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Research Article

ELECTROCHEMICAL OXIDATION AND DETERMINATION OF AN ANTIgCANCER DRUG PEMETREXED DISODIUM

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ABSTRACT

Objective: This study was undertaken to propose electro-oxidation mechanism and to develop a selective and sensitive method for the determination of an anti-cancer drug, pemetrexed disodium (PTD).

Methods: The electrochemical oxidation of anti-cancer drug PTD has been investigated at glassy carbon electrode using voltammetric techniques. The dependence of current on potential, pH, concentration, scan rate, and excipients were investigated to optimize the experimental conditions.

Results: According to the liner relation between peak potential, peak current, scan rate and PTD concentration, differential pulse voltammetric method for the quantitative determination in phosphate buffer solution was developed. The linear response was obtained in the range of 10 μM to 0.75 μM with a detection limit of 0.19 μM. The electrochemical oxidation of mechanism of anti-cancer drug PTD was proposed.

Conclusion: The proposed method is rapid and does not include any time-consuming steps. The simplicity, sensitivity, and low cost of analysis are the main features of the proposed method for the determination of PTD.

Keywords: Pemetrexed disodium, Cyclic voltammetry, Electrochemical studies, Glassy carbon electrode.

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INTRODUCTION

Pemetrexed disodium (PTD), an anticancer drug, is a folate anti-metabolite that primarily inhibits thymidylate synthase (TS) [1]. Pemetrexed is used for the treatment of patients with lung cancer after prior chemotherapy. Pemetrexed shows activity against a variety of solid tumor in clinical trials, that is, non-small cell lung [2,3] and breast cancers [4,5]. It also inhibits both dihydro folatereductase and glycinamide ribonucleotide formyl transferase (GARFT) [6]. Mechanisms of action such as 5-fluorouracil and raltitrexed, pemetrexed primarily inhibits TS resulting in decreased thymidine available for DNA synthesis. Pemetrexed also inhibits dihydrofolatereductase and GARFT, which are key enzymes required for the *de novo* bio-synthesis of thymidine and purine nucleotides [7-9]. Once pemetrexed gains entry to the cell, through the reduced folate carrier, it is polyglutamated. Glutamation increases cellular retention and the intracellular half-life of pemetrexed, as well as making the polyglutamated metabolites greater than 60-fold more potent in their inhibition of transferase. Pemetrexed is a radiation-sensitizing agent [10]. Pemetrexed induces cell cycle arrest in the G1/S Phase 1.

In February 2004, PTD was approved by the Food and Drug Administration (FDA) for use in combination with cisplatin in the treatment of mesothelioma. (US FDA News Online, February 5, 2004). On September 26, 2008, FDA approved PTD for injection for use in combination with cisplatin therapy for the initial treatment of patients with locally advanced or metastatic non-squamous non-small cell lung cancer (NSCLC) and on July 2, 2009 the FDA approved PTD injection (Alimta, made by Eli Lilly and Company) for maintenance treatment of patients with locally advancedlocally advanced or metastaticmetastatic non-squamous non-small cell lung cancerNSCLC whose disease has not progressed after four cycles of platinum-based first-line chemotherapy.

In preclinical studies, PTD showed activity against a wide range of tumor types including lung carcinoma, mesothelioma and breast, colon, and bladder carcinomas [7,11-14]. A few analytical methods, high-performance liquid chromatography (HPLC) [15], reversed phase-HPLC [16], and LC [17] have been reported for the determination of PTD. Besides, a few spectrophotometric methods were also reported for its determination in drug samples [18].

Investigation of the redox behavior of biologically occurring compounds by means of electrochemical techniques have the potential for providing valuable insights into the biological redox reaction of these molecules. Due to their high sensitivity, voltammetric methods have been successfully used to the redox behavior of various biological compounds [19-23]. Since the development of modern computer based electrochemical instrumentation, electroanalytical techniques, especially modern pulse technique, such as differential pulse voltammetry (DPV) have been used for the sensitive determination of a wide range of pharmaceuticals. The use of carbon based electrodes for electroanalysis has gained popularity in recent years because of their applicability to the determination of substances that undergo oxidation reaction [24,25].

The purpose of this study is to investigate the electro-oxidation mechanism and the determination of an anticancer drug, PTD using voltammetric techniques. Determination of PTD in real samples without any time-consuming extraction or evaporation steps prior to PTD assay. The GCE has been widely used in electro analysis for various substrates for a long time because of its stability, wide potential window, and fast electron transfer rate. The influences of some interfering species will also be investigated. In addition, an electrochemical behavior of PTD is investigated with cyclic voltammetry and DPV.

