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Voltammetric analysis and electrochemical behavior of an antibiotic drug Nifuroxazide on a boron-doped diamond electrode in the existence of a cationic surfactant

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ABSTRACT: In this work, the voltammetric sensing of nifuroxazide (NFX) was assessed utilizing a boron-doped diamond (BDD) electrode that underwent electrochemical pretreatment, enhancing its surface activity. Cyclic voltammograms of NFX revealed well-defined, single, and irreversible peaks at 0.94 V (vs. Ag/AgCl) in a 0.04 M Britton-Robinson (BR) buffer solution at pH 6.0. The oxidation peaks of NFX were found to be pH-dependent across a range from 2.0 to 12.0. Introducing a cationc surfactant (CTAB; cetyltrimethylammonium bromide) into the selected electrolyte increased the anodic peak currents of NFX. Under optimized conditions, a linear relationship was established for NFX quantification in 0.04 M solution of BR buffer (pH 6.0) containing 2×10^{-4} M CTAB at +1.02 V (vs. Ag/AgCl). The linear range spanned from 0.05 to 2.0 µg mL^{-1} (1.8×10^{-7} – 7.2×10^{-6} M), with a detection limit of 0.013 µg mL^{-1} (4.7×10^{-8} M). The NFX concentration in the drug formulation was successfully quantized using this method.

KEYWORDS: Nifuroxazide; voltammetry; cationic surfactant; boron-doped diamond electrode; drug form.

1. INTRODUCTION

Gastroenteritis is a leading cause of medical visits globally, affecting an estimated 2 to 3 billion people annually, particularly in areas with poor sanitation and healthcare. Acute gastroenteritis typically involves softer or watery stools and/or frequent bowel movements, occurring three or more times within 24 hours, and lasting fewer than 7 to 14 days [1]. Diarrhea, characterized by three or more liquid or loose or stools per day, can be categorized as acute, chronic, or persistent. Globally, the most common pathogens responsible for diarrhea include rotavirus, enterotoxigenic *E. coli* (ETEC), *Campylobacter*, *Vibrio cholerae*, *Shigella*, and non-typhoidal *Salmonella*. Diarrhea remains a leading cause of illness and mortality, responsible for 1.5 million child deaths annually, accounting for 15% of all deaths in children. Infectious diarrhea, a major global public health concern, is caused by pathogens that produce non-inflammatory or inflammatory types of diarrhea[2-4].

Nifuroxazide (NFX) is a nitrofuran-based chemotherapeutic agent and broad-spectrum antibacterial with the chemical name (E)-4-hydroxy-N-[(5-nitrofuran-2-yl)methylidene]benzohydrazide. It acts as a fast-acting anti-infective agent in the intestine, effectively targeting various pathogens that cause diarrhea [5]. NFX has long been recognized as an effective anti-diarrheal medication. However, its routine use has declined in favor of probiotics, despite limited evidence supporting the superior efficacy of probiotics over NFX in treating acute diarrhea. The broad-spectrum activity of NFX makes it suitable for treating colitis and diarrhea, and it is particularly advantageous in that it does not disrupt intestinal flora, even at high doses. Despite its clinical benefits, NFX is contraindicated for pregnant and breastfeeding women as a precaution, despite the absence of evidence for teratogenic effects [6,7].

Electroanalytical techniques, particularly voltammetric methods, offer practical alternatives for drug analysis due to their simplicity, cost-effectiveness, rapid analysis, and minimal use of toxic reagents. These methods provide adequate sensitivity and selectivity, which largely depend on the type of working electrode employed. Notably, voltammetry excels in elucidating the oxidation-reduction behavior of target molecules through their redox-active groups, a unique advantage over non-electrochemical methods. This capability is especially valuable for developing strategies to assess the potentially harmful or therapeutic

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effects of pharmaceutical compounds [8,9]. Boron-doped diamond (BDD) electrodes have emerged as a superior choice in electrochemical drug analysis due to their exceptional properties. BDD electrodes offer the widest potential window among electrode materials, particularly in anodic potentials, and exhibit long-term stability, mechanical robustness, minimal fouling, low capacitive currents, and low background noise. These characteristics make them ideal for sensitive detection and quantification of pharmaceutical compounds, addressing the need for precise and reliable measurements in drug analysis. Moreover, the versatility and efficacy of BDD electrodes have been demonstrated across various fields, including analytical chemistry, biomedical sciences, and environmental science. Their ability to handle complex matrices and maintain high performance under challenging conditions has been well-documented in numerous studies. Additionally, flow-based systems incorporating BDD electrodes have gained recognition for reducing waste generation and reagent consumption while maintaining efficient analytical performance and sample throughput, effectively mechanizing chemical assays [10-18].

Surface-active agents, or surfactants, are amphiphilic molecules essential in interfacial research and colloid chemistry. They possess both hydrophobic (water-repelling) and hydrophilic (water-attracting) components, enabling them to adsorb at the interface between immiscible phases such as water and oil, reducing interfacial tension and promoting stable colloidal structures. Even at low concentrations, surfactants can alter the surface or interfacial free energy within a system. The term 'interface' refers to the boundary between two immiscible phases, while 'surface' refers specifically to an interface where one phase is gas, typically air [19]. The hydrophilic end of cationic surfactants holds a positive charge. Cetyltrimethylammonium bromide (CTAB), a notable cationic surfactant, has a 16-carbon chain and a quaternary ammonium group with three attached methyl groups. CTAB is frequently utilized in wastewater treatment to enhance pollutant absorption [20]. Additionally, material modification with CTAB improves sensing performance, resulting in enhanced detection limits and sensitivity due to increased hyperchromicity and probe sensitization [21,22].

