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Electrochemical characterization and determination of the anticancer drug, Flutamide by cyclic voltametry

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Abstract

In the present work, Cyclic Voltammograms(CV) of Flutamide were obtained using hanging drop mercury electrode under different experimental conditions Viz solution concentration and pH, voltage scanning rate and range. At solution pH=9 and a scanning rate of 100mV/s, the CVs were exhibited only one reported cathodic peak due to the electrochemical reduction of the nitro-aromatic moiety of the Fluitamide molecule. Calibration curves between the concentration of Flutamide and the cathodic reduction peak current, in drug form, one of its pharmaceutical formulation and in human urine and serum, were obtained.

Keywords: Flutamide, Cyclic voltametry, calibration curves

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1.0 Introduction

Nitro group-containing drugs and pharmaceuticals have always good biological importance especially for biological systems including proper growth and metabolic connections [1]. Flutamide, 4-nitro-3-trifluromethyl-isobutilanilide, is a synthetic antiandrogenic agent devoid of- hormonal agonist activity. It has been reported to be useful to improve urine flow in begin prostatic enlargement and gynaecomstia [2-5]. Flutamide, on the other hand, is an unusual example of antiandrogenic drugs lacking a steroidal structure [6].

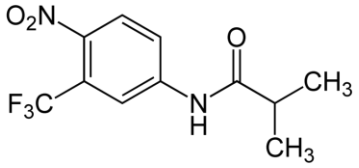
Several instrumental methods are widely used for the identification and the quantitative determination of anticancer drugs especially Flutamide as alkaylated type chemotherapy. High-performance liquid chromatography was used by Nunes et al [7] to assay flutamide in pharmaceutical preparations. On the other hand, a sensitive and simple spectrophotometric method for determination of the reduction product of Flutamide (Flu) was described by Nagaraja et al [8]. The method was based on the interaction of the diazotized flutamide reduction product with N-(1-naphthyl) ethylenediamine dihydrochloride (NEDA) in neutral or resorcinol (RSL) in alkaline media. Absorbance of the resulting chromophores was measured at 525 and 480 nm, respectively, and is stable for at least 7 days. The two coupling reagents were applied successfully for the determination of Flutamide in tablets. Results from the analysis of pure Flutamide and its commercial tablets by the proposed methods agreed well with the other reported method.

The first flow injection (FI) method for the determination of flutamide potent antiandrogen was reported by Tzanavaras et al[9]. The method was based on the direct measurement of the absorbance of the analyte at 310 nm under flow condition. Parameters affecting the determination such as detection wavelength, sample injection volume and flow rate were studied and optimized. The assay was validated (linearity, limits of detection and quantitation, accuracy, repeatability, reproducibility and selectivity) for the dissolution studies of flutamide–containing tablets.

In recent years, interest in the electrochemical behavior and determination of biological compounds has increased. In the present part of the thesis, the simple technique of cyclic voltammetry is used for the characterization and determination of flutamide, FLU, in drug form and in one of its pharmaceutical formulation, as well as, in human urine and serum.

2.0 Materials and methods

The investigated pharmaceutical drug used in this work is Flutamide {2-methyl-N-(4-nitro-3 (trifluoro methyl) phenyl) propanamide}. The structural formula of the compound is:



Flutamide(2-Methyl-N-(-(4-nitro-3 trifluoro methyl) phenyl)propanamide)

Preparation of drug solutions

Stock solution of flutamide was prepared every day by dissolving the appropriate weight in the specific volumes of tri-distilled water in stopper dark glass bottles to give solutions of different concentrations. Photodecomposition of the drug solutions was avoided by means of aluminum foil protecting the electrolytic cell and volumetric flasks.

