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Investigation of electrochemical behavior and voltammetric determination of the anti-inflammatory agent bufexamac

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ABSTRACT: This study aimed to develop an electrochemical method for the electrochemical determination of bufexamac (BUF), an important anti-inflammatory drug. For this purpose, the electrochemical properties of BUF were elucidated using a glassy carbon electrode (GCE) and cyclic voltammetry. The electrochemical performance of BUF was examined by the differential pulse voltammetry (DPV) method. It was observed that the response of GCE to DBT was linear in the concentration range of 6.0×10^{-6} M and 1.0×10^{-4} M. The limit of detection (LOD) was found to be 2.64×10^{-8} M; The limit of quantification (LOQ) was calculated as 8.79×10^{-8} M. The dexketoprofen tromethamine (DEX), diclofenac (DIC), and ibuprofen (IBU) were used as model active compounds to illustrate the oxidation mechanism of BUF. A simple, rapid, and sensitive electrochemical method for quantitatively determining BUF in a commercial sample of human serum was developed and validated. The calibration curve was linear in the 8.0×10^{-6} – 1.0×10^{-4} M in the biological media. The LOD for the commercial sample of human serum was set at 1.59×10^{-7} M. The electrochemical response of BUF in the presence of interferent was evaluated. The data obtained from the study showed that the developed method can be used in sensitive and selective BUF determination.

KEYWORDS: Bufexamac; determination; electrochemical sensor; glassy carbon electrode; validation.

1. INTRODUCTION

With the isolation of salicylic acid in early 1829, non-steroidal anti-inflammatory drugs (NSAIDs) began to form a significant part of the drug treatment of pain (at low doses) and inflammation (at high doses) [1]. NSAIDs are widely used due to their analgesic, antipyretic, and anti-inflammatory effects, and they show their effect by inhibiting the cyclooxygenase (COX) enzyme [2]. In recent years, it has been discovered that NSAIDs have many different therapeutic effects in addition to their well-known classical effects [3]. Its impact on preventing stroke, heart attack, cancer, Alzheimer's, and Parkinson's diseases is the most remarkable of these. NSAIDs are among the most prescribed drug groups in the world [4]. Bufexamac (BUF) is an anti-inflammatory used topically in the symptomatic treatment of pruritis manifested in skin inflammation [5,6].

Various analytical techniques, such as high-performance liquid chromatography (HPLC) [7] and liquid chromatography-tandem mass spectrometry (LC-MS/MS) [8], are used in BUF determinations. In a study by Kamata and his team in 1986, they developed and validated a simple, prompt, and sensitive HPLC method for quantitatively determining BUF in cream and ointment. Calibration curves were linear in the 0.2-1.0 mg/mL range in both media [7]. Woo I.S. et al. developed a high-performance LC-MS/MS method to determine 10 therapeutic compounds for atopy in toner and lotion. The technique has been validated in the concentration range of 0.16−30.05 ng/mL BUF in toner and lotion. The limit of detection (LOD) values for toner and lotion were determined as 50.08 pg/mL and 45.07 pg/mL, respectively. The limit of quantification (LOQ) values for toner and location were determined as 150.24 pg/mL and 135.21 pg/mL, respectively [8]. With this study, an electrochemical sensor was developed for the first time.

The voltammetric sensors record the current as a function of potential. In the measurement, the potential is variously applied either step by step or continuously to obtain a voltammogram. Several

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electrochemical sensors based on the voltammetry technique include cyclic, differential pulse, square wave, linear sweep, stripping, and hydrodynamic [9]. Electrochemical sensor has several advantages such as high sensitivity and selectivity to analyze the drugs, rapid response time, small sampling volume, and cost-effective protocols. There is no need for expensive equipment for signal transduction since the electron-transfer process directly generates an electronic signal [10].

A triple-cell electrode system was used during the experiments. These electrodes are used as working electrodes; glassy carbon electrode (BASi, U.S.A.), platinum wire (BASi, U.S.A.) as the counter electrode, and Ag/AgCl (BASi, U.S.A.) was used as the reference electrode.

