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Electrochemical Study of Anti-Cancer Drug Exemestane in Pharmaceutical Formulation by Voltammetric Techniques Using Multiwalled Carbon Nanotubes Modified Glassy Carbon Electrodes

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Abstract: ***A continuous cyclic voltammetric study of exemestane at glassy carbon electrode was carried out. The exemestane gives one well-defined reduction peak in the potential range -0.7 V to -0.8 V (Ag/Agcl) reference electrode in Britton Robinson buffer (BR) of pH 7.0.Under these conditions, a linear response was obtained between 4.7 x 10^{-6} M and 1.0 x 10^{-6} M in non-aqueous media for various voltammetric techniques. Moreover, the drug was analysed using Square Wave Cathodic adsorptive stripping voltammetry (SWCAdSV). Each determination method was fully validate and applied for the analysis of anti-cancer drug exemestane in its pharmaceutical dosages form. The influences of the pH, concentration, scan rate, volume of MWCNTs suspension, accumulation potential and time on peak current and peak potentials were examined.***

Keywords: Cyclic Voltammetry, Exemestane, Glassy Carbon Electrode, Modified electrode, Multiwalled carbon nanotubes, Square Wave Voltammetry

I. INTRODUCTION

Exemestane (Fig. 1), 6-methylideneandrosta-1,4-diene-3,17-dione, an oral chemotherapeutic drug was used to treat cancerous growth or malignancies. Exemestane known as an irreversible steroidal aromatase inactivator which is typically used to deal with breast cancer. Aromatase is an enzyme that synthesizes estrogen, aromatase inhibitors block the synthesis of estrogen. Exemestane is in a class of medications called non-steroidal aromatase inhibitors. When this drug is used , it inhibits the production of estrogen. Therefore, the estrogen level will be lowered and growth of cancers is reduced. The EXE- aromatase molecular interaction, when EXE interacts with aromatse. During interaction the drug was converted into reactive compound that can lead to bind enzyme through irreversible and covalent bonds.[1-4]

The worldwide use of this compound and the need for clinical and pharmaceutical studies. Although spectrophotometry and chromatography are most commonly used techniques, but these reported methods for the determination of exemestane required pretreatment and time consuming extraction or evaporation steps prior to the analysis and are not sufficiently sensitive for convenient application to pharmaceutical formulations to final analysis Electrochemical methods have long been used for determination of a wide range of drug compounds due to their simplicity, low cost and relatively structure of exemestane short analysis time in comparison to other analytical techniques like reverse phase extraction, chromatography, LC-MS, high performance liquid chromatography (HPLC), solid phase extraction (SPE) and salting out liquid extraction. So the voltammetric techniques are very good and appropriate for the analysis of exemestane.[5-9]

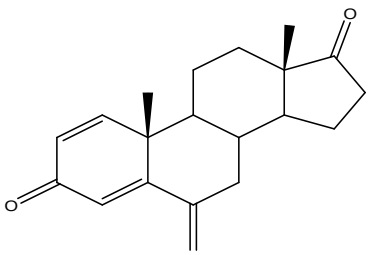


FIG. 1 Structure of Exemestane

II. MATERIALS AND METHODS

Exemestane was obtained under the trade name Aromasin and was used after purification through the centrifugation. A stock solution of exemestane (10^{-3} mol/ L) was prepared in dimethyl formamide (DMF). Doubly distilled water obtained from Electrochemical Research Laboratory distillation assembly, was used for entire studies. For recording voltammograms solutions were prepared by mixing accurate volume of stock solution and buffers. In this process the buffer is used that is Britton- Robinson (BR) buffer. All chemicals and reagents were of analytical grade and obtained from Sigma-Aldrich (St Louis, MO, USA).

