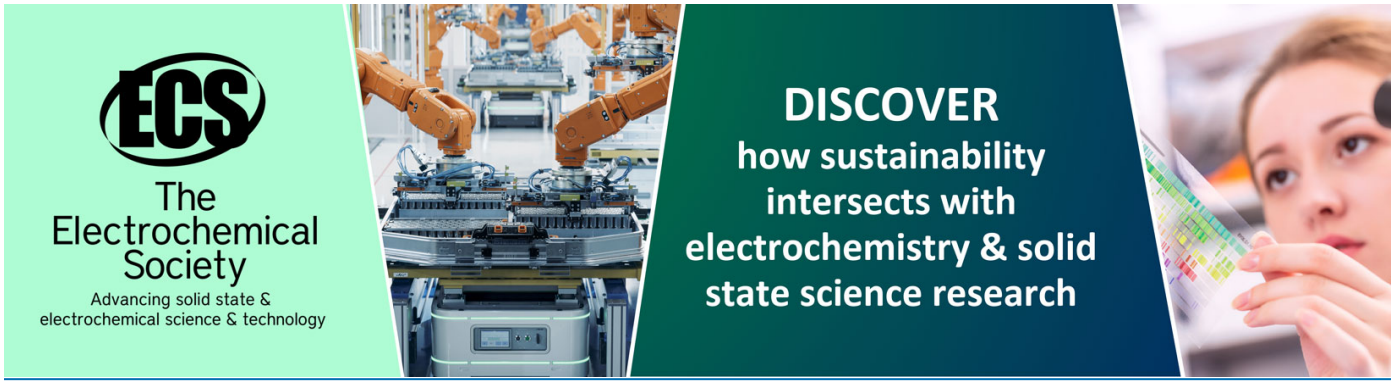
Electrochemical Biosensors for Determination of Anticancer Medicine Etoposide in Human Blood by Glassy Carbon Modified Electrode Based on Film of Poly (L-Lysine) with MWCNTS



Electrochemical Biosensors for Determination of Anticancer Medicine Etoposide in Human Blood by Glassy Carbon Modified Electrode Based on Film of Poly (L-Lysine) with MWCNTS

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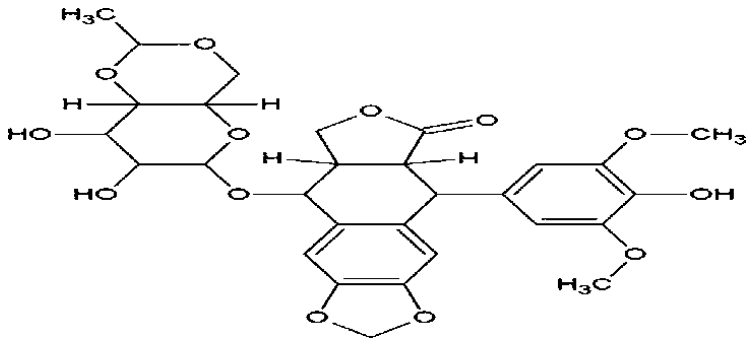
Abstract. Sensitive, selective and reproducible electrochemical sensors were developed for the electroanalysis of Etoposide (anticancer drug) based on L-lysine film, using the PLY-MWCNTS/GCE sensor. The PLY-MWCNTSs films on modified electrode exhibited very good conductivity. Cyclic voltammetry (CV) was applied to examine the electrochemical behavior of PLY film and electrochemical response toward ETO. The PLY-MWCNTS/GCE sensor for detection of the ETO and also the experimental parameters such as film thickness, solution pH, time and accumulation potential were optimized. The obtained LOD of 1.6×10^{-11} M is the lowest LOD, compared to LODs reported in the literature [31] for detection of ETO, using electrochemical techniques. This method was successfully applied for direct determination of ETO, and tested for human blood sample with high specificity, and sensitivity.

Keywords. Etoposide, poly L-lysine, voltammetry, modified electrode, electrochemical sensor.

1. Introduction

(ETO) is currently one of important drugs for cancer chemotherapy (Scheme I) [1, 2]. Etoposide is used in chemotherapy treatment of specific types of cancer, such as an essential and a standard part of treatment for some cancers, such as testicular cancer, lymphomas and acute lymphoblastic leukemia [3-5]. ETO is mostly combined with the other antitumor agents such as cisplatin, carboplatin or cyclophosphamide in the treatment regimens, in combination chemotherapy [2].

The following Scheme (1) illustrates the chemical composition of ETO.



Scheme 1. The etoposide chemical structure.

ETO was evaluated in the treatment of neoplasms [5, 6]. During past years, ETO become a standard component of combination therapy, used in front-line for germinal-cell cancer [6, 7]. ETO has been substituted for vinblastine in front line [8], having an equivalent efficacy with an associated superior tolerance [9]. Consequently, the combination of bleomycine, ETO, cisplatin (BEP) is commonly used in the germinal type of cancers with up to 80% efficiency [10, 11].

ETO is a semi synthetic derivative of podophyllotoxin from the rhizome of the wild mandrake (Podophyllum peltatum) (Scheme 2).

Scheme 2. Podophyllum peltatum, semi- synthetic derivative of podophyllotoxin from the rhizome of the wild mandrake**.**

Different methods applied to assess ETO, including High Performance Liquid Chromatography (HPLC) [12-18], spectrofluorimetry [12], mass spectrometry, liquid chromatography [19, 20], and micellar chromatography electrokinetic [21]. Electrochemical methods are useful for development of selective and more sensitive methods for detection of medicines [22-25]. Electrochemical biosensors have a nanostructure, which can be applied in the pharmaceutical industry.

Multiwall carbon nanotubes (MWCNTs) have been successfully used for the alteration of the electrodes [26-28], because of intrinsic electrocatalytic properties.

In addition to the specific properties, MWCNTSs have the capability to transfer the electron in reactions, improved sensitivity in electrochemistry, and applied previously as working electrode for drug analysis [29]. Voltammetric study of ETO has been examined in details on MWCNTS-modified GCE.

Poly L-lysine (PLY), with advantages such as rapid and simple preparation, reproducibility and good stability, has been used as a conductive polymer in manufacturing of some sensors, i.e. glucose [29], oxygen dissolved [30], DNA [31], trace metals [32], protein [33] and cancer cells [34]. Recently, PLY was used for alteration of several types of electrodes (pyrolytic graphite, glassy carbon, gold and platinum), and applied for assessment of pharmaceutical compounds, biological molecules, metals and dyes [35]. Despite the high sensitivity and selectivity of modified electrode by PLY films, and the great significance of ETO as an electrochemical sensor, few investigations were carried out on the application of this method.

In the present study, the fabrication and characterization of a voltammetric sensor, and application with the selective optimum concentration of ETO on GCE by PLY, were investigated. Furthermore, the

high specificity for ETO detection without interference of endogenous components of the biological fluids was approved, using human plasma samples.

2. Materials and Methods

2.1. Instrumentation

Voltammetric measurements were carried out on 797 VA (Computrace Metrohm, Switzerland).

A conventional three-electrode system comprised of a bare electrode of GC and a modified electrode as working electrode, Ag/AgCl with saturated KCl and a wire of platinum as auxiliary was used in all electrochemical experiments. All experiments were performed at temperature 25.0±0.5℃. The pH measurements were accomplished, using a pH meter (digital HANNA, Portugal). For cleaning the working electrode, an ultrasonic cleaner (model CD-4820, China), and for separating a serum from human blood, a centrifuge (HERMLE-Z200A) were used.

2.2. Chemical and materials

The ETO and its pharmaceutical dosage were provided by Koçak Farma Inc. (Istanbul, Turkey). L-lysine and L- phenylalanine were of analytical grade, and purchased from Fluka.

Phosphate, Tris-HCl and Britton Robinson buffers were used as supporting electrolytes. All solutions were kept in dark and consumed in a day to prevent any decay. Chemicals were of analytical grade (Fluka, BDH), and used as received without any further purification. Double distilled water (DDW) was used throughout the experiments.