METHODS

Apparatus

A stock solution of PTD (5×10^{-4} M) was prepared in milli-pore water and stored in a refrigerator at 4°C. In this study, phosphate buffer

(pH = 3-10) was used. All the solutions were prepared in milli-pore water and all other chemicals used were of analytical reagent grade [26].

Instrumentation

The voltammetric experiments were performed with instruments, USA (model CHI1112C Version 9.03). A three electrode system including a glassy carbon electrode (3 mm diameter) as the working electrode, an Ag/AgCl (3M KCl) reference electrode and a platinum wire as the auxiliary electrode was used. To provide a reproducible active surface and to improve the sensitivity and resolution of the voltammetric peaks, the glassy carbon electrode was polished to a mirror finish with 0.3 micron alumina on a smooth polishing cloth and then rinsed with milli-pore water before each electrochemical measurement. The cleaning procedure of the electrode required less than 3 minutes. The solutions were purged with nitrogen gas. All measurements were carried out at room temperature 25°C. DPV conditions maintained were: Pulse amplitude 50 mV; pulse width 60 ms and scan rate 20 mV/s.

The area of the electrode was calculated using 1.0 mM $K_3(Fe[CN]_6)$ as a probe at different scan rates [27]. For a reversible process, the Randles - Sevcik formula has been used [28-31].

$$I_p=(2.69\times10^5)n^{3/2}AD_0^{1/2}V^{1/2}C_0^* \quad (8)$$

Where, n = number of electrons transferred i.e., 1, A = surface area of the electrode, D_0 = diffusion coefficient, ν = sweep rate (0.1/Vs.) and C_0^* = concentration of electro active species (1 mM). The surface area of the electrode was found to be 0.04 cm^2.

Analytical procedure

For good reproducible results, improved sensitivity and resolution of voltammetric peaks, the working electrode was polished carefully with 1 µm, 0.3 µm, 0.05 µm α-alumina on smooth polishing cloth and then washed in a milli-pore water. The 3 electrode system consisting of a glassy carbon electrode (3 mm diameter) as the working electrode, an Ag/AgCl (3 M KCl) reference electrode and a platinum wire as the auxiliary (counter) electrode was used. Electrolyte solutions were prepared by diluting the stock solution as required with relevant buffer of required pH. For DPV studies, the following parameters were maintained: Sweep rate-20 mV/s, pulse amplitude-50 mV, pulse width-60 ms, pulse period-500 ms for analytical applications. All experiments were carried out at 25±1°C [32-35].

RESULT AND DISCUSSION

Voltammetric behavior of PTD

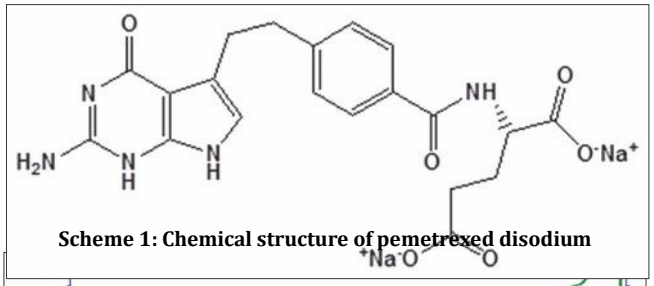
We have carried out the electrochemical oxidation of PTD in different buffers solutions. Acetate and phosphate were used in this study. Since, phosphate buffer gave a good peak response (peak shape and peak current), it was selected for further studies. For this, we prepared phosphate buffers of different pH (2.68, 4.2, 4.43, 5.43, 6.5, 7.4, 8.07, 9.27, and 10.4) [28]. The phosphate buffer solution of pH 2.68 (Fig. 2) offered improved sensitivity.

Effect of pH

PTD exhibited oxidation peaks at 0.642V (a_1) and 1.454V (a_2) in phosphate buffer of pH 7.4 (Fig. 1). The pH of the electrolyte solutions also affected the PTD oxidation peak potential. With increase in pH (from 3 to 10), a rapid shift in peak potential toward more negative side was observed. This indicated that the reduction would occur with difficulty. With increase in pH of the supporting electrolyte, the oxidation peak became weaker (Fig. 2). The plot of I_p of PTD versus pH showed maximum peak current at pH 2.68 with a scan rate of 100 mv/s (Fig. 3a). The results indicated the participation of electrons in the electrode process. Further, the shift in peak potential with increase in pH indicated that the pH of supporting electrolyte exerted a significant effect on electro-oxidation of PTD at glassy carbon electrode. A good linear relationship between E_{pa} and pH of the medium at glassy carbon electrode was noticed and the same is shown in Fig. 3b.

