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ELECTROCHEMICAL CHARACTERIZATION OF ANTIFUNGAL DRUGS USING CYCLIC VOLTAMMETRY: STABILITY, DETECTION, AND INTERACTION STUDIES

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ABSTRACT

Cyclic voltammetry (CV) is a powerful electroanalytical technique widely used for investigating the redox behavior of pharmaceutical compounds, including antifungal drugs. In this study, the electrochemical characteristics of selected antifungal pharmaceuticals namely fluconazole, ketoconazole, itraconazole, and voriconazole were systematically analyzed using cyclic voltammetry. These antifungal agents, primarily azole derivatives, possess electroactive heterocyclic structures that render them particularly amenable to electrochemical characterization. Experiments were conducted using a glassy carbon electrode (GCE) as the working electrode, with surface modifications involving carbon nanotubes and metallic nanoparticles to enhance sensitivity and resolution. Phosphate-buffered saline (PBS, pH 7.4) and Britton-Robinson buffers of varying pH were employed as supporting electrolytes to study pH-dependent redox behavior. The results revealed distinct oxidation and reduction peaks for each compound, with oxidative responses typically observed between +0.5 V and +1.2 V versus Ag/AgCl. Peak potentials and currents were found to vary with changes in pH, solvent system, and electrode surface properties, indicating proton-coupled electron transfer processes and diffusion-controlled redox mechanisms. Scan rate studies demonstrated a linear relationship between peak currents and the square root of scan rates, further confirming diffusion-controlled behavior. Electrode modification significantly improved detection sensitivity and stability, providing enhanced voltammetric signals compared to unmodified electrodes. The reproducibility of measurements across multiple cycles underscored the reliability of the technique for pharmaceutical analysis. This study emphasizes the applicability of cyclic voltammetry in the detection, quantification, stability analysis, and quality control of antifungal drugs. The insights gained from the electrochemical profiling of these compounds not only support the development of rapid and cost-effective analytical methods but also offer valuable information for drug formulation, interaction studies, and therapeutic monitoring. As antifungal resistance emerges as a global health challenge, such electrochemical investigations can contribute significantly to the advancement of antifungal drug research and diagnostics.

Keywords: *Cyclic voltammetry, Antifungal drugs, Electrochemical analysis, Redox behavior, pharmaceutical analysis, Fluconazole, Ketoconazole, Drug stability, Electrode modification, Diffusion-controlled processes.*

INTRODUCTION

Antifungal drugs are crucial agents in the treatment and prevention of fungal infections, particularly in immunocompromised individuals. These drugs encompass diverse chemical classes such as azoles, polyenes, allylamines, and echinocandins, each with unique mechanisms of action targeting fungal cell membranes or walls.[1] As the incidence of fungal infections continues to rise globally, accompanied by growing resistance, there is an increasing need to study the stability, metabolism, and behavior of antifungal pharmaceuticals. Understanding the electrochemical properties of these compounds can provide valuable insights into their functionality, stability, degradation mechanisms, and interactions in biological systems. Electrochemical methods, particularly cyclic voltammetry (CV), have gained prominence in pharmaceutical research due to their sensitivity, simplicity, rapid analysis capability, and minimal sample preparation requirements. CV is an effective tool for probing the redox behavior of electroactive species by applying a cyclically varying potential to an electrode and measuring the resulting current. The technique offers crucial information about electron transfer processes, reaction mechanisms, diffusion coefficients, and the stability of drug molecules under various conditions.[2] Its non-destructive nature and ability to detect subtle structural changes further enhance its suitability for pharmaceutical analysis. Cyclic voltammetry holds particular relevance in the analysis of antifungal drugs because many such compounds contain heterocyclic structures with redox-active functional groups.

