Table (3-8) Real, imaginary permittivity and a.c. conductivity of f-MWCNT-GOT nanocomposites and a.c. conductivity of MWCNT.

Freq.(Hz)	Log f	f-	MWCNT		
		Real Permittivity (ε')	Imaginary permittivity (ε")	(σ) (S.m <sup>-1</sup> )	$(\sigma) (S.m^{-1})$
100	2	42250847	4286770983	23.8	0.02
120	2.1	24474576	2787654237	18.6	0.022
200	2.3	13568361	1716397740	19	0.03
400	2.6	-17346892	848436519	18.9	0.05
1000	3	-1339661	338666305	18.8	0.13
2000	3.3	-79177	168917067	18.8	0.20
4000	3.6	-961807	84379407	18.7	0.37
10000	4	94463	34176813	19	0.74
20000	4.3	163163	17017988	18.9	1.11
40000	4.6	123661	8519007	18.9	1.2
100000	5	76944	3370944	18.7	2.53

The real permittivity for f-MWCNT-GOT nanocomposites [Fig (3-34)] started at high positive values at 100Hz then, decreased sharply crosses zero (negative permittivity) and increased at 10 KHz with slightly increasing with increasing frequency.



Figure (3-34) Variation of real permittivity (ἐ) with frequency of f-MWCNT-GOT nanocomposites.

The values of the imaginary permittivity [Fig. (3-35)] were higher than real values. This difference is due to the high conductivity of the f-MWCNT, this reason caused the leakage of the electric charge from the material and appeared the imaginary permittivity higher than the real permittivity [204].



Figure (3-35) Variation of imaginary permittivity  $\hat{\epsilon}$  of f-MWCNT-GOT nanocomposite with frequency.

 $(S.m^{-1})$ Fig. (3-36)shows the ac conductivity of nanocomposites f-MWCNT-GOT and MWCNT as function of logarithm frequency. The nanocomposites exhibit higher conductivity at 100Hz, with increasing the frequency the conductivity varied little. The conductivity of the composite was higher than the other prepared nanomaterial and MWCNT without functionalized.





# 3.7 Electrochemical characterization of the modified SPCE with GO and GOT using ferri-ferro cyanide.

The CV technique was used to investigate the effect of different carbon nanomaterials and theirs functionalization on the oxidation reduction reactions [Fig.(3-37)] shows the cyclicvoltammograms (CVs) for bare SPCE and modified SPCE with GO and GO functionalzed with (ASTT) (GOT)obtained in 0.1M KCl solution containing 0.5 mM Potassium ferricyanide  $K_3$ [Fe(CN<sub>6</sub>]<sup>-3</sup> at 0.1V.s<sup>-1</sup> scan rate.

Potassium ferricyanide (red crystalline salt contains the octahedral coordinated ion) was chosen as an electroactive probe to account the advantages of the modified electrode [205] and for its electrochemical response particularly for carbon materials (implies a single electron transfer and produce a quasi-reversible outer sphere kinetic behavior) [127]. An optimum potential range was determined by using a wide range (-1.0V to+1.0V) at slow scan rate  $(0.01V.s^{-1})$ before performing the measurements on the electrodes. From this experiment, a lower potential range, from -0.3V to +0.6V was selected. In this range the redox peaks of Potassium ferricyanide was At bare SPCE and modified GO-SPCE appeared [127]. well-defined redox peaks were observed and the peak-to-peak separation ( $\Delta E_p$ ) 0.24V with small redox peak current, which was mainly due to slow electron transfer rate at the surface of electrode/solution interface.

At the modified GOT- SPCE the pair of redox peak current of ferricyanide was increased and the  $\Delta E_p$  value decrease to 0.18V due to functionalize (ASTT) at the edge of GO sheet. According to K.R.

Kneten and R.L. McCreery, the electron transfer rate is much faster at the edge plane than at the basal plane [206].

The ratio of the anodic and cathodic peak currents reached unity (*I*pa/*I*pc =0.983) indicating that GOT could effectively increase the electron transfer rate of potassium ferricyanide. A large peak separation (>300 mV) means a resistance to electron transfer [207] these findings indicate that the fastest electron transfer electrochemical reaction occurs at GOT -SPCE than bare SPCE and GO-SPCE. Thus a significant electrochemical behavior refinement could be carried out in the modification of SPCE with GOT.



Figure (3-37) CV curves of bare SPCE, GO and GOT-SPCE in 0.1M KCL solution containing 0.5 mM  $K_3$  [Fe (CN)<sub>6</sub>]<sup>-3</sup>at 0.1V.s<sup>-1</sup> scan rate

The effective area of the electrodes a bare SPCE ,GO-SPCE and GOT-SPCE was calculated in a solution of  $0.5 \text{mM}_3$ [Fe(CN)<sub>6</sub>]<sup>-3</sup> in 0.1M KCl, using Randles Sevick equation [208] at different scan rates ranging from 0.01 to  $0.1 \text{V.s}^{-1}$ 

$$I_{v} = 2.69 \times 10^{5} \text{A} n^{3/2} D_{R}^{1/2} c v^{1/2}$$
(3-6)

Where (I<sub>p</sub>) refers to the peak current, (A) is the surface area of electrode cm<sup>2</sup>, n is the number of electrons ,D<sub>R</sub> is diffusion coefficient, C is the concentration of K<sub>3</sub> [Fe (CN)<sub>6</sub>]<sup>-3</sup> and v refers to the scan rateVs<sup>-1</sup>.For Potassium ferricyanide , D<sub>R</sub> = 7.6 × 10<sup>-6</sup> cm<sup>2</sup> s<sup>-1</sup>[209],and n=1.The redox behavior of 0.5mM K<sub>3</sub>[Fe(CN)<sub>6</sub>]<sup>-3</sup> in 0.1M KCl was investigated by cyclic voltammetry. The redox peak currents at bare SPCE, GO-SPCE and GOT-SPCE increased linearly with scan rate ranging from 0.01 to 0.1V.s<sup>-1</sup> as shown in Figs. (3-38), (3-39) and (3-40) respectively.



Figure (3-38) Cyclic voltammogram for bare-SPCE in potassium ferricyanide



Figure (3-39) Cyclic voltammogram for GO-SPCE in potassium ferricyanide



Figure(3-40)Cyclic voltammogram for GOT-SPCE in potassium ferricyanide.

The current obtained from voltammograms for electrodes recorded and tabulated in Table (3-9).

Scan rate	bare	SPCE	GO-SPCE		GOT-SPCE	
Vs <sup>-1</sup>	current µA		current µA		current µA	
	I <sub>Pa</sub>	I <sub>pc</sub>	I <sub>Pa</sub>	I <sub>Pa</sub>	I <sub>Pa</sub>	I <sub>pc</sub>
0.01	3.062	-2.822	2.742	-2.614	2.969	-2.482
0.02	3.952	-4.057	3.726	-4.712	4.147	-3.66
0.04	4.747	-5.126	5.208	-4.920	5.606	-4.738
0.06	5.482	-5.936	6.059	-6.020	6.341	-4.712
0.08	5.797	-6.472	6.716	-6.654	6.939	-4.920
0.1	5.940	-6.801	7.008	-6.913	7.331	-6.032

Table (3-9) Oxidation reduction peaks current for bare, GO and GOT-SPCE at different scan rates

Plotting the  $I_{Pa}$  and  $I_{pc}$  for bare SPCE, GO-SPCE and GOT-SPCE versus  $v^{1/2}$  produced a straight line. Surface areas were calculated from the slope of these linear graphs table (3-10). The apparent electroactive area values was found for the electrodes bare SPCE, modified electrode GO-SPCE and GOT-SPCE to be 0.04 cm<sup>2</sup>, 0.05 cm<sup>2</sup> and 0.06 cm<sup>2</sup> respectively. This enlargement in the effective surface area proved the modification of bare SPCE with GO and GOT.

Table (3-10) Slope and correlation coefficient for plotting redox peaks current vs square root of scan rate

	Bare-SPCE	GO-SPCE	GOT-SPCE
Slope of $I_{pa}$ vs v <sup>1/2</sup>	15.732	20.308	22.012
$\mathbb{R}^2$	0.955	0.983	0.977
Slope of $I_{pc}$ vs v <sup>1/2</sup>	-15.946	-19.625	-23.547
$\mathbb{R}^2$	0.982	0.968	0.982

## **3.7.1 Electrochemical behavior of GOT modified SPCE with the antibiotics AMP, AMOX and TET**

The change in the pH value affected the electrochemical oxidation process through changing the proton concentration. The modified electrode GOT-Nafion/SPCE examined with 0.1 mM ampicillin AMP, Amoxicillin AMOX and tetracycline TET in phosphate buffer solution PBS at different pH using cyclic voltammetry CV technique at scan rate 0.1V.s<sup>-1</sup>as shown in Figs.

(3-41) and (3-42) there was no redox peaks for AMP or TET respectively at the modified electrode.



Figure (3-41) Cyclic voltammogram of 0.1mM AMP in 0.1M PBS (different pH) at GOT-Nafion/SPCE. Scan rate 0.1v.s<sup>-1</sup>



Figure (3-42) Cyclic voltammogram of 0.1mM TET in 0.1M PBS (different pH) at GOT-Nafion/SPCE. Scan rate 0.1v.s<sup>-1</sup>

AMOX shows significant response [Fig (3-43)]. This varying behavior of the modified SPCE with GOT nanomaterial toward these analytes was unclear. The redox peaks current varied with pH values, the redox peak at pH3 was appeared with higher oxidation and reduction current values.



Figure (3-43) Cyclic voltammogram obtained at GOT-Nafion/ SPCE with 0.5mM AMOX in PBS with different pH values at scan rate  $0.1 \text{v.s}^{-1}$ 

There was no response of blank PBS in GOT-Nafion/ SPCE in all pH range as shown in Fig. (3-44) indicated that the current and potential value of the oxidation and reduction were due to the AMOX only.



Figure (3-44) Cyclic voltammogram obtained at GOT-Nafion/ SPCE in PBS only with different pH values at scan rate 0.1v.s<sup>-1</sup>

The anodic peak current values of AMOX at the GOT-SPCE were plotted versus pH values of the supporting electrolyte (PBS). Fig. (3-45) reveals that the sharp increase in the oxidation peaks in the pH range of 2-3 this may be due to fast electron process then decreased in the range of 3 to 4 this can be related to change of AMOX interaction with surface of modified SPCE (GOT) [210]. Gradually increase in current was observed as the pH values increased from 4 to 6 due to oxidation ability of AMOX, where the

electrochemical response of AMOX in pH7 was low. Considering all these points, the pH3 was with highest current value and show clear redox peak than the other pH values. Therefor this value was used for the determination the limit of detections LOD.



