Sensitive electrochemical determination of ketoconazole and its application in pharmaceutical formulations and real samples using pretreated graphite pencil electrode

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Abstract

A simple and rapid electrochemical determination of ketoconazole(KTZ), at pretreated graphite pencil electrode(PGPE) was investigated. After the pretreatment, GPE exhibited excellent electrocatalytic activity with good reproducibility and more stability. The voltammetric behaviour of KTZ for physiological buffer at pH 7.4 was observed, sharper and better oxidation peak was obtained. From the scan rates (v) of both linear sweep and cyclic voltammeric techniques, KTZ was found to be irreversible and diffusion controlled electrode process, and there was transfer of unequal number of proton and electrons. Differential pulse voltammetry exhibits a good linear range of 5.0×10^{-8} M to 5.5×10^{-7} M with a detection limit of 3.98×10^{-9}M and Square wave voltammetry in concentration range from 1.0×10^{-8} M to 5.0×10^{-6} M with a detection limit of 0.96×10^{-9} M for KTZ. The proposed method was applied to the determination of ketoconazole in real and pharmaceuticals samples.

Key words: Voltammetric, Ketoconazole, Pretreatment, DPV, Pharmaceuticals Samples

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1. Introduction

Ketoconazole, *cis*-1-acetyl-4-{4-[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}piperazine(Scheme 1), is most effective when administered orally, for the antifugal drug treatment against a wide variety of superficial and systemic mycoses[1,2]. Ketoconazole(KTZ) is active against specific dermatophytes and yeast, so used in the preparation of pharmaceuticals like tablets, shampoos and ointments, etc. They are good oral bioavailability and fewer side effects and small fraction of

the administered dose was excreted and there is no change in the urine. Various analytical methods have been developed for the determination of ketoconazole, among them some include visible spectrophotometry[3,4], UV spectrophotometry[5], spectrofluorimetry[6], thin-layer chromatography[7], and capillary electrophoresis with diode array detection[8,9]. These methods suffer from some disadvantages, such as complex operating process, expensive instruments and strict pre-disposal. The use of electrochemical procedures have been extensively investigated in the determination of biologically important drugs in recent years because of their simplicity, ease of miniaturization, high sensitivity and relatively low cost as compared to other analytical methods.

"Here Scheme 1"

In the electrochemical determination process electrode modifications usually involve laborious preparation and the modified electrodes can exhibit long-term instability, loss of sensitivity and chemicals highly expensive, overcome the regeneration drawback of the modified electrodes. Electrochemical pretreatment of graphite pencil electrode (PGPE) seems to be a simpler, less time consuming and more applicable strategy in comparison with other modification procedures of the electrode surface. The graphite pencil electrode has been successfully used as a sensor in modern electroanalytical field due to its high electrochemical reactivity, good mechanical rigidity, low cost, low technology and ease of modification, renewal and low back ground current[10,11]. The pretreated GPE has good application in the analysis of drugs and detection of traces of metal ions. To the best of our knowledge no methods were reported in literature based on electrochemical redox properties of KTZ using PGPE. Hence in the present work, the voltammetric oxidation behavior of KTZ at PGPE was studied using CV, DPV and SWV techniques and the optimum conditions for quantitative analysis were determined. The proposed method was applied for determination of KTZ in real and pharmaceuticals samples.

2. Experimental Section

2.1. Reagents and Chemicals

The pencil-lead rods (HB 0.5 mm in diameter and 6 cm length) were purchased from local bookstore. Ketoconazole was purchased from Sigma Aldrich and used as such. A stock solution of Ketoconazole (1.0 mM) was prepared by using methanol. The phosphate buffers from pH 3.0 to 10.2 were prepared according

to the method of Christian and Purdy [12]. Other reagents used were of analytical grade. All solutions were prepared with millipore water.

2.2 Instrumentation

Electrochemical measurements were carried out on a CHI 630D electrochemical analyzer (CH Instruments Inc., USA). The voltammetric measurements were carried out in a 10 mL single compartment three-electrode glass cell with Ag/AgCl as a reference electrode, a platinum wire as counter electrode and a pretreated graphite pencil electrode as working electrode. All the potentials are given against the Ag/AgCl (3.0 M KCl). pH measurements were performed with an Elico LI 120 pH meter (Elico Ltd., India).

