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# Studies of mechanism, kinetic model and determination of bupivacaine and its application pharmaceutical forms

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ABSTRACT

Bupivacaine, a local anesthetic that is often preferred in many wide ophthalmology applications, was investigated from different aspects such as electrochemical behavior, kinetic model, mechanism illumination, and analytical applications by cyclic voltammetry (CV) and square wave stripping voltammetry (SWSV) methods on glassy carbon electrode (GCE). Bupivacaine exhibited a well-defined anodic signal at nearly 850 mV, an irreversible and adsorption-controlled electrode process. Furthermore, a sensitive and selective SWSV method with a linear working range of 0.3–12.5 mg/L was developed for bupivacaine determination. Limits of detection (LOQ) and that of determination (LOD) were analyzed as 85 and 282 μg/L, respectively. In addition, the most probable study mechanism was carried out for the first time by this study. The selectivity of some co-existences such as heavy metals, ketotifen, moxifloxacin, ciprofloxacin, cyclopentanate and tropicamide was studied in 1 mg/L bupivacaine determination. They did not have a serious effect on bupivacaine determination at 5% tolerance limits. Otherwise, bupivacaine was successfully analyzed in matrix samples without any pretreatment by the proposed SWSV. Consequently, a fast, reliable, inexpensive, portable, eco-friendly, and sensitive new method has been developed to analyze bupivacaine in real samples.

## 1. Introduction

In recent years, extensive research has been focused on pharmaceutical and biological compounds, demonstrating the importance of paying attention to these compounds [1–4]. Bupivacaine is a local anesthetic frequently preferred in ophthalmology in a broad spectrum from vitrectomy to strabismus, dacryocystorhinostomy, glaucoma filtering surgery, cataract surgery, and eyelid surgery which is so-called blepharoplasty [5–10]. Besides, another way to use subconjunctival bupivacaine injection was described as an effective analgesic after silicon oil removal surgery [11]. Intraoperative sub-tenon bupivacaine injection was found to be effective in reducing pain and irritation in pterygium excision surgery [12]. Another study investigating the oculocardiac reflex effects on sub-tenon's Bupivacaine in strabismus surgery revealed that bupivacaine injection as a local anesthetic can decrease bradycardia [13]. In a study, bupivacaine was used as an intrathecal agent in neuropathic pain [14]. Moreover, there are numerous animal studies concerning the use of bupivacaine [15].

The analytical methods available upuntil now for the determination of bupivacaine, a vital drug for human health, have been commonly done by chromatography, spectrophotometry, and electrophoresis [16–26]. The classical analytical methods used in the investigation of bupivacaine drug included high performance liquid chromatography (HPLC) combined with ultraviolet (UV) and diode array detector (DAD) (HPLC-UV or HPLC-DAD)[20,21,23,26–30], high-performance liquid chromatography-tandem mass spectrometric (HPLC-MS-MS) [24], reverse phase high performance liquid chromatography (RP-HPLC) [19], liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) [16,31,32], capillary electrophorese (CP) [19,33,34], capillary gas chromatography (CGE)[22,35], capillary electrophoresis-mass spectrometry (CE-MS) [25] and gas chromatography-mass spectrometry (GC/MS) [36]. In addition, apart from these studies, only one ion-selective electrode has been developed for bupivacaine analysis [37]. However, these methods have basic shortcomings such as

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equipment cost, long pretreatment application, use of a lot of organic solvent, and expertise. Electrochemical methods can be the alternative options to avoid the shortcomings of these methods, such as cost, time, and chemical surplus [38–44]. Furthermore, electroanalytical techniques have proven to be a good alternative according to existing methods in the analysis of substances with low detection limits, high dynamic range, and sensitivity [45–52]. Furthermore, due to its portability and ease of use, the instrument used in electrochemical methods has attracted great attention in recent years [53–60]. In addition to valuable studies performed on bupivacaine determination by chromatography and spectrophotometry, there is only one study in the literature using the electrochemical technique as a differential pulse voltammetry (DPV) by using polyimide membrane-based electrode [61]. However, basic method validation studies such as electrochemical behavior, kinetic model, mechanism, support electrolyte effect, selectivity, and analytical application for the bupivacaine have not been performed. In order to prove the accuracy and selectivity of the method, analytical applications in complex biological systems and the effects of interference species in bupivacaine determination should be investigated.