The pH scanning was performed to obtain the appropriate buffer and pH to determine the electrochemical properties of BUF. The medium was determined as a pH 3.0 phosphate buffer. The results obtained as a result of rate scanning studies provided information that the oxidation reaction of BUF is diffusion or adsorption-controlled. The slope of the log v and log Ip equation was found to be 0.2953, which shows that the oxidation reaction of BUF is diffusion-controlled.For the calibration study, the current values of the BUF peaks obtained in the +0.2 to +1.2 V potential scanning range using the DPV method were read and entered into the calibration graph. LOD and LOQ values for the BUF were obtained using the calibration equation's slope and the lowest concentration's standard deviation. The analytical performance of the GCE was evaluated under optimized conditions, and the linear range of BUF was obtained as 6.0×10^{-6} – 1.0×10^{-4} M, with a limit of detection (LOD) of 2.64×10^{-8} M and a limit of quantification (LOQ) of 8.79×10^{-8} M using DPV. The applicability of the developed method was evaluated in the commercial serum sample. In this study, where the electrochemical properties of BUF were examined, the %RSD value was evaluated to ensure the precision of the developed method. The recovery study was carried out using the DPV method to ensure the accuracy of the developed method. The recovery (99.94-99.71%) and RSD%(1.53-1.97%) values were evaluated to ensure the accuracy and precision of the developed method. Moreover, interference studies were performed. Measurements were obtained by cyclic voltammetry (CV) for BUF and its compounds with similar chemical structures to BUF to elucidate the oxidation mechanism of BUF structure.

The objective of this research is to evaluate the electrochemical behavior and identify BUF. This study is the first work in the literature to investigate the electrooxidation behavior and determination of BUF through the use of rapid, sensitive, cost-effective, and environmentally friendly electrochemical analyses on GCE.

2. RESULTS & DISCUSSION

2.1. Investigation of BUF's electrochemical behavior on bare GCE and effect of the supporting electrolyte pH

This current study is the first to examine BUF's electrochemical oxidation and reduction behavior on GCE. The electrooxidative behavior of BUF was examined in pH 3 PBS, which was selected as the optimum environment as a result of measurements between pH 3 and 12. As displayed in Figure 1, the three anodic peaks obtained by the CV of $1x10^{-4}$ M BUF for pH 3 PBS are located at approximately 0.79 V, 1.14 V, and 1.4 V. The absence of the reduction peak in the reverse scanning indicates that the process of BUF electrooxidation is irreversible.

The effect of the supporting electrolyte pH was studied over a range of pH between 0.3 and 12.0 using H_2SO_4 solution, ABS, PBS, and BRB. An increase in pH causes the oxidation peak potential (Ep) to shift to lower potential values, indicating that protons are involved in the electrochemical oxidation process and that oxidation is easier (Figure 2A and 2C). As a result, taking into account the highest peak currents (I_p) while selecting the supporting electrolyte, the peak at 0.64 V in pH 3 PBS was chosen as the main focus of the determination (Figure 2A and 2B). Since it has a higher and more consistent Ip, the peak at 0.64 V (1^{st} peak) has been considered as the main peak from this part of the study. The relationship between E_p and pH is described in the equation below (Equation 1):

$$Ep\ (mV) = -66.794\ pH + 823.26;\ r=0.992\ (pH\ 0.3 – 12)\quad \text{(Equation 1)}$$

The slope value of this equation was found to be ~67 mV/pH, which is close to 59 mV/pH (theoretically calculated value). This data suggests that the number of electrons and protons involved in the oxidation process of BUF is equal [11].

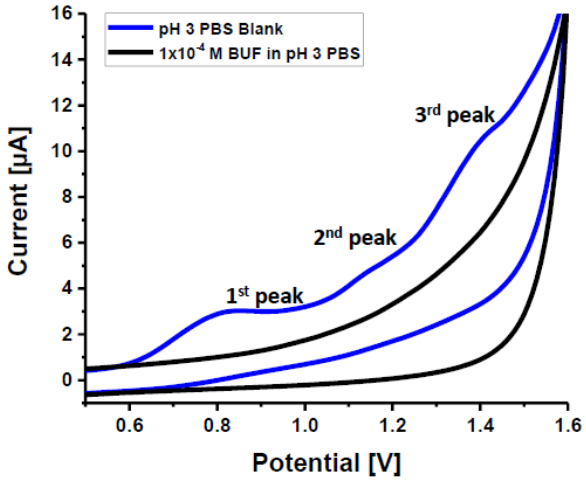

Figure 1. Cyclic voltammograms of 1x10^{-4} M BUF in pH 3 PBS (blue) and pH 3 PBS (black) on bare GCE (potential scan range: -0.2 – 1.6 V scan rate: 100 mV/s, step potential: 0.01 V).