Flutamide pharmaceutical formulation (Eluxin)

Eluxin Tablets, supplied by Merk Sharp & Dohme (Australia), suggested to contain 250 mg of flutamide per tablet were used as pharmaceutical flutamide formulation. 10 tablets were thoroughly grounded and mixed uniformly. A weighed portion of the powder equivalent to contain $1x10^{-3}$ M flutamide was transferred to a 50 ml measuring flask and completed with methanol/tridistilled water,v/v. Appropriate solutions with different concentrations were prepared by dilution with the supporting electrolyte.

Buffer solution

Buffers in the pH range 2-12 were prepared by mixing stock solutions of 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4 and adjusting the pH by the addition of either H_3PO_4 or NaOH solutions.

Urine and serum treatment

Human urine and serum were obtained from healthy donors and used directly after collection. Urine samples were centrifuged and filtered before used. The serum samples, on the other hand, were treated with 2 ml of methanol as a serum-protein precipitating agent. The precipitated proteins were separated by centrifugation for 15 minutes at 1500 rpm. The clear supernatant layer was filtered to produce a protein-free spiked serum. The standard method was applied by adding successive concentrations of the drugs.

The electrolytic cell used throughout the present work had a capacity of 100 ml. It had a double-walled jacket through which water at the adjusted temperature was circulated. A conventional three electrodes system was used. A hanging mercury drop electrode (HMDE) was the working electrode), Ag/AgCl saturated KCl as a reference electrode and a platinum wire as a counter electrode, Purified nitrogen gas was bubbled through the solution for 20 minutes before polarization started. All measurements were carried out at 25°C. Cyclic voltammograms, CVs, were performed using a potentioscan Type POS73, and the current-potential curves were recorded on an X-Y recorder Type Adance A-2000.

Statistical analysis

Statistical analysis of the calibration curves parameters e.g. slopes, intercepts, regression coefficients and standard deviations was done. The variation of cathodic peak current, I_P, with the concentration, C, of the studied drugs is based on the linear relations:

$$\mathbf{I_p = K + n C} \qquad \text{---------- (1)}$$

where K and n are constants.

3.0 Results and discussion

Effect of cyclization

Figure-1 shows three typical cyclic voltammograms, CVs, of 60µM FLU at the HMDE in phosphate buffer solution at pH=9, in the potential range 0.0V to -

0.8V versus the Ag/AgCl reference electrode, at a scanning rate, √, of 100 mV/s.

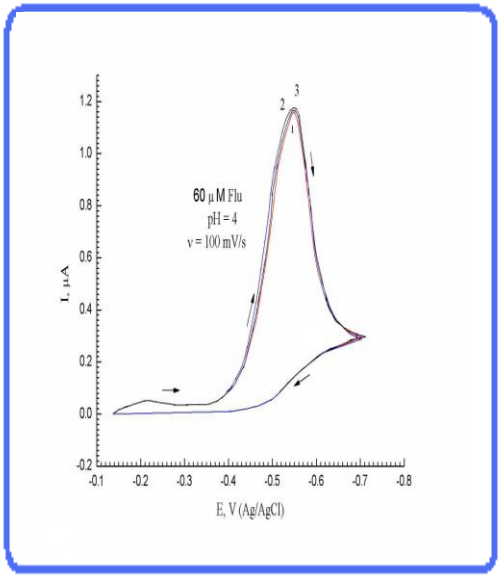


Figure-1: Repeated Cyclic Voltyammograms, CVs, of 60 µM Flutamide at pH=9 and 100mV/s

It is quite clear that repeated cycling of the electrode gave rise to essentially similar cyclic voltammograms except that the current of the only reported cathodic peak, I_p, increased only slightly with the number of cycles, while the cathodic peak potential, E_p, remains more/or less unchanged. It is of importance to note that for the rest of results, only the first cycle is taking in consideration.