We herein present a simple, fast, portative and eco-friendly square wave stripping voltammetric method for determination of bupivacaine drug with high precision and selectivity on glassy carbon electrode (GCE). This study aims to investigate electrochemical behavior and develop a new analytical method for the bupivacaine drug, whose electrochemical properties have not been studied in detail by using cyclic voltammetry (CV) and square wave stripping voltammetry (SWSV) methods on GCE. The kinetic model, mechanism, and electrode process studies of bupivacaine were performed for the first time. Moreover, studies of pH effect, selectivity, and analytical application, which are essential for method validation, were carried out by SWSV. Furthermore, in the presence of coexistence substances, bupivacaine determination was performed with high recovery with less than 5% error by SWSV. In addition, analytical applications were successfully performed at pharmaceutical dosage and synthetic serum by developed SWSV. Consequently, the electrochemical properties, electrode mechanism, selectivity and applicability of the bupivacaine substance were fully illuminated in detailed.

## 2. Materials and methods

### 2.1. Apparatus

All the electrochemical measurements were carried out by using a Vertex. One-Ivium electrochemical analyzer. These measurements were performed in a chamber electrochemical cell containing 10 mL supporting electrolyte and consisting of a three-electrode system. This system consists of triple electrodes, a reference electrode (Ag/AgCl (BASi, MF-2052), an auxiliary electrode (platinum wire, BASi, MW-1032) and an indicator electrode (glassy carbon electrode (GCE), BASi MF-2012, d = 3 mm). Furthermore, alumina powder (size 58 Å, ~150 mesh, purchased from Sigma-Aldrich) was used to polish the surface of the GCE before each electrochemical measurement to obtain a clean, smooth, and reproducible surface of the working electrode. The cyclic voltammetry (CV) and square wave stripping voltammetry (SWSV) methods were used to improve a new electroanalytical methodology. All pH measurements were carried out by using Mettler Toledo pH meter with measurements at an accuracy of ± 0.05. Besides, all measurement applications were performed at room temperature.

### 2.2. Reagents

Bupivacaine as an analytical standard substance (United States pharmacopeia (USP) reference standard) and its pharmaceutical dosage form as a Marcaine® were obtained from Sigma-Aldrich and a drugstore, respectively. The stock solution of bupivacaine was prepared by dissolving 0.0050 g of bupivacaine in 5 mL acetone and 5 mL distilled water at the concentration of 500 mg/L. The carbon paste electrode was made by using a mixture of 30% mineral oil (suitable for preparation of Nujol mulls for infrared spectroscopy) and 70% graphite (powder $< 20$ µm, synthetic) supplied from Sigma Aldrich. The two supporting electrolytes were used to obtained different pH values. The HCl solution for the pH 1.0, and Britton Robinson (B–R) buffer solution, 0.04 mol/L acetic acid (glacial, ReagentPlus®, $\geq$99%), orthophosphoric acid (85%) and boric acid ($\geq$99.8%), for the pH between pH 2.0 to pH 8.0 were prepared by adjusted 2 M NaOH or 2 M HCl. In all solution preparations and cleaning processes, distilled water was used. Furthermore, all solutions were stored in a refrigerator at 4 °C when not used in the experiment.

### 2.3. Analytical procedures

Firstly, the sensitivity of CPE and GCE electrodes to bupivacaine at pH 5.0 was examined and GCE was found to be the most ideal for the determination of bupivacaine. The voltammetric response of bupivacaine to GCE was investigated in different solutions of support electrolytes and the optimum pH buffer was found as a pH 7.0 B-R buffer solution. To find the type of transport for the agent to the electrode surface, studies of different scan rates were determined by CV. Parameters on the SWSV instrumental moderated such as pulse amplitude ($\Delta$E), frequency ($f$), accumulation time ($t_{acc}$), accumulation potential ($E_{acc}$), and step potential ($\Delta E_s$) which significantly affect the peak current and potential have been optimized in detail. Under instrumental conditions for the SWSV, $\Delta E = 60$ mV, $t_{acc} = 60$ s, $E_{acc} = 200$ mV, $f = 125$ *Hz and* $\Delta E_s = 7$ mV, calibration curves of bupivacaine were performed by the standard addition method. Furthermore, the interfering effect of some organic substances and heavy metals were evaluated on the peak current of bupivacaine to show the selectivity of the SWSV method.

### 2.4. Analytical application to pharmaceutical dosage and synthetic serum

The pharmaceutical dosage form as a Marcaine®, commonly used in local anesthetics applications, was obtained from a local drugstore. The drug solution contains bupivacaine of bupivacaine hydrochloride as a 0.5% w/v. Because the dosage of the drug is liquid, the amount of direct analysis by SWSV using standard addition method on GCE was performed without any pretreatment. Before taking electrochemical measurements, the drug dosage was centrifuged at 3500 rpm for 5 min. Besides, bupivacaine was analyzed by high performance liquid chromatography (HPLC) method, and also *F* test and Student *t*-tests were performed for both methods to compare SWSV results.

