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Original Article

Electrochemical Behaviour and Quantitative Determination of Clomifene in Pharmaceutical Formulations

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ABSTRACT

The electrochemical behaviour of clomifene was studied and determined in pharmaceutical formulations. The drug of clomifene was electrochemically examined by cyclic and differential pulse voltammetry using a glassy carbon electrode (GCE). The obtained results showed that, the Britton Robinson buffer with pH 4.0 was the best medium for reduction of clomifene on the glassy carbon electrode at the peak potential -1.01 V. The range of linearity was found to be from 0.20 µg mL^{-1} to 60.0 µg mL^{-1} (R^2 = 0.994) with limit of detection (LOD) 0.168 µg mL^{-1} and limit of quantification (LOQ) was 0.511 µg mL^{-1}. Differential pulse voltammetric method was successfully applied for the electrochemical determination of clomifene in pharmaceutical formulations

***Keywords*:** Clomifene, drug, voltammetry, pharmaceutical formulations.

1. INTRODUCTION

An alkenyl group containing molecules play a vital role in drug chemistry.[1] There are innumerable chemical reactions that involve or produce a carbon-carbon double bond group as an intermediate or as an end produce.[1] Consequently the discovery of the first non-steroidal antiestrogen, several studies have been conducted to determine the mechanism of action of this classes of compounds. However, despite expressive efforts in many laboratories, there exists no mechanism, which adequately could explain both the

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agonistic and antagonistic actions of the triphenyl ethylene compounds. Clomifene [2-(4-(2-chloro-1,2-diphenylethenyl)phenoxy)-N,N-diethyl-ethanamine ($C_{26}H_{28}ClNO$)], acts as a selective estrogen receptor modulator (SERM) inhibitor of estrogen binding to estrogen receptors, have mixed agonist and antagonist activity depending upon the target tissue.[2] It's generally used as a stimulant of ovarian function and it is listed as a doping substance on the World Anti-Doping Agency (WADA) prohibited list due to its selective estrogen receptor modulator capabilities.[3-5] Treatment of negative effects of anabolic androgenic steroid abuse (e.g., gynecomastia), as well as a negative feedback to testosterone metabolism causing an indirect enhancement of the serum testosterone concentration is the main reason for the ban of this substance.[6]

Excretion of several clomifene metabolites and clomifene itself has been described after in vivo and in vitro studies.[7-9] Clomifene citrate has been found very effective in the treatment of secondary male hypogonadism in many cases.[10-12] This has shown to be a much more attractive option than testosterone replacement therapy (TRT) in many cases because of the reduced cost and convenience of taking a pill as opposed to testosterone injections or gels.[13] Unlike traditional TRT also does not shrink the testes and as a result, can enhance fertility. Traditional TRT can render a man sterile (although with careful monitoring and low-dose hCG as an adjunct, this is both preventable and reversible for most men).

Since clomifene citrate has not been FDA approved for use in males it is prescribed off-label. According to Craig Niederberger, because this drug is now generic, no drug company would pursue FDA approval for use in men now because of a limited profit incentive, mostly due to the relatively small market potential. However, the single isomer of clomifene, enclomifene under the brand name Androxal, is currently under phase 2 trials for use in men.[14,15] Mechanism of action of clomifene has both estrogenic and anti-estrogenic properties, but its precise mechanism of action has not been determined. Isotope dilution mass spectrometry for micro- and trace-element determination of clomifene,[16] determination by liquid chromatography–mass spectrometry of clomiphene isomers in the plasma of patients undergoing treatment for the induction of ovulation.[17-22] Direct current polarography and cyclic voltammetry were used to study tamoxiphene (TX), clomiphene (CM) and chlorotrianisene (ChT). The dependences of the limiting currents and half-wave potentials on the pH of the solution, temperature, mercury head, ionic strength and surface tension of the solution were studied.[23] Here, a modest and rapid an electrochemical method has been established for the investigation of clomifene. The proposed method describes the investigations on the electrochemical behaviour, mechanism of clomifene at glassy carbon electrode (GCE) using and development of a differential pulse voltammetric method for its determination in pharmaceutical formulations.

