Full Paper

Development of Ofloxacin Electrochemical Sensor in Milk Sample using Boron-doped Diamond Electrode Decorated by Zinc Nanoparticles

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Abstract- The study of Ofloxacin (OFL) electrochemical sensor on the surface of zinc modified boron-doped diamond (Zn-BDD) electrode is reported. OFL has been known as one of the fluoroquinolone antibiotics which is used in treating infectious diseases and also in animal agriculture. Despite its effectiveness, long-term consumption could promote antimicrobial resistance. Therefore, a sensitive and selective detection method is important. Meanwhile, BDD has been known for its low background current and high stability, in which, it is necessary for sensor application. Besides, Zn nanoparticles have been widely used for sensor applications due to their low toxicity and low cost. In this work, Zn-BDD electrode was prepared through electrodeposition. The S/B value and sensitivity for OFL analysis were 9.5 and 0.42 respectively on Zn-BDD, in which, it is higher compared to the use of a bare BDD electrode. The analysis in milk was possible with the recovery of 83.05% and 101.20% for BDD and Zn-BDD electrodes respectively. It is suggested that Zn-BDD electrode could be applied for OFL analysis for further real application.

1. INTRODUCTION

Ofloxacin (OFL), namely (±)-9-fluro-2,3dihydro-3-methyl-10(4-methyl-1-piperazynyl-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxacine-6-carboxilic acid is one of the synthetic antibiotics used to treat urinary tract infection, pneumonia, and respiratory infection (Scheme 1) [1,2]. OFL, as one of the fluoroquinolone antibiotics also has been used in animal agriculture and companion animal medicine, due to its ability to promote animal growth and prevent various diseases [3,4]. The release of this fluoroquinolone antibiotics to the environment, even in small quantities, further could stimulate the antimicrobial resistance. Moreover, problem related to food safety is an important point to be considered for human health. Therefore, the need of OFL monitoring is mandatory
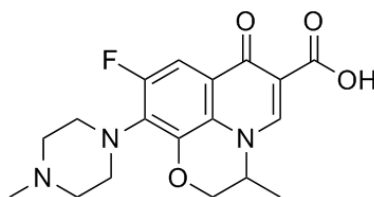
Scheme 1. Chemical structure of OFL

Various methods have been developed such as spectrofluorometric [5], electrophoresis [6], flow-potentiometric system [7] and chemiluminescence [8]. Meanwhile, electrochemistry has been known as a green analysis technique due to its less reagent requirement, short time, and simple analysis skill. In electrochemistry, it has been known that electrocatalytic performance is greatly influenced by electrode selection. Various types of modified and unmodified electrodes such as glassy carbon electrode (GCE) [9], graphene/zinc oxide composite modified on GCE [10], graphene/GCE [11], graphene oxide (GO)/carbon nanotube (CNT) composite [12], multiwalled-carbon nanotube (MWCNT) [13], and carbon paste electrode (CPE) [14], have been applied for electrochemical analysis of OFL. Meanwhile, boron-doped diamond (BDD) electrode is one of the promising carbon electrodes for electroanalytical application due to its low background current, wide potential window, and also good physical and chemical stability [15–19]. A modification on BDD surface has been known to improve its catalytic activity, selectivity, and lower background current [20–22]. Wahyuni et al reported the improvement of the limit of detection (LOD) in an analysis of Zanamivir using gold-modified BDD electrode [23]. The work by Zheng et al also reported the improvement of the sensitivity by 103 times by modifying BDD electrodes with gold and nickel nanoparticles [24].

Meanwhile, Zinc (Zn) and ZnO nanoparticles have been widely used for electroanalytical sensor applications due to their excellent properties such as low toxicity, biocompatible, chemically stable, and low cost [25-27]. ZnO nanoparticles had been used for ascorbic acid, dopamine, and uric acid electrochemical detection, resulting in a very low limit of detection [28]. Besides, nanosized ZnO has been reported for trimethoprim detection with low lit of

detection in pharmaceutical dosages and real samples [29]. Therefore, utilizing Zn/ZnO nanoparticles is promising due to their excellent properties. Modification of Zn nanoparticle through electrodeposition method on the surface of BDD electrode was then suggested, in order to improve the sensitivity of the ofloxacin analysis. In this report, various studies such as pH dependence, linearity, sensitivity, reproducibility, and analysis of the commercial production of antibiotics in fresh milk samples will be presented.