2.3 Area of electrode

The area of the electrode was obtained by the cyclic voltammetric method using 1.0 mM $K_4Fe(CN)_6$ as a probe at different scan rates. For a reversible process, the following Randles - Sevcik formula was used [13].

$$I_{pa} = (2.69 \times 10^5)\, n^{3/2}\, A_0\, D_o^{1/2} C_0^* v^{1/2}$$

where I_{pa} refers to the anodic peak current, n is the number of electrons transferred, A_0 is the surface area of the electrode, D_0 is diffusion coefficient, v is the scan rate and C_0^* is the concentration of $K_4Fe(CN)_6$. For 1.0 mM $K_4Fe(CN)_6$ in 0.1 M KCl electrolyte, n = 1, $D_o = 7.6 \times 10^{-6}$ cm^2s^{-1}, then from the slope of the plot of I_{pa} vs. $v^{1/2}$, the electro active area was calculated. In our experiment electro active area for pretreated graphite pencil electrode was found to be 0.298 cm^2 and for glassy carbon electrode was found to be 0.0377 cm^2 and carbon paste electrode was found to be 0.0856 cm^2. Electro active area of PGPE is higher than the electro active area of GCE and CPE, hence greater response of peak current was observed for PGPE towards ketocanazole.

2.4. Preparation of pharmaceutical sample

Commercially available Nizoral® tablets each containing 200 mg ketocanazole, were weighed accurately and made a homogeneous fine powder in a mortar. An equal amount of calculated average tablet mass was transferred to a 25ml flask, and dissolved by using methanol. The mixture was sonicated for 15 min to achieve complete solubility and there after filtered the solution into calibrated flask and used for further analysis.

Nizoral® shampoo and Keto-B® cream each containing 2% ketocanazole were dissolved each in 10ml of 0.1 M HCl and filtered the solution, collected the filtrate and transfered the solution in separate 50 ml calibrated flask and diluted by using Millipore water to collect a stock solution for further study.

2.5. Procedure

Cyclic voltammetric measurements were carried out each time by using a new graphite pencil lead. Electrochemical pretreatment was done by electrode immersed in 1.0 $\times 10^{-3}$ M HCL solution and sonicated for 10 minutes. The electrode was washed cleanly with millipore water and dried in room temperature. After the pretreatment, GPE was activated in 10 ml phosphate buffer (0.2 M, pH=7.4) by cyclic voltammetric sweeps between +0.0 to +1.4 V, until a stable cyclic voltammogram was obtained. After open-circuit accumulation for 150 sec with constant stirring and following quiet for 20 sec, potential scan was initiated and cyclic voltammograms were recorded between +0.2 and +1.0 V, with a scan rate of 50 mV s^{-1}. All measurements were carried out at room temperature of 25±0.1 °C.

3. Results and Discussion

3.1. Electro-oxidation of Ketoconazole

The electrooxidation of Ketoconazole at pretreated GPE was studied over the pH range of 3.0 – 10.2 in 0.2 M phosphate buffer solutions by cyclic voltammetry. The cyclic voltammogram obtained for 1.0mM Ketoconazole solution at a scan rate v = 50 mVs^{-1} shows maximum anodic peak potential at E_p= + 0.693 V and peak current I_p = + 5.12 A at pH = 7.4 (Fig. 1). On scanning in the negative direction, no reduction peak was observed, showing that the oxidation of ketoconazole is an irreversible process.

“Here Figure 1”

3.2. Effect of pH

The electrode reaction might be affected by pH of the medium. The electro-oxidation of 1.0mM ketoconazole was studied over the pH range of 3.0 – 10.2 in phosphate buffer solution by cyclic voltammetry which is as shown in Fig. 2(a). On variation of pH there is an increase in the peak current from pH 3.0 to 7.4, at pH 7.4 gives maximum peak intensity thereafter gradually decreases (Fig. 2(b)). Hence we consider the pH 7.4 as optimum pH to study the further experimental analysis. The pH dependence on the peak potential obtained when cyclic voltammetry was used is shown in Fig. 2(c). With the increase in pH of the solution, peak potential shifted to less positive value, and obeys the following equation:

E_p (V) = 0.848 - 0.025pH (r = 0.979)

The slope plot of Ep versus pH was found to be 25 mV/pH, which is close to the theoretical value of 30 mV/pH. This confirms the number of protons transferred in the electrode mechanism is half of the total number of electrons transferred in the rate determining step [14-16].