The synthetic blood serum was purchased from Aldrich-Sigma. 1 mL of the bupivacaine stock solution was added to 9 mL of the synthetic blood serum samples to obtain a sample having a 50 mg/L of bupivacaine. It was stirred at room temperature for about 10 min in an ultrasonic bath, then analyzed by voltammetric techniques with standard addition method.

## 3. Results and discussion

### 3.1. Electrochemical behavior of bupivacaine

Initially, the working electrode type was determined as a GCE and CPE to detect bupivacaine. For this purpose, SWS voltammograms were obtained in the presence of 8.41 mg/L bupivacaine in pH 5.0 B–R buffer solutions using both of the working electrodes. The GCE was found to be quite sensitive to the CPE for the analysis of bupivacaine, and further studies were carried out only with the GCE in the novel electroanalytical methodology to deduce bupivacaine.

Herein, cyclic voltammetry (CV) is preferred to investigate the electrochemical behaviors, kinetic model, and the illumination of the mechanism for the bupivacaine substance. Scan rate studies by CV provide important information, especially in determining whether substance transport is controlled by diffusion or adsorption in the electrode reaction process. Therefore, cyclic voltammograms were obtained at different scan rates on GCE in the presence of 10.0 mg/L bupivacaine in pH 7.0 B-R buffer solutions. As shown in Fig. 1, when scanning in anodic directions, the bupivacaine exhibited a single peak at nearly +950 mV, but in the opposite directions as cathodic potentials, no signal was obtained in pH 7.0 B-R buffer solutions on GCE. Basically, the electrode reaction of bupivacaine indicates that oxidation has an irreversible electrochemical process. Moreover, with increasing scanning rates by CV, bupivacaine's peak potential values shifted to more positive regions on GCE. Considering the linear equation between the obtained Ep and log *ν*, approximately 5.83 mV shift was observed in the positive anodic direction against the change in every 100 mV scanning rate by CV on GCE. Therefore, it can be said that the shift of peak potential for signal exhibited by bupivacaine depends on the scanning rates.

$$E_p\ (\mathrm{V}) = 0.038 \log \nu\ (\mathrm{V/s}) + 1.004\ \ r = 0.9955$$

Furthermore, the effect of the scan rate provides clear ideas about the bupivacaine's electrode process where diffusion or adsorption is controlled. Therefore, cyclic voltammogram measurements of bupivacaine were collected at different scanning rates from 25 mV to 500 mV/s in pH 7.0 support electrolyte on GCE (Fig. 1). The relationship between the logarithm of the scan rate and that of the peak current was calculated for the single anodic peak of 10 mg/L bupivacaine. A linear equation with a slope of 0.960 was obtained between the logarithm of the scanning rate (*ν*) and the peak current ($I_p$). Since this value is very close to 1.0 value accepted for adsorption control electrode processes, we can easily say that the bupivacaine electrode process is adsorption controlled.

$$\log I_p\ (\mu\mathrm{A}) = 0.960 \log (\nu)\ (\mathrm{V/s}) - 1.223\ (r = 0.9949)$$

An important parameter of the kinetic model studies with CV is the square root of the scan rate ($\nu^{1/2}$) and the peak current ($I_p$) relationship of the substance. Here, we examined the effect of the square root for the different scanning rates in the range of 25–500 mV/s on the oxidation peak currents of the bupivacaine substance. These two values exhibited a linear slope and the linear equation between the peak currents and the square root of the scan rates that can be expressed as follows:

$$I_p\ (\mu\mathrm{A}) = 14.972\ \nu^{1/2} - 2.590\ (r = 0.9791)$$

### 3.2. *The influence of pH*

A crucial point in electrochemical studies is also the study of the effect of support electrolytes, since it causes significant changes not only in peak current, but also in peak potential. Two different solutions were used to find the optimum support electrolyte solution. 0.1 M HCl was used for pH 1.0 and between 2.0 and pH 8.0, Britton Robinson (B-R) buffer solution was preferred. Each assay was performed twice in the presence of 12.62 mg/L bupivacaine to examine the pH effect with SWSV on GCE (Fig. 2). No peaks of bupivacaine were observed in strongly acidic regions such as pH 1.0 and pH 2.0 in the working potential range on GCE by SWSV. An oxidation peak of bupivacaine occurred at nearly 1.1 V in pH 3.0 onwards. This peak current increased liner up to pH 7.0, and also the peak potential of bupivacaine shifted to less positive regions by increasing pH value. The signal of bupivacaine at pH 8.0 and later in the alkaline regions disappeared. The peak potential of bupivacaine versus each unit pH change shifted to 86 mV less positive range. The optimum supporting electrolyte was chosen as a pH 7.0 for further experiments due to the maximum signal obtained for the bupivacaine on GCE by SWSV. Furthermore, the shift of peak potential by increasing pH values is considered as evidence of the presence of protons in the oxidation electrode reaction. Thus, it can be said that the electrochemical oxidation of bupivacaine contains proton ($H^+$). The following equation was obtained from the graph of the peak potential ($E_p$) versus changed pH value between pH 3.0 and pH 7.0 on GCE by SWSV.