Figure (3-45) Plots of peak current Ip for oxidation 0.5mM AMOX at GOT-Nafion / SPCE vs pH of electrolyte solution PBS (pH3) in 0.1KCl

Cyclic voltammetry CV is a powerful tool for identifying and studying proton and electron transfer (PET). If a process involving m-protons and n-electrons as shown in equation  $A + mH^+ + ne \Rightarrow B$  (3-7)

For Nernst equation, assumption the electrode process was electrochemically reversible, we can write [211]

$$E = E_{f}^{*}(A/B) - RT/nF \ln [B]/[A][H^{+}]^{m}$$
(3-8)  
$$E = E_{f}^{*}(A/B) + RT/nFln[H^{+}]^{m} - RT/nFln[B]/[A]$$
(3-9)  
$$E = E_{f}^{*}(A/B) - 2.303 mRT/nFpH - RT/nF \ln [B]/[A]$$
(3-10)

For a chemically reversible half reaction, the pH dependence of a PET couple is given by eq (3-12) [211]

$$E_{f,eff}^{*} = E_{f}^{*} (A/B) - 2.303 mRT/nF \, pH \qquad (3-11)$$

 $E_{f,eff}^{\circ}$  was an effective formal potential, the potential midway between the peaks for redox peaks for A and B. The dependence formal redox potential  $E^{\circ}$  of AMOX on the pH was investigated by cyclic voltammetry. The  $E^{\circ}$  value was obtained from the average value of anodic potential and cathodic potential. Fig. (3-46) shows that the formal potential (midpoint) of AMOX was highly pHdependent .However, It was found that the E<sup>°</sup> values shifted to more negative values (decrease linearly) with increase in pH buffer solution. Between pH 2 and 3, a slope has value agreement with the theoretical value of 0.06V [212 and 213] according to the equation.

$$E^{\circ} = -0.065pH + 0.26$$
 (3 - 12)

Suggesting that electrochemical oxidation of Amoxicillin involved equal number of electrons and protons [214] then the potential returend to be shifted to more positive values between pH 3-7 with a slope 0.0315 V/pH according to the equations.

$$E^{\circ} = 0.0315pH - 0.0375$$
 (3 - 13)

Sugguseted that two electrons and one proton involved in electrochemical oxidation of AMOX in the this pH range [215]. The intersection of the curve was located at pH 3 corresponding to the apparently pKa. The value of pKa is in agreement with A.T.suji et al [216] and GN. Rolinson [217]



### Figure (3-46) Plots of formal potential E<sup>°</sup> for oxidation 0.5mM AMOX at GOT-Nafion/ SPCE *vs* pH of electrolyte solution PBS ranging from (pH2-pH7) in 0.1KCl.

The cyclic voltammograms for bare SPCE, GO-SPCE and GOT-Nafion/SPCE in 0.1M blank phosphate buffer solution (pH 3.0) and in the presence of 0.5 mM AMOX is shown in Fig. (3-47). The potential was swept from - 0.5V to 0.6V at scan rate 0.1V.s<sup>-</sup> <sup>1</sup>. When the SPCE coated with GO (orange curve) redox peaks for AMOX were observed at 0.12 V with anodic peak current  $(13.186\mu A)$  and at -0.05V with cathodic peak current  $(15.904\mu A)$ . The cyclic voltammogram of GOT-Nafion/SPCE in blank 0.1 M phosphate buffer solution (pH 3.0) showed broad anodic and cathodic peaks (red curve). But in the presence AMOX (green curve), the anodic peak current was appeared at about 0.08 V(anodic current16.443 $\mu$ A), with cathodic peak on the reverse scan at -0.3V ( cathodic current 17.393µA). The increace in redox signal may be attributed to the functionalization of GO with 4-amino-3mercapto1, 2, 4-triazol at the edge of GO sheet which facilitate the electron movment between AMOX and the electrode surface and also could be attributed to GOT which has higher effective surface on this,GOT-Nafion/SPCE can area. based be used as electrochemical sensing for sensitive determenation of AMOX. At the bare SPCE in presence AMOX (black curve) no redox peak can be seen indicating oxidation process of AMOX hard to occur due to surface of SPCE has small amounts of oxygenated functionalities [218]. On the other hand, the bare SPCE have no electrochemical response in blank PBS (pink curve).



Figure (3-47) cyclic voltammogram of 0.5mM AMOX in 0.1M PBS (pH 3.0) at scan rate 0.1v.s<sup>-1</sup>at bare and GO-T-Nafion/SPCE.

### **3.7.2** Effect of amount of the modifier GOT-Nafion mixture on electrochemical detection AMOX

As described in experimental section (2-12) the sensor was prepared by drop a small volume of the GOT/Nafion suspension on the WE of the screen-printed electrode. CV was carried out in presence of 0.5mM of AMOX in PBS (pH 3.0) at 0.1V.s<sup>-1</sup> scan rate with different amounts of GOT/Nafion deposited onto the electrode surface, Fig. (3-48).



Figure (3-48) Cyclic voltammogram of amount of GOT suspension at SPCE in PBS (pH3) containing 0.5mM AMOX .scan rate 0.1V.s<sup>-1</sup>

Many compounds show slow charge transfer rate with SPCE leading to weak defined voltammograms. Several recipes were employed to enhanced the sensitivity and electrochemical properties of carbon-based electrodes such as oxygen plasma treatment [218], preanodized [219], electrochemical cycling [220], these recipes enhanced the electron movement of the modified electrode (WE) through increasing activated surface sites, increasing carbon-oxygen functional (carbonyl and carboxyl) groups and remove contaminants.

This treatment made oxidation process readily occurred and increased peaks current [163]. electrochemical cycling method was used to activate the modified electrode using different volumes of modifier GOT 2, 4, 6, and 8µL by cycling the potential to extreme anodic (positive) potentials from 0 to 1.9V for 19 number of scan Figure (3-49). Using 2µL GOT suspension, no redox response was found probably due to the entire surface wasn't covered, when 4µL casted, an oxidation peak current increase little. An increase in the oxidation peak current was recorded when the deposition volume was up to 6µL (this volume gets higher surface area) which also significantly increased .This volume was almost similar to literature [213]



The oxidation peak current decreased when the amount of GOT-Nafion dispersion increased to (8µL), this may be due to a hindrance electron transfer result from an excessive GOT suspension [213]. From this result, the investigations will be carried out by modification SPCE with 6µL GOT–Nafion suspension. The surface morphology of pretreatment GOT-SPCE was characterized by SEM. From Fig. (3-50A) it is obvious the presence of pores, the surface of SPCE is smoother (nanoscale roughness) and well covered (spread uniformly) with GOT this may provide active sites (edge planes) to interact the AMOX molecules with the surface when compared with bare SPCE Fig. (3-50B)





Figure (3-50) Scanning electron microscope (SEM) images of the surface (A) SPCE modified with GOT. (B) bare SPCE.

### 3.7.3 Effect of scan rate

The effect of the potential scan rate on the electrochemical process was studied to understand the electrode behavior and the reversibility of electrode reactions. Cyclic voltammograms of GOT-Nafion/SPCE in the supporting electrolyte solution PBS (pH3) containing 0.5 mM AMOX at different scan rates from 0.01 to  $0.1 \text{V.s}^{-1}$  which are shown in Fig. (3-51)



Figure (3-51) Cyclic voltammogram of 0.5mM AMOX in pH3 PBS obtained with various scan rates (0.01-0.1 V.s<sup>-1</sup>).

With increasing the scan rate, the oxidation and reduction peak current represent good linear relationship with the scan rates, correlation coefficient 0.9921 and 0.9976 respectively Fig. (3-52)



Figure (3-52) Variation of the peak current  $(I_{pa})$  and  $(I_{pc})$  with the sweep rate for 0.5 mM AMOX.

The peak current is proportional to, scan rate v, surface coverage concentration  $\Gamma$  of electroactive species and number of electrons n according to the following equation. [221]

$$I_{Pa} = n^2 F^2 \Gamma A \upsilon / 4RT \tag{3-14}$$

A is the surface area (cm<sup>2</sup>). The electroactive coverage must be high enough for a current to be observed. the surface coverage concentration of 0.1mM (AMOX) at the surface of GOT-SPCE can be estimated to be about  $5.39 \times 10^{-10}$  mol cm<sup>-2</sup> (n=2) or  $3.246 \times 10^{14}$  molecules.cm<sup>-2</sup>.

In addition, the relationship between  $I_{pa}$ ,  $I_{pc}$  and  $v^{1/2}$  was studied [Figure (3-53)]. The redox peaks current were proportional to the square root of scan rate which proposed the process was a diffusion-controlled in solution [222] when transport the redox species of AMOX in solution to and from electrode.

At scan rates between 0.01 and 0.1 V.s<sup>-1</sup> .the peak heights of the anodic and the cathodic signals were proportional to scan rate and the ratio was almost equal. The difference of the peak potentials is significantly bigger than 59 mV and the separation of the two signals is constant at 0.35 V  $\pm$  3 V. Therefore, the redox reaction of AMOX can be considered to be reversible this means the electrode

practically remain in equilibrium with an oxidized form of AMOX as shown in table (3-11) [207].

Table (3-11) Peak to peak separation and peaks current ratio for AMOX at different scan rate obtained from Fig. (3-51)

Scan rate (V.s <sup>-1</sup> )	$E_{pa}$ - $E_{pc}(V)$	$I_{pa}/I_{pc}$	
0.01	0.33	1.06	
0.02	0.32	0.85	
0.04	0.35	1.05	
0.06	0.36	1.01	
0.08	0.38	1.005	
0.1	0.38	0.96	

The slope of the curve for the two processes, almost the same which mean the redox process occurs with the same electron transfer rate. [223]



Figure (3-53) Relationship between  $I_{pa}$  and  $I_{pc}$  vs. square root of scan rate for GOT modified SPCE.

The diffusion and adsorption of electrode process were studied and a plot of a logarithm of peaks current *vs.* logarithm of the scan rate gave a straight lines with a slope of 0.9494 for foreword scan and 0.8597for reverse scan is shown in Fig. (3-54).This value is close to that found in the literature [224]. The slope of the straight lines were intermediate value between 1 the theoretical value of the adsorption controlled electrode process and the theoretical value of 0.5 which was expressed for the diffusion controlled electrode process this indicated that the electrode process was controlled simultaneously both by diffusion and adsorption [225] for oxidation and reduction process.

The peak current arises not only of AMOX molecules which were already adsorbed on the GOT-SPCE surface but also of those which reach to the electrode by means of diffusion. These results reveal that the anodic process was dominated by partially adsorption (adsorbed AMOX at the electrode) and partially diffusion of AMOX through the diffusion layer simultaneously.



Figure (3-54) Log current vs.  $\log v$  for GOT modified SPCE in presence AMOX.