The literature review on NFX revealed that there are few analytical methods currently available for the determination of NFX in different samples. For example, electroanalytical [23–27], spectrophotometric [28], and chromatographic methods [29]. It is important to develop a simple, inexpensive, environmentally friendly, and sensitive analytical method for the determination of NFX. Based on the available research, five studies have documented the use of electroanalytical methods to quantitatively analyze NFX in various forms. NFX content in capsules was analyzed using differential pulse polarography on a static mercury drop electrode [23]. The NFX compound was examined using adsorptive stripping voltammetry and polarography techniques with both carbon paste and mercury electrodes [24]. NFX concentration in human serum was determined by surface of a hanging mercury drop electrode using adsorptive stripping voltammetry [25]. A voltammetric investigation assessed the behavior of NFX at carbon paste electrodes, both unmodified and treated with Sephadex [26]. Finally, NFX concentration was measured using flow injection linear adsorptive stripping voltammetry on an electrode modified with screen-printed carbon nanofibers [27].

The primary aim of this study is to design and create and implement an efficient approach for the analysis of NFX using a cost-effective, rapid, and user-friendly voltammetric technique. The proposed methodology was rigorously tested and its effectiveness demonstrated by successfully applying it to the analysis of NFX in pharmaceutical formulations under optimal laboratory conditions. This approach not only simplifies the process but also ensures reliable and reproducible results, making it suitable for routine analysis.

2. RESULTS AND DISCUSSION

The electrochemical behavior of NFX was studied using cyclic voltammetry (CV) on a cathodically pretreated boron-doped diamond electrode (CPT-BDD). The three consecutive CV curves for a 100 µg mL^{-1} NFX solution in 0.04 M BR buffer (pH 6.0) were recorded at a scan rate of 100 mV s^{-1}, as depicted in Figure 1. In the oxidative direction extending from 0 to +1.6 V, during the first scan NFX shown an anodic peak at approximately 0.94 V, while during the reverse cathodic scan no decreased peak identified. The oxidation of NFX appears to be an electrochemically irreversible process, as indicated by the absence of a reduction peak. The signal strength decreased with each subsequent scan, likely due to the adsorption of NFX byproducts onto the electrode surface, resulting in electrode blockage. The impacts of scan rate on the electro-oxidation response for 100 µg mL^{-1} NFX was further investigated to gain insight into the electrode process nature, using the CV technique in the same buffer solution. Figure 1B illustrates the corresponding CVs for voltage

scan rates ranging from 50 to 400 mV s^{-1}. A subtle change towards more positive potential values was observed with enhancing rate of scan, corroborating the irreversibility of the electrode process mentioned earlier. Additionally, a linear association was found between the square root of scan rates ($v^{1/2}$) and the peak current (I_p), indicating that the NFX electrode reaction is diffusion-controlled. The corresponding equation is presented below:

I_p (µA) = 0.037 $v^{1/2}$ (mV s^{-1}) + 0.845 r= 0.998

Furthermore, the equation describing the correlation between the logarithm of the peak current (log I_p) and the logarithm of the voltage (log v) was evaluated, yielding the subsequent formula: log I_p (µA) = 0.172 log v (mV s^{-1})- 0.252, with a correlation coefficient (r) of 0.991. The obtained slope (~0.172) is roughly in agreement with the theoretical value of 0.5 anticipated for diffusion-controlled processes.

