Electrochemical Sensor of Levofloxacin on Boron-Doped Diamond Electrode Decorated by Nickel Nanoparticles

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Abstract: *Levofloxacin (LEV) was known as one of the fluoroquinolone antibiotics that widely used as an antibacterial agent. Monitoring of LEV is important due to its negative side effect on humans. The determination of LEV was studied for the first time on nickel modified on a boron-doped diamond (NiBDD) electrode using the square wave voltammetry (SWV) method to improve the catalytic and sensitivity of the sensor. The response was linear in the range of 30–100 μM LEV. LEV sensor on NiBDD was found to be selective in the presence of urea, glucose, and ascorbic acid interferences. Good reproducibility with % a relative standard deviation of 1.45% (n = 10) was achieved. Therefore, the NiBDD electrode could be potentially applied for the real detection method of LEV.*

Keywords: *levofloxacin; electrochemical sensor; nickel; boron-doped diamond; environmental pollution*

■ INTRODUCTION

Levofloxacin (LEV), $C_{18}H_{20}FN_3O_4$, is one of the fluoroquinolone drugs which has an optical S(-) isomer of ofloxacin. This drug is commonly used as an antibacterial agent against most aerobic gram-positive and gram-negative organisms that cause respiratory, skin, and genitourinary tract infections [1]. It is also known to be more active against gram-positive organisms than other fluoroquinolone drugs, such as ciprofloxacin. LEV works by inhibiting bacterial deoxyribonucleic acid (DNA) gyrase. Thus, it is usually used as an alternative antibiotic to heal the disease if other antibiotics cannot give a positive response.

On the other hand, besides the effectiveness of LEV in treating infections, LEV may cause serious heart problems, tendon rupture, abdominal discomfort, and bacterial resistance in long-term use [2-5]. Besides that, improper waste treatment of the pharmaceutical companies of health care centers could promote environmental pollution caused by the high concentrated LEV. Therefore, it is important to monitor LEV in biological fluids and the environment. Various methods have been reported as effective methods to detect LEV, such as using HPLC [6-7], UV detection [8-9], and chemiluminescence [10]. Despite their sensitivity, these methods require expensive instrumentation, high-skilled

professionals, and are time-consuming.

One of the promising methods used is electrochemistry, which is known to have a short time analysis, and only a small amount of reagent is needed. To support the optimum analysis using the electrochemical method, a suitable electrode is mandatory. Many types of research on the detection of LEV have been reported by using various electrodes such as carbon nanotubes [11], nickel oxide porous films [12], modified screen-printed electrode (SPE) [13], and modified glassy carbon electrode (GCE) [14]. Among those electrodes, boron-doped diamond (BDD) electrode is famous for its excellent properties for sensor applications such as detecting arsenic (III) [15], neuraminidase [16], chemical oxygen demand (COD) [17], glucose [18], and many more applications such as for CO_2 reduction [19-20] and synthesis application [21]. This wide range of BDD sensor applications is known due to its low background current that supports the analysis in a very low concentration, wide potential window in aqueous solution, and high physical and chemical stability suitable for real applications [22-23].

In this report, we modified nickel nanoparticles on the surface of the BDD electrode (NiBDD) to be used as a working electrode. A previous study shows that the use of nickel oxide (NiO) modified on GCE could enhance the voltammetric signal [12]. Thereafter, we propose to use Ni NPs decorated on the BDD surface to act as a catalyst for detecting LEV in order to improve sensitivity and selectivity.

■ EXPERIMENTAL SECTION

Materials

LEV, Na_2SO_4 (99.5 %), and $NiSO_4$ (99.99%) were purchased from Sigma Aldrich. H_2SO_4 (98%), NaOH (98%), HCl (37%), and HNO_3 (65%) were purchased from SAP chemicals. Ascorbic acid (99%), D-glucose (99.8%), and ammonium sulfate (99.5%) were purchased from Merck. All solutions were used without further pretreatment and purification.

