Electrochemical behavior and differential pulse voltametric determination of an antineoplastic drug bicalutamide and pharmaceuticals formulations using glassy carbon electrode

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Abstract- The redox characteristics of the drug Bicalutamide at a glassy-carbon electrode (GCE) was investigated for the first time in aqueous media by differential-pulse voltammetry (DPV) and cyclic voltammetry (CV). In Britton–Robinson (BR) buffer of pH 2.0–10.0, an irreversible and diffusion-controlled reduction voltagram were developed. The dependence of the CV response of the developed cathodic peak on the sweep rate (v) and on depolarizer concentration was typical electrode-coupled chemical reaction mechanism (EC) in which an irreversible reaction is interposed between the charges. In BR buffer of pH 6 - 7, a well defined cathodic peak was developed and the plot of peak current height of the DPV against Bicalutamide concentration at this peak potential was linear in the range $1 \times 10^{-8} - 2.6 \times 10^{-5}$ M with lower limits of detection (LOD) and quantization (LOQ) of 8×10^{-8} M and 1×10^{-7} M, with good selectivity and sensitivity. A relative standard deviation (RSD) for Bicalutamide at a concentration of 4.5×10^{-5} M was 2.48% (n=6). The proposed method was successfully applied to Bicalutamide determination in pharmaceutical samples and results are very good correlations with previously reported methods.
Key words: Bicalutamide, Glassy carbon Electrode, Britton Robinson Buffer, Cyclic Voltammetry and Differential Pulse Voltammetry

1. INTORDUCTION

The chemical name of bicalutamide is (*RS*)-*N*-[4-cyano-3-(trifluoromethyl) phenyl]-3-[(4-fluorophenyl) sulfonyl]-2-hydroxy-2-methyl-propanamide, under the trade name of Casodex [1–3]. It belongs to a class of drugs called antiandrogens that includes flutamide (Eulexin) and nilutamide (Nilandron). Androgens (an example of which is testosterone) are hormones that are produced and released by the adrenal glands. Bicalutamide is thought to prevent the growth of prostate cancer by blocking the effects of androgens on the cancer cells. The FDA approved bicalutamide in 1995. Bicalutamide is available as 50 mg tablets. It binds preferentially to receptors located outside the central nervous system and causes little increase in testosterone levels with little agonist activity [4–6]. The pharmacokinetics of Bicalutamide was studied thoroughly [7, 8].
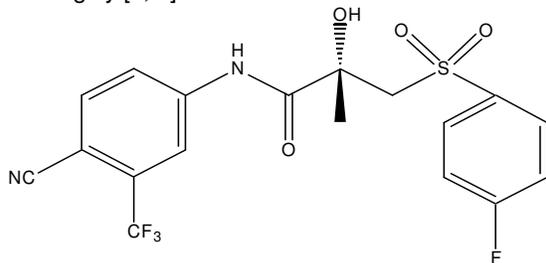
Structure of Bicalutamide

CASODEX is a racemate with its antiandrogenic activity being almost exclusively exhibited by the R-enantiomer of Bicalutamide; the S-enantiomer is essentially inactive. The inactive ingredients of CASODEX tablets are lactose, magnesium stearate, methyl hydroxypropyl cellulose, polyethylene glycol, polyvidone, sodium starch glycollate, and titanium dioxide. CASODEX is a non-steroidal antiandrogen. It competitively inhibits the action of androgens by binding to cytosol androgen receptors in the target tissue. Prostatic carcinoma is known to be androgen sensitive and responds to treatment that counteracts the effect of androgen and/or removes the source of androgen. When CASODEX is combined with luteinizing hormone-releasing hormone (LHRH) analogue therapy, the suppression of serum testosterone induced by the LHRH analogue is not affected. However, in clinical trials with CASODEX as a single agent for prostate cancer, rises in serum testosterone and estradiol have been noted [9,10]. Bicalutamide has a molecular weight of 430.37. The pKa' is approximately 12. Bicalutamide is a fine white to off-white powder which is practically insoluble in water at 37°C (5 mg per 1000 mL), slightly soluble in chloroform and absolute ethanol, sparingly soluble in methanol, and soluble in acetone and tetrahydrofuran. A thorough literature search has revealed that, there are only very limited analytical methods i.e. HPLC and Spectrophotometric methods has been reported for determination of Bicalutamide in Bulk, Pharmaceutical formulations and in human plasma [11-16]. A few chiral HPLC methods have been reported for the determination of enantiomers of BCT [17–21]. There is no electrochemical procedure for the assay of Bicalutamide reported so far [22]. However, these techniques required expensive apparatus and reagents as well as skilled operators. It is also a time consuming one

and tedious pre as well as post-column derivatization procedure is often required. For clinical investigation such as pharmacokinetic studies, development of sensitive and specific analytical techniques is highly required for the determination of Bicalutamide in biological samples. The clinical and pharmacodynamics of Bicalutamide in human have been recently reported. Electrochemical methods are found to be effective for the determination of several pharmaceutical compounds as they are faster, cheaper, easier and sensitive than spectrometric and HPCL methods. Moreover, they can be successfully employed for the analysis of material in complicated systems (tablets and biological fluids). There is no study done on the determination of Bicalutamide using electrochemical techniques. The present paper describes a systematic study of the voltametric behavior and quantification of Bicalutamide using Glassy Carbon electrode using Cyclic Voltammetry and differential pulse voltammetry. Among the electrochemical techniques presently used voltametric pulse techniques proposed by barker and Jenkins [23] has been found to be highly sensitive from the analysis of various electro active specious as they allow quantitative determinations of concentrations down to 10^{-7} to 10^{-9}M. The differential pulse voltammetry (DPV) is a pulse technique that offers the advantage of highly sensitivity and the resultant signals obtained in this technique are very sharp and well defined. The improvement in the sensitivity is achieved by a significant increase of ratio between Faradaic and capacitive current [24].