A. Apparatus

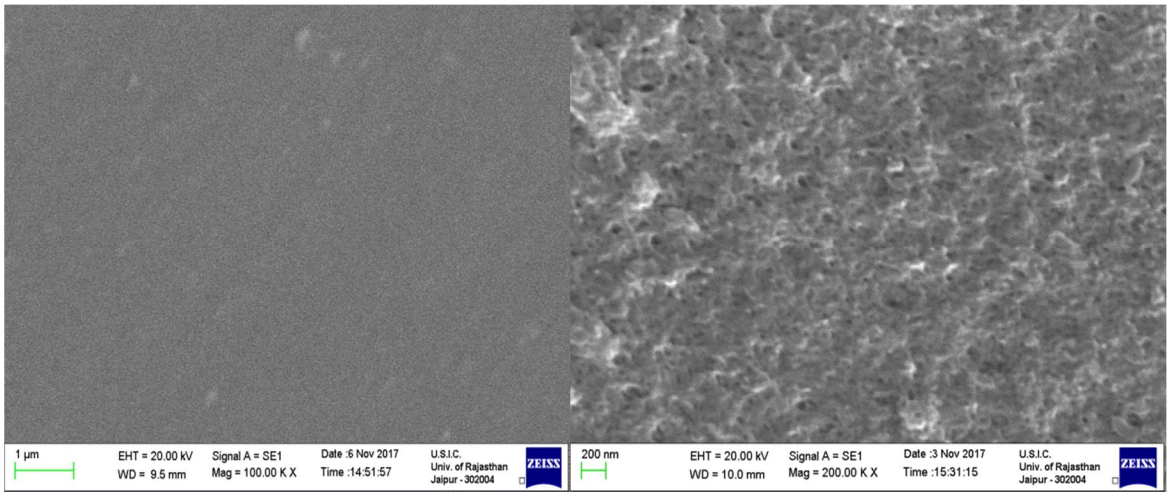
Voltammetric experiments were performed on a 1230A [SR 400] electrochemical analyser (CHI Instrument, Bee Cave, TX, USA), with a totally automated attached to a PC for total control of the experiments and data collection and treatment purchased from Sinsil International, Mumbai, India. The three electrode system consisted of Ag/Agcl (3.0 mol/L KCl) as reference electrode, platinum electrode as auxiliary electrode and the modified glassy carbon electrode (GCE) as working electrode were used.

All the solutions examined by electrochemical techniques were purged for 10–15 minutes with purified nitrogen gas in which a continuous stream of nitrogen was passed over the solutions before each of the measurements. Nitrogen gas was deoxygenated by passing it through acidic sodium (meta) vanadate solution. All pH-metric measurements were made on a CHINO (DB-10110) (Chino Scientific Instruments Mfg, Ajmer, Rajasthan, India) digital pH meter fit with a glass electrode standardized with buffers of known pH.

B. Construction Of Mwcnts/Gce

The working GCE was polished with 0.05 µm alumina slurry until a mirror-like surface to remove impurities on the electrode surface was obtained and further subjected to sonication for a short duration with doubly distilled water then allowed to dry at room temperature (Fig. 2a) . A 1 mg/ml MWCNTs dispersion was prepared by dissolving 1 mg MWCNTS in 0.5ml Nafion ethanol solution with the help of hot sonication for 3 hours in an ultrasonic bath. A drop of 10 µl of this dispersion was cast onto the surface of pretreated GCE using a micropipette and then it was dried at room temperature.

The multiwalled carbon nanotubes modified electrode (MWCNTs/GCE) was obtained (Fig. 2b).



(a) (b)

Fig. 2 SEM images of bare glassy carbon electrode and MWCNTs modified glassy carbon electrode. (a) SEM image of bare GCE, (b) SEM Image of MWCNTs modified GCE

C. Experimental Procedure

The finely grounded substance equivalent to 10 mg of exemestane was accurately weighed and dissolved in dimethyl formamide (DMF). Total 10 ml of the solution containing BR buffer of pH 7.0 and the appropriate concentration of the exemestane was transferred into the electrochemical cell, deoxygenated solution is obtained from nitrogen stream.

The accumulation of Exesmestane at the working electrode was carried out for a selected time while the solution was stirred at 2000 rpm. After optimization of operational parameters the cyclic voltammograms and other techniques were recorded.

