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Investigation of the Electrochemical Behavior of the Drug Active Ingredient Pirfenidone and Its Analytical Application in Natural Samples

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ABSTRACT

Pirfenidone is an active pharmaceutical ingredient used orally in the treatment of idiopathic pulmonary fibrosis and possesses antifibrotic, antioxidant, and anti-inflammatory properties. Studies conducted on both animals and humans have observed that pirfenidone halts the progression of fibrosis, stabilizes lung function, and reduces the number of acute exacerbations in IPF patients. However, the drug is also associated with side effects such as elevated liver enzymes, liver damage, gastrointestinal problems, photosensitivity reactions, and rashes. Therefore, monitoring liver function during treatment is essential.

The analysis of pirfenidone in physiological samples such as blood and urine is of great importance. According to the literature, chromatographic and spectrofluorimetric, spectrophotometric, and capillary electrophoresis methods are generally used for the determination of pirfenidone. Techniques such as high-performance liquid chromatography (HPLC) are also common, but these methods have disadvantages such as requiring expensive equipment, large amounts of organic solvents, long pretreatment times, and expert analysts.

In recent years, electroanalytical methods have gained significant interest in the scientific community due to their speed, cost-effectiveness, and practicality. In this project, a new analytical method has been developed for the analysis of pirfenidone in natural and pharmaceutical dosage samples, which had not been previously determined by any electrochemical methods. The electrochemical properties of pirfenidone were investigated using voltammetric methods, and a sensitive, reliable, and reproducible analytical method was developed using square wave voltammetry (SWV) and differential pulse voltammetry (DPV) techniques. This newly developed method has successfully achieved the analytical application of pirfenidone in natural samples and commercial formulations and has been introduced into the literature as a novel method.

KEYWORDS

Differential pulse stripping voltammetry, electrochemistry, ıdiopathic pulmonary fibrosis, pirfenidone

1. INTRODUCTION

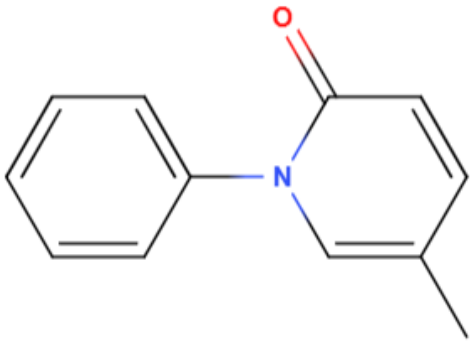
Pirfenidone is a synthetic pyridone compound that inhibits the progression of fibrosis and is administered orally to treat idiopathic pulmonary fibrosis (IPF). Although classified as an immunosuppressant, the mechanism of action of pirfenidone is not fully elucidated; it exhibits antifibrotic, anti-inflammatory, and antioxidant properties (1). This novel antifibrotic agent has demonstrated inhibition of fibrosis progression in animal studies. Promising results from select clinical parameters suggest its potential to stabilize lung function in individuals with IPF and to prevent the occurrence of acute exacerbations (2). Orally administered

pirfenidone, also known as 5-methyl-1-phenyl-2-[1H]-pyridone, is a small molecule with high oral bioavailability. Peak plasma concentrations are achieved within 30 minutes post-administration, and the compound is primarily bound to serum albumin in plasma. The majority of pirfenidone, approximately 70-80%, undergoes hepatic metabolism via the cytochrome P450 pathway, particularly via the CYP1A2 enzyme. Caution is warranted when administering pirfenidone in severe and end-stage cases. Metabolized pirfenidone is retained as 5-carboxy-pirfenidone and eliminated through excretion. While dose adjustments are made for mild and moderate renal impairment, pirfenidone use is contraindicated in cases of severe renal dysfunction (3).

Pirfenidone (PFD) demonstrates significant clinical efficacy against idiopathic pulmonary fibrosis (IPF); however, it exhibits poor tolerability due to substantial gastrointestinal adverse effects, including nausea, vomiting, anorexia, and severe phototoxicity (4,5). As observed in PFD medication, drug-induced phototoxicity is precipitated by exposure of the skin and/or eyes to topical or systemic administration of pharmaceutical substances, followed by exposure to sunlight. PFD is predominantly (82%) excreted in unchanged form through the urine. It requires repeated administration due to its relatively short half-life of 2.4 h (6,7). According to official monographs, a drug or drug product is considered stable if its physical, chemical, therapeutic, and toxicological characteristics remain unaltered. Consequently, the determination of pirfenidone in biological samples is of considerable importance.

A variety of analytical techniques have been commonly used for the quantification of pirfenidone in natural and pharmaceutical dosage samples. These techniques include chromatographic, spectrofluorometric, spectrophotometric, and capillary electrophoresis methods (8). Furthermore, high-performance thin layer chromatography (HTPLC) and combined high-performance liquid chromatographic (HPLC) approaches, incorporating diverse detectors such as ultraviolet (UV), mass spectrophotometric (MS), and composite array detector (CAD), are also utilized (9). These methodologies generally circumvent the need for costly instrumentation, excessive organic solvent consumption, prolonged sample preparation, and specialized analytical expertise. Consequently, researchers have initiated investigations into novel analytical techniques for substance allocation in pharmaceutical dedecetion. Recent years