2. EXPERIMENTAL
2.1 Apparatus
The Voltammetry experiments were performed with CH Instrument, USA (model 1100A Series and 760C). A conventional single compartment three-Electrode System consisting of a Glassy carbon Electrode (3 mm Diameter) as working electrode, a Saturate Calomel electrode used as reference Electrode and a Platinum wire as the auxiliary electrode was used. All the reported potentials were referenced to the SCE electrode. In order to provide a reproducible active surface and to improve the sensitivity and resolution of the voltametric peaks, the Glassy Carbon Electrode was polished to as mirror finish with 0.3 micron alumina on smooth polishing cloth and then rinsed with DMF and Milli-Q- Water prior to each electrochemical measurement [25, 26]. The electrode cleaning requires less than 5 minutes. All the solutions examined were carried out at room temperature 25±2°C. The pH measurements were made on a Schott Gerate pH meter CG 804.

2.2 Reagents and Solutions
All the reagents were analytical grade. Solutions were prepared in Milli-Q-Water. Bicalutamide, Active Pharmaceutical ingredient were supplied by M/S.Kekule Pharma, Hyderabad on courtesy. A standard stock solution was prepared by dissolving an accurately weighed amount Bicalutamide Bulk drug in dimethylformamide. The solution was stored under refrigeration at less than 10°C. More dilute solutions of the drug were prepared daily in volumetric Flask by diluting the stock solution with buffer to get desired concentration. The Britton Robinson buffers were prepared by using 0.04 mol L^{-1} of Boric Acid, Ortho Phosphoric Acid, and Acetic acid and pH were adjusted by 0.2 mol L^{-1} NaOH for the use of supporting electrolytes [27]. Desired concentration of solution were prepared daily from stock solution.

3. RESULTS AND DISCUSSIONS.
3.1 Electrochemical Behavior of Bicalutamide
Preliminary studies on the electrochemical behavior of Bicalutamide in DMF with Tetra Butyl Ammonium per chlorate as supporting electrolyte at a Glassy Carbon electrode by use of Cyclic Voltammetry have shown that the drug has well defined Reduction and Oxidation peak developed in the potential range −2.0 V to +1.80V relative to SCE reference electrode. Also, the preliminary study carried out, on the pH of the electrolysis medium is one of the variables that often severely affect the shape of the voltammograms, the peak potential and peak current, the effect of pH was investigated in detail. A stream of oxygen-free nitrogen gas was passed into solution for 15 minutes before measuring the Volta metric study and all the measurements were carried out at 22±2°C [28]. **Fig-(1)** shows a Typical Cyclic Voltammogram of Bicalutamide shows two peaks one at Negative potential and another one at Positive potential, i.e. Cathodic peak with peak potential E = -1.2637 V and peak current i = -4.209x10^{-5} A and Anodic peak with peak potential E = +1.0422 V and peak current i = +2.179x10^{-5} A. This is clearly shows that Bicalutamide under go both Reduction and oxidation at the Glassy Carbon Electrode surface. Anodic peak may be due to oxidation of secondary amino group in the irreversible and diffusion controlled oxidation [29]. Cathodic peak may be due to reduction Nitrile group involves four protons and four-electron transfer reaction or reduction of Carbonyl group, which involves 2 electrons and 2 protons involves [30,31] or reduction Sulphonyl group, which involves 6 electron and 6 protons [32-38]. Further to study the electrochemical behavior of Bicalutamide, only reduction property is selected, CV is carried out with the potential sweep rate in the range of 25 to 300 mV s^{-1} and Potential ranges from -0.2V to -2.2V and well defined cathodic peak obtained in between -1.0555V to -1.2189 V. **Fig – (2)** shows Cyclic Votammogram of Bicalutamide 1x10^{-4}M in DMF and Tetra Butyl ammonium per

