Electrochemical Behavior and Determination of Ketoconazole from Pharmaceutical Preparations

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Abstract
The oxidation of ketoconazole (KC) was investigated by rotating disk electrode voltammetry, cyclic voltammetry, and controlled potential coulometry in chloroform at platinum (Pt), gold (Au), and glassy carbon (GC) electrodes using tetrabutylammonium perchlorate as supporting electrolyte. Based on the electrochemical results obtained, the following possible mechanism was proposed to explain the electrochemical oxidation of KC

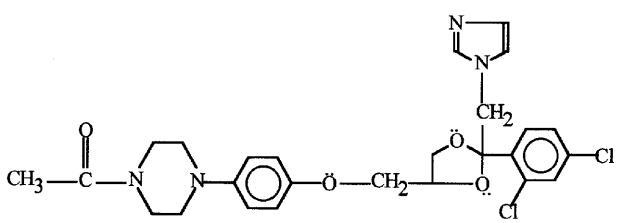
$$KC \underset{}{\overset{-e}{\rightleftharpoons}} KC^{\cdot+} \xrightarrow{-e} P$$
$$\downarrow$$
$$P'$$

A differential pulse voltammetric method at a Pt electrode was developed for the determination of KC from pharmaceutical preparations in the concentration of 3.0×10^{-6}–1.0×10^{-4} M. The procedure was applied to the determination of KC in its tablets and creams as well as its recovery from blood serum and urine samples.

Keywords: Ketoconazole, Electrochemical behavior, Voltammetry, Determination

1. Introduction

Ketoconazole, *cis*-1-acetyl-4-[4-[2-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-ylmethyl)-1,3-dioxolon-4-yl]methoxy]piperazin (KC, Scheme 1), as a highly effective broad spectrum antifungal drug [1] has been widely used to treat a wide variety of superficial and systematic mycoses [2, 3]. It possesses the advantage over other imidazole derivatives of producing adequate sustained blood levels following oral administration [4]. Due to the vital importance of ketoconazole determination in pharmaceutical preparations and in biological fluids, several chromatographic [5–11] and spectroscopic methods [12–20] for its quantitative determination have been reported.



Scheme 1. Ketoconazole.

Despite the analytical importance of the electrochemical behavior of ketoconazole, the literature report on electrode processes of the drug is quite sparse [21]. In 1992, Fijalek et al. described a polarographic method for the determination of ketoconazole on dropping mercury electrode in the range of 1–30 μg/mL using a phosphate buffer pH 7.45 [21]. In this article we studied the voltammetric oxidation of ketoconazole in chloroform solution on the glassy carbon, platinum, and gold electrodes. The electrode reaction mechanism was investigated by rotating disk electrode voltammetry, cyclic voltammetry and controlled potential coulometry. Differential pulse voltammetry on a platinum electrode was also employed to develop a new electroanalytical method for the quantitative determination of ketoconazole in pharmaceutical preparations, after extraction of the drug into chloroform, as well as the recovery of ketoconazole from biological fluids. It should be noted that, despite its potential toxicity and relatively high volatility, chloroform is reported as a very suitable solvent for the extraction and determination of ketoconazole from pharmaceutical (tablets and creams) and biological samples [12, 16, 20].

2. Experimental

2.1. Reagents

All chemicals used in this study were of the highest purity available and used without further purification except for vacuum drying over P_2O_5. Triply distilled deionized water was used throughout.

Reagent grade ketoconazole and tablets and creams containing 200 mg and 2% of the drug, respectively, were obtained from Behvazan Pharmaceutical Company, Rasht, Iran. Analytical grade tetrabutylamonium perchlorate (TBAP, Fluka) and chloroform (Merck) were used as received. N_2 gas with purity 99.99% was used to deaerate the solutions during the experiments.

