

Sensitive electrochemical sensor of levofloxacin using boron-doped diamond (BDD) electrode modified with $\text{Ti}_3\text{C}_2\text{T}_x$ (MXene) material

Prastika Krisma Jiwanti ^{a,b,*}, Indah Harindy Putri ^c, Grandprix T.M. Kadja ^{d,e,f}, Yasuaki Einaga ^g, Laurencia Gabrielle Sutanto ^a, Siti Wafiroh ^c, Dewi Kartika Azizah Sukardi ^c, Anis Puspita Sari ^c, Tahta Amrillah ^{a,b}, Ilma Amalina ^{a,b}, Qonita Kurnia Anjani ^h, Wan Jeffrey Basirun ^{i,j}

^a Nanotechnology Engineering, Faculty of Advanced Technology and Multidiscipline, Universitas Airlangga, Surabaya 60115, Indonesia

^b Airlangga Functional Nanomaterials Research Group, Faculty of Advanced Technology and Multidiscipline, Universitas Airlangga, Surabaya 60115, Indonesia

^c Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia

^d Division of Inorganic and Physical Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung, Jl. Ganesha No. 10, Bandung 40132, Indonesia

^e Center for Catalysis and Reaction Engineering, Institut Teknologi Bandung, Jl. Ganesha No. 10, Bandung 40132, Indonesia

^f Research Center for Nanosciences and Nanotechnology, Institut Teknologi Bandung, Jl. Ganesha No. 10, Bandung 40132, Indonesia

^g Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan

^h School of Pharmacy, Medical Biology Centre, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK

ⁱ Nanotechnology and Catalysis Research Center (NANOCAT), Universiti Malaya, Kuala Lumpur 50603, Malaysia

^j Department of Chemistry, Faculty of Science, Universiti Malaya, Kuala Lumpur 50603, Malaysia

Abstract

Overuse of levofloxacin (LEV) is often associated with bacterial resistance and serious health problems, underscoring the need for reliable sensing and monitoring of LEV molecules. Therefore, this study aimed to investigate LEV using boron-doped diamond (BDD) and boron-doped diamond modified with MXene ($\text{Ti}_3\text{C}_2\text{T}_x$) (BDD-MXene) electrode. The successful deposition of MXene on the BDD surface was confirmed using scanning electron microscope (SEM). Cyclic voltammetry (CV) and square wave voltammetry (SWV) methods were also applied to evaluate the electrochemical behavior. The results showed that both electrodes had a linear response in the range of 30–100 μM . The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 1.0×10^{-6} M and 3.37×10^{-6} M for bare-BDD, while on BDD-MXene, the values were 3.90×10^{-7} M and 1.30×10^{-6} M, respectively. Furthermore, both electrodes showed good responses on selectivity tests with glucose and another fluoroquinolone antibiotic such as ciprofloxacin. The results also indicated good precision with %RSD less than 5%. In real sample applications using wastewater, bare-BDD and BDD-MXene produced excellent %recovery of 92.96% and 101.29%, respectively.

Keywords: Boron-doped diamond, Good health & well-being, Levofloxacin, MXene, Sensor

1. Introduction

Levofloxacin (LEV) is also recognized by the chemical name of (S)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid.

The mechanism of action is to inhibit topoisomerase II (DNA gyrase) and topoisomerase IV, which prevents bacterial DNA from replicating and transcribing, thereby causing bactericidal effects [1]. LEV is used for the treatment of urinary tract infections, respiratory tract infections, skin and soft

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* Corresponding author at: Nanotechnology Engineering, Faculty of Advanced Technology and Multidiscipline, Universitas Airlangga, Surabaya 60115, Indonesia.

E-mail address: prastika.krisma@ftmm.unair.ac.id (P.K. Jiwanti).

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tissue infections, bone and joint infections, intra-abdominal infections, as well as sexually transmitted diseases [2]. It has a different mechanism compared to most antibiotics, including β -lactams, macrolides, tetracyclines, or aminoglycosides. Therefore, some organisms that are resistant to other antibiotics remain sensitive to LEV. Previous studies also reported that LEV is prone to overuse due to the widespread application [3,4].

Several methods for detecting LEV have proven to be effective, such as UV-VIS spectrophotometry [5], Flow Injection Analysis (FIA) [6], Nuclear Magnetic Resonance Spectroscopy (NMR) [7], High-Performance Liquid Fluorimetry (HPLC) [8], and Capillary Electrophoresis (CE) [9]. Despite the sensitivity, some of these methods require expensive instruments, high expertise, and prolonged analysis. One promising method is electrochemistry, known for simplicity, higher sensitivity, shorter analysis time, and lower cost compared to traditional methods [10].

Numerous electrochemical sensors have been developed for detecting LEV, including carbon nanotubes [11], modified nickel oxide porous films [12], screen-printed electrodes (SPE) [13], and modified glassy carbon electrodes (GCE) [14]. Among these electrodes, boron-doped diamond (BDD) is known to be excellent for sensors due to the wide potential window, low background current, and inertness to surface adsorption, which resists electrodes from fouling [15]. Additionally, BDD is chemically more durable and mechanically strong, leading to wide applications for electroanalysis, electrocatalysis, and electrosynthesis [16].