III. RESULT AND DISCUSSION

The electrochemical behavior of Exemestane was studied by cyclic voltammetry (CV), square wave adsorptive stripping voltammetry (SWCADSV) and differential pulse adsorptive stripping voltammetric (DPCADSV) techniques on glassy carbon electrode. In all electrochemical methods exemestane gave on well defined reduction peak in BR buffer at -0.77 V(Ag/Agcl) in pH 7.0.

A. Cyclic voltammetric behaviour

A typical cyclic voltammogram of exemestane were recorded within a wide range of potential 0 to -1.0 v at different scan rate, pH values and concentration. Exemestane gave one well defined reduction peak, it indicate by the voltammogram there is no peak were observed in the anodic direction of the reverse scans, suggesting the irreversible nature of the process, as shown in Fig. 3.

1) *Influence of scan rate:* The electrochemical behavior of exemestane at different scan rate were observe. A linear relationship between the peak current and the scan rate in the range of 0.02 to 0.2 vs^{-1} was observed, corresponding to the following equation, shown in Fig. 4:-

$Ip\ (\mu A) = 0.341v^{1/2}\ (vs^{-1}) + 2.075 \qquad R^2 = 0.996$

Which indicate that the reduction of exemestane was an adsorptive controlled step. The peak potential negatively shifted with increasing the scan rates. It confirms the irreversible nature of the reduction process.[10,11]

The linear relation between peak potential (Ep/v) and natural logarithm of scan rate (log v) was Ep (v) = 0.102 logv (vs^{-1}) + 0.243 R^2 = 0.992 shown in Fig. 5. Furthermore, peak current (Ip) was found to be linearly dependent on square root of scan rate related with the Randles - Sevcik equation, which can be expressed as

$$Ip = (2.99x10^5)\ n\ [\alpha n]^{1/2}\ A\ CoDo^{1/2}\ v^{1/2} \qquad (1)$$

Where n is the number of electrons exchange in reduction, α is the charge transfer coefficient, A (cm^2) is the apparent surface area of the electrode, Co (mol/L) is the concentration of the electro-active species, Ip (μA) is the cathodic peak current, Do (cm^2/s) is the diffusion coefficient of the electroactive species and v (mV/s) is the scan rate.[12-14]

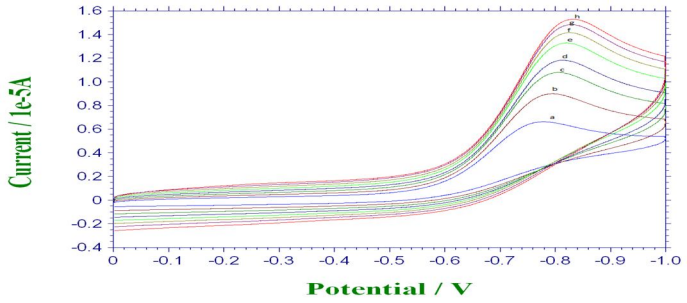


Fig. 3 Cyclic voltammogram of 2.5x10-6 molL-1 Exemestane in Britton-Robinson buffer at different scan rates: (a) 25 mV-1 (b) 50 mV-1 (c) 75 mV-1 (d) 100 mV-1 (e) 125 mV-1 (f) 150 mV-1 (g) 175 mv-1 (h) 200 mv-1 at pH 7.0