Instrumentation

The following instrumentations were used in this work are microwave plasma-assisted chemical vapor

deposition system (Model AX 5400, CORNES Technology Corp.) to synthesize BDD electrode, Potentiostat Emstat3⁺Blue from Palmsen, to run electrochemical methods such as cyclic voltammetry (CV), linear sweep voltammetry (LSV), and square wave voltammetry (SWV). The scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) (Hitachi SU 3500). to characterize the topography of electrode surface and metal analysis.

Procedure

Electrode preparation and characterization

One percent (B/C) BDD electrode was deposited on the surface of Si (111) using a microwave plasma-assisted chemical vapor deposition system (Model AX 5400, CORNES Technology Corp.). A detailed procedure was described by Watanabe et al. [24]. Prior experiment, the BDD electrode was ultrasonicated in ultrapure water for 5 min. Follow-up treatment was carried out by applying CV in 0.1 M H_2SO_4 in a potential range of -2.5 V to 2.5 V with a scan rate of 1 V/s for 40 times repetitions. A potential of 2.5 V for 300 s was applied using the chronoamperometry technique to prepare oxygen terminated BDD (OBDD) electrode. NiBDD electrode was prepared by depositing Ni nanoparticles on the surface of the OBDD electrode in 1 M $NiSO_4$ for 250 s at a potential of -1.2 V. OBDD and NiBDD electrodes were then characterized using CV and SEM-EDS.

Electrochemical measurement

All measurements were performed in one compartment cell (5 mL). Pt spiral and Ag/AgCl (saturated KCl) were used as counter and reference electrodes, respectively. Working electrodes, OBDD and NiBDD, were used with a geometric area of 0.04 cm². Prior to the electroanalytical measurement, the working and counter electrodes, including electroanalytical cells, were ultrasonicated in ultrapure water for 10 min for cleaning. The supporting electrolyte used in this experiment was 0.1 M Na_2SO_4. An electrochemical method such as CV was used to characterize the electrode, and electrochemical detection measurements were performed using LSV and SWV methods. All

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measurements were performed using Emstat3+Blue potentiostat.

■ RESULTS AND DISCUSSION

Preparation and Characterization of NiBDD

The pretreatment of the BDD electrode was carried out before electroanalysis was performed. BDD was anodically oxidized by applying potential from 0 V to +2.5 V for 40 cycles in 0.1 M H_2SO_4. Anodic oxidation is reported to be able to convert the hydrogen functional group to an oxygen functional group on the surface of the BDD electrode [25-26]. Accordingly, it minimizes the conversion of hydrogen- to the oxygen-terminated surface during measurement, which may interfere with the LEV detection performed in positive range potentials. Fig. 1 shows the SEM image and EDS of the NiBDD electrode. The topography of the NiBDD surface was revealed, as can be seen in the white spot of Ni particles. From the EDS data, 1% Ni particles were found on the surface of the NiBDD electrode (96% C and 3% O). This result shows that Ni nanoparticles have successfully deposited on the surface of the BDD electrode.