The models developed by Laviron were developed to provide a quick estimate of the electron transfer rate constant  $\kappa$  using cyclic voltammetry. This method count on the electron transfer coefficient ( $\alpha$ )( dimensionless parameter), which was a measure of the symmetry of the energy barrier of the redox reaction. To determine  $\alpha$ , the peak potential E<sub>p</sub> is plotted vs. In scan rate v [226 and 227]. The peak potentials can be described by following equations.

$$E_{pa} = E^{\circ} + \frac{RT}{(1-\alpha)nF} \ln \upsilon \qquad (3-15)$$
$$E_{pc} = E^{\circ} + \frac{RT}{\alpha nF} \ln \upsilon \qquad (3-16)$$

 $E^{\circ}$  is formal potential, R is the gas constant, n was the number of electrons involved in the redox reaction, T is the absolute temperature in Kelvin, F is the Faraday constant, and v is the scan rate. The cathodic peak potential (E<sub>pc</sub>) is changed linearly as the function of scan rate in range from 0.01 to 0.1 V.s<sup>-1</sup>. A linear regression equation is shown below

$$E_{vc} = -0.0277 \ln v + 0.4096 \qquad (3 - 17)$$

From the slope of Fig. (3-55) and taking the electron transfer coefficient 0.5 according to R.Guidelli *et al* [228] the number of electrons was estimated to be (n~2) this value is in agreement with value obtained from pH measurement. The E <sub>pa</sub> effort non-linear relationship .The electron transfer constant rate  $k_{et}$  was obtained by introducing the values  $\alpha$ , a scan rate 0.1V.s<sup>-1</sup>, and  $\Delta Ep = 0.38V$  in the following equation [229].

$$lnk_{s} = \alpha ln(1-\alpha) + (1-\alpha)ln\alpha - ln\frac{RT}{nFv} - \frac{\alpha(1-\alpha)nF\Delta E}{RT} (3-18)$$

The heterogeneous electron transfer rate constant is  $2.45 \times 10^{-3}$  cm.s<sup>-1</sup>. This value indicate a slow kinetics and longer time requirement for equilibrium [226]





The modified SPCE with GOT was used to estimate lower concentration of AMOX presence in solution. Using differential pulse voltammetry (DPV) technique. Fig. (3-56) showed differential pulse voltammogram under optimized conditions for varying concentrations of AMOX. The concentrations varying in the range of 1-19  $\mu$ M. The voltammogram showed that with increasing concentration of the drug the peak current linearly increased



Figure (3-56) Differential pulse voltammograms of AMOX (PBS pH 3) at different concentrations, (down to up; 1, 4, 7, 10, 13, 16,  $19\mu$ M) scan rate 0.1 V.s<sup>-1</sup> volume of GOT suspension  $6\mu$ L.

Over concentration ranges of  $1-19 \mu M$  [Fig. (3-57)] the calibration curve was linear regression which can be expressed as following equation.

 $I_{v}(\mu A) = 0.411c(\mu M) + 9.6118$   $R^{2} = 0.9744$  (3-19)



Figure (3-57) Values of current versus the concentration of AMOX which plotted based the data of Fig. (3-56).

The limit of detection (LOD) was calculated using the following equation [230 and 231]

$$LOD = 3S_{y/x} / b \qquad (3-20)$$

where b and  $S_{y/x}$  are the slope of the related calibration curve and standard deviation of peak current respectively

The limit of detection (LOD) for AMOX was obtained as 2  $\mu$ M. According to the obtained results, it is possible to apply this technique to the quantitative analysis of AMOX. Table (3-12) showed LOD of other method of determination AMOX.

Table (3-12) Comparison of the efficiency of some methods in the determination of AMOX.

method	рН	LOD(µM)	Reference
Adsorptive Stripping voltammetry MWCNT/GCE		0.2	[232]
Ni/Curcumin/CPE Amperometric		5	[233]
Glutaraldehyde/Glutamic Acid /GCE/SWV	5.2	0.92	[234]
SWV –MWCNT/PCE	10.5	0.0087	[235]
DPV-GOT/SPCE	3	2	This work

### 3.8 Electrochemical characterization of RGO and RGOT-SPCE with potassium ferricyanide.

CV was used to characterize the fabricated modified SPCE with carbon nanomaterials RGO and RGOT electrochemically using redox probe 0.5 mM potassium ferricyanide in 0.1 KCl. RGO-SPCE exhibited electron-transfer reactivity towards ferri/ferro with anodic potential( $E_{pa}$ ) and cathodic ( $E_{pc}$ ) peak potential about 0.18 V and 0.04 respectively[Fig.(3-58)]. The anodic current (Ipa) about 9.630  $\mu$ A and cathodic peak current (Ipc) about -9.293  $\mu$ A.The redox couple gave a  $\Delta$ Ep value of 0.19 V with RGOT-SPCE lower than

bare SPCE ( $\Delta Ep \ 0.24 \text{ V}$ ) and higher than RGO-SPCE ( $\Delta Ep \ 0.14 \text{ V}$ ) this value is in a good agreement with literature [236]. The anodic  $(I_{pa})$  and cathodic  $(I_{pc})$  peak current is the highest (12.358  $\mu$ A) and (-12.23  $\mu$ A) respectively in RGOT-SPCE with I<sub>pa</sub>/ I<sub>pc</sub>=1.0103. A higher peak current and a lower peak potential of the modified SPCE electrode could provide preferable flow of electron transfer and oxidative reaction can be a more sensitivity and reactivity. Thus the presumed electrochemical response improvement could be carried out in the modification of SPCE with RGO and RGOT. It is possible to note a higher and more shaped peak after modified SPCE with RGOT than RGO. Electro catalytic activity of RGO was further enhanced by introducing the groups 4-amino,3-substituted 1H,1,2,4 Triazole 5(4H) thion or thiol (ASTT) which provided the conductive bridges to electron-transfer of ferrocyanide between the sheets. However, RGOT-SPCE modified electrodes show better results than that bare-SPCE.



Figure (3-58) CV curves of bare SPCE, RGO-SPCE and RGOT-SPCE in 0.1M KCL solution containing 0.5 mM  $K_3$  [Fe(CN)<sub>6</sub>]<sup>-3</sup>at 0.1V.s<sup>-1</sup> scan rate.

## **3.8.1** Surface area study for modified SPCE with RGO and RGOT suspension

The real surface area of modified electrode RGOT-SPCE was estimated [237] as the electrode reaction rate was extensive quantities. So the electrochemically active surface areas of the RGO and RGOT modified SPCE were measured. The CV of 0.5mM K<sub>3</sub> Fe (CN)<sub>6</sub> were recorded Figs. (3-59) and (3-60).



Figure (3-59) cyclic voltammogram for RGO-SPCE in potassium ferricyanide at different scan rate.



Figure (3-60) cyclic voltammogram for RGOT-SPCE in potassium ferricyanide at different scan rates.

Under diffusion control and using Randles Sevick equation [207] at different scan rates ranging from 0.01 to  $0.1 \text{V.s}^{-1}$ . Since the value of the diffusion coefficient  $D_R$  of ferricyanide was known the value of A can be obtained, from the slope of plotting of  $I_p$  versus v<sup>1</sup>/<sub>2</sub> Fig. (3-61) and (3-62) for RGO and RGOT respectively. The electrochemically active area of bare, the modified RGO and RGOT electrodes were found to be 0.04 cm<sup>2</sup>, 0.079 cm<sup>2</sup> and 0.10076 cm<sup>2</sup>

respectively .The enhancement in surface area resulted from modified the SPCE with RGO and RGOT.



Figure (3-61) Current versus square root of scan rate for RGO-SPCE.



Figure (3-62) Current versus square root of scan rate for RGOT-SPCE.

Cyclic voltammograms of RGOT-SPCE was measured in various supporting electrolytes such as PBS, NaOH and  $H_2SO_4$  to show the dependence of the electrocatalytic current of AMP .When 0.1M NaOH solution was used no obvious peak was appeared as shown in Fig. (3-63). With  $H_2SO_4$  solution AMP shows weak oxidation peak current at 0.17V [Fig. (3-64)].



Figure (3-63) Cyclic voltammogram of RGOT-SPCE in AMP 0.1mM in 0.1NaOH



Figure (3-64) Cyclic voltammogram of RGOT-SPCE in AMP 0.1mM in 0.1M H<sub>2</sub>SO<sub>4.</sub>

However when used PBS as supporting electrolyte at different pH ranging from 2 to 10 AMP gave well define redox peaks with high peak current Fig. (3-65). At pH<sub>2</sub> the highest current value and an excellent signal enhancement Fig. (3-65) where the best deprotonation of 5-substitution1,2,4 triazole 3-mercapto (ASTT)functional group that attached to RGO sheet.





at RGOT-SPCE.



Figure (3-66) Effect of the pH on the peak current of 0.1mM AMP in phosphate buffers using CV at RGOT-SPCE ; scan rate,  $0.1V \text{ s}^{-1}$ 

To study the proton and electron transfer (PET) for AMP, the formal peak potential *vs.* pH at scan rate of  $0.1V \text{ s}^1$  was plotted .Fig. (3-67) showed that, there are two segments, the first corresponds to slope 0.05V/pH over the pH range of 2–5. The value of slope is close to the expected of the theoretical value of a reversible proton-coupled single electron transfer for Nernst equation [211] as shown in equation below

 $dE^{\circ}/d_{pH} = 0.0592 \ m/n \tag{3-21}$ 

Suggests that the number of electrons is equal to hydrogen ions in the electrode reaction and base on this we predicated the mechanism interaction between AMP and the SPCE substrate modified with RGOT. In the second segment no obvious dependence on pH in a pH window of 6.0–9.0



Figure (3-67) Formal potential, Ep for oxidation0.1mM AMP.at RGOT/Nafion modified SPCE *vs* pH of PBS (pH2) in 0.1KCl.

#### 3.8.2 Effect of drop casted volume

Voltammetric response of AMP was investigated, by drop casting different volumes of RGOT suspension on WE. Increasing the volume suspension of RGOT from 2.0 to  $4\mu$ L enhanced the peak current but at volumes more than 4  $\mu$ L the peak current decrease this may be attributed to the thickness of the suspension film which blocked the surface of electrode [213]. Therefore,  $4\mu$ L was chosen as the optimum amount of RGOT suspension for fabrication of the sensor.

### **3.8.3** Voltammetric behavior of AMP, AMOX and TET at RGOT-SPCE

The modified electrode RGOT-SPCE was examined with 0.1mM TET, AMOX as shown in Fig. (3-68) and (3-69). AMOX and TET revealed a poorly (broad) oxidation peak with low current compared with AMP [Fig. (3-71)], that is due to slow electron transfer. This means there was specificity for GOT-SPCE toward the AMP. So this electrochemical behavior will investigate in detailed in the following section


Figure (3-68) Cyclic voltammogram of RGOT-SPCE with TET in different pH



Figure (3-69) Cyclic voltammogram of RGOT-SPCE with AMOX in different pH.

Figure (3-70) illustrates the cyclic voltammogram of electrochemical response of AMP at bare SPCE by sweeping the potential from- 0.5V to 0.6V at scan rate  $0.1V.s^{-1}$ . The response of bare SPCE in presence of 0.1 mM AMP (PBS pH2) exhibits electron transfer resistance and in which AMP showed broad and poor oxidation peak (0.12µA) with a few small features on both the anodic and cathodic scans which may belong to the carbon ink itself and/or indicates low conductivity because of the existence of small amounts of oxygenated functionalities on the surface of SPCE [218].