chlorate as supporting electrolyte. As the potential sweep rate increased, both peak potential and peak current increased. Also peak potential shifted to more negative value and no peak appeared in the reverse sweep, indicating irreversible nature of the reaction process. It is observed that in continuous scanning of CV of the drug significantly reduced the peak current and signal was hardly discernible from the base line, indicating passivation of the surface of the GCE electrode [39-41]. It must be noted that current changes [result of the analyte] at the voltammogram can be caused by various process. Those processes which take place at the electrode surface includes,
a. Oxidation and reduction of adsorbed analyte and
b. inhibition of oxidation and reduction of the electrode surface by adsorbed analyte.
Indeed, in order to the influence of the adsorbed analyte on the electrode surface, scan rate must be set at very high rates. However, during the scan, some of the adsorbed analyte molecules desorbed. Depending on the rate of those processes and scan rate, the amount of the desorption analyte molecule (during the scan) can be changed. The important point here is that part of the adsorbed analyte molecule still remaining on the electrode surface that can inhibit the reduction / oxidation process of the electrode surface. In this method, ΔQ is calculated based on the current changes [41-46]. The peak potential shifted to more negative value and the loss of anodic diffusion peak in reverse scan showed that the reaction was not reversible and diffusion controlled. Diffusion controlled nature of the electrode process is evidence from the linear plot of i_p vs $v^{1/2}$, Which is pass through the origin indicating the adsorption controlled [47,48]. The experimental consistency of $i_p / Cv^{1/2}$ with scan rate [v] has shown the electrode process to be free from any kinetic complications. In addition, the peak currents depend linearly on the corresponding square root of potential sweep rate with regression equation of y=3E-06x-4E-05, R=0.9898, **Graph-(1)**, indicating diffusion-Controlled process. From the linear dependency of the peak current on the square root of potential sweep rate, i_p can be calculated using the Randles-Sevicl equation for totally irreversible electron transfer process[47].

$$i_p = [2.99 \times 10^5] \, n \, \alpha^{0.5} A C^* D^{0.5} v^{0.5}$$

Where α - is the Electron transfer coefficient, n is the number of electron exchanged, A surface area of the working electrode, C^* and D are the bulk concentration and diffusion coefficient of the electro reactant species, respectively, and v is the potential sweep rate.

3.1.1 Effect of pH
The effect of pH on the electrochemical reduction behavior of Bicalutamide was investigated by cyclic voltametry of 1×10^{-4}M of Bicalutamide using Britton Robinson buffer at various pH ranging from 2 to 10 with the variation in potential sweep rate from 15 to 300 mV s^{-1}. It clearly shows the change in peak

potential and shifted to negative potential with respect to pH of the solution and change in potential sweep rate. **Fig –(3)** is Typical Cyclic voltammogram over lay of 1×10^{-4}M of Bicalutamide using Britton Robinson buffer at 2 to 10 with the potential sweep rate 100 mV s^{-1}. It clearly shows that, there is steep increase in peak potential at lower pH. It clearly shows lower pH favors the reduction of compound due to higher concentration of H^+ ions and peak potential decrease at higher pH. There is well-defined peak in the pH range 4 to 10, and the peaks are sharper between pH 4 to 8. Further more it is also possible to conclude that when an accumulation [adsorption] step is used before the potential scan, the adsorption occurring at -1.20 V increase the Bicalutamide concentration in the region of the solution adjacent to the electrode surface with respect to that in the bulk of the solution. Nevertheless, the difference between these concentrations decreases in the successive scan and, eventually, both concentrations coincide. In relation to the behavior observed in acidic medium, Bicalutamide is adsorbed on the electrode when its reduction begins and the resulting current has contribution from both species weakly adsorbed on the electrode surface and solution species diffusing to this surface. From these results, one conclude the Cathodic peak current obtained in the irreversible reduction of Bicalutamide at the Glassy carbon electrode either acidic or in alkaline medium has a contribution from both surface species adsorbed on the electrode and solution species diffusing to electrode surface.

3.2 Differential Pulse Voltammetry (DPV)
3.2.1 General DPV procedure
An accurate volume (10 mL) of the BR buffer at the required pH (2 – 10) was transferred to the electrochemical cell and the electrodes were immersed in test solutions through which a stream of nitrogen is purged for 15 min before recording the voltammograms. The scans were initiated in the Negative direction of the applied potential from +0 V to -2 V. After recording the voltammogram of the blank solution, an accurate volume (0.5–2.0 mL) of the drug solution was added. The Cathodic potential sweep was then recorded under different operating conditions of pH, sweep rate, and pulse amplitude. Before each measurement the GCE was polished manually with a paste of 0.5 mm alumina in distilled water on a smooth polishing cloth and gently dried with a tissue paper **[24,25]**. The effect of scan rate (v =10 – 300 mV/s) on the voltammograms was determined using the same solution. At each scan rate the initial conditions at the electrode surface were restored by polishing manually as already described.

3.2.2 Recommended procedures for the analysis of Bicalutamide in tablets
Preparation and Analysis of Pharmaceutical Samples

Ten Casodex tablets, each containing 50mg of Bicalutamide, crushed to fine power, an appropriate portion of the finally ground material was accurately weighed and dissolved in the minimum volume of DMF by sonication for 10 min. The solution of the drug was shaken for 20 min in a mechanical shaker to achieve complete dissolution of the active material, accurately transferred to 10 mL volumetric flask, and the solution was diluted to volume with DMF. An accurate volume of the clear supernatant liquor was then transferred to the electrochemical cell containing 10 mL of BR buffer, pH 6.4 to yield a final concentration of approximately 1.0×10^{-8} mol L^{-1} Bicalutamide. The DPV was then recorded under the optimum experimental conditions and the unknown amount of the drug in the test solution was then determined by the developed procedures using the standard curve. A typical Differential pulse Volta gram recorded in various concentration ranges from 1×10^{-4} to 1×10^{-8} are shown in **fig-(4)**.