have witnessed a surge of interest in electroanalytical methods within the scientific community, attributed to their notable advantages, including rapidity, cost-effectiveness, practicality, and reliability (10). The primary objective of this study is to investigate the natural and pharmaceutical dosage forms of pirfenidone, which has not been previously characterized electrochemically, in order to develop a novel analytical method for its analysis in various samples. To achieve this goal, the electrochemical properties of pirfenidone were examined using voltammetry techniques. Subsequently, a sensitive, reliable, and reproducible analytical method was developed, and method validation studies were conducted using voltammetry techniques such as square wave (SW) and differential pulse (DPV). The most sensitive electroanalytical studies were performed for the analytical application of pirfenidone in natural samples and commercial formulations. Consequently, this research contributes to the analytical method literature for the quantification of the active pharmaceutical ingredient in pirfenidone-based medications. Based on these findings, the developed electroanalytical method for pirfenidone demonstrates potential for practical applications in pharmaceutical quality control and clinical monitoring.



Scheme 1. Molecular structure of pirfenidone

2. EXPERIMENTAL

2.1. Apparatus and reagents

The electrochemical cell section employed a potentiostat/galvanostat model V01205 from IviumSoft for electrochemical data acquisition, functioning as an electrochemical analyzer in combination with a three-electrode system. This configuration incorporated a working electrode (BASi, MF-2010), an Ag/AgCl reference electrode (BASi, MF-2052), and a platinum wire counter electrode (BASi, MW-1032). pH measurements of the necessary solutions were conducted throughout the experiment using a portable pH meter from ISOLAB. Solution preparation involved precise weighing using an AXIS model balance (±0.0001 g precision) from ACN220. Homogeneous sample preparation was achieved through the use of an ultrasonic bath.

Analytical grade reagents were employed for the precise and accurate preparation of requisite stock solutions. Pirfenidone (Scheme 1), an analytical grade reagent, was procured from Merck at ≥97%. The stock solution of pirfenidone was formulated at 500 mg/L by dissolving 0.0050 g of pirfenidone in 10 mL of methanol. For pH 1.0, a 0.1 M hydrochloric acid (HCl) solution served as the supporting electrolyte, whereas a 0.04 M Britton Robinson (BR) buffer solution was utilized as the supporting electrolyte for pH values between 2.0 and 12.0. The BR buffer solution was composed of 2,5 g boric acid (99.8%), 2,7 mL acetic acid (Glacial, ReagentPlus®, ≥99%), and 2.3 mL phosphoric acid (85%). The supporting electrolyte was adjusted to desired pH values using 5 M NaOH or 0.1 M HCl solutions. Distilled water was utilized for the preparation, dilution, and cleaning of all solutions. The electrode was cleaned using a 1:1 methanol-water mixture. When not in use, stock solutions were stored in a dark refrigerator. All electrochemical measurements were conducted at room temperature (25 °C±2).

2.2. Preparation of the electrode

The electrodes require cleaning prior to use. This is accomplished by removing the paste from the electrode surface using a wire and spare parts. Subsequently, the electrodes are immersed in a 1:1 ratio methanol-distilled water solution and subjected to ultrasonic cleaning for 10 minutes. Following this, the electrodes are dried in an oven for 10 minutes. Once dry, the electrodes are filled with carbon paste using spatula tools.

For the production of a bare carbon paste electrode (CPE), 30% mineral oil by mass

was initially weighed in a mortar, and graphite was subsequently added to approximately 70% by mass. This mixture was then homogenized through press-mixing for approximately 2 hours to ensure complete uniformity. The prepared homogenized carbon paste was subsequently inserted into the BASi MF-2010 hollow Teflon electrode, which had been thoroughly cleaned and dried with water and methanol, using a plastic syringe needle. Finally, the bare CPE was polished with zero-grit sandpaper to obtain a smooth electrode surface.

2.3. General Lines for the preparation of pharmaceutical samples

Pirfect®, a commercial introduction of a novel detailed electroanalytical method for accuracy and precision studies and investigations for recyclable studies on synthetic blood serum samples. Initially, DPV measurements of the supporting electrolyte solution at pH 1.0 (0.1 M HCl) were conducted. The commercial formulation (6 mg/L) was subsequently added to this solution, and three measurements were performed using DPV technology.

To prepare spiked samples in the synthetic human serum, 9 mL of synthetic blood serum and 1 mL of 500 mg/L pirfenidone stock solution were combined, using human male AB plasma obtained from Sigma Aldrich. The spiked blood samples were subjected to homogeneous treatment in an ultrasonic bath for approximately 30 minutes without any pre-treatment. The synthetic human serum was subsequently stored in darkness at 4 °C until analysis by DPV on CPE in pH 7.0 BR buffer solutions.

3. RESULTS AND DISCUSSION

3.1. Comparing the Sensing of Nanosensors

To determine which electrode exhibits greater sensitivity, GCE, CPE, and MWCNTPE sensors were compared in the analysis of pirfenidone. Under identical experimental conditions (Pulse time: 5ms, pulse amplitude: 50mV, step potential: 5mV, scan rate: 100mV/s), three replicate measurements were conducted using Differential Pulse Voltammetry (DPV) for all three electrodes. The current value of pirfenidone was measured at 2.65 mA with CPE, 1.60 mA with GCE, and 1.80 mA with MWCNTPE. The peak potentials of pirfenidone were observed at 1360 mV, 1310 mV, and 1315 mV, respectively.