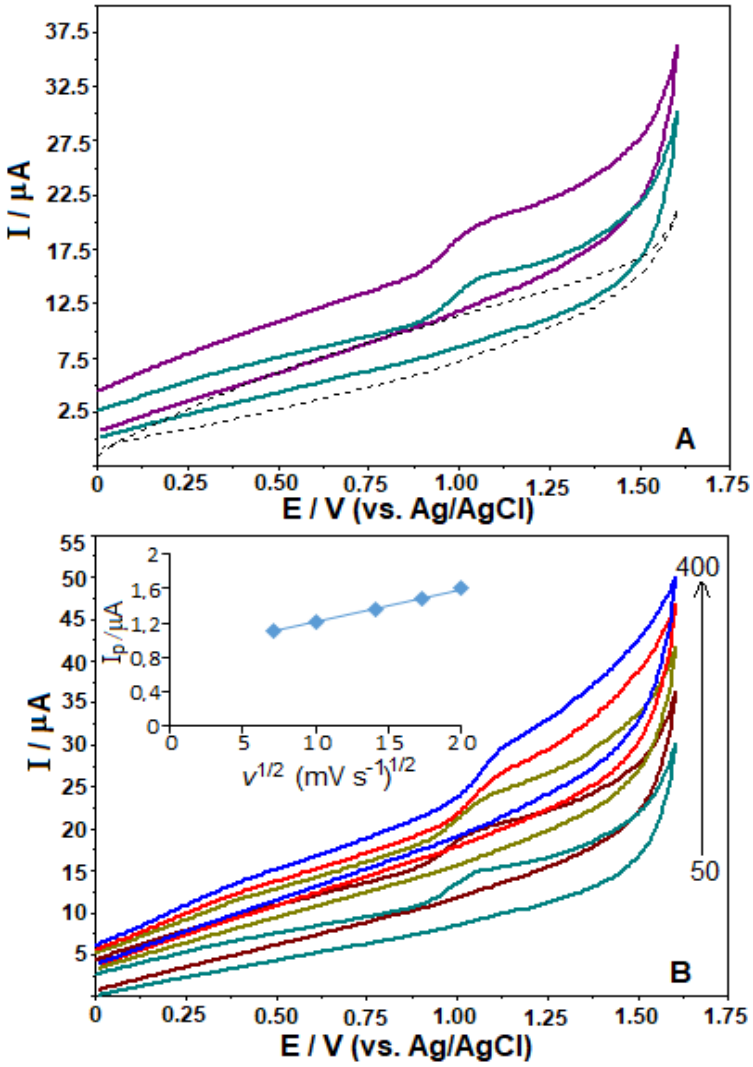

Figure 1. Performance of CVs for 100 µg mL^{-1} NFX in BR buffer solution (pH 6.0) on the BDD electrode at the scan rate of 100 mV s^{-1} (A), and CVs undergo analysis at various scan rates, including 50, 100, 200, 300 and 400 mV s^{-1}. The background current is visually represented using dashed lines (A). The plots of I_p versus $v^{1/2}$ are included as insets (B).

Initial investigations indicated that the untreated surface of BDD electrode was not successful in overcoming passivation difficulties, especially when dealing with large concentrations of NFX, before conducting sensitive voltammetric technique study on NFX. The BDD electrode that was not prepped could not provide good findings in terms of sensitivity and reproducibility owing to this adverse situation. In order to tackle this problem and improve the pretreatment procedures for the BDD electrode, we used square-wave voltammetry (SWV) to assess the effectiveness of three different pretreatment strategies for a 2.0 µg mL^{-1} concentration of NFX in a BR (pH 6.0) solution. The effectiveness of a BDD electrode that underwent anodic pretreatment (APT) was evaluated by conducting voltammetric tests. The APT included

applying a voltage of +1.8 V for 180 seconds in a solution of 0.5 M H_2SO_4. Subsequently, the effectiveness of the BDD electrode underwent cathodic pretreatment (CPT, -1.8 V for 180 s in 0.5 M H_2SO_4) was evaluated. Lastly, on the BDD electrode both cathodic and anodic pretreatment procedures were sequentially performed, and voltammetric analysis results were recorded. Comparative analysis revealed that the cathodic pretreatment procedure yielded more repeatable and sensitive findings for NFX measurement (refer to Fig. 2). This procedure likely enhanced the electron transfer rate of NFX on the surface of electrode compared to the other techniques. Consequently, this method was deemed the most efficient pretreatment method and was adopted for electrode pretreatment at the onset of every testing day. To date, no comprehensive scientific study on the pKa values of NFX has been published. The DrugBank database reports its pKa value to be approximately 8.33. It is important to note that NFX is a highly hydrophobic and pH-sensitive compound, exhibiting low solubility under strongly acidic conditions. At lower pH levels (pH ≤ pKa), the uncharged, hydrophobic form of NFX predominates. It can be hypothesized that hydrophobic interactions occur between CPT-BDD electrode (as the surface of the BDD electrode becomes hydrophobic after this treatment procedure) and the analyte, which may explain the highest peak intensity for NFX oxidation observed at the CPT-BDD electrode.

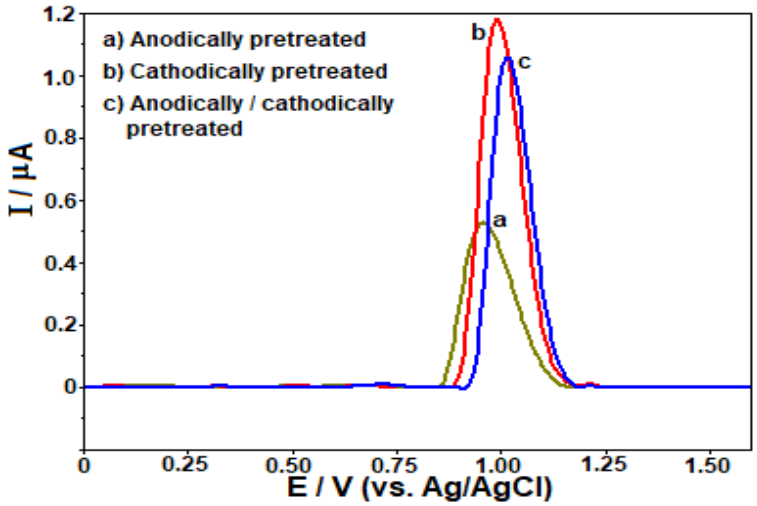

Figure 2. Recorded in BR buffer solution (pH 6.0) using the BDD electrode after various electrochemical pretreatments, the SW voltammogram shows 2 µg mL^{-1} NFX. The SWV parameters were as follows: step potential, 10 mV pulse amplitude, 40 mV; frequency, 50 Hz.