Standard Addition Method
Alternatively, the standard (spiking) method was used. Known volumes (0.25–1.0 mL) of the test drug solution at pH 6.4 were transferred to the electrochemical cell, with the current height being measured before and after addition of the standard. The change in the current height was recorded and used to determine the drug.

3.3 Electrochemical Determination of Bicalutamide
Electrochemical behavior of Bicalutamide Preliminary studies on the electrochemical reduction of Bicalutamide in DMF with TBAP as supporting electrolyte at a GCE by use of DPV have shown that the drug has one well defined reduction wave in the potential range 0 to -2.0 V relative to the SCE reference electrode. pH of the electrolysis medium is one of the variables that often severely affects the shape of voltammograms, the peak potential, and the peak current, the effect of pH was investigated in detail. The DPV voltammograms of Bicalutamide 1×10^{-7} M (0.043μg ml^{-1}) at the GCE over a wide range of pH (2.0–10) had a well-defined reduction peak. The reduction peak observed is most probably attributed to proposed reduction mechanism. It is observed that the cathodic peak potential was shifted to negative values on increasing the solution pH and the plot of pH against peak potential was found to be linear. The data also revealed that the plot of pH against the cathodic peak current was maximum at pH 6 -7. Thus, in subsequent analytical determination of the drug solution pH was kept in the range 6 -7, with BR buffer as supporting electrolyte to optimize the pH for the determination of Bicalutamide. The cyclic voltammogram obtained for Bicalutamide $(1.0 \times 10^{-4}$ mol $L^{-1})$ at the GCE in BR at pH 6.4 had one well-defined Cathodic peak at -0.764 V. On reverse scanning no Anodic peak was observed at the scan rates investigated (25–300 mV s–1),

confirming the irreversible nature of the observed electrochemical reduction of Bicalutamide in the potential window investigated. The effect of sweep rate (25–300 mV/s) on $E_{p,c}$ and $i_{p,c}$ of Bicalutamide 1×10^{-7} M (0.043μg ml^{-1}) at pH 6.4 in cyclic voltammetry was critically investigated on freshly polished GCE. In this investigation the GCE was polished thoroughly before each sweep, because decrease in the peak current was observed if the electrode surface was not polished. The cathodic peak current was also increased steadily by raising the scan rate, suggesting the occurrence of slow chemical reactions and limited mass transfer after the electrochemical process [47,48] . Finally, the peak current function, $ip/v1/2$ decreased continuously on increasing the scan rate, indicating the Reduction process followed an electrode-coupled (EC) chemical reaction mechanism [47,48].

3.4 Validation of the Method
Validation of the method for the quantitative assay of Bicalutamide was examined via evaluation of linearity range, limit of detection (LOD), limit of quantification (LOQ), reproducibility, precision, accuracy, recovery, specificity, robustness, ruggedness, and stability.

3.4.1 Specificity & Selectivity
Commercial Bicalutamide tablets in dosage form are usually coated with excipients, e.g. lactose, Sodium starch gylcollate, povidone, Magnesium stearate, Titanium dioxide, Hypromellous. It was therefore essential to analyze the uncoated tablets by the proposed DPV method at a GCE at pH 6.4 to determine whether the coating materials would interfere with the analysis. Each excipient (0.25–0.75 g) was added according to the manufacturer's batch formula to known amounts of Bicalutamide 1×10^{-7} M (0.043μg ml^{-1}). The tolerable limit was defined as the concentration of the excipient causing a deviation of less than ±3% in the cathodic peak current of the solution containing the pure active ingredient. The recovery data indicated that the magnitude of the peak current of Bicalutamide did not deviate by more than ±3% from the peak current of the solution containing no interfering additives, confirming that the developed DPV method is free from serious interferences from the tested excipients. The developed DPV procedure can therefore be regarded as a selective method for determination of the analyte.

3.4.2 Analytical Application
The high adsorption and sensitivity of the developed Reduction peak (E_{pc} = -0.764 V) of Bicalutamide at the GCE at pH 6.4 suggests possible application of the developed DPV method for analysis of Bicalutamide in dosage forms. Under the optimum experimental conditions of pH 6.4, pulse amplitude 50 mV, pulse width 50 ms, and 20 mV/s scan rate, DPV of Bicalutamide showed that

the peak current increased linearly on increasing the drug concentration in the range $1 \times 10^{-7} - 2.6 \times 10^{-5}$ M (0.043µg ml^{-1} - 11.19 µg ml^{-1}) **Fig –(5)** and the regression equation $I = 64.051C - 79.6$ with $r = 0.9904$, Graph-2. A lower limit of detection (LOD) of 8×10^{-8} M (0.034µg ml^{-1}) was obtained by use of the formula LOD=3Sy/x/b **[49]** (where Sy/x is the standard deviation of y-residuals and b is the slope of the calibration plot). The LOQ was found to be 1×10^{-7} M (0.043µg ml^{-1}) and the relative standard deviation (RSD) for Bicalutamide at a concentration of 4.5×10^{-5} M (19.79µg ml^{-1}) was 2.51% (n=6).