Optimization of Electrochemical Technique for LEV Determination

The first optimization for LEV determination was carried out using LSV analytical method and then compared to SWV (Fig. 2). A significant improvement in the LEV oxidation peak was observed using the SWV method. Moreover, a correlation between LEV concentration and the current response was also conducted to know the sensitivity of measurement by using LSV and SWV methods. Linear calibration of LEV was obtained with $R^2 = 0.9927$ and sensitivity of 0.1216. Although the correlation between LEV concentration and current shows linear, the sensitivity is much lower compared to the use of the SWV analytical method, which shows a value of 1.095 with $R^2 = 0.9955$. The result was also supported by the limit of detection (LOD) value of LEV, in which 11.62 and 5.11 μM were calculated by using LSV and SWV analytical methods, respectively. In this report, this result of LOD for the SWV technique is higher compared to the report by Farias et al. using reduced graphene oxide/glassy carbon electrode [11]. This confirms that the SWV method is a sensitive analytical
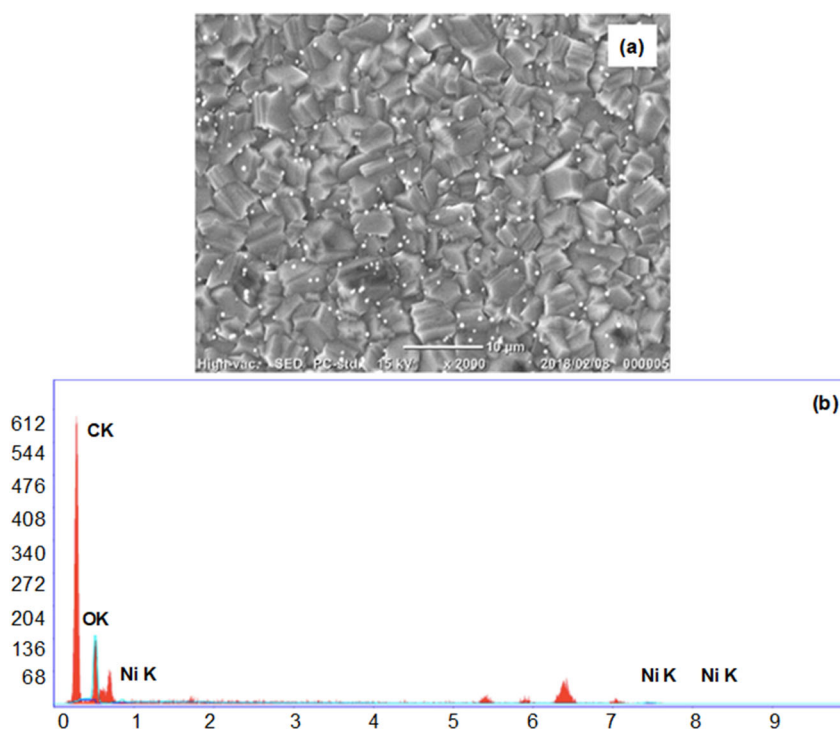
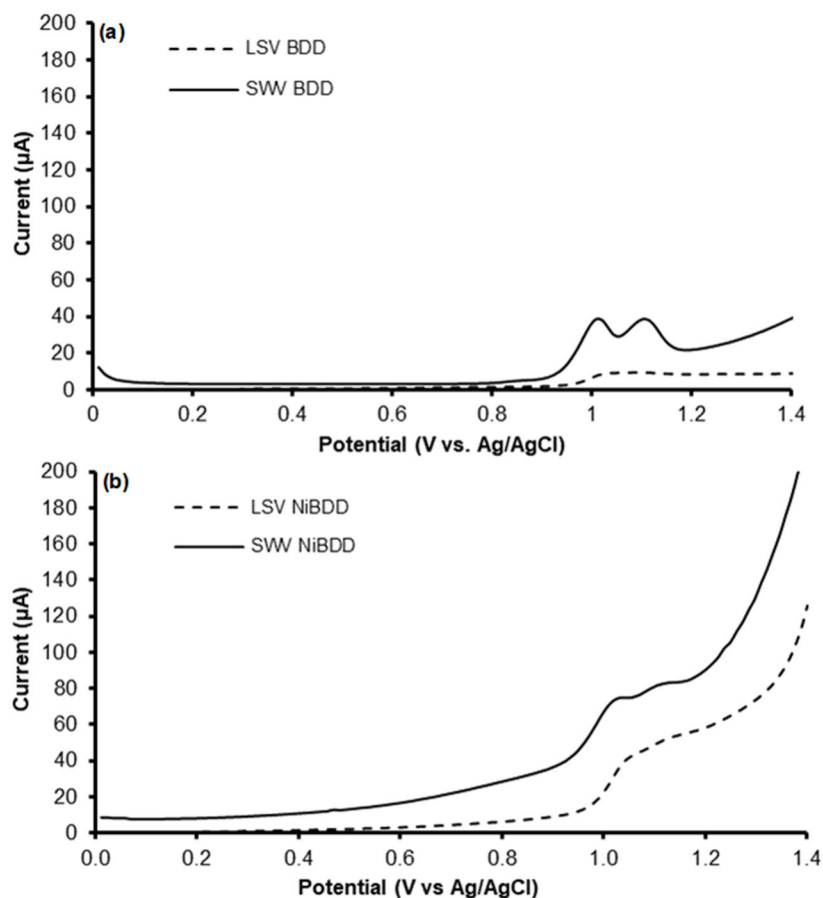

Fig 1. SEM image (a) and EDS (b) of NiBDD

Fig 2. Voltammetric curves of potential vs. current of 50 µM LEV in 0.1 M Na_2SO_4 performed on OBDD (a) and NiBDD (b) electrodes with LSV and SWV method

method that is usually used for many sensing methods in electrochemistry, such as for environmental pollutant analysis [27]. Therefore, SWV was used for further analysis.