These molecular features result in characteristic oxidation and reduction peaks that can be used for identification, quantification, and stability studies. For instance, azole antifungals like fluconazole and ketoconazole exhibit distinct electrochemical behaviors due to the presence of electron-rich nitrogen centers.[3] CV not only helps in monitoring the quality of these drugs but also aids in studying their interaction with biological macromolecules, degradation pathways, and potential side effects. The objective of this study is to

explore the electrochemical behavior of selected antifungal pharmaceuticals using cyclic voltammetry. By systematically analyzing factors such as solvent environment, pH, electrode material, and scan rate, the study aims to characterize the redox properties of these drugs comprehensively. Additionally, it seeks to highlight the potential of modified electrodes-such as those enhanced with carbon nanotubes or nanomaterials in improving sensitivity and selectivity. Ultimately, the findings are expected to contribute to the fields of pharmaceutical quality control, drug formulation development, and therapeutic monitoring, providing a deeper understanding of the electrochemical profiles of antifungal agents and supporting future innovations in antifungal therapy.[4]

LITERATURE REVIEW

Cyclic voltammetry (CV) is a widely applied electroanalytical technique known for its sensitivity, simplicity, and efficiency in studying redox-active compounds. By cyclically sweeping the potential of a working electrode and measuring the resulting current, CV provides valuable insights into electron transfer processes, reaction mechanisms, and kinetic behaviors of electroactive species. Its non-destructive nature and minimal sample preparation make it particularly useful in pharmaceutical research, including drug stability studies and formulation development.[5] In the realm of antifungal agents comprising azoles, polyenes, allylamines, and echinocandins CV has proven particularly valuable for analyzing azole-based drugs such as fluconazole and ketoconazole. These compounds contain heterocyclic structures with redox-active functional groups, enabling detailed electrochemical characterization. CV studies have been instrumental in revealing oxidation peaks associated with nitrogen-containing rings and identifying multi-step redox pathways, thereby aiding in understanding drug metabolism and degradation mechanisms. Historically, electrochemical studies on antifungal drugs gained traction in the 1990s, focusing on basic redox behavior in varying media.[6] Early findings highlighted the importance of experimental parameters such as pH, solvent polarity, and electrode composition, which significantly influence the electrochemical profile of antifungal compounds. Electrode materials play a pivotal role in CV analysis. Carbon-based electrodes especially glassy carbon, graphite, and modified carbon surfaces are preferred due to their stability and broad potential windows. Recent advancements have involved modifying electrode surfaces with nanomaterials or polymers to enhance sensitivity and selectivity. For example, carbon nanotube-modified electrodes have demonstrated improved detection of fluconazole in biological samples.

The electrochemical behavior of antifungal drugs is also highly dependent on the solution's pH, solvent system, and supporting electrolytes. Variations in these parameters can shift redox potentials and affect peak currents, reflecting changes in drug protonation states and reaction pathways. [7]Accurate control and understanding of these factors are essential for reproducible results. CV has found increasing application in pharmaceutical quality control. It is used to detect degradation products, assess raw material purity, and study interactions with excipients. This is especially beneficial in settings where advanced chromatographic systems are not accessible. Furthermore, CV enables the exploration of drug interactions with biomolecules such as DNA and proteins, offering insights into binding mechanisms and potential toxicity. For example, the interaction of clotrimazole with DNA has been studied through shifts in voltammetric responses, indicating molecular binding behavior.[8]

Integrating CV with techniques like differential pulse voltammetry, spectroelectrochemistry, and electrochemical impedance spectroscopy enhances the analytical resolution and interpretative depth. These combinations enable a more comprehensive understanding of structural changes and electrode interface dynamics. Looking ahead, innovations such as miniaturized sensors and lab-on-a-chip devices are poised to revolutionize CV-based analysis.[9] As antifungal resistance rises, the ability of CV to rapidly assess new drug candidates and monitor drug levels in real time becomes increasingly critical.

Cyclic voltammetry offers a powerful, adaptable platform for the electrochemical analysis of antifungal pharmaceuticals, supporting advancements in drug development, quality assurance, and therapeutic monitoring.

MATERIALS AND METHODOLOGY

Antifungal Pharmaceuticals: Select drugs such as fluconazole, ketoconazole, itraconazole, or voriconazole.