Effect of scan rate

The effect of the potential scan rate (between 2.5 and 50 mV/s) on the peak current was evaluated. Scan rate studies were carried out to assess whether the process at the glassy carbon electrode was under diffusion or adsorption controlled. Cyclic voltammograms of 5×10^{-4} M PTD at different scan rates were recorded and are shown in Fig. 5. It was observed that when the scan rate was varied from 2.5 to 50 mV/s, a linear relationship dependence of the peak current I (µA) on the square root of the scan rate, $V^{1/2}$ mV/s (Fig. 6). The slope 0.0041 mV/s is close to theoretically expected value 0.005 mV/S with a correlation co-efficient of 0.9938 demonstrating that, the electrode process was diffusion controlled [36].



Scheme 1: Chemical structure of pemetrexed disodium

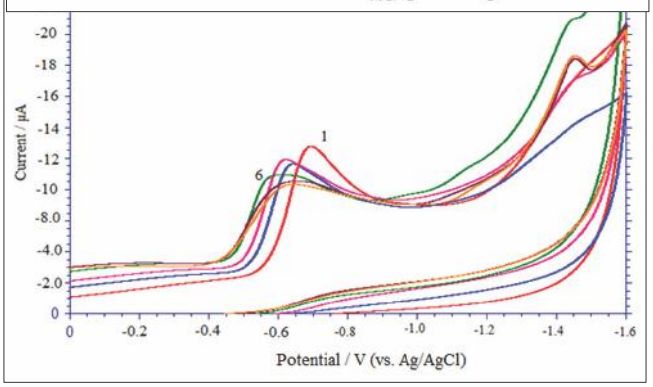


Fig. 1: Cyclic voltammograms of 5×10^{-4} M pemetrexed disodium on glassy carbon electrode phosphate buffer (pH 7.4) at a scan rate of 100 mVs^{-1}

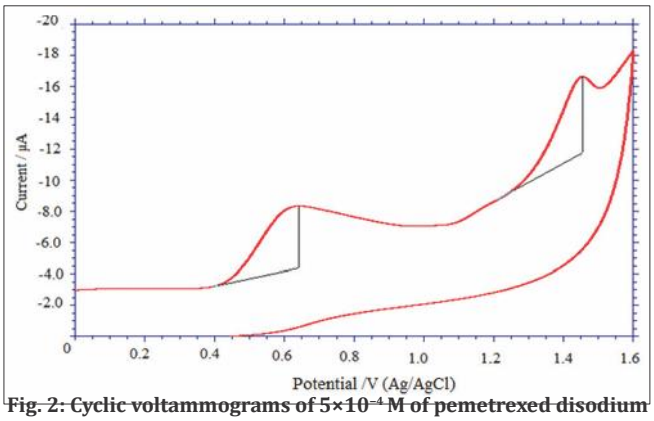


Fig. 2: Cyclic voltammograms of 5×10^{-4} M of pemetrexed disodium in phosphate buffer of pH (1) 2.68, (2) 4.2, (3) 4.43, (4) 5.43, (5) 6.5 and (6) 7.4 at a scan rate of 100 mV/s

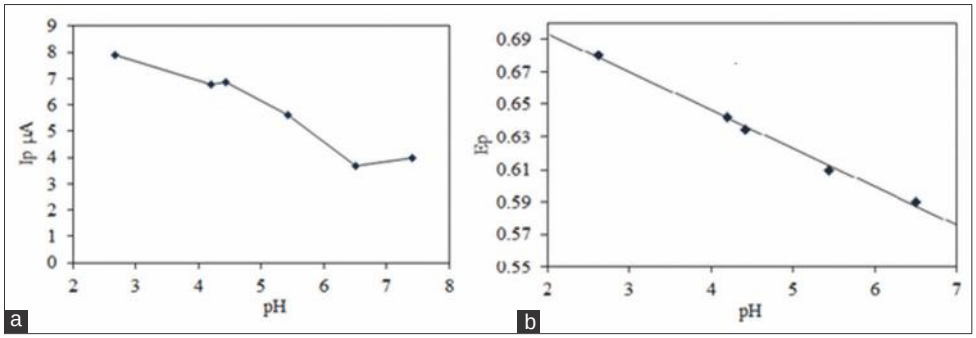


Fig. 3: (a) Influence of pH on the peak current I_p/µA of pemetrexed disodium, (b) Variation of peak potential E_p/V of pemetrexed disodium with pH