Based on the characteristic properties of the electrodes, CPE was identified as the most sensitive sensor, exhibiting the highest anodic signals. However, regarding catalytic properties, GCE demonstrated the highest activity, with the most pronounced anodic signal observed in the positive

region at 1310 mV. Due to the dispersed nature of the signals obtained in quantitative analysis, CPE was selected for its sensitivity and high flow characteristics. Subsequent investigations were conducted using this CPE.

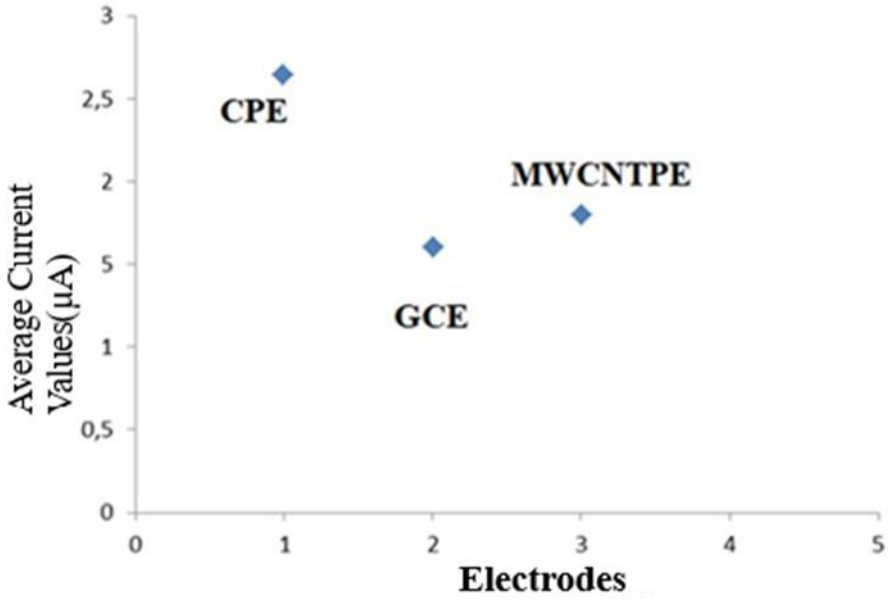


Figure 1. CPE 2,65 mA (1360 mV) GCE 1,60 mA (1310 mV), MWCNTPE 1,80 mA (1315mV)

3.2. Investigation of Electrochemical Properties of Pirfenidon

To investigate the pirfenidon electrode behavior, cyclic voltammograms from 0 V to 1.4 V potentials were obtained at various scan rates in pH 7.0 BR buffer solutions utilizing the CPE (Figure 2). As illustrated in Figure 2, a distinct, characteristic, and well-defined peak of 20 mg/L pirfenidon was observed in a single direction at scan rates ranging from 5 mV/s to 1000 mV/s. While a well-characterized anodic peak of approximately +1.06 was detected in the oxidation direction at different scan rates, no reduction peak of pirfenidon was observed in the reverse scan on the CPE by CV. This phenomenon serves as a strong indication that pirfenidon undergoes an irreversible electrochemical reaction.

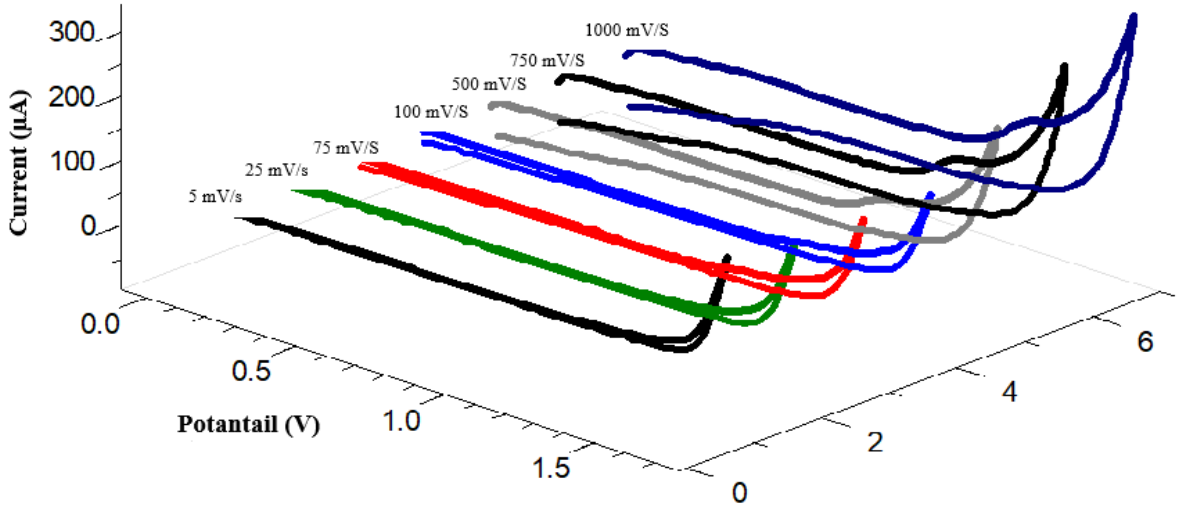


Figure 2. Cyclic voltammograms at different scan rates on CPE with 20 mg/L Pirfenidone

