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Highly Specific Voltammetric Detection of Cephalexin in Tablet Formulations and Human Urine Samples Using a Poly(2,4,6-2′,4′,6′-hexanitrodiphenylamine)-Modified Glassy Carbon Electrode

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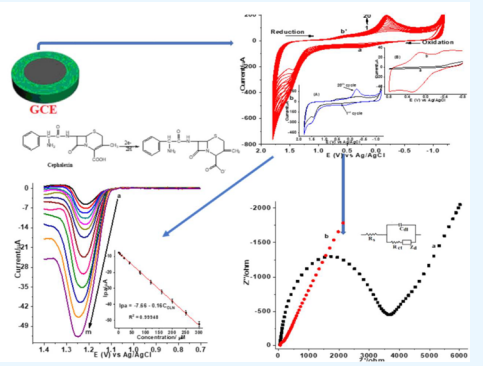
ABSTRACT: β-Lactam antibiotics are employed to treat bacterial illnesses. Despite a high level of clinical success, they have encountered serious resistance that demands a high-dose regimen and a new pharmacokinetic combination. This requires continuous monitoring of their levels in pharmaceutical and biological samples. In this study, an electrochemical sensor was developed for the determination of cephalexin (CLN) in pharmaceutical formulations and biological fluid samples. The sensors were developed by modifying a glassy carbon electrode (GCE) using a conducting polymer (dipicrylamine) by potentiodynamic electropolymerization. Characterization (using cyclic voltammetry and electron impedance spectroscopy) results revealed modification of the electrode surface, leading to an enhanced effective electrode surface area and their conductivity. The appearance of an irreversible oxidative peak at much-reduced potential with 5-fold current enhancement at a poly(dipicrylamine)-modified glassy carbon electrode (poly(DPA)/GCE) verified the electrocatalytic role toward CLN. Under optimized conditions, a wider linear concentration range (5×10^{-8} to 3.0×10^{-4} M), lowest limit of detection (LoD) (2.5 nM), detected amount of each tablet brand above 97.00% of the labeled value (showing excellent agreement between the detected amount and company label), and excellent % recovery results in pharmaceutical and biological samples were obtained with an excellent interference recovery error of less than 4.05%. Its excellent accuracy, selectivity, reproducibility, and stabilities and only requiring a simple electrode modification step combined with its readily available and nontoxic modifier, which sets it apart from most previously reported methods, have validated the present method's potential applicability for determining CLN in biological and pharmaceutical samples.

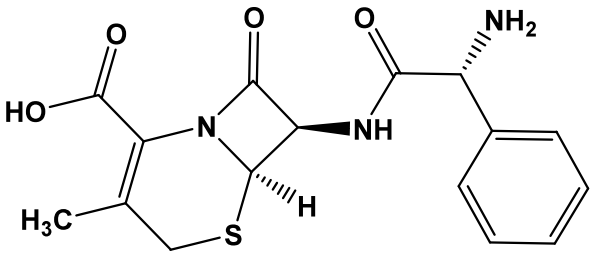


1. INTRODUCTION

One of the most significant groups of antibacterial drugs is the β-lactam class of antibiotics, which is distinguished by the presence of a β-lactam ring in their structure.[1] The mechanism of action of cephalosporins, a class of antibiotics, involves interfering with the production of the bacterial cell wall through the fusion of a β-lactam ring with a six-membered ring containing a sulfur atom.[1,2] Cephalexin (CLN, (6*R*,7*R*)-7-{[(2*R*)-2-amino-2-phenylacetyl]-amino}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid) (Scheme 1) belongs to the first-generation semisynthetic broad-spectrum cephalosporin class of antibiotics, which is effective against susceptible bacteria causing, for example, urinary tract and respiratory tract infections (sinusitis, otitis media, pharyngitis, tonsillitis, and bronchitis) caused by Gram-positive and Gram-negative bacteria.[3−5]

Common adverse drug reactions associated with use of CLN includes stomach pain, vomiting, nausea, diarrhea, joint pain, unpleasant taste, restlessness, and vaginal itching.[4,5] According to the World Health Organization, cephalosporins are considered as the most common drugs that have developed bacterial resistance,[5,6] thus is the most current health challenge for professionals in the area of antibacterial resistance. The development of a fast, selective, sensitive, environmentally

Scheme 1. Chemical Structure of CLN



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friendly, and stable method for detection and quantification of CLN in trace levels in different matrices is essential. Hence, the design of a rapid, sensitive, and accurate method for CLN determination in human urine and tablet formulations is highly needed.