2. EXPERIMENTAL SECTION

2.1. Reagents and Chemicals

OFL was obtained from Sigma Aldrich. The 1 % (B/C) BDD electrode was prepared by growing it on a silicon wafer (Si <111>) using microwave plasma-chemical vapor deposition (MP-CVD, Model AX-5400, CORNES Technology Corp.) as described in a previous report [30]. Other reagents used were of analytical grade. H_3PO_4 (85%), $NaH_2PO_4.H_2O$ (99-102%), $Na_2HPO_4.2H_2O$ ($\geq$99.5%), and $ZnCl_2$ (98-100%) were obtained from Merck. All experiments were performed in a 0.1 M phosphate buffer solution (PBS). Fresh milk was purchased from the local supermarket in Surabaya, Indonesia. All chemicals were used without further purification

2.2. Preparation of the modified electrode and electrochemical analysis

The bare BDD electrode was cleaned using the ultrasonication method in distilled water for 5 min. Thereafter, cyclic voltammetry (CV) from -2.5 V to +2.5 V for 40 cycles was applied with a scan rate of 1 V s-1 in 0.1 M H_2SO_4 solution to clean and ensure the same surface condition for all BDDs. The Zn nanoparticles modified BDD (Zn-BDD) electrode was obtained by electrodeposition at a potential of -1.6 V for 60 s in a solution of 0.6 mM $ZnCl_2$ (optimum condition). Finally, Zn-BDD was obtained and allowed to stand at room temperature to dry for further use. Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) (Zeiss, Bruker, EVO MA 10) characterization was performed to know the topography of Zn-BDD. All electrochemical experiments were carried out using a potentiostat (Emstat3+Blue Palmsens). A conventional three-electrode electrochemical system, consisting of a BDD and Zn-BDD as a working electrode, Ag/AgCl (saturated KCl) as a reference electrode, and a Pt wire as a counter electrode, were used for all electrochemical analysis.

3. RESULTS AND DISCUSSION

3.1. Characterization of Zn-BDD

The Zn-BDD electrode was further characterized using SEM-EDX (Figure 1). Zinc particles has been successfully deposited on the surface of the BDD electrode. The EDX mapping shows the

presence of atoms C, O, and Zn, with compositions of 82.49%, 14.94%, and 2.56% respectively. The SEM image shows that the average particle size of Zn nanoparticles was around ~100 nm.
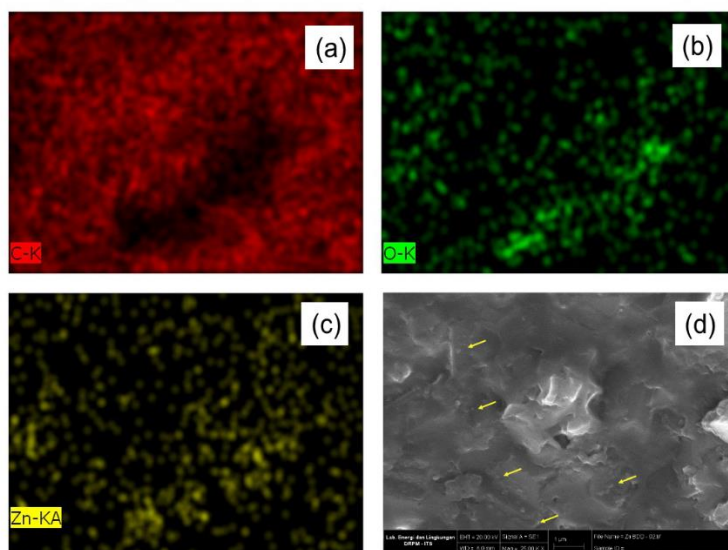

Figure 1. EDX mapping (a-c) and SEM image (d) of Zn-BDD electrode

3.2. Electrochemical behaviors of OFL on BDD and Zn-BDD

3.2.1. Signal per background (S/B)

Measurement of the S/B was performed on both BDD and Zn-BDD electrodes. CV was applied from potential 0 V to 1.6 V, with and without 60 µM OFL in 0.1 M PBS pH 5 (Figure 2). OFL showed anodic peak potential and peak current of 1.06 V and 90.18 µA respectively on the BDD electrode, whereas on Zn-BDD electrode obtained anodic peak potential and peak current of 1.10 V and 127.28 µA respectively. Therefore, S/B obtained on the BDD electrode was 8.1, while on the Zn-BDD electrode was 9.5. It is shown that Zn-BDD provides a higher S/B ratio compared to bare BDD electrode which provides better sensitivity.
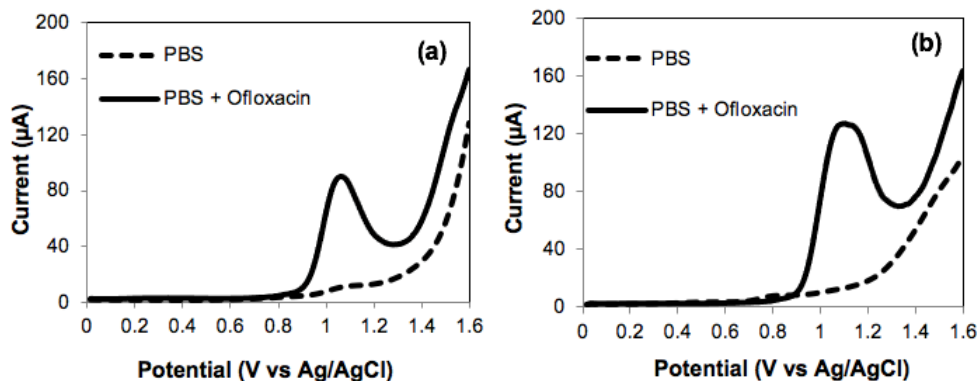