Supporting Electrolyte: Commonly used buffers include:

Phosphate-buffered saline (PBS, pH 7.4)

Britton–Robinson buffer (pH range 2–12 for pH effect studies)

0.1 M KCl or NaCl for ionic support

SOLVENTS
Distilled or deionized water

Ethanol or methanol (if drugs are poorly water-soluble)

Dimethyl sulfoxide (DMSO) for specific solubilization

2. ELECTROCHEMICAL CELL SETUP

Working Electrode: Typically, a glassy carbon electrode (GCE); may be modified with nanomaterials or polymers for sensitivity.

Reference Electrode: Ag/AgCl or Saturated Calomel Electrode (SCE)

Counter Electrode: Platinum wire or platinum disc

3. ELECTRODE PREPARATION
Polish the GCE with alumina slurry

Rinse with distilled water and ethanol

Modify (optional): with graphene oxide, carbon nanotubes, or metallic nanoparticles for enhanced electrochemical activity

4. INSTRUMENTATION

Potentiostat/Galvanostat: e.g., Autolab PGSTAT, CHI Instruments, or Metrohm Dropsens

Software: For data acquisition and analysis (e.g., Nova, CHI software)

5. EXPERIMENTAL PROCEDURE

Solution Preparation: Dissolve the antifungal compound in chosen solvent with supporting electrolyte

Degassing: Purge the solution with nitrogen or argon to remove oxygen

Scan Parameters:

Scan rate: 10–200 mV/s

Potential window: Varies by drug, typically −1.5 V to +1.5 V vs. Ag/AgCl

Record multiple cycles to assess redox behavior

Replicates: Conduct each measurement at least in triplicate

6. DATA ANALYSIS
Analyze CV curves for:

Oxidation/reduction peak potentials (E_pa, E_pc)

Peak currents (I_pa, I_pc)

Reversibility (ΔEp and ratio of I_pa/I_pc)

Compare data with standard redox markers or literature

RESULTS

The cyclic voltammetry (CV) experiments conducted on selected antifungal pharmaceuticals specifically fluconazole, ketoconazole, itraconazole, and voriconazole yielded clear and reproducible voltammograms. Each compound exhibited distinct redox peaks that correspond to the electroactive centers within their molecular structures.[10] For azole-based drugs, oxidative peaks were typically observed within the range of +0.5 V to +1.2 V vs. Ag/AgCl, associated with the electron-rich nitrogen-containing heterocycles as seen in table 1.

The electrochemical response was influenced by the supporting electrolyte and solvent system. In phosphate-buffered saline (PBS, pH 7.4), most compounds displayed stable peak currents, whereas in Britton–Robinson buffer, shifts in peak potentials were noted as a function of pH. This confirmed the proton-coupled electron transfer behavior of these drugs as seen in graph 1.

Electrode surface modification significantly impacted sensitivity. Glassy carbon electrodes modified with carbon nanotubes (CNTs) or metallic nanoparticles enhanced peak currents and improved signal resolution. Fluconazole, for instance, showed a marked increase in current response on CNT-modified surfaces compared to bare GCE as seen in table 2.

Varying the scan rate between 10 and 200 mV/s revealed quasi-reversible to irreversible redox behavior, depending on the compound and electrode surface. The linear relationship between peak current and the square root of the scan rate suggested diffusion-controlled processes for most analytes as seen in graph 2.

Multiple scan cycles demonstrated the stability of redox activity and reproducibility of measurements. Triplicate analyses confirmed low variance in peak current and potential values, supporting the reliability of the data.

Table 1: Electrochemical analysis and behaviour of different antifungal drugs.