Figure (3-70) Cyclic voltammograms of 0.1mM AMP at bare SPCE in 0.1M PBS (pH =2) and 0.1M PBS only at scan rate  $0.1v.s^{-1}$ .

By adding 0.1 mM AMP, a significant enhancement in the cathodic and anodic peak current was observed with RGOT-SPCE so that the modified electrode attractive for applications in the field of electrochemical sensing Fig.(3-71).



Figure (3-71) cyclic voltammogram for bare, RGOT and RGO-SPCE in presence AMP in PBS pH<sub>2</sub>.

The anodic and cathodic peak potential for the oxidation and reduction of AMP at the modified SPCE with RGOT were about 0.19 V and -0.19V respectively. The RGO-SPCE didn't show any response for the AMP test. This result may be because the RGO doesn't contain enough deprotonation groups in its structure. Functionalize RGO with (ASTT) make the electrochemical redox of AMP was stronger than RGO. However, the conductance of the prepared RGOT-SPCE displayed higher conductivity than the bareSPCE, this may be due to the interaction between the AMP with functional group ASTT, and lead us to propose that the deprotonation induced by ASTT and the presence of the hydrogen ion affected the  $\beta$ -lactam group (Azetidine-2-one) of AMP in aqueous solution to yield the carboxylic acid group instead of ketone group, because the AMP has an electrochemically amide functional group which was as in Azetidine-2-one group. Therefore it was expected to show hydrolysis with opening of the four membered ring azetidine-2-one, in a H+/1ē process. This redox behavior closely resembles the quinine/imine redox process in polyaniline. This case of mechanism can be applied on all antibiotic compounds which have a  $\beta$ -lactam group in its structure.

Therefore, consider that both the deprotonation of ASTT, the interaction between the carbone materials of the screen printed electrode and the functionalized carbone nanomaterials (RGOT) and in addition to the hydrogen bonding between the  $\beta$ -lactam group and the amine groups of the ASTT facilitate better sensing performance for AMP Figs.(3-72) and (3-73).



Figure (3-72) The redox process for AMP with applied CV





Series of concentrations of AMP were examined at pH=2 in the range from 1 to 5  $\mu$ M using CV technique [Fig. (3-74)].The oxidation peak current was increased with the increasing of AMP concentration. At present, there was a slight shift in the peak position, it may be said that the adsorption of the side-product of the electrochemical oxidation products on the electrode surface



Figure (3-74) Cyclic voltammogram of AMP (PBS PH 2) at different concentration (0.5,1, 1.5, 2, 2.5, 3, 3.5, 4  $\mu$ M) scan rate 0.1 V.s<sup>-1</sup> volume of RGO-T suspension 4 $\mu$ L.

Under optimal experimental conditions, the calibration plot for detecting AMP with the proposed modified electrode RGOT-SPCE was shown in Fig. (3-75). A linear relationship between the current change and the concentration of AMP was obtained. The linear regression equation was shown below with the correlation coefficients of 0.8139  $I_p = 1.4648c(\mu M) + 26.264$  (3 - 22)

The detection limit was estimated to be 0.5  $\mu$ M. It was noted that the research is focusing on identifying and determination of the LOD of the ampicillin with the CV technique very few. For comparison, kloth *et al.* [238] mentioned 1.1  $\mu$ g/L for different antibiotics in raw milk, J. Adrian *et al* [239]mentioned LOD value of 58-190 nmol/L and Khalilzadeh *et al*[240] mentioned 0.67  $\mu$ mol L<sup>-1</sup>by using carbon-paste electrode modified with Ferrocendicarboxylic acid.



Figure (3-75) The relationship between current and the concentration of AMP

### **3.8.4 Effect of scan rate**

Cyclic voltammograms of the RGOT-SPCE in 0.1mM AMP at different scan rate displayed evident voltammetric waves Fig. (3-76). The separation of the peak potential,  $\Delta E = E_{pa} - E_{pc}$ , increased according to the increasing of the scan rate from 0.01 to 0.1V s<sup>-1</sup> indicating that the velocity of the electron transfer is not high enough. This system is called quasi-reversible [241].



Figure (3-76) Voltammograms obtained from the RGOT-SPCE with 0.1mM AMP in PBS with pH= 2 at different scan rate.

Plotting the anodic peak current  $I_{pa}$ , and cathodic peak current  $I_{pc}$ , against the square root of the sweep rates (v<sup>1/2</sup>) are a linear correlation at all scan rates (0.01-0.1V s<sup>-1</sup>). Figure (3-77), suggesting that the diffusion of the electroactive species on the surface of the electrode was fast to give a linear relation for Ip against the square root of the sweep rate. The slope of the curve  $I_{pa}$  versus square root of scan rate of oxidation process is greater than that corresponding to the reduction process, meaning that the oxidation process occurs with a higher electron transfer rate and the current depends on the concentration of AMP which is oxidized as soon as it reaches the surface of the RGOT-SPCE [224].



Figure (3-77) Linear relationship between Ip *vs*. square root of scan rate for AMP at RGOT-SPCE.

As shown in table(3-13), the peak heights of the anodic signal and the cathodic signal are not completely equal when the scan rate varies between 0.01-0.1V.s<sup>-1</sup> indicated that the process is not

completely reversible[242] anymore Also the difference of the peak potentials was significantly bigger than 59 mV and the separation of the two signals increases with faster scan rates revealing that electron transfer kinetics of AMP at higher scan rates can be considered to be quasireversible behavior [242].

Table (3-13) Potential separation  $\Delta E_p$  with ratio of anodic and cathodic peaks current for RGOT-SPCE in presence AMP at different scan rate

Scan rate (V.s <sup>-1</sup> )	$E_{pa}$ - $E_{pc}(V)$	$I_{pa}/I_{pc}$
0.01	0.26	1.26
0.02	0.29	1.12
0.04	0.34	1.28
0.06	0.33	1.25
0.08	0.42	1.44
0.1	0.46	1.71

Furthermore, from the slope of the linear plot of  $I_{Pa}$  vs. scan rate for RGOT-SPCE and bare SPCE Fig. (3-78) and Fig. (3-79) respectively, the surface coverage concentration  $\Gamma$  of the electroactive species 0.1mM (AMP) that adsorbed at the surface of RGOT-SPCE and bare SPCE can be estimated(using eq.3-14) to be about 6.73×10<sup>-10</sup> molcm<sup>-2</sup> and 3.37×10<sup>-11</sup> molcm<sup>-2</sup> (n=1) respectively.

This high surface concentration for RGOT-SPCE is attributed to the functionalized RGOT deposited in SPCE this means there is strong interaction of AMP to modify electrode than bare SPCE.





Figure (3-78) Variation of the peak current  $(I_{pa})$  and  $(I_{pc})$  with the sweep rate for 0.1mM AMP at RGOT-SPCE



A plot of logarithm the peaks of current *vs.* logarithm of scan rate gave a straight line with a slope of 0.833 for foreword scan and 0.718 for reverse scan Figure (3-80). The slope of the straight lines was intermediate value between 1 the theoretical value of the adsorption controlled electrode process and the theoretical value of 0.5 which is expressed for the diffusion controlled electrode process, this indicated that the electrode process was controlled simultaneously both by diffusion and adsorption [226] and depended on the scan rate [243].



# Figure (3-80) Log current vs. log scan rate $\upsilon$ (V/s) for 0.1mM AMP at RGOT- SPCE

The electron-transfer coefficient ( $\alpha$ ) and electron-transfer rate constant k can be easily determined according to Laviron's equations (3-16) and (3-17).

Fig. (3-81) shows the relationship between the peaks potential ( $E_p$ ) and the natural logarithm of the scan rate of the RGOT-SPCE. The cathodic peak potential ( $E_{pc}$ ) and anodic peak potential ( $E_{pa}$ ), in the range from 0.01 to 0.1Vs<sup>-1</sup>, was changed linearly as the function of scan rate with a linear regression equation of y =-0.0543x-0.035 and y=0.0302x+0.0124 respectively. The slope for cathodic peak equals to  $-RT/\alpha nF$  and for anodic peak equals to  $RT/(1-\alpha) nF$ .  $\alpha n$  can be calculated as 0.47. According to Ref. [244], it is usual that the value of  $\alpha$  is 0.3 <  $\alpha$  < 0.7. Thus, with n = 1 the value of  $\alpha$  will be 0.47. This indicates that the redox reaction between AMP and RGOT-SPCE was a single electron transfer process. This result corresponds with value predicated from pH measurement. The value of apparent heterogeneous electron transfer rate constant ( $k_s$ ) could be calculated using Eq. (3-18) [244]. The value of  $k_s$  is 5.4×10<sup>-2</sup> cm.s<sup>-1</sup>.

RGOT-SPCE showed a faster electron transfer rate, having the higher slopes of 105.41  $\mu$ A (V. s<sup>-1</sup>)<sup>-1/2</sup> compared with RGOTM-SPCE of 32.92  $\mu$ A (V. s<sup>-1</sup>)<sup>-1/2</sup> which have value of k<sub>s</sub> about 4.2×10<sup>-3</sup>.



Figure (3-81) Plot of variation of peaks potential with natural logarithm of scan rate for AMP at RGOT-SPCE.

## **3.8.5 Stability of SPCE modified with RGOT**

The stability of RGOT-SPCE was investigated during storage in dray situation in 25°C shown in Fig. (3-82), by cycling the potential forty times of CV measurement at RGOT-SPCE in 0.1mM AMP in 0.1M PBS (pH2). The peak current intensities, I <sub>pa</sub> and I <sub>pc</sub>, measured between the potentials – 0.5 and 0.6V at scan rate of 0.1V s<sup>-1</sup>.the RGOT-SPCE offers good measurement stability. Little decrease of peaks current was observed with increase of scanning cycle. The cathodic peaks current hold 90% of its initial current after 10days. The electrode can be considered to have a good chemical stability, therefore it can be use without significant variation in response.





#### 3.8.6 Surface characterization of modified SPCE with RGOT

The WE surface of the SPCE plays a very important role, because the area of the electrode was one of the major causes to increase or decrease the efficiency of the sensor and its results. The coated surface of the WE with nanomaterial (RGOT) increase the total surface and added the new properties of the nanomaterial. Fig. (3-83) shows SEM image of bare and modified WE with (RGOT) in microscale (A and B) and in nanoscale (C and D) respectively. The images show that the surface was slightly rough and disordered in bare-SPCE Fig.3-83 (C), while modified electrode with RGOT seems to be an overall smoother than the bare-SPCE. However, the granule sizes appeared smaller than for the bare electrode on the existence of accumulated RGOT sheets.





### 3.8.7 Surface characteristics by AFM measurement

The characterization of the bare and modified WE surface with RGOT was achieved by measuring the roughness parameters extracted from AFM topography using PHYWE software. Quantitative morphological characteristics such as peak height Rp, peak-valley height (Ry), valley depth (Rv) average roughness (Ra), and root mean square roughness (Rq) were obtained and tabulated in table (3-14). Nanoscale surface features were visualized as shown by 3D AFM images in Fig. (3-84), (A) represents the bare SPCE and (B) the modified SPCE respectively. The modified SPCE has lower values for all parameters than bare SPCE.