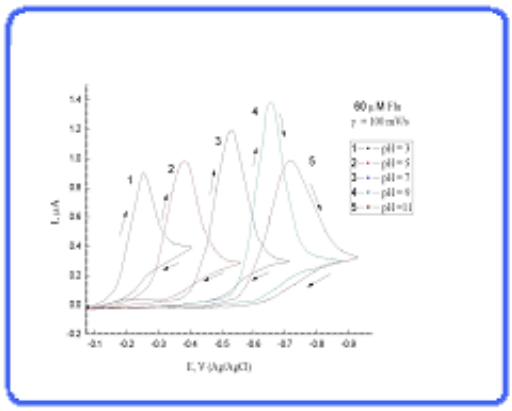


Figure-2: Cyclic Voltammograms, CVs, of 60µM Flutamide at different pH's and 100mV/s

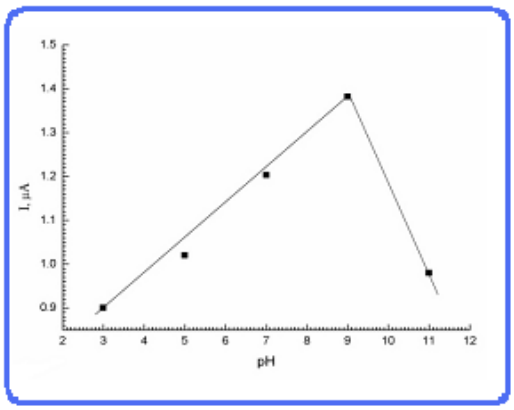


Figure-3: Variation of cathodic peak current, Ip, with the pH of Flutamide solution

Further inspection of the CVs of Figure-1 reveals only one cathodic reduction peak at a potential around -0.55V versus the Ag/AgCl reference electrode. Along the obtained cyclic voltammograms, no anodic oxidation peaks were

reported indicating the irreversibility of the electrochemical reduction process of FLU.

Effect of solution pH

Figure-2 shows the cyclic voltammograms, CVs, of 60 µM FLU, in phosphate buffer solution in the pH range 3 – 11, at the HMDE in the potential range 0.0 to – 1.0 V versus the Ag/AgCl reference electrode and at a scanning rate, √, of 100 mV/s.

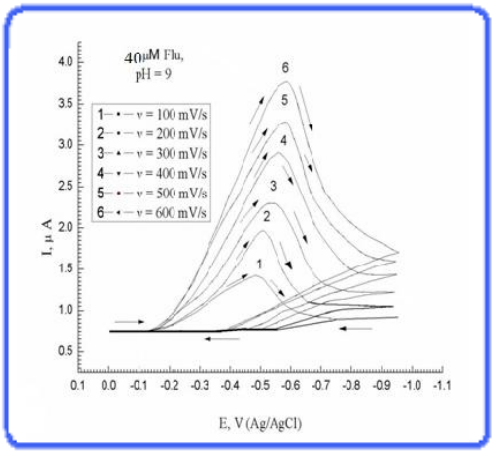


Figure-4: Cyclic Voltammograms, CVs, of 40 µM Flutamide at pH=9 and different scanning rates.

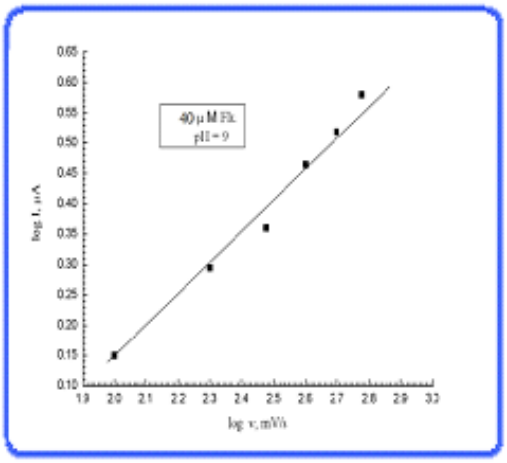


Figure-5: Variation of the cathodic peak current, Ip, and the logarithm of scanning rate

Investigation of the CVs of Figure-2 indicates also that over the studied pH range, the CVs are characterized by the only one cathodic reduction peak, which was previously reported in Figure-1, with no indicative anodic oxidation ones.