3.4.3 Recovery test of Bicalutamide
The recovery tests of Bicalutamide ranging from 1.0×10^{-7} to 8.0×10^{-5} M were performed using DPV. The results are listed in **Table 1.** The recoveries lie in the range from 96.0% to 101.7%. The relative standard deviation was 2.07%.

3.4.4 Precision
The precision of a method is defined as the closeness of agreement between independent test results obtained under prescribed conditions. The precision around the mean value should not exceed 15% of the RSD **[50]**. The intra assay precision for Bicalutamide (calculated from repeated analyses during one working day) was ≤ 2.32% within the range 0.60–7.65 mg ml^{-1}. The inter-assay precision (calculated from repeated analyses on different days) was ≤ 3.09%.

3.4.5 Accuracy
The accuracy of a method is defined as the closeness of agreement between the test result and the accepted reference value. It is determined by calculating the percentage relative error between the measured mean concentrations and the added concentrations. The results obtained for intra- and inter-day accuracy were ≤ 0.48 and ≤ 4.5%, respectively.

3.4.6 Ruggedness
The effect of different analysts (two) on the results for 0.6 and 5.84 mg ml^{-1} standard samples was evaluated. The results showed good agreement, with RSD in the range 0.5–5.75%.

3.4.7 Robustness
The robustness of the method was examined by evaluating the influence of small variations of some of the most important procedure variables including pH and potential range. The results indicated that none of these variables significantly affect the recovery of Bicalutamide. This provided an indication of the reliability of the proposed procedure for the assay of Bicalutamide and the proposed method could be considered robust.

3.4.8 Stability
The stability of Bicalutamide stock solutions was tested by keeping them in the dark at 4℃ and analyzing them daily for a period of a week. Results showed that solution was stable for at least a week.

3.5 Analysis of Bicalutamide in Dosage form
The proposed DPV method was successfully applied to the determination of Bicalutamide in the tablet form. There was no need for any extraction step priors to the drug assay. The percentage recovery of Bicalutamide in Casodex tablets, based on the average of seven replicate measurements was found as 99.28+0.72% . The results were obtained in acceptable limits.

The proposed DPV procedure for analysis of Casodex tablets containing Bicalutamide (50 mg) was validated by direct determination and by standard addition procedures. Tablets were processed as described in the experimental section and analyzed under the optimum experimental conditions. Use of the direct DPV procedure for the quantification of Bicalutamide in five samples furnished results in good agreement with the label claim and with results obtained by HPLC and spectrophotometric methods at the 99 and 95% probability level **(Table -2)**. The average recovery **(Table - 1)** was 99.34%, in good agreement with the data obtained by HPLC & Spectrophotometric methods. At 99% probability there were no differences between the mean results and RSD values in the range 2.5– 3.20% were acceptable. Statistical evaluation involving t and F-tests revealed good agreement between the means and the variances from these methods **(Table-1).** The standard addition method was successfully used for analysis of the drug in tablets by the DPV method. Excellent correlation of 0.999 and recovery of 98.34±2.9% were achieved. These values are in good agreement with the label claim and the results obtained by HPLC and spectrophotometric methods **(Table - 2)**. The accuracy of the DPV method was evaluated by determination of the recovery of added Bicalutamide. On plotting the amount of drug added against the amount recovered, a regression line was obtained with a slope of 0.997±0.003% and a correlation coefficient of 0.999. The result from F-test at the 95% confidence level did not exceed the tabulated (theoretical) value, so there were no significant differences between the averages and the variances of the DPV method and previously reported HPLC **[51]** & spectrophotometric methods **[16]**.

4. CONCLUSION
Present investigation studied the electrochemical reduction behavior of Bicalutamide by varying pH using Britton Robinson buffer and Scan rate on Glassy Carbon electrode using Cyclic Voltammetry. This clearly confirms that Bicalutamide get reduced at the electrode surface with totally irreversible process and most probable overall mechanism for cathodic reduction was proposed. With the present investigation, a well defined DPV method enable simple, inexpensive, rapid, selective, and accurate

analysis of bicalutamide**.** The method compares favorably with reported HPLC and spectrophotometric methods. The method is therefore an excellent alternative means of analytical determination of Bicalutamide because of its low cost, repeatability and adequate precision. The sensitively and selectivity of the procedure could be improved by the pre concentration of the drug from large sample volumes under the optimum experimental condition. Moreover, because of the low LOD of the proposed procedure, it could be applied in clinical laboratories and pharmacokinetic studies.