2.3. Preparation of the sensor by L-lysine electropolymerization

GCE was polished with 0.3 and 0.05 μm alumina slurry, and subsequently sonicated in acetone and DDW, to become a mirror-like surface and left to air dry. Then 5 μl of MWCNTs suspension was added to pretreated GCE and dried at room temperature (denoted as MWCNTs/GCE). A L-lysine polymer film modified electrode was made by L-lysine electropolymerization on the MWCNTs/GCE. The experiment was carried out in PBS, containing 2.0×10^{-3} M L-lysine, at pH 8.0, by CV sweeps, and potential ranging from −1.5 to +2.0 V. As shown in Fig 1, the reduction and oxidation currents increased as scanning cycles increased. Following ten cycles at scan rate of 50 mVs^{-1}, the electropolymerization process was aborted and subsequently the polymer film electrode was rinsed with DDW to physically eliminate the adsorbed substances. After air-drying, clearly a yellow poly-L-lysine film could be observed on the GCE surface. At a higher positive potential, monomer of L-lysine oxidized to free radical form of amino, can be associated with GCE surface. Then, poly-L-lysine films can be created.

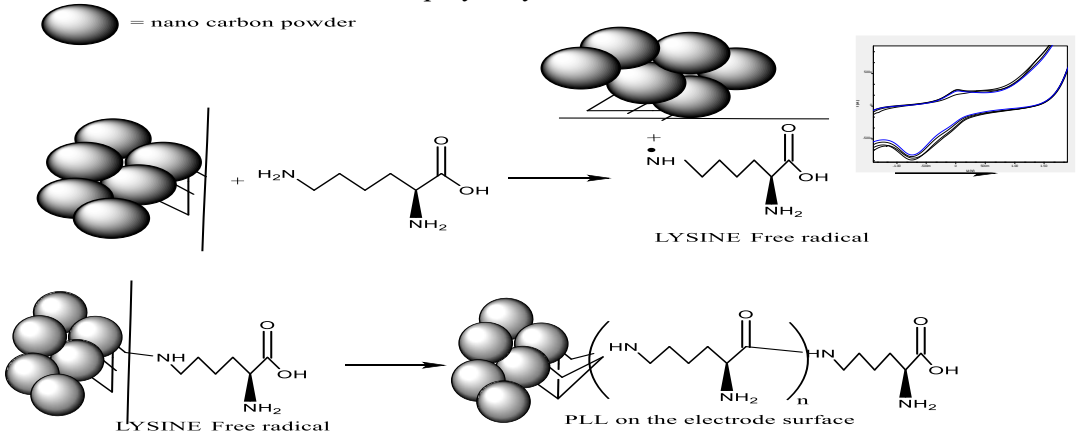


Figure 1. The proposed process of the l-lysine electropolymerization.

3. RESULTS AND DISCUTION

3.1.1. Voltammetric study of ETO on GCE

The properties of ETO as a drug for the cancer treatment, was investigated for further application of biodetection in 0.2 M PBS at pH 7.0 by CV. In this process, the potential was changing from −1500 *mV* to +2500 *mV vs*. Ag/AgCl, using a scan rate of 100 *mV/s*. The oxidation potential was 420 *mV* under default conditions of the instrument. Fig 2-c shows the CV for ETO concentration, ranging from 0.5×10^{-6} to 5.0×10^{-6} M in 10 ml of 0.2 M PBS, at pH 7.0 on GCE.

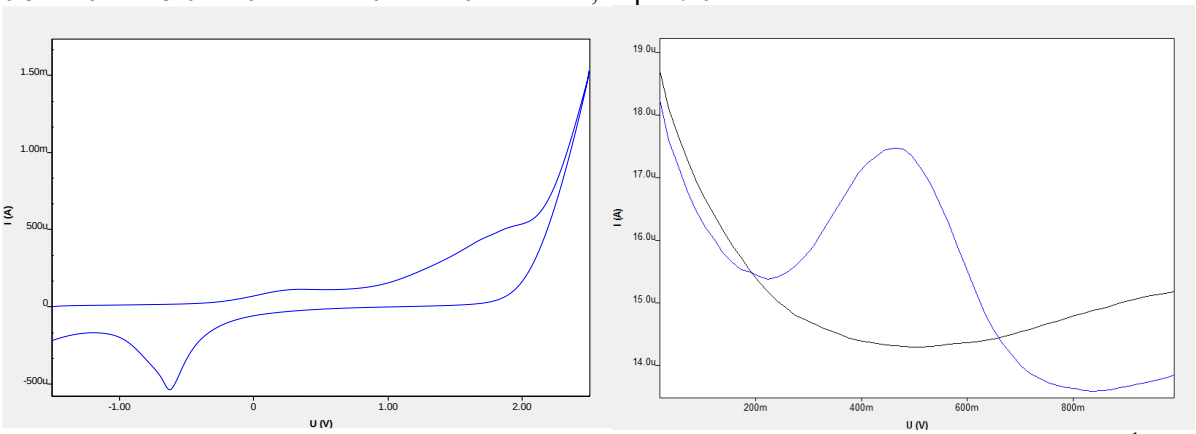


Figure 2-a. cyclic voltammetry in 0.2 M PBS at pH 7.0 of ETP with a scan rates of 100 mV s^{-1}, **2-b** square wave voltammetry of ETP .

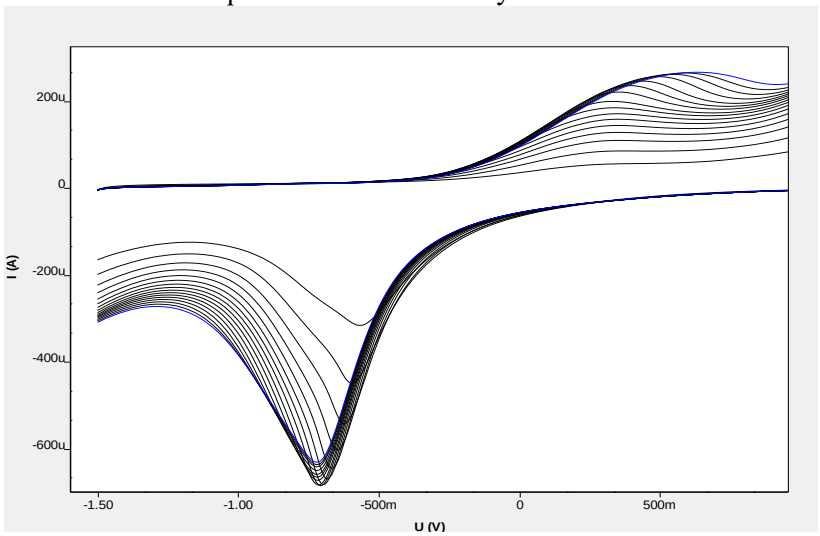


Figure 2-c. cyclic voltammograms for etp (0.5×10^{-6} to 5.0×10^{-6} M) in 10 mL of 0.2 M PBS, pH 7, at a GCE.

3.1.2. Optimum effective conditions of ETO on the GC electrode

These conditions could affect ETO concentration on bare GCE and MWCNTs-PLY/GCE. The optimum concentration of 5.0×10^{-9} M ETO was studied in PBS at pH 7.0 by CV. Deposition potential is considered as an important parameter, which is used to detect the insertion and de-insertion of doping anions into polymer structure, through increasing the anodic and cathodic currents' values, during reduction and oxidation processes, respectively. This gives us well-defined redox waves in CV. The deposition potential of 0.70 V was optimum effective conditions for modified GCE. The optimum and effective time deposition, ranging from 0 to 60 s was also studied. The oxidation current reached to maximum after 5 s and decreased, indicating a saturation of MWCNTs-modified electrodes. To find the optimal equilibration time, the range from 0 to 11 s was tested. The current increased gradually with an optimum at 10 s, using 0.008 V for voltage step. Therefore, the oxidation current was optimal at 10 s equilibration time.

3.1.3. Effect of pH

The effect of pH of the solution on the response of 5.0×10^{-9} M ETO was assessed, at pH, ranging from 4.0 to 9.0 by CV, at a scan rates of 100 mVs^{-1}. As the pH of the solution increased, oxidation potential was switched to more negative value, and became immeasurable at pH 9.0.

The linear correlation between pH and Ep_a can be presented as follows:

Ep_a=−58.3 pH+845.8 between pH 4.0 and 9.0, E in mV and r=0.997 as shown in Fig 3-a.The pH was effective to oxidation current considerably. The PBS at pH 7.0 was selected for ETO assessment at the highest current, compared to the rest of pH that resulted in less current as shown in Fig 3-b.