2.2. Apparatus

A three electrode system with a Pt wire counter electrode, an Ag/AgCl, 3 M KCl reference electrode and a solid state working electrode was employed. The working electrodes made of platinum (Pt), gold (Au), and glassy carbon (GC), with a diameter of 2 mm, were purchased from Metrohm. The electrodes were polished with alumina powder (0.05 μm) for 10 min and washed with water and acetone before use. The

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working electrode used in controlled potential coulometry experiments was a platinum gauze cylinder (2 cm diameter × 3 cm, Metrohm). All voltammetric measurements were carried out on a Metrohm multipurpose instrument Model 693 VA processor, equipped with a Metrohm 694 VA stand and a thermal printer.

2.3. Voltammetric Procedure

Ten mL of 0.5 M TBAP solution in 95 : 5 (v/v) chloroform-acetic acid solution containing appropriate amount of KC was placed in the voltammetric cell. Then, a stream of oxygen-free nitrogen was bubbled through the solution for 2 min to deaerate it (although it is not of urgent need). The Pt electrode was placed in the cell and the KC determination was conducted via the differential pulse voltammetry (DPV) technique. The potential was scanned at a rate of 10 mV/s between 0.2 and 1.2 V (vs. Ag/AgCl). A pulse amplitude of 50 mV was applied to the DC ramp and the peak current was measured at 850 mV.

2.4. Analysis of Tablets and Creams

An accurately weighed portion of the KC cream or finely powdered KC tablet sample equivalent to about 25 mg of the drug was transferred into a 100-mL separatory funnel, 2 mL HCl 1 M and 8 mL water was added and the solution was vigorously shaken for about 10 min. Then 1 mL of NaOH 10 M was added and after thorough mixing the solution was extracted with three 10-, 10- and 5-mL portions of chloroform. An accurate μL-volume of the chloroform extract was pipetted into a 10-mL volumetric flask, diluted to the mark with a 0.5 M TBAP solution in 95 : 5 (v/v) chloroform-acetic acid and the voltammetric procedure was followed.

2.5. Recovery of Ketoconazole from Biological Fluids

The deprotonization and elimination of polar serum constituents were accomplished using a C-18 reverse-phase sample preparation cartridge as described in the literature [22]. A 5 mL portion of the sample serum containing 20 μg added KC was introduced to the preconditioned cartridge. The cartridge was then washed with 5 mL of water followed by 5 mL of methanol. The eluent from the final 4.5 mL of methanol was collected and evaporated to dryness using a gentle air stream at 40 °C. The residue was dissolved in 5 mL water containing few drops of 1 M hydrochloric acid. Then the KC content was extracted into 5 mL chloroform and the voltammetric procedure was followed.

A 5-mL portion of the urine sample containing 20 μg added KC was introduced to a 25-mL separatory funnel and 5 mL chloroform was added and the mixture was shaken for about 10 min. The solution was allowed to stand for clear separation of the two phases. The chloroform layer was separated and the KC determination was conducted by the recommended voltammetric procedure.

3. Results and Discussion

3.1. Rotating Disk Electrode Voltammetry

Current–potential curves for the oxidation of 0.5 mM KC in the presence of 0.5 M TBAP were recorded at polished GC, Pt and Au electrodes as shown in Figure 1. Under these conditions, two anodic waves were obtained. Figure 1 shows that, while the half-wave potential of the first electrooxidation step is more or less the same on the three solid electrodes used, that of the second step largely changed in the order GC < Au < Pt.

Plots of diffusion limiting current, i_l, vs. rotation speed, $\omega^{1/2}$, for the first oxidation waves at the concentration range 10^{-5}–10^{-4} M are straight lines with a correlation coefficient very close to unity and the height of the waves are linearly dependent on the concentration of KC. Such observations agree well with the Levich predictions and clearly indicate an electrochemical process governed by diffusion. On the other hand, the plots of log $[i/(i_d - i)]$ against E for the first oxidation waves are also straight lines with a slope close to that expected for an electrochemically reversible behavior. The characteristic data of the DC voltammograms are summarized in Table 1.