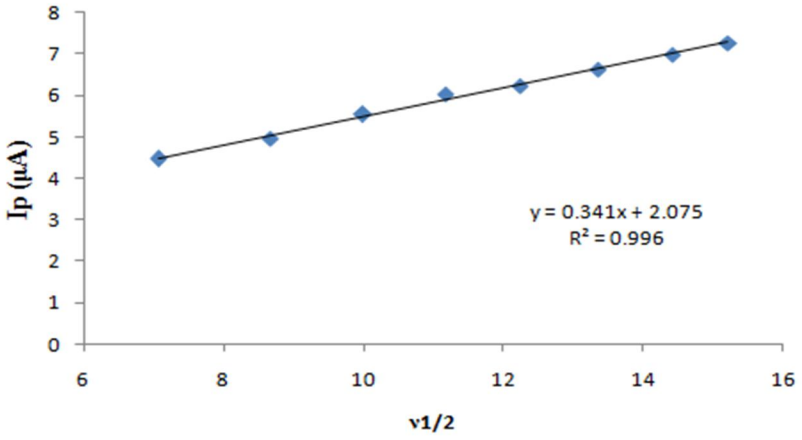


Fig. 4 Plot of peak current (Ip/μA) *vs.* root of scan rates $v^{1/2}$

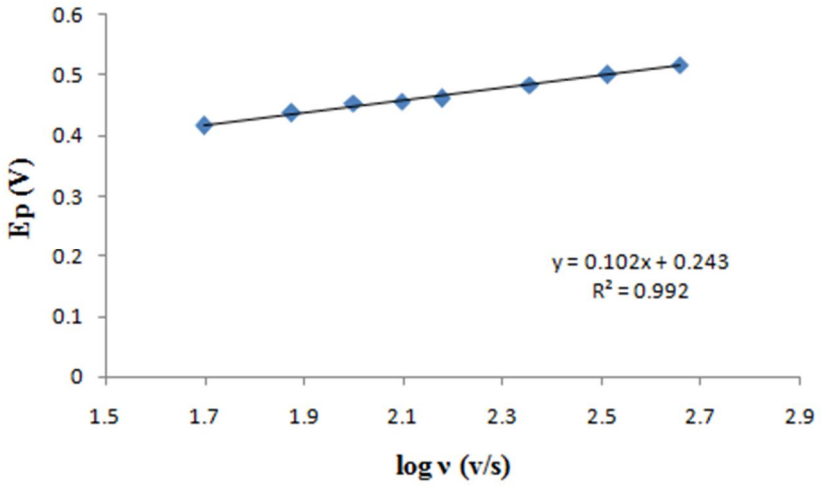


Fig. 5 Plot of peak potential (Ep/V) *vs.* Logarithm of scan rates log ν

B. Enhancement of experimental conditions

1) Selection of supporting electrolyte solution: Several buffer solutions such as Britton Robinson buffer, Phosphate buffer, carbonate buffer were examined as a supporting electrolyte by cyclic voltammetric measurements. The best results showed by the Britton-Robinson buffer and also the maximum peak current is observed in BR buffer. Therefore, BR buffer is the prime supporting electrolyte for the study of exemestane. The effect of BR buffer concentration was also observed. The current responses of exemestane increased sharply from 0.08 to 0.4 M and then decreased with further increase of the BR concentration shown in Fig. 6. The most appropriate valve of BR buffer concentration in the experiments is 0.4 M due to maximum reduction of resistance of the solution and abolish electromigration action. Hence, 0.04 M BR buffer was opt for the following experiments[15-17].

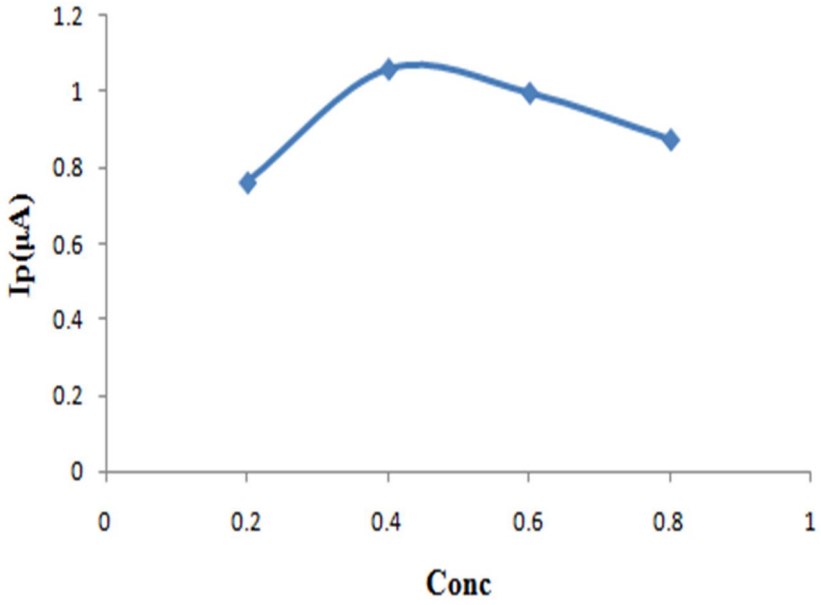


Fig. 6 Effect of concentration of BR buffer on peak current to 1.0 x 10^{-6} M Exemestane