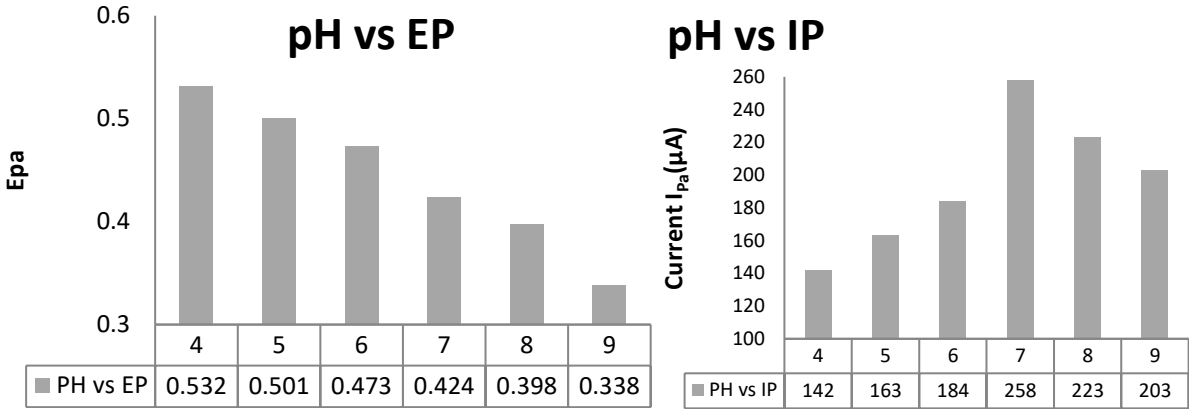


Figure 3-a. pH effect of ETP peak potentials and ETP concentration is 5.0×10^{-9}M.

Figure 3-b. E pH effect of ETP peak currents and ETP concentration is 5.0×10^{-9}M.

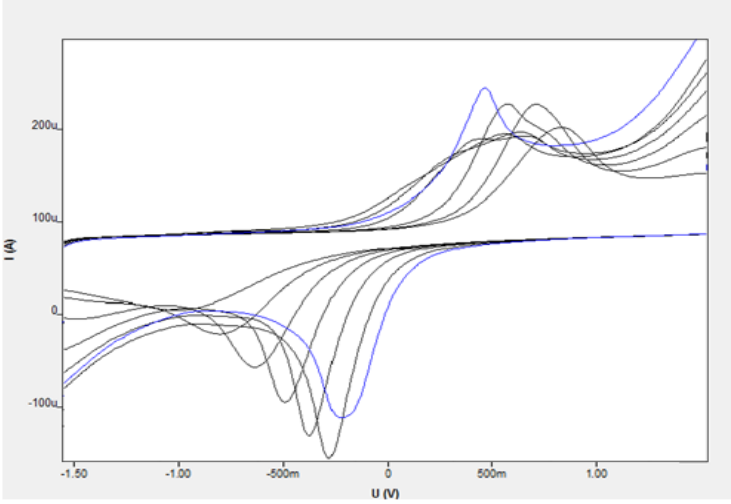


Figure 3-c. Cyclic voltammograms for 5.0×10^{-9}M ETP solutions with different pH: (a) 4.0; (b) 5.0; (c) 6.0; (d) 7.0; (e) 8.0; (f) 9.0(0.2M PBS).

3.1.4. Effect of concentration of ETO compound on the optimum current at different pH

For this aim, the effect of pH on both oxidation potential and current was studied within pH 4.0 to 9.0, using CV at various ETO concentrations, and by applying the optimal conditions established and the reference electrode Ag / AgCl. The peak currents were increased at pH 7.0, in comparison to the pH 4.0, 5.0, 6.0, 8.0 and 9.0. The linear correlation between Ip and pH for concentrations, ranging from 0.5×10^{-9} to 5×10^{-9} M was plotted as a calibration curve. The experimental results indicated that the proper linear curves and the maximum oxidation currents obtained at pH 7.0 of PBS, and due to the highest sensitivity and selectivity, the oxidation current obtained at pH 7.0 PBS was chosen for analytical purposes as shown in Fig 4.

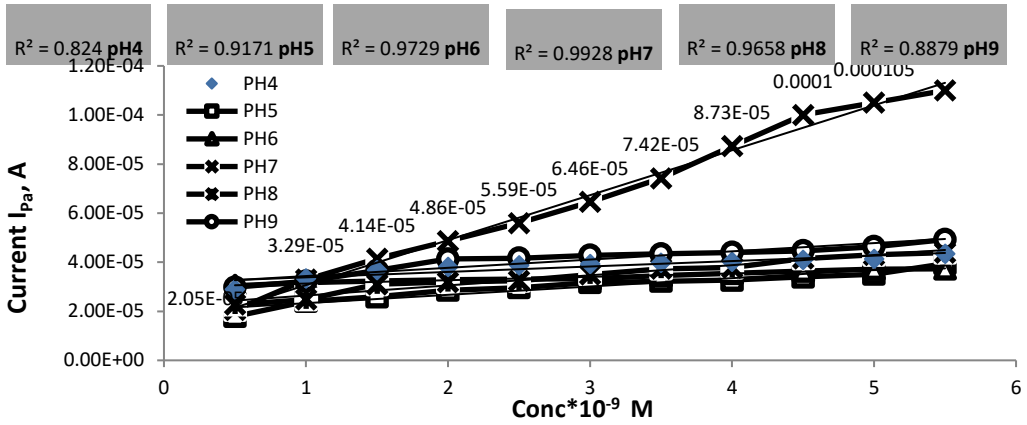


Figure 4. Effect of concentration of ETP(0.5×10^{-9} to 5×10^{-9} M) with different pH 0.2M PBS.

3.1.5. Effect of different concentrations of ETO (calibration graph of ETO) by CV

Calibration graph of ETO was plotted, using CV, under optimum conditions. Cyclic Voltammograms were recorded for successive additions of 10^{-6} M ETO in 10 ml PBS at pH 7.0 and 25˚C.Oxidation current was plotted as a function of ETO concentration in Fig5.

It is very clear that the graph shows two straight lines, the first one between concentration (0.5×10^{-5} - 5×10^{-5}) M with a correlation coefficient (R= 0.975), and the second correlation line between the concentration 0.5×10^{-9}–5×10^{-9} M with a correlation coefficient (R= 0.992), this may be due to molecular association at high concentrations.

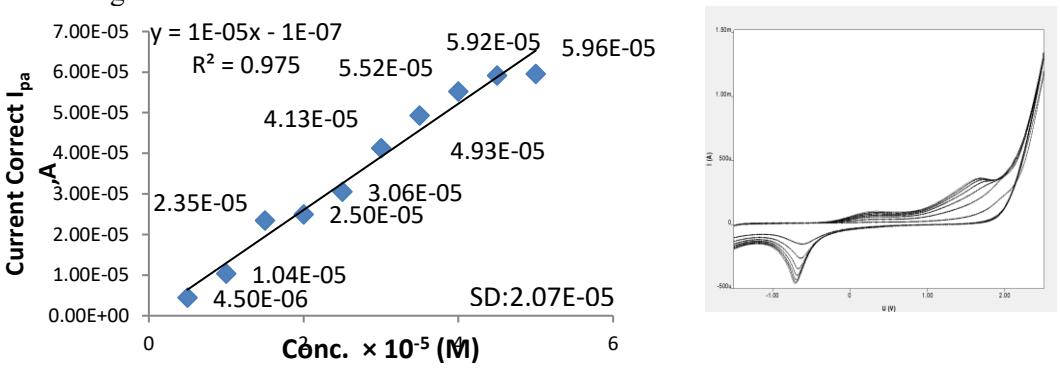


Figure 5-a. Plot of correct current of measurement (***ip***) versus concentration range ($0.5e^{-5}$ - $5e^{-5}$ M) of ETP.

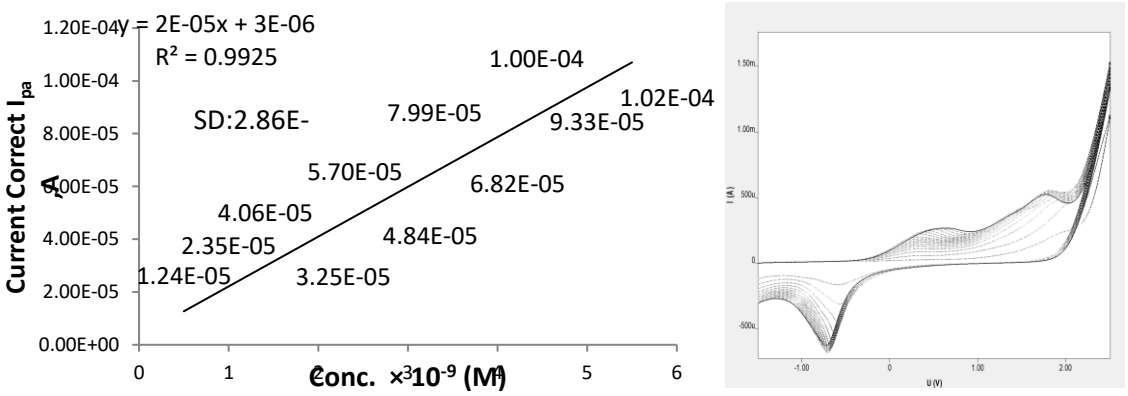


Figure 5-b. A plot of correct current of measurement (*ip*) versus concentration range (0.5e-9 - 5e-9 M) of ETO.