Previous studies focused on improving the electroanalytical performance of bare electrodes. Carbon-based materials, nanomaterials, and polymers-based composites have been used to prepare and modify the electrochemical sensor application [17]. For example, MXene is a family of 2D layered materials with a general formula of $M_{n+1}X_nT_x$. Specifically, M represents an early transition metal, X denotes carbon or nitrogen with $n = 1, 2, 3$, and T signifies surface termination such as oxygen, hydroxyl, or fluorine ($-O$, $-OH$, $-F$) [18]. MXene offers several advantageous properties such as high conductivity, electroactive surface area, ease of fabrication with other materials, and superior hydrophilicity compared to other 2D materials [19]. These characteristics make MXene an ideal candidate for enhancing the sensitivity of BDD sensors.

This study focused on the integration of MXene with BDD for sensor modification, aiming to improve the detection performance of the sensor for LEV. Specifically, MXene is expected to enhance the sensor's sensitivity towards LEV detection, while

also potentially improving other key performance metrics such as limit of detection (LOD), linear response time, and long-term stability [20]. This modification addresses the demand for sensors with high sensitivity, low LOD, fast response times, and prolonged operational durability, which are essential for various applications including environmental monitoring and biomedical diagnostics.

2. Materials and methods

2.1. Materials and instrumentations

Levofloxacin (>98%), Ciprofloxacin (>98%), and Ofloxacin (>98%) were purchased from Sigma Aldrich, NaH_2PO_4 (>99%), Na_2HPO_4 (>99.5%), D-glucose and buffer phosphate solution (PBS) with a pH range of 4.0–8.0 were acquired from Merck, while H_2SO_4 (>98%) was purchased from SAP Chemical. All solutions were used without further pretreatment and purification.

BDD (1% B/C) was synthesized following a previous study [21] by depositing the layer on the surface of silicon wafer (111) for 6 h using a microwave plasma-assisted chemical vapor (Model AX6500X, CORNES Technology Corp). Electrochemical measurements such as cyclic voltammetry (CV) and square wave voltammetry (SWV) were run using Potentiostat Emstat³⁺ Blue Palmsens with an electrolyte cell, Ag/AgCl as a reference electrode, and platinum wire spiral for support. Furthermore, scanning electron microscopy (SEM, Hitachi SU-3500) was used to characterize the modified electrode.

2.2. Synthesis of MXene

The synthesis of $Ti_3C_2T_x$ (MXene) was performed following the procedures used in previous studies [22,23] with slight modifications. In a typical preparation, 1.5 g of Ti_3AlC_2 was added to the LiF–HCl solution, containing 2 g LiF within 15 mL of 10 M HCl solution, at 50 °C under vigorous stirring overnight. Subsequently, the mixture was centrifuged at a spinning rate of 4000 rpm to obtain deposited black solids. The solids were washed until the pH of the supernatant was below 6. This was followed by dispersion in dimethyl sulfoxide (DMSO), followed by centrifugation at 4000 rpm. The deposited solids were collected, redispersed in demineralized water, and ultrasonicated for 2 h. The resulting suspension was then centrifuged at 4000 rpm to yield an MXene suspension with a concentration of ~2 mg/mL. The characterization of MXene was performed using X-ray diffractometer

(XRD, Bruker D8 Advance) and SEM (Hitachi SU-3500).

2.3. Electrode preparation and characterization

Pretreatment of the electrode started by sonicating the electrode with isopropanol and deionized water for 300 s. This was followed by performing CV in the potential range of -2.0 to 2.0 V at a scan rate of 1 V/s for 40 cycles in a 0.1 M H_2SO_4 electrolyte solution. The bare-BDD electrode was modified using the drop cast method on the active side of the electrode. Using MXene concentration of 1 mg/mL, the BDD-MXene was allowed to dry at room temperature for 24 h to ensure complete deposition on the BDD. Subsequently, the electrode was characterized using SEM.

3. Results and discussion

3.1. Characterization of MXene and BDD-MXene

The XRD pattern of the $\text{Ti}_3\text{C}_2\text{T}_x$ MXene in Fig. 1a shows characteristic peaks observed as the typical reflections of (001) crystalline planes of two

dimensional $\text{Ti}_3\text{C}_2\text{T}_x$, i.e., (002), (004), (006), and (008) planes [22,23]. Moreover, Fig. 1b shows the direct observation of $\text{Ti}_3\text{C}_2\text{T}_x$ through SEM, presenting a layered structure with the combination of unilamellar nanosheets. The morphology of polycrystalline BDD and BDD-MXene modification was observed by using SEM (Fig. 1c–d). The deposition of MXene on bare-BDD was successfully achieved, as indicated by brighter (white) spots or grains scattered among the BDD lattice. After the incorporation of MXene, the surface of BDD-MXene became very dense and smooth compared to before modification.