Additionally, scanning rate studies using cyclic voltammetry provide valuable information about the transport process of pirfenidon, specifically whether it is diffusion or adsorption controlled. Consequently, cyclic voltammograms of pirfenidon were obtained at various scanning rates between 5 mV/s and 1000 mV/s at pH 7.0 BR buffer solution on CPE (Figure 2). Moreover, the logarithm of the scanning rate (log v) was calculated in relation to the logarithm of the peak current (log Ip) over a wide range of scanning potentials for pirfenidon. A linear equation with a slope of 0.6852 was obtained between these two parameters (log v and log Ip). For the transport process type, when these slope values are 0.5, it is considered to be diffusion controlled, and when 1.0, it is considered to be adsorption controlled [REFERENCES]. The d(Ip)/d (log (v)) slope value for the anodic peak of pirfenidon was calculated as 0.685. Given that this slope value is closer to 0.5, it can be concluded that the pirfenidon electrode process is diffusion controlled.

$$\log Ip\ (\mu A) = 0.685 \log v\ (V/s) - 0.980 \quad R^2=0.9901\ (1)$$

In addition, when the scanning rate was increased from 5 mV/s to 1000 mV/s, the anodic peak potential (Ep) value of pirfenidon also shifted to more positive regions. The slope of the linear curve obtained for Ep versus log v was found to be 53 mV on the CPE. The peak potential shift depending on the scanning rates

indicates that electrons are involved in the electrode reaction of pirfenidon.

$$Ep\ (V) = 0.053 \log v\ (V/s) + 1.328$$

$$R2=0.9863\ (2)$$

An additional parameter to elucidate the electrochemical behavior of pirfenidon, specifically its kinetic model, is the relationship between the square root of the scan rate and the peak currents. The effect of the square root of the scanning rates, varying in the range of 5 mV/s to 1000 mV/s, on the peak current flow demonstrated a linear relationship. This linear relationship between peak currents and the square root of the scanning rate can be expressed mathematically.

$$Ip\ (\mu A) = 14.55\ \sqrt{v}\ (V/s) - 1.661$$

$$R2=0.9717\ (3)$$

3.3. Studying the pH effect

One of the most significant factors influencing the electroanalytical quenching peak current peak potential is pH. To determine the optimal supporting electrolyte and ideal pH solution, supporting electrolytes at various pH levels between 1.0 and 8.0 were investigated using Differential Pulse Stripping Voltammetry (DPSV) with Carbon Paste Electrode (CPE). For pH 1.0, a 0.1 M HCl solution was utilized, while Brittson-Robinson supporting electrolyte was employed for buffer solutions between pH 2.0 and 8.0. BR buffer recordings at different pH levels were obtained for 2M NaOH solutions. Subsequently, Differential Pulse Voltammetry (DPV) measurements were conducted for 10 mg/L pirfenidone in various pH environments under identical experimental conditions.

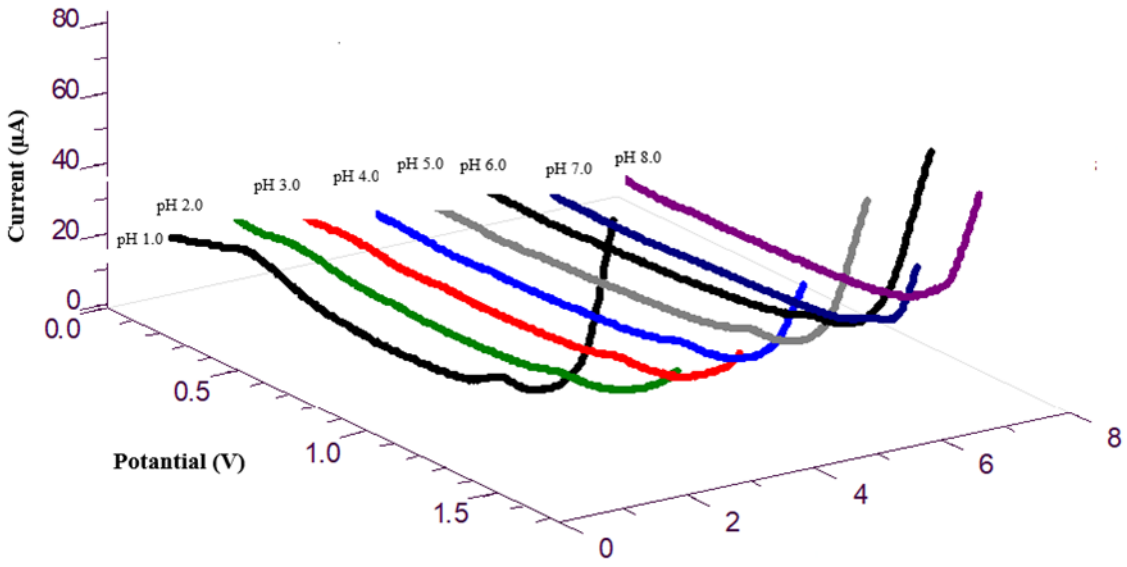


Figure 3. pH Investigation in DPV

As illustrated in the figure, the anodic signal of pirfenidone was distinctly recorded between pH 1.0 and pH 7.0, whereas no peak was observed at pH 8.0 and higher. For precise detection of pirfenidone, pH 1.0 (0.1M HCl) support solution, which yielded the highest peak, was selected as the optimum pH. Furthermore, it was observed that the anodic potential of pirfenidone shifted towards less positive components with increasing pH. This phenomenon demonstrates the hydronium (H+) ion strength of pirfenidone at electrode temperatures. As the pH value increased, the peak potential of pirfenidone shifted towards lower potentials, and consequently, equations with two distinct slopes were derived as shown below.