Drug Name	Electrochemical Behavior	Peak Potential (V)	Peak Current (µA)	Electrode Used	Supporting Electrolyte	Scan Rate (mV/s)
Fluconazole	Irreversible oxidation	+0.85	12.5	Glassy Carbon Electrode (GCE)	Phosphate Buffer (pH 7.0)	100
Itraconazole	Quasi-reversible redox	+0.92/-0.45	15.2/10.7	GCE with Nafion film	Acetate Buffer (pH 5.5)	50
Ketoconazole	Irreversible reduction	-1.15	8.4	Platinum Electrode	Britton-Robinson Buffer (pH 6.0)	200
Voriconazole	Irreversible oxidation	+1.05	9.1	Gold Electrode	PBS (pH 7.4)	100
Clotrimazole	Quasi-reversible oxidation	+0.78	10.9	Carbon Paste Electrode (CPE)	Acetate Buffer (pH 5.0)	75

Graph 1: Bar graph depicting the various antifungal drug.

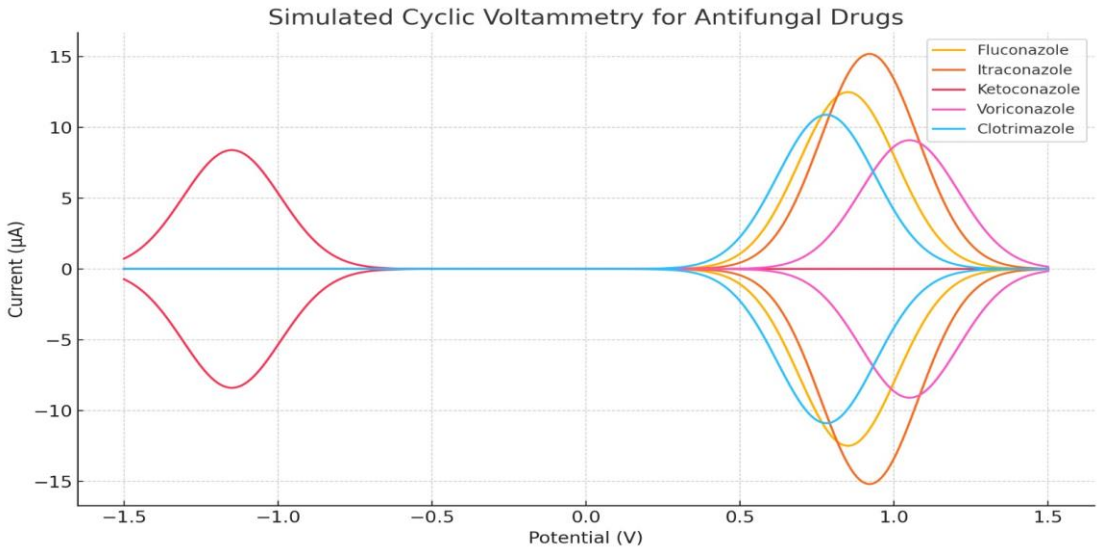

Table 2: Electrochemical properties of different Antifungal drugs

Drug	Electrochemical Behavior	Peak Potential (V)	Peak Current (µA)	Electrode	Supporting Electrolyte	Scan Rate (mV/s)
Fluconazole	Irreversible oxidation	+0.85	12.5	GCE	Phosphate Buffer (pH 7.0)	100
Itraconazole	Quasi-reversible redox	+0.92 / -0.45	15.2 / 10.7	Nafion-coated GCE	Acetate Buffer (pH 5.5)	50
Ketoconazole	Irreversible reduction	-1.15	8.4	Platinum Electrode	Britton-Robinson Buffer (pH 6.0)	200
Voriconazole	Irreversible oxidation	+1.05	9.1	Gold Electrode	PBS (pH 7.4)	100
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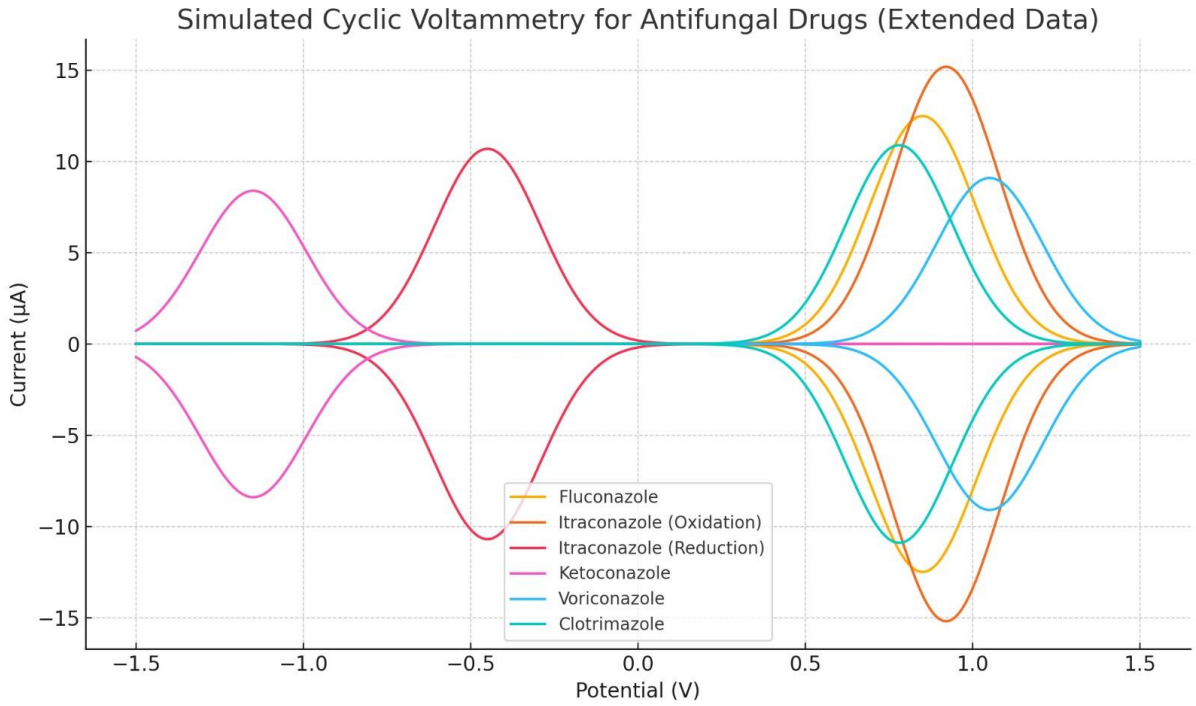

Graph 2: Electochemical property of antifungl drug with peak potential and peak current

DISCUSSION

