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Study of levofloxacin electrochemical sensors on screen-printed carbon electrodes

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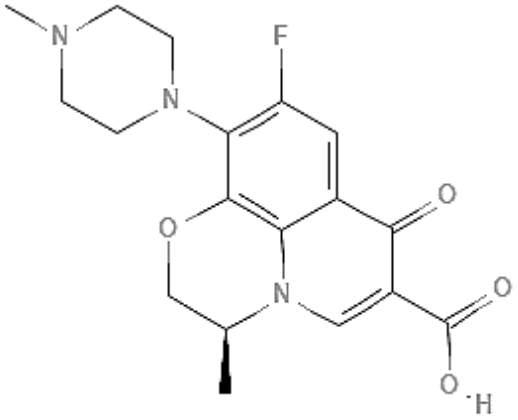
Abstract
Levofloxacin (LEV) is a type of fluoroquinolone antibiotic that usually used for treating the bacterial infection. The released of LEV in environment may impact a significant risk to the ecosystems. Thus, a fast and sensitive sensor device is required. In this work, the detection of LEV is carried out using a screen-printed carbon electrode (SPE). The measurement methods used were square wave voltammetry and cyclic voltammetry. The limit of detection and limit quantitation were 4.34 µM, 14.4 µM, respectively. The relative standard deviation was obtained at 5.4%. The %recovery results obtained using screen printed electrode in drug, milk, and wastewater were in the range of 95-110%. The validated method was successfully applied to detect the levofloxacin and resulted in a sensitive and efficient measurement.
Keywords: human & health; levofloxacin; screen printed electrode; square wave voltammetry

1. Introduction

Levofloxacin (LEV) is the third generation of the fluoroquinolone class of antibiotics (Sitovs et al., 2021) (Scheme 1). Most of the LEV antibiotics will be released into aquatic ecosystems due to the limitations of traditional wastewater treatment plants (Rodriguez-Mozaz et al., 2020). LEV released in aqueous solution will pose a significant risk to aquatic ecosystems and human health (Altaf et al., 2021). The dose of LEV consumed in the form of tablets and syrup is around 250 — 500 mg (Noel, 2009). Excessive use of LEV can cause body resistant and other side effects such as headache, dizziness, restlessness, tremor, insomnia, hallucinations, convulsions, anxiety, and depression (Moorthy et al., 2008). Therefore, analytical techniques to detect and determine the LEV are needed. Until now, there are numbers of analytical techniques have been reported for the determination of LEV including high-performance liquid chromatography (HPLC) (Szerkus et al., 2017)-(Szerkus et al., 2016) , capillary electrophoresis (CE) (Tsai et al., 2007)-(Liu et al., 2008), UV-vis spectrophotometry (Maleque et al., 2012), flow injection analysis (FIA) (Altiokka et al., 2002), and nuclear magnetic resonance (NMR) (Salem et al., 2012).



Scheme 1. Levofloxacin structure

However, most of these methods are less simple, cannot be applied for routine analysis, relatively expensive, require sophisticated instrumentation. Meanwhile, another technique, namely electrochemical techniques have the advantage of simplicity, low cost and fast compared to other methods (Ostojić et al., 2017). In the electrochemical technique, type of the working electrode is very important, as it may provide a sensitive and selective measurement. Carbon-type electrode has been used for sensor application due to its advantages, such as cheap, wide potential window, strong, high durability, and easily operated (Jiwanti et al., 2022). There are various carbon-based electrodes applied for LEV sensor application, such as polycrystalline boron-doped diamond electrode (Jiwanti et al., 2022; Rkik et al., 2017) , graphene (Wang et al., 2014), and glassy carbon electrode (Tang et al., 2014).

As for sensor application, flexibility, fast, low-cost, and high reproducibility are some of the advantages to be fulfilled by a sensor device other than its sensitivity and selectivity. Screen-printed electrode (SPE), and electrode device that may contain working electrode, counter electrode, and reference electrode in one device, is currently developed by researcher for in-situ sensor application (García-Miranda Ferrari et al., 2021). One of working electrode types in SPE is carbon material (SPCE). In this study, due to the effectiveness and good properties of SPCE for sensor application, SPCE will be used as a sensor to detect levofloxacin in drug, wastewater, and milk samples. In addition, square wave voltammetry (SWV) method will be used in this work as it has been known for its sensitivity in electrochemical analysis.