$$Ep = -0.0073\ pH + 1.264\ R^2 = 0{,}9099 \text{ for the from pH 1.0 to 5.0} \quad (4)$$

$$Ep = -0.0015\ pH + 1.320\ R^2 = 0{,}9985 \text{for the from pH 6.0 to 8.0} \quad (5)$$

3.4. Optimum Electroanalytical Modules

Electroanalytical parameters such as step potential, pulse amplitude, pulse time, scanning rate, accumulation time and accumulation potential in DPSV technique were optimized. The voltammetric

measurements were conducted using CPE at pH 1.0 BR buffer solutions in the presence of 10 mg/L pirfenidone to determine optimal values.

To ascertain the optimal step potential, DPS voltammograms with varying step potentials ranging from 1 mV to 7 mV were obtained. Step potentials up to 5 mV resulted in significant increases in pirfenidone peak current. However, no significant change in the pirfenidone peak current was observed in subsequent stepwise potential increases. Consequently, 5 mV was selected as the optimal step potential, yielding the highest current and most uniform peak shape.

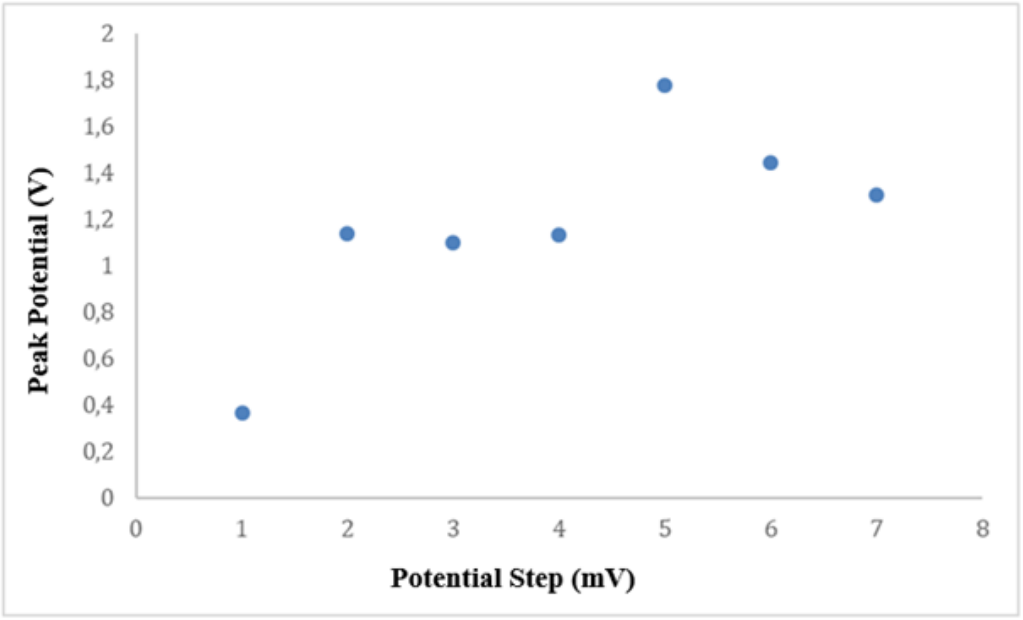
To determine the optimal pulse amplitude, DPS voltammograms at different pulse amplitudes ranging from 20 mV to 180 mV were obtained. Significant increases were observed in the peak signal of pirfenidone at pulse amplitudes up to 160 mV. However, subsequent decreases were noted in the pirfenidone peak current at higher pulse amplitudes. Therefore, 160 mV was selected as the optimal pulse amplitude, providing the highest current and most uniform peak shape.

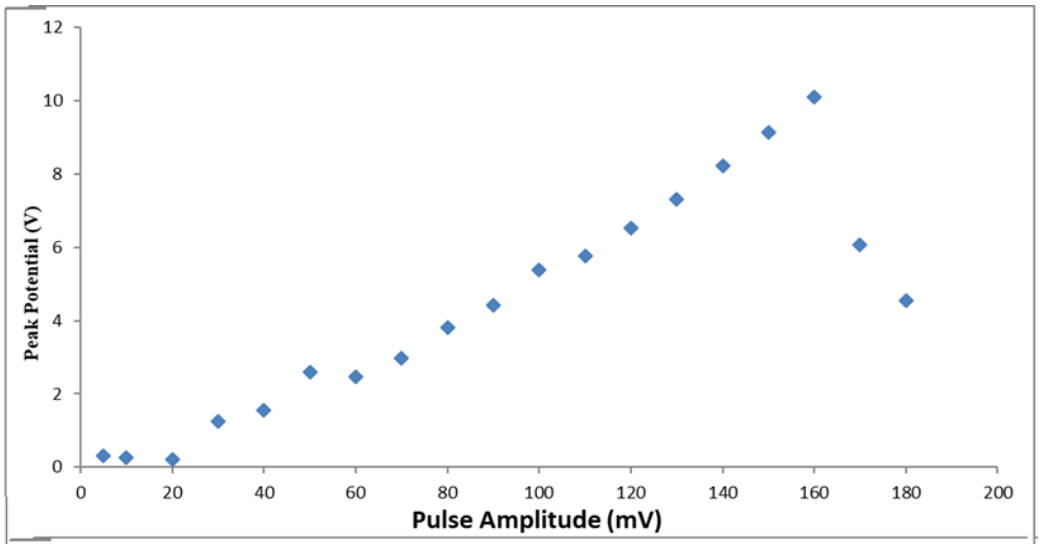
To optimize the pulse time parameter, electrochemical measurements at different pulse times ranging from 1 ms to 11 ms were conducted by DPSV on the CPE in pH 1.0 BR buffer solutions. Steady increases in pirfenidone peak current were observed with increasing pulse times up to approximately 10 ms. Subsequently, an insignificant and declining change in pirfenidone peak current was noted at longer pulse times. Consequently, 10 ms was selected as the optimal pulse time, yielding the highest current and most uniform peak shape.