The values in table (3-14) show a decrease in the values of Rp, Ry, Rv, Ra and Rq for RGOT-SPCE, these results occur through the filling of the valley depth with RGOT nanomaterials and this reduced the valley height and the average roughness became smaller, this indicate to a well distribution of the carbone

nanomaterials (RGOT-SPCE) within the carbone surface of the bare SPCE and came to an agreement with the SEM images results [Fig.(3-83)].

Table (3-14) Surface parameter for bare and modified SPCE with RGOT

parameter	Bare SPCE	Modified SPCE
peak height Rp	47.817 nm	11.835 nm
peak-valley height Ry	84.272 nm	34.602 nm
valley depth Rv	-36.455 nm	-22.767 nm
average roughness (Ra)	12.921 nm	4.9568 nm
root mean square roughness (Rq)	16.592 nm	6.0457 nm





# 3.9 Electrochemical characterization of RGOTM-SPCE using ferricyanide as probe.

Fig. (3-85) shows the electrochemical response of 0.5mM K<sub>3</sub>Fe (CN) <sub>6</sub> in 0.1 KCl, this redox pair is very sensitive to the state of the surface and has been used in the investigations of electrocatalytic activity of carbon electrodes [245]in potential

window from -0.3V to + 0.6V. At bare SPCE well defined redox peaks were observed and the peak-to-peak separation  $\Delta Ep$  was 0.24 with the small redox peak current. When the RGOTM solution immobilize on SPCE a pair of redox peak current increased clearly than bare SPCE and  $\Delta Ep$  value decrease to 0.22 Vindicating that RGOTM increased the electron transfer rate of potassium ferricyanide. Also the anodic ( $I_{pa}$ ) and cathodic ( $I_{pc}$ ) peak current is highest with  $I_{pa}/I_{pc}=1.08$ . This value was identical for simple reversible couple. In light of the above results, the fastest electron transfer electrochemical reaction occurs at RGOTM-SPCE than the bare SPCE. We found the  $\Delta Ep$  value for RGOT lower than RGOTM suggesting that the charge transfer resistance was a little less than RGOTM.



Figure (3-85) Cyclic voltammogram of RGOTM and bare SPCE in presence 0.5mM K<sub>3</sub>Fe (CN) <sub>6</sub> in 0.1 KCl

#### 3.9.1 Surface area study

Surface area study shows a clear evidence for the effective modification of Bare SPCE with modifier GOTM, 0.5 mM  $K_3Fe(CN)_6$  was used to measure the effective surface areas of both bare and modified SPCE by CV at different scan rates Fig.(3-86).



Figure (3-86) cyclic voltammogram for RGOTM-SPCE in potassium ferricyanide at different scan rate.

In the reversible system, the peaks current should follow Randles Sevick equation [207]. The areas of bare SPCE and modified RGOTM-SPCE were estimated from the slope of peaks Ip ( $\mu$ A) vs square root of scan rate v<sup>1/2</sup> (V.s<sup>-1</sup>)<sup>1/2</sup> [Fig. (3-87)] and found to be 0.04 cm<sup>2</sup> and 0.0768 cm<sup>2</sup> respectively. Thus, there was an enhancement in the effective surface area when SPCE was modified with RGOTM, which is a strong evidence for the successful and effective modification of SPCE using RGOTM.



Figure (3-87) Current versus square root of scan rate RGOTM-SPCE.

# 3.9.2 Voltammetric behavior of AMP, AMOX and TET at RGOTM-SPCE.

The influence of pH on the oxidation peak current and potential of AMP, AMOX and TET in PBS pH range 2 to 10 at the GOTM-Nafion modified SPCE was investigated by CV at scan rate 0.1V.s<sup>-1</sup>. Fig. (3-88) shows the cyclic voltammogram for

AMOX, the peak anodic current was broad. The peaks current that started to generate at potential lower 0.4 V with peaks around 0.6 v may belong to the presence of  $MnO_2$ . The modified SPCE with RGOTM doesn't show any response with TET as shown in Fig. (3-89)



Figure (3-89) Cyclic voltammogram of RGOTM –SPCE with TET in different pH. Fig. (3-90) shows existence of well-defined a pair of redox peaks of AMP. Greater current response is observed at pH 2 as shown in Fig. (3-91)





The formal peak potential *vs.* pH was plotted at scan rate of 0.1V.  $s^1$  Fig. (3-92) showed that, there were two segments correspond to slope 0.01V/pH over the pH ranges, suggesting that electron transfer is not accompanied with a proton transfer



Figure (3-92) Plots of peak potential, Ep for oxidation 0.1mM AMP.at RGOTM-Nafion modified SPCE vs pH of PBS in 0.1KCl.

# 3.9.3 Electrochemical behavior of AMP at RGOTM /Nafion-SPCE

The cyclic voltammograms for RGOTM/Nafion-SPCE in 0.1M blank phosphate buffer solution (pH 2) and in the presence of 0.1m M AMP by sweeping the potential from - 0.3V to 0.6V at scan rate 0.1V.s<sup>-1</sup> are shown in Figure (3-93). The response of RGOTM/Nafion-SPCE showed in presence of PBS (pH2) two anodic and cathodic peaks current with height about 7.2  $\mu$ A and - 5.577  $\mu$ A respectively. This redox couple was associated with modified SPCE with RGOTM. The test of AMP show the anodic peak current (height 13.98  $\mu$ A) and significantly appeared at about 0.13 V, with cathodic peak (height -13.198 $\mu$ A) on the reverse scan at -0.21 V( $\Delta$ E<sub>p</sub> =0.34 V). this increace in electrochemical signal come from precence of AMP.



Figure (3-93) CV for RGOTM-SPCE in presence AMP in PBS pH=2

Scan rate studies were carried out to appreciate whether the processes at the GOTM modified SPCE under diffusion or adsorption controlled therefore, the electrochemical behaviors of AMP at different scan rates were investigated with CV [Fig.(3-94)]





A linear relationship was observed between the peak current and the scan rate in the range 0.1 to 0.1V.S<sup>-1</sup> Figure (3-95). The regression equation obtained from CV analysis is:

$$I_{va} = 80.2x + 5.544$$
  $R^2 = 0.9871$   $(3-23)$ 



Figure (3-95) Variation of the peak current  $(I_{pa})$  and  $(I_{pc})$  with the sweep rate for 0.1mM AMP.

The peak heights for the anodic and the cathodic signal are almost equal. Also, the potential difference between the anodic and cathodic potential is almost constant at  $0.36V \pm 2$  V at scan rates between 0.01 and  $0.1V \cdot s^{-1}$ .so AMP fulfills the criteria of a reversible reaction [223] as shown in table (3-15).

Table (3-15) Potential separation  $\Delta E_p$  with ratio of anodic and cathodic peaks current for RGOTM-SPCE in presence of AMP at different scan rates.

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Scan rate (V.s <sup>-1</sup> )	$E_{pa}$ - $E_{pc}(V)$	I <sub>pa</sub> /I <sub>pc</sub>	
0.01	0.35	1.2	
0.02	0.35	0.96	
0.04	0.36	0.95	
0.06	0.37	1.03	
0.08	0.38	1.00	
0.1	0.35	1.06	

The surface coverage concentration  $\Gamma$  of 0.1mM (AMP) that adsorbed at the surface of RGOTM-SPCE was estimated from slope to be  $3.04 \times 10^{-10}$  mole.cm<sup>-2</sup>also a linear dependence of the peak current upon the square root of the scan rate was found Fig. (3-96), demonstrating a diffusional behavior that is, the rate of electron transfer is controlled by diffusion the redox species (AMP) from the bulk to the electrode [246].



Figure (3-96) Linear relationship between  $I_{pa}$  and  $I_{pc}$  of AMP vs. square root of scan rate.

The regression equation obtained from CV analysis is

$I_{pa} = 32.921v^{1/2} + 2.6347$	$R^2 = 0.946$	(3 – 25)
$I_{vc} = -33.37v^{\frac{1}{2}} - 2.2394$	$R^2 = 0.9872$	(3 - 26)

The slope of the curve  $I_p$  versus square root of scan rate were almost similar in oxidation and reduction process, meaning that the redox process occurs with the same electron transfer rate. A plot of logarithm the peaks of current *vs*. logarithm of scan rate showed a straight line with a slope of 0.3153 for foreword scan and 0.3594 for reverse scan Fig. (3-97).



Figure (3-97) log current vs. log scan rate  $\upsilon$  (V/s) for AMP at RGOTM-SPCE.

The slope of the straight lines was close to the theoretically expected value of 0.50 for a diffusion controlled process [226].

Therefore, the electrode process has a diffusion control without any adsorption phenomenon complicating the electron transfer. Transfer coefficient  $\alpha$ n can be determined from the slope of the plot of the peak potential of a cyclic voltammogram against the natural logarithm of the scan rate, v, where slope is equal to the following

$$slop = RT/2\alpha nF$$
 (3 – 27)

This equation hold for reacting species that diffuses toward the electrode without being specifically adsorbed [229].the linear regression of relation above was

$$y = 0.0136x + 0.154 \tag{3-28}$$

From slope an can be estimated as 0.94. Substituted value of a 0.5[229]. Number of electron can be found as 2. The E  $_{pc}$  effort non-linear relationship. The value of apparent heterogeneous electron transfer rate constant (k<sub>s</sub>) could be calculated using Eq. (3-18). The value of k<sub>s</sub> calculated as  $4.2 \times 10^{-3}$  cm<sup>2</sup> s<sup>-1</sup>

## 3.9.4 SEM characterization of SPCE modified with RGOTM

The surface morphology of modified working electrode WE with RGOTM was studied by SEM, from Fig. (3- 98) it clear the presence of RGOT sheets with particles of  $MnO_2$  as they were shown in oval and circle, A represented the carbone substrate of the SPCE, where B represented the RGOT mono layer sheet and spread on it the  $MnO_2$  nanoparticles which appeared in the oval and circle



Figure (3-98) SEM image of WE modified with RGOTM

# 3.10 Electrochemical characterization of GOTM -SPCE Electrode using ferricyanide.

CV is an effective and convenient technique for probing the feature of the modified electrode surface. Here in, CV was employed to investigate the electrochemical properties of GOTM-SPCE electrode .CV experiment have been conducted in 0.1M KCl solution containing 0.5mM  $K_3[Fe(CN)_6]^{-3}$ at 0.1V.s<sup>-1</sup> scan rate [Fig.(3-99)].



Figure (3-99) cyclic voltammogram of bare and GOTM-SPCE in0.8mM potassium ferricyanide at 0.1V.s<sup>-1</sup>.