Figure 2. Linear sweep voltammogram for determining the S/B of OFL measurement on (a) BDD electrode and (b) Zn-BDD electrode

3.2.2. Scan Rate Variation

The analysis of OFL was performed in various scan rates from 20 to 150 mV s-1 at a potential range of 0 V to 1.6 V on BDD and Zn-BDD electrodes (Figures 3 and 4). The oxidation peaks of OFL at potential +1.1 V were increased as the scan rate was increased. A linear relationship between the logarithm of the current and the logarithm of the scan rate was obtained with a slope of 0.5189 for BDD electrode and 0.6205 for Zn-BDD electrode. This value is close to the theoretical one (0.50), indicating that the electrochemical reaction of OFL is controlled by diffusion. The linear relationship existing between the oxidation peak current and the square root of the scan rate showed with the regression equation shown below (Eq. (1) and (2)).

$$\text{Current } (\mu A) = 1.6636 \, \sqrt{(\text{scan rate})} \, (\text{mV/s}) - 1.735 \quad R^2 = 0.9611 \tag{1}$$

$$\text{Current } (\mu A) = 2.5138 \sqrt{(\text{scan rate})} \, (\text{mV/s}) - 6.542 \quad R^2 = 0.9006 \tag{2}$$
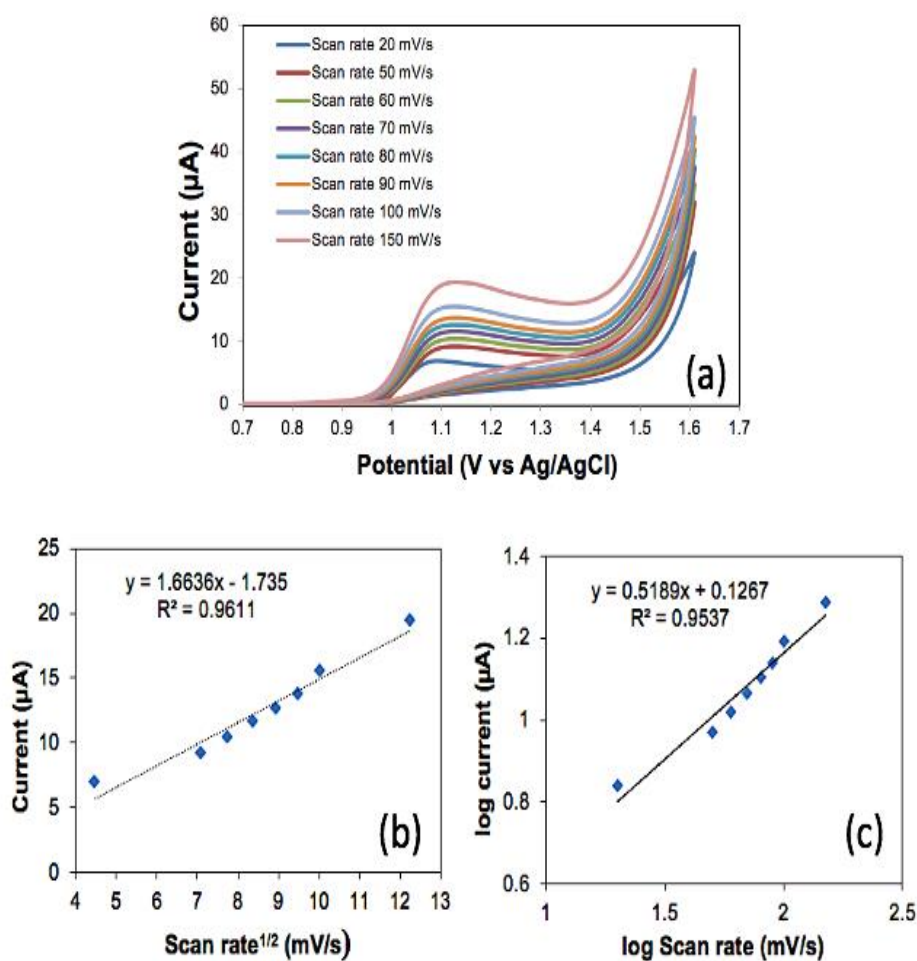

