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RESEARCH ARTICLE

# Detection of Anticancer Drug by Electrochemical Sensors at Modified Electrode (MWCNT/polyEosin-Y)

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**ABSTRACT**

In this research, a biosensor was developed to evaluate VP-16 Which is used in the treatment of several tumors, including small lung cancers, testicular cancer, The sensor is generated by modifying bare GCE with Eosin-Y(yellowish)(EOY) using MWCNT electropolymerization (PEOY-MWCNT / GCE) at the pH 6.0 by cyclic technique voltammetry with 50 mV.s−1 of a scanning rate of with 14 number of cycles for the optimum cycles . A number of variables were analyzed, which in turn controlled the sensor 's efficiency and functioning and were mounted for use in experiments while developing the sensor, One of these variables is to choose the best pH at which the material on the surface of the GCE electrode can be calculated to choose the best pH 7 as the best measuring variable . To ensure completion of electrochemical polymerization on the electrode surface by visualizing the electrode surface properties by scanning an electron microscope (SEM), which describes PEOY/GCE and MWCNTs/PEOY/GCE morphology, The variables related to the EOY polymerization process were tested for more accuracy in the analysis, and the best values were selected to produce the best result. The electrodes used for the analysis were then tested separately by analyzing the findings and it was noticed that the PEOY / VP-16 / GCE electrode provides a better measurement of the bare electrode and in effect the presence of multi-walled carbon nanotubes gives a higher measurement of all the electrodes.

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## INTRODUCTION

VP-16 is a semi-synthetic glycoside derivative of podophyllotoxin, and initially an extract of the mandrake plant [1]. VP-16 is active against several tumors, including small lung cancers, testicular cancer [2], lymphoma, leukemia and Karposi's sarcoma associated with AIDS. It is used in patients with advanced hematological malignancies as part of a bone marrow preparatory regimen[3, 4]. VP-16 undergoes an easy metabolism by numeral enzymatic processes, *in vitro* and *in vivo* to make reactive products. Our group was the first to discover that VP-16 is o-demethylated by horseradish peroxidase, cytochrome P450, and tyrosinase, to o-dihydroxy VP-16 (Fig. 1) and highly reactive o-quinone-VP-16 [5, 6].

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One electron oxidation is required for formation of intermediates 4-hydroxyl group on VP16 to create a phenoxyl radical, which can be detected by EPR (Fig. 2) [7].

Methods used to assess VP-16 are HPLC [8-15], spectrofluorimetry [16], liquid chromatography–mass spectrometry [17, 18], fluorescence spectroscopy [19, 20], with chromatography of electrokinetic micellar [21]. Electrochemical techniques are sensitive in selective development methods for drug assessment. Voltammetric methods are sufficient for studying the redox properties of pharmaceutical active components [22]. Electronic properties suggest that MWCNTs have promoted the transfer of electron and the sensitivity has been improved in electrochemistry, therefore are used as working electrodes for drug analysis. Eosin-Yellowish (EOY) with IUPAC name of

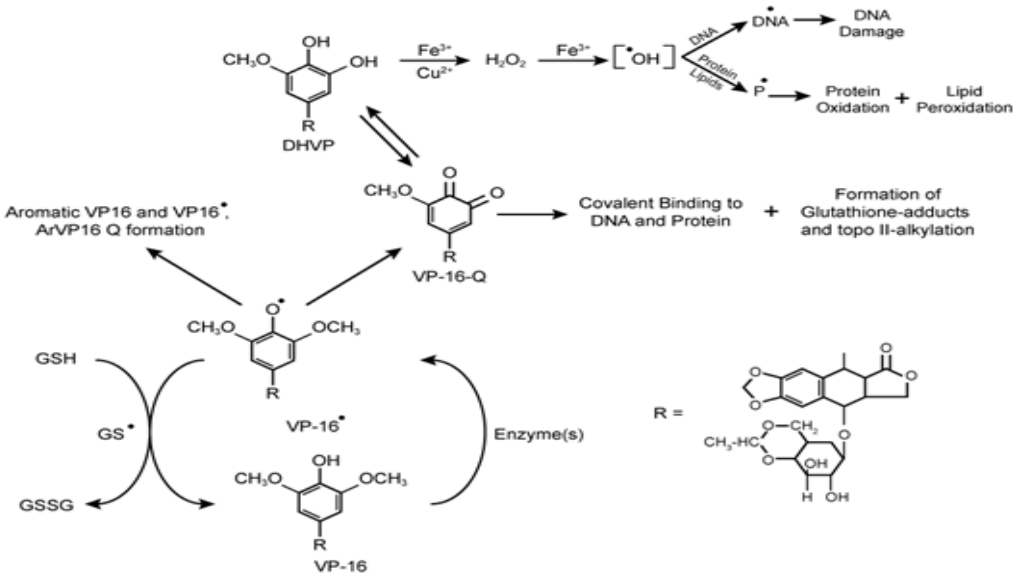


Fig. 1. Formation of VP-16 phenoxy radical and one-electron oxidation of VP-16.