DPS voltammograms were recorded at various deposition potentials ranging from -300 mV to 300 mV to determine the optimal deposition potential, a critical parameter in stripping techniques. No significant variations were observed in the pirfenidone peak current at different accumulation potentials. The difference between the highest and lowest peak currents was merely 2.42 percent of the anodic signal of pirfenidone obtained at different accumulation potentials. In determining the optimal accumulation potential, the most well-defined signal was considered, rather than the peak current value. Consequently, 0 mV was selected as the optimal accumulation potential, at which the most refined DPS voltammogram was obtained.

Consequently, the operating parameters of the developed differential pulse voltammetry (DPV) method for pirfenidone determination were optimized sequentially on the carbon paste electrode (CPE) in pH 1.0 Britton-Robinson (BR) buffer solutions. The optimized parameters

included a pulse amplitude of 160 mV, a step potential of 5 mV, and a pulse time of 10 ms.





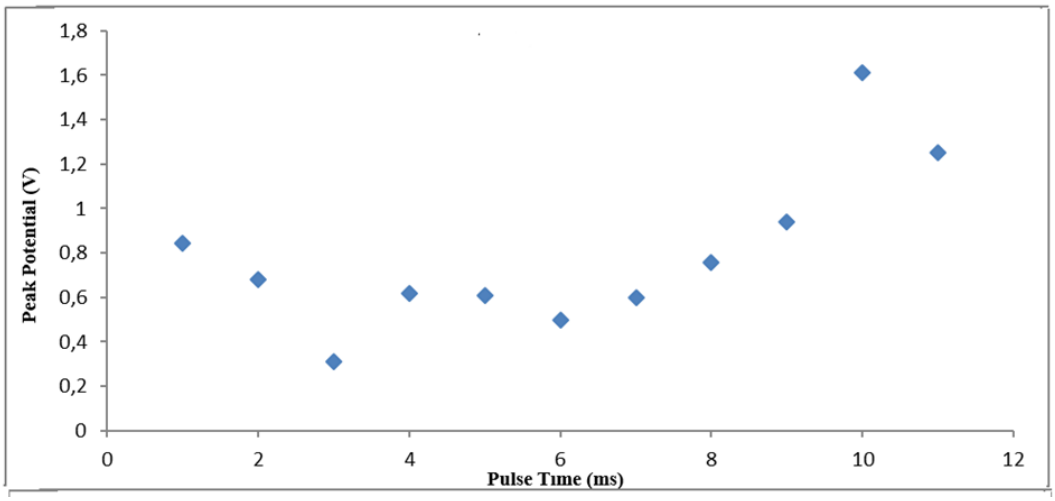


Figure 4. Optimized DPV operating models for the determination of pirfenidone on CPE in pH 1.0 BR buffer solutions

3.5. Calibration in DPV

Differential Pulse Voltammetry (DPV) was performed under optimal conditions (pulse time: 10ms, step potential: 5mV, pulse width: 160mV, scan rate: 70mV/s) in 0.1M HCl using the standard addition method. The resulting voltammograms were used to plot anodic signals against concentration. Analysis of this graph revealed a linear working range spanning from 0.8 mg/L to 38 mg/L.

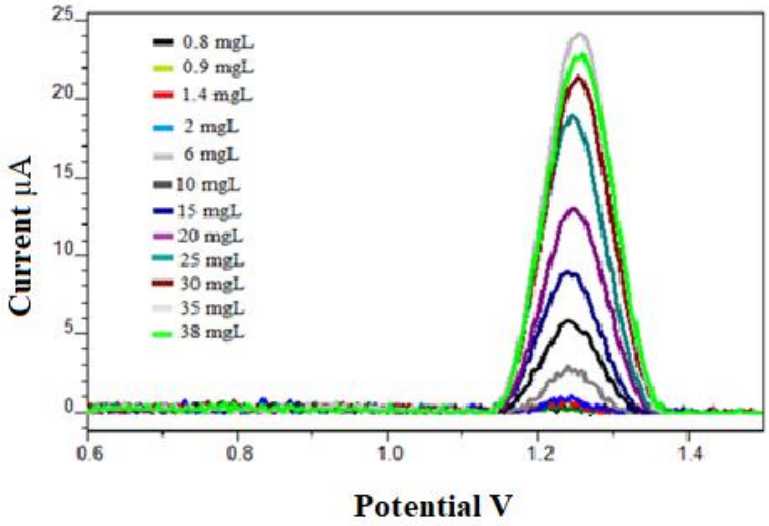


Figure 5. Calibration in DPV

Ip(mA) =0.6456c – 0.3857 R2 = 0.9936

The determination limits (LOD and LOQ) were subsequently derived from the calibration graph. The formulas 3 s/m and 10 s/m were applied to calculate LOD and LOQ, respectively, where “s” represents the standard deviation and m denotes the slope of the calibration graph equation. Application of these formulas yielded values of 0.173 for TS and 0.577 for TAS. Moreover, the proximity of the correlation coefficient (R2) to unity in the calibration graph suggests that the developed DPV method exhibits a high degree of sensitivity.