2) Effect of Ph: The electrochemical retaliation of exemestane in 2.5 x 10^{-6}M concentration with different pH values were studied. The peak current (Ip) is highest at pH 7.0, as seen in Fig. 7. Hence pH 7.0 of BR buffer was used for the determination of exemestane. A linear negative shift in potential with increase of pH value for anodic peak, as shown in Fig. 8.

The linear equation is Ep (V) = -0.009pH + 0.541 R^2 = 0.987 for a irreversible process.

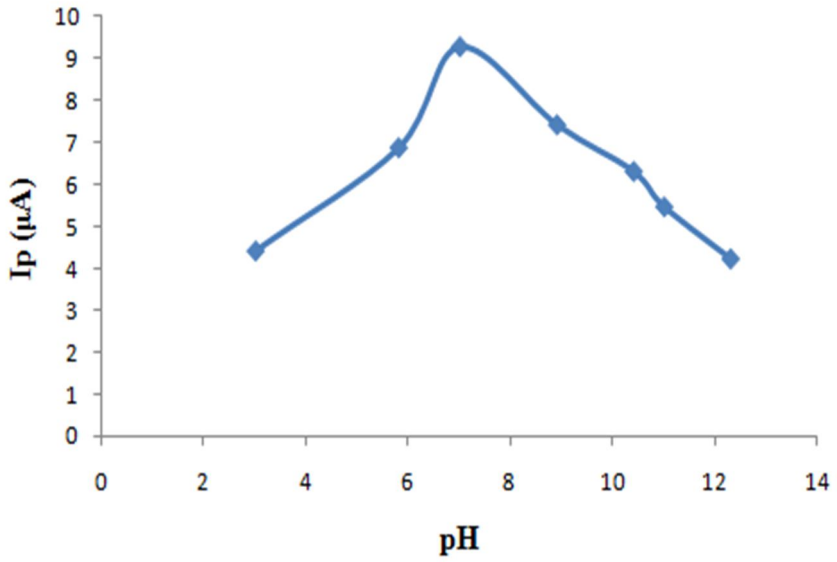


Fig. 7 Effect of pH of medium on cathodic peak current

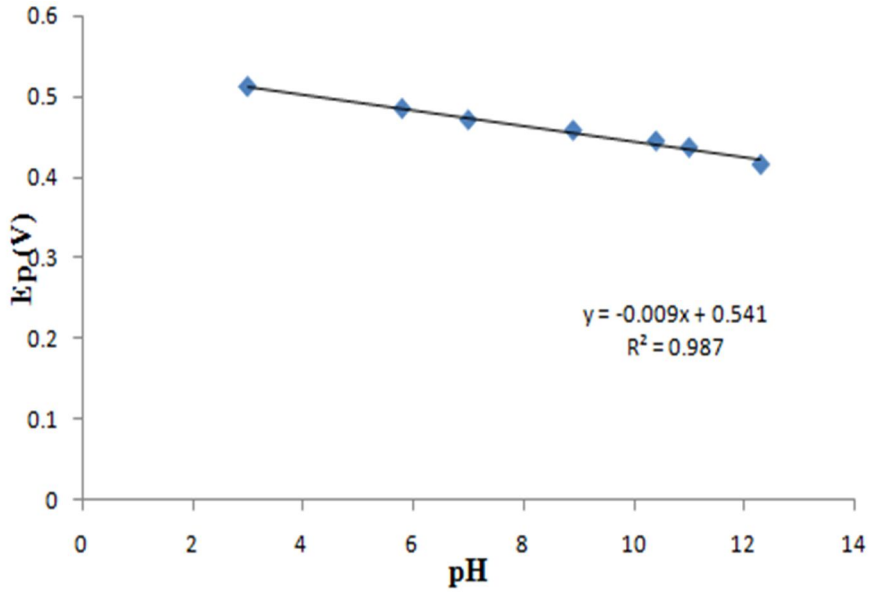


Fig. 8 Plot of peak potential (Ep/V) *vs*. pH

3) *Effect of accumulation potential & time:* The study of linear sweep voltammetry shows the effect of accumulation potential and time on peak current response. It is observed that the peak current changes moderately as changing accumulation potential from 0.8 to -0.35V, indicate that there is no effect of accumulation potential on peak current of exemestane. Consequently, the -0.0 V accumulation potential versus Ag/Agcl as reference electrode was used for successive determination. The peak current increased very quickly with increasing accumulation time from 2 to 200 s, which promoted rapid absorption of exemestane on the surface of modified electrode. The peak current reached at