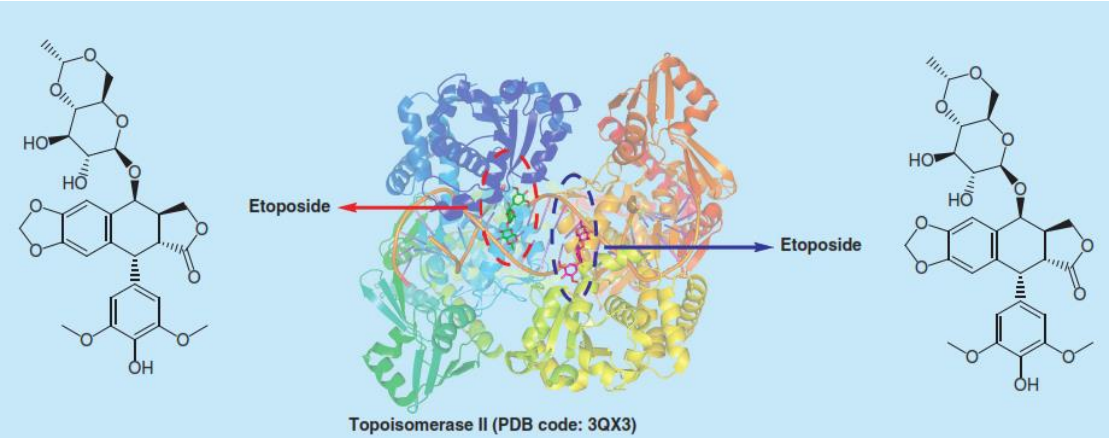


Fig. 2.topoisomerase II effective with complex with DNA and VP-16

2′,4′,5′,7′-tetrabromo fluorescein disodium salt , due to light absorption, high stability and versatility [23-25].

## EXPERIMENTAL DETAILS

*Instrumentation*

Voltammetric measurements were recorded, using an electrochemical analyzer, Computrace 797 VA (AG, Metrohm , CH-9101 Herisav, Switzerland).A cell with 3 types of electrode, including a reference electrode with an Ag/AgCl with KCl saturated, a platinum wire counter electrode, and a Glassy C working electrode were used. The pH meter (digital HANNA, Portugal) was used to measure the pH [26].

*Materials*

The pharmaceutical dose of VP-16 was provided by Koçak Farma Inc (Istanbul, Turkey). EOY was acquired from Fluka (BDH), and was in analytical grade. Britton Robinson and Tris-HCl buffers were applied by supporting electrolytes. The analytical grade (Fluka, BDH) chemicals and reagents used in this study were used with no further purification. Throughout the experimental work, double distillated water (DDW) was used.