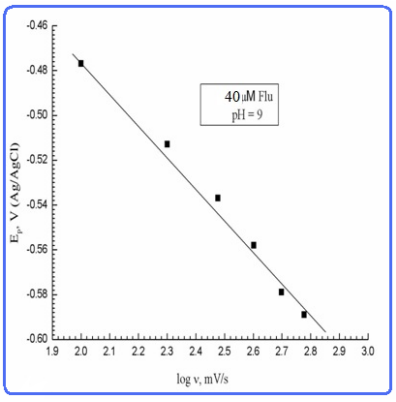


Figure-6: Variation of the cathodic peak potential, Ep, and the logarithm of scanning rate

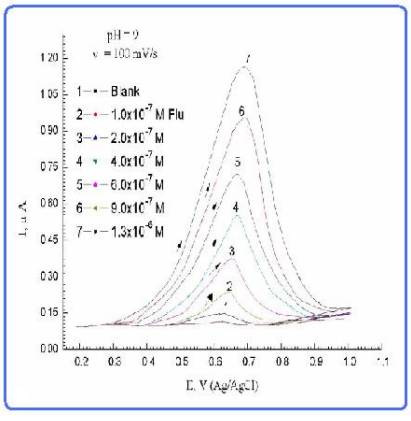


Figure-7: Cyclic Voltammograms, CVs, of different concentrations of Flutamide at pH=9 and 100 mV/s

This could be also attributed to the irreversible nature of the electrochemical reduction process of FLU [10].
Furthermore, it is quite clear that upon increasing the pH of the solution, the cathodic peak potential is shifted to the active (negative) direction. This phenomenon is an indication of a protonation step during the reduction process of FLU.

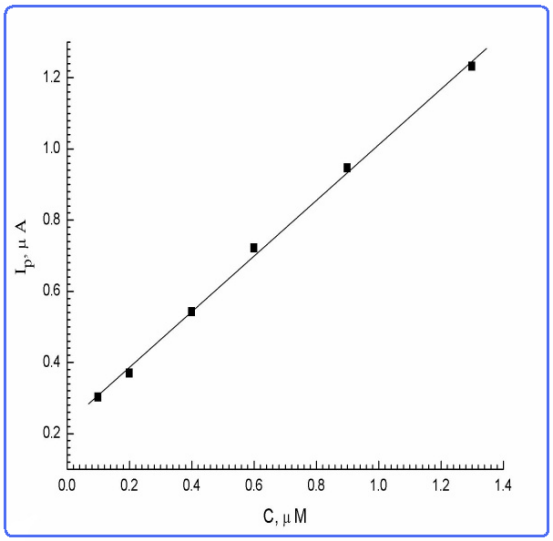


Figure-8: Variation of the cathodic peak current, Ip, and the concentration of Flutamide

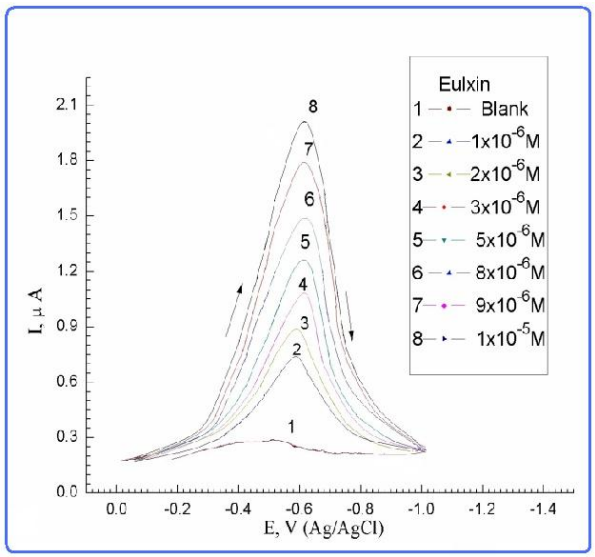


Figure-9: Cyclic Voltammograms, CVs, of different concentrations of the Flutamide Drug Eulxin at pH=9 and 100mV/s

