ELECTROCHEMICAL OXIDATION AND DETERMINATION OF AN ANTICANCER 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 anticancer drug, pemetrexed disodium (PTD).

Methods: The electrochemical oxidation of anticancer 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 anticancer 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.

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 folate reductase and glycaminide ribonucleotide formyltransferase (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 dihydrofolate reductase 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 (3 M 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 K₃[Fe(CN)₆] as a probe at different scan rates [27]. For a reversible process, the Randles- Sevcik formula has been used [28-31].

$$I_p=\left[2.69+10^n\right]A^nD^n/2\nu^{1/2}C^0$$

Where, \(n\) = number of electrons transferred i.e., \(1\), \(A\) = surface area of the electrode, \(D_i\) = diffusion coefficient, \(\nu\) = scan 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 cm².

**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. Lactate 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 (a₁) and 1.454V (a₂) 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 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 Epa 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, V¹/₂ 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].
The corresponding equation is
$$I_p = 0.0051v^{1/2} (mV/s)^{1/2}$$
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].

$$LOD = 3s/m \quad LOQ = 10s/m$$

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 $0.1918 \times 10^{-4}$ M and $0.6396 \times 10^{-4}$ 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 $5 \times 10^{-4}$ M PTD. The relative standard deviation (RSD) values for intra- and inter-day assay were calculated using the relation

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

Where, $s$ is standard deviation, $x$ 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 values were calculated and these values are shown in Table 1. Low values of RSD values were calculated and these values are shown in Table 1.

**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.
Linearity was noticed between the peak current and concentration in the concentration range of $1 \times 10^{-5}$ to $7.5 \times 10^{-5}$ M through which slope (0.0136) intercept ($3 \times 10^{-3}$) and the correlation coefficient 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 ($I_p$) 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 PTD and concentration in the range of $1 \times 10^{-5}$ to $7.5 \times 10^{-5}$ 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 $7.5 \times 10^{-5}$ 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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