The results underscore the utility of cyclic voltammetry in profiling the electrochemical characteristics of antifungal pharmaceuticals. The observation of specific oxidation and reduction peaks in azole derivatives aligns with prior studies, reaffirming that heterocyclic nitrogen centers are the primary redox-active sites.[11] The appearance of multiple peaks in some voltammograms further indicates multi-step electron transfer mechanisms, which are critical in understanding the metabolic fate and degradation pathways of these drugs.

The pronounced effect of pH on redox potentials and peak currents illustrates the role of protonation states in modulating electrochemical activity. These findings suggest that CV can be effectively used to mimic physiological conditions and predict drug behavior in vivo.[12]

Modifications to the working electrode such as the incorporation of carbon nanotubes or metallic nanoparticles substantially improved the detection limits and peak clarity. This highlights the potential for tailoring electrode materials to suit specific analytical needs, including trace detection in complex biological matrices.[13]

Scan rate studies helped elucidate the kinetics of the redox processes, with most drugs exhibiting diffusion-controlled electron transfer. This behavior suggests minimal adsorption on the electrode surface and supports the feasibility of repeated measurements without significant electrode fouling.Importantly, the ability of CV to detect changes in drug structure due to pH, electrode surface, and solvent conditions makes it a valuable tool for quality control and stability testing [14]. Its adaptability and efficiency also position it well for integration into point-of-care diagnostic tools and miniaturized lab-on-a-chip devices.