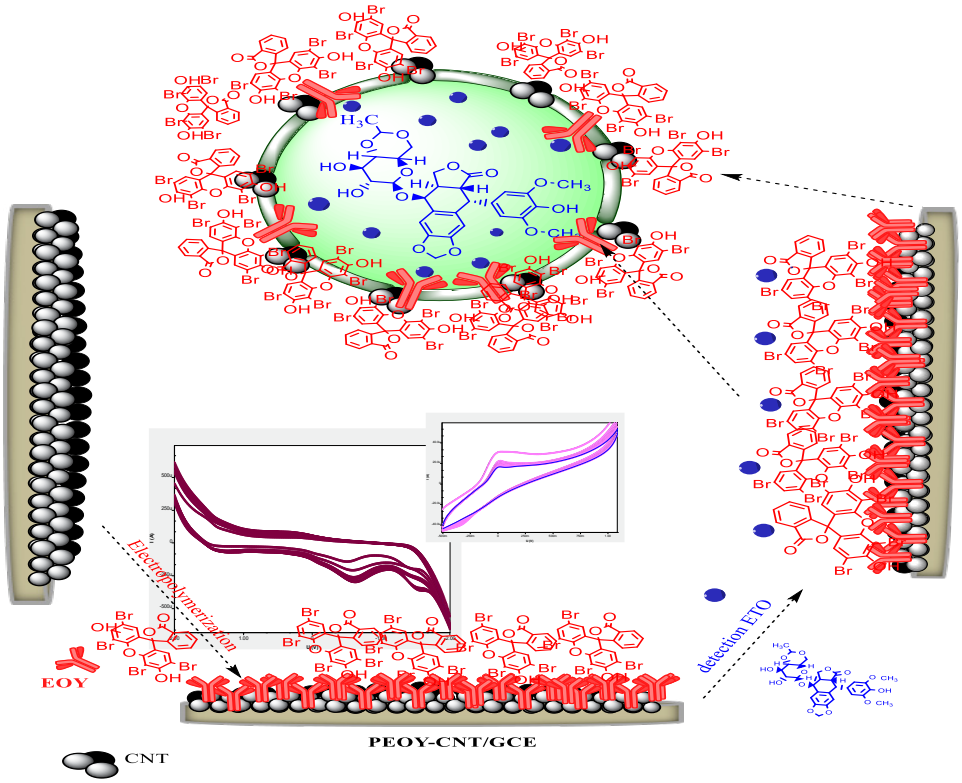


Fig. 3. The electropolymerization of $1\times10^{-3}$M EOY in 0.2 M PBS of pH 6.0 for 14 cycles

*of EOY-MWCNTs/GCE preparation*

GCE polished with 0.3 and 0.05 µm alumina slurry consecutively and ultrasonically cleaned in nitric acid: acetone (1:1), for five minutes, reagent water type IV (ASTM D1193) [27-28]. On the pretreated GCE, 5 µl of MWCNT suspension was then applied and dry. Electropolymerisation of EOY was performed on the surface of bare GCE, using cyclic voltammetric technique, in solution harboring $1\times10^{-3}$ M EOY in 0.2 M PBS at pH 6.0. The electropolymerisation was accomplished by the creation of a film, using (−1.5V to +2.0 V) potential at 50 $mV\times s^{-1}$ of scan rate for 14 cycles (Fig. 3).

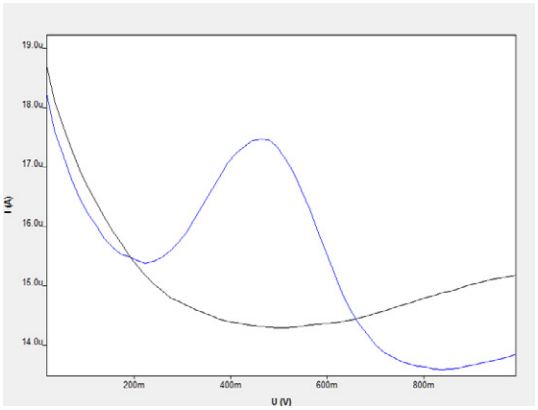

Fig. 4. SWV of VP-16 in PBS at pH 7.0

## RESULTS AND DISCUSSION

*Electrochemistry of VP-16*

The redox mechanism of VP-16 , the further application of bio-detection to 0.2 M PBS at pH 7.0 has been investigated, by CV sweeps between (+2.0 to −1.5 V)until stable voltammograms were acquired(Fig. 4).

*Optimization of condition for VP-16*

To optimize the conditions for measurements through CV technique, the voltammogram of 3.0 nM VP-16 in PBS at pH 7.0 [29], which was studied previously, used as supporting electrolyte. The table below shows optimal conditions for the parameters involved in the measurement of the VP-16 (Table 1) .

*pH effect*

Effect of pH on the $3.0\times10^{-9}$ M VP-16 was assessed in the pH range of 4.0–9.0 in PBS by CV

Table 1. The optimal conditions for 3.0 nM VP-16 in PBS for optimum peak resolution with higher current

| Optimum Condition | Values |
|---|---|
| Potential deposition potential (V) | 0.7 |
| Equilibration deposition time (s) | 10 |
| Deposition potential time (s) | 5 |
| Start deposition Potential (V) | -1.5 |
| End deposition Potential (V) | 2.0 |
| Voltage step (V) | 0.008 |
| Scan rate (V/s) | 0.1 |

