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Anodic voltammetric behavior of hydroxyurea and its electroanalytical determination in pharmaceutical dosage form and urine

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ABSTRACT

A simple and a novel electroanalysis of hydroxyurea (HU) drug at pencil graphite electrode (PGE) has been investigated by using cyclic, linear sweep and differential pulse voltammetric techniques. The oxidation of HU was irreversible and exhibits a diffusion controlled process and is of pH dependence. The oxidation mechanism was proposed. The dependence of the current on pH, the concentration, nature of buffer, and scan rate was investigated to optimize the experimental conditions for the determination of HU. It was found that the optimum buffer for the determination of HU was pH of 8.0. In the range of 0.01 to 1.0 mM, the current measured by differential pulse voltammetry presents a good linear property as a function of the concentration of HU with a limit of detection of 7.89 μM. The developed method was successfully applied to HU determination in pharmaceutical formulation and human urine.

1. Introduction

Synthesis, chemical analysis and testing of the drugs are important events in pharmaceutical laboratories. Analysis of the drugs obtained from synthetic and natural source is of immense importance to determine the concentration of its active ingredient, helping to establish the proper dose of the drug. The past decade has many reports of potent drug molecules which require the development and validation of analytical methods for their analysis. The requirements of the quality of the drugs have increased tremendously due to the regulations led by regulatory departments like FDA and ICH. Conventional methods have some shortcomings like time consumability and use of costly and hazardous chemicals. This in turn has motivated the analyst to develop and establish newer and faster method of analysis giving the indication of the quality of the drug substance and drug products. Hydroxyurea (HU), the simplest, 1-carbon organic antitumor agent, is a member of the substituted urea group and is chemically known as hydroxycarbamide [1]. In 1981 it was reported to have antineoplastic activity against sarcoma [2]. At present, the primary role of hydroxyurea (Scheme 1) in chemotherapy is the management of granulocytic leukemia and thrombocytosis. It has been used in combination with radiotherapy for carcinomas of the head and neck [3]. HU is used in the treatment of cancer [4], sickle cell anemia [5] and infection with the human immunodeficiency virus (HIV) [6]. HU is a potent, nonalkylating myelosuppressive agent that inhibits DNA synthesis [7].

Only a few analytical procedures have been reported for the determination of HU. Nuclear magnetic resonance spectroscopy [8], liquid chromatographic (LC) procedures have been recommended by the U.S. Pharmacopeia [9] and others [10–12] for determination of hydroxyurea in pharmaceutical formulations and biological fluids. Capillary gas chromatography (GC) with thermionic (N–P) specific detection has also been reported [13]. A few electroanalytical methods have been reported for the determination of HU [14]. The main problems encountered in using such methods are either the need for derivatization or the need for time-consuming extraction procedures.

Electrochemical methods may offer certain advantages, such as requiring easier sample preparation, being less time-consuming and offering detectivity and dynamic range comparable to other analytical methods [15,16]. These methods have proven to be useful for development of very sensitive and selective methods for the determination of organic molecules including drugs. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmaceutical activity [17].

Electrochemical methods, especially differential pulse voltammetry (DPV) make it possible to decrease the analysis time as compared to the time exhaustive chromatographic methods [18]. The advantages of DPV over other electroanalytical techniques are greater speed of analysis, lower consumption of electroactive species in relation to the other electroanalytical techniques, and fewer problems with blocking of the electrode surface.

The pencil graphite electrode (PGE) (Scheme 2) has been successfully used as a biosensor in modern electroanalytical field. A porous composite

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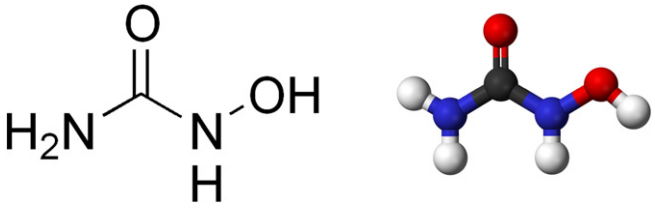


Scheme 1. Structural formula of hydroxyurea.

consists of graphite particles, polymeric binder and other additives such as clay. Due to high electrochemical reactivity, high electrical conductivity, good mechanical rigidity, low cost, low technology, high electrochemical reactivity, ease of modification, renewal, low background current, and miniaturization, the PGE has good application in analysis of drugs and in the detection of traces of metal ions [19]. PGE has a larger active electrode surface area and is therefore able to detect low concentrations of the analyte. This type of electrode has been successfully applied to design various biosensors.

As per the literature survey reveals that, till date there is no report on the electroanalytical method for the determination of HU using PGE. The purpose of this work is to develop a sensitive, simple, rapid and selective voltammetric method for the determination of HU in real samples like the pharmaceuticals and human urine. Our aim of this study is to establish the suitable experimental conditions, to investigate the voltammetric behavior and oxidation mechanism of HU at PGE by cyclic, linear sweep and differential pulse voltammetric methods.