2. EXPERIMENTAL

2.1 Apparatus and reagents

Electrochemical measurements were carried out in Auto lab, three electrode systems consisting of glassy carbon electrode as a working electrode, Ag/AgCl, Cl^- (3.0 M KCl) was used as a reference electrode and a platinum wire as an auxiliary electrode. All the solutions were degassed prior to analysis by bubbling purified nitrogen gas through the cell for 10 min. All the experiments were performed at 25±1 ^oC, pH measurements were carried out with a Hanna instruments (Italy) pH meter.

Clomifene (99%) and pharmaceutical formulations were purchased from Micro Labs Ltd. Acetonitrile, H_3PO_4 (≥85%), KH_2PO_4, and K_2HPO_4 were acquired from Sigma-Aldrich. CH_3COOH, CH_3COONa, H_2SO_4, CH_3OH, CH_3CH_2OH, HCl procured from S.D fine chemicals, and all other chemicals and reagents were of analytical reagent grade and used as received. The solutions and subsequent dilutions were prepared with deionised water. Clomifene pills were accurately weighed and finely powdered. The powder equivalent to 100 mg of clomifene was accurately weighed and transferred to a volumetric flask of 100 ml containing 50 ml of the acetonitrile and sonicated for 1 hr. This solution was carefully filtered through Whatmann filter paper (No. 1) and the final solution was made up with acetonitrile to get the 1000 ppm of stock solution.

2.2 Electrochemical procedure

The electrochemical behavior of clomifene was investigated by cyclic, differential pulse voltammetry performed in the potential window of -0.0 to -1.8 V versus Ag/AgCl, Cl^-. The differential pulse measurement of clomifene was carried out by AdSV. The accumulation at the open circuit of clomifene (5.0 µg/ml) at the working electrode immersed in 10 mL of BR buffer solution (0.05 mol/L, pH 4.0) containing clomifene and kept under stirring for detection of accumulated clomifene. An accumulation time of 3 min at the open circuit was used for quantitative measurements. The scan parameters included a differential potential frequency of 20 Hz, a scan rate of 50 mV/s, and a step potential of 10 mV. Consequently, for each measurement the electrode was cleaned by stirring in distilled water until obtaining a plane voltammogram. All experiments were performed at room temperature.

3. RESULTS AND DISCUSSION

3.1 Characterization of peaks

Well-defined cathodic peak was observed for clomifene (5.0 µg/ml) in the supporting electrolyte Britton-Robinson buffer systems studied over the pH range 2.0-12.0. This peak is attributed to the reduction of carbon-carbon double bond in two electron process to its corresponding saturated product. According to the cyclic voltammograms, we observed that the solutions of greater alkalinity, the reduction are not well facilitated owing to the less availability of protons. When the

potential was scanned from -0.0 to -1.80 V by cyclic voltammetry, clomifene showed a well-defined peak at -1.01 V on GCE (Fig.1). Considering the peak in order to establish the optimum conditions, the experimental parameters are evaluated after the signal. Corresponding peaks are not observed in anodic scan indicating an irreversible reduction process. Subsequent scans exhibit a dramatic decrease of peak current to a stable value representing the response of the solution species. This is obvious indication that clomifene has a differential characteristic at the glassy carbon electrode. Such anodic signal is attributed to the cyclisation reaction to form the corresponding derivative.

The reduction process in clomifene is diffusion controlled adsorption process on electrode surface was observed by the linear plot i_p vs. $v^{1/2}$. Value of E_p is found to increase with concentration of the clomifene to more negative potentials. This phenomenon has been observed in the reduction of many organic compounds and is the characteristic of the irreversible process. An increase in the percentage of clomifene in the voltammetric test is seen to shift the peak potentials towards more negative values with a simultaneous decrease in differential pulse current.