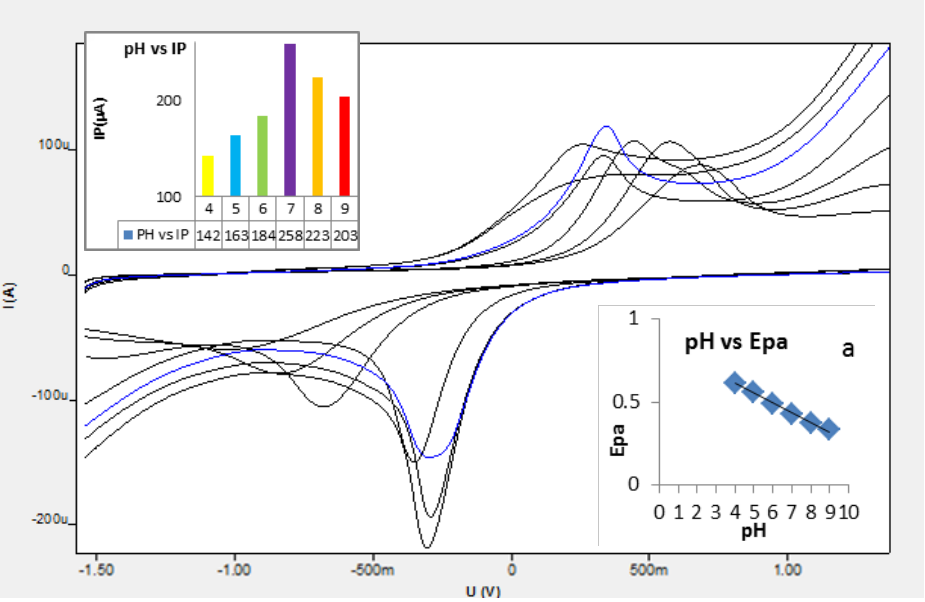


Fig. 5. a. The Effect of pH on VP-16 peak potentials and peak currents, using VP-16 concentration of 3.0×10-9 M. b. Cyclic voltammogram of VP-16 in PBS (pH 4.0-9.0)

with a 100 mV×s$^{-1}$ scan rate (Fig. 5-a). Increased pH shifted the oxidation potential to less positive values till pH 9.0, up until it became immeasurable. The correlation linear between oxidation potential and pH presented as follows:

$$\text{Epa} = -58.3\,pH + 845.8 \qquad (1)$$

Where Epa is expressed in mV, with r = 0.997.

*Characterization of morphology of PEOY/GCE&MWCNTs/EOY/GCE by SEM*

Fig.-6 (a-c) and Fig.-6 (d-f) show the morphology of PEOY/GCE and MWCNTs/PEOY/GCE by SEM respectively, using scanning electron microscope (SEM).

*Electropolymeriztion of EOY on GCE*

After 14 cycles(pH 6.0 PBS)( Fig. 7), the constant peak currents remain; thus, 14 no. of cycles are selected for the modification procedure. A consistent film is created on the GCE surface, indicating that the EOY has been placed on the GCE surface by electropolymeriztion technique.

*Effect of pH*

In the pH range of 4.0 to 8.0, the effect of pH on the current response of EOY was examined. The oxidation current slowly elevated with the increase of pH from 4 to 6. Nevertheless, when pH reached at 8, the peak current diminished. Thus, polymerization sensitivity of EOY at pH 6 was selected as the optimum pH for polymerization of EOY on the GC electrode surface. The relation between the peak current and pH was also shown in Fig 8-a, demonstrating an increase in oxidation current with increasing pH until pH 6.0, then a decrease was observed with increasing of pH value more than 6. For more accurate calibration curve,