the maxima after200s and then tends to decreases the level of peak current. This shows the saturation accumulation. The accumulation time is long then the stability of the modified film might reduce, so 200 s was chosen as accumulatiom time[18-20].
4) *Effect of the volume of MWCNTs suspension:* The volume of MWCNTs suspension is increased from 5.0to 19.0 µL, the peak current is gently increases but further increases the volume the peak current is slowly decreased are shown in Fig. 9. This observation may be shows due to stagnant mass transfer process to the electrode surface. So, the optimized amount was selected 15 µL.

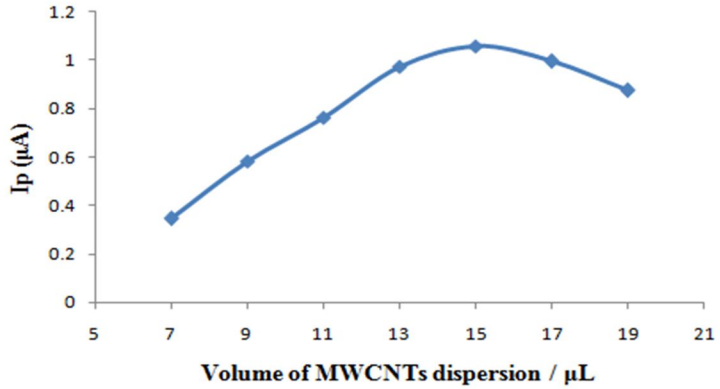


Fig. 9 Effect of volume of MWCNTs suspension on the peak current

C. Effect of concentration

The effect of concentration of exemestane in Britton-Robinson buffer was investigated by LSV. A linear variation of the peak current (Ip) with concentration of bulk exemestane was analysed within the concentration range of 1.0 x 10^{-6} to 4.7 x 10^{-6}. At different concentrations, linear sweep voltammograms of exemestane are shown in Fig. 10. On increasing the concentrations of exemestane the LSV peak current linearly increased, as shown in Fig. 11. [21] and corresponding regression equation for the graph between Ip (µA) versus concentration (mol/Lit) as follows -