2. Experimental

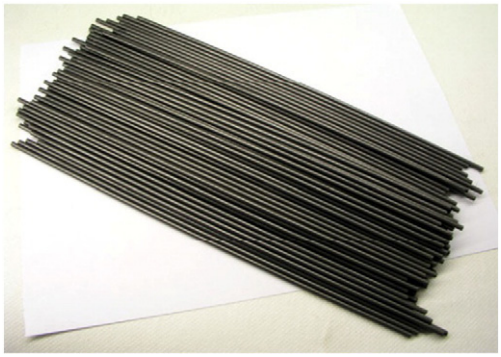
2.1. Reagents and chemicals

Hydroxyurea (HU) was obtained from Sigma Aldrich and stock solution of HU (1.0 mM) was prepared in water and stored in a refrigerator at 4 °C. Standard working solutions were prepared by diluting the stock solution with the selected supporting electrolyte. The phosphate buffers from pH 3.0–11.2 were prepared according to the method of Christian and Purdy [20]. The HU containing pharmaceutical product, HYDROX-L, was purchased from a local pharmacy. Other reagents used were of analytical grade. All solutions were prepared with millipore water.

2.2. Instrumentation and analytical procedure

Electrochemical measurements were carried on a CHI 630D electrochemical analyzer (CH Instruments Inc., USA). The voltammetric measurements were obtained in a 10 ml single compartment three-electrode glass cell with Ag/AgCl as a reference electrode, a platinum wire as counter electrode and pencil graphite electrode as working electrode. All the potentials are given against the Ag/AgCl (3 M KCl). pH measurements were performed with Elico LI120 pH meter (Elico Ltd., India). All experiments were carried at an ambient temperature of 25 ± 0.1 °C.

The differential pulse voltammetry (DPV) was performed at initial potential: 0.2 V; final potential: 1.2 V; increase potential: 0.004 V; amplitude: 0.05 V; frequency: 15 Hz; quiet time: 2 s; sensitivity: 1×10^{-4} A/V.



Scheme 2. Pencil graphite electrode (PGE).

2.3. Sample preparation

Two pieces of HU containing tablets were weighed and ground to a homogeneous fine powder in a mortar. A portion equivalent to a stock solution of a concentration of about 1.0 mM was accurately weighed and dissolved in water. The contents were sonicated for 20 min to affect complete dissolution. The excipient was separated by filtration and the residue was washed three times with water. The filtrate was transferred into a 100 mL calibrated flask and diluted to a final volume with the water. Appropriate solutions were prepared by taking suitable aliquots from this stock solution and diluting them with the phosphate buffer solutions. Each solution was transferred to the voltammetric cell. The differential pulse voltammograms were subsequently recorded following the optimized conditions. The content of the drug in tablet was determined referring to the calibration graph or regression analysis. To study the accuracy of the proposed method and to check the interferences from excipients used in the dosage form, the concentration of HU was calculated using standard addition method and recovery experiments were carried out.

3. Results and discussion

3.1. Electrochemical characterization of PGE using standard potassium ferricyanide system

The cyclic voltammograms of freshly prepared 1 M KCl and 1 mM potassium ferricyanide with 1 M KCl (Fig. 1) were recorded with PGE at scan rate of 50 mV s^{-1} to know the electrode activity. The anodic and cathodic peak potentials were located at 238 mV and 161 mV. The redox peak potential difference (ΔE_p) was 77 mV. This shows the electrode activity of PGE.

3.2. Cyclic voltammetric behavior of hydroxyurea

The electrochemical behavior of HU at pencil graphite electrode was studied using cyclic voltammetry (CV) at pH = 8.0. The cyclic voltammograms obtained for 1.0 mM HU solution at a scan rate of 50 mVs^{-1} exhibits well-defined irreversible anodic peaks at 0.63 V (peak A) and

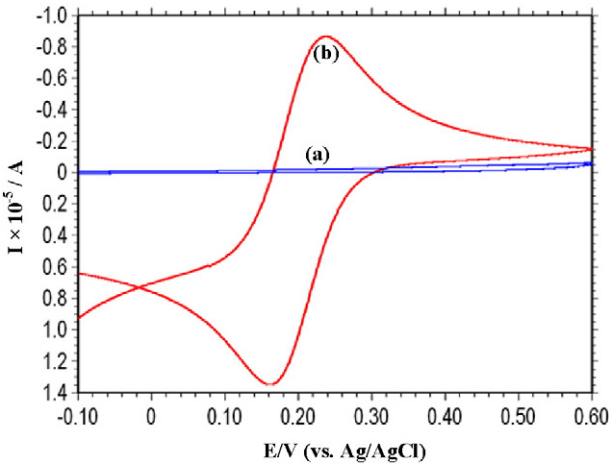


Fig. 1. Cyclic voltammograms on Pencil graphite electrode (PGE) at $\upsilon = 50$ mVs^{-1} of (a) 1 M potassium chloride and (b) 1 mM potassium ferricyanide in 1 M potassium chloride.