At the same time, it is quite clear that the cathodic peak current, I_p, increased with the increase of the pH of the solution to reach a maximum value at pH=9, then started to decrease on further increase in pH. Consequently, a pH=9 was chosen to be the pH at which all other experimental results were obtained. The curves of Figure-3 shows the variation of the cathodic peak current, Ip, with the pH of solution which shows a maximum at pH=9.

Effect of voltage scanning rate

The curves of Figure-4 represent the cyclic voltammograms, CVs, of 40 µM FLU at the HMDE at pH=9 and 100mV/s scanning rate, in the potential range 0.0V to -1.0V versus the Ag/AgCl reference electrode, at different scanning rates (√), in mV/s.
As could be easily indicated from the CVs of Figure-4, as the scanning rate, √, was increased the cathodic peak current, I_p, increased while the cathodic peak potential, E_p, was shifted to more active (negative) values. This shift of peak potential in the negative direction could be attributed to the irreversibility of the electrochemical cathodic reduction process of FLU [11,12]. The dependence of the cathodic peak current, Ip, and cathodic peak potential, Ep, on the voltage scanning rate,√, could be seen in Figure-5 and 6, respectively. The

cathodic peaks current, I_p, and potential, E_p, varied with the scanning rate, √, according to:

$$\log I_p = a_1 + b_1 \log \sqrt{} \quad \text{----------} \quad (2)$$

while E_p varied with √ according to the relation:

$$E_p = a_2 - b_2 \log \sqrt{} \quad \text{----------} \quad (3)$$

where (a_1, a_2) and (b_1, b_2) are constants.

The slope, b_1, of Figure-5 amounts to about 0.81 which is near to the expected value for an ideal electrochemical reduction of a surface adsorbed species [13]. The slope, b_2, of the E_p /log √ relation, Figure-6, is found to be -0.019 indicating that the number of electrons involved in the electrochemical reduction of FLU (n = 1) (13). For the subsequent experimental results, a scanning rate of 100 mV/s was chosen.

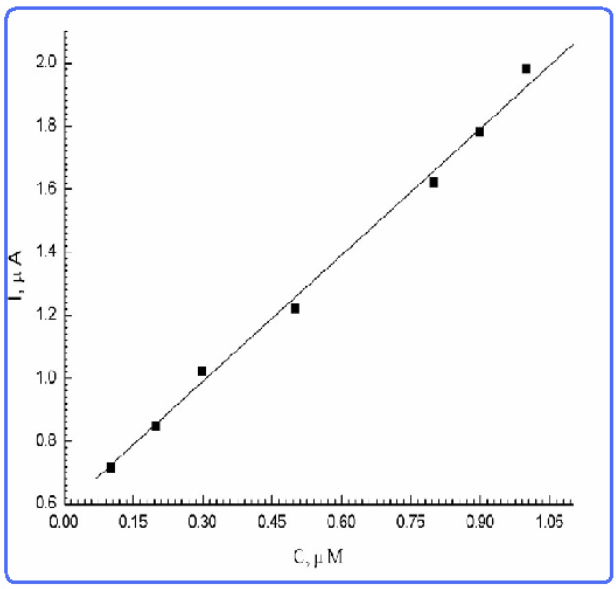


Figure-9: Variation of the cathodic peak current, Ip, and the concentration of the Flutamide Drug Eulxin