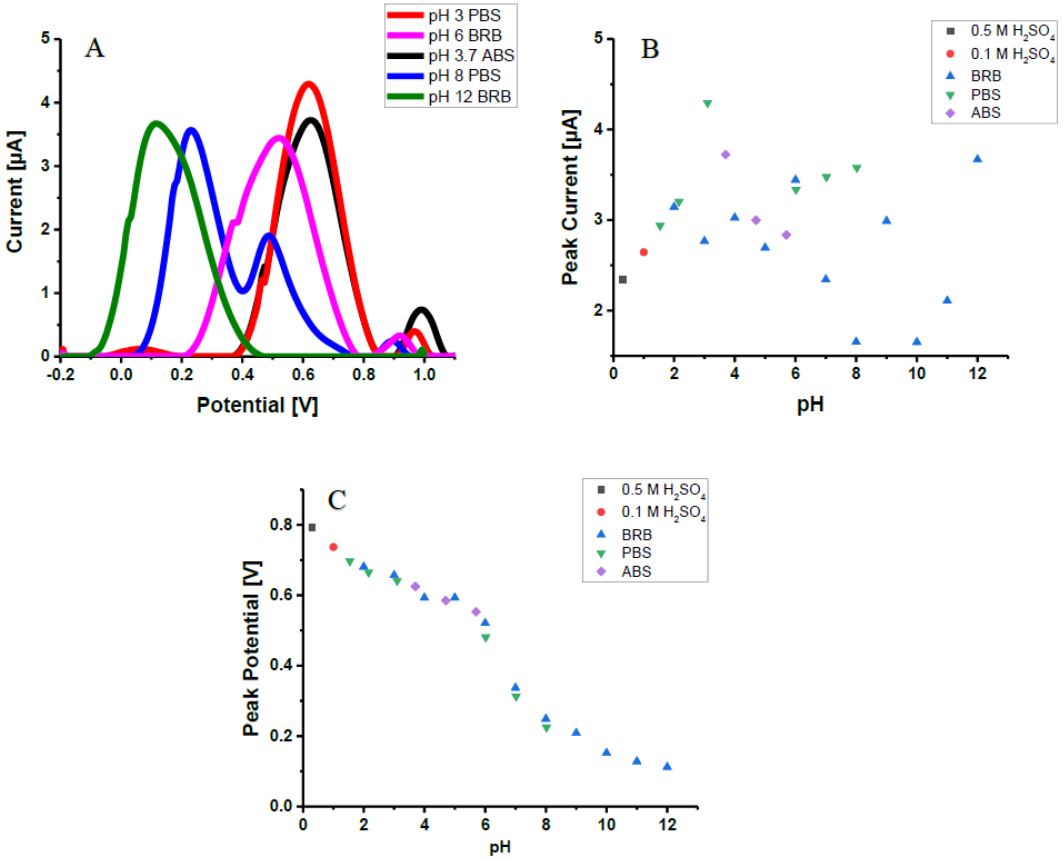

Figure 2. A) DPV voltammograms of 1x10^{-4} M BUF on GCE in pH 3 PBS, pH 8 pBS, pH 6 BRB, pH 12 BRB, and pH 3.7 ABS; the plots of B) I_p versus pH and C) E_p versus pH (The measurements are performed in 1x10^{-4} M BUF using DPV, pH range is 0.3 – 12.0, DPV Parameters; potential scan range: -0.2 – 1.6 V, scan rate: 0.1 V/s, step potential: 0.008 V, E_{pulse}: 0.2 V, t_{pulse}: 0.02 s).

2.2. Effect of scan rate

In order to better understand the electrochemical redox mechanism and identify whether the process is either adsorption or diffusion-controlled, the effect of scan rate (υ) was examined (Figure 3). CV responses of 1x10^{-4} M BUF on GCE in pH 3 PBS were analysed by applying the υ values changing between 0.005 and 1 V/s. The relationships of I_p versus $\upsilon^{1/2}$ (Figure 3A) and logI_p versus logυ (Figure 3B), and I_p versus υ (Figure 3C) are expressed via the equations below (Equation 2-4):

I_p (μA) = 0.0557 $\upsilon^{1/2}$ + 0.3372; r = 0.985 (for pH 3 PBS) (Equation 2)

logI_p (μA) = 0.2953 logυ – 0.5968; r = 0.991 (for pH 3 PBS) (Equation 3)

I_p (μA) = 0.0015 υ + 0.7086; r = 0.980 (for pH 3 PBS) (Equation 4)