$$E_p\ (\mathrm{V}) = -0.086\ \mathrm{pH} + 1.452\ \ R^2 = 0.9676\ (\mathrm{pH}\ 3.0\text{–}7.0)$$

### 3.3. *Optimization of SWSV modules*

In the square wave voltammetric technique, the effect of stripping modules such as accumulation time, accumulation potential, step potential, amplitude, and frequency with a significant effect on peak current and peak potential were examined in detail. Each of these instrumental modules is studied as one variable, while the other parameters are fixed. Optimization of all SWSV modules was performed in pH 7 B-R buffer solution in the presence of 8.41 mg/L bupivacaine on the GCE.

Different pulses ranging from 10 mV to 80 mV were applied to find the optimal pulse amplitude for the SWSV module. The peak currents of bupivacaine were increased with pulse amplitudes up to 60 mV in pH 7 B-R buffer solution on the GCE. However, pulse amplitudes after 60 mV decreased suddenly on peak current of bupivacaine exhibited at nearly 0.85 V. The highest peak current was selected as the optimum pulse amplitude of 60 mV and this value was always used in further studies. Similarly, to find the step potential effect, different step potentials ranging from 1 mV to 9 mV were applied. A steady increase was

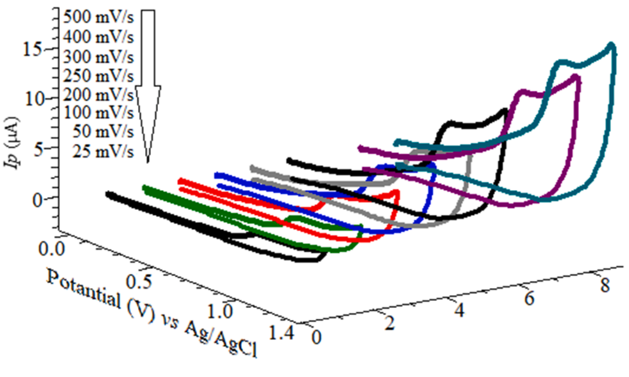

**Fig. 1.** Cyclic voltammograms of 10 mg/L bupivacaine at different scan rate from 25 mV/s to 500 mV/s on GCE at pH 7.0.

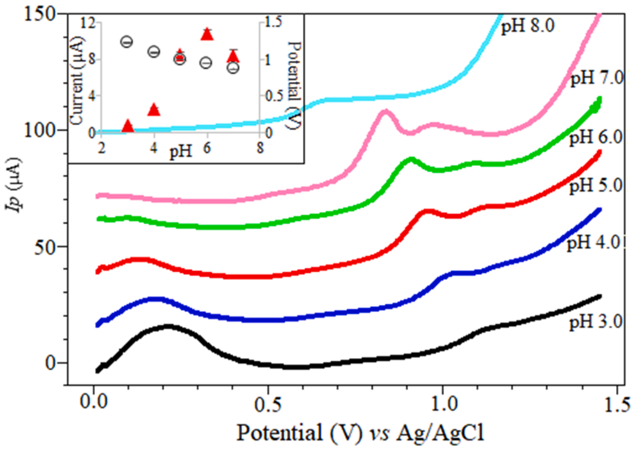

**Fig. 2.** Different pH values effect on the oxidation peak of bupivacaine by SWSV.

observed in the peak current of bupivacaine with step potential up to 7 mV. However, there was no significant change in peak current of bupivacaine at the next step potentials. The most sensitive peak current is selected as the optimum step potential of 7 mV. The SWSV module as a frequency was investigated between 10 Hz and 250 Hz to find the optimum value in pH 7 B-R buffer solution on the GCE. There was a steady and significant increase in peak currents of bupivacaine up to 150 Hz. At a frequency of 150 Hz, the peak intensity of the bupivacaine gradually decreased. However, although there was a slight increase between 125 Hz and 150 Hz, the optimum and uniform peak shape was chosen as the optimum frequency of 125 Hz mV. In addition, due to increasing frequencies (from 10 mV to 250 mV), peak potentials of bupivacaine shifted to more positive regions (Fig. 3).