3.2. Cyclic Voltammetry

Cyclic voltammograms of 0.5 mM KC solution in chloroform in the presence of 0.5 M TBAP were measured between 0.4 and 1.6 V at various scan rates. Sample voltammograms on the GC electrode are shown in Figure 2. As seen, the voltammograms consisted of three peaks, at 790 mV in the cathodic scan and at 870 and 1320 mV (at a scan rate of 250 mV/s) in the anodic scan. The cathodic peak is formed as a consequence of the reduction of the first oxidation product of KC. The piperazine ring of the KC molecule is oxidized at high positive potentials (close to the solvent evolution) in a similar process to that reported for the oxidation of the piperazine rings of trazodone [23] and doxazosin molecules [24]. The anodic peak at 870 mV is due to the oxidation of the cathodic peak. As is obvious, the second oxidation

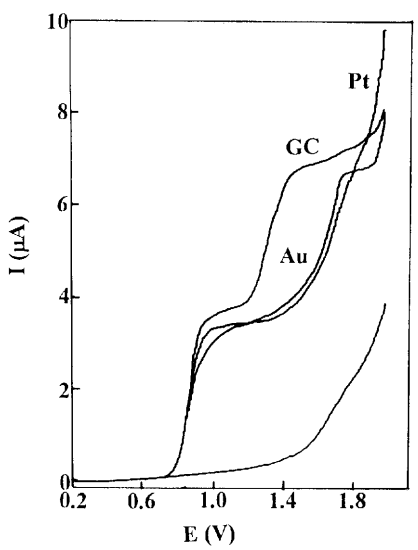


Fig. 1. DC voltammograms of 0.5 mM KC in the presence of 0.5 M TBAP in chloroform at rotating disk Pt, Au, and GC electrodes. Rotation speed $\omega = 500$ rpm and scan rate $\nu = 10$ mV/s.

Table 1. DC voltammetric data for KC at Pt, Au, and GC electrodes in 0.5 M chloroform solution of TBAP. Slope: $S = dE/d\log[(i_d - i)/i]$.

	First wave			*Second wave*
Electrode	$E_{1/2}$ *[mV]*	*S*	$E_{3/4}$-$E_{1/4}$ *[mV]*	$E_{1/2}$ *[mV]*
Pt	858	65 ± 3	62 ± 5	–
Au	863	65 ± 3	63 ± 5	1520
GC	867	64 ± 2	60 ± 4	1230

process is chemically irreversible. A similar irreversible behavior was reported for the electrooxidation of the piperazine rings of trazodone and doxazosin drug molecules [23, 24].

The corresponding cyclic voltammograms were obtained while the scan is reversed after the first anodic peak (i.e., at the potential range 0.2–1.2 V, Fig. 3). In this case, the cathodic peak appears to a greater extent in comparison with that observed in Figure 2. The current function values $(i_{pa})_1 \nu^{1/2}$ for the first electrooxidation step were found to be independent of ν, at low scan rates, but at higher scan rates (i.e., higher than 250 mV/s) decreased slightly with ν. Moreover, at the scan rates > 100 mV/s, $(\Delta E_p)_1$ is very close to the theoretical value of 59/n mV at all solid electrodes used, while at low scan rates it was slightly altered by changing the scan rates. Moreover, $(\Delta E_p)_1$ was negligibly altered by changing the scan rates. It is also noteworthy that, at rates lower than 25 mV/s, the anodic peak potential shows a shift of about 15–25 mV towards more negative values. The characteristic data of the resulting cyclic voltammograms for KC at a scan rate of 100 mV/s without IR compensation, obtained at all three solid state electrodes used, are given in Table 2. As seen, the electrochemical behavior of the KC/KC^+ system seems to be independent of the nature of the electrodes used. As seen from Figure 3B, the i_{pc}/i_{pa} ratio for the KC/KC^+ system changes from about zero at a scan rate of 5 mV/s to 0.96 at a scan rate of 2000 mV/s. The observed electrochemical behavior most probably suggests that the product of the first reversible electrooxidation step (KC^+) participates in a slow chemical reaction [25].