High value of redox peaks current was obtained for GOTM/Nafion-SPCE compared with bare SPCE. The cyclic voltammogram of K<sub>3</sub>Fe (CN)<sub>6</sub> at bare SPCE has low current signal [ $\Delta$ Ep for bare SPCE was 0.24V]. However, the voltammetric response was apparently improved at GOTM/Nafion-SPCE reflected by the enlargement of the peak currents (Ip) and the ramp of the potential difference [ $\Delta$ Ep GOTM/Nafion-SPCE is 0.23V] between the reduction and the oxidation peaks curve. The ratio of the anodic and cathodic peak currents reached unity ( $I_{pa}/I_{pc} = 1.02$ ), indicating that GOTM could promote electron transfer of [Fe (CN)<sub>6</sub>]<sup>-3/4</sup> quite well due to its high surface area.

### 3.10.1 Surface area study

The microscopic areas of the bare SPCE and GOTM/Nafion-SPCE was measured. Cyclic voltammograms were recorded at different scan rates for GOTM/Nafion-SPCE in presence of 0.5 mM  $K_3$  [Fe (CN)<sub>6</sub>]<sup>-3</sup> as shown in Fig. (3-100).



Figure (3-100) Cyclic voltammogram for GOTM-SPCE in potassium ferricyanide

The obtained current were plotted against the square root of scan rates in case GOTM-SPCE Figure (3-101). The regression equations obtained for GOTM/Nafion-SPCE were

$I_{pa}(\mu A) = 19.321\nu^{\frac{1}{2}} + 1.0876$	$R^2 = 0.9861$	(3 – 29)
$I_{pc}(\mu A) = -23.498v^{\frac{1}{2}} - 0.3677$	$R^2 = 0.9975$	(3 - 30)

Randles-Sevcik equation [207] was used to measure the effective surface area of bare and modified electrode from the slope of the straight line. There was a difference in values of the slopes for both anodic and cathodic currents for ferricyanide in GOTM/Nafion-SPCE indicated a difference in the diffusion rate [224]. The effective surface area increased to 0.05 cm<sup>2</sup> when SPCE modified with GOTM. This enhancement in surface area was resulted in the remarkable catalytic activity of the modified electrode.





## 3.10.2 Voltammetric behavior of Tetracycline at GOTM-SPCE

Fig.(3-102) illustrates the cyclic voltammogram of electrochemical response of TET at the GOTM-SPCE electrode and bare SPCE by sweeping the potential from- 0.6V to 0.8V at scan rate 0.1V.s<sup>-1</sup>. The response of bare SPCE in presence of 0.1 mM TET (PBS pH2) exhibits electron transfer resistance and in which TET showed broad and poor oxidation peak with a few small features on both the anodic and cathodic scans, which may be due to the carbon materials itself



Figure (3-102) Cyclic voltammograms of 0.1mM TET at bare SPCE in 0.1M PBS (pH 2) and 0.1M PBS only at scan rate 0.1v.s<sup>-1</sup>.

From slope of plotting current versus different scan rates Fig.(3-103) The surface coverage concentration ( $\Gamma$ ) of TET at bare



-SPCE was found by using eq.(3-14) [221]As  $3.979 \times 10^{-12}$  mole.cm<sup>-2</sup> or  $26.26 \times 10^{11}$  molecules.cm<sup>-2</sup>



Fig. (3-104) demonstrates the cyclic voltammogram of GOTM-SPCE and bare SPCE in presence of 0.1mM of TET in PBS (pH2) and PBS (pH2) only. The voltammogram exhibited a well-defined two anodic peaks at  $E_p^{a1}$ =0.698V and  $E_p^{a2}$ = 0.08V with a pair of cathodic peaks  $E_p^{c1}$ = -0.2197V and  $E_p^{c2}$ = -0.3841V. The features that are present in bare electrode tend to be dwarfed in modified SPCE. An oxidation peak was appeared at 0.698 V corresponding to presence of GOTM on SPCE. This oxidation peak confirmed presence of MnO<sub>2</sub> on GOT sheet as reported in literature [247]. Cathodic peak at -0.2197 v was assigned to the MnO<sub>2</sub> reduction [248] as shown in the following equation

$$MnO_2 + H^+ + e^- \rightarrow MnOOH$$
 (3-31)

The other two redox peaks were assigned to oxidation and reduction of TET at GOTM-SPCE, as can be seen [Fig. (3-104)] the cathodic reduction peak was not distinctly visible (much broader). The anodic peak current clearly increased compared with bare SPCE this may be attributed to  $MnO_2$  nanoparticles which have catalytic properties for oxidation TET. In addition GO functionalized with TCH may enhanced catalytic activity through synergies with  $MnO_2$  nanoparticles all this could accelerate the transfer rate of electron.



Figure (3-104) Cyclic voltammograms of modified SPCE with GOTM and bare SPCE at scan rate 0.1V.s<sup>-1</sup>in PBS only and in presence TET

### 3.10.3 Effect of amount of the GOTM-Nafion dispersion.

The amount of modifier compounds related to voltammetric response, so the relation between the volume of suspension (GOTM/nafion) and the redox peaks current was examined Fig.(3-105) when the amount of GOTM suspension is  $2\mu$ L no change in current values would be detected (a very thin film). In addition, the higher current is obtained when amount of modifier increases to  $4\mu$ L (high surface area). With further increase in the amount of GOTM-Nafion dispersion, the oxidation peak current showed a drastic decrease, this indicates the hindrance to the electron transfer of TET due to excessive GOTM [214].



Figure (3-105) cyclic voltammogram of amount of suspension GOTM at SPCE in PBS (pH 2) containing 0.1mM TET .scan rate  $0.1 \text{V.s}^{-1}$ 

#### 3.10.4 Effect of pH

The influence of pH value was studied to show electrochemical response of 0.1 mM TET with GOTM modified electrode with CV technique. Fig. (3-106) shows the effect of pH in 0.1 mM TET in buffer solution (PBS) ranging from pH2 to 8 with increasing the pH values of the solution, the cathodic peaks were disappeared of  $MnO_2$ . Also anodic peaks current corresponding to the oxidation of  $MnO_2$ , decreased progressively with increasing pH solution.



Figure (3-106) Cyclic voltammogram obtained from presence 0.1  $\mu$ M TET with modified SPCE with GOTM at varying pH of PBS at scan rate 0.1Vs<sup>-1</sup>.

The oxidation peak current of TET was decreased significantly from pH2to pH8 as shown in Fig. (3-107) .At pH 2, an excellent signal enhancement accompanied by greater current response was obtained. Thus, in subsequent work, a supporting electrolyte PB of pH 2.0 will chose.





The high peak current at pH2 may be attributed to the TET in high acid conditions undergoes dehydration to yield anhydrotetracycline as shown in following figure [249] [250]:



Figure (3-108) dehydration of TET in acid condition

The possible oxidation sites are present in anhydrotetracyclin molecule hydroxide groups, carbonyl and amide. In addition the electrochemical reduction of  $MnO_2$  (introduction of protons and electrons in its structure) [251] makes TET oxidized and gives high peak current. The disappearing of oxidation peak of TET with increasing pH to 8 may be due to transform TET to isotetracycline [252]. From this behavior, it was possible to deduce, that the anodic peak current of TET attributed to the hydroxyl group not to amide group.



Figure (3-109) Transform TET to isotetracycline in base condition

## 3.10.5 Effect of scan rate

The effect of varying potential scan rate on the peak potential and peak current of TET was studied by CV in the scan rate range  $0.02-0.1 \text{ V.s}^{-1}$  Fig. (3-110)



Figure (3-110) Cyclic voltammogram of 0.1mM TET in pH2 PBS obtained with various scan rate (0.02-0.1 V.s<sup>-1</sup>).

The peaks at potentials > - 0.2 V and 0.6 V belong to redox of  $MnO_2$ . There was no corresponding reduction peaks observed at the reverse scan, indicating that the electrochemical oxidation of TET were totally irreversible reaction. This may be due to that oxidized form of TET will be converted to product as in the case of an electron transfer – chemical reaction ( $EC_{irr}$ ) mechanism *and* the rate constant k of reaction (3-37) is finite. I<sub>pc</sub> wasn't appeared at slow and fast scan rates. This means the constant rate reaction for oxidized form was large (the chemical reaction step might be relatively faster than the electron transfer for the oxidation reaction)

Estep	$R \leftrightarrow OX + ne^{-}$	(3 - 32)
Cstep	$OX \leftrightarrow P$	(3 - 33)

By increasing the scan rate, the oxidation peak current shows a good linear relationship [Fig. (3-111)]. The average surface coverage ( $\Gamma$ ) of TET on the surface of modified electrode GOTM could be estimated based on the slope of current I<sub>p</sub> vs. scan rate v [253].the surface concentration of TET at GOTM-SPCE was found to be  $1.60 \times 10^{-9}$  mole cm<sup>-2</sup> or  $10.56 \times 10^{14}$  molecule cm<sup>-2</sup> by the involvement of two electrons in the process. This high surface concentration (higher than bare) can be attributed to modify the electrode with GOTM nanomaterial. The larger surface area of the GOTM nanomaterial may be helpful for more of TET molecules to take part in the electron transfer process.





The dependence of the oxidation peak current  $I_p^{ox}$ , on the square root of the scan rate  $v^{1/2}$  at the GOTM electrode was presented in Fig. (3-112). Anodic peak current at the GOTM electrode increases linearly with  $v^{1/2}$  indicating a diffusion-controlled process in solution, according to the Randles–Ševčik equation.



Figure (3-112) Linear relationship between I  $_{pa}$  vs. square root of scan rate v V.s<sup>-1</sup>

The plotting of log-log peak current versus scan rate considers a primary quantitative to distinguish simple adsorption peaks from diffusion peaks. However, adsorption and diffusion peaks may be overlapped and difficult to distinguish. A plot of log ( $I_p \mu A$ ) vs log (v Vs<sup>-1</sup>) Fig.(3-113) gave straight line with a slope of 1.002 correlation coefficient 0.9927. The slope of the straight lines was similar to the theoretical value of the adsorption controlled electrode process [226].





This means the TET molecules that were adsorbed at the electrode will undergo electron transfer process [226] and has significant interaction with the electrode surface. Additionally, the peak potential  $E_{pa}$  of oxidation TET gradually shifted to the positive direction with increasing the scan rates Fig. (3-114) which confirms the irreversibility of the process [254].



Figure (3-114). Linear relationship between E (V) vs. Scan rate v  $(V.s^{-1})$ 

The  $E_p$  of the oxidation peak was also dependent on scan rate Fig. (3-115) shows the relationship between the peak potential ( $E_p$ ) and the logarithm of scan rate (logv) for the modified electrode GOTM in 0.1 mM TET.