The effects of accumulation time and accumulation potential, which are crucial for the stripping module in the SWSV method, were investigated due to the fact that these two stripping modules have a profound effect on the peak density of the target agent. Different accumulation potentials between –0.1 V and +0.4 V were applied to examine the effect of deposition potential in pH 7 B-R buffer solution in the presence of 8.41 mg/L bupivacaine on the GCE. Among the applied accumulation potentials, the peak current of bupivacaine exhibited at 0.85 V had no significant effect as much as the other optimum parameter. The optimum accumulation potential of 200 mV was selected, snice the most sensitive peak current was obtained. Furthermore, to examine the effect of accumulation time, which is one of the most important parameters of stripping methods, accumulation studies were performed between 10 s and 80 s. There was a significant increase in peak intensity of bupivacaine up to 60 s accumulation times. After 60 s accumulation time, there was no significant change in the peak current of the agent. This is an indication that the electrode surface is completely covered with the substance during accumulation times of 60 s or more. The 60 s, at which the best peak height was obtained, was chosen as the optimum accumulation time. Consequently, the SWSV modules can be summarized as pulse amplitude (ΔE) of 60 mV, accumulation time ($t_{acc}$) of 60 s, accumulation potential ($E_{acc}$) of 200 mV, frequency ($f$) of 125 Hz and step potential ($\Delta E_s$) of 7 mV in pH 7.0 B-R buffer solution on the GCE.

Square wave stripping voltammetry (SWSV) is a highly sensitive, accurate, reliable, and fast technique compared to conventional analytical methods. Therefore, it has attracted attention for the last two decades and also many studies have been carried out by using this technique. Furthermore, one of the most important advantages of the SWSV well-established module is the quantitative determination of the target analyte, even at very low concentrations.

SWSV, selected to be the best instrumental modules for the analytical studies, was improved for the quantitative determination of bupivacaine in pH 7.0 B-R buffer solution on GCE. The calibration graph was constructed by using the well-defined anodic peak of bupivacaine at around 0.85 V by standard addition methods under SWSV modules (Fig. 4). Fig. 4 presented an appropriate linear concentration of bupivacaine ranging from 0.3 mg/L to 12.5 mg/L. The linearization equation from 0.3 to 12.5 mg/L was expressed as:

$$I_p\ (\mu A) = 2.789C\ (mg/L) + 1.564;\ (0.3\ to\ 12.5\ mg/L,\ R^2 = 0.9937)$$

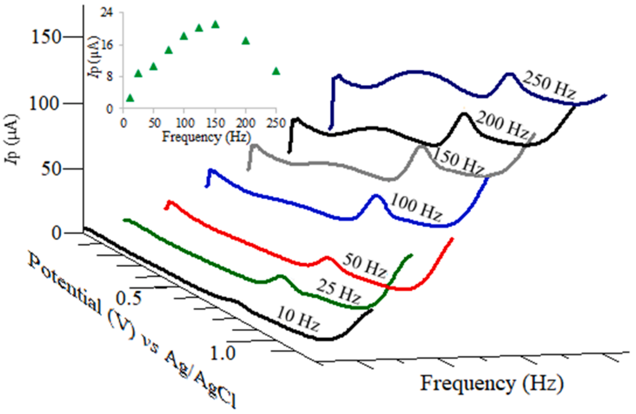

**Fig. 3.** SWSV voltammograms at different frequencies in pH 7 B-R buffer solution in the presence of 8.41 mg/L bupivacaine on the GCE.

The validation modules such as the limit of detection (LOD), the limit of quantitation (LOQ), repeatability of peak current, and potential for a novel SWSV which is the first-time analysis of bupivacaine, were evaluated to show precision and accuracy of the proposed methods. The LOD and LOQ were calculated by using "3 *m/s*" and "10 *m/s*" equations, respectively [62–64]. The "*m*" is the slope of calibration graph and "*s*" is the standard deviation of 7 measurements in the presence of minimum concentration as a 0.3 mg/L. The LOD and LOQ of bupivacaine were calculated as 85 and 282 µg/L, respectively. In addition, repeatability studies, which are indicative of the accuracy, precision, and reliability of the novel SWSV method, were performed on the same day. Relative standard deviation (RSD%) was used to calculate repeatability of the anodic peak intensity and peak potential exhibited at nearly 0.85 V in pH 7.0 B-R buffer solution on GCE by SWSV and the RSD% value was found to be less than 4.0%. Consequently, full validated parameters of the developed SWSV module for the analysis of bupivacaine are detailed in Table 1.