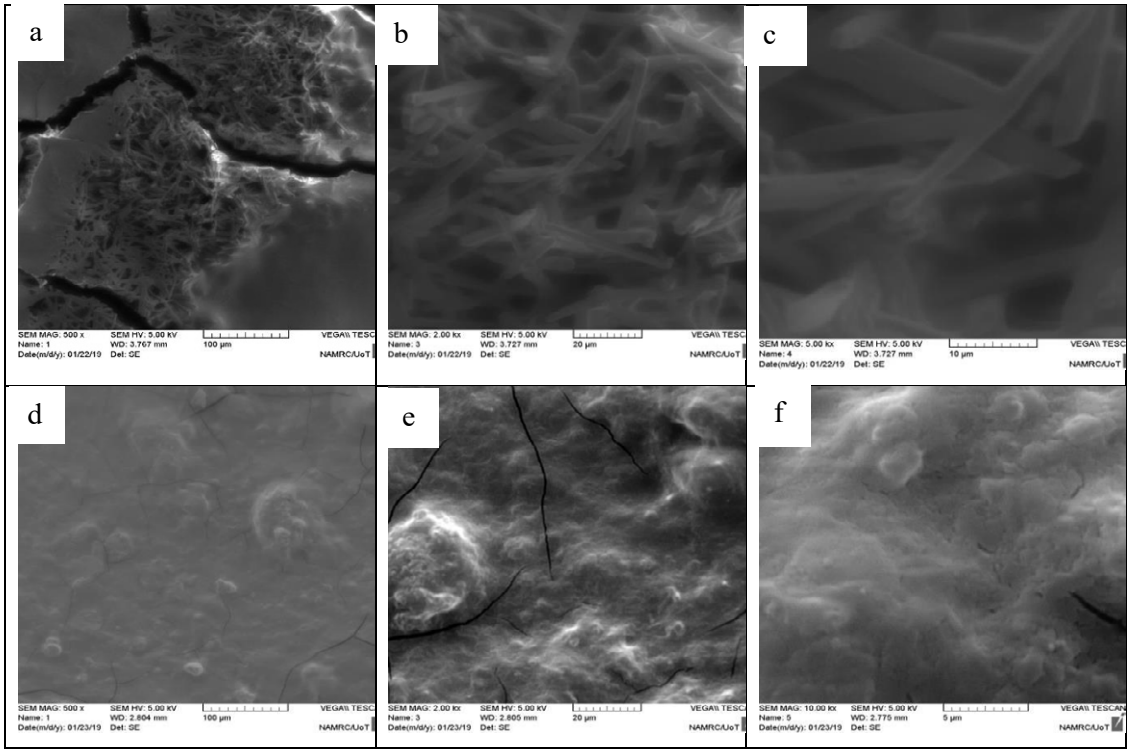


Fig. 6. Morphology characterized by SEM of PEOY/GCE&MWCNTs/PEY/GCE

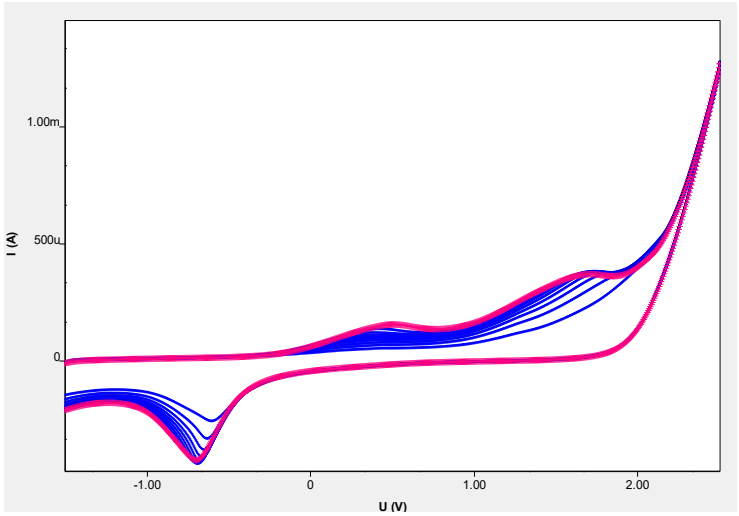


Fig. 7. Electropolymerization of $1.0 \times 10^{-3}$M EOY in 10ml pH 6.0 PBS at GCE

the correlation between EOY concentrations and oxidation current is presented in (Fig. 8-b).

*Effect of cycle numbers and the layer thickness*

Thickness can be overseen by altering the cycle number from 5 to 15 multiple cycles on GCE, corresponding to the electrocatalytic activity in regard to the oxidation of $3.0 \times 10^{-8}$ M to $3 \times 10^{-7}$ M VP-16 in 0.2 M PBS at pH 7.0.

14 cycles were selected as a representative typical example for the transformation of naked GCE. The (Fig. 9-b) shows the relation between