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Table 1- Recovery test of Bicalutamide

Added in (M)	Found in (M)	Recovery (%)
1.00×10^{-7}	0.96×10^{-7}	96.0
2.00×10^{-7}	2.03×10^{-7}	101.5
2.00×10^{-6}	1.97×10^{-6}	98.5
4.00×10^{-6}	3.89×10^{-6}	97.3
6.00×10^{-6}	6.05×10^{-6}	100.8
3.00×10^{-5}	3.05×10^{-5}	101.7
6.00×10^{-5}	5.92×10^{-5}	98.7
8.00×10^{-5}	8.03×10^{-5}	100.4

Table 2- Results from determination of Bicalutamide (n=6) in dosage forms (Casodex tablets) by the proposed direct DPV procedure, HPLC [51] and spectrophotometric [16] procedure

	HPLC [51]	Spectrophotometric [16]	Present method
Label Claim (mg)	50	50	50
Amount Found	49.94	49.82	49.98
RSD (%)	-	2.87	2.48
Added	-	50%	4
Found	99.88	97.98%	3.98
Recovered (%)	99.64	98.27	99.5
% of recovery RSD	-	0.35	0.35
LOD	0.045 (µg / ml)	0.0116 (µg / ml)	8×10^{-8} M (0.034 µg / ml)
LOQ	0.656 (µg / ml)	0.0395 (µg / ml)	1×10^{-7} M (0.043 µg / ml)
Linearity	25 150 (µg / ml)	2.5 12.5 (µg / ml)	1×10^{-8} - 2.6×10^{-5} M (0.043 to 11.19 µg / ml)

Graph 1- Linear plot of Current Vs scan rate for of $1x10^{-4}$M Bicalutamide solutions in DMF and Tetra butyl ammonium per chlorate as supporting electrolyte
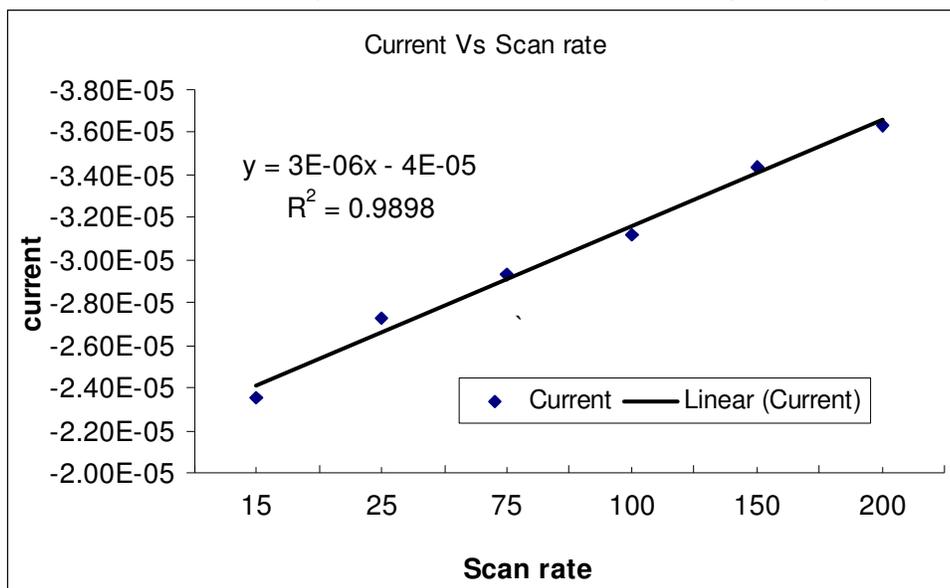
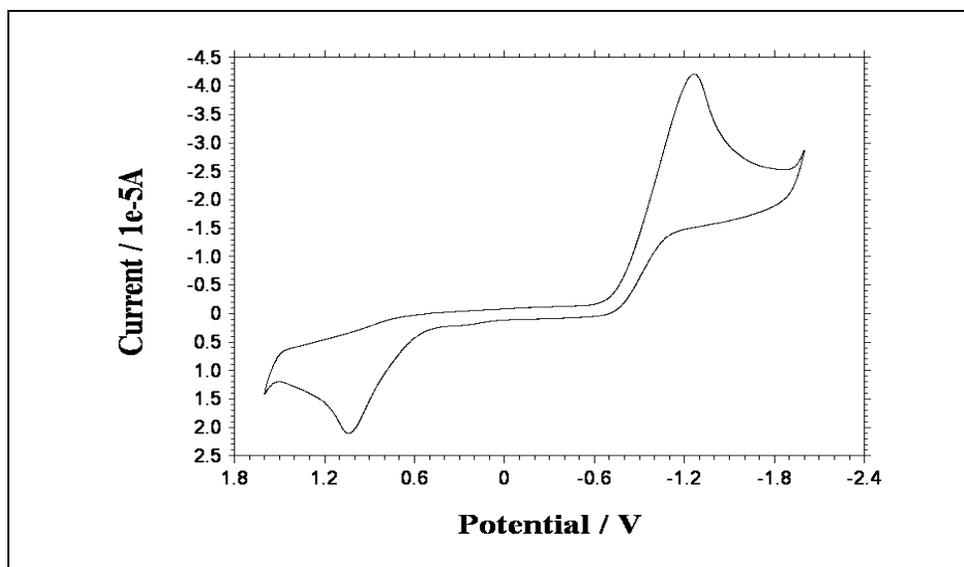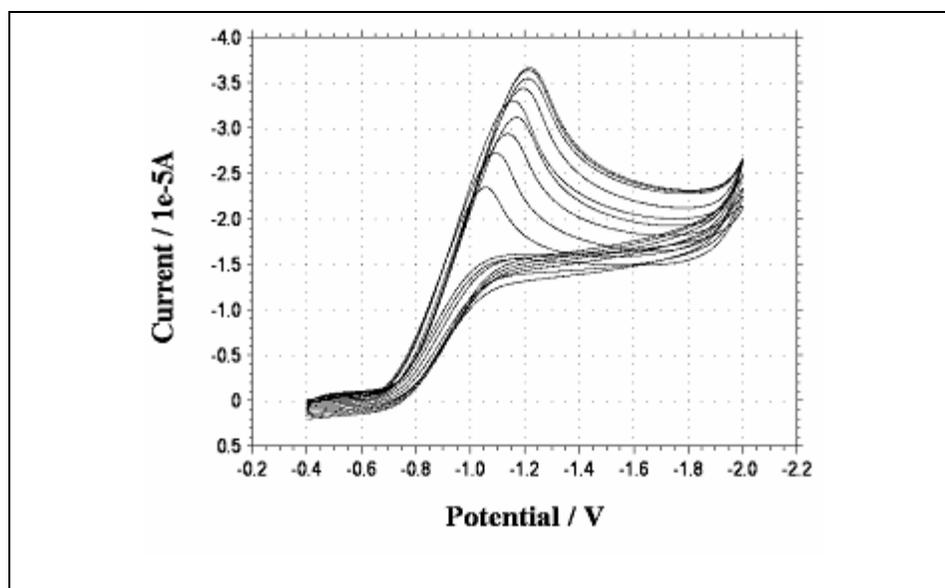Graph 2- Calibration curve for Bicalutamide obtained in DPV
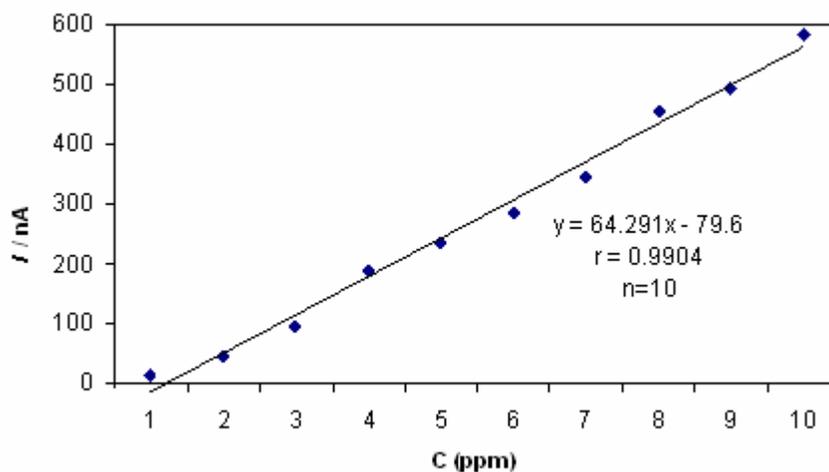
Fig. 1- Cyclic voltammogram of 1×10^{-4}M Bicalutamide solutions in DMF and Tetra butyl ammonium per chlorate as supporting electrolyte with scan rate of 100 mV/s