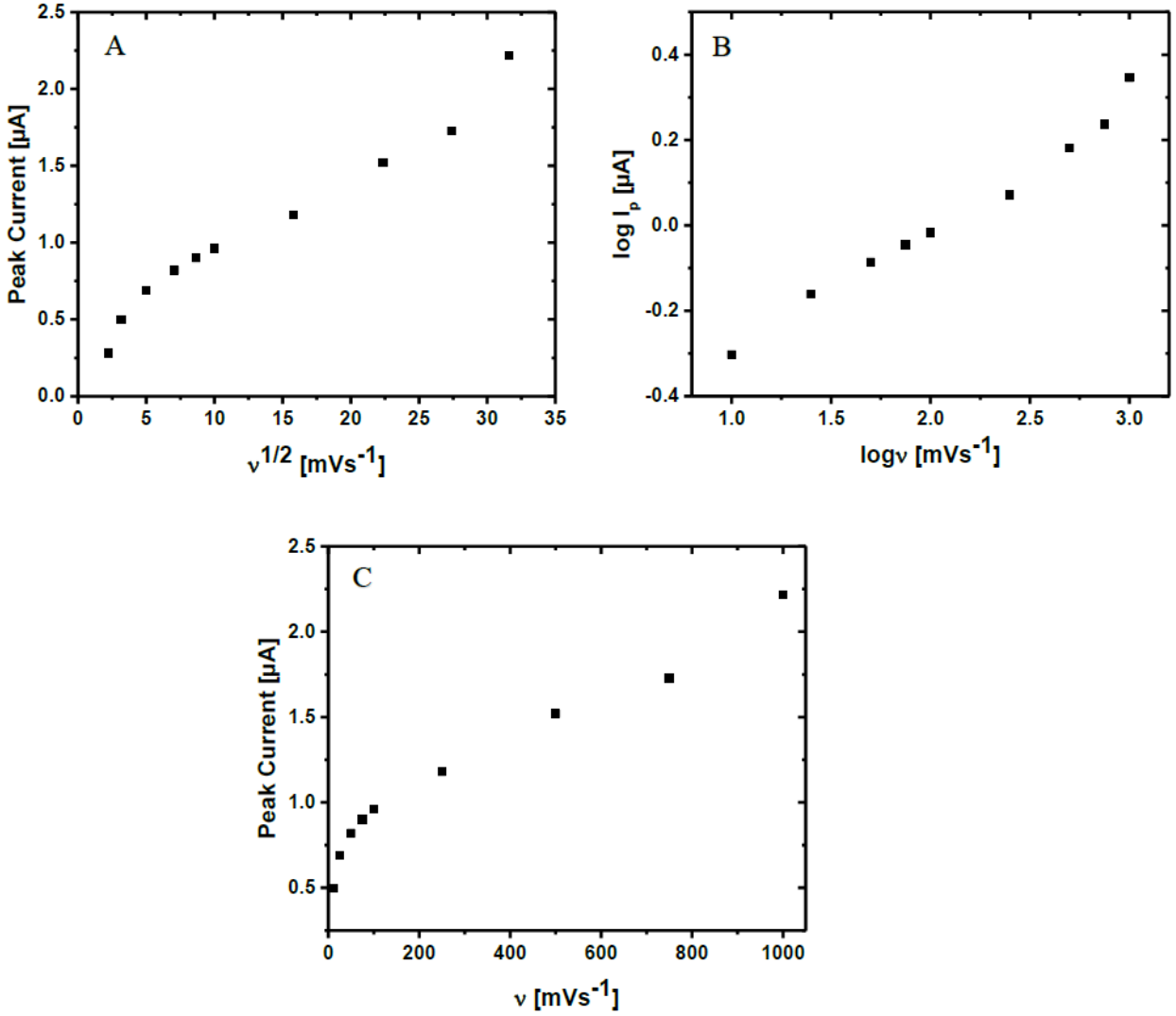

Figure 3. The plots of A) I_p versus $\upsilon^{1/2}$, B) logI_p versus logυ, and C) I_p versus υ in pH 3 PBS on GCE (The measurements are performed in 1x10^{-4} M BUF).

The diffusion-controlled oxidation process of BUF is demonstrated by the computed slope value of the logIp against logυ equation (~0.3) being near to the theoretical value of 0.5 [12,13]. Moreover, the number of transferred electrons (n) for the BUF oxidation process in pH 3 PBS on GCE was determined using the equation below (Equation 5) [14]:

The slope value of the E_p vs logυ equation = $\frac{2.3RT}{2(1-\alpha)nF}$ (Equation 5)

(R: Universal gas constant, T: Absolute temperature, α: Transfer coefficient -if it is an irreversible oxidation reaction, it is calculated as 0.5-, F: Faraday constant)

Upon performing the requisite calculations, it was determined that n≅3.