3.2. Signal per background (S/B)

The S/B ratio served as a valuable metric for analyzing the electrochemical behavior of LEV on both the bare-BDD and BDD-MXene electrodes. This measurement was conducted by SWV method and comparing the peak current of the LEV sample with the background current (blank). As shown in Fig. 2, the LEV on bare-BDD and BDD-MXene had a peak current in the potential range of 0.4 – 1.6 V

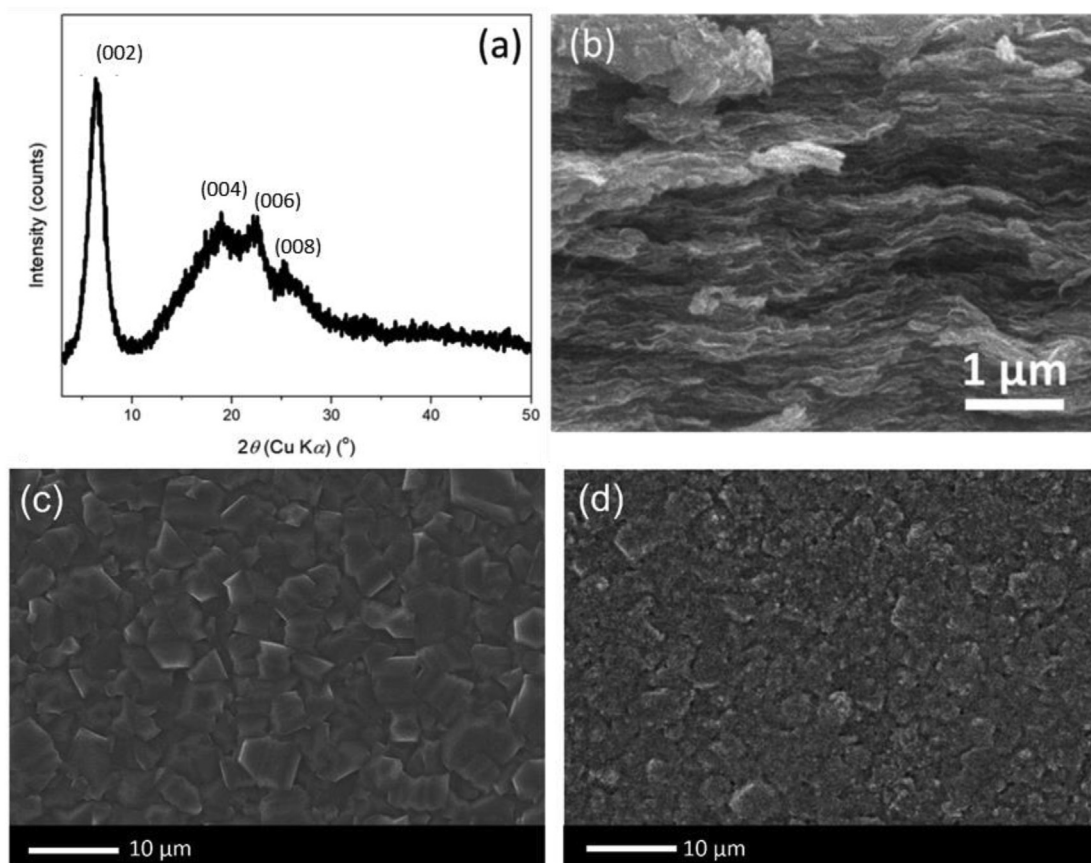


Fig. 1. (a) XRD pattern, (b) SEM image of $\text{Ti}_3\text{C}_2\text{T}_x$ MXene, (c) SEM image of bare BDD and (d) SEM characterization results of BDD-MXene.

(vs Ag/AgCl). The peak current on BDD-MXene was 105.37 μM , which was higher than the bare-BDD, with a value of 83.87 μM , while the S/B was 13.7 and 8.9 respectively. The higher oxidation peak of LEV indicated that MXene material on the BDD surface successfully enhanced the electrocatalytic activity of the sensor. BDD-MXene facilitated a higher electron transfer rate compared to the inert surface of bare-BDD [24]. The background current observed on BDD-MXene was also smaller, contributing to the heightened sensitivity. Consequently, the electrode had high sensitivity relative to the bare-BDD counterpart.

3.3. Scan rate

The determination of scan rate was observed within various predefined values of 20, 50, 80, 100, and 120 mV/s. The measurements were conducted to study the electro-oxidation mechanism of LEV [24]. The potential range of 0–1.6 V (vs Ag/AgCl) using CV method was applied for bare-BDD and BDD-MXene electrodes. The peak current of LEV in each electrode is shown in Fig. 3. The oxidation peak increased and shifted linearly to a more positive potential with higher scan rates. A peak reduction was not observed, indicating that the LEV oxidation was irreversible. Moreover, the peak current and the square root of the scan rate were plotted to observe the linearity, resulting in coefficient of determination (R^2) values of 0.9923 and 0.9972 for bare-BDD and BDD-MXene electrodes respectively. Based on this linearity graph, the oxidation reactions of LEV were diffusion-controlled.