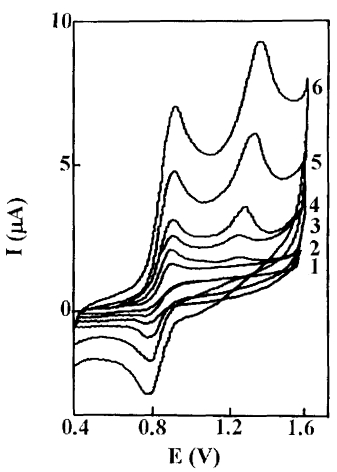


Fig. 2. Cyclic voltammograms of 0.5 mM KC in the presence of 0.5 M TBAP in chloroform at a GC electrode. Scan rates [mV/s] are: 1) 5; 2) 10; 3) 25; 4) 50; 5) 100; 6) 250.

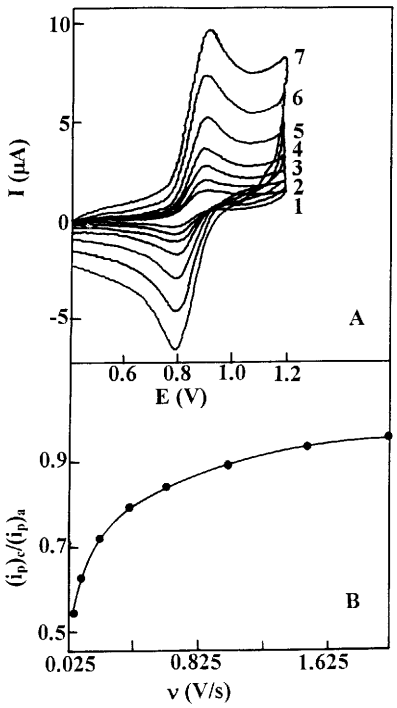


Fig. 3. A) cyclic voltammograms of 0.5 mM KC in the presence of 0.5 M TBAP in chloroform at a GC electrode, while the scan is reversed after the first anodic peak. Scan rates [mV/s] are: 1) 25; 2) 50; 3) 100; 4) 200; 5) 400; 6) 625; 7) 1000. B) Plot of $(i_p)a/(i_p)_c$ against scan rate ν.

3.3. Controlled Potential Coulometry

The electrolysis of 12 mL of 0.42 mM KC in chloroform in the presence of 0.5 M TBAP on a Pt gauze cylinder at a fixed potential of −900 mV (vs. Ag/AgCl) related to the top the first anodic peak resulted in a red solution. The evolution of the UV-vis spectrum during the course of electrolysis is shown in Figure 4. As is seen, while the initial KC solution is colorless and has no absorption in the visible region, the final electrolysis product is a red solution possessing a maximum absorption at 520 nm. The quantity of electricity consumed is 0.461 coulombs, confirming the occurrence of a one electron process ($n = 0.96$) for the fist electrooxidation step.

The resulting RDE voltammograms on a GC electrode during the course of electrolysis are shown in Figure 5. As is obvious from Figure 5, at the beginning of coulometry, two anodic waves with nearly equal heights are observed (curve 1). However, during coulometry, the height of the first anodic wave decreases in the expense of the appearance of a cathodic wave with a height nearly equal to the height loss of the first anodic wave (curve 2). Finally, at the end of coulometry, the first anodic wave has almost disappeared and only a cathodic wave and the second anodic

Table 2. Cyclic voltammetric data for KC at Pt, Au, and GC electrodes in 0.5 M chloroform solution of TBAP at a scan rate of 100 mVs.

Electrode [mV]	$(E_{p1})_a$ *[mV]*	$(E_{p1})_c$ *[mV]*	ΔE_{p1} *[mV]*	$(E_{p2})_a$
Pt	880	810	70	–
Au	860	790	70	1420
GC	860	790	70	1250

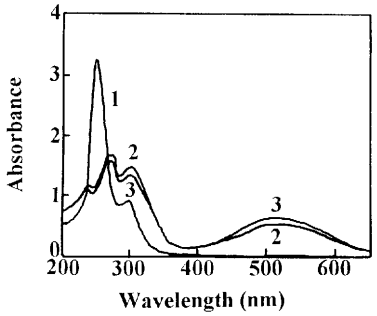


Fig. 4. UV-vis. spectrum evolution of 0.5 mM KC in chloroform (0.5 M TBAP) during controlled potential coulometry: 1) before electrolysis; 2) during electrolysis; 3) at the end of electrolysis.