2.3. Possible oxidation mechanism for BUF

The most widely used technique to elucidate the mechanisms of the electrooxidation process of electroactive drugs is CV. Oxidation peaks of DEX, DIC, and IBU as the molecules that share structural similarities with BUF were investigated using the CV method in pH 3 PBS, pH 7 PBS, and pH 9 BRB. The molecular structures of the BUF, DEX, DIC and IBU are given in Figure 4. According to the data obtained in the scan rate study, the n value for BUF was found to be 3. Additionally, the pH scan study revealed that equal numbers of electrons and protons were involved in the BUF oxidation process. Accordingly, the oxidation reaction for BUF is a process involving three electrons and three protons.

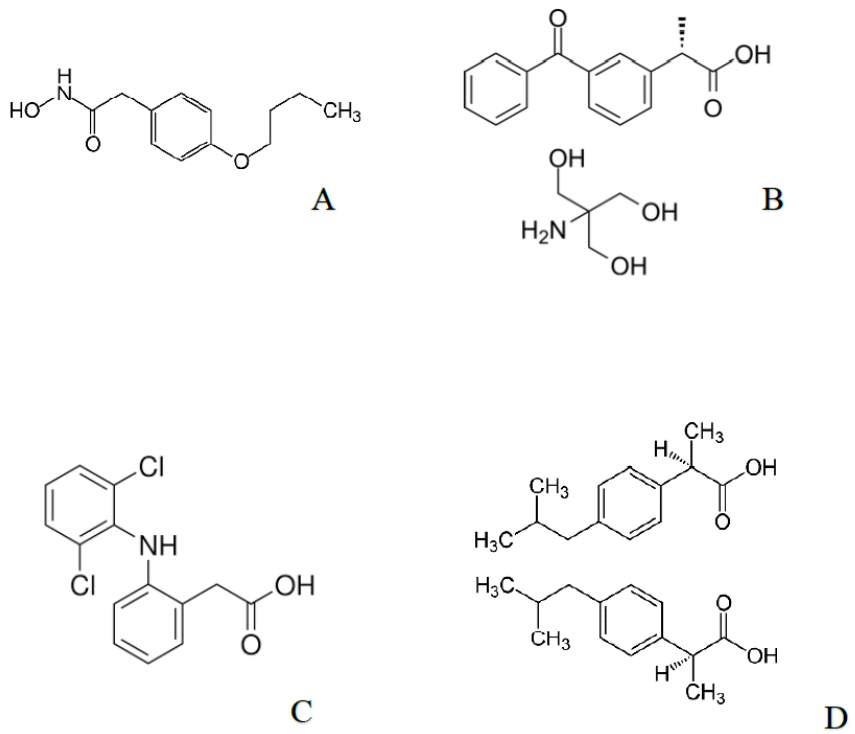

Figure 4. The molecular structures of BUF (a), DEX (b), DIC (c), and IBU (d)

It can be suggested that oxidation of BUF occurs from hydroxyl amine and ether groups after examining the oxidoreduction behavior of similarly structured drugs in selected buffer environments and studies that have previously been conducted on these drugs (Figure 5) [15–18].

2.4. Analytical performance evaluation and validation

GCE's analytical performance assessment was performed using DPV measurements in increasing concentrations of BUF solutions containing pH 3 PBS as the electrolyte. When the obtained I_p values against the concentrations are plotted, it is seen that the linear response is obtained between 6.0×10^{-6} M and 1.0×10^{-4} M (Figure 6A). An overlay of the differential pulse voltammograms corresponding to each concentration is shown in Figure 6B. The equation (Equation 6) corresponding to the calibration curve in Figure 6A is:

$$I_p\ (\mu A) = 3.94 \times 10^4\ (\mu A/M) \times C\ (M) - 0.318\ (r = 0.999)\ \text{(Equation 6)}$$

The LOD and LOQ values found using the calculations in the ICH guidelines (see below for equations, Equation 7 and 8) are 2.64×10^{-8} M and 8.79×10^{-8} M, respectively [19]. Table 1 contains the remaining regression data. Repeatability and reproducibility calculations were performed for $6x10^{-5}$ M BUF. Repeatability is based on intra-day measurements, and reproducibility is based on inter-day measurements.