"Here Figure 2(a), 2(b), 2(c)"

3.3. Effect of scan rate

The effect of scan rate on the electro-oxidation of Ketoconazole was studied by cyclic voltammeric and linear sweep voltammetric methods. The useful information involving electrochemical mechanism generally can be acquired from the relationship between peak current and scan rate. The voltammetric behavior of Ketoconazole was examined at different scan rates from 0.025 mVs^{-1} to 0.250 mVs^{-1} using linear sweep voltammetry (Fig. 3(a)) and scan rates from 0.025 mVs^{-1} to 0.300 mVs^{-1} for cyclic voltammetry (Fig. 3(b)).

"Here Figure 3(a), 3(b)"

Scan rate studies were carried to assess whether the process on pretreated graphite pencil electrode was diffusion or adsorption controlled. A linear relationship was observed between log I_{pa} and log *v*, (Fig. 3(c), 3(d)) corresponding to the following equations:

$\log I_{pa}(\mu A) = 0.344 \log v\ (V\ s^{-1}) + 1.000$; (r = 0.979) for LSV

$\log I_{pa}(\mu A) = 0.380 \log v\ (V\ s^{-1}) + 0.945$; (*r* = 0.976) for CV

"Here Figure 3(c), 3(d)"

The peak potential shifted to more positive values on increasing the scan rate. The linear relationship between log I_{pa} and log *v* for LSV and CV with the slope values of 0.344 for LSV and 0.380 for CV which were near to theoretical value 0.5 indicating typical diffusion- controlled process [17].

For an irreversible electrode process, according to Laviron [18], E_p is defined by the following equation (1),

$$E_p = E^{0'} + \left(\frac{2.303RT}{\alpha nF}\right) \log \left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) \log v \quad (1)$$

where 'α' is the transfer coefficient, 'k^0' the standard heterogeneous rate constant of the reaction, 'n' the number of electrons transferred and the 'E$^{0'}$' is formal redox potential. Other symbols have their usual meanings. Thus the value of "αn" can be easily calculated from the slope of E_p versus log *v*. In this system,

the slope was 0.061 for LSV and 0.059 for CV taking T = 298 K, R= 8.314 J $K^{-1}mol^{-1}$, and F=96480 C mol^{-1}.

According to Bard and Faulkner [19], α can be expressed as

$$\alpha = \frac{47.7}{E_p - E_{p/2}} \text{ mV}$$

where $E_{p/2}$ is the potential where the current is at half the peak value. So we calculated the value of 'α' for an irreversible system as 0.562 and the value of 'αn' were 0.969. The number of electron 'n' transferred in the electro oxidation of Ketoconazole calculated was 1.73 ≈ 2. The value of k^0 can be determined from the intercept of the above plot if the value of $E^{0'}$ are 0.085 and 0.087. The value of $E^{0'}$ in Eq. (1) can be obtained from the intercept of E_p vs. *v* curve by extrapolating to the vertical axis at *v* = 0 [20]. In our system the intercept for E_p vs. log *v* plot was 0.788 for LSV and 0.798 for CV methods. The values of 'k^0' obtained are found to be $k^0 = 7.58 \times 10^2$ s^{-1} and $k^0 = 8.12 \times 10^2$ s^{-1}, for LSV and CV methods respectively.

3.4. Calibration Curve

In order to develop a voltammetric method for determining KTZ, we selected the differential-pulse voltammetric (DPV) mode and square wave voltammetric (SWV) mode, because these techniques produce voltammograms with sharper and better defined peaks at lower concentration of Ketoconazole, than those obtained by CV mode. According to the obtained results, it was possible to apply these techniques to the quantitative analysis of Ketoconazole. The phosphate buffer solution of pH = 7.0 was selected as the supporting electrolyte for the quantification of Ketoconazole. Differential pulse voltammograms and square wave voltammograms obtained at different concentration of KTZ with peak currents increased linearly with increasing concentrations, as shown in Fig. 4(a) and 5(a).