Fig. 2- Cyclic voltammogram of 1×10^{-4}M Bicalutamide solutions in DMF and Tetra butyl ammonium per chlorate as supporting electrolyte with scan rate from outer arc to inner arc a). 300 mV/s, b). 250 mV/s, c). 200 mV/s, d). 150 mV/s, e). 100 mV/s, f). 50 mV/s, f). 25 mV/s, g). 15 mV/s
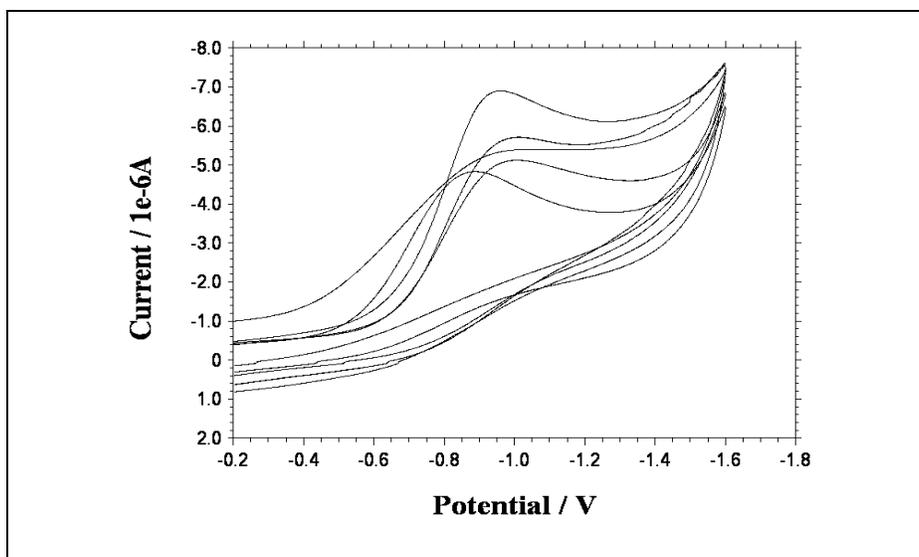
Fig. 3- Cyclic voltammogram of $1x10^{-4}$M Bicalutamide solution in Britton Robinson Buffer of various pH (10 to 2) with scan rate of 100 mV/s from outer arc to inner arc a).10, b). 8, c). 6 d). 4 & e). 2
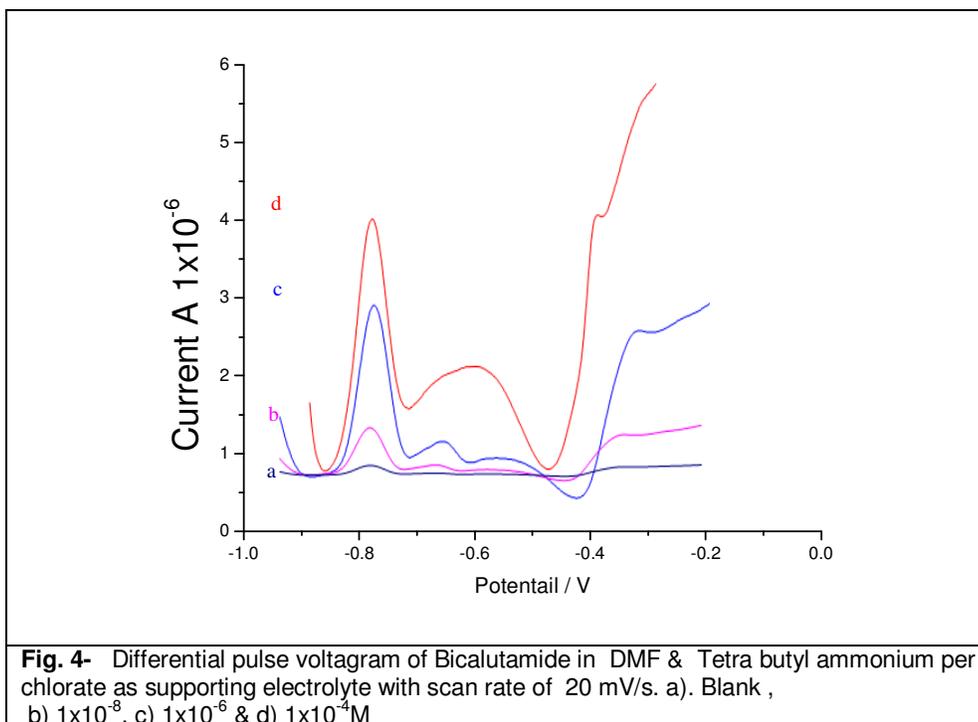