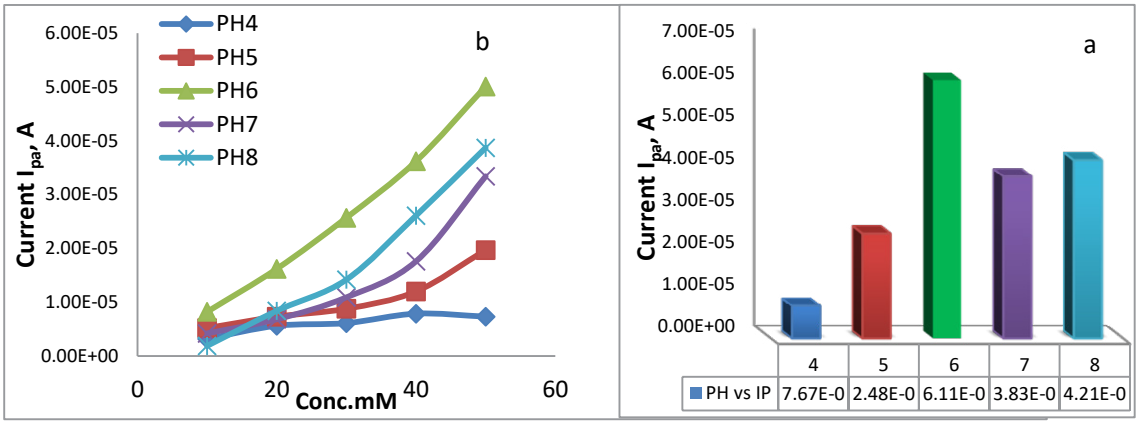


Fig. 8. a) Relation between pH value of 4.0-8.0 and oxidation current of EOY on GCE, b) Calibration curve of EOY in concentration of $1\times10^{-3}$ to $5\times10^{-3}$ M and peak oxidation current for pH ranging from 4.0-8.0

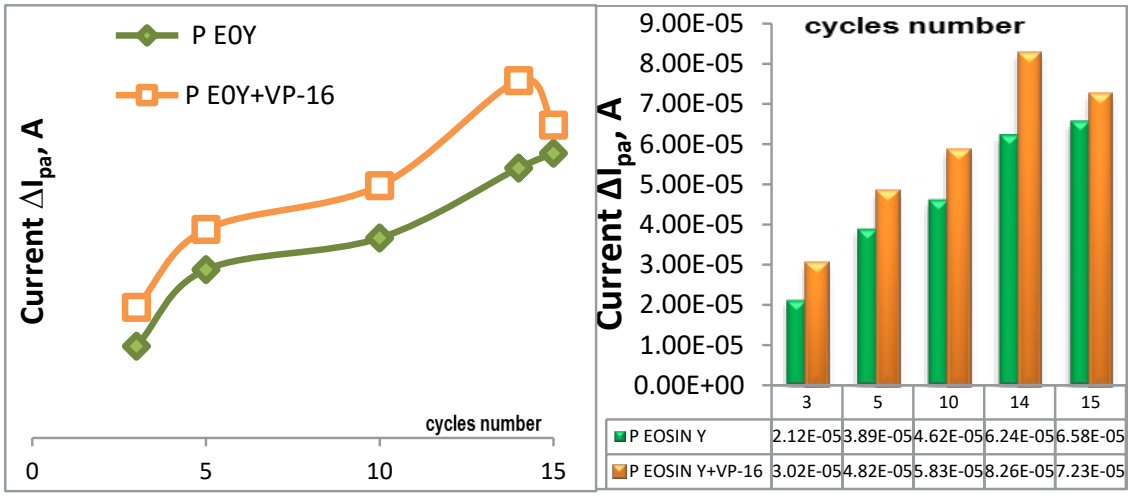


Fig. 9. cycle number and oxidation current relation of PEOY on GCE and oxidation current of VP-16 on PEOY/GCE

cycle numbers and the peak current in the potential range of (−1.5 V to 2.0 V). As the positive potential is lower than the 2.0 V, no polymer film would be obtained, because of the lack of the monomer free radical EOY. As the cycle number increases more and reaches to the 15, the electrochemical activity of EOY film decreases(Fig. 9).

*Effect of Scan Rate*

The CVs of the response of the PEOY-MWCNTs/GC modified electrode were examined in PBS solution containing 5×10-8 M of VP-16 at pH=7(Fig. 10)., at various scan rates, and the potentials ranging from (-0.5V to 1.2 V). The results are illustrated in Fig 11-a. As it appeared, the peak currents have a linear relation with rate of scan between 100 and 400 mV×$s^{-1}$.

*Detection of VP-16 at poly (eosin yellow)/MWCNTs/GCE*

Fig. 11 displays the PEOY-MWCNTs/GC electrode with injection of different concentrations of VP-16 at a working potential of 0.7 V, in comparison with a reference electrode. These data show that the modified electrode is stable and efficient. A linear relationship between response currents and VP-16 concentrations in the range of $3.0\times10^{-8}$ M to $3\times10^{-7}$ M has been found (Fig. 12). The linear equation for this linear range is an

$$Ipa(\mu A) = 0.434[VP-16]\mu M - 0.361 \quad (2)$$

With a R=0.996 correlation coefficient and a 0.434 μA/μM sensitivity.