Optimization of SWV parameters such as frequency (Hz), pulse amplitude (mV), and step potential (mV) was performed in 0.1 M Na_2SO_4 containing 50 µM LEV. Pulse amplitude was examined by applying various amplitudes from 10 mV to 50 mV. The current response increased as the pulse amplitude increased. This trend was similar to frequency optimization. On the other hand, at step potential optimization, the current was decreased to 14 mV. The maximum step potential was 12 mV. For all these three optimizations, the peak potentials did not shift (at 1.09 V), indicating that the peak potentials were insensitive to frequency, pulse amplitude, and step potential. Therefore, for all analytical measurements in

this experiment, pulse amplitude 50 mV, frequency 50 Hz, and 12 mV step potentials were used.

Electrochemical Detection of LEV

In Fig. 3, a solid line shows the SWV of the NiBDD electrode with 50 µM LEV in 0.1 M Na_2SO_4. Two oxidation peaks at +1.0 and +1.1 V are detected and belong to the oxidation peak of LEV [28]. The oxidation of LEV occurs in two steps of electron transfer to form cation and followed by hydrolysis and deprotonation steps. This oxidation peak shows the catalytic activity of Ni nanoparticles on the detection of LEV in Na_2SO_4 solution. Ni nanoparticles increase the adsorption of LEV on the active surface of the working electrode. Thus, for further analysis, we use these oxidation peaks to study the detection of LEV on OBDD and NiBDD electrodes.
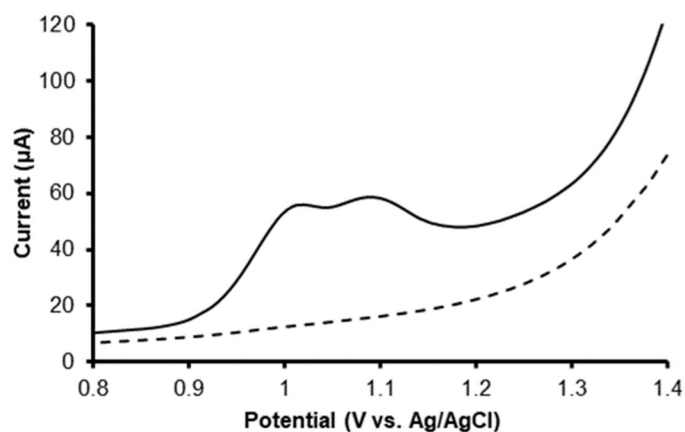
Fig 3. SWV on NiBDD electrode with (solid line) and without (dash line) 50 μM LEV in 0.1 M Na$_2$SO$_4$. The scan rate of 100 mV/s

The influence of the scan rate on the LEV determination was also studied on NiBDD electrodes. In Fig. 4(a), 50 μM LEV in 0.1 M Na$_2$SO$_4$ was measured with the CV analytical method at various scan rates ranging from 10 mV/s to 50 mV/s. The voltammogram shows the irreversible process of LEV, in which only the oxidation peak of LEV was detected. In Fig. 4, a graph of the square root of scan rate versus current at potential around +1.0 V shows a linear regression equation of y = 9.1786x + 14.485 (R^2 = 0.9927). It is suggested that the electrochemical oxidation of LEV on NiBDD was diffusion-controlled.