Spectrophotometry,[7] hyphenated methods,[8] and chromatography[9] are among the common techniques reported for the determination of CLN in different samples. Unfortunately, all these methods need tedious sample preparation, are time-consuming, require complex analytical equipment, and consume large amounts of organic solvents (non-eco-friendly).[10,11] On the contrary, electrochemical methods are more appealing than conventional methods because they are easy to use, inexpensive, repeatable, very sensitive, quick to analyze, and environmentally safe.[12−14]

There have been attempts to use voltammetric techniques to determine CLN in various real samples.[10,15] Via increasing its compatibility, electron conductivity, and surface area, surface modification often enhances the electrochemical performance of a working electrode.[10−13] In this regard the use of conducting polymers as electrode modifiers has drawn a lot of interest due to their good chemical stability, electrical conductivity, reproducibility, improved sensitivity, excellent biocompatibility, electrocatalysis, and strong adhesion to the electrode surface compared with a conventional electrode.[10,16−19] Dipicrylamine (2,4,6-2′,4′,6′-hexanitrodiphenylamine) is a conducting polymer containing two trinitrobenzene units with secondary amine functional groups (−NH−), available for electropolymerization, and can be used as a sensor in electrochemical techniques;[20−22] for sensing purposes only limited work is found.

A few studies have been conducted on CLN's electrochemical behavior in diverse real samples at various modified electrode surfaces, including a pencil graphite electrode,[23] poly-(resorcinol)/GCE,[10] a heated glassy carbon electrode (HGCE),[24] a molecularly imprinted polymer (MIP),[25] a mercury electrode,[15] and a bare boron-doped diamond electrode.[26] The majority of these reported electrochemical techniques require the use of expensive modifiers like Au nanoparticles with complex preparation procedures and mercury as a working electrode, making them still not ecologically friendly.

In this work, we reported the synthesis of a novel method for the potentiodynamic electropolymerization of a conducting polymer (dipicrylamine) at the surface of glassy carbon electrodes. To date, no electrochemical method using a poly(dipicrylamine)-modified glassy carbon electrode has been reported for the determination of CLN, which is an electrode fabricated easily from an available material, with an easy electrode modification procedure using a modifier with a low chip cost, low toxicity, high electrocatalytic activity, electrical conductivity, reproducibility, improved sensitivity, larger surface area, wide concentration range, and low detection limit. The modified glassy carbon electrode surface property was examined by using cyclic voltammetry and electrochemical impedance spectroscopy methods. A square wave voltammetric method was used for the determination of CLN in tablet formulations with a complex matrix and in biological fluid samples.

2. MATERIALS AND METHODS

2.1. Chemicals and Apparatus. Cephalexin monohydrate (≥99.0%) from Sigma-Aldrich; potassium hexacyanoferrate-(III) (98.0%), potassium hexacyanoferrate(II) (98.0%), and dipicrylamine (≥98.0%) from BDH Laboratories Supplies, England; potassium chloride (99.5%), sodium monohydrogen phosphate (≥98.0%), and sodium dihydrogen phosphate (≥98.0%) from Blulux Laboratories (p) Ltd.; hydrochloric acid (37.0%), nitric acid (70%), and sulfuric acid (98%) from Fisher Scientific; and sodium hydroxide (extra pure) from Lab Tech Chemicals, all of analytical grade, were utilized with no additional purification. A CHI 760E potentiostat (Austin, TX, USA), pH meter (Adwa, AD8000, Romania), refrigerator (Lec Refrigeration PLC, England), deionizer (Evoqua Water Technologies), centrifuge (1020D, Centurion Scientific LTD, UK), and electronic balance (Nimbus, ADAM Equipment, USA) were among the instruments used.

2.2. Procedures. *2.2.1. Preparation of Poly(DPA)/GCE.* A highly polished glassy carbon electrode with a mirror finished was placed in a 0.1 M phosphate buffer solution (PBS) of pH 7.0 containing 1.0 mM poly(dipicrylamine) and scanned for 20 cycles at a scan rate of 100 mV s^{-1}, over an optimum potential range of −1.2 to +1.8 V. Afterward, the modified GCE was rinsed with deionized water and stabilized in 0.5 M H_2SO_4 within a potential range of −0.80 V to +0.80 V until a consistent cyclic voltammogram was obtained. After that, the altered electrode was allowed to dry properly and was made ready for more tests.

2.2.2. Preparation of Standard CLN Solutions. A stock solution of standard CLN with a concentration of 5.0 mM was prepared by dissolving an accurately weighed 91.35 mg in 50 mL of deionized water, respectively. Working standard solutions were then prepared in 0.1 M PBS of the appropriate pH from the stock solution by serial dilution.

2.2.3. Preparation of Analyzed Real Samples. 2.2.3.1. Pharmaceutical Tablet Sample Preparation. A mortar and pestle was used to grind and homogenize five randomly chosen tablets from each of the four brands of CLN that were studied: Remedica Ltd. (Felexin), Cyprus; Sterling Lab. (Cephast), India; (Salexin), India; and CT Ger (Cephalex), Germany. The tablets were labeled as 500 mg of CLN/tablet, with average mass/tablet of 574.3, 572.2, 571.5, and 568.4 mg/tablet, respectively. Stock solutions of tablet samples with a nominal concentration (2.0 mM) for each tablet brand of CLN were prepared by transferring a mass of tablet powder (41.97, 41.82, 41.77, and 41.54 mg, respectively (equivalent to 36.54 mg)) to a 50 mL volumetric flask and filling up to the mark with deionized water. Further working tablet sample solutions of CLN in pH 6.5 PBS for each tablet brand were prepared from the respective tablet stock solution through serial dilution.