"Here Figure 4(a), 5(a)"

Using the optimum conditions described previously, linear calibration curves were obtained for Ketoconazole in the range of 5×10^{-8} M to 5.5 $\times10^{-7}$ M for DPV and1.0×10^{-8} M to 5.0 $\times10^{-6}$ M for SWV (Fig. 4(b) and 5(b)) and the linear equations were;

$I_{pa}(\mu A) = 15.05$ [KTZ] M + 3.12; (r = 0.98) for DPV

$I_{pa}(\mu A) = 11.25$ [KTZ] M + 0.65; (r = 0.99) for SWV

"Here Figure 4(b), 5(b)"

The deviation from linearity was observed for more concentrated solutions, due to the adsorption of oxidation product of Ketoconazole on the surface of pretreated pencil electrode. Related statistical data of the calibration curves were obtained from the five different determinations. For DPV the limit of detection (LOD) and quantification (LOQ) were 3.98×10^{-9} M and 1.32×10^{-8} M, and for SWV the detection limit (LOD) and quantification (LOQ) were 0.961×10^{-9} M and 3.20×10^{-9} M respectively. The LOD and LOQ were calculated using the following equations, comparisons of these two method square wave voltammetry is good and sensitive tool.

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

where, 's' is the standard deviation of the peak currents of the blank, and 'm' is the slope of the calibration curve [21]. The comparison of concentration range and detection limits for Ketoconazole using different analytical methods are tabulated in **Table.1**. The proposed method was compared with the previous literature methods [22-28] and the proposed method is more sensitive and selective, with good precision and with low detection limit.

"Here Table 1"

3.5. Effect of interferences

The possible several interfering substances on the determination of 10.0 µM ketoconazole, was investigated. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than ±5% for determination of ketoconazole. Effect of the interfering ions was verified by comparing the anodic peak current of ketoconazole after 100-fold excess addition of Glucose, citric acid, dextrose, gum acacia, lactose, oxalic acid, sucrose, starch, ascorbic acid, tartaric acid and uric acid. The experimental result showed that foreign substances did not interfere with the voltammetric signals listed in **Table 2**. Thus, the procedures were able to assay ketoconazole in presence of excipients, and hence the method can be considered as specific.

"Here Table 2"

3.6. Pharmaceuticals sample analysis

The applicability of proposed method in commercially available ketoconazole pharmaceuticals by using differential pulse voltammetry was subsequently recorded employing the optimized conditions. The

procedure for the preparation of pharmaceutical samples was as described in the exprimental section. To quantify the unknown amount of ketocanazole in solutions, the concentration of the pharmaceutical formulations was determined with the help of calibration curve. The obtained results are in good agreement with the content marked in the label (Table 3). Recovery studies were carried out after the addition of known amount of drug for various pre-analyzed formulations of KTZ. The recoveries are found to lie in the range of 96.8% to 98.5%.

"Here Table 3"

3.7. Determination of Ketocanazole in urine sample and human serum plasma

The developed differential pulse voltammetric method for the Ketocanazole determination was applied to urine samples. The recoveries from urine were measured by spiking drug free urine with known amounts of Ketocanazole. The urine samples were diluted 100 times with the phosphate buffer solution before analysis without further pretreatments. A quantitative analysis can be carried out by adding the standard solution of Ketocanazole into the detection system of urine samples and the peak intensity linearly increased. The calibration graph was used for the determination of spiked Ketocanazole in urine samples with a recovery range of 99.4 to 100.2 %. The results of three urine samples obtained are listed in Table 4.

The determination of Ketocanazole in a human serum plasma sample was investigated. Methanol was added to plasma sample and then centrifuged. Sample was diluted with PBS pH=7.4 and spiked with solutions of Ketocanazole by standard addition method. Differential pulse voltammetric method was applied to the analysis of samples. The recovery determined was in the range from 99.5 to 100.7 %, and the RSDs are listed in Table 4. Thus, satisfactory recoveries of the analyte from the real samples make the developed method applicable in clinical analysis.

"Here Table 4"

3.8. Stability and repeatability of electrode

To investigate the repeatability of the treated pencil electrode, electrochemical oxidation of ketocanazole at the same conditions were performed by using different electrodes. The results showed that an acceptable repeatability with a RSD of 3.77% was obtained. The results indicate that the pretreated electrode has a good stability and reproducible.

4. Conclusion

A new analytical tool was developed for the determination of ketoconazole which is very simple, high sensitive with a low cost and quick analysis. The pretreatment increased the electrochemical activity of the pencil graphite electrode significantly. The limit of detection value obtained for KTZ was 3.98×10^{-9}M for DPV and 0.961×10^{-9} M for SWV. The PGPE exhibited a stable and reproducible response for ketoconazole determination without any influence of physiologically common interferences. The usefulness of the method was demonstrated by applying it to the analysis of pharmaceutical preparations and real samples. The technique is suitable for quality control laboratories.