3.1.6. Influence of volume of MWCNTs

Three concentrations of MWCNTs were tested. The suspension volume between 5 and 15 µl was expanded over the electrodes, and left to dry overnight. The CV was used for experiments optimization of the carbon nanotubes volume. With the increase of MWCNTs suspension volume (in microliter), oxidation current increases due to enhancement of surface area of electrode. When the MWCNTs suspension volume was 5 µl, oxidation current reached to the maximum value. The electrode effective area did not increase further for 15 µl of MWCNTs suspension volume. In contrast, oxidation current diminished with an elevation of film thickness, and decreased with electron transfer rate. Thus, optimum MWCNTS suspension volume was estimated as 10 µl.

3.2. Electrochemical sensor of LY

3.2.1. Film surface morphology characterized by SEM

In this study, SEM was used to illustrate the surface morphology of the PLY film based on the GC-modified electrode, which was prepared, using electrochemical polymerization. Fig 6 (a,b) shows the microporous images in the PLY film, while Fig 7 (a,b) shows the effect of WMCNTs when added to PLY/GCE.

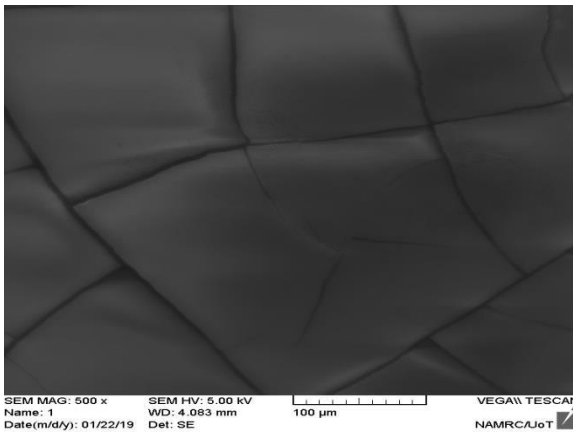


Figure 6-a

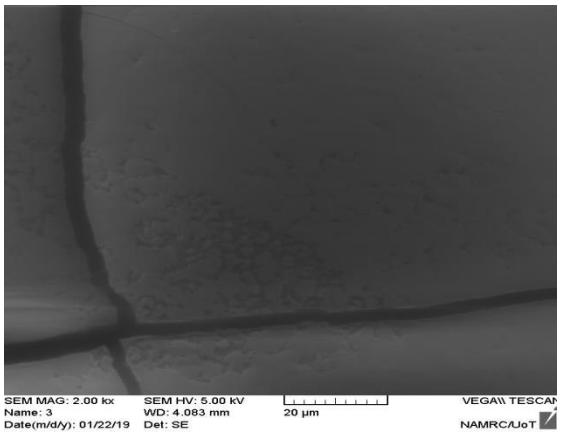


Figure 6-b

Figure 6 (a,b). The microporous images for the PLY film.

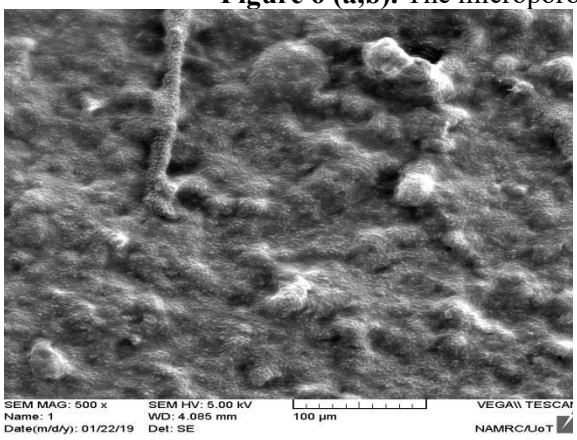


Figure 7-a

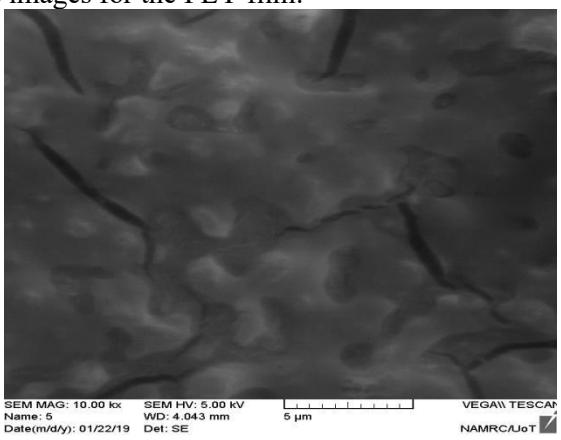


Figure 7-b

Figure 7 (a,b). The effect of MWCNTs added to the PLY/GCE by SEM images

3.2.2. Influence of the film thickness of PLY on GCE

The cycle number is called layer number, defined as film number of formed layers that are deposited onto the surface area of electrode, and should be equal to10 cycles.

A relationship between redox currents and the cycle number, as shown in Fig. 8, indicated that 10 cycles were sufficient for a thin PLY modified film with a homogeneous surface, and a good adherence onto a GC working electrode surface.

Moreover, greater numbers of cycles > 10, for the synthesis of PLY modified film on a GCE is required i.e., 15 cycles were led to an increased film thickness, but also to a poorer adhesion of the additional material to the surface of the working electrode.

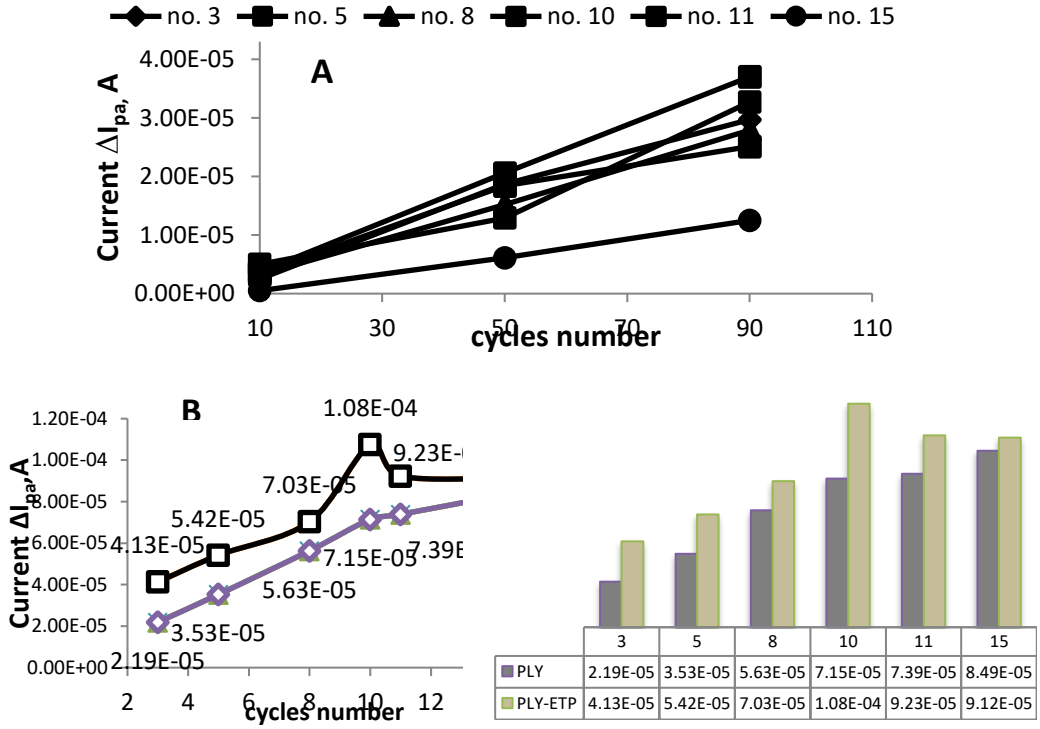


Figure 8. (A) The Effect of cycle number on oxidation currents while increasing the concentrations (B) Comparison of oxidation currents vs cycle number between PLY on surface electrode and PLY-ETO, and shown that 10 cycles is the optimum cycles for detection of the ETO

The electropolymerization procedure was influenced by the monomer concentration and also the scan cycles, which both can be controlled by the film thickness, in the electropolymerization solution. The cycle number was 10 and the electropolymerization solution was a PBS at pH 8.0, containing 2.0 $\times 10^{-3}$ M L-lysine, as shown in Fig 9.