In addition, the influence of pH on the LEV determination was also performed. Fig. 4(b) shows the pH
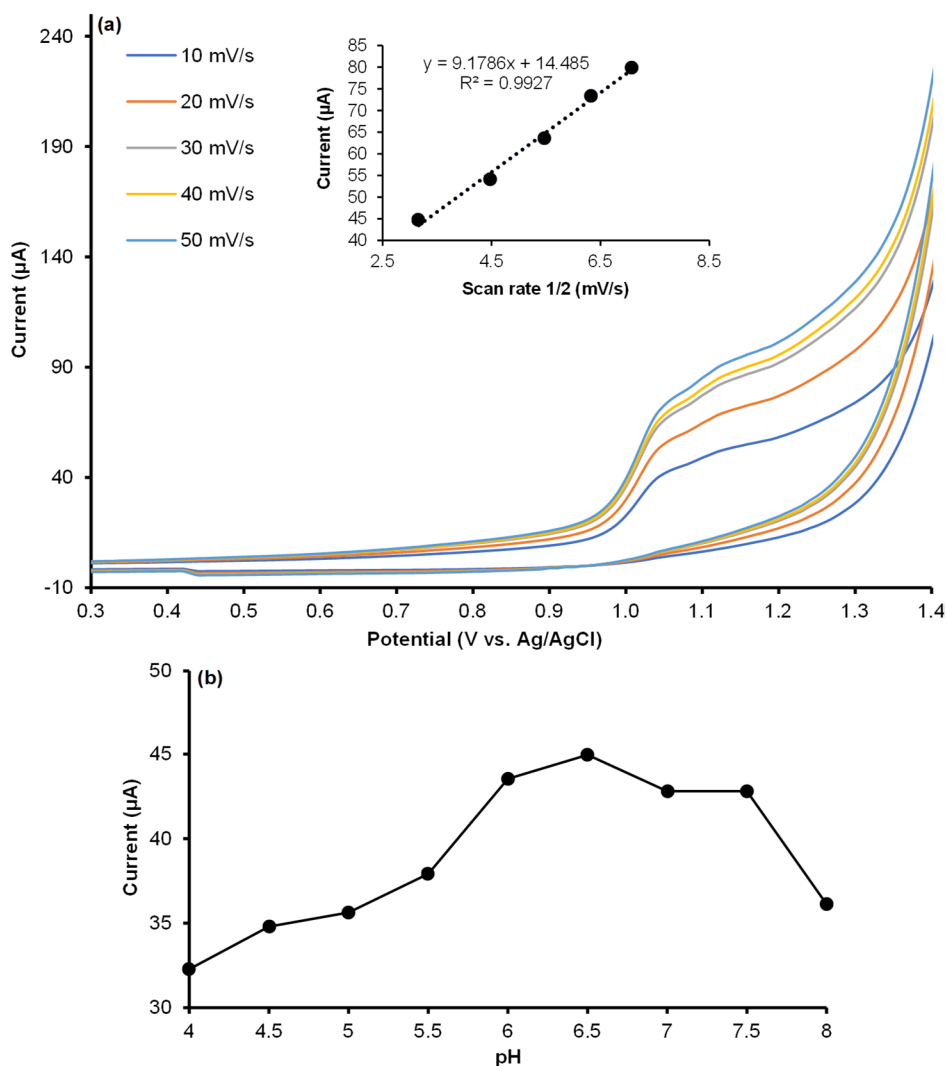