Figure 3. Cyclic voltammograms of OFL with various scan rate (a). Plot between square root of scan rate vs current (b) and plot of logarithmic of scan rate and logarithmic of current (c) measured on BDD electrode from potential 0 V to 1.6 V
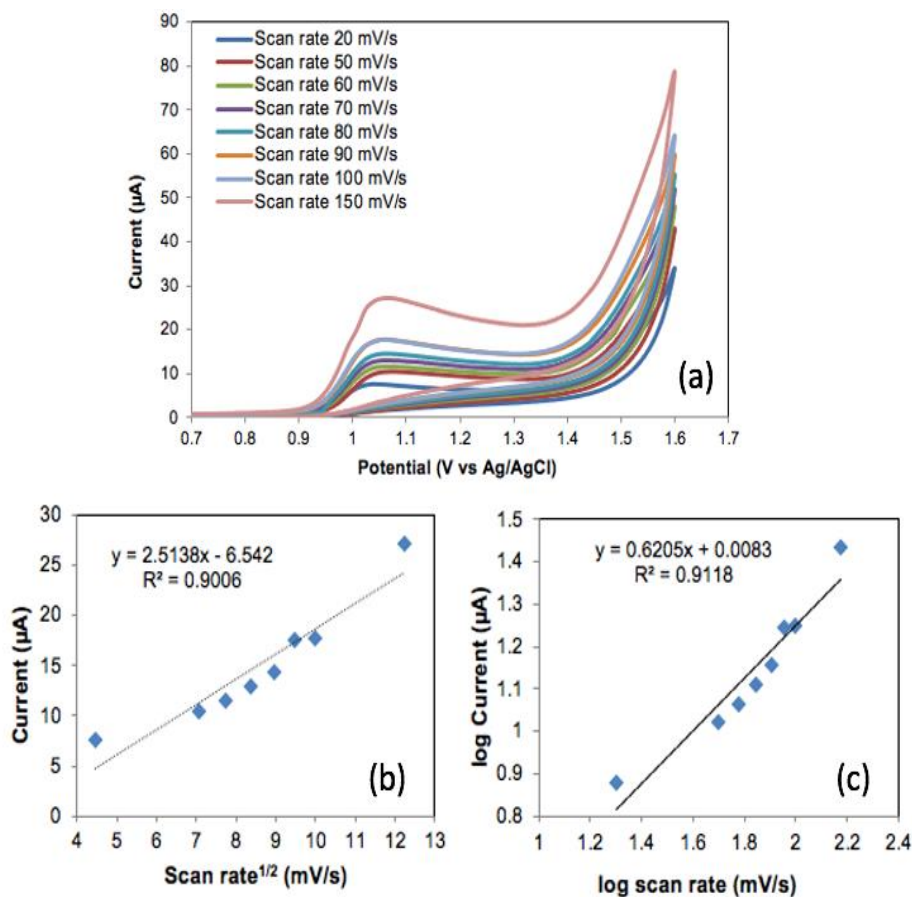
Figure 4. Cyclic voltammograms of OFL with various scan rate (a). Plot between square root of scan rate vs current (b) and plot of the logarithm of scan rate and the logarithm of current (c) measured on Zn-BDD electrode from potential 0 V to 1.6 V.

To determine the electron number (n) that is involved in the OFL oxidation process at BDD and Zn-BDD electrode, the n value was determined by voltammogram using the equation (Eq. (3)).

$$\left| E_p - E_{p/2} \right| = \frac{47{,}7 (mV)}{an} \tag{3}$$

where E_p and $E_{p/2}$ represent peak potential and half-peak potential respectively in the CV. The obtained values of $E_p - E_{p/2}$ are 42 mV for BDD and 48 mV for Zn-BDD. a is the charge transfer coefficient (generally, assumed as 0.5 for a totally irreversible system) and n is the number of electrons. Therefore, the value of n for BDD is 2.14 ≈ 2, while for Zn-BDD the value of n is 1.88 ≈ 2. These results indicate that the irreversible oxidation of OFL involves two electrons per molecule on the BDD and Zn-BDD electrodes in PBS solution.

3.2.3. Influence of pH

In this study, pH dependence was studied between pH 2 and pH 9 using the square wave voltammetry (SWV) method. SWV method was selected due to its clearly defined peaks at low

concentrations. SWV parameters such as frequency (Hz), pulse amplitude (mV), and step potential (mV) were optimized in 0.1 M PBS containing 60 μM OF. The optimum frequency of 50 Hz (10 to 50 Hz), pulse amplitude of 0.05 V (0.01 to 0.05 V), and step potential of 0.014 V for BDD and 0.016 for Zn-BDD (0.008 to 0.016 V) were used for further analysis, taking into account its repeatability, stability, accuracy, and magnitude of analytical signal at the BDD electrode for OFL determination.