### 3.4. Electrode reaction mechanism

Electrode reaction mechanism is the most important pillar of scanning rate studies performed with cyclic voltammetry. In general, the effects of scanning rates on peak current and peak potential at various values are examined and investigated whether the substance is involved in the electrode reaction or not. Therefore, as previously mentioned in Section 3.1, cyclic voltammograms were obtained at different scanning rates from 25 mV/s to 500 mV/s in the presence of 10 mg/L bupivacaine in GCE pH 7.0 B-R buffer solution. A linear equation with a slope of 0.038 was obtained between the peak potential and the scan rate of the logarithm at scanning rates from 25 mV/s to 500 mV/s by CV. Also, the Laviron equation which is most suitable for the irreversible electrode was employed to find the number of electrons transferred in the anodic process of the bupivacaine [63,64].

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

In this equation, icons of R, T, n, F, k°, α, and $v$ are related to universal gas constant (8.314 J/K·mol), temperature (K), number of

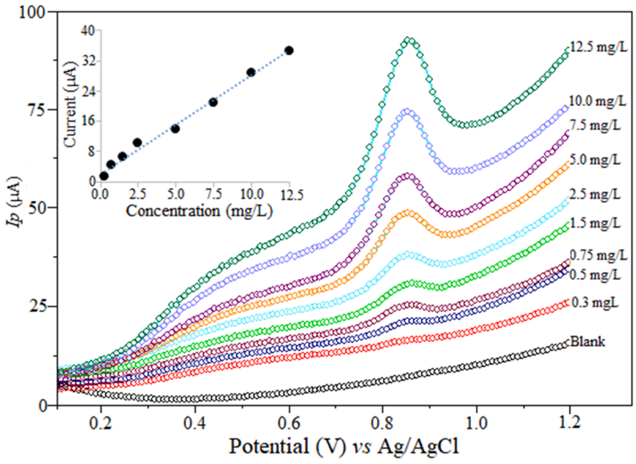

**Fig. 4.** SWSV voltammograms at various bupivacaine concentrations obtained by standard addition in pH 7 B-R buffer solution on the GCE (ΔE = 60 mV, $t_{acc}$ = 60 s, $E_{acc}$ = 200 mV, $f$ = 125 Hz and $\Delta E_s$ = 7 mV).

**Table 1**
Regression data of the calibration graph of bupivacaine by obtained SWSV.

| Parameters (unit) | Value |
|---|---|
| Peak potential (mV) | 850 |
| Linearity range (mg/L) | 0.3–12.5 |
| Slope (μA L/mg) | 2.789 ± 0.074 |
| Intercept (μA) | 1.564 ± 0.441 |
| Correlation coefficient | 0.9937 |
| Limit of determination (LOD) (μg/L) | 85 |
| Limit of quantification (LOQ) (μg/L) | 282 |
| Repeatability of peak current (RSD%)[a] | 3.79 |
| Repeatability of peak potential (RSD%)[a] | 1.12 |

Relative standard deviation, $\mathrm{RSD} = \left(\frac{s}{\bar{x}}\right) 100$: [a]n = 7.

transferred electrons, Faraday constant (96485 C/mol), standard heterogeneous constant rate of reaction, electron transfer coefficient, and scanning rate, respectively.

When all known values were substituted in the Laviron equation, the value "αn" was found to be 1.56 for the oxidation process of bupivacaine. For irreversible electrochemical reactions, the value of "α" is usually taken as 0.5. Here, the number "n" was directly calculated as 3.12. On the other hand, the number of electrons (n) transferred in the bupivacaine electrode reaction was accepted as 3. Furthermore, the peak potentials of bupivacaine, with increasing pH head, shifted to less positive regions. The signal shift against each pH unit change was approximately 85 mV. This can easily be said that it includes a proton ($H^+$) in the bupivacaine electrode reaction. Consequently, a possible oxidation mechanism of bupivacaine can be proposed as follows (Fig. 5).

### 3.5. Method selectivity study

One of the most vital parameters for a novel analytical method that is newly developed or offered as an alternative is that the method needs to be highly selective. Moreover, the selectivity of the method is also a major factor that has a direct impact on its accuracy and precision. For these reasons, the interference effects of substances that have high co-existence potential in the samples should be investigated in the presence of bupivacaine. To interpret the applicability and selectivity of the proposed SWSV, analysis of bupivacaine was carried out in the presence of metal ions at 5:1 and 10:1 (mass (interfering agent)/mass (bupivacaine)) by adding 1.0 mg/L bupivacaine on GCE in an optimum supporting electrolyte solution. The selectivity study of a novel SWSV method for the proposed method in determining bupivacaine was calculated as the recovery percentage of the anodic peak signal by four times repeating of measurements through standard deviation (Table 2). Interferences effect of the heavy metals such as Ba(II), Pb(II), Cu(II), Zn (II) and Fe(III) on the anodic peak were evaluated in the presence of 1.0 mg/L bupivacaine. The interference agents did not dramatically influence on the anodic signal of bupivacaine at both concentrations in pH 7.0 B-R buffer solution on GCE. Percent recovery of bupivacaine was determined within 10% limit values in presence of co-existence heavy metal by SWSV. Whence, the selectivity of the SWSV method proposed for the determination of bupivacaine is very satisfactory (Fig. 6).

