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Electrochemical oxidation behavior of itraconazole at different electrodes and its anodic stripping determination in pharmaceuticals and biological fluids

Abdalla Shalaby [a], Wafaa S. Hassan [a], Hassan A.M. Hendawy [b], A.M. Ibrahim [b,*]

[a] Department of Analytical Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt
[b] National Organization for Drug Control and Research (NODCAR), P.O. Box 29, Cairo, Egypt

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ABSTRACT

The oxidation of itraconazole at three different electrodes; ultra-trace graphite (UTGE), pencil graphite (PGE) and carbon paste (CPE) electrodes were studied voltammetrically. After the optimization of solution pH, accumulation potential and accumulation time, anodic stripping differential pulse and anodic stripping square wave voltammetric peaks were obtained. The oxidation mechanism was proposed. Based on these findings, a simple and not time-consuming procedure was successfully applied for the determination of itraconazole in pharmaceutics and biological fluids.

1. Introduction

Fungal infections are important causes of morbidity and mortality. As a consequence, early and appropriate antifungal chemotherapy is pivotal for successful management and survival [1]. The azoles are the largest single source of synthetic antifungal agents. As a group, they are broad spectrum in nature and mostly fungistatic. The mechanism of action of these compounds is the inhibition of lanosterol demethylase, a cytochrome P450 enzyme [2]. Itraconazole is an orally administered triazole antifungal agent [3]. It is chemically 4-(4-{4-[4-({(2RS,4SR)-2-(2,4-dichlorophenyl)-2-[(1H-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-[(1RS)-1-methylpropyl]-2,4-dihydro-3H-1,2,4-triazol-3-one, ITZ (Fig. 1) [4]. ITZ is soluble only at low pH and is better absorbed when the patient is not fasting, therefore, this drug should be taken with food and/or acidic fluids [2].

Various analytical methods have been developed for the determination of Itraconazole, among them include polarographic [5], voltammetric [6], spectrophotometric [7–10], spectrofluorimetric [11] and chromatographic methods [12–32].

In past decades, modern voltammetric techniques have been used to determine drug ingredients. Modern electroanalytical techniques, such as square wave voltammetry (SWV) and differential pulse voltammetry (DPV), have been utilized for the sensitive and quick determination of a wide range of drug ingredients with the advantages that there is no need for derivatization or time-consuming extraction steps.

The stripping voltammetry (SV) is an excellent technique for the determination of drug ingredients at trace levels [33]. SV is insensitive to matrix effects [34].

This work aimed to complete a point by point examination on the electrochemical behavior and possible oxidation mechanism of ITZ by using cyclic voltammetry (CV) as well as the determination of trace amounts of ITZ by anodic stripping differential pulse voltammetry (AS-DPV) and anodic stripping square wave voltammetry (AS-SWV) at different electrodes. The procedures did not require sample pretreatment or any time-consuming extraction. The proposed methods might be alternatives to chromatography techniques. No electroactive interferences from the excipients and endogenous substances were found in the pharmaceutical dosage forms and biological samples, respectively.

2. Experimental

2.1. Instrumentation

Voltammetric experiments were performed using Metrohm electroanalyzers Model 797VA Computrace. The measurements were recorded using VA Computrace version 1.3.1. The three electrode system consisted of an ultra-trace graphite electrode (UTGE) as the working electrode, a Ag/AgCl (3 M KCl) electrode as the reference electrode, and a platinum wire as the auxiliary electrode. A JENWAY 3510 pH

* Corresponding author.

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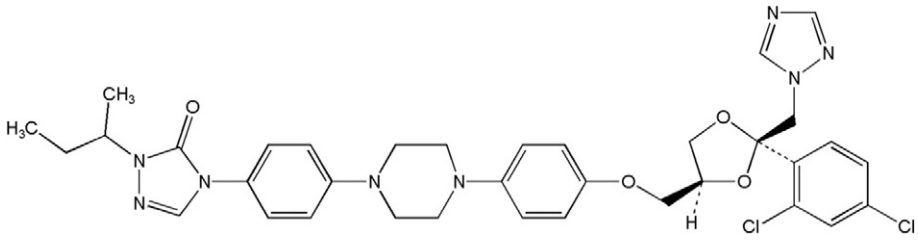

Fig. 1. Chemical structure of ITZ.

meter was used for pH measurements. All experiments were carried at an ambient temperature of 25 ± 0.1 °C.

2.2. Chemicals and reagents

All reagents were of analytical-reagent grade and used without further purification. Deionized water was used throughout the work. High purity nitrogen was used for deaeration.

ITZ standard was obtained from NODCAR. Itranox was obtained from the local market. A stock solution of ITZ (1.0 mg mL^{-1}) was prepared in methanol and stored in a refrigerator at 4 °C. ITZ working standard solutions were prepared daily by serial dilution of the stock solution. A 0.04 M Britton–Robinson (BR) buffer [35] was used. An appropriate amount of 0.2 M NaOH was added to the BR buffer to obtain solutions of pH values varying from 2 to 10. Graphite powder (particle dimension 20 μm) was purchased from Sigma–Aldrich.