2. Methods

The materials used in this study were levofloxacin 98% (Sigma Aldrich), NaH_2PO_4 99% and Na_2HPO_4 99.5% from Merck, H_3PO_4 98%, ethanol 99.9% were purchased from Millipore Corporation, and ultrapure water. All chemicals were used without further purification. All electrochemical measurement were carried out by taking 1.33 µL of 60 µM levofloxacin in 60 µL of 0.1 M phosphate buffer solution (PBS) and drop casted on the SPCE electrode (working and counter electrodes are carbon, reference electrode is Ag). The electrode device was used without pretreatment. Cyclic voltammetry (CV) and SWV were performed with an Emstat^{3+} Blue Palmsens potentiostat. The mixed solution was allowed to equilibrate for 5 seconds to sweep from 0 V to 1.6 V at amplitude of 0.05 A, frequency of 50 Hz and step potential of 0.05 V in SWV mode. Electrochemical sensor for determination of levofloxacin was examined in various LEV concentrations ranging from 30 to 100 µM, and the optimization of parameters such as signal per background, scan rate, linearity, selectivity, and optimum pH. This method was applied in drugs, milk and wastewater.

3. Results and Discussion

First, the LEV determination was measured using SWV in PBS pH 6 and recorded over the potential window from 0 V to 1.6 V with the addition of 1.33 µL LEV 60 µM. An oxidation signal at potential around +0.8 V is attributed to the oxidation of LEV due to the 2 electrons and 2 protons H^+ transfer, leading to LEV N-oxide as reported in previous work (Rkik et al., 2017). Signal per background (S/B) is performed to determine the background current of each electrode used and to test the performance of the electrodes. The background value of the blank measurement on SPCE is obtained at a current of 19 µA. Meanwhile, the signal measurement from the LEV measurement at SPE obtained a current peak of 190 µA. The S/B ratio was calculated to be 10 (Fig.1). The appearance of a peak signal indicates that levofloxacin is electroactive in SPE.

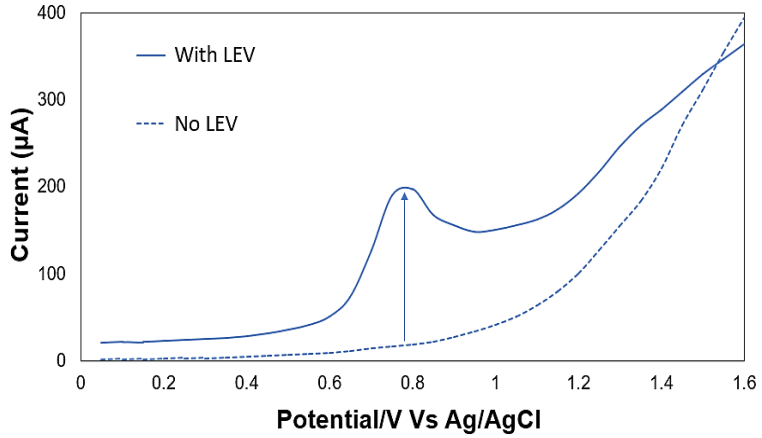


Figure 1. SWV of 60 µM LEV on SPCE in 0.1 M PBS pH 6 for determining background current of levofloxacin

Furthermore, LEV was also observed at various scan rates. The SPCE scan rate was determined using the CV method, by measuring 60 µM LEV samples at various predetermined scan rates of 40 mV/s - 100 mV/s using a current range of 0 V – 1.6 V (vs Ag/AgCl). The measurement results show that the LEV peak current increases linearly as the scan rate increases with y = 15.675x - 78.698 and R^2= 0.9924 (Fig.2). The linear increase as the square root of the scan rate with increasing current indicates a diffusion control process. Thus, the LEV oxidation process occurs under a diffusion-controlled process.