The effect of varying pH values on the anodic peak current responses of NFX was assessed on the CPT-BDD electrode using SWV, aiming to optimize voltammetric performance for analytical applications involving NFX samples. Figures 3A and B display the baseline-corrected SW voltammograms in BR buffer, tested across pH values ranging from 2.0 to 12.0. The voltage range of NFX was from 0 V to +1.60 V and the concentration was 2 µg mL^{-1} (the pH value was higher than 9 and worked up to +1.4 V). As shown in Fig. 3A, a single oxidation peak was seen across the pH range of 2.0-6.0, with an increase in anodic peak currents from pH 2.0 to 6.0. This peak voltage shifted to more negative potentials with enhansing pH values. A graph of E_p against the solution pH gave a linear relationship (E_p (V) =-0.068 pH + 1.349, r = 0.997) at the CPT-BDD electrode. This observation indicates that the oxidation of NFX at the BDD electrode depends on the pH within the working pH range. On the other hand, a shoulder in the signal at pH 7, peak splits, and a second signal was observed at higher pH values (Fig. 3B). Also, it was observed that the basic signal shifted to more negative regions as the pH value increased. Based on the obtained signal, a plot of Ep versus solution pH yielded a linear relationship (E_p (V) =-0.053 pH + 1.231, r = 0.998) at the CPT-BDD electrode. The slopes of 0.068 and 0.053 V/pH, which closely approximate the projected value of 0.059 V, indicate that in the electrode reactions, the total amount of and protons and electrons is equal. Figures 3A and B illustrate the most noticeable SW voltammogram of 2 µg mL^{-1} NFX, exhibiting a single peak potential and the best-shaped peak at pH 6.0 (in BR buffer solution) on the CPT-BDD electrode. This optimized condition was selected for future research endeavors.

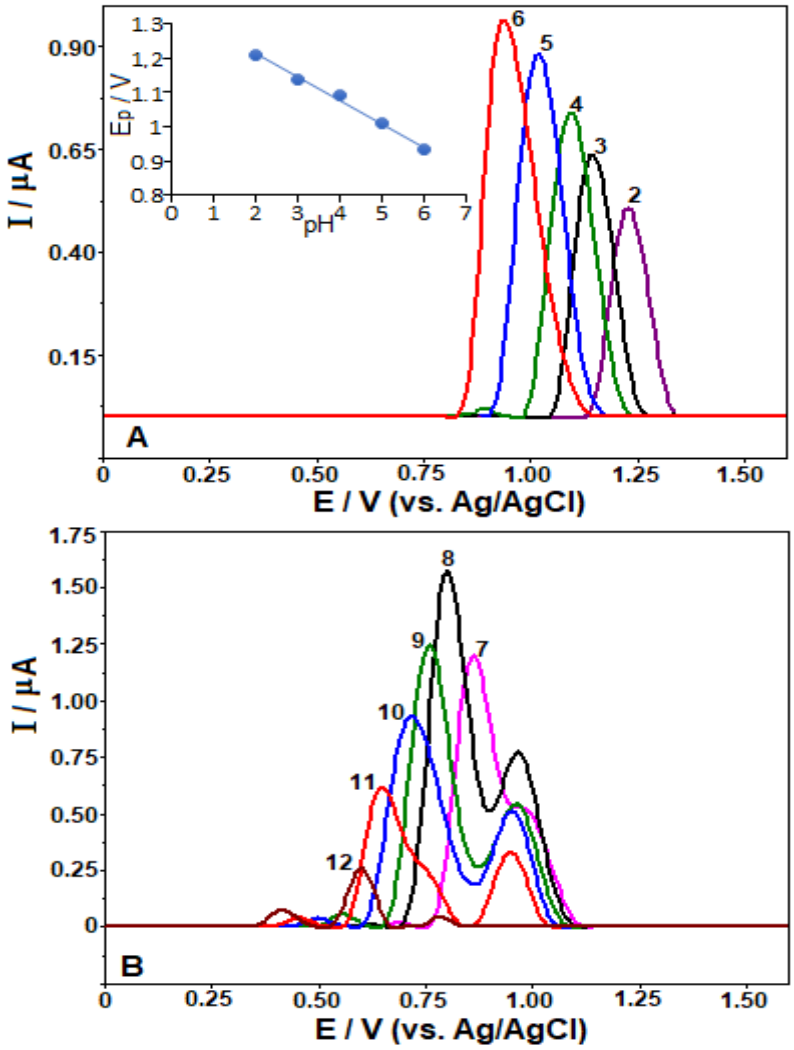

Figure 3. SW voltammograms in BR buffer for 2 µg mL^{-1} NFX between pH 2.0-6.0 (A), and between pH 7.0-12.0 (B) at the CPT-BDD electrode. Other working situation are illustrated in Figure 2.

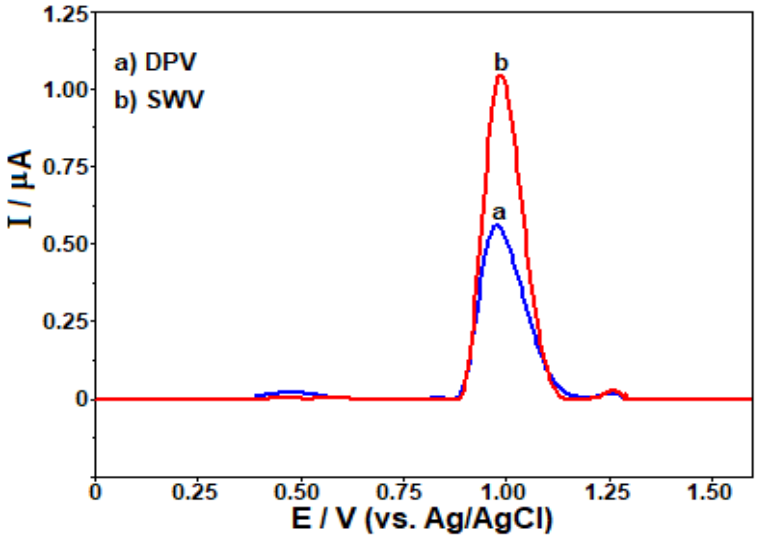

Figure 4. DP (a) and SW (b) voltammograms of 2 µg mL^{-1} NFX in BR buffer solution (pH 6.0) at the CPT-BDD electrode. DPV parameters: modulation time 0.05 s; step potential, 8 mV and modulation amplitude, 50 mV. Other working situation are illustrated in Figure 3.