2.3. Electrochemical measurement

A 10 mL volume of BR buffer solution containing a suitable amount of ITZ was added to the sample cell. The test solutions were purged with nitrogen for 5 min. The UTGE was kept at the desired accumulation potential for a given time period, while the solution was stirred at about 2000 rpm throughout the selected accumulation period. The stirring was then stopped and the solution was allowed to rest for 5 s, after which a scan was carried out towards positive potentials over the range 0.3 to 1.0 V, and the voltammograms were recorded. The experimental conditions for anodic stripping differential pulse voltammetry (AS-DPV) were: pulse amplitude, 0.050 V; pulse time, 0.04 s; sweep rate, 0.010 V s^{-1}; voltage step, 0.0065 V; and voltage step time, 0.65 s. The experimental conditions for anodic stripping square wave voltammetry (AS-SWV) were: amplitude, 0.020 V; frequency, 1.0 Hz; voltage step, 0.0065 V; and sweep rate, 0.0065 V s^{-1}.

2.4. Preparation of the working electrodes

a) *Ultra-trace graphite electrode* (*UTGE*): the UTGE was polished manually with 0.5 μm alumina powder on a smooth polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed with distilled water.
b) *Carbon paste electrode* (*CPE*): the carbon paste was prepared by mixing of 0.5 g graphite powder with 0.3 mL of paraffin oil in a mortar. A portion of carbon paste was packed into the hole of the insulin syringe body with a 3.0 mm diameter which contained a copper wire that was in contact with the apparatus.
c) *Pencil graphite electrode* (*PGE*): Rotring HB pencil leads with a diameter of 0.5 mm and a length of 60 mm were employed. Electrical contact with the pencil was achieved by soldering a metallic wire to the metallic part fixing the lead inside the pencil.

2.5. Itranox capsule analysis

Ten capsules of Itranox each one containing 200 mg of ITZ were crushed in a glass mortar. An adequate amount of this powder corresponding to a stock solution of 1.0 mg mL^{-1} was accurately weighed and dissolved in methanol. The flask content was sonicated for 30 min until complete dissolution. The excipients were separated by filtration. The filtrate was transferred into a 100 mL measuring flask and diluted to a final volume with methanol. Working solutions was prepared by taking suitable aliquots from this stock solution and diluting them with methanol. 50 ng mL^{-1} was then transferred to a voltammetric cell. An accumulation potential was applied and after 5 s as equilibrium period the voltammograms were recorded.

2.6. Spiked serum analysis

0.5 mL ethanol and 0.3 mL 5% $ZnSO_4$ were added to 0.3 mL serum and the mixture was centrifuged for 15 min at 13,000 rpm. An aliquot (1 mL) of the clear solution was added to a mixture of 9 mL BR buffer. The solution was deaerated for 5 min, and spiked with 50 ng mL^{-1} of ITZ. Then follows the procedure described above.

2.7. Spiked urine analysis

A mixture of 0.5 mL urine, 0.5 mL ethanol and 0.5 mL of 5% $ZnSO_4$ was adjusted to pH 11 by 0.1 mL of 1 M NaOH. The mixture was centrifuged for 15 min at 13,000 rpm. An aliquot (1 mL) of the clear solution was added to a mixture of 9 mL BR, and spiked with 50 ng mL^{-1} of ITZ. Then follows the procedure described above.

3. Results and discussions

3.1. Area of electrodes

The area of the electrode was obtained by the cyclic voltammetric method using 20.0 mM $K_3Fe(CN)_6$ as a probe at different scan rates. For a reversible process, the following Randles–Sevcik equation can be used [36,37]

$$I_p = \left(2.69 \times 10^5\right) A n^{3/2} D_R{}^{1/2} C_0 v^{1/2}$$

where, I_p refers to the anodic peak current, n is the number of electrons transferred, A is the surface area of the electrode, D_R is diffusion coefficient, v is the scan rate and C_0 is the concentration of $K_3Fe(CN)_6$, respectively. For 20.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte, T = 298 K, R = 8.314 J K^{-1} mol^{-1}, F = 96,480 °C mol^{-1}, n = 1, D_R = 7.6×10^{-6} cm^2 s^{-1}, then from the slope of the plot of I_{pa} vs. $v^{1/2}$, the electrode surface area was calculated [38,39]. In our experiment the slope was 1×10^{-6}, 2×10^{-5} and 6×10^{-6} A $(V\ s^{-1})^{1/2}$ respectively, and the area was calculated to be 0.007, 0.135 and 0.04 cm^2 for UTGE, PGE and CPE respectively.

3.2. Cyclic voltammogram

The cyclic voltammetric behavior of ITZ at UTGE, PGE and CPE was studied (Fig. 2). ITZ gave only an oxidation peak. On reverse scan, no reduction peak was observed; indicating that oxidation of ITZ is an irreversible process at UTGE, PGE and CPE respectively. It was found that