LOD= 3 x standard deviation/slope (Equation 7)
LOQ = 10 x standard deviation/slope (Equation 8)

2.5. Analytical application in the biological media

In order to test the applicability and accuracy in a biological environment, the determination of BUF in GCE was analyzed with the commercial sample of human serum. A calibration curve was created by plotting the Ip values of BUF against concentration at increasing concentrations in the commercial serum sample (Figure 6C). BUF had a linear range of 8.0 × 10^{-6} M to 1.0 × 10^{-4} M with the corresponding differential pulse voltammograms shown in Figure 6D. The relevant calibration equation (Equation 9) was found as follows, and other regression data are included in Table 1:

$$I_p\ (\mu A) = 2.87 \times 10^4\ (\mu A/M) \times C\ (M) + 0.149\ (r = 0.997)\ \text{(Equation 9)}$$

The LOD and LOQ values found according to the necessary calculations are 1.59 × 10^{-7} M and 5.32 × 10^{-7} M, respectively. After that, the sample of commercial human serum was spiked with two known quantities of standard BUF solution to carry out the recovery assay. When calculating recovery%, five measurements were performed, and the average was calculated. The accuracy and viability in biological samples were validated by the average recovery% and RSD% values obtained in Table 2.

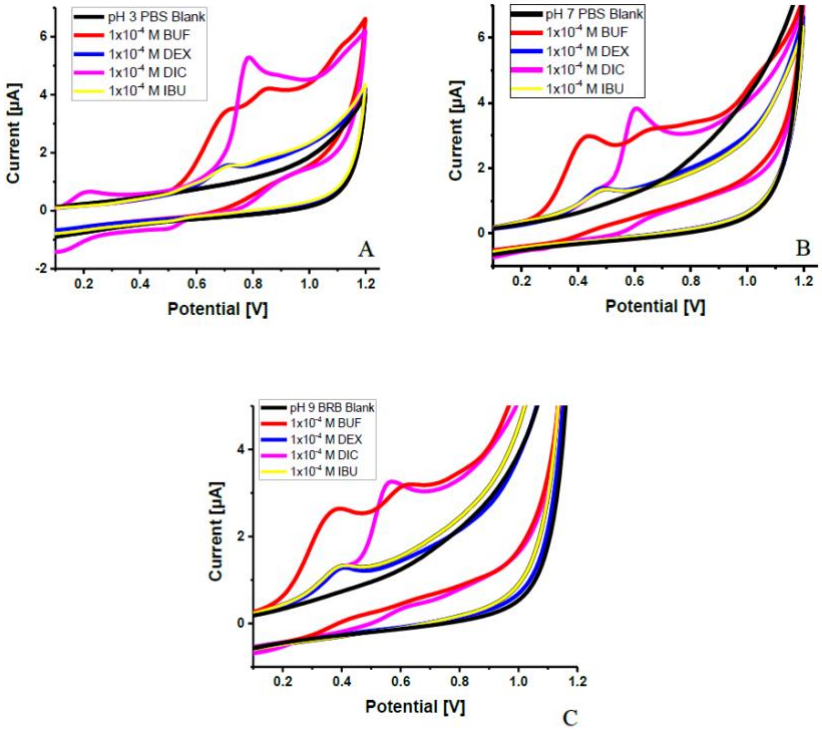

Figure 5. Cyclic voltammograms of 1x10^{-4} M BUF, DEX, DIC, and IBU in A) pH 3 PBS, B) pH 7 PBS, and C) pH 9 BRB on GCE (potential scan range: -0.2 – 1.2 V scan rate: 100 mV/s, step potential: 0.01 V).