Fig 4. CV of NiBDD electrode with various scan rates. Inset: linear relationship between the square root of scan rate and current (a). Effect of pH on the anodic current (b). 50 μM LEV in 0.1 M Na$_2$SO$_4$ with a scan rate of 100 mV/s
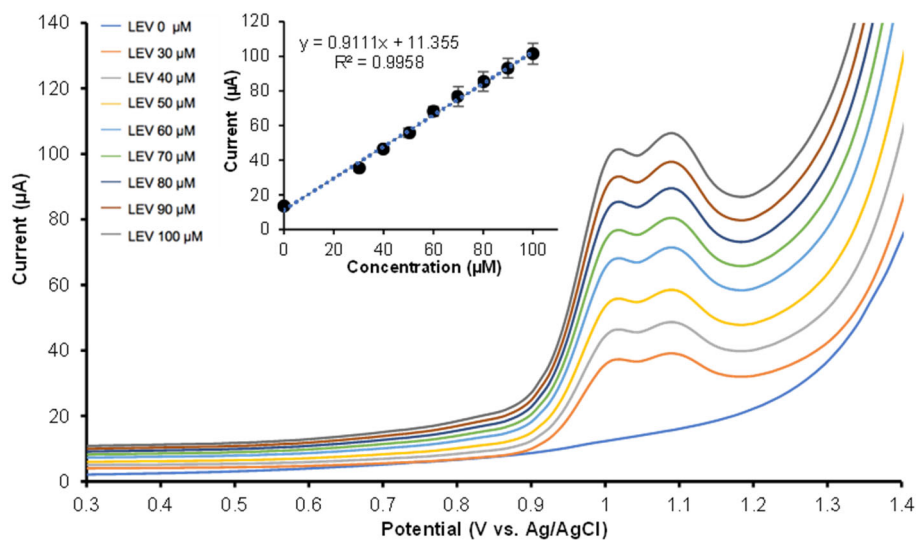
Fig 5. SWVs of LEV with various concentrations in 0.1 M Na₂SO₄ on NiBDD electrode. The scan rate was 100 mV/s. Inset: linear correlation of LEV correlation and current at a potential of +1.0 V
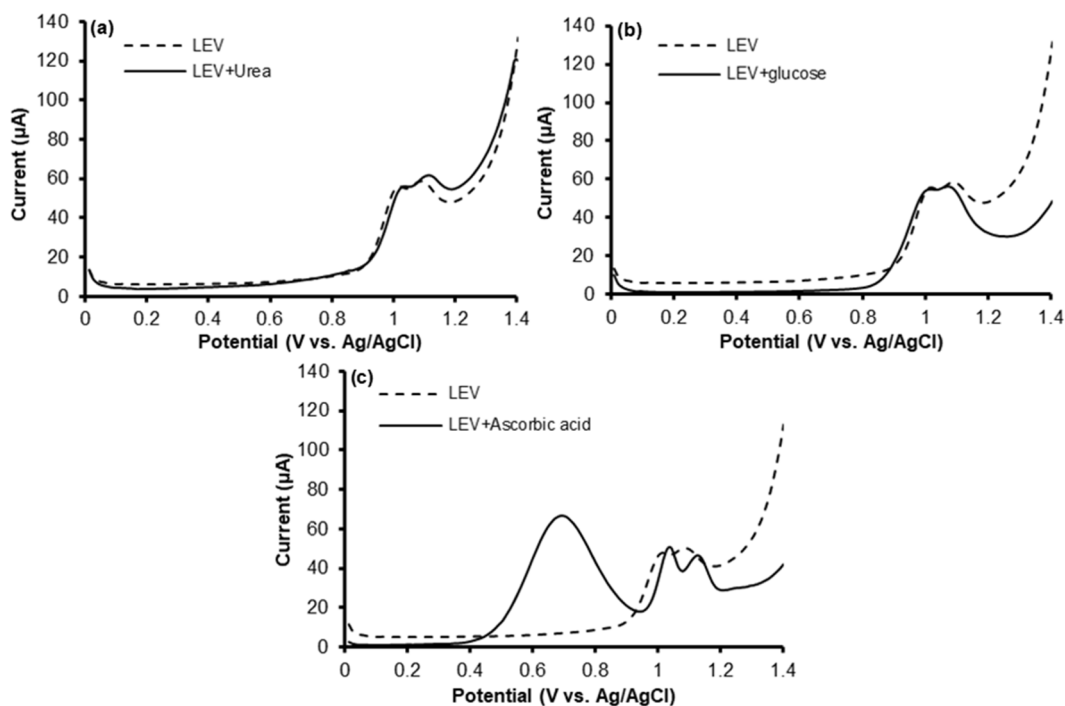

Fig 6. SWVs of 50 μM LEV with interferences of (a) urea, (b) glucose, and (c) ascorbic acid with the same concentration on NiBDD electrode

dependence on the current response of LEV oxidation studied by the SWV method. pH was varied from 4.0 to 8.0. As can be seen, the current responses increased from pH 4 to 5.5 and then decreased over 5.5. Thus, the optimal pH to measure LEV in this experiment was pH 5.5.