The diffusion coefficients for both bare-BDD and BDD-MXene electrodes were determined using the Randles–Sevcik equation,

$$I_{pa} = (2.69 \times 10^5) \times n^{3/2} \times D^{1/2} \times v^{1/2} \times A \times C$$

Where I_{pa} is the peak current (A), n is the number of electrons transferred, D is the diffusion coefficient, v is the scan rate, A is the geometrical surface area (cm^2), and C is the concentration of bulk solution. Following the equation, the diffusion coefficient was found to be 0.1868 and 0.264 cm^2/s for bare-BDD and BDD-MXene, respectively. The diffusion coefficient represents the speed of electroactive molecule transfer from the diffusion layer in the solution to the electrode surface. Based on the results, BDD-MXene had a larger diffusion coefficient, leading to more sensitivity than bare-BDD.

3.4. Optimum pH

The optimum condition of LEV oxidation was observed at various pH of 4.0–8.0 using PBS 0.1 M. This measurement was performed using SWV method and the relation between the potential, pH, and current are shown in Fig. 4. Based on the results, LEV had the highest peak current at pH 5.5 on both the Bare-BDD and BDD-MXene electrodes. This observation suggests that at pH 5.5, there is an enhanced electron transfer efficiency, leading to the optimization of the sensor's performance. The optimal performance underscored the importance of pH control in achieving the highest sensitivity and efficiency of the sensor for LEV detection, hence, pH 5.5 was used as the optimum pH of this study. LEV is a zwitterion that possesses a carboxylic group with $pK_a = 5.5$ and a piperazinyl group with $pK_a = 8.0$ [25]. In this condition, a transfer of 2 electrons and 2 protons H^+ occurs, leading to LEV N-oxide as shown in Fig. 5. The current increased linearly with higher pH, then after reaching the optimum pH, the current started to decrease. This pH also affected the potential shift, where at low pH (acidic), the oxidation potential shifted towards the positive direction, while at high pH (basic), there

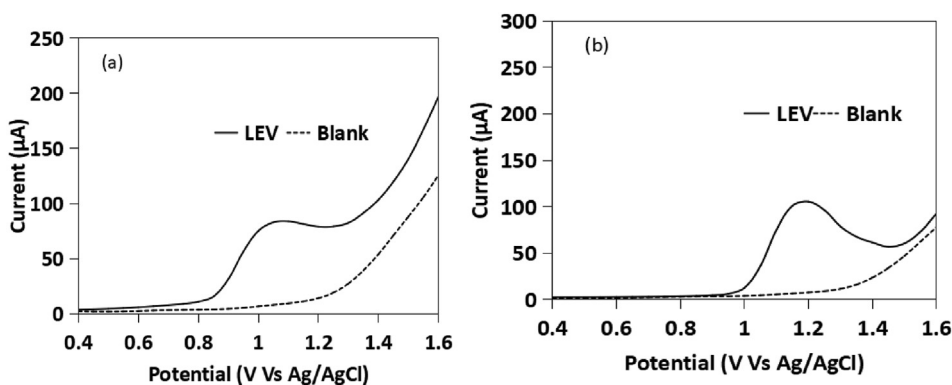


Fig. 2. SWV on (a) bare-BDD (b) BDD-MXene electrode with LEV (solid line) and without (dash line) 60 μM LEV in 0.1 M PBS 6.5.

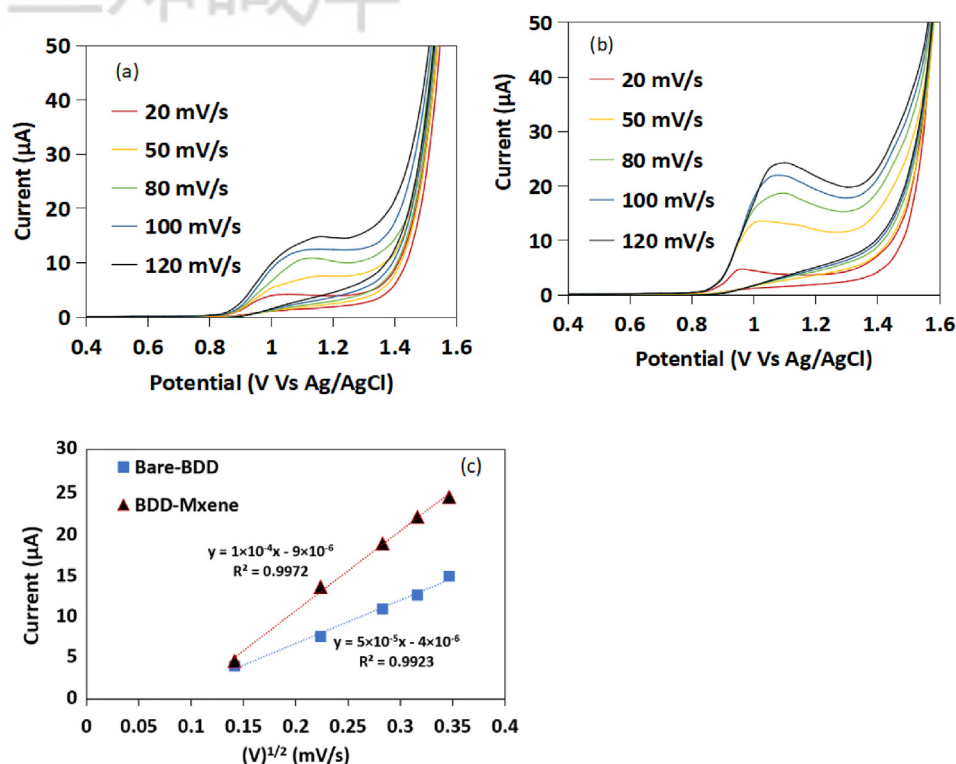


Fig. 3. Various scan rates of potential vs. current of 60 μ M LEV in 0.1 M PBS 6.5 performed on (a) bare-BDD (b) BDD-MXene electrode and (c) linear relationship between the square root of scan rate and current.