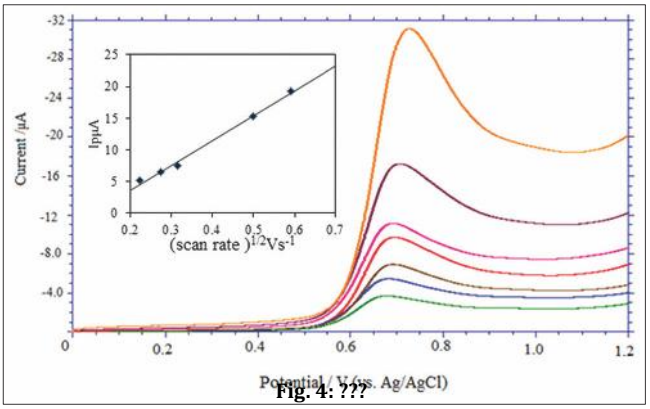


Fig. 4: ???

Fig. 5: Cyclic voltammograms of 5×10⁻⁴ M pemetrexed disodium in phosphate buffer of pH 2.6 at different scan rates: (1) 2.5, (2) 5.0, (3) 7.5, (4) 10, (5) 15, (6) 25, and (7) 50 mV/s (A) Dependence of Peak Current I_p/µA on the scan rate υ/Vs

The corresponding equation is

I_{pa} (µA) = 0.0051$v^{1/2}$ $(mV/s)^{1/2}$-0.0041

Further, the linear relationship between square root of scan rate and peak current also indicated irreversible nature of electrode processes (Fig. 6).

Electro-oxidation mechanism

PTD showed two well resolved anodic signals in a limited range of pH studied. In acid media, the oxidation of PTD at GCE follows a proton-dependent mechanism while in alkaline media protons were not involved in the rate determining step or before. In the acid media, an increase of the peak current with the increase of pH was observed. On the other hand, in the basic media decrease in the peak current with the increase of pH was observed. By the calculation, we found that the oxidation mechanism involves two proton- two electrons at GCE. Based on all these observations, we postulated the mechanism as shown in Scheme 2.

Calibration curve

Limit of detection (LOD) and limit of quantification (LOQ)

Validation of the optimized procedure for the quantitative assay of PTD was examined through evaluation of LOD, LOQ, accuracy, precision, and recovery (Fig. 7). Values of LOD and LOQ were calculated based on the peak current using the following equations [37].

LOD = 3s/m LOQ=10s/m

Where, s is the standard deviation of the peak current (five replicates), m is the slope of the calibration plot (Fig. 8). The LOD and LOQ values were calculated to be 0.1918×10⁻⁶ M and 0.6396×10⁻⁶ M, respectively. Low values of both LOD and LOQ values confirmed the sensitivity of the proposed method. The process of validation was studied by analyzing five replicates of 5×10⁻⁴ M PTD. The relative standard deviation (RSD) values for intra- and inter-day assay were calculated using the relation

$$RSD = \frac{s}{\bar{x}} \times 100$$

Where, s is standard deviation, $\bar{x}$ is mean deviation. They are found to be 3.4% and 2.88% respectively indicating good reproducibility of the method. The corresponding results are shown in Table 1.

Precision

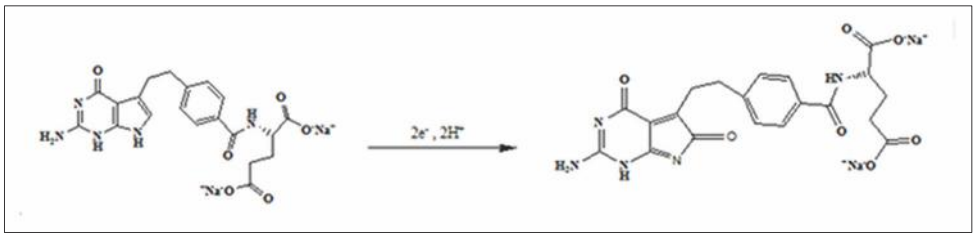
To examine the reproducibility of results on the same day and on different days, cyclic voltammograms of PTD were recorded. The corresponding RSD values were calculated and these values are shown in Table 1. Low values of RSD highlighted the precision of the proposed method for the assay of PTD [38].

Accuracy

Accuracy of the method was demonstrated at three different concentration levels by spiking a known quantity of the drug into a previously analyzed sample in triplicate. The results of analysis revealed that the method was more accurate.