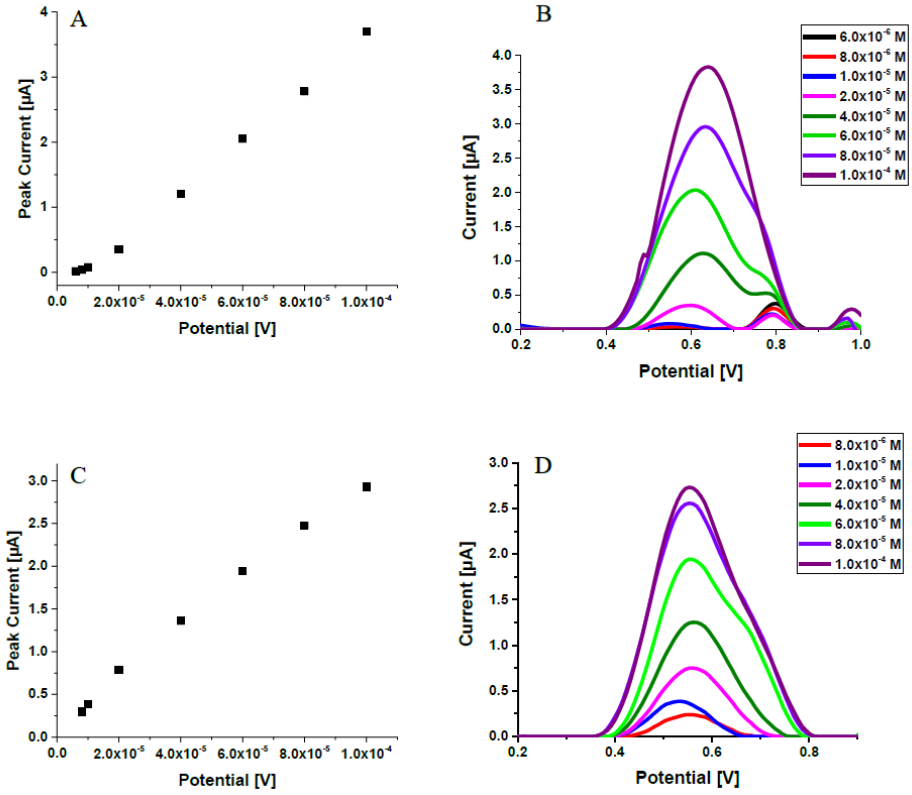

Figure 6. Calibration dependence on GCE A) in pH 3 PBS; C) in the commercial sample of human serum; differential pulse voltammograms of increasing BUF concentrations B) in pH 3 PBS; D) in the commercial sample of human serum (DPV curves are given after baseline correction. DPV Parameters; potential scan range: -0.2 – 1.0 V, scan rate: 0.1 V/s, step potential: 0.008 V, E_{pulse}: 0.2 V, t_{pulse}: 0.02 s).

Table 1. Regression data of the calibration line for BUF on GCE in standard solution and commercial human serum sample

Parameter	Standard solution	Commercial human serum sample
Linearity range (M)	6.0×10^{-6} – 1.0×10^{-4}	8.0×10^{-6} – 1.0×10^{-4}
Slope (µA/M)	3.94×10^{4}	2.87×10^{4}
SE of slope (µA/M)	8.19×10^{2}	9.08×10^{2}
Intercept (µA)	0.318	0.149
SE of intercept (µA)	0.043	0.051
Correlation coefficient (*r*)	0.999	0.997
LOD (M)	2.64×10^{-8}	1.59×10^{-7}
LOQ (M)	8.79×10^{-8}	5.32×10^{-7}
Repeatability of response (RSD%)*	1.54	1.04
Reproducibility of response (RSD%)*	1.70	1.39

*Each value is the mean of three experiments.

Table 2. Recovery results of commercial human serum samples for BUF on GCE

Sample concentration (M)	$2.00x10^{-5}$	$4.00x10^{-5}$
Spiked amount (M)	$4.00x10^{-5}$	$2.00x10^{-5}$
Found amount (M)*	$5.99x10^{-5}$	$5.98x10^{-5}$
Average recovery%	99.94	99.71
RSD% of recovery	1.53	1.97
Confidence intervals of recovery*	±1.34	±1.72
Bias%	-0.06	-0.29

*Each value is the mean of five experiments.

2.6. Interference study

The interference study assesses how well GCE can measure the target drug in the presence of exogenous or naturally occurring chemicals in biological fluids without being impacted by their signals. The most well-known potential interfering substances, ascorbic acid, KNO_3, $MgCl_2$, paracetamol, dopamine, Na_2SO_4, and uric acid, were chosen for this reason. In the interference study, for the determination of $6x10^{-5}$ M BUF, analysis was performed in the presence of an interference agent at concentrations equal to BUF, 10 and 100 times higher than BUF. According to the results, an interference effect was observed for BUF determination in the presence of paracetamol, dopamine, and uric acid and at 100 times higher concentrations of $MgCl_2$ and ascorbic acid. On the other hand, no interference effect occurred with KNO_3 and Na_2SO_4. Calculated recovery% and RSD% values are between 98.24% - 100.77% and 0.77% - 1.91%, respectively.

3. CONCLUSION

This study represents the first electrochemical analysis of BUF in standard solution and commercial human serum samples, employing a bare GCE. In the initial phase of the study, it was demonstrated that BUF exhibits an anodic peak at 0.79 V, 1.14 V, and 1.4 V in pH 3.0 phosphate buffer. The pH scanning was performed to obtain the optimal pH media, and The medium was determined as a pH 3.0 phosphate buffer.

According to the calculation, the number of electrons and protons involved in the oxidation process of BUF is equal. The number of transferred electrons (n) for the BUF oxidation process in pH 3 PBS on GCE was determined using the equation 5 and n was found as 3.

The slope of the log v and log ip equation was found to be 0.2953, which shows that the oxidation reaction of BUF is diffusion-controlled. The oxidation mechanism of BUF was investigated by recording the cyclic voltammetry of compounds with similar structures (DEX, DIC, and IBU) in pH 3 PBS, pH 7 PBS, and pH 9 BRB.