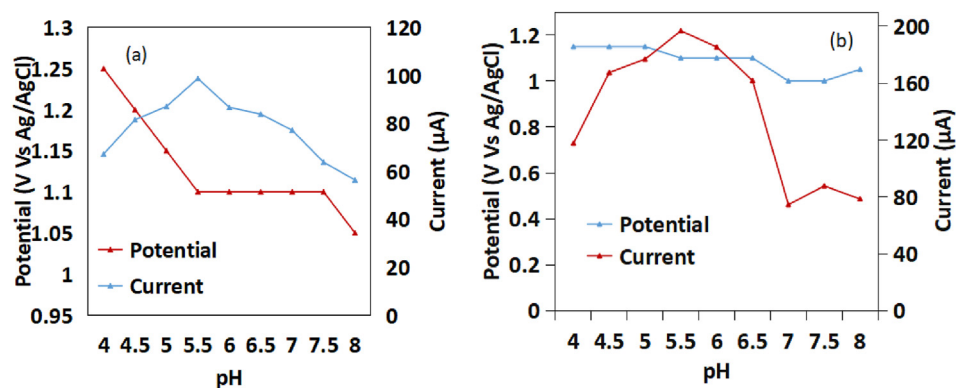


Fig. 4. Relationship between pH, potential, and current of LEV measurement on the (a) bare-BDD and (b) BDD-MXene electrodes.

was a shift towards the negative. This property is due to the addition of many H^+ ions at acidic pH, causing the peak current to shift towards the positive potential [25].

3.5. Linearity

The effect of various concentrations was measured to study the linearity of both bare-BDD and BDD-MXene electrodes. The concentration range of 30–100 μ M was applied and measured using SWV method with optimum parameters condition,

namely optimum frequency of 50 Hz, optimum amplitude of 50 mV, and optimum step potential of 50 mV. As shown in Fig. 6, the peak current increased with higher LEV concentrations. Therefore, the peak current and the various concentrations are plotted to be calibration curves and show linear response with correlation coefficient, $R^2 = 0.9926$ and $R^2 = 0.9975$ for bare-BDD and BDD-MXene, respectively.

LOD and limit of quantitation (LOQ) were calculated according to the slope of the calibration curve of each electrode. Specifically, LOD was calculated

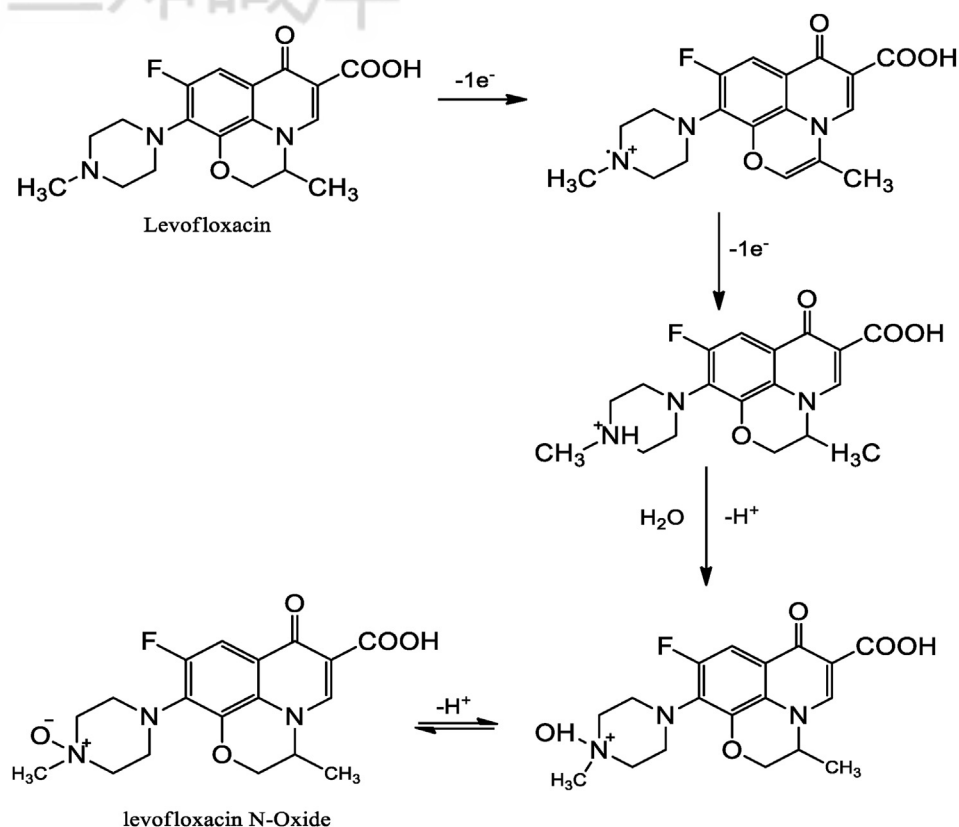


Fig. 5. Electrochemical oxidation from LEV to LEV N-Oxide [25].