The effects of two sensitive pulse methods, namely, SWV and differential pulse voltammetry (DPV), on the oxidation peak currents of NFX were compared under identical conditions. The results indicated that SWV produced more sensitive responses compared to DPV (Figure 4). Consequently, future investigations could be performed utilizing the SWV approach.

Optimizing pulse parameters including frequency (f), pulse amplitude (ΔE_{sw}), and step potential (ΔE_s) has a significant impact on the sensitivity of NFX detection. This optimization process involved adjusting one pulse parameter while maintaining the other two constant, then measuring the resulting signal. Initially, the f variable was varied from 25 to 150 Hz, while ΔE_{sw} and ΔE_s were held constant at 40 mV and 10 mV, respectively. The optimal sensitivity and peak shape were achieved at a frequency of 100 Hz. Subsequently, the ΔE_{sw} was varied between 20 and 70 mV, while ΔE_s and f were maintained at 10 mV and 100 Hz, respectively. Similarly, optimization was carried out with ΔE_s ranging from 6 to 18, while f and ΔE_{sw} were held constant. Ultimately, the optimal parameters for SWV on the CPT-BDD electrode for detecting 2 µg mL^{-1} NFX in BR buffer solution (pH 6.0) were determined as follows: f = 100 Hz, ΔE_s = 16 mV, and ΔE_{sw} = 50 mV.

In electrochemical analysis, surfactants play three crucial roles. Firstly, they influence the reaction mechanism of compounds at the surface of electrode. Secondly, surfactants accelerate the transfer of electrons between the solution and electrode surface. Lastly, they often enhance sensitivity, leading to more robust results [30,31]. To develop a methodology for NFX determination, we focused on three kinds of surfactants: Tween 20 (non-ionic), CTAB, (cationic), and SDS (sodium dodecyl sulfate, anionic), aiming to maximize the peak current of NFX oxidation. Using a fixed NFX concentration of 0.75 µg mL^{-1} and BR buffer solution (pH 6.0), each surfactant at a concentration of 1 x 10^{-4} M was separately tested under optimal conditions. CTAB notably increased the NFX oxidation signal by approximately 2.0 times, while SDS and Tween 20 showed no significant impact on the NFX oxidation signal. Consequently, CTAB yielded the highest peak current of NFX, leading to subsequent experiments being conducted in its presence. Ionic surfactants adsorb onto hydrophobic surfaces (notably, the CPT-BDD surface is hydrophobic) from aqueous solutions, forming a low-density monolayer with surfactant tails aligned parallel to the surface at low concentrations. Below the critical micelle concentration (CMC), phenolic compounds can interact with these surface micelles without penetrating them, instead adhering to their surface through a mechanism similar to solubilization in micellar solutions—referred to as coadsorption or adsolubilization. This process is largely independent of the substrate's electric charge, requiring only sufficient hydrophobicity. The surface activity of surfactants, influenced by tail length, is highest for CTAB among common anionic and cationic surfactants [32]. To further enhance sensitivity, the impact of CTAB levels in the electrolyte solution on the peak current of NFX was evaluated under optimal conditions. With a fixed NFX level of 0.75 µg mL^{-1}, the working solution was supplemented with CTAB within the range of 5x10^{-5} M to 3x10^{-4} M. As depicted in Figure 5, increasing the CTAB concentration gradually augmented the intensity of NFX peak current, reaching a maximum at a CTAB concentration of 2x10^{-4} M. Beyond this concentration, further increases in CTAB levels resulted in minimal changes in the peak currents of NFX.

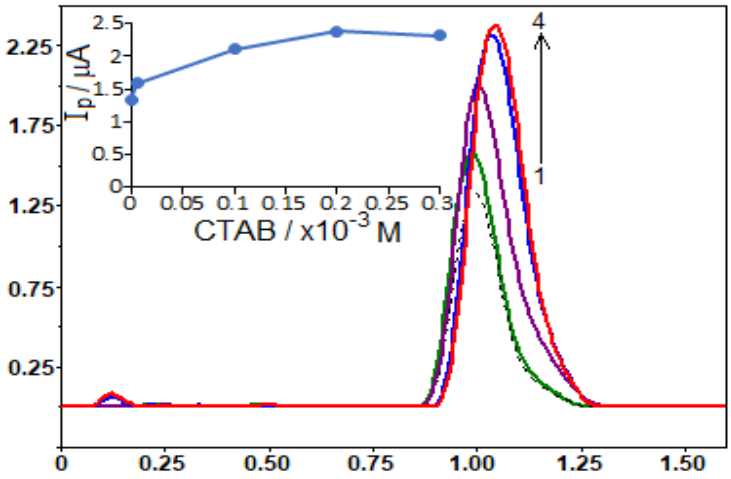

Figure 5. SW voltammograms of 0.75 µg mL^{-1} NFX in the solution of BR buffer at pH 6.0 with the existence of different CTAB concentrations (from 1 to 4; 5.0×10^{-5} -3×10^{-4} M) at the CPT-BDD electrode. Dashed lines depict the voltammograms obtained without CTAB. Inset: a graph of I_p versus CTAB concentration. SWV parameters; SWV parameters; step potential, 16 mV; pulse amplitude, 50 mV; frequency, 100 Hz.