Determination of LEV on NiBDD using SWV was performed in optimum conditions with different LEV concentrations ranging from 0 to 100 μM (Fig. 5). Each LEV concentration was measured three times and showed the increase of current response linearly with a correlation coefficient of 0.9958. The LOD was measured and showed a value of 11.13 μM.

Selectivity, Reproducibility, and Real Sample Analysis

The selectivity of LEV measurement was evaluated. LEV with a concentration of 50 μM was measured in 0.1 M Na_2SO_4 containing the same concentration of interference (urea, glucose, and ascorbic acid). Fig. 6 shows the SWV with and without interferences. There are no significant changes in the current response, 3.06%, 4.29%, and 7.47% for the addition of urea, glucose, and ascorbic acid, respectively, according to the acceptable precision in AOAC (11%). The exception is for ascorbic acid, in which a new peak was detected at around potential +0.7 V. However, this peak does not promote a significant effect on the presence of LEV oxidation peak. Meanwhile, reproducibility is one of the important aspects to be considered in the sensor. To evaluate the reproductivity of LEV, NiBDD was fabricated, and the measurement was conducted for 10 days in the same condition, in which one measurement was carried out each day. The results show a satisfactory result, with an RSD of 1.45%, which this result is better than the report of LEV analysis (1.70%) in urine samples by Radi et al. [29] and is an acceptable % RSD value according to AOAC [30].

Determination of LEV in the real sample was conducted in a human urine sample (Fig. 7). LEV with a concentration of 50 μM was added into the urine sample and measured using the SWV method. An oxidation peal of LEV was detected at potential around +1.0 V, showing a good recovery of 93.91 ± 0.02% (acceptable limit of 80–110%) [30], indicating that this method of measurement on
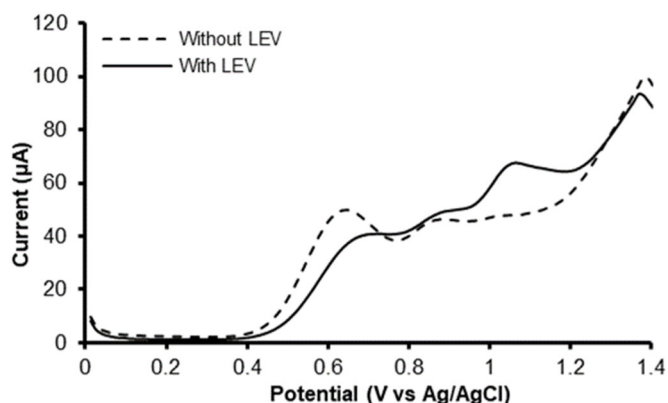

Fig 7. SWVs of LEV in urine sample on NiBDD electrode

on NiBDD electrode can be applied to detect LEV in the real sample.

■ CONCLUSION

LEV was successfully detected on the NiBDD electrode by analyzing the LEV oxidation peak at around +1 V at maximum pH of 5.5. The detection of LEV was sensitive whether in Na_2SO_4 (which contains ultrapure water) or in a real urine sample. SWV method shows a 56% lower LOD value compared to the LSV method. Optimized conditions of SWV with pulse amplitude of 50 mV, frequency of 50 Hz, and step potentials of 12 mV were used to detect LEV accurately. Analysis in real urine sample shows good antibiotic % recovery of 93.91 ± 0.02. The LEV electrochemical detection on the NiBDD electrode is fast, simple, and sensitive, which has a potential for further development in antibiotic detection.

■ ACKNOWLEDGMENTS

■ AUTHOR CONTRIBUTIONS

Prastika Krisma Jiwanti conceptualized, supervised, wrote, and revised the manuscript, Irfansyah Rais Sitorus conducted the experiment, Grandprix T.M. Kadja conducted the experiment, Siti Wafiroh supervised, and Yasuaki Einaga supervised. All authors agreed to the final version of this manuscript.

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