wave are observed (curve 3). It is interesting to note that the height of the cathodic wave at the end of coulometry (curve 3) is much lower than that of the first anodic wave before coulometry (curve 1). This is also in support of the idea that the product of the first electrooxidation step (KC^{+}) participates in a subsequent chemical reaction. The product P′ seems to be electrochemically inactive in the potential window of 0.4 to 1.2 V (vs. Ag/AgCl) according to the cyclic voltammograms given in Figure 3A. However, the RDE voltammogram of the controlled potential electrolysis product (curve 3 in Fig. 5) shows a significant cathodic current, which seems to indicate that the product P′ should display some sort of voltammetric behavior, at slower scan rates in Figure 3. This discrepancy is most probably due to the low reaction rate of $KC^{\cdot+}$ to P′ in chloroform solution. This was confirmed by obtaining the RDE voltammogram of the final solution after 2 h, where the cathodic wave was completely removed, indicating that after such a prolonged period of time all $KC^{\cdot+}$ is converted to the electrochemically inactive product P′.

3.4. Mechanism

The voltammetric and coulometric data thus obtained clearly indicated that KC is initially oxidized reversibly with the loss of one electron to form a $KC^{\cdot+}$ cation radical, which gradually decays via a chemical reaction to form some stable products.

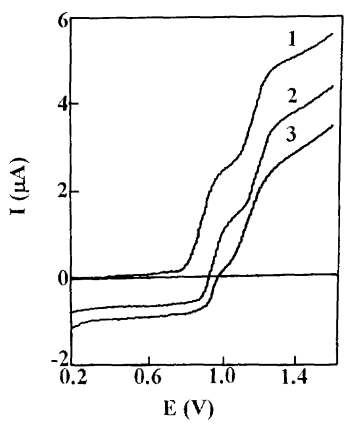


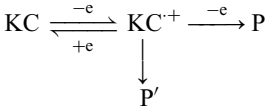
Fig. 5. DC voltammograms of 0.5 mM KC in the presence of 0.5 M TBAP in chloroform at a rotating GC disk during controlled potential coulometry (scan rate: 10 mV/s and rotation speed: 600 rpm): 1) before electrolysis; 2) during electrolysis; 3) at the end of electrolysis.

Table 3. Results of determination of ketoconazole in its formulations.

	Amount		
		Found [a]	
Sample	*Labeled*	*Proposed method*	*Official method*
Tablet	200 (mg/tablet)	202.2 ± 1.1	201.9 ± 1.0
Cream	2 % (wt.% drug)	2.05 ± 0.02	1.99 ± 0.01

[a] Average of three replicate measurements.

Meanwhile, the drug can be further oxidized irreversibly at increased applied potentials with the loss of a second electron. Thus, the following scheme can be proposed as possible mechanism to explain the electrochemical oxidation KC in chloroform:



The products P and P′ may presumably resemble the metabolites of KC, oxidation, cleavage and degradation of the imidazole and piperazine rings, *o*-dealkylation and aromatic hydroxylation are the reported pathways of KC metabolisms [26].

As was mentioned in Section 3.2., the most possible route for the irreversible oxidation of $KC^{\cdot+}$ cation radical is the loss of an electron from the piperazine ring of the KC molecule [23, 24]. However, the initial reversible oxidation step may occur as a result of the loss of an electron from either the imidazole ring or the methoxyl group of the drug molecule. Our preliminary voltammetric study of imidazole and clotrimazole revealed that no electrochemical oxidation takes place at the potential range studied (i.e., 0.4–1.6 V vs. Ag/AgCl). Thus, the only possibility is the loss of an electron from the methoxy group of KC. The resulting cation radical, which can be stabilized by resonance, results in the observed red color of the chloroform solution (Fig. 4).

3.5. Determination of Ketoconazole

The determination of the KC concentration in a formulation was achieved using the differential pulse voltammetric (DPV) response obtained for the initial electron transfer process at a Pt electrode. In order to increase the conductivity of the solutions, a 5 % (v/v) of acetic acid was added to chloroform solutions; thus, the reproducibility of KC determination was improved. The calculated limit of detection with a pulse amplitude of 50 mV was 1.0×10^{-6} M (3σ). Typical differential pulse voltammograms obtained at various KC concentrations are shown in Figure 6.