The performance of the developed procedure using the CPT-BDD electrode was assessed under optimal experimental and instrumental conditions. As illustrated in Figure 6, there was a notable enhancement in anodic peak currents with the addition of NFX standards ranging from 0.05 to 2.5 µg mL^{-1} (1.8×10^{-7} - 9.1×10^{-6} M) in the presence of 2×10^{-4} M CTAB. In reaction to the sequential additions of NFX, a highly linear association was observed among the SW voltammograms obtained (around +1.03 V), expressed by the equation:

I_p (µA) = 2.868 C (µg mL^{-1}) + 0.008 (r = 0.999), where I_p represents the peak current, and r is the correlation coefficient.

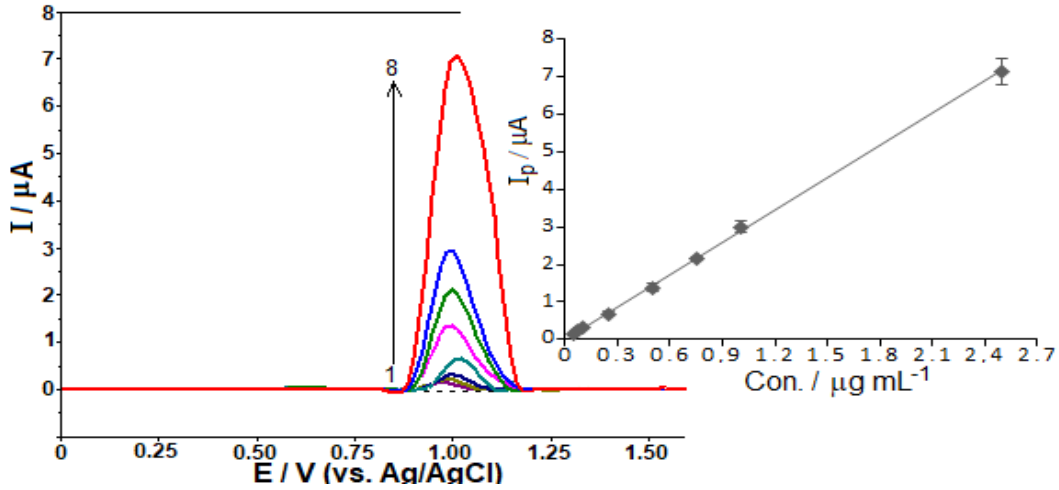

Figure 6. SW voltagramms for NFX levels of (1-8) 0.05, 0.075, 0.10, 0.25, 0.50, 0.75, 1.0 and 2.5 µg mL^{-1} in the solution of BR buffer at pH 6.0 with 2×10^{-4} M CTAB. Other working situation are illustrated in Figure 5.

The developed methodology has achieved a limit of detection (LOD) of 0.013 µg mL^{-1} (4.7×10^{-8} M) and a limit of quantification (LOQ) of 0.043 µg mL^{-1} (1.6×10^{-7} M) after subjecting the electrode to a simple pretreatment process in the presence of CTAB. Using the three s/m formula, the LOD was determined, where m represents the slope of the calibration curve and the standard deviation of ten measurements at the lowest concentration within the calibration range is represented by "s".

As far as we know, no previous research has utilized unmodified BDD electrodes for the sensing of NFX. In comparison to earlier studies, our results using an unmodified BDD electrode (with a LOD of 4.7×10^{-8} M) show lower sensitivity than the static mercury drop electrode, but similar sensitivity (based on LOD values) to other methods. Furthermore, the proposed method offers environmental benefits (compared to toxic mercury electrodes), along with enhanced cost-effectiveness, practicality, and efficiency for NFX measurement. Table 1 provides a comparative analysis of the developed technique against various other reported electroanalytical methods.

Table 1. Evaluation of the analytical performance of the suggested method with other results electrochemical NFX sensors in the previous studies.

Electrode(s)	Linearity range (M)	LOD (M)	Reference
Static mercury drop	1.0×10^{-7}-7.5×10^{-5}	1×10^{-5}	23
Mercury and carbon paste	3.6×10^{-7}- 3.6×10^{-5}	3.6×10^{-8}	24
Hanging mercury drop	2.0×10^{-8}-1.0×10^{-7}	5.0×10^{-9}	25
Sephadex-modified carbon paste	5.0×10^{-8}- 4.0×10^{-7}	2.0×10^{-8}	26
Screen- printed carbon nanofiber modified	3.6×10^{-7}-1.0×10^{-5}	3.6×10^{-8}	27
Boron-doped diamond	1.8×10^{-7}- 9.1×10^{-6}	4.7×10^{-8}	This work

The intra-day (ten repeated consecutive measurements with electrode surface cleaning between each measurement using the CPT procedure) and inter-day (three replicates performed over five consecutive days) repeatability of 0.05 µg mL^{-1} NFX were evaluated under identical conditions to assess the method's precision. The calculated relative standard deviation (RSD) values were 7.42% and 8.55%, respectively, indicating that the CPT-BDD electrode provides reliable NFX measurements. Additionally, the RSDs for 0.5 and 1.0 µg mL^{-1} NFX (ten repeated consecutive measurements with electrode surface cleaning between each CPT procedure) were 5.76% and 4.75%, respectively, demonstrating excellent repeatability for medium and high NFX concentrations.