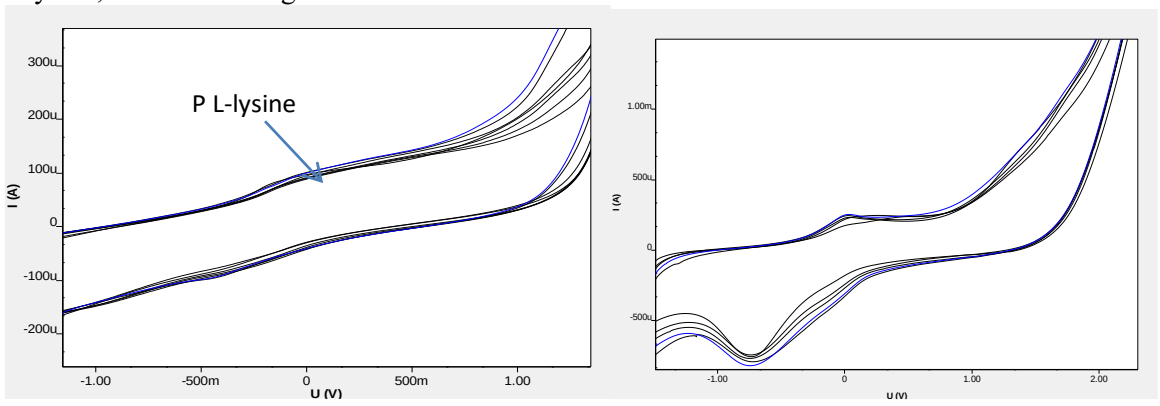


Figure 9. The cyclic voltammograms for L-lysine electropolymerization of. supporting electrolyte : PBS (pH 8.0); scan rates: 50 mV/s; L-lysine concentration: 2.0 $\times 10^{-3}$M.

Electropolymerization cycles for the electro oxidation of sulfide ions was optimized by CV (Fig.10). Oxidation current significantly increased in the polymerization cycle range of 3–15 cycles. However, an oxidation sharp peak and higher peak current value were observed in 10 successive polymerization

cycles. A decrease in oxidation current was observed by thin layer formation of PLY on the electrode surface. By comparison, considering the oxidation current, peak potential and peak shape, we have chosen 10 cycles as optimal number, for the preparation of PLY-modified electrode.

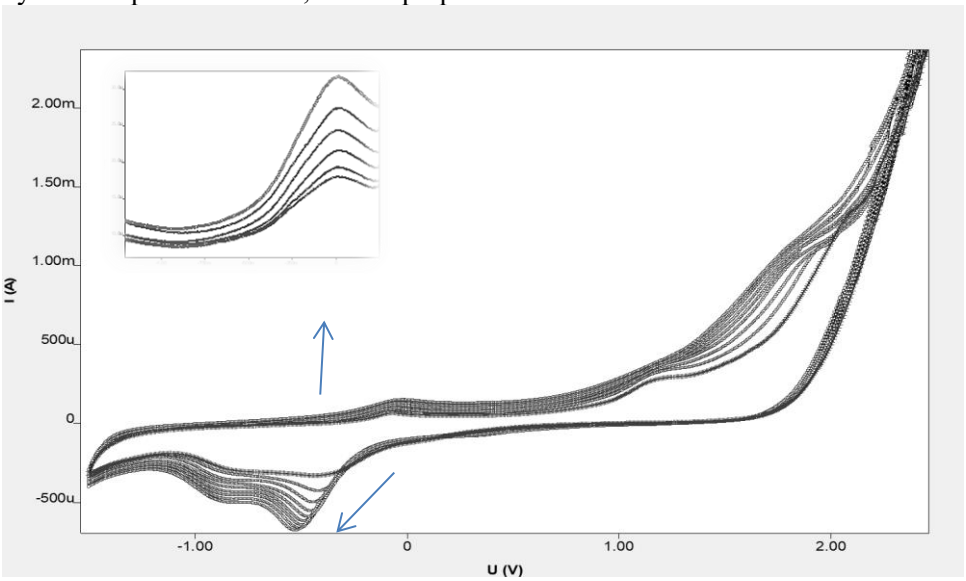


Figure 10. The CV response of GCE on 2 mM L-lysine in PBS (pH 8) at an applied range of potential −1.5 to +2.5V for 10 cycles, and scan rates: 50 mV s^{-1}.

3.2.3. Electroactivity of the prepared film

The electroactivity of the film is characterized by the number of cycles on the GCE, and can be controlled based on the thickness of the layer on the surface of the electrode. The electrochemical activity of developing polymeric film was assessed in acidic and basic media. The modified film electrode was detected extremely active in basic medium, especially at pH 8.0 by observing the redox peaks and higher currents, compared with the other pH ranging from 3.0 to 9.0. To identify an optimum condition for obtaining a reactive and steady film electrode, the pH factor was also examined.

In general, the current (Ip) is increasing with increasing the concentration. There is evidence that the reduction process is controlled by diffusion, but increasing oxidation current was proportional with pH 8.0 more than the other pH, as it is evident, by drawing the concentration as a function of propagation current, as shown in Fig 11.

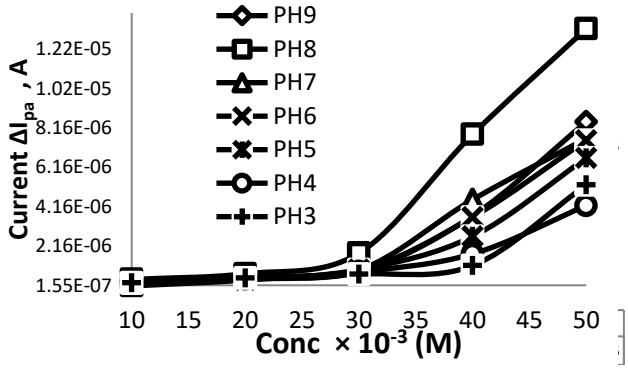


Figure 11-a. Graph shows the relationship between Ipa vs. 0.2 M PBS (pH 3.0 – 9.0) to PLY/GCE.
11-b. A plot of oxidation current and the concentration of PLY at different pH range (3.0 to 9.0) in 0.2 M PBS.

3.2.4. Effect of pH after elctropolymerization of PLY for estimation of ETO

Optimization of experimental conditions

The pH was an important factor to investigate the electro-oxidation behavior of ETO on the PLY-MWCNTS$_S$ /GCE. Oxidation currents and the pH values (from 3.0 to 9.0) were displayed in Fig. 12. The oxidation currents of ETO after L-lysine polymerization gradually increased with increasing pH, the maximum current response at pH 7.0 was used for optimal pH condition. The oxidation potentials of PLY- MWCNTS$_S$ /GCE shifted to more negative values with increasing pH, indicating that protons directly participate in the electrode reaction. The linear-regression equations of *E*pa (mV) = 0.6944-0.0408 pH (R^2 = 0.9649) Fig (12-c), and the linear equations for oxidation currents at different pH are as follows:

Ipa (µA)= 1E-07pH + 5E-07 ; r = 0.9994 pH3.0
Ipa (µA)= 1E-07pH + 9E-08 ; r = 0.9692 pH4.0
Ipa (µA)= 2E-07pH + 3E-08 ; r = 0.9688 pH5.0
Ipa (µA)= 2E-07pH + 2E-07 ; r = 0.9967 pH6.0
Ipa (µA)= 3E-07pH + 8E-08 ; r = 0.9983 pH7.0
Ipa (µA)= 3E-07pH + 2E-07 ; r = 0.9777 pH8.0
Ipa (µA)= 1E-07pH + 4E-07 ; r = 0.9943 pH9.0

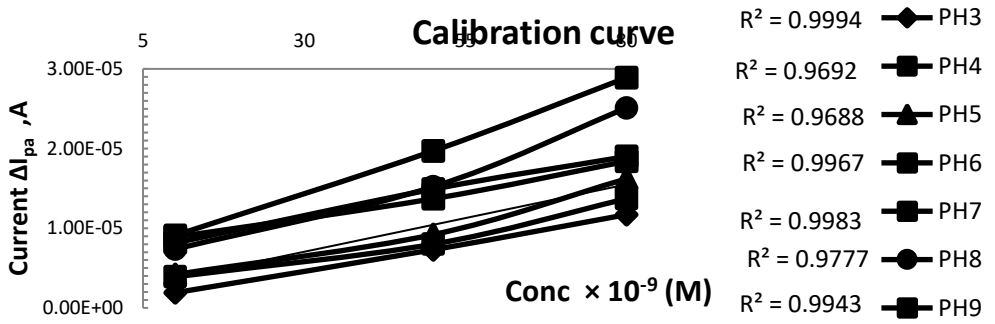


Figure 12-a. The calibration curve Ip correct vs the concentration in different pH.