OFL has a carboxylic group with $pk_{a1} = 5.97$ and a piperazinyl group with $pk_{a2} = 9.28$, in which, OFL will become cationic at pH conditions below pka1, and anionic above pka2, and zwitter ion at pH conditions between pka1 and pka2. The results show pH has an influence on the high and low of the peak currents produced in the measurement of OFL. This can be seen from the difference in peak currents resulting from each pH. The highest peak current produced by measuring OFL using BDD as well as on Zn-BDD electrode was at pH 5.5. The difference in the measured peak current value was due to the charge produced by large number of OFL molecules measured on the electrode surface. The higher the peak current value, the greater the number of charged from measured OFL on the electrode surface and the faster the electron transfer process. On the other hand, the lower the peak current value, the smaller the charged from the measured OFL on the electrode surface and the slower the electron transfer process. In this study, there was a shift in the peak potential towards the negative potential with increasing pH until it reached pH 7. This was due to the OFL as a zwitter ion. As a result, at a fairly low pH, high amount of H^+ addition will promote the equilibrium reaction to shift towards a negative potential, therefore the oxidation process requires a larger potential. In conclusion, the lower the pH, the more the response of the oxidation current shifts towards a positive potential, and the higher the pH, response of the oxidation current shifts towards a negative potential (Figure 5).
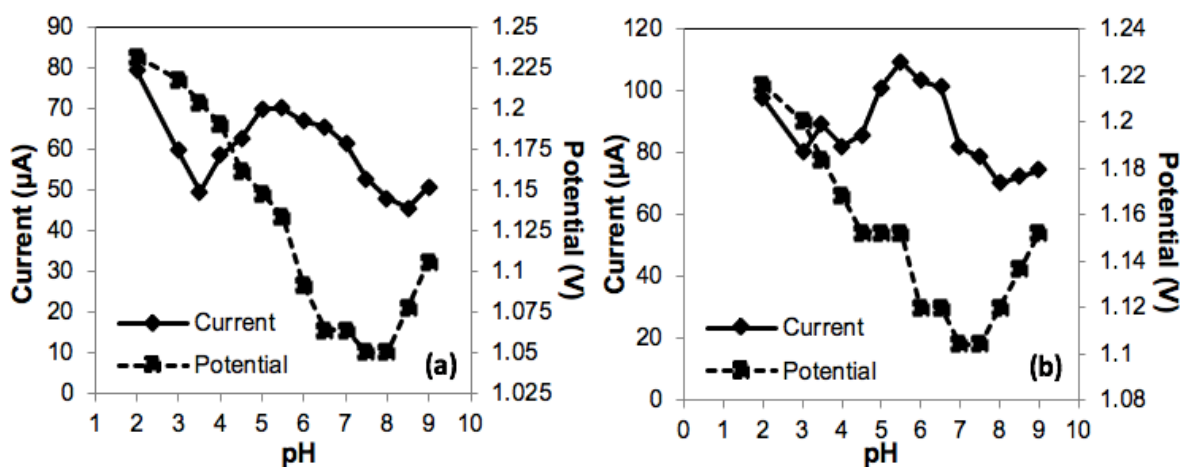

Figure 5. Effect of pH on the current and peak potential of OFL on (a) BDD electrodes and (b) Zn-BDD electrodes

3.2.4. Temperature Dependence

The effect of temperature on the electrochemical analysis of 60 µM OFL in 0.1 M PBS on the surface of BDD and Zn-BDD electrode was studied at temperatures between 20 °C and 60 °C (Figure 6). The results on the BDD and Zn-BDD electrodes showed an increase of the peak currents along with the increase of the solution temperature. Moreover, the potential of these peaks shifts to negative values as the temperature increases. This result is due to the decreasing of the medium viscosity which facilitates the diffusion of the species towards the electrode.