Furthermore, some organic types such as ketotifen, moxifloxacin, ciprofloxacin, cyclopentanate, and tropicamide which are coexistent agents at 5 mg/L concentration were investigated in the presence of 1 mg/L bupivacaine. Among these coexistent substances, moxifloxacin and ciprofloxacin were electroactive and exhibited peaks nearly at 1115 mV and 1160 mV on GCE in pH 7.0 B-R buffer solutions, respectively. The effects of interference agents were found lower than 5% for the peak signal of bupivacaine. As a result, the selectivity of the proposed SWSV which is used for the analysis of bupivacaine is very satisfactory in the presence of all the coexistent agents.

### 3.6. Application of pharmaceutical dosage and synthetic serum

The new method is the most important analytical performance for

**Table 2**
Interference effects of some cations and organic substances the determination of bupivacaine by SWSV.

| Interfering agent | 1:5 (m/m) | 1:10 (m/m) |
|---|---|---|
| Ba (II) | 99.87 ± 3.50 | 95.77 ± 0.60 |
| Cu (II) | 97.04 ± 1.94 | 93.55 ± 0.82 |
| Fe (III) | 97.12 ± 1.89 | 95.90 ± 3.18 |
| Pb (II) | 99.72 ± 2.35 | 92.01 ± 2.41 |
| Zn (II) | 102.15 ± 1.76 | 99.34 ± 3.78 |
| Ketotifen | 100.09 ± 3.01 | — |
| Moxifloxacin | 96.78 ± 2.97 | — |
| Ciprofloxacin | 95.05 ± 1.48 | — |
| Cyclopentanate | 96.66 ± 2.84 | — |
| Tropicamide | 100.41 ± 2.43 | — |

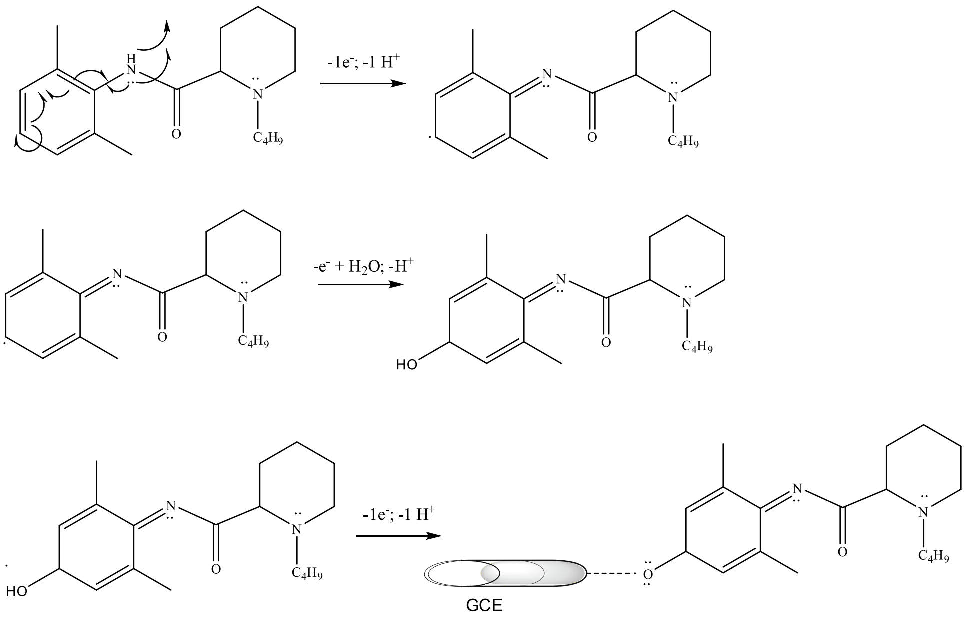

**Fig. 5.** The possible oxidation mechanism of bupivacaine on GCE.

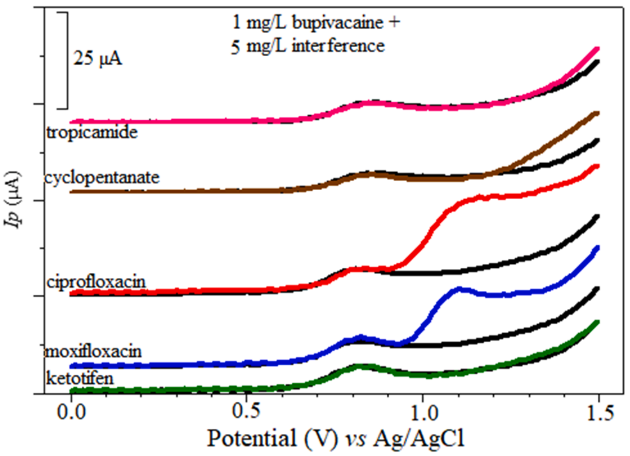

**Fig. 6.** Interference effects of organic compounds at 5 mg/L in the determination of 1 mg/L bupivacaine by SWSV on GCE.