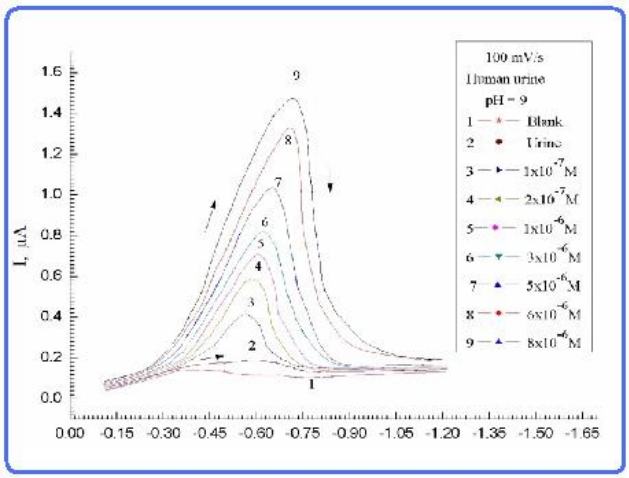


Figure-10: Cyclic Voltammograms, CVs, of different concentrations of Flutamide in Human Urine at pH=9 and 100mV/s

Effect of Flutamide concentration

The curves of Figure-7 represent the cyclic voltammograms, CVs, of increasing concentrations of FLU ($1x10^{-7}$ M to $1.3x10^{-6}$ M), at pH=9 and a scanning rate, √ , 100 mV/s in the potential range 0.0 to – 1.0 V versus the Ag/AgCl reference electrode.

Inspection of the CVs of Figure-7 reveals that as the concentration of FLU increased, the cathodic peak current, Ip, increased while the cathodic peak potential, Ep, was slightly shifted into the negative (active) direction. Figure-8 represents the calibration curve obtained, over the FLU concentration range $1x10^{-7}$ M to $1.3x10^{-6}$ M, when the cathodic peak current, I_p, in μA is plotted versus the concentration of FLU. The data for five replicated measurements were subjected to the least square refinement and the values of the regression coefficient, R, were computed and shown in Table-1.

The cathodic peak current, I_p, Figure-8 was found to vary with the FLU concentration, C, according to the relation:

$$I_p = a' + b' C \quad \text{---------- (4)}$$

where a' and b' are constants. The values of the limit of detection, LOD, and limit of quantitation, LOQ, as well as, the regression coefficient, R, were computed for the reduction of FLU and summarized also in Table-1.

Table-1: Analysis of Flutamide (FLU) in Bulk Form, Eulxin, Human Urine and Human Serum

	Lineriity	Molar concentraion	R	LOD	LOQ
Bulk form	$1x10^{-7}$	$1.3x10^{-6}$M	0.993	$1.78x10^{-7}$M	$1.02x10^{6}$M
Eluxin	$1x10^{-6}$	$1.0x10^{-5}$M	0.995	$8.46x10^{-6}$M	$2.8x10^{-6}$M
Human Urine	$1.0x10^{-7}$	$8.0x10^{-6}$M	0.997	$1.37x10^{-7}$ M	$4.53x10^{-6}$M
Human serum	$5x10^{-7}$	$3.25x10^{6}$M	0.991	6.5×10^{-7} M	$2.1x10^{-6}$ M

Mechanism of the electrochemical reduction of Flutamide

The effect of the reactant activity (concentration, C) and of the voltage scanning rate, √, on peak potential and peak current, under conditions of semi-infinite linear diffusion, is a diagnostic criterion frequently used for mechanistic determination [14]. Thus, for irreversible reactions, under the same conditions, E_p is independent of reactant concentration and varies with the voltage scanning rate, √, according to [15]

$$E_p = -1.14 (RT/ \alpha nF) + RT/ \alpha nF \ln (K^o/D^{1/2}) - RT/2\alpha nF) \ln (\surd) \quad \text{---- (5)}$$

where α is the transfer coefficient of the electrode reaction, n is the number of electrons involved in the reduction process, D is the diffusion coefficient (cm/sec^2) F is the Faraday constant (96500Coulmb/mole), R is the molar gas constant (8.314 Joule/deg,/mole). and K^o is a constant.