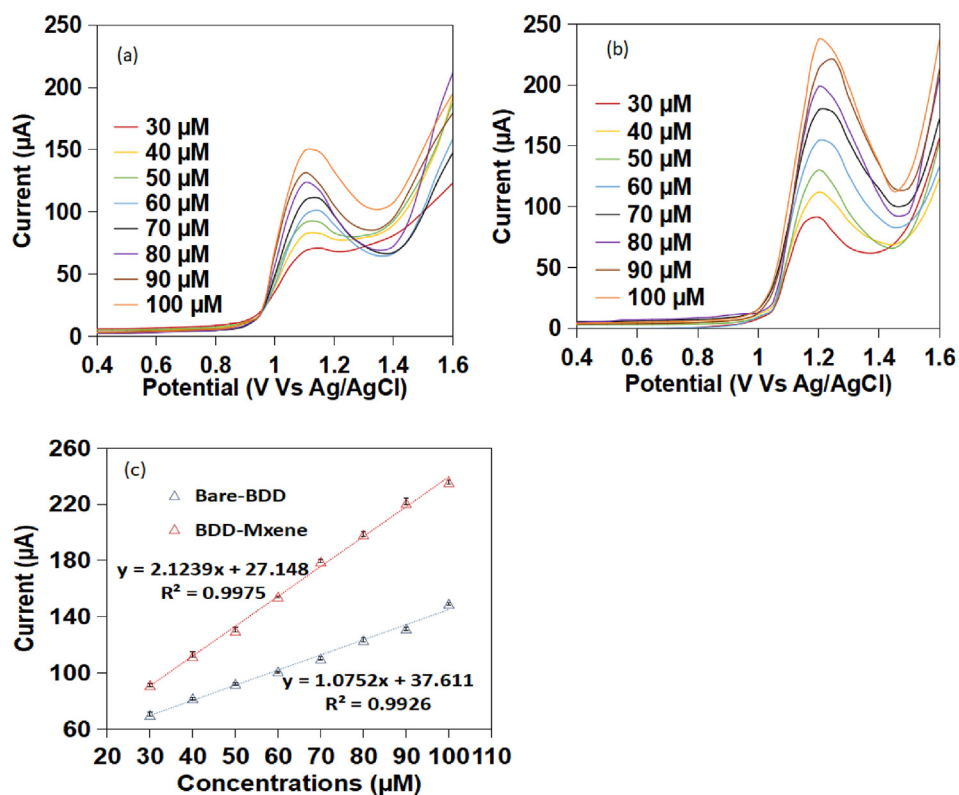


Fig. 6. Voltammogram curve for signal determination per background in the measurement of LEV using electrodes (a) bare-BDD electrode (b) BDD-MXene electrode and (c) plot calibration curves for both electrodes.

Table 1. Comparison between LEV for electrochemical sensors with others.

Methods	Electrodes	LOD (μM)	References
SWV	Ni/BDD	5.11	[28]
SWV	BDD	2.88	[25]
DPV	CP/NFS	9.00	[29]
SWV	BDD	1.00	This Work
SWV	BDD-MXene	0.39	This work

as three times the slope of calibration curve divided by the standard deviation of the intercept. On the other hand, LOQ was calculated following the equation which divided ten times the slope calibration curve against the standard deviation of the

intercept [26]. Based on the results, LOD and LOQ were found to be $3.90 \times 10^{-7} \text{ M}$ and $1.30 \times 10^{-6} \text{ M}$ for BDD-MXene, while the values for bare-BDD electrode were $1.00 \times 10^{-6} \text{ M}$ and $3.37 \times 10^{-6} \text{ M}$ for, respectively. In addition, the sensitivity of both electrodes was $2.1239 \times 10^{-6} \text{ M}/10^{-6} \text{ A}$ and $1.0752 \times 10^{-6} \text{ M}/10^{-6} \text{ A}$ respectively. This result indicated that the modified BDD using MXene enhanced the sensitivity of LEV detection.

BDD-MXene had a lower LOD, showing better sensitivity compared to bare-BDD. Consequently, it requires a lower concentration to be detected in actual samples, translating to more effective in real-world applications. The comparison of LEV sensor