Linearity

To establish linearity of the proposed method, five separate sets of drug solutions were prepared and analysed. Calibration graph was constructed by plotting the values of peak current versus concentration.



Scheme 2: Possible electrode reaction mechanism of pemetrexed disodium

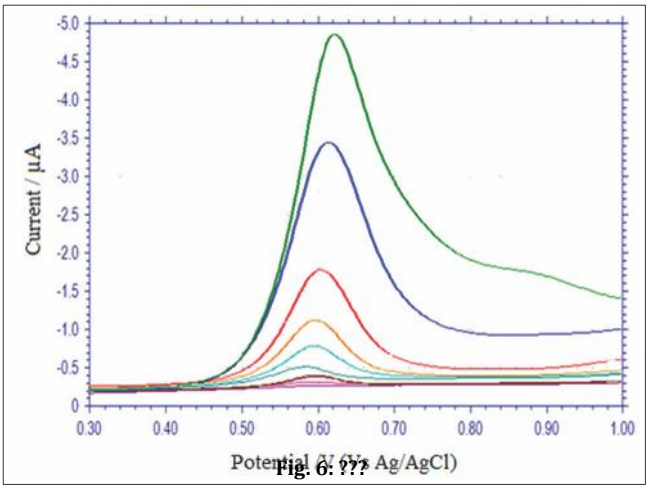


Fig. 6: ???

Fig. 7: Differential pulse voltammetry for increasing concentrations of drug, pemetrexed disodium in phosphate buffer at pH 6.0. Scan rate, 20 mV s^{-1}; pulse amplitude, 50 mV and pulse width, 60 ms (1) Blank, concentration of drug: (2) 7.5×10^{-7}, (3) 1×10^{-6}, (4) 2.5×10^{-6}, (5) 7.5×10^{-6},(6) 1×10^{-5}, (7) 2.5×10^{-5}, (8) 7.5×10^{-5}, (9) 5×10^{-5} and (10) 1×10^{-4} M

Linearity was noticed between the peak current and concentration in the concentration range of 1×10^{-4} to 7.5×10^{-7} M through which slope (0.0136) intercept (3×10^{-8}) and the correlation coefficent were determined, which can be used to determine unknown concentration.

Detection of PTD by DPV

The analytical method was developed involving DPV for the determination of the drug. For this, the variation of peak current (i_{pa}) with the concentration of PTD was investigated. The DPV of different concentrations of PTD are shown in Fig. 8. Under the optimized experimental conditions, a linear relation between the peak current of

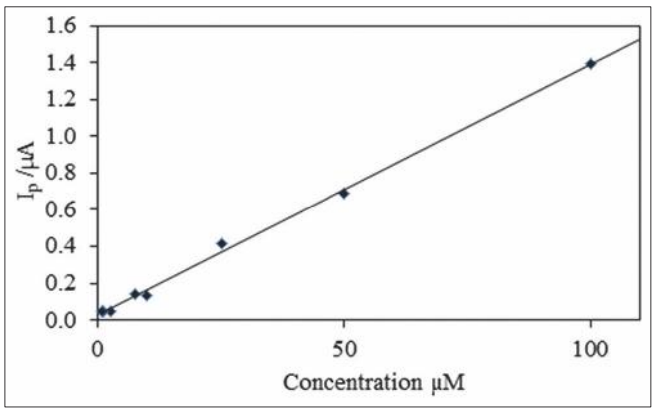


Fig. 8: Plot of peak current versus concentration

Table 1: Characteristics of calibration plot for pemetrexed disodium

Parameters	DPV
Linearity range (µM)	0.75-10
LOD (µM)	0.1918
LOQ (µM)	0.6396
Intra-day assay RSD (%)	3.40
Inter-day assay RSD (%)	2.88

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation, DPV: Differential pulse voltammetry

PTD and concentration in the range of 1×10^{-4} - 7.5×10^{-7} M was observed. In this concentration range, the response was found to be diffusion controlled. The analytical characteristics of the calibration plot are summarized in Table 1.

CONCLUSIONS

The electrochemical behavior of PTD on glassy carbon electrode was studied for the first time. The cyclic voltammogram was found to be irreversible and pH dependent. Two electrons were found to participate in the electrode process. By selecting the anodic peak of PTD, DPV were recorded. The proposed method is rapid, requiring <3 minutes to run a sample and does not include time consuming steps. By the proposed method, as low as 7.5×10^{-7} M of PTD can be accurately determined with sufficient precision and accuracy. The simplicity, sensitivity and low cost of analysis are the main features of the proposed method for the determination of PTD.

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