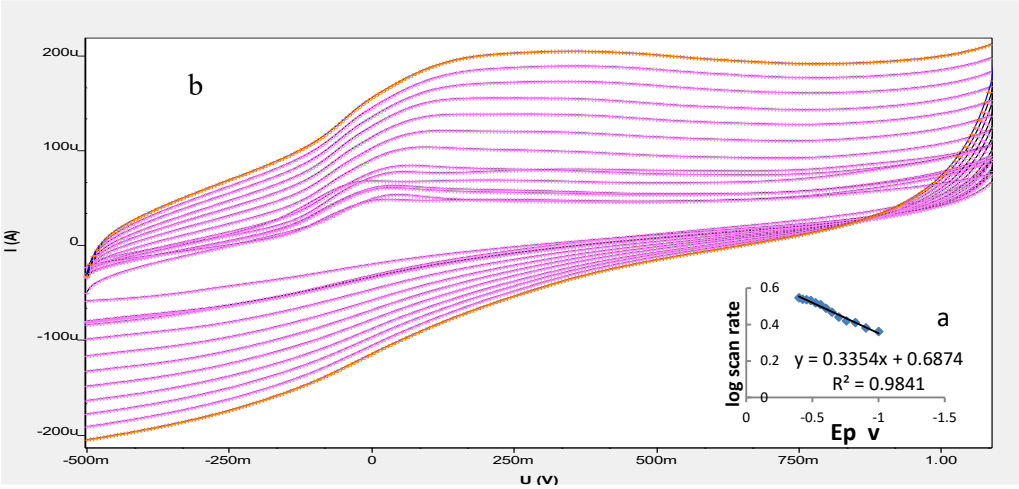


Fig. 10 a) peak potentials proportional to the logarithm of the scan rates. b) Cyclic voltammetry of VP-16 for different scan rates .

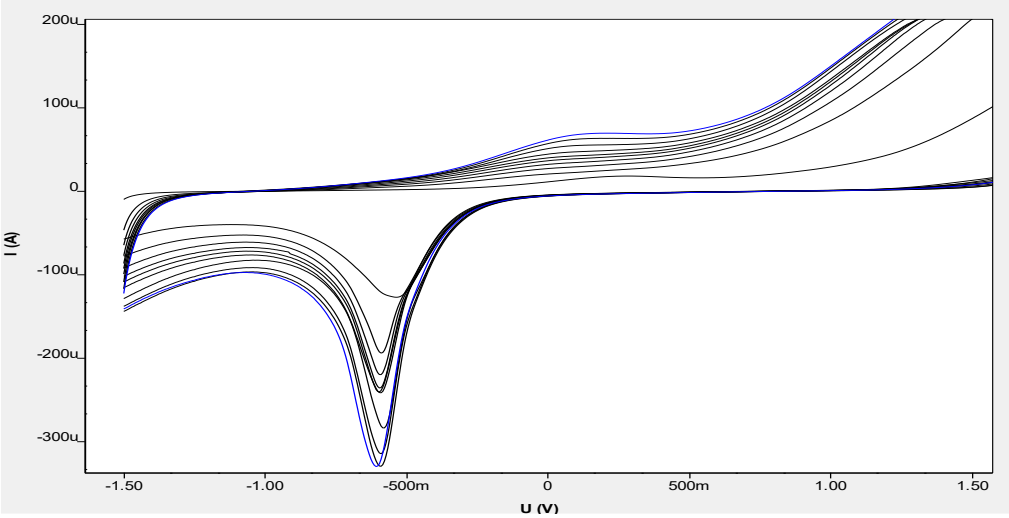


Fig. 11. CVs for VP-16 concentrations on the surface of PEOY/GC

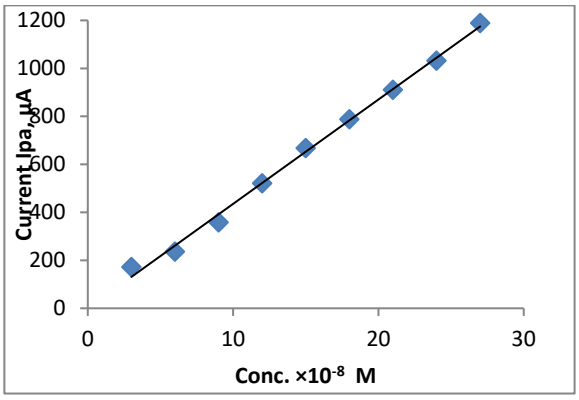


Fig. 12. VP-16 concentrations in the ranges of 3.0 ×$10^{-8}$ M to 3×$10^{-7}$ M on POY/MWCNTs/GCE