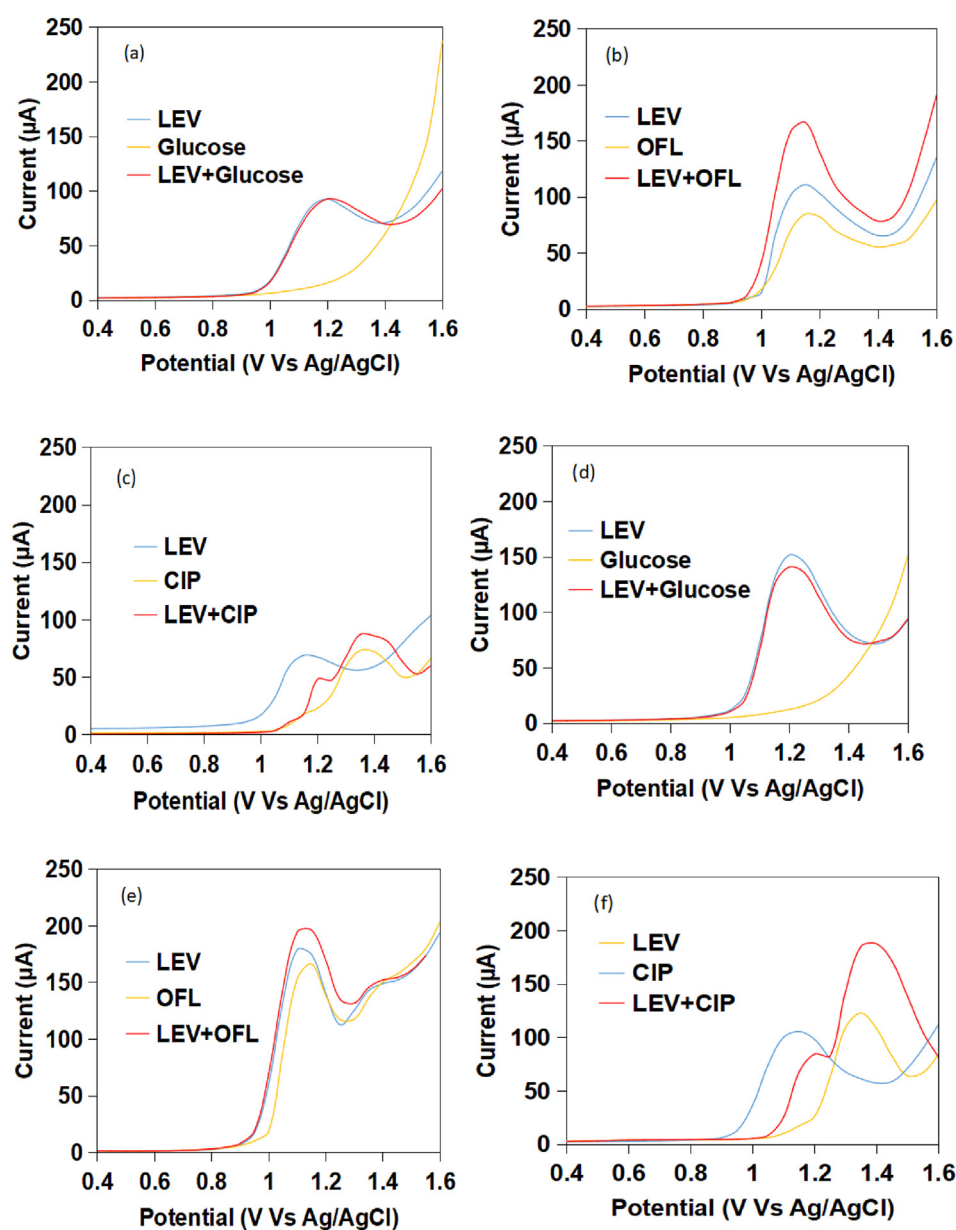


Fig. 7. SWV of 60 μM LEV with interferences of D-glucose, OFL, and CIP (a), (b), (c) on bare-BDD and (d), (e), (f) on BDD-MXene.

with various electrodes is summarized in Table 1. BDD-MXene had better LOD compared to previous studies. The presence of MXene material indicates that the electrochemical sensor for LEV produces a lower LOD. This sensitivity and lower LOD sensor are supported by the excellent characteristics of MXene which has good chemical stability, high surface areas, and is environment friendly [27]. Therefore, the BDD-MXene has promising potential and can be further developed for antibiotic sensor detection.

3.6. Selectivity and repeatability

The selectivity parameter was evaluated by measuring LEV at 60 μM in 0.1 M PBS at pH 5.5 with interference solutions, including D-glucose, ofloxacin (OFL), and ciprofloxacin (CIP). D-glucose interference is potentially present in the actual sample, while OFL and CIP have similar chemical structures with LEV. The selectivity parameter was measured using a 1:1 v/v ratio of LEV and each

interference solution through SWV methods, with the corresponding result presented in Fig. 7. D-glucose and CIP did not demonstrate interference in the LEV sample. However, OFL showed an inseparable peak due to LEV being one of the isomeric racemic mixture forms [14]. Both bare-BDD and BDD-MXene electrodes showed high selectivity against D-glucose in measuring LEV 60 μM . In addition, both electrodes successfully detected LEV and CIP in separated oxidation peak currents.