Good calibration plots, covering the KC concentration range 3.0×10^{-6}–1.0×10^{-4} M, were obtained with DPV. The regression equation obtained is $i_p[\text{nA}] = 6.516 \times 10^6\, C[\text{M}] + 11.38$ ($n = 15$, $r = 0.9992$). The reproducibility of the method was examined by analyzing five samples containing 5.0×10^{-6} M KC. The relative standard deviation of the method thus obtained was 1.5 %.

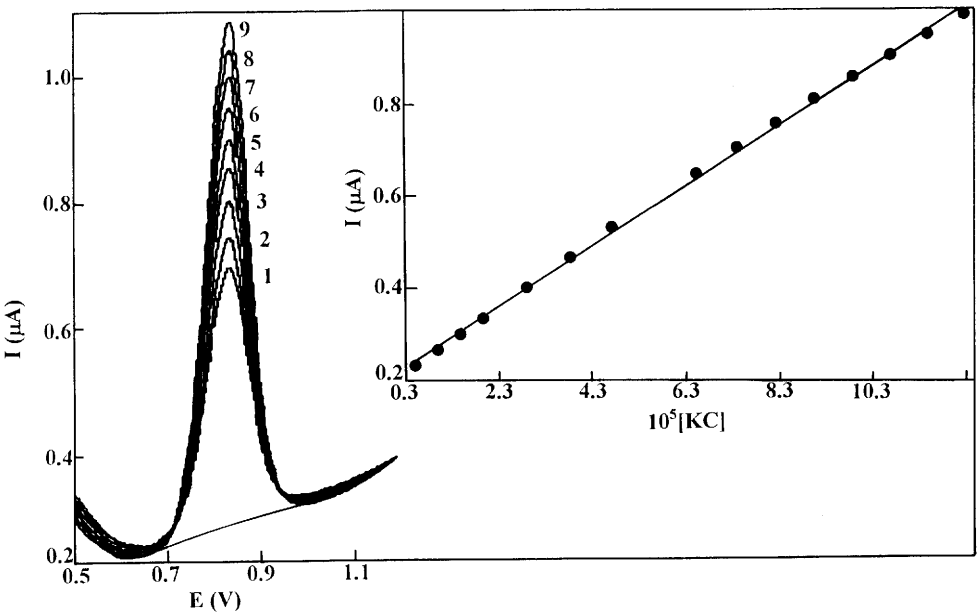


Fig. 6. Differential pulse voltammograms obtained for varying concentration of KC in 0.5 M solution of TBAP in 95 : 5 (v/v) chloroform-acetic acid. The KC concentration [M] is: 1) 5.66×10^{-5}; 2) 6.54×10^{-5}; 3) 7.41×10^{-5}; 4) 8.26×10^{-5}; 5) 9.09×0^{-5}; 6) 9.91×10^{-5}; 7) 1.07×10^{-4}; 8) 1.15×10^{-4}; 9) 1.23×10^{-4}. Inset is the corresponding calibration plot.

The PVC determination of KC in its powdered tablets and creams was carried out by the standard additions methods and the results (Table 2) were compared by those obtained by the official potentiometric method [14, 20]. The data given in Table 2 clearly indicate a good agreement between KC contents determined by the proposed and official methods as well as the drug declared amounts in the drug preparations used. This is indicative of noninterference of the other ingredients and the excepients which are present in the formulations.

In order to investigate applicability of the proposed method to the determination of ketoconazole in the biological fluids, it was applied to the recovery of KC from blood serum and urine samples. Thus, 20 μg ketoconazole was added to the samples and the recommended recovery and voltammetric procedures followed. The percent recovery obtained from three replicate measurements by the standard addition method were found to be $(102.5 \pm 1.1)\%$ and $(97.5 \pm 1.1)\%$ for the blood serum and urine samples, respectively.

4. References

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