According to the Arrhenius equation (Eq (4) and (5)), the activation energy (E_a) was found to be 5.83 kJ/mol for BDD and 6.14 kJ/mol for Zn-BDD. This value <40 kJ/mol corresponds to the diffusion properties.

$$k = Ae^{-\frac{Ea}{RT}} \tag{4}$$

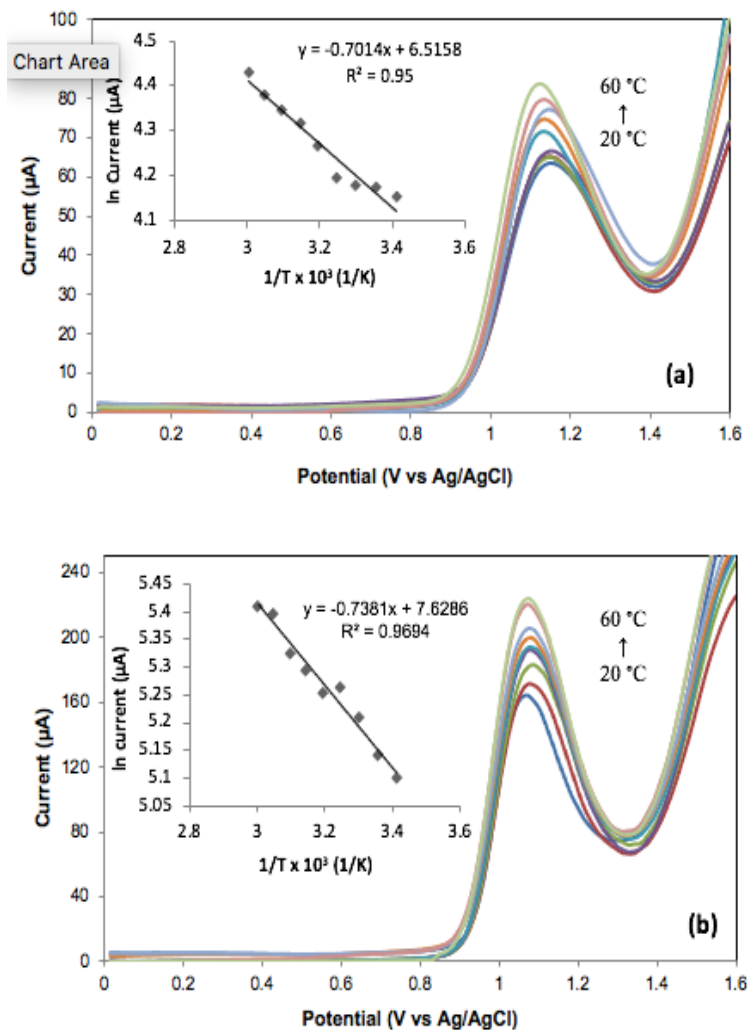$$\ln k = \ln A - \left(\frac{Ea}{R}\right)\left(\frac{1}{T}\right) \tag{5}$$

Figure 6. Square wave voltammogram of the (a) BDD electrode (b) Zn-BDD electrode at various temperatures. Inset: plot between ln current vs 1/T

3.2.5. Various OFL Concentration

The effect of ofloxacin concentration was performed by measuring the OFL in 0.1 M PBS using the SWV method, by varying the concentration of OFL from 30 µM to 100 µM. As seen in Figure 7, the peak current increased proportionally with the increase of OFL concentration. The corresponding equation is given in (Eq. (6) and (7)) indicating a diffusion-controlled process.

$$\text{Current (µA)} = 0,4068 \text{ concentration (µM)} + 55.924 \qquad R^2 = 0.9922 \qquad (6)$$

$$\text{Current (µA)} = 0,4174 \text{ concentration (µM)} + 87.566 \qquad R^2 = 0.9947 \qquad (7)$$

The relation coefficient (R^2) in the measurement of OFL using BDD electrode was 0.9922, while for the Zn-BDD electrode was 0.9947. This means that the measurement of OFL using BDD and Zn-BDD electrodes has good linearity. In addition, as the concentration increases, the peak potential shifts to a more positive value, confirming the irreversible oxidation process.
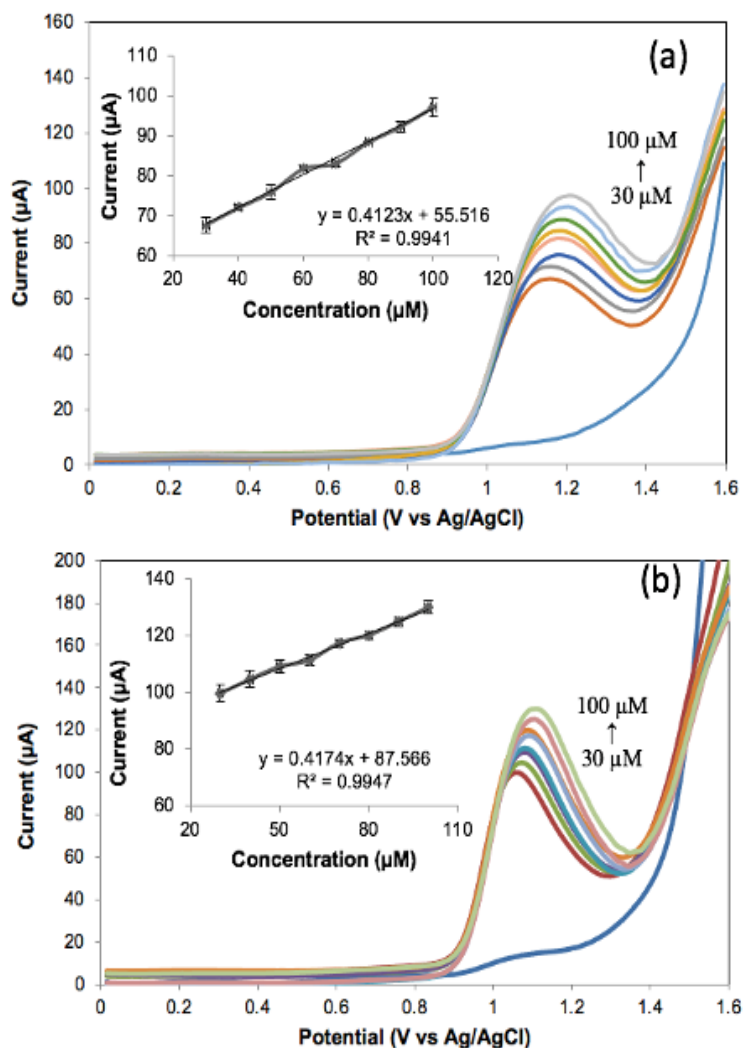