Ip (µA) = 0.450 C (µM) + 4.503 (µA) $R^2 = 0.985$

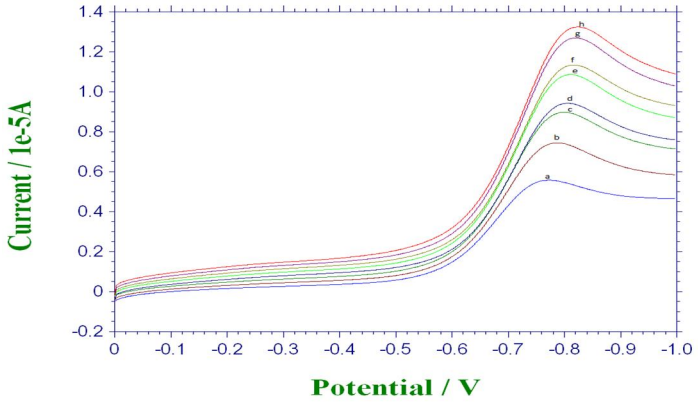


Fig. 10 Linear Sweep voltammogram of Exemestane at different concentrations

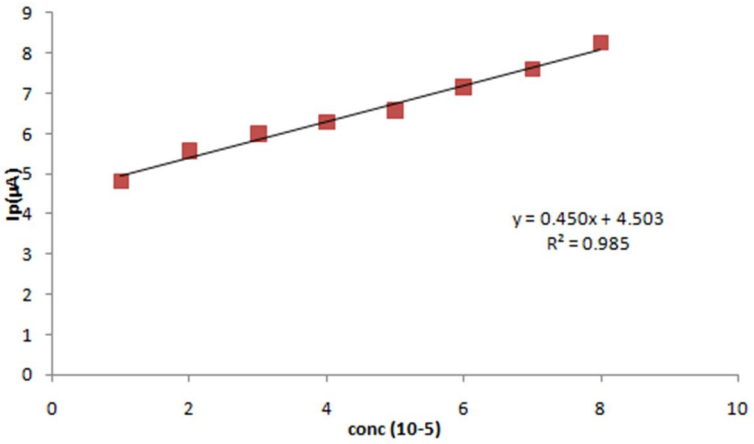


Fig. 11 Plot of peak current *vs*. Concentration of Exemestane from Linear Sweep voltammogram

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IV. VALIDATION OF THE ANALYTICAL PROCEDURE

The linear correlation between the peak current and concentration is the base of the quantitative determination. The validation of proposed method for quantification in bulk form of exemestane was carried out via evaluation of linearity range of concentration, limit of detection (LOD), limit of quantification (LOQ).

A. LOD and LOQ

The smallest concentration of the sample that can be detected with appreciable certainty was calculated using the equation:

$$LOD = 3s/m$$

where *s* is the standard deviation of intercept and *m* is the slope of the calibration curve (*I*p versus concentration). LOD for thestandard solution of the sample using the technique SWCAdSV was found to be 1.502×10^{-6} M.
The lower LOQ for precise measurements was determined usng the equation:

$$LOQ = 10s/m$$

The LOQ for the proposed method was found to be 5.006×10^{-6} M. The low values of LOD and LOQ proved the good sensation of the method.
Similarly, low value of % relative standard deviation (RSD) indicates less spread of sets of data, which shows a good precision in the method [22]. All data are tabulated in table 1.

Table 1. Characterisation data of Exemestane calibration plots in BR buffer of pH value 7.0 for LSV methods

Parameters	LSV
Linearity Range, M	4.7×10^{-6} to 1.0×10^{-6}
Slope	0.450
Intercept	4.503
LOD, M	1.502×10^{-6}
LOQ, M	0.5006×10^{-7}
Standard Deviation	0.2253
Correlation Cofficient (R^2)	0.985
% RSD	0.0675
Measure Potential (V)	0.775

B. Repeatability and stability

The repeatability of the modified electrode was investigated with the same modified electrode in the presence of 2.5×10^{-6} M exemestane. The relative standard deviation (RSD) was 0.20647 for 5 successive assays, indicating that the modified electode have good repeatability. Furthermore, after accumulating the MWCNTs/GCE in air for 5 days, its initial peak current responces retained 98.9% for exemestane concentration 2.5×10^{-6} M. That suggests this electrode has long standing stability [23]. All data are tabulated in table 2.

Table 2. Result for repeatability

Serial No.	Conc. (mg/L)	Peak current (µA)	Mean +- SD	% RSD
1	2.5	5.330	4.835+0.4779	0.09884
2	2.5	5.051	4.835+0.4779	0.09884
3	2.5	4.982	4.835+0.4779	0.09884
4	2.5	4.546	4.835+0.4779	0.09884
5	2.5	4.267	4.835+0.4779	0.09884