3.6. Selectivity Study

The selectivity of a newly developed method is a critical aspect of its validation process. To evaluate the selectivity of the DPV method, an investigation was conducted to assess the interference effects of various organic and inorganic compounds, including iron, copper, cadmium, magnesium, lead, zinc, dopamine, uric acid, ascorbic acid, mepivacaine, and rosuvastatin. The percentage recoveries of pirfenidone were determined at mass ratios of 1:1, 1:5, and 1:10. Results indicated that interference species had minimal impact at 1:1 and 1:5 mass ratios. However, at 1:10 concentrations, dopamine and rosuvastatin exhibited significant interference in pirfenidone determination, primarily due to their electroactive properties. Despite these challenges, the data presented in the Table demonstrates that pirfenidone can be reliably quantified even in the presence of potential interfering substances.

Table 1. Selectivity Study in Organic and Inorganic Compounds

Mass ratio ($m_{pirfenidon}/m_{\text{affecting substance}}$)			
Interference Substance	1:1	1:5	1:10
	Effect (%)	Effect (%)	Effect (%)
Fe (III)	96,136	56,637	55,242
Cu (II)	89,868	74,042	65,915
Cd	96,347	69,312	66,438
Mg	95,990	76,800	78,723
Pb (II)	100,041	96,710	97,204
Zn (II)	93,122	82,380	97,831
Dopamine	104,450	77,398	40,241
Uric acid	104,826	85,900	80,464
Ascorbic acid	79,857	105,808	84,851
Mepivacaine	106,517	84,450	62,821
Rosuvastatin	103,541	145,804	173,46

3.7. Analytical Application of Pirfenidone in Samples

To evaluate the accuracy and precision of a novel electroanalytical method, recovery analyses were performed utilizing commercial Pirfect® formulations and synthetic blood serum samples. The procedure commenced with differential pulse voltammetry (DPV) measurements of a 0.1 M HCl support electrolyte solution. Subsequently, a 6 mg/L commercial formulation sample was introduced to this solution, followed by three DPV measurements. Upon addition of a standard pirfenidone solution, the anodic current increases of pirfenidone were used to calculate a recovery of 98.50±4.23% and a relative error of -1.50%.

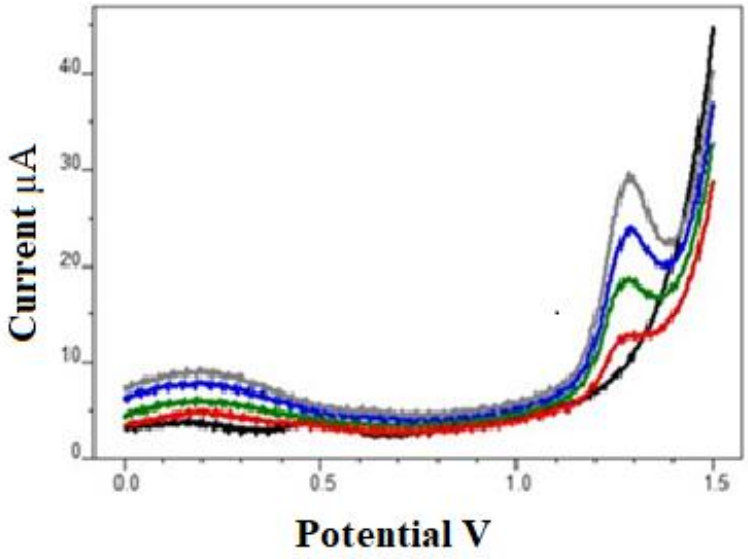


Figure 6. Analytical Application in Natural Samples

Table 2. Analytical Application in Natural Samples

Samples	Affected	Added (µM)	Found(µM)	Recovery (%)	RSD (%)	Relative Error (%)
Commercial medicine (6 mg/L)	6mg/L	0	5,91±0,14	98,50±4,23	4,30	-1,50
Blood serum	0	12	11,63±0,11	96,95±5,45	5,64	-3,07

Synthetic blood serum was obtained from Sigma-Aldrich. Initially, DPV measurements of 0.1M HCl support electrolyte solution were conducted. Three replicate measurements were performed on the blood serum sample. Standard pirfenidone solution (12mg/L) was subsequently added to the blood serum sample, and anodic current increases were determined, yielding a recovery of 96.95±5.45% and a relative error of -3.07%.