Fig. 4- Differential pulse voltagram of Bicalutamide in DMF & Tetra butyl ammonium per chlorate as supporting electrolyte with scan rate of 20 mV/s. a). Blank , b) $1x10^{-8}$, c) $1x10^{-6}$ & d) $1x10^{-4}$M

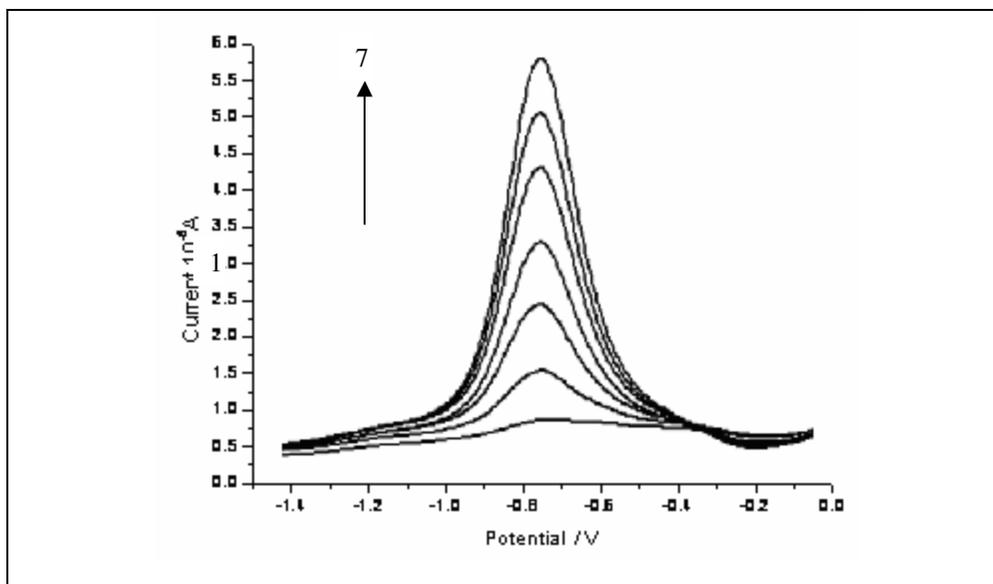**Fig. 5-** Effect of concentration on the peak current of   Bicalutamide using DPV. (a) Supporting electrolyte, at pH – 6.4. 1). Blank, 2).1×10^{-8} 3). 2×10^{-7} 4). 6×10^{-7} 5). 2×10^{-6} 6). 4×10^{-6}M & 7). 6). 6×10^{-5}M