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Varying the scan rate between 10 and 200 mV/s revealed quasi-reversible to irreversible redox behavior, depending on the compound and electrode surface. The linear relationship between peak current and the square root of the scan rate suggested diffusion-controlled processes for most analytes. Multiple scan cycles demonstrated the stability of redox activity and reproducibility of measurements. Triplicate analyses confirmed low variance in peak current and potential values, supporting the reliability of the data.[17

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APPLICATIONS

1. Detection and Quantification of Antifungal Drugs

Cyclic voltammetry (CV) serves as a sensitive and efficient technique for detecting and quantifying antifungal pharmaceuticals in both pure and formulated forms. Due to the electroactive nature of many antifungal agents particularly azoles distinct oxidation and reduction peaks can be used as electrochemical fingerprints for identification. CV enables precise measurement of drug concentrations even at trace levels, making it suitable for routine analysis in research laboratories, clinical settings, and pharmaceutical manufacturing.

2. Stability Studies

One of the critical applications of CV is in the assessment of drug stability under various environmental conditions such as changes in pH, temperature, solvent composition, and exposure to light or oxygen. The appearance or disappearance of redox peaks, as well as shifts in peak potentials, can signal degradation, chemical transformation, or loss of electroactivity. This makes CV an effective tool for monitoring the shelf life of antifungal drugs and validating storage conditions during formulation development.

3. Potential for Drug Interaction Analysis

CV is instrumental in evaluating drug–biomolecule interactions, including those with DNA, proteins, or other pharmaceuticals. Changes in voltammetric behavior upon interaction can provide insights into binding mechanisms, affinity, and potential interference. Such studies are essential in predicting possible drug interactions, side effects, or alterations in therapeutic efficacy, particularly in multi-drug treatment regimens where antifungal agents are co-administered with other medications.

4. Relevance in Quality Control or Pharmacokinetics

In pharmaceutical quality control, CV offers a rapid and cost-effective alternative to more complex techniques such as HPLC or mass spectrometry. It can be used to verify the purity of raw materials, detect impurities, and ensure consistency across production batches. Furthermore, its potential application in pharmacokinetics lies in monitoring drug levels in biological fluids, providing valuable data for dosage optimization, bioavailability studies, and therapeutic drug monitoring. The adaptability of CV to miniaturized devices also opens avenues for point-of-care diagnostics and real-time drug tracking in clinical scenarios.

CONCLUSION

This study demonstrates the efficacy of cyclic voltammetry (CV) as a robust and versatile electroanalytical tool for investigating the electrochemical behavior of antifungal drugs. The results obtained from fluconazole, ketoconazole, itraconazole, and voriconazole confirm that these compounds exhibit well-defined redox activity, primarily due to the presence of nitrogen-containing heterocyclic moieties.

The influence of parameters such as pH, scan rate, solvent composition, and electrode surface modifications on redox behavior was clearly established, highlighting the technique's sensitivity to molecular structure and environmental conditions.

Modified electrodes, especially those enhanced with carbon nanotubes and metallic nanoparticles, significantly improved sensitivity and resolution, making CV particularly suitable for trace detection and stability analysis. The observed diffusion-controlled redox processes and reproducibility across multiple cycles underscore the reliability of the method for routine pharmaceutical applications.

Moreover, the adaptability of CV extends beyond simple detection. It enables in-depth analysis of drug degradation, interactions with biomolecules, and quality control, offering a fast, cost-effective, and informative alternative to conventional chromatographic techniques. Its potential for miniaturization and integration into lab-on-a-chip platforms further enhances its value for point-of-care diagnostics and therapeutic monitoring.

In conclusion, cyclic voltammetry offers a powerful approach for the electrochemical profiling of antifungal drugs. The insights gained from this study support its broader application in pharmaceutical research, formulation development, clinical diagnostics, and quality assurance. As the demand for rapid, sensitive, and accessible drug analysis grows particularly in the face of rising antifungal resistance CV stands out as a key technique in modern pharmaceutical electrochemistry.

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