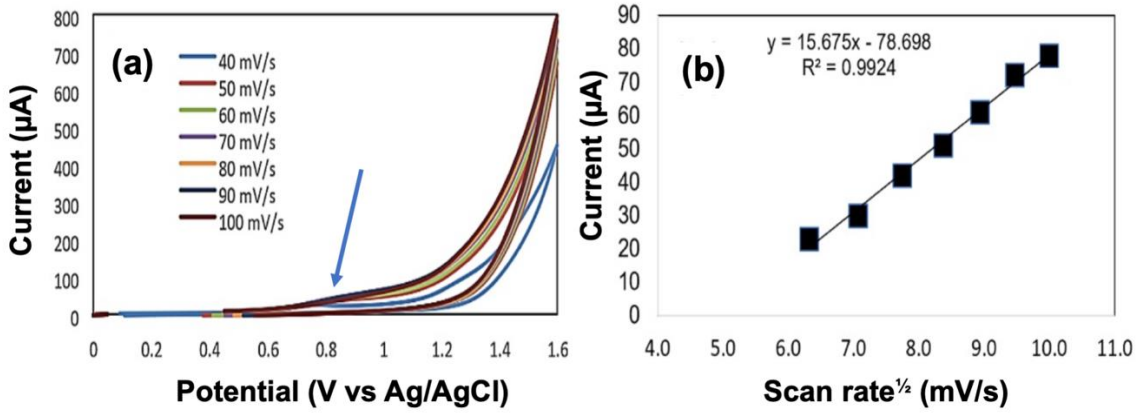


Figure 2. CV voltammogram of LEV at (a) various scan rates (b) Linear correlation of square root of scan rate

LEV was also analyzed in various concentrations to determine limit of detection (LOD) and limit quantitation (LOQ). LOD was determined by three times of standard

deviation divided by the slope of the calibration curve. Whereas LOQ was determined by ten times of standard deviation divided by the slope of the calibration curve. The linearity obtained at concentration measurements of 30-100 µM showed good results with R^2=0.9956 (Fig.3). LOD and LOQ were calculated to be 4.34 µM and 14.4 µM, respectively, with sensitivity of 1.594 µM/µA. This study provides a good repeatability test performed by conducting seven analyzes on the same day. The measurement results show a pretty good %RSD value of 5.4%.

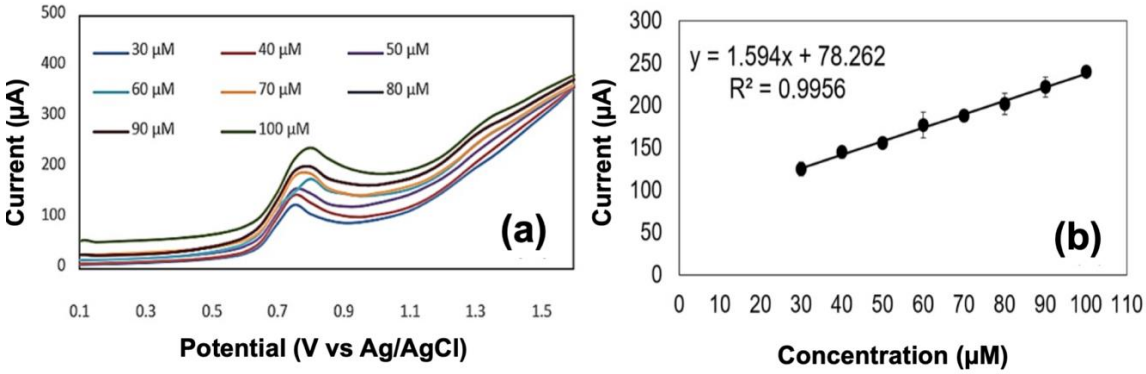


Figure 3. SWV voltammogram of 30-100 µM LEV on SPE in 0.1 M PBS pH 6 (a) and correlation of various concentrations and current responses (b).

Determination of the optimum pH at LEV was carried out using SWV. 60 µM levofloxacin solution was added to 0.1 M PBS at various pH from pH 5 to pH 9 (Fig. 4). Measurements were conducted using a potential range of 0 V - 1.6 V, amplitude 0.05 V, frequency 50 Hz, and E-step potential 0.05 V. LEV has a carboxyl group with a pKa = 5.5 so that the optimum pH of SPE is adjusted to pH 6. This is because LEV has a carboxyl group (Michot et al., 2005). The increase in linear pH with increasing current reaches the optimum pH and the current decreases after the optimum pH applied. This is due to repulsive electrostatic interaction of the molecule with the electrode surface caused by the oxidation of the analyte, so it has poor kinetics (Kingsley et al., 2016).