Assuming that the transfer coefficient, α, of FLU is 0.5, and from the slope of the straight line of Figure-6, the number of electrons involved in the electrochemical reduction of FLU is found to be one (n=1). Therefore, and according to Lueje et al [16], one can assume that the only cathodic peak reported is due to the electrochemical reduction of the nitro-aromatic moiety of the flutamide molecule. The presence of this irreversible one electron charge transfer reduction peak in basic media is well known for several nitro-aromatic compounds [17].

The validity of the technique of cyclic voltammetry for the quantitative determination (assay) of FLU is evaluated by the determination of the limits of detection, LOD, and the limit of quantitation, LOQ, which were calculated from the calibration curve of Figure-7, using the equation (17):

$$LOD = 3SD / b \quad \text{---------- (5)}$$

$$LOQ = 10\ SD/b \quad \text{---------- (6)}$$

where SD is the standard deviation of the intercept and b is the slope of the calibration curve. The values of LOD and LOQ were calculated and listed in Table-1 which confirm the sensitivity of the proposed electrochemical analytical method for the trace determination of FLU drug in clinical analysis (pharmaceutical formulation (Eluxin) and the physiological liquids (humane urine and serum).

Determination of Flutamide in pharmaceutical formulation

The technique of cyclic voltammetry was further applied for the determination of FLU in the pharmaceutical formulation Eluxin. Eluxin tablets were provided to contain 250 mg of FLU per tablet. Solutions of increasing concentrations of FLU were prepared by dissolving the appropriate weights of the powdered drug in methanol/tridistilled water,v/v. The curves of Figure-9 show the cyclic voltammograms, CVs, of Eluxin in the concentration range 1x10 $^{-6}$ M - 1x10^{-5} M at pH=9 and at a scanning rate of 100mV/s in the potential range 0.0 to -1.0 V versus the Ag/AgCl reference electrode.

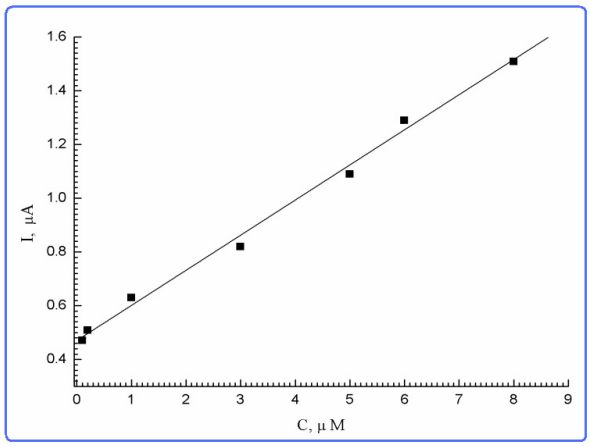


Figure-11: Variation of the cathodic peak current, Ip, and the concentration of Flutamide in Human Urine