accurate and precise studies. For these reasons, the validity of the recommended SWSV method was investigated in real samples. First, the determination of bupivacaine in Marcaine® drug purchased from the pharmacy was carried out by SWSV on GCE in pH 7.0 B-R buffer solutions (Fig. 7). Based on the fact that Marcaine contains 0.5% bupivacaine indicated on the label of the drug, target analytical analysis was successfully performed with the standard addition method (Table 3). Furthermore, spiked bupivacaine in synthetic serum at a concentration of 50 mg/L was analyzed by SWSV on GCE under the optimized conditions. The proposed electrochemical method was directly carried to evaluate bupivacaine on GCE without any pretreatment applications in synthetic serum. According to the analytical applications, the proposed SWSV method is very precise, accurate, reliable, costly, and suitable for the analysis of bupivacaine in real samples.

## 4. Conclusion

This article includes the electrochemical behavior, kinetic model, mechanism illumination, and analytical applications in real samples of bupivacaine using GCE electrode by cyclic voltammetry (CV) and square wave stripping voltammetry (SWSV) methods. For this purpose, it was determined that the bupivacaine substance only exhibits anodic peak, has an irreversible electrode reaction and an adsorption-controlled electrode process. Moreover, it was proved that it contains 3 electrons and protons in the electrode reaction according to the CV. In addition, the most probable mechanism was proposed for the first time in this study. Validation parameters of bupivacaine were determined using the proposed SWSV method. A highly sensitive, reliable and precise method has been developed for the determination of bupivacaine at nanomolar level as 261 nM in natural and real samples. In addition, the selectivity of the SWSV method was examined in the presence of some cations and organic substance on CPE. It was observed that these co-existence substances did not affect the bupivacaine detection almost at 5% tolerance limit level. Moreover, with the developed method, bupivacaine can be determined simultaneously with electro active substances such as ciprofloxacin and moxifloxacin. Finally, with the developed SWSV, simple, fast, reliable, and precise determination of bupivacaine was performed on matrix samples such as pharmaceutical dosage and synthetic serum without any pretreatment. As a result, for bupivacaine, an important local anesthetic drug, a fast, reliable, inexpensive, portable, eco-friendly and sensitive new method has been developed to analyze.

## CRediT authorship contribution statement

**Ersin Demir:** Formal analysis, Investigation, Methodology. **Onur İnam:** Formal analysis, Investigation, Methodology. **Hülya Silah:** Writing - original draft. **Hassan Karimi-Maleh:** Writing - review & editing.

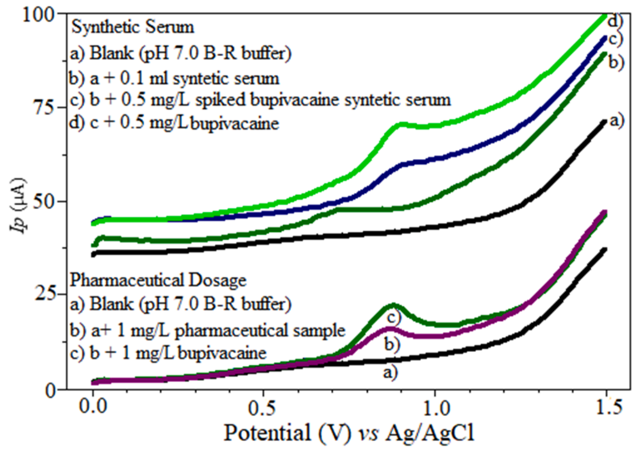

**Fig. 7.** Analytical performance of SWSV method in pharmaceutical dosage and synthetic serum on GCE in pH 7.0 B-R buffer solutions.

**Table 3**
Analysis of bupivacaine in pharmaceutical dosage and synthetic serum by SWSV on GCE n = 3.

| | Pharmaceutical dosage | Synthetic serum |
|---|---|---|
| Labeled Claim | 5 g/L | 50 mg/L (spiked) |
| Found | 4.94 ± 0.08 g/L | 48.95 ± 1.2 mg/L |
| RSD (%) | 1.62 | 2.45 |
| Relative error (%) | −1.20 | −2.10 |

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2020.105531.

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