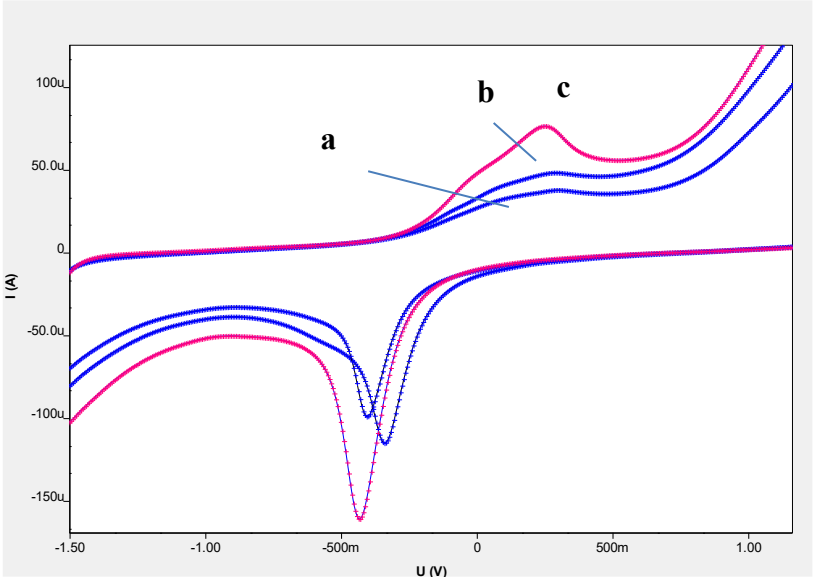


Fig. 13. The CV of (a) GCE, (b) PEOY/GCE and (c) PEOY-MWCNTs /GCE

Table 2. Analytical qualities of 5 sensors for VP-16 estimation

| Exp.no | sensor | Ip, A(CV) |
|---|---|---|
| 1 | EOY-MWCNTs/GCE | 2.95E-05 |
| 2 | EOY-MWCNTs/GCE | 2.99E-05 |
| 3 | EOY-MWCNTs/GCE | 3.08E-05 |
| 4 | EOY-MWCNTs/GCE | 2.97E-05 |
| 5 | EOY-MWCNTs/GCE | 3.02E-05 |
| | SUM | 1.50E-04 |
| | MEAN | 0.00003002 |
| | SD | 5.06952E-07 |
| | RSD% | 1.688713105 |
| | LOD | 8.36E-08 |
| | LOQ | 2.53E-07 |

*Characterization of the electrochemical behavior of PEOY-MWCNTs/VP-16 /GCE*

PEOY-MWCNTs/VP-16 was added to GC electrode, an oxidation peak for the analyte VP-16 /GCE was noted(Fig. 13); its oxidation current elevated and oxidation potential switched to negative values (curve c), which may be the consequence of electrocatalytic activity of PEOY /VP-16 /GCE. However, the anodic peak of VP-16 occurred at a greater negative potential in relation to PEOY-MWCNTs/VP-16 /GCE. Modification of PEOY-MWCNTs / GCE diminished the oxidation potential with an elevation of the peak current in comparison to PEOY-MWCNTs /GCE, which may be due to the synergic effect of both mediators, used in the PEOY-MWCNTs/VP-16 /GCE.

*Analytical properties*

Through the use of biosensors to check the viability of the biosensor through the use of five different biosensors in the presence of the sensors EOY-MWCNTs/GCE in five experiments forVP-16 estimation , the results gave value 8.36E-08(M) for LOD and gave 2.53E-07(M) for LOQ estimation with 5 experiments was obtained in (Table 2).

## CONCLUSION

A new modified electrode was developed in this study using EOY electropolymerization to evaluate the VP-16 (anti-cancer drug); the PEOY-MWCNT / VP-16 / GCE sensor has good sensitivity and selectivity of EOY to VP-16. Low concentrations were estimated at (0.836 nM), following the polymer treatment of the electrode. By using MWCNTs, the electrode showed a high sensitivity as it improved the sensitivity of the electrode to measure the drug compared to the polymer alone, so it was very important to perform a practical application for measuring pharmaceutical models in patient samples

## CONFLICT OF INTEREST

The authors of this have declared there is no conflict of interest.

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