Figure 7. Square wave voltammograms of various concentration of OFL on (a) BDD electrode and (b) Zn-BDD electrode

The limits of detection (LOD) and limit of quantification (LOQ) were calculated from the calibration curve using the equation of $3\times$ (s/m) and $10\times$ (s/m) criteria, respectively, where m is the slope of the calibration curve and s is the standard deviation of the intercept. The LOD and LOQ were found to be 1.09 µM and 3.65 µM for the BDD electrode, respectively, while for the Zn-BDD electrode the LOD and LOQ were 1.25 µM and 4.15 µM, respectively.

3.2.6. Selectivity and reproducibility

Selectivity is the ability of a method to accurately and specifically measure an analyte in the presence of other components that interfere the measurement. The determination of selectivity in the measurement of OFL on BDD and Zn-BDD electrodes was carried out using several interfering solutions that could potentially exist in the actual sample, namely ascorbic acid, urea, ammonium sulfate, D-glucose and other antibiotics such as ciprofloxacin. Measurements were carried out by measuring 60 µM of OFL and 0.6 mM of interference in 0.1 M PBS. The result is the measurement of OFL on BDD and Zn-BDD electrode size selective to analyse OFL in the presence of interferences. Figure 8 shows the voltammogram of OFL in the presence of ascorbic acid on BDD and Zn-BDD electrodes. It is shown that OFL can be detected without a significant shifted of potential and current, especially on Zn-BDD electrode. OFL and ascorbic acid (AA) exhibited anodic peak potentials at 1.10 V and 0.61 V, respectively.
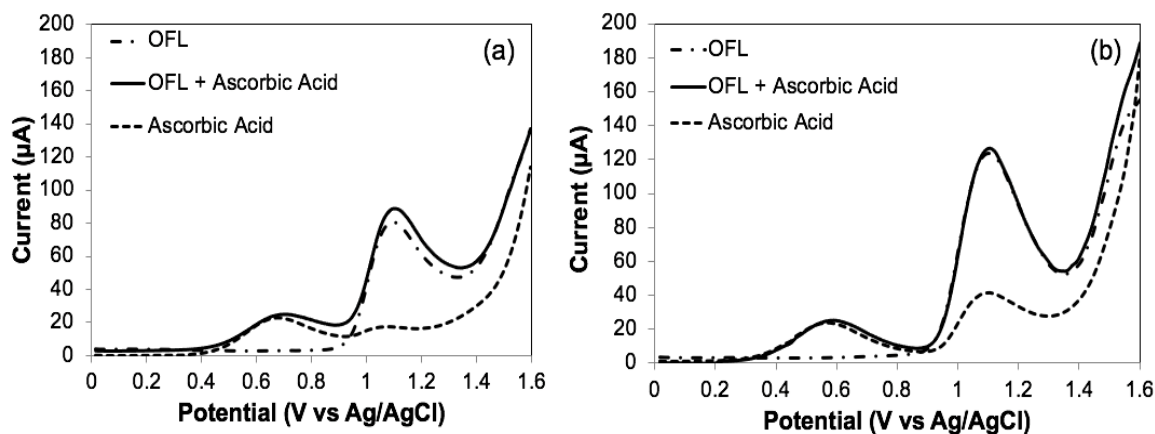

Figure 8. Square wave voltammogram of 60 µM OFL with and without 0.6 mM ascorbic acid

Meanwhile, the reproducibility test was investigated by measuring 60 µM of OFL in 0.1 M PBS using SWV method for 10 consecutive days. The results obtained relative standard deviations (RSD) of 4.01% for the BDD electrode and 2.29% for the Zn-BDD electrode. This means that the reproducibility of OFL measurement using the Zn-BDD electrode is better, and has a higher precision than the measurement using the BDD electrode. A good and well-

prepared Zn nanoparticle deposition process on BDD surface, allowing good reproducibility results.