Reference

1. F. C. Odds, L. J. R. Milne, J. C. Gentles and E. H. Ball, *J. Antimicrob. Chemother*, 1980, **6**, 97-104.
2. E. W. Gascoigue, G. J. Barton, M. Michaels, W. Mendlermans and J. Heykants, *Clin. Res. Rev*, 1981, **1**, 177-182.
3. F.M. Abdelgawad, *Farmaco*, 1997, **52**, 119-122.
4. F.M. Abdelgawad, *J. Pharm. Biomed. Anal*, 1997, **15**, 1679-1685.
5. K. Kelani, and L.I. Bebawy, *Anal. Lett*, 1997, **30**, 1843-1860.
6. S.R. Elshabouri, K.M. Emara, P.Y. Khashaba, and A.M. Mohamed, *Anal. Lett*, 1998, **31**, 1367-1385.
7. A. El-Bayoumi, A.A. El-Shanawany, M.E. El-Sadek, and A. Abd El-Sattar, *Espectrosc. Lett*, 1997, **30**, 25-46.
8. M. Asrhaf-Khorassani, and J.M. Levy, *Chromatographia*, 1995, **40**, 78-84.
9. C.X. Zhang, and F. Vonheeren, W. Thormann, *Anal. Chem*, 1995, **67**, 2070-2077.
10. M.R. Majidi, K. Asadpour-Zeynali, and B. Hafezi, *Electrochim. Acta*, 2009, **54**, 1119–1126
11. Jayant I. Gowda and Sharanappa T. Nandibewoor, *Electrochim. Acta*, 2014, **116**, 326– 333.
12. G.D. Christian, and W.C. Purdy, *J. Electroanal. Chem*, 1962, **3**, 363-367.
13. B. Rezaei, and S. Damiri, *Sens. Actuators B*, 2008, **134**, 324 – 331.
14. B. Dogan-Topal, B. Bozal, B. T. Demircigil, B. Ulsu, and S. A. Ozkan, *Electroanalysis*, 2009, **21**, 2427–2439.
15. Wang, J. Electroanalytical Techniques in Clinical Chemistry and Laboratory Medicine; VCH: New York, 1988.
16. P. T. Kissinger, and W. R. Heineman, Laboratory Techniques in Electroanalytical Chemistry, 2nd ed.; Marcel Dekker: New York, 1996
17. D.K. Gosser, Cyclic Voltammetry: Simulation and Analysis of Reaction Mechanisms. New York; VCH. 1993
18. E. Laviron, *J. Electroanal. Chem*, 1979, **101**, 19-28
19. A. J.Bard, and L. R. Faulkner, Electrochemical Methods Fundamentals and Applications, second ed., Wiley, New York, 2006
20. W. Yunhua, J. Xiaobo, and H. Shengshui, *Bioelectrochem*, 2004, **64**, 91-97.
21. E. Swartz, and I.S. Krull, Analytical Method Development and Validation. New York; Marcel Dekkar. 1997.
22. Mojtaba Shamsipur and Khalil Farhadi, *Analyst*, 2000, **125**, 1639–1643.
23. M.A. El Ries, M.F. Abdel Ghany, L.A. Hussin, F.M. El-Anwar, and A.M. Mohamed, *Bulletin of Faculty of Pharmacy, Cairo University*, 2013, **51**, 49–55.
24. Mojtaba Shamsipur and Khalil Farhadi, *Electroanalysis*, 2000, **12**, No. 6
25. Katarzyna Mielech-Łukasiewicz and Katarzyna Rogi´nska, *Anal. Methods*, 2014, **6**, 7912–7922.

26. Allan Nilson de Sousa Dantas, Djenaine De Souza, Janete Eliza Soares de Lima, Pedro de Lima-Neto, and Adriana Nunes Correia, *Electrochimi. Acta*, 2010, **55**, 9083–9089.
27. Katarzyna Mielech-Lukasiewicz, Helena Puzanowska-Tarasiewicz, and Anna Niedzielko Anal. Lett, 2011, **44**, 955–967,
28. Lida Fotouhi, and Farzaneh Bahmani, *Electroanalysis*, 2013, **25**, 757 – 764.