As can be seen, in the range from 20 to 100 mV.s<sup>-1</sup>, the anodic peak potential ( $E_{pa}$ ) changed linearly versus *logv* which is expressed by the equation

$$E_{v}(V) = 0.045 \log v(V S^{-1}) + 0.0113 \qquad (3-34)$$



Figure (3-115) Variation of peak potential with logarithm of scan rate

For an adsorption-controlled and irreversible electrode process, Ep was defined according to Laviron equation [255,256]

$$E_{p} = E^{\circ} + \left(\frac{2.303RT}{\alpha nF}\right) \log\left(\frac{RT\kappa^{\circ}}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) \log \upsilon \quad (3-35)$$

The slope of the linear regression of Ep vs. logv Fig. (3-115) was equal to  $2.303RT/\alpha nF$ . The evaluated value of  $\alpha n$  can be calculated to be 1.3111.  $\alpha$  was assumed as 0.5 for a totally irreversible electron transfer, it could be concluded that  $n \cong 2$ . The value of  $\kappa^{\circ}$  was determined from the intercept of the above plot if the value of E<sup>°</sup> was known. The value of E<sup>°</sup> can be obtained from the intercept of Ep vs v curve [Fig. (3-114)] by extrapolating to the vertical axis at v=0 [257]. E<sup>°</sup> can be obtained to be 0.0425 V.

So the apparent electrochemical rate constant  $k^{\circ}$  was calculated to be 10.41 cm.s<sup>-1</sup>.

### 3.10.6 Calibration curve and detection limit

Differential pulse voltammetry (DPV) was employed as a technique for the evaluation of the performance of the GOTM-SPCE sensor for the determination of TET concentration under the optimized experimental conditions. The increasing of TET concentration on the WE increased the peak current linearly and shows a little change in the potential values with DPV measurements Fig. (3-116).



Figure (3-116) Differential pulse voltammograms at a GOTM-SPCE for TET concentrations ranging 1-9µM.

The calibration curve was linear over concentration ranges of  $1-9 \mu M$  Fig. (3-117).The linear regression equations can be expressed as





The LOD was determined to be  $0.2 \times 10^{-6}$  M. The TET detection in other ways is shown in Table (3-16).

Table (3-16) Comparison	with other	methods for	the deter	rmination
	of TE	Г		

Used method for detection	LOD (µM)	Ref.
Fluorescence optical fiber sensor	0.106	[258]
Molecularly Imprinted Technique	0.0558	[259]
High-throughput suspension array technology	0.00034	[260]
High pressure liquid chromatographic	0.021	[261]
Aptasensor	0.0003	[262]
Electrochemical sensor	0.2	This work

The modified electrode GOTM-Nafion /SPCE also was examined with AMP and AMOX. There was no redox peaks for

AMP or AMOX in this modified electrode even after cleaning the electrode surface in phosphate buffer (in absence of the target analyte) by high anodic (+2V) potential. The peaks current at around 0.6 V belonged to  $MnO_2$  that immobilized at GOT sheet as shown in Figs. (3-118 and 3-119) respectively. This means there was specificity for GOTM-Nafion /SPCE toward the TET.



Figure(3-118) Cyclic voltammogram of 0.1mM AMP in 0.1M PBS (pH2) at scan rate 0.1v.s<sup>-1</sup>at GOTM-Nafion/SPCE



Figure (3-119) Cyclic voltammogram of 0.1mM AMOX in 0.1M PBS (pH 2) at scan rate 0.1v.s<sup>-1</sup>at GOTM-Nafion/SPCE.

# 3.11 Electrochemical properties of (f-MWCNT) and its nanocomposites using potassium ferricyanide.

Carbon nanotubes (CNTs) have received great attention especially in the field of sensors due to their unique properties. In

this section the electrocatalytic properties of f-MWCNT and its composite with GOT were studied.

### 3.11.1 Surface area study.

Drops casted method was used to study voltammetric response of modified SPCE with different volumes of MWCNT without treatment. MWCNT with treatment (f-MWCNT) and its nanocomposites with GOT (f-MWCNT-GOT) using ferri/ferro redox couple system using CV technique. Fig (3-120) shows the modifier f-MWCNT and f-MWCNT-GOT nanocomposites exhibit higher current response in presence of ferric/ferro couple and can be use more volumes  $(18\mu L)$  and  $(14\mu L)$  respectively compared with plain MWCNT (2µL). Also response of f-MWCNT/GOT didn't change. This enhanced in heterogeneous electron transfer rate for f-MWCNT is due to presence oxygens functional moieties at the tips of MWCNT according to reference [263], Also presence of the ASTT groups functionalized attached at the edge of GO sheets in nanocomposite accelerate electron transfer between ferro/ferric and electrode surface. The higher amount of f-MWCNT and its nanocomposite didn't make the film removed from the electrode surface.



Figure (3-120) Volume of MWCNT, f-MWCNT and f-MWCNT/GOT modifiers in presence 0.8 mM Potassium ferricyanide in 0.1M KCl

The Randles Sevick equation [207] was used to estimate effective surface area of the modified SPCE with MWCNT, f-
MWCNT and f-MWCNT-GOT using 0.5mM potassium ferrocyanide as probe. Cyclic voltammetric measurements were performed at various scan rates ranging from 0.03 to 0.18V.s<sup>-1</sup>.by plotting Ip versus square root of scan rates fig. (3-121), (3-122) and (3-123) surface of MWCNT-SPCE, f-MWCNT-SPCE and f-MWCNT-GOT-SPCE was founded as 0.04, 0.119 and 0.12 respectively.



Figure (3-121). Linear relationship between I pa *vs.* square root of scan rate of MWCNT-SPCE.



Figure (3-122) Linear relationship between Ipa *vs*. square root of scan rate of f-MWCNT-SPCE.



Figure (3-123) Linear relationship between I<sub>pa</sub> vs. square root of scan rate for f-MWCNT-GOT.

According to method by Sharp [221] eq. (3-15) and from the slope of the anodic peak currents *versus* various scan rate as shown Fig. (3-124) an approximate of the surface coverage  $\Gamma$  (mol cm<sup>-2</sup>) of the modified electrode was estimated for MWCNT-SPCE, f-MWCNT-SPCE and f-MWCNT-GOT/SPCE and found as 9.447×10<sup>-10</sup>, 9.449×10<sup>-10</sup> and 9.3×10<sup>-10</sup> mol cm<sup>-2</sup>, for MWCNT, f-MWCNT and f-MWCNT-GOT respectively.



Figure (3-124) Variation of  $(I_{pa})$  and  $(I_{pc})$  with the sweep rate for modified SPCE MWCNT, f-MWCNT and f-MWCNT-GOT in 0.8mM potassium ferrocyanide.

The modified electrode electrochemically tested to investigate its electroactivity and to improve it for a variety of applications. The standard solution of  $Fe(CN)_6^{3^-}/Fe(CN)_6^{4^-}$  couple was used for comparing the voltammetric behavior of modified screen-printed electrodes. This redox couple shows a simple and well-defined response .The cyclic voltammograms of the redox couple 0.8mM potassium ferrocyanide at scan rate 0.1V/s at a bare/SPCE, MWCNT, f-MWCNT and f-MWCNT-GOT /SPCE are shown in Fig.(3-125).



Figure (3-125) Cyclic voltammograms for modified electrodes MWCNT, f-MWCNT, f-MWCNT-GOT and bare in 0.5mM ferri/Ferrocyanide in 0.1M KCl supporting electrolyte.

The peak separations and anodic ( $I_{pa}$ ) and cathodic ( $I_{pc}$ ) peak current height are also illustrated in Fig. (3-126) and (3-127) respectively. The f-MWCNT electrode, yielded good reversibility with  $\Delta E_p$  values of 0.14V and higher peak current  $I_{pa}$  values of 14.07  $\mu$ A. This behavior was attributed to the good charge transfer of the electrode. The redox peak current of ferro couple for plain MWCNT was lower than the bare SPCE although the peak separation for MWCNT is lower. This may belong to absence of functionalities that accelerate electron transfer. Thus the peak current was dependent in MWCNT being treated or not. The modified SPCE with f-MWCNT-GOT was exhibited electron-transfer towards ferri/ferro, with a  $\Delta E_p$  value of 0.17V.

The values of  $\Delta E_p$  were lower than the  $\Delta E_p$  for vertically aligned (VA) CNTs and horizontally/randomly oriented (HRO) CNTs by 163 mV and 277 mV [264] respectively. The reduction current of f-MWCNT-GOT nanocomposites was a little higher than f-MWCNT. The anodic peak potentials that caused to oxidation ferricyanide at the modified electrodes MWCNT, f-MWCNT and f-MWCNT-GOT

are almost the same 0.21, 0.20 and 0.21 V  $\,$  respectively and lower than bare SPCE (E  $_{pa}$  0.24 V) .



Figure (3-126) The seperation peak potential for bare-SPCE, modified electrodes MWCNT,f-MWCNT and f-MWCNT-GOT/SPCE in 0.8mM ferri/ferrocyanide





The half-wave potentials  $E_{1/2}$  were determined from the anodic ( $E_{pa}$ ) and cathodic ( $E_{pc}$ ) peak potentials according to equation.

$$E_{1/2} = (E_{pa} + E_{pc})/2 \tag{3-37}$$

And the peak current ratio  $I_{pa}/I_{pc}$  was estimated for all the modified electrodes MWCNT, f-MWCNT, f-MWCNT-GOT and bare are listed in Table (3-17).

Table (3-17) Electrochemical parameters characterizing the redox couple0.8mM ferrocyanide on bare SPCE, MWCNT, MWCNT/GOT, F- MWCNT /GOT and F-CNT/GOT electrodes at scan rate  $0.1 \text{ Vs}^{-1}$ 

electrodes	$E_{1/2}(V)$	$I_{pa}/I_{pc}$
Bare	0.12	0.87
MWCNT	0.125	1.03
f- MWCNT	0.13	1.34
f-MWCNT -GOT	0.125	0.94

## 3.12. The modified SPCE with MWCNT and its composite for the detection test of AMP.

### 3.12.1 The effect of pH

The electrochemical behavior of ampicillin antibiotic was dependent on the pH value of solution. To evaluate the optimum pH for ampicillin detection on the surface of f-MWCNT-GOT modified SPCE, its electrochemical behavior was investigated in the buffered solutions with various pH in the range of 2-8. A buffer solution of Ph= 2 was chosen as an optimum value in order to obtain the best sensitivity in all voltammetric measurements [Fig. (3-128)].



Figure (3-128) Electrochemical behavior of AMP in f-MWCNT-GOT modified SPCE at different pH value of PBS at sweep rate  $0.1 \text{V s}^{-1}$ .

For probing the feature of the modified surface of SPCE, CV was employed to investigate the electrochemical properties of each modified electrode. For bare SPCE very weak andic peak current was observed in pH2 was (0.6 $\mu$ A) and in presence of AMP (0.67  $\mu$ A) [Fig. (3-129)].



Figure (3-129) Cyclic voltammograms for bare-SPCE in presence AMP in buffer pH2 and in PBS pH2 only.