Before analyzing actual samples, the specificity of the SWV method was evaluated in the presence of various potential interfering substances. These included carbohydrate compounds (saccharin, glucose, sucrose, fructose, and lactose), uric acid (UA), dopamine (DOP), ascorbic acid (AA), and inorganic ions such as nickel (Ni^{2+}), zinc (Zn^{2+}), magnesium (Mg^{2+}), calcium (Ca^{2+}), potassium (K^{+}), sodium (Na^{+}), nitrate (NO_3^-), chloride (Cl^-), and sulfate (SO_4^{2-}) (Table 2). Matrix interference was carefully assessed at molar ratios of 1:1, 1:10, and 1:50 (NFX: interference species) in supporting electrolytes containing 0.5 µg mL^{-1} NFX. The impact of each interferent was investigated by comparing the signal from a solution containing both NFX and the interfering species with the signal from a pure NFX solution. To manage interference, the tolerance limit was established as the maximum concentration of an interfering substance that caused a deviation in the NFX signal of less than ±5%. It was discovered that increasing the concentration of carbohydrate compounds by 50 times did not have a noticeable impact on the oxidation peak current of NFX. Similarly, the presence of inorganic ions did not have any effect on the anodic peaks of NFX, even when present at a 50-fold higher concentration. An investigation was conducted to evaluate the possible disruption caused by AA, DOP, and UA (a significant constituent of urine) in order to determine the practicality of managing these compounds in biological samples. This might be advantageous in forthcoming investigations on pharmacodynamics and pharmacokinetics. It was noted that when UA and AA were used at concentrations suitable for work, their separate solutions did not have any impact on the anodic peak currents of NFX. Nevertheless, the NFX signal was affected by DOP when it was present at a 50-fold excess concentration. These results demonstrate that the designed method exhibits proper sample and holds promise for application in real samples.

Table 2. Effects of possible interference-causing substances on the current response of 0.5 µg mL^{-1} NFX

Interfering agent	Concentration ratios (NFX: interfering agent)	Signal change (%)
Inorganic ions	1:50	<6
Lactose	1:50	<5
Fructose	1:50	<4
Glucose	1:50	<4
Sucrose	1:50	<6
Saccharin	1:50	<5
Uric acid	1:50	<9
Dopamine	1:50	<45
Ascorbic acid	1:50	<7

Inorganic ions: K^+, Na^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Fe^{3+}, Cl^-, SO_4^{2-}, NO_3^-

The proposed electroanalytical methodology was practically validated for determining NFX in commercially available pharmaceutical formulations using the calibration curve method. The Experimental section contains the details regarding the procedures for sample preparation and measurement. Representative SW voltammograms of samples and standard additions are depicted in Figure 7. The average NFX content of the sample was determined to be 190.8 mg per capsule using a series of dilutions. This number closely aligns with the manufacturer's claimed label value of 200 mg per capsule, with a relative standard deviation (RSD) of 4.2%. In order to confirm the effectiveness of the developed method, a recovery investigation was performed. This involved adding known amounts of an NFX standard solution (at final concentrations of 0.1, 0.5, and 1.0 µg mL^{-1}) to the sample solution that had been previously analyzed. The mixture was then analyzed using the established procedure in an electrochemical cell. Table 3 displays the average results from three replicate measurements, showing acceptable RSD values. The high recovery rates suggest that there is minimal matrix interference in the capsule samples.

Table 3. Outcomes of measuring the incorporation and recovery of NFX from the capsule sample utilizing the newly designed voltammetric technique.

Added[a] (µgmL[1])	Expected[a] (µg mL^{-1})	Found[a,b] (µg mL^{-1})	Recovery (%) ± RSD (%)
0	–	0.954	0 ± 4.2
0.1	1.054	0.988	93.7 ± 3.8
0.5	1.454	1.383	95.1 ± 3.5
1.0	1.954	2.036	104.2 ± 3.1

[a]Concentration in the measured solution
[b]Average of three measurements of replicate

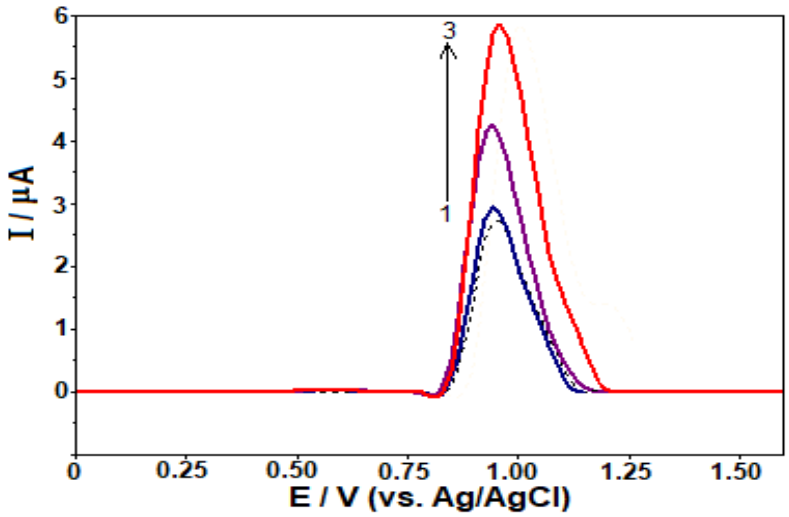

Figure 7. SW voltammograms of the diluted capsule sample (dashed line) and after standard additions of 0.1, 0.5, and 1.0 µg mL^{-1} NFX (1-3) in BR buffer solution at pH 6.0 with 2×10^{-4} M CTAB on the CPT-BDD electrode. Other working situation are illustrated in Figure 6.