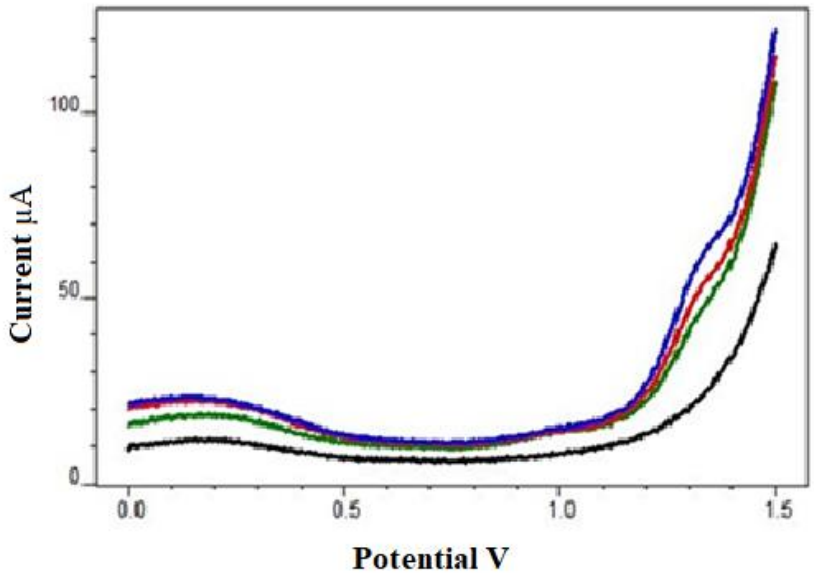


Figure 7. Analytical Application in Natural Samples

4. Conclusions

This investigation explored the electrochemical properties of pirfenidone utilizing differential pulse voltammetry. Key parameters of the DPV technique, encompassing pulse amplitude, step potential, and scan rate, underwent individual optimization. A prominent peak, suitable for analytical applications, was detected in the range of 1300 mV-1400 mV (vs. Ag/AgCl). The efficacy of the proposed methodology was validated through successful implementation in natural samples. Experiments conducted on commercial pharmaceutical preparations and blood serum yielded recovery percentages of 98.50±4.23 and 96.95±5.45, respectively. The method's precision and accuracy were substantiated by low relative standard deviations (4.30% and 5.64%, respectively) and minimal relative errors (-1.50% and -3.07%, respectively). To assess the method's selectivity, potential interference from various organic and inorganic compounds, including Fe (III), Cu (II), Cd, Mg, Pb (II), Zn (II), dopamine, uric acid, ascorbic acid, mepivacaine, and rosuvastatin, was evaluated. Pirfenidone exhibited favorable selectivity among the interacting substances at both 1:1 (m/m) and 1:5 (m/m) ratios. In conclusion, this research culminated in the development of an innovative electroanalytical technique for the quantification of pirfenidone, a pharmaceutically significant active ingredient.

5. Ethics approval and consent to participate

This study does not need any Ethics report.

6. Consent for publication

The Authors give consent for publication.

7. Availability of data and materials

All data and materials of the paper are available to the public.

8. Authors' contributions

Nuran KÖKENER: Methodology, experimental, validation, writing—original draft preparation, supervisor,

Barkın TUNAY: Methodology, experimental, validation, supervisor,

Ersin DEMİR: Methodology, experimental, validation, writing—original draft preparation, supervisor,

Nida AYDOGDU: Experimental, writing—review and editing,

Murat MISIR: Methodology, experimental, validation, writing—original draft preparation, supervisor,

Disclosure statement

No potential conflict of interest was reported by the author(s).

REFERENCES

1. Oku, H., Shimizu, T., Kawabata, T., Nagira, M., Hikita, I., Ueyama, A., … Arimura, A. (2008). Antifibrotic action of pirfenidone and prednisolone: different effects on pulmonary cytokines and growth factors in bleomycin-induced murine pulmonary fibrosis. European journal of pharmacology, 590(1–3), 400–8. doi:10.1016/j.ejphar.2008.06.046
2. Bando, M. (2016). Pirfenidone: Clinical trials and clinical practice in patients with idiopathic pulmonary fibrosis. Respiratory Investigation, 54(5), 298–304. doi:10.1016/j.resinv.2016.03.007
3. Özer, M. (2022). Üretra Yaralanması Oluşturulan Sıçanlarda Pirfenidonun Üretral Fibrozise Etkisi. Tıpta Uzmanlık Tezi, Sağlık Bilimleri Üniversitesi, Üroloji Kliniği, İstanbul.
4. H. Taniguchi, et al., Eur. Respir. J., 35, 821 – 829 (2010).
5. C. Schaefer, D. Ruhrmund, L. Pan, et al., Eur. Respir. Rev., 20, 85 – 97 (2011).
6. K. R. Flaherty, et al., Eur. Respir. J., 52 (2018).
7. L. Richeldi, U. Yasothan, P. Kirkpatrick, Nat. Rev. Drug-Discov., 10, 489 – 491 (2011).
8. Dural, E., Köz, S., Köz, S. (2021). Development and validation an YPSK - UV method for determination of esomeprazole and pirfenidone simultaneously in rat plasma: application to a drug monitoring study. İstanbul Journal of Pharmacy, 51(1), 16–25. doi:10.26650/istanbuljpharm.2020.0091

9. Bodempudi, S. B., Babur, R., Reddy, K. S. (2015). Development and Substantiation of a RP-YPSK Method for Monitoring of Impurities in Pirfenidone Drug Substance. American Journal of Analytical Chemistry, 06(13), 1019–1029. doi:10.4236/ajac.2015.613097

10. Kasar, K., Demir, T., Akin, M. M., Gungor Kobat, S. (2021). The effect of halofugınone and pirfenidone on wound healing in experimental glaucoma filtration surgery. Journal francais d'ophtalmologie, 44(3), 340–349. doi:10.1016/j.jfo.2020.04.067