C. Electrochemical Behavior Of Exemestane On The Mwcnts/Gce

1) Cyclic voltammogramms of exemestane at different electrodes: The cyclic voltammetric study of exemestane at bare GCE and MWCNTs/GCE were shown in Fig 12. A cyclic voltammogram was recorded at bare GCE and MWCNTs/GCE without containing the solution of exemestane in Fig 12a and c. at this time, the potential was applied from 0.0 to -1.0 V, no peak is observed in reverse direction. After that cyclic voltammogram were recorded in the presence of 2.5 X 10^{-6} M exemestane solution, a irreversible reduction peak is observed at -0.80 V at bare GCE (Fig 12b) and same peak is appeared at -0.775 V on the MWCNTs / GCE (Fig 12d). Moreover, There was significant amplification in the peak current and also increased the sensitivity. Due to the use of modified glassy carbon electrode by the MWCNTs surface area increased. There are ascribed to the magnificent electrocatalytic abilities of MWCNTs. Further validation of the totally irreversible process there is no corresponding oxidation peak was observed neither at the bare GCE nor on the MWCNTs / GCE in the reverse scan.[24-28]

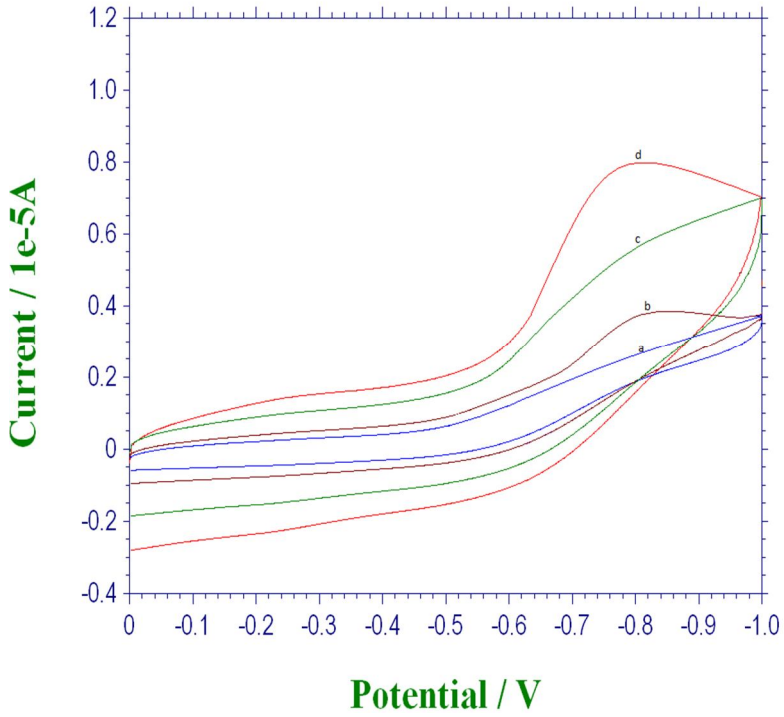

Fig. 12 Cyclic voltammogram of different electrodes in BR buffer. (a) The bare GCE in the absence of exemestane and (b) in the presence of 2.5 x 10^{-6} M exemestane; (c) the MWCNTs/GCE in the absence of exemestane and (d) in the presence of 2.5 x 10^{-6} M exemestane. Accumulation time :200s; scan rate: 0.50 Vs^{-1}

V. CONCLUSION

The investigated voltammetric behavior of exemestane at a modified glassy carbon electrode has been successfully fabricated by using MWCNTs. Exemestane can produce a sensitive irreversible cathodic peak at -0.775 V in pH 7.0 in BR buffer. Under the optimize conditions, the peak current was propostional to the concentration of exemestane in the range of 1 x 10^{-6} to 4.7 x 10^{-6} M with a detection limit (LOD) of 1.502 x 10^{-6}M. The limit of quantification (LOQ) is 0.5006 x 10^{-7}M. The MWCNTs exhibits god catalytic activity and surface area of GCE is increased. It could be found that the electrochemical reduction of exemestane had been greatly improved. The MWCNTs exhibited superior performance in terms of peak current, linear working range, sensitivity and limit of detection. The modified electrode shows good repeatability. The proposed method is efficient and more sensitive compared to the other electrochemical methods like HPLC, UV- Vis spectrometry and other chromatographic methods. Thus, the proposed method can be adapted for pharmacokinetic studies as well as for real samples in quality control laboratory studies.

VI. ACKNOWLEDGEMENT

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