Fig. (3-130) shows the CVs of modified electrodes, MWCNT, f-MWCNT and f-MWCNT-GOT in PBS buffer (pH = 2) at scan rate 0.1V/s. MWCNT-SPCE showed oxidation peak of AMP at 0.12 V with oxidation current about (6.273 $\mu$ A). The current of AMP clearly increased with f-MWCNT-SPCE, 46.072  $\mu$ A for anodic and -19.26  $\mu$ A for cathodic, indicating that the carboxylic group significantly enhanced the electron transfer between AMP and surface of f-MWCNT-SPCE with redox peaks at -0.19 and 0.13 V respectively. Based on the comparison in terms of the peak current, SPCE modified with f-MWCNT-GOT nanocomposite produced the highest peak currents (105.279  $\mu$ A at potential 0.19V) for anodic and (-23.976  $\mu$ A at potential -0.36V) for cathodic. Since MWCNT and f-MWCNT have the lowest peak current, it was not considered further in the study.



Figure (3-130) Cyclic voltammograms for bare and modified electrode MECNT, f-MWCNT and f-MWCNT-GOT in presence and absence of 0.1mM AMP.

### 3.12.2 Effect of scan rate

Cyclic voltammograms of the AMP were recorded at different scan rates (from 0.03 to 0.18 V s<sup>-1</sup>) [Fig. (3-131)].



Figure (3-131) Cyclic voltammograms for modified electrode f-MWCNT-GOT in presence of 0.1mM AMP at different scan rates.

The dependence of anodic peak current  $(I_{pa})$  and cathodic peak current  $(I_{pc})$  on the scan rate showed, a linear relationship .Fig (3-132) illustrates that the anodic and cathodic peak currents  $(I_p)$  were linearly dependent on scan rate (v).



Figure (3-132) Variation of the peak current  $(I_{pa})$  and  $(I_{pc})$  with the sweep rate for 0.1mM AMP at f-MWCNT-GOT nanocomposite modified SPCE.

The peak heights for the anodic signal was higher than the cathodic signal  $I_{pa}/I_{pc} \neq 1$ . Also, the potential difference between the anodic and cathodic potential increased with varying scan rates between 0.03 and 0.18V.s<sup>-1</sup>.As the reverse wave is noticed at  $\Delta E_p > 0.4V$  [265]for this AMP have irreversible charge transfer process  $E_{irr}$  as shown in table (3-18).

Table (3-18) Potential separation  $\Delta E_p$  with ratio of anodic and cathodic peaks current for f-MWCNT-GOT in presence of AMP at different scan rates

unterent sean fates.			
Scan rate $(V.s^{-1})$	$E_{pa}$ - $E_{pc}(V)$	$I_{pa}/I_{pc}$	
0.03	0.25	4.4	
0.05	0.34	4.5	
0.07	0.39	4.52	
0.09	0.48	3.35	
0.12	0.49	3.24	
0.14	0.54	4.38	
0.16	0.63	4.18	
0.18	0.55	4.68	

According to the eq.(3-42) for an irreversible electron transfer ET [265]

$$dEp/d\log v = 1.15RT/F\alpha \tag{3-38}$$

From Fig (3-133) The values of the electron transfer coefficient  $\alpha$ , which have been estimated from the peak potential slope, and found to be 0.15 this diminished due to breaking bonds (high activation energy).



Fig (3-133) variation of potential with log scan rate

As for the irreversible electrode process of the oxidation reaction of AMP, the Laviron's equation eq. (3-43) [36] was used to estimate n and k<sub>s</sub> values as follows:

### $E_{p}=E^{0}+(2.303RT/\alpha nF)\log(RTk_{s}/\alpha nF)+(2.303RT/\alpha nF)\log v$ (3-39)

Where  $\alpha$  is the electron transfer coefficient,  $k_s$  is the standard rate constant of the surface reaction, v is the scan rate, n is the electron transfer numbers and  $E^0$  is the formal potential.  $K_s$  and n values can be concluded from the intercept and slope of the linear plot of  $E_p$  with respect to log v Fig. (3-133), if the value of  $E^0$  was known. The  $E^0$  value at f-MWCNT-GOT nanocomposite of the modified SPCE can be deduced from the intercept of Ep *vs.* v [Fig. (3-134)] plot on the ordinate by extrapolating the line to v=0. Knowing  $E^0$ , and from the graphical representations of  $E_p$  *vs.* Log vfor AMP [Fig. (3-133)]



Figure (3-134) The variations of peak potential *vs. v* at f-MWCNT-GOT/SPCE.

The value of n=2 obtained from the slope indicated that two electron was involved in the oxidation of AMP at the modified SPCE with f-MWCNT-GOT nanocomposite. Also the values of  $k_s$ =432.68 cm.s<sup>-1</sup> was obtained from the intercept. This high enhancement in value of  $k_s$  compared with other modified electrode clearly demonstrate that the introduction of a nanocomposite f-MWCNT-GOT in the surface of electrode opens an efficient ET between AMP and the modified WE.

Linear relationships were observed between log  $I_p$  and log v over the scan range 0.03-0.18 V.s<sup>-1</sup> with slope of 0.6442 and 0.6781 respectively as shown in Fig. (3-135). The slope of the straight lines are intermediate value between 1 the theoretical value of the adsorption controlled electrode process and the theoretical value of 0.5 which was expressed for the diffusion controlled electrode process, this indicated that the electrode process was controlled simultaneously both by diffusion and adsorption [226]for redox process.



Figure (3-135) Plot of log current vs. log scan rate  $\upsilon$  (V.s<sup>-1</sup>) for f-MWCNT-GOT modified SPCE in the presence of AMP.

## **3.12.3 Limit of detection for AMP using f-MWCNT-GOT modified SPCE**

The DPV of the f-MWCNT-GOT modified SPCE in the presence of various concentration of AMP are shown in Fig. (3-136) the oxidation of AMP was accompanied by an increase in the anodic current which could significantly improve the detection limits of electrochemical measurement.



Figure (3-136) Differential pulse voltammogram for modified SPCE with f-MWCNT-GOT nanocomposite in presence of different concentrations of AMP in range of  $1.52 \times 10^{-7}$  to  $1.46 \times 10^{-5}$  M.

Under optimum experimental conditions the calibration curve was obtained. The peaks current increase linearly with the increase of AMP concentration in range of  $1.52 \times 10^{-7}$  to  $1.46 \times 10^{-5}$  M [Fig. (3-137)].



# Figure (3-137) The relationship between current and the concentration of AMP at modified SPCE with f-MWCNT-GOT nanocomposite.

The detection limit was found as  $1.46 \times 10^{-7}$ M using the same equation (3-21) according to this equation the f-MWCNT-GOT modified electrode has lower detection limit.

## **3.12.4** Surface characterization of the modified SPCE with f-MWCNT and its composite f-MWCNT-GOT.

Fig. (3-138) shows the SEM image of f-MWCNT. The SEM image reveals that MWCNT has well distribution on the SPCE surface. From Fig. (3-139) it was clearly that the composite is well distributed even though some MWCNT still visible. There are several proposals that clarify the mechanism of dispersed the MWCNT with nanosheets. S.Chen *et al* [266] proposed the sheets can wrap around the CNTs while M.Shin *et al* [267] proposed the CNTs are attached to the edges and surfaces of the graphene sheets.



Figure (3-138) SEM image of surface of f-MWCNT



Figure (3-139) SEM image of surface of f-MWCNT-GOT nanocomposite.

### 3.13 Conclusion

1. In this work carbon nanomaterials (GO and RGO) and functionalized with -4-amino,3-substituted 1H,1,2,4 Triazole 5(4H) thion(ASTT) (GOT and RGOT) was prepared, moreover the carbon nanomaterials made a composite with  $MnO_2$  nanorod (GOTM and RGOTM).

2. A commercial multiwalled CNT, was carboxylate with strong acid (f-MWCNT) and nanocomposites with GOT was prepared successfully.

3. All the carbon nanomaterials were characterized with FT-IR and XRD and compatible with references.

4. The diffraction at  $2\theta$  (11°) was disappeared this indicated a reduction process was happened and at the same time appeared the (002) diffraction again in  $2\theta$  (27.3°) this may be due to the rearrangement of the sheets in form nearly like in graphite structure and caused the high conductivity values for RGO.

5. The morphology of the GO, RGO nanosheets and  $MnO_2$  nanorods were studied with AFM technique. The GO shows sheet thickness 3.71 nm and this represented mono sheet thickness, which means a very good syntheses of the carbon nanomaterial.

6. Image of the  $MnO_2$  nanorods showed the development of the rods from the spherical  $MnO_2$  nanoparticles and this image is very uncommon in the references.

7. The number of moles of  $KMnO_4$  played important role on the preparation and controlled diameter of  $MnO_2$  nanorods.

8. The prepared carbon nanomaterials shows different behaviors and values in the electrical measurements.

9. The real permittivity was in the following sequence from the high to the low value at 400 Hz GOT 27187345 >RGO 8598418> RGOT 8290508>GOTM 5970998 > GO 8376 > RGOTM 0.45 > f-MWCNT -17346892

10. The electrical conductivity at 400Hz was found in the following sequence from the high to low values.

f-MWCNT-GOT (18.86) > GOT (0.67) > RGO(0.21) > RGOT (0.17)> GOTM (0.15) > GO (0.0002) > RGOTM (1.22E<sup>-09</sup>)

11. The electrical conductivity of GOT was higher than the RGO and RGOT.

12. Carbon nanomaterials showed a relatively different cathodic and anodic current values using cyclic voltammetry technique, due to different electrochemical activities and the speed of electron movement between the examined antibiotic and the working electrode.

13. The following carbon nanomaterials show a sensing and the detection of the antibiotics.

f-MWCNT-GOT composite detected AMP

f-MWCNT detected AMP

GOT detected AMOX

RGOT detected AMP

GOTM detected TET

RGOTM detected AMP

The above carbon nanomaterials were arranged from top to bottom according to the electrical conductivity values from the highest to the lower one.

14. The f-MWCNT-GOT composite and f-MWCNT showed the highest current peak in comparison with other nanomaterials.

15. The f-MWCNT-GOT composite showed higher apparent heterogeneous electron transfer rate constant  $(k_s)$ 

#### 3.14 Future Study

1- The obtained results encourage the researcher to work in the wider area of the different families of antibiotics.

2- Development of new carbon nanomaterials with other functional groups were not used in this work.

3- Functionalization of the carboxylate MWCNT with TCH and examine the LOD values of different antibiotic and agricultural pesticides.

4- Study the effect of the real and imaginary values of permittivity and the electrical conductivity of the prepared carbon nanomaterials on the LOD values.

5- The synthesized carbon nanomaterial in this work can be studied the environmental pollution with agricultural pesticides, those containing amide groups.

6- Preparation of new carbon nanomaterials with nano-metal oxide, nano metal and or conductive polymer, to detect the different organic and inorganic materials.

7- Functionalize the prepared carbon nanomaterials with azo compounds and studying the photoelectric effect of the cis-trans isomerization in photo electric cells.

8. Studying the aqueous suspension of the reduced graphene oxide and graphene oxide functionalized with thiocarbohydrazide RGOT, GOT respectively alone or with MWCNT by incorporate enzymes to using them in biological applications.

9. Using this novel functionalities with hydrogel to study drug delivery.

10. Using this nanomaterials as adsorbent surface to many pollutants.

11. Studying Influence of this novel functionalities on dielectric behavior of some polymers

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