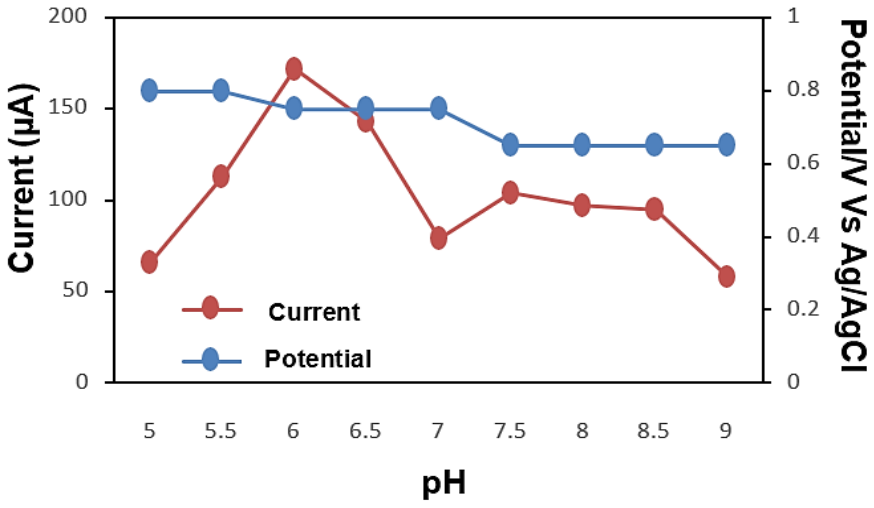


Figure 4. Effect of pH on the current density and peak potential LEV using SPCE by square wave voltammetry (pH 5-9). The measurement was carried out in 60 µM LEV in 0.1 M PBS from potential of 0 to 1.6 V.

Table 1. The result of determination analysis of levofloxacin in drugs, milk, and wastewater

Samples	Concentration [µM]		%Recovery
	Expected	Found	
Drug	70	70.72	101.00
Milk	70	67.60	96.60
Wastewater	70	66.70	95.30

Finally, LEV was determined in real sample, such as in drugs, milk sample, and wastewater. Due to the low concentration of LEV in the sample, standard addition technique was used by adding 70 µM of LEV into the samples. The results showed good recovery at the acceptable range of 85%-110%. Thus, it is suggested, that the analysis of LEV using SPCE electrode device can be applied directly in real sample (Table 1). The result of this work was compared against the previous result as shown in Table 2, revealing it is comparable with the previous work, showing good LOD.

Table 2. Comparison of LEV determination with previous published work

Method	Electrode	Linier range (µM)	LOD (µM)	Ref.
CV	BDD	48-100	10.1	(Rkik et al., 2017)
SWV	BDD	30-100	11.13	(Jiwanti et al., 2022)
DPV	Graphene-AuNP/MIP	1-100	0.53	(Wang et al., 2014)
SWV	SPCE	30-100	4.34	This work

4. Conclusion

The LEV electrochemical detection study was successfully investigated on SPCE. The screen-printed electrode successfully oxidized LEV optimally at a potential of 0.8 V in PBS pH 6 with a current of 190 µA. This research was successfully applied for the determination of LEV levels in drugs, milk and wastewater with a %recovery range of 95-110%. The result showed comparable detection limit against previous result. Thus, the results from the validation method carried out by SPCE exhibited good sensitivity, good precision and good accuracy when applied in the real sample.

Author Contribution

Conceptualization, P.K.J.; Methodology, P.K.J., A.B.Z.R.R; Formal Analysis, P.K.J., A.B.Z.R.R .; Investigation, A.B.Z.R.R.; Writing – Original Draft Preparation, I.Z.D.P; Writing – Review & Editing, P.K.J., I.Z.D.P.

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Conflicts of Interest

The authors declare no conflict of interest

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