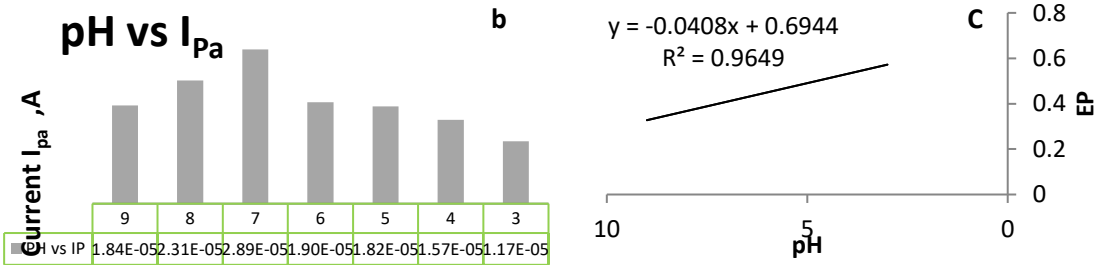


Figure 12-b. The relationship between oxidation current and the pH on PLY/GCE. **12-c.** The relationship between oxidation potential and the pH on PLY/GCE

3.2.5. Calibration curves for the PLY on MWCNTs/GCE modified electrodes for detection of ETO

Calibration curve method was the best technique for ETO electrochemical determination. A relationship between ΔIp and the ETO concentrations on PLY /GCE, and on PLY-MWCNTs/GCE, is demonstrated in Fig13-a, and Fig 13-b, respectively.

The reduction peak currents of Ip (I) for PLY/ETO /GCE and Ip (II) PLY-MWCNTs/ETO/GCE gradually increase, and the increase in oxidation current Ip (I) of the PLY/ETO /GCE and Ip (II) of PLY-MWCNTs/ETO/GCE is directly proportional to the ETO concentration. The linear equations of ΔIp (I), and ΔIp (II) are: y = 2E-05x + 5E-06; and y = 184.2x + 209.97, respectively.

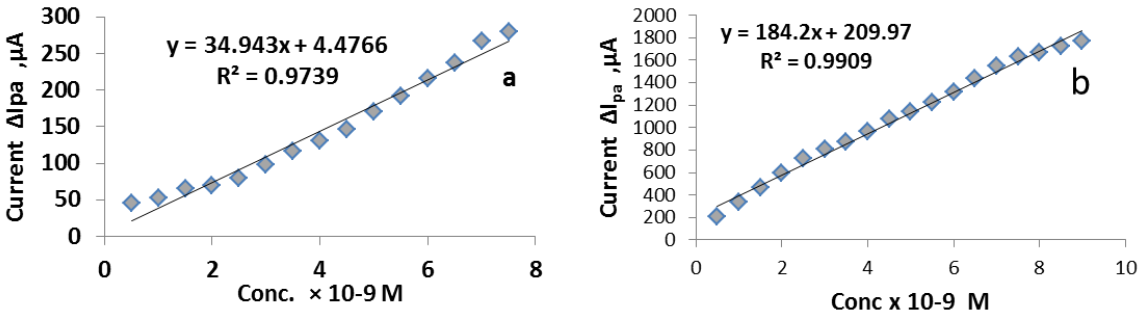


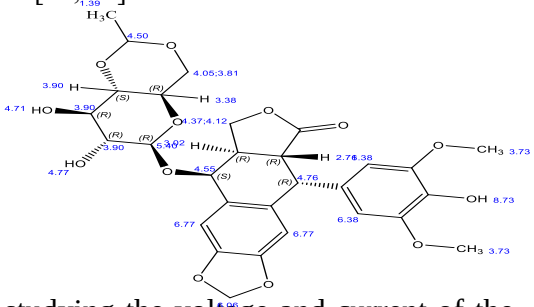
Figure 13-a. The relationship between the ΔIp and the ETO concentrations on the PLY /GCE**, b.** The relationship between the ΔIp and the ETO concentrations on the PLY-MWCNTs /GCE

3.2.6. Measurement of ETO by CV

CV is known as a suitable method for redox study of the pharmaceutical application in the metabolic pathways in the human body, and ETO is determined by CV on MWCNTs-PLY-GCE through the electrode.

The ETO structure has a phenol moiety. The redox couple of phenol oxidation is known as an anodic process. It is accepted on the first step in the oxidation of phenols leading to the formation of a radical phenoxy. Then, oxidized radical of phenoxy changes to a quinone with a new redox couple at lower potential, compared to the principal oxidation step of phenols [36, 37].

The new couple was observed in voltammogram scans (see Fig. 14) and the second one was attributed to the oxidation of the radical of phenoxy to quinone. They formed a product after 2e⁻oxidation in cation, unstable form, which converted rapidly into more stable form of ortho-quinone [38].



The electrochemical process can be examined by studying the voltage and current of the drug.

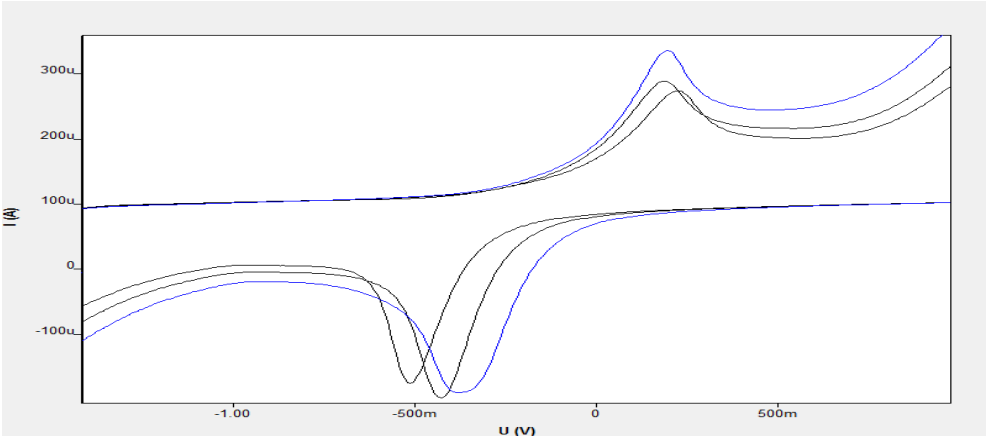


Figure 14. The cyclic voltammograms for the ETO.

3.2.7. Electrochemical behavior of ETO on modified GC electrode

The electrochemical synthesis of PLY modified film on a GCE was performed by CV. PLY film easily formed on electrode surface by electropolymerizations. PLY was mostly as a linker to attach bioactive molecules or nanomaterials, due its numerous amino groups or electrical property. Thus in this study,

PLY not only could improve the stability and reproducibility in the nano carbon materials modified electrode, but also gives an excellent current for identification and diagnosis of ETO. The electrochemical behaviors of linear range of 5.0×10^{-10} to 9.0×10^{-9} M ETO were investigated on modified electrodes by CVs. As shown in Fig.15, the results of three electrodes are compared, with bare GCE (curve a) oxidation currents of ETO in PLY /GCE (curve b), this result is due to the presence of PLY on the surface of the electrode, and PLY- $MWCNT_S$ /GCE show oxidation peak with higher currents as presented (curve c).

The CV of PLY- $MWCNT_S$ /GCE represented a remarkably increased oxidation peak. The good results illustrated that the novel sensor not only could enhance the oxidations of ETO, but also low concentrations of ETO relate to the high currents of PLY- $MWCNT_S$ /GCE as shown in (Fig 15).

Above all, PLY-$MWCNT_S$ /GCE has provided large surface, good electronic conductivity and stability on the surface electrode and also:

1- The synergistic effect of ETO-MWCNTs facilitated an electron transfer between the substrate and electrode; as a result, redox peaks of ETO can be obviously increased
2- The random coil structures of L-lysine polymer prevented the over potential use of the electrode in PBS, this will be a benefit for ETO detection with low quantity.