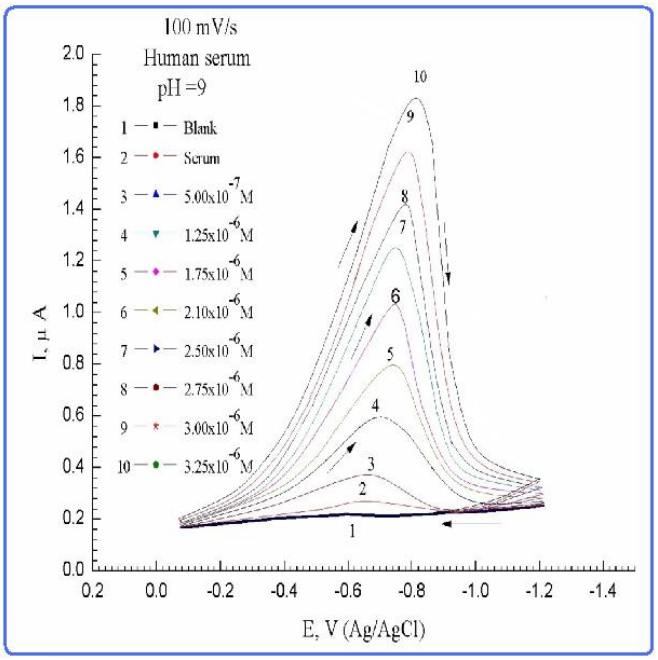


Figure-12: Cyclic Voltammograms, CVs, of different conmcentrations of Flutamide in Human Serum at pH=9 and 100mV/s

Inspection of the CVs of Figure-9 indicates that as the concentration of FLU in Eluxin drug increased , the cathodic peak current, I_p, increased while the cathodic peak potential remains more/or less unchanged. Plotting of the peak current, I_p, versus the concentration of FLU in Eluxin gave rise to the calibration straight line of Figure-10. The limit of detection, LOD, and limit of quantitation, LOQ, of FLU in the Eluxin drug, as well as, the regression coefficient, R, were calculated and shown in Table-1.

Determination of Flutamide in human urine

In this part of the work, the cyclic voltammetry technique was extended to be applied in the direct determination of FLU in human urine.The cyclic voltammograms, CVs, shown in Figure-10 represent the electrochemical reduction of increasing concentrations of FLU in human urine. As could be easily seen the cathodic peak current, Ip, increased markedly with increasing the concentration of FLU, in urine, while the cathodic peak potential remained more/or less unchanged. The cathodic peak current, Ip, varied with the concentration, C, of FLU according to the straight line relationship of Figure-11. The limit of detection, LOD, and limit of quantitation, LOQ , as well as, the regression coefficient, R, of FLU spiked in human urine were calculated and shown also in Table-1.

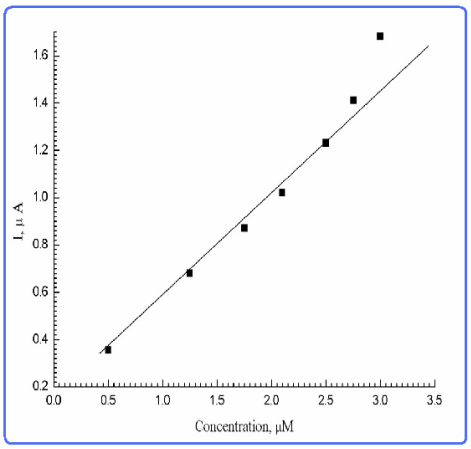


Figure-13: Variation of the cathodic peak current, Ip, and the concentration of Flutamide in Human Serum

Determination of Flutamide in human serum

Cyclic voltammetry was extended for the direct determination of FLU in human serum. The serum was only subjected to centrifugal separation of proteins prior to the determination of FLU. The curves of Figure-12 show the CVs of FLU in human serum in the concentration range 5x10 $^{-7}$ M to 3.25 x 10 $^{-6}$ M at pH=9 and a scanning rate of 100mV/s in the potential range 0.0 to -1.0 V versus the Ag/AgCl reference electrode. As could be indicated from the CVs of Figure-12, the cathodic reduction peak current, I_p, increased markedly with the increase of the concentration of FLU in human serum, while the cathodic peak potential, Ep, was slightly shifted in the negative direction. The current peak, I_p, varied also with the concentration of FLU, in human serum, to give the calibration straight line of Figure-13. The limit of detection, LOD, and limit of quantitation, LOQ, as well as the regression coefficient, R, of FLU in human serum were calculated and shown also in Table-1.

4.0 Conclusion

In the present work an attempt has been done to carryout the electrochemical characterization and determination of the Flutamide which is an anticancer drug by cyclic voltametry. Results from the present study indicate that the developed method is simple and accurate and can be employed for determination in various pharmaceutical formulations successfully.

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