3. CONCLUSION

In the literature, five publications discuss the voltammetric measurement of NFX, as noted in the introduction. These studies primarily use chemically modified or mercury electrodes, which are time-consuming, costly, and pose chemical hazards while being environmentally unfriendly. This investigation explores the suitability of a BDD electrode, with only simple electrochemical pretreatment, in conjunction with SWV to analyze the electrochemical behavior of NFX. The inclusion of the cationic surfactant CTAB in the working electrolyte greatly increases the anodic peak currents of NFX. The validation of this new approach for measuring NFX in commercially available pharmaceutical formulations showed excellent recoveries. Additionally, the proposed method is immediately applicable without generating waste, requiring complex sample extraction, increasing chemical consumption, or needing expensive equipment.

4. MATERIALS AND METHODS

4.1. Chemicals

ChemScene LLC (USA) supplied a reference standard of NFX (ReagentPlus®, ≥98.55%). The conventional method for preparing a 1 mg mL^{-1} solution of NFX involves dissolving it in dimethyl sulfoxide (DMSO) and thereafter keeping it in a volumetric flask at a temperature of 4 0C to avoid deterioration during periods of non-use. The manufacture of the Britton-Robinson buffer (BR, 0.04 M, pH 2-12) solution also involves the use of analytical-grade chemicals and purified water obtained from the Millipore Milli-Q system (Millipore, resistivity > 18.2 MΩ cm). The CTAB (99% purity, Sigma) is obtained by dissolving the required amount of solid in a water-methanol mixture at a 90:10 (v/v) ratio. Prior to use, the stock solution was diluted with the appropriate electrolyte to prepare the calibration and working solutions of NFX. All analyses were conducted under controlled laboratory settings.

4.2. Instruments and operating procedures

Using GPES software (Version 4.9), the voltammetric measurements were conducted with a µAutolab Type III instrument (Metrohm Autolab B.V., Utrecht, The Netherlands). The Savitzky-Golay method was applied to smooth all square wave voltammograms, while the baseline was corrected using the moving average algorithm with a peak width of 0.01 V. A 10-mL, one-compartment voltammetric cell, equipped with the standard three-electrode configuration, was employed for the experiments. The working electrode was a BDD electrode with a diameter of 3 mm and a boron doping level of 1000 ppm, sourced from Windsor Scientific Ltd. (UK). The reference electrode, an Ag/AgCl electrode immersed in a 3 M NaCl solution, was supplied by BAS (Model RE-1, USA). The counter electrode, made of platinum (Pt), was also supplied by BAS (MW-4130, USA). Each working day began with the activation of the BDD electrode in 0.5 M H_2SO_4 by applying a potential of -1.8 V (unless specified otherwise) for 180 seconds. This activation

program was reapplied to the BDD electrode for 60 seconds under the same conditions as before each experiment. The surface of BDD electrode was subsequently utilized for electrochemical analysis, yielding consistent and reproducible signals.

The electrochemical behavior of NFX was studied, and its reaction kinetics surface of the BDD electrode in the selected electrolyte were assessed using CV. Following this, SWV was applied to fine-tune experimental variables, covering the supporting electrolyte, SWV parameters, and surfactant content, with the objective of improving the sensitivity and selectivity of NFX detection. The technique's practical usefulness and analytical performance were assessed using the same pulse methodology. This procedure included immersing the three electrodes into electrochemical cells filled with NFX and solution of BR buffer at pH 6.0, with CTAB. The SWV method was employed to perform anodic scanning ranging 0 V to +1.4 V. NFX was investigated using optimal values of step potential (ΔE_s), pulse amplitude (ΔE_{sw}), and frequency (f) at 16 mV, 50 mV, and 100 Hz, respectively.

4.3. Sample preparation

The effectiveness of the developed approach was assessed in the quantitative analysis of NFX in the drug form. Diafuryl Fort® capsules (Abdi İbrahim Co., Turkey) which contain 200 mg of NFX as indicated on the label, were sourced from a local pharmacy (Abdi İbrahim Co., Turkey). First, ten of these capsules were weighed sensitively. Then, the shell of the capsules were removed and the weight of the empty capsules was weighed. The net weight of the sample per capsule was determined by deducting the weight of the empty capsules from the overall net weight. A suitable quantity of the sample was placed into a 50-mL volumetric flask, and DMSO was added up to the marked line. The mixture was sonicated for 15 min to ensure complete dissolution. To prepare suitable NFX concentrations, this mixture was taken from the upper clear part and diluted with a BR buffer solution (pH 6.0). This final prepared solution was added to the same solution in the voltammetric cell containing solution of BR buffer (pH 6.0). The amount of NFX in the capsule content was calculated from the regression equation with the help of the calibration curve of the method developed for the standards.

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