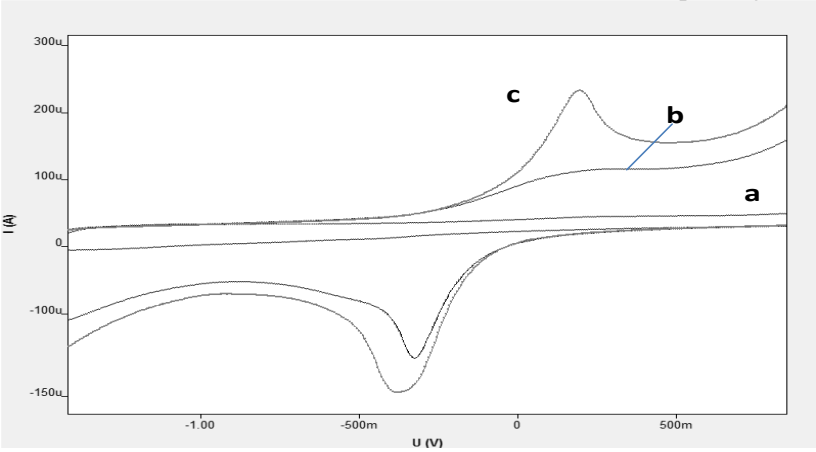


Figure 15. The CV of oxidation currents of the ETO in 0.2M PBS (pH 7.0) on a bare GCE (curve a), PLY- /GCE (curve b) and PLY- $MWCNTS_S$ /GCE (curve c).

3.2.8. Effective scan rates

In the electrochemical study of ETO, optimum e scan rates were investigated (Fig. 16). The redox current linearly increased with the square root of scan rates, in the range of 25–200 mV/s. The regression equations can be expressed as

I_p (μA) = 7.5484 $v^{1/2}$ (mV) + 2E-05 (R^2 = 0.9579) and

Log I_p (μA) = 0.9412 log$v^{1/2}$ (mV) + 2E-05 (R^2 = 0.9743), which indicated that the electrochemical reaction of ETO at controlled adsorption process in the selected scan rate range.

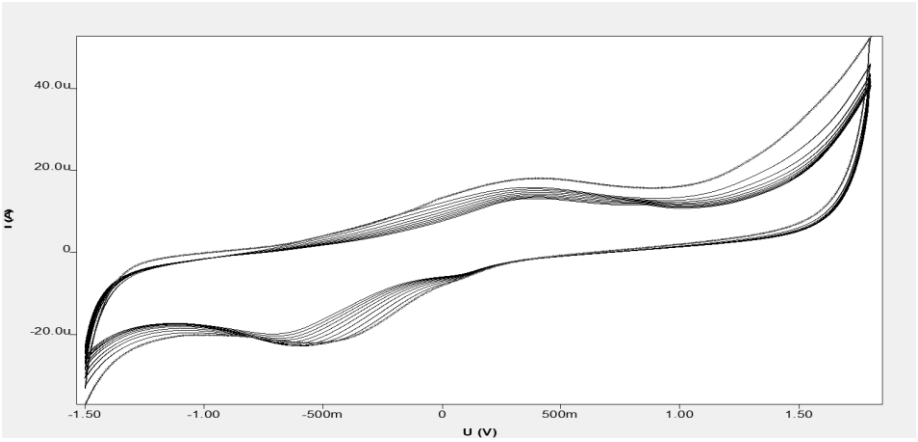


Figure 16. The cyclic voltammograms of oxidation currents of the ETO on GCE with scan rates of (a) 0.025; (b) 0.05; (c) 0.075; (d) 0.1; (e) 0.125; (f)0.15; (g)0.175; (h)0.2V s^{-1}.

Linearity of oxidation potential (E_P) against scan rates natural logarithm (lnv) was observed with a linear equation and correlation coefficient of

$E_P\ (V) = 0.001v\ (Vs^{-1}) + 0.015\ V$ and $R^2 = 0.9741$

$E_P\ (V) = 0.0823lnv\ (Vs^{-1}) + 0.0331\ V$ and $R^2 = 0.9604$, respectively. The equation was used to calculate standard rate constant [36] , where E_P is the peak potential, Eo is the formal potential, α is the transfer coefficient, K_o (s^{-1}) is the electrochemical rate constant. The value of Eo, which was obtained from the intercept of the E_P versus v plot (Fig. 17) was 0.001 V.

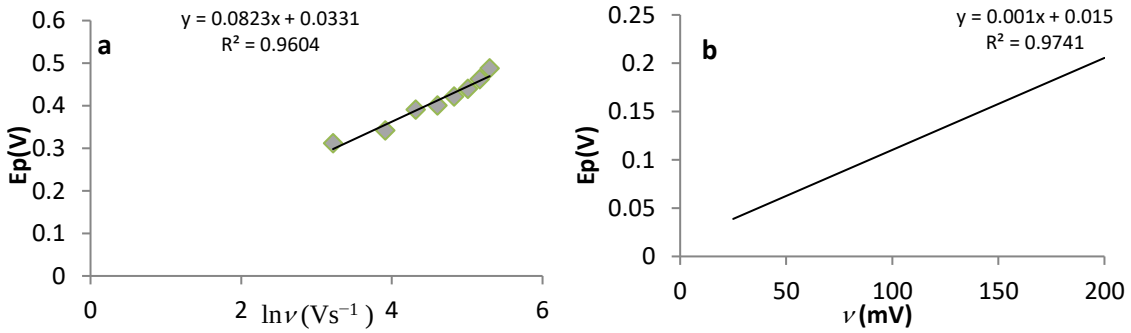


Figure 17. The plot of oxidation current (I_P) versus (a)scan rates ,(b)scan rates log versus log of oxidation current (I_P)

3.2.9. Linearity, detection limit, stability and sensitivity of ETO sensor

A comparison was made between the signal of ETO at a GC bare electrode, PLY-GCE and COOH-MWCNTs-PLY-modified GCE in PBS at pH 7.0, as shown in Fig.18 (curve-a). The optimal conditions of GC- modified electrode as studied previously, were for GC bare electrode, and CV of ETO had an oxidation small wave at 0.42 V, and oxidation current increased with PLY-GCE larger than bare electrode. Finally, oxidation current of ETO at MWCNTs-PLY modified GCE, showed a large oxidation peak at 0.42V.

As seen in Fig.19 (curve-b), oxidation current of ETO increased almost 20 times on MWCNTs-PLY-modified GCE, compared with bare GCE, which may suggest the larger surface area of MWCNTs-PLY-modified GCE. In addition, the increased surface area of electrode, improved efficiency adsorption of surface electrode, due to the presence of MWCNTs and played an important role in improvement of ETO voltammetric signal [45]. Our data confirm that MWCNTs is highly effective, showing ETO enhancement due to surface area and electrical special properties, which made the ETO adsorption easier by providing effective sites of reaction.

In order to assess the potency of MWCNTs-PLY-GCE for analytical purposes, the CV method was applied for ETO evaluation.

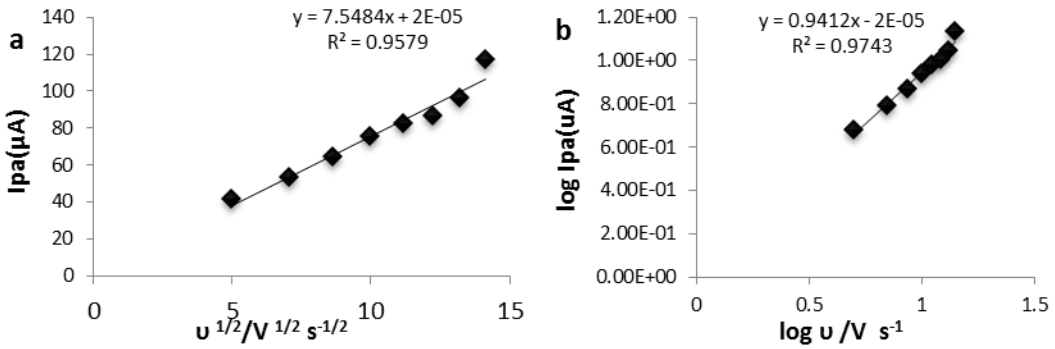


Figure 18. The plot of oxidation potential (E_P) versus (a) ln of scan rates and (b) scan rate.