3.2 Differential pulse voltammetric studies

Electrolysis of the electroactive substance has been carried out from -0.0 V to -1.8 V vs. Ag/AgCl, Cl^- and the product formed after the controlled potential electrolysis are identified and confirmed as the saturated product of the corresponding electroactive species. Typical voltammograms are shown in Fig. 2. The determination of a number of electrons (n) involved during the electrode process in clomifene has been carried out by millicoulometry. According to this technique, 'n' is found to be two for clomifene in acidic as well as neutral media. Controlled potential electrolysis is employed to get the reduction product. It has been carried out in a modified cell with a three electrode system. The obtained product has been studied by FTIR spectroscopy. The disappearance of IR peak at 1675 cm^{-1} indicates that the double bond in the clomifene has undergone reduction in the final product. As per the experimental results, a possible electrochemical reduction mechanism has been suggested on the basis of protons and electrons involved in the reduction as shown in Fig. 3.

3.3 Optimum Parameters

The influence of pH on the differential pulse voltammetric response for 5.0 µg mL^{-1} clomifene was examined in Britton Robinson buffer of pH 2.0 to 12.0 after pre-concentration of the clomifene onto the GCE for 180 s. A single irreversible peak was generated in solution of pH 4.0. It can be observed from Fig. 4, when the pH was increased above 4.0, it shifts to more negative potentials.

The effect of accumulation potential on AdSV current of the peak of clomifene has been investigated after pre-concentration of the drug onto the GCE for 180 s. over the potential range 0.0 to -1.18 V. A much more peak developed for peak current was achieved at potential range -1.0 V. Therefore, a pre-concentration potential of -1.0 V was chosen over the rest of the study. The scan rate has been varied from 20 to 200 mVs^{-1}, the peak current increased linearly with the square root of scan rate indicating diffusion controlled reduction reaction. However, the variation of peak current with accumulation time was studied for a concentration of 5.0 µg mL^{-1} clomifene solution. As the pre-concentration time increased the peak current increases slowly and reaches a maximum value at 180 s. With further increase in accumulation time, no effect on the peak signal was observed indicating that the electrode surface was saturated with the analyte molecules.

3.4 Application

A stock solution was prepared by dissolution of the appropriate amount of the electroactive species in acetonitrile. 1.0 mL of standard solution is transferred into a voltammetric cell and diluted with 9.0 mL of supporting electrolyte and deoxygenated with N_2 gas for 10 min. After the voltammogram is a recorded, small increments (0, 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 µg mL^{-1}) of standard solution are added and then voltammograms are recorded after each addition under similar condition. In the present study, the best precision is obtained at pH 4.0 with rest time of 2 sec, pulse amplitude of 25 mV and an applied potential -1.0 V. Under these conditions the current was linear function with concentration of electroactive species in the range of 0.2 to 60.0 µg mL^{-1} (R^2 = 0.994) with limit of detection (LOD) 0.168 µg mL^{-1} and limit of quantification (LOQ) was 0.511 µg mL^{-1} respectively.

The described procedure has been successfully employed for the determination of clomifene in tablet forms. In the present study, the pharmaceutical formulation of namely clomifene is employed for assay results. The required quantity of pharmaceutical formulation is prepared corresponding to the known amount is accurately measured and transferred to a volumetric flask and the amount of pharmaceutical formations was determined as described in the experimental section. Assay results for clomifene in formulations are given in Table 1. The recoveries of clomifene in pharmaceutical formulations ranging from 96.00% to 99.80% are obtained.

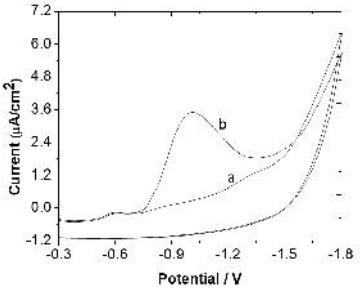

Fig 1: Cyclic voltammograms clomifene at GCE (a) blank (b) Concentration: 5.0 µg/mL using GCE at pH 4.0; frequency: 20 Hz, scan rate of 50 mV/s.