3.3. Analytical Application in real sample

3.3.1. Determination in pharmaceutical formulations of OFL

BDD and Zn-BDD electrodes were used to measure the voltammetry of OFL in three types of commercial pharmaceutical samples. These commercial pharmaceutical sample were prepared by pounding and dissolving in electrolyte to a concentration of 3 mM and then analyzed directly using the SWV technique. The results obtained on BDD electrode and Zn-BDD electrode are presented in Table 1. These results indicate that the use of Zn-BDD electrode in the analysis of OFL pharmaceutical samples can be applied and the results are better when compared to the analysis using the BDD electrode.

Table 1. Analysis of OFL pharmaceutical product samples

Sample	Electrode	Added (µM)	Founded (µM)	% Recovery
1	BDD	60	49.33	82.22
	Zn-BDD	60	65.10	108.49
2	BDD	60	49.35	82.29
	Zn-BDD	60	63.98	106.64
3	BDD	60	46.89	78.15
	Zn-BDD	60	61.45	102.42

3.3.2. Determination of OFL in Milk

The determination of OFL in the milk sample (2% v/v) was performed using the proposed method on the BDD and Zn-BDD electrode (Figure 9). There is no additional peak was found, and the OFL oxidation peak was clearly observed at potential +1.1 V. The recovery for the BDD electrode was 83.05% and for the Zn-BDD electrode was 101.20%. Finally, Table 2 shows the comparison of the performance of OFL detection on BDD and Zn-BDD electrode. It was concluded that the presence of Zn nanoparticles could improve the analysis of OFL. Although the LOD value is slightly lower than on BDD electrode, it has a better value of S/B and sensitivity, which is essential in sensing applications.
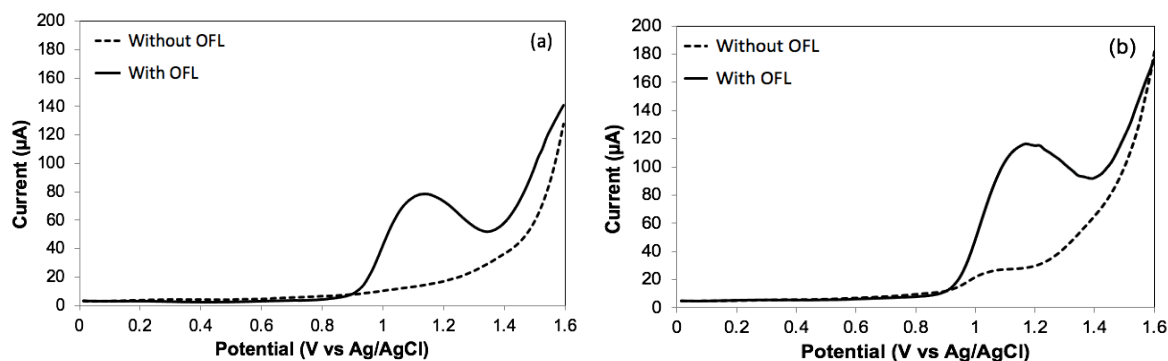
Figure 9. SWV of OFL analysis in real sample on BDD (a) and ZnBDD (b) electrodes

Table 2. Comparison of OFL detection performance on BDD and Zn-BDD

	BDD	Zn-BDD
S/B	8.1	9.5
Sensitivity (μA/μM)	0.4068	0.4174
Linear Range (μM)	30–100	30–100
Linearity (R^2)	0.9922	0.9947
Recovery (%)	83.05	101.20
RSD (%)	4.01	2.29

4. CONCLUSION

Zn-BDD electrode was applied for OFL detection, in comparison to BDD electrode in its optimum condition such as SWV method parameters, pH, temperature, and concentration. The analysis on Zn-BDD shows better results compared to the analysis on bare BDD electrodes. It shows better S/B, sensitivity, linearity, and reproducibility. This sensor also shows high selectivity in the presence of ascorbic acid, urea, ammonium sulfate, D-glucose, and ciprofloxacin. OFL analysis has been successfully performed in commercial pharmaceutical samples, as well as in milk. Therefore, Zn-BDD electrode could be a promising electrode used in the electroanalysis of OFL, and an alternative compare to expensive and longer analysis methods such as chromatography.

Declarations of interest

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