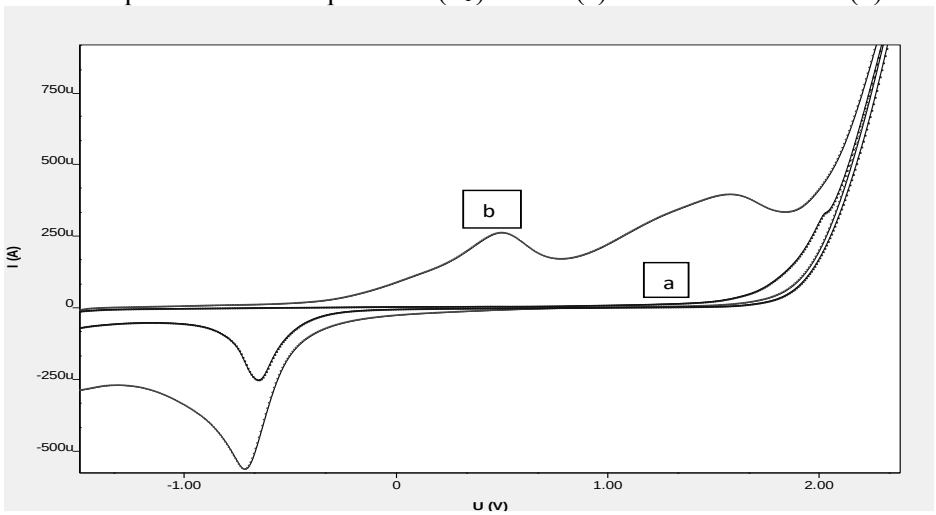


Figure 19. The CV of oxidation currents of ETO in 0.2M PBS (pH7.0) on a bare GC electrode (curve a) and PLY- $MWCNT_S$ /GCE (curve b)

We immersed the MWCNTs-PLY modified GCE in PBS, containing an increasing concentration of ETO, at pH 7.0.

The plot of the current of ETO oxidation peak as a function of ETO concentration, ranging from 5.0×10^{-10} to 9.0×10^{-9}M, are shown in Fig. 13-b1. Attributes of calibration curve are outlined in Table 1. Using this calibration curve, the limit of detection (LOD) of 1.6×10^{-11}M was acquired. This LOD is the lowest one from value documented for the ETO detection in the literature [28].

The LOD and LOQ were calculated by equations LOD=3.3 s/m 5.40×10^{-9} M and LOQ=10 s/m, using standard deviation (s) and calibration curve slope (m).

The overall validation attributes such as LOQ, LOD, and precision and accuracy are demonstrated in Table 1, according to the literature [28]. In addition, a new HPLC method was established for comparison with the validity of other electroanalytical technique. Data were compared with HPLC, using variance ratio F test and t test (Table 2).

Table 1. Regression data of the calibration for quantitative determination of ETO by CV using standard solution and comparison with AdSDPV for determination of ETO from literature [28].

	CV	**AdSDPV**
Measured potential (V)	0.42	0.42
Linearity range (M)	5.0×10^{-10} to 9.0×10^{-9}M	2.0×10^{-8} to 2.0×10^{-6}M
Slope (µA M^{-1})	0.184×10^{3}	$(2.696\pm0.002)\times10^{6}$
Intercept (µA)	1.02	0.015±0.005
Correlation coefficient	0.9929	0.998
LOD (M)	1.6×10^{-11} M	5.40×10^{-9} M
LOQ (M)	4.9×10^{-11} M	1.80×10^{-8} M

SD	0.9055385	
SRD%	0.4093755	1.55
Number of tests	n=10	n=5

Table 2. Results from HPLC determination and recovery experiments in Etoposide [28].

	HPLC
Labeled claim (mg per mL)	20.00
Amount found (mg)	19.43
RSD (%)	1.33
Bias (%)	2.85
Calculated t value	0.02
Calculated F value	0.51

3.2.10. ETO determination in serum samples

Blood Samples were collected from patients who undergo the chemotherapy, using the chemical doses (chemotherapy) from the study drug. The serum was separated from the blood samples by centrifugation, and preserved by deep-freezing for later measurement. The potency of the suggested techniques for ETO determination was evaluated, using the serum samples. Serum samples were spiked with ETO to reach the final concentration of 9.68×10^{-10} M and 9.91×10^{-7} M. The ETO concentration was computed in serum of human from associated linear regression equations (Table 3). The typical CV curve of ETO was assessed in serum (Fig. 20).

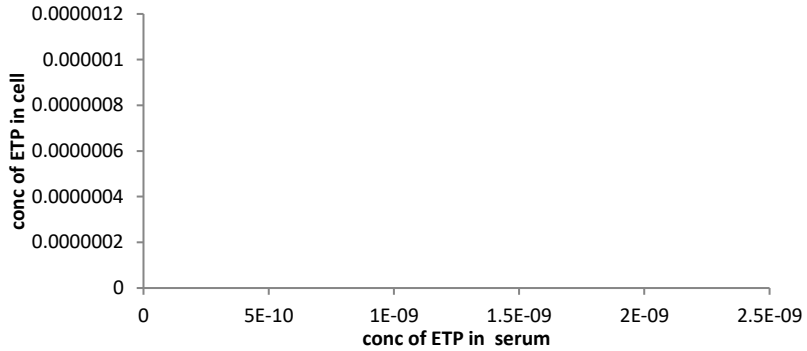


Figure 20. The plot of concentration of the ETO in human serum with spiked ETO

Data for the assessment and recovery of the known amounts of ETO added to the serum samples, were estimated by CV technique, and was equal to 99.1% with relative standard deviation (RSD) value of 0.72. Good recoveries were reached, using serum samples in both methods (Table 3).

Table 3. Results obtained for ETO analysis from spiked serum samples

Technique (CV)	ETO added (M)	ETO founded (M)	Average recovery (%)
Blood samples	1.97418×10^{-9}	9.91039×10^{-7}	99.10394265

3.2.11. Repeatability and stability

ETO concentration of 5.0×10^{-10} to 9.0×10^{-9} M was chosen to assess the repeatability of the voltammetric measurements of the modified electrode

(MWCNTs-PLY/ETO/GCE) in PBS (pH 7.0), as shown in Fig. 21. The RSD was calculated as 0.4 for ten successive assays. The long-term storage stability of the sensor was tested for 120-day period. After 120 days of storage, by storing the sensor at room temperature and measuring intermittently, the sensor maintained about 76% of its initial activity. These data demonstrate good stability repeatability for the suggested modified electrode for ETO determination.

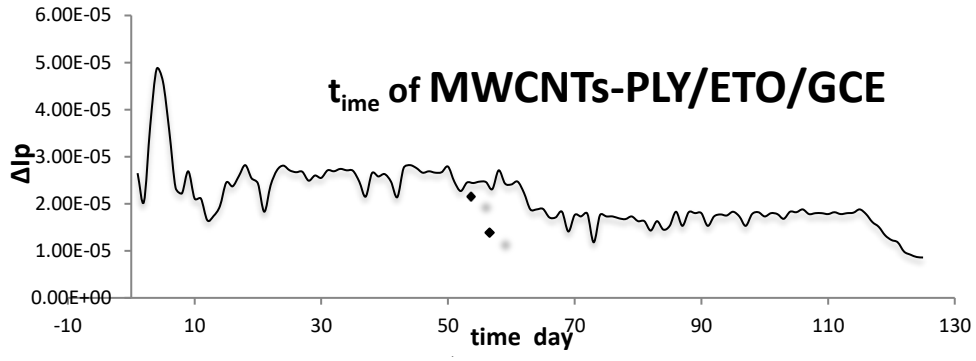


Figure 21. A plot of MWCNTs-PLY/ETO/GC electrode showing the relationship between ΔIp and time measurement

4. Conclusion

A MWCNTs-PLY-GCE utilized for ETO determination by CV in standard laboratory in PBS at pH 7.0. A potential oxidation mechanism was proposed. The oxidation current of ETO was enhanced 20 times on MWCNTs-PLY-GCE by CV. LOD of 1.6×10^{-11} M was obtained. To the best of our knowledge, this LOD is the lowest one from value reported for the ETO detection in the literature [28] using electrochemical techniques.

The suggested method, using a MWCNTs-PLY-GCE has more advantages, compared to the other methods in the literature, including very low LOD, high sensitivity, reasonable reproducibility, simple handling, resistant surface, and cost-effectiveness. The previously reported method [28] is less sensitive and time-consuming technique. This method has a potential to be used for ETO determination in human blood sample.

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