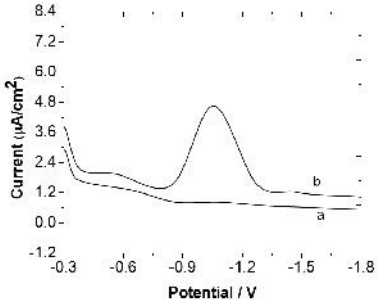

Fig 2: Differential pulse voltammogram of clomifene a) blank and b) 5.0 µg mL^{-1} at GCE at pH 4.0; frequency: 20 Hz, scan rate of 50 mV/s.

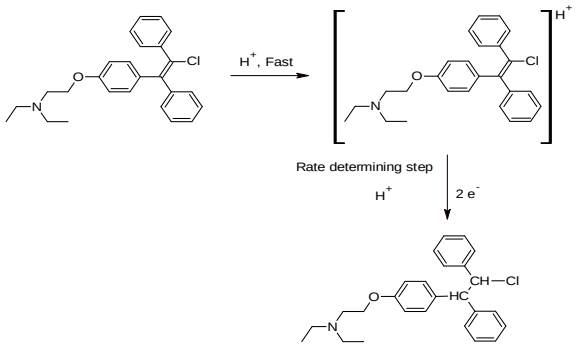

Fig 3: Electrochemical mechanism of clomifene at pH 4.0; Concentration: 5.0 µg mL^{-1}; frequency: 20 Hz, scan rate of 50 mV/s.

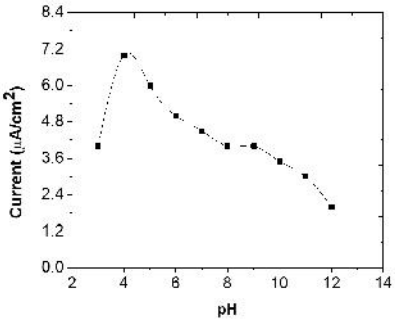

Fig 4: Effect of pH on the peak current of clomifene of conc. 5.0 µg mL^{-1} at pH 4.0; frequency: 20 Hz, scan rate of 50 mV/s.

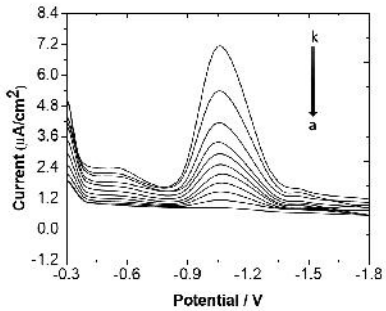

Fig 5: Differential pulse voltammograms of clomifene at GCE obtained on various concentration from (A) to (k): 0, 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 µg mL^{-1} at pH 4.0; frequency: 20 Hz, scan rate of 50 mV/s.

Table 1: Voltammetric assay of clomifene pharmaceutical formulations at pH 4.0; frequency: 20 Hz, scan rate of 50 mV/s

Formulation	Amount taken (µg mL^{-1})	Amount found* (µg mL^{-1})	% Recovery	% RSD
Siphene	10	9.70	97.00	1.02
	25	24.90	99.60	0.60
	50	49.70	99.40	0.80
Clomid	10	9.60	96.00	0.46
	25	24.90	99.60	1.12
	50	48.90	97.80	0.10

*** No. of determinations = 6**

4. CONCLUSION

In this article, the electrochemical behaviour of clomifene at glassy carbon electrode was investigated in BR buffer by cyclic and differential pulse voltammetric techniques. A fully validated, modest, sensitive and rapid differential pulse voltammetry and cyclic voltammetric methods were established for the determination of clomifene in pharmaceutical formulations. The selected method could be recommended for use in trace analysis, quality control and clinical laboratories.

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