For the calibration study, the current values of the BUF peaks were obtained in the range of 6.0×10^{-6}-1.0×10^{-4} M with an LOD of 2.64×10^{-8} M. The applicability of the developed method was evaluated in the commercial serum sample. The recovery (99.94-99.71%) and RSD%(1.53-1.97%) values were evaluated to ensure the accuracy and precision of the developed method. The interference study was performed. No interference effect occurred with KNO_3 and Na_2SO_4. Calculated recovery% and RSD% values are between 98.24% - 100.77% and 0.77% - 1.91%, respectively.

4. MATERIALS AND METHODS

4.1. Reagents & chemicals and apparatus

BUF, dexketoprofen tromethamine (DEX), diclofenac (DIC), and ibuprofen (IBU) active pharmaceutical ingredients were supplied from Sigma-Aldrich (St. Louis, Missouri, USA). Their 10^{-3} M stock solutions were prepared in methanol (MeOH). All other reagents and chemicals used in this study were obtained from Sigma-Aldrich (St. Louis, Missouri, USA) and can be listed as follows with intended uses: MeOH (≥99.8%) as stock solution solvent of drugs; acetonitrile (ACN; ≥99.9%) for the precipitation of proteins in the preparation of biological samples; sulfuric acid (H_2SO_4; ≥99.99%), acetic acid (≥99.0%), sodium acetate trihydrate (≥99.0%), phosphoric acid (≥99.0%), sodium dihydrogen phosphate dihydrate (≥98.0%), disodium hydrogen phosphate dihydrate (≥99.0%), and sodium hydroxide (≥97.0%) for buffer solution preparation;

ascorbic acid, dopamine, uric acid (≥99.0%), paracetamol, magnesium chloride ($MgCl_2$; ≥99.0%), sodium sulfate (Na_2SO_4; ≥99.0%), potassium nitrate (KNO_3; ≥99.0%) as interference agents; drug-free commercial human serum as biological sample.

The intended buffer solutions were used to prepare the measurement solutions, with the MeOH ratio maintained at 20%. Electrochemical studies were performed using a range of buffer solutions, including Britton-Robinson buffer (BRB, pH 2.0–12.0), phosphate buffer solution (PBS, pH 1.5–8.0), acetate buffer solution (ABS, pH 3.7–5.7), and H_2SO_4 solution (pH 0.3, 1.0).

PSTrace 5.9 software was utilized to operate a potentiostat manufactured by PalmSens BV (Houten, Netherlands) for the electrochemical measurements of CV and DPV. The potentiostat was coupled to a three-electrode cell system made up of a platinum wire counter electrode, an Ag/AgCl reference electrode (3 M KCl), and a GCE (diameter = 3.0 mm). Every one of them was bought from BASi Inc. (West Lafayette, USA). Solid chemicals were weighed using a precision balance from Ohaus Instruments (Shanghai, China). A pH/ion meter S220 manufactured by Mettler-Toledo in Switzerland was employed to monitor and modify the pH levels of buffer solutions. A vortex mixer and ultrasonic bath were provided by J.P. Selecta Corporation (Barcelona, Spain) and ISOLAB Laborgerate GmbH (Eschau, Germany).

There were two steps involved in cleaning the GCE surface: First, it was placed in an ultrasonic bath for 15 minutes after being submerged in a mixture of double distilled water and MeOH (1:1, v/v). Following that, alumina slurry and a polish pad were used to polish it. Distilled water was used for washing, and it was dried at 25°C.

The following are the ideal conditions for measuring CV: Potential range: -0.2 V to 1.2 V, scan rate: 0.1 V/s, step potential: 0.01 V; and DPV: Potential range: -0.2 V to 1.0 V; step potential: 0.008 V; pulse potential: 0.2 V; pulse duration: 0.02 s; scan rate: 0.1 V/s.

4.2. Preparation steps of biological sample

Mixing $5x10^{-3}$ M BUF solution (500 µL), drug-free commercial human serum (1.8 mL), and ACN (2.7 ml) $5x10^{-4}$ M serum stock solution was prepared. This prepared mixture was centirfuged (5000 rpm, 20 min) in a centrifuge tube to allow the serum proteins to fully precipitate and separate. Measurement solutions were made with the appropriate dilutions using the resultant supernatant. By maintaining a constant MeOH ratio of 20% during the preparation of these solutions, the ideal buffer solution was employed. Then, standard BUF solutions were spiked at two distinct known concentrations to conduct recovery experiments.

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Conflict of interest statement: The authors declared no conflict of interest.

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