In the repeatability test, LEV was measured in 0.1 M PBS at pH 5.5 using the SWV method on bare-BDD and BDD-MXene electrodes. Measurements were carried out 7 times in a row for each electrode. The precision of the two electrodes was determined by the stability of the current and peak potential produced. The results showed that both electrodes had quite good precision when used for LEV measurements as presented in Fig. 8, with %RSD values of 1.28% and 1.59% for bare-BDD and BDD-MXene respectively. BDD-MXene had a higher %RSD due to the addition of MXene material, making the

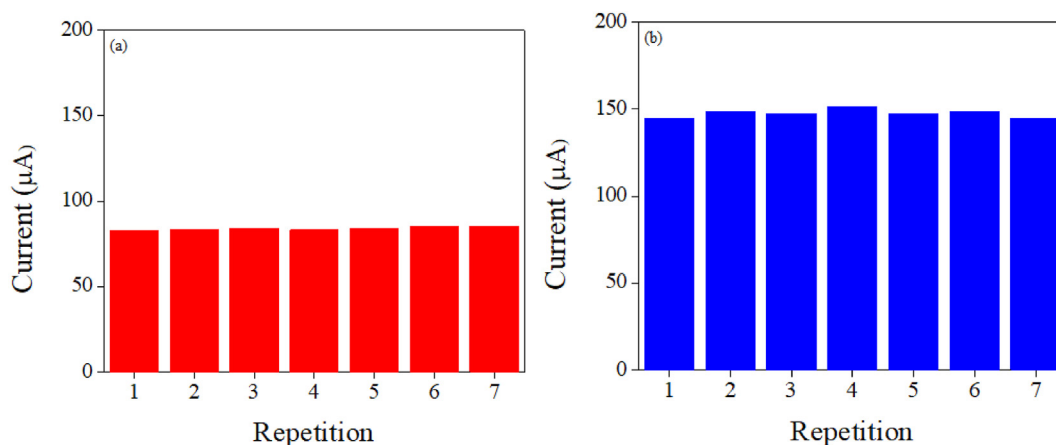


Fig. 8. Repeatability of 60 μM LEV (a) on bare-BDD and (b) on BDD-MXene.

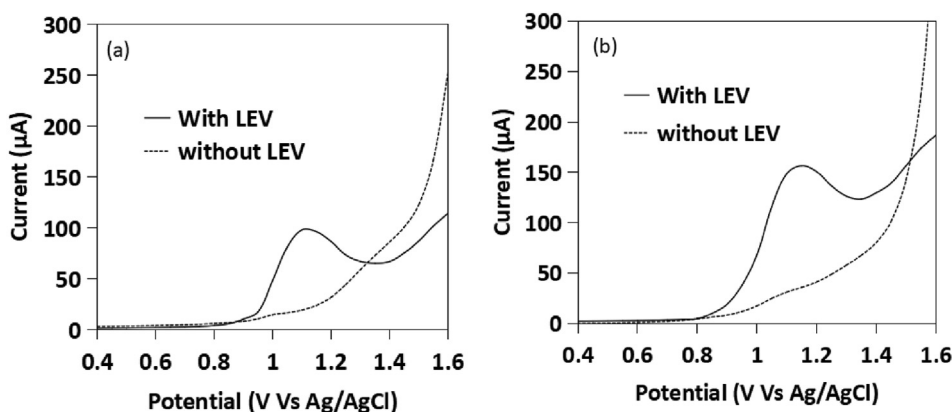


Fig. 9. Voltammogram curve for wastewater sample in the measurement with LEV (solid line) and without (dash line) 1:1 using (a) bare-BDD and (b) BDD-MXene electrode.

Table 2. The summary of %recovery measurement in real sample application.

Sample	Electrodes	LEV added (μM)	LEV found (μM)	% Recovery	% RSD
Wastewater in river	Bare-BDD	60	55.77	92.96	0.89
	BDD-MXene	60	60.77	101.29	0.76

electrode less stable. However, this %RSD suggested acceptable precision because the value was less than 5%.

3.7. Real sample analysis

The determination of LEV in actual samples was carried out on wastewater samples on the bare-BDD and BDD-MXene electrodes in the potential range of 0–1.6 V (vs Ag/AgCl). This analysis was performed to evaluate the validation methods of electrochemical sensors using bare-BDD and BDD-MXene electrodes. The measurements on actual samples were performed by standard addition methods. The solution of 60 μM was added to the wastewater with a 1:1 ratio in 0.1 M PBS 5.5. As shown in Fig. 9, the LEV current peaks on bare-BDD and BDD-MXene were 98.83 μA and 156.62 μA , respectively. The obtained data were then calculated for recovery as shown in Table 2. The recovery value was 92.96% and 101.29% for bare-BDD and BDD-MXene, respectively. These values represent good recovery, falling within the range of 85–110%. Consequently, both electrodes demonstrate excellent accuracy in determining LEV for future application in a real sample.

4. Conclusion

In conclusion, the MXene material was successfully modified on the BDD electrode. The electrochemical behavior of LEV was observed through S/B ratio with bare-BDD and BDD-MXene electrodes producing values of 8.85 and 13.7, respectively. BDD-MXene yielded a lower LOD at 0.39 μM compared to bare-BDD at 1.00 μM in the linear concentration range of 30–100 μM . Furthermore, the %RSD values were 1.28% and 1.59%, respectively, indicating that both electrodes had good precision. The real sample application was carried out by measuring wastewater samples and the recovery value obtained was 92.96% and 101.29% for bare-BDD and BDD-MXene respectively. These results indicate that BDD-MXene has promising potential for future applications in LEV detection.

Conflict of interest

The authors declare no conflict of interest.

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