2.2.3.2. Human Urine Sample. A fresh human urine sample was obtained from an adult volunteer and centrifuged at 4000 rpm for 10.0 min, and a 0.5 mL portion of the supernatant portion was transferred to a 25 mL volumetric flask and diluted with pH 5.5 PBS. Urine samples spiked by standard ASA having different concentrations (0.0, 20.0, 40.0, and 80.0 μM) were prepared for spike recovery analysis.

2.3. Electrochemical Measurements. Electroanalytical measurements were performed using a conventional three-electrode system with a Pt coil as a counter electrode, Ag/AgCl (3.0 M KCl) as a reference electrode, and bare GCE (3 mm diameter) or poly(DPA)/GCE as a working electrode. EIS and CV techniques were used to characterize the modified electrode and/or investigate the electrochemical behavior of CLN at the surface of the poly(DPA)/GCE at various scan rates and pHs. Square wave voltammetry (SWV) was employed for quantitative

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determination of CLN in four tablet brands and human blood serum samples.

3. RESULTS AND DISCUSSION

3.1. Preparation of the Poly(DPA)/GCE. When a material on the electrode surface is altered potentiodynamically, one of the most important variables is the thickness of the layer. Therefore, the film thickness may be regulated by looking at the peak growth and optimizing it by analyzing the changed electrode's current response to CLN as a probe and adjusting for the number of scan cycles. Presently, Figure S1 shows the poly(DPA)/GCE response for CLN at different polymerization scan cycles (10−25). The anodic peak current of CLN is enhanced with different slopes; as a compromise between the current, overpotential, and analysis time advantages, 20 scan cycles were taken as the optimum in this experiment (inset of Figure S1).

Figure 1 presents repetitive CVs of GCE in pH 7.0 PBS containing 1.0 mM DPA scanned between −1.2 and +1.8 V for

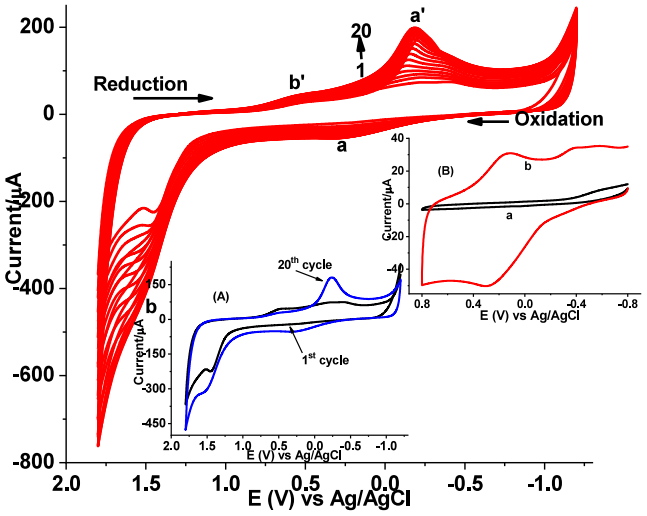

Figure 1. CVs of 1.0 mM DPA in pH 7.0 PBS at a GCE scanned between −1.2 and +1.8 V at a scan rate of 100 mV s^{-1} for 20 cycles. Inset: CVs of (A) the 1st cycle and 15th cycle and (B) (a) unmodified GCE and (b) stabilized poly(DPA)/GCE both between −0.8 and +0.8 V at 100 mV s^{-1} in 0.5 M H_2SO_4.

20 cycles. As evidenced in the figure, both the anodic peak (a and b) and cathodic peak (a′ and b′) current increased with scan cycles, revealing the deposition of the polymer film on the electrode surface (inset A of Figure 1). A further indication that an electroactive polymer layer had been deposited on the electrode surface was the appearance of numerous oxidative and reductive peaks of poly(DPA)/GCE (curve b of inset B), compared to just one broad reductive peak of bare GCE in 0.5 M H_2SO_4, assigned to reduction of molecular oxygen (curve a of inset B).

Therefore, poly(DPA)/GCE synthesized from DPA monomer in pH 7.0 PBS scanned between −1.2 and +1.8 V for 20 cycles was selected for determination of CLN in tablets, human urine, and blood serum samples.

3.2. Modified Electrode Surface Characteristics. The electrode surface was characterized using cyclic voltammetry and an electrochemical impedance spectroscopy technique using $Fe(CN)_6)^{3-/4-}$ as a probe.

3.2.1. Electrochemical Impedance Spectroscopy. Figure 2 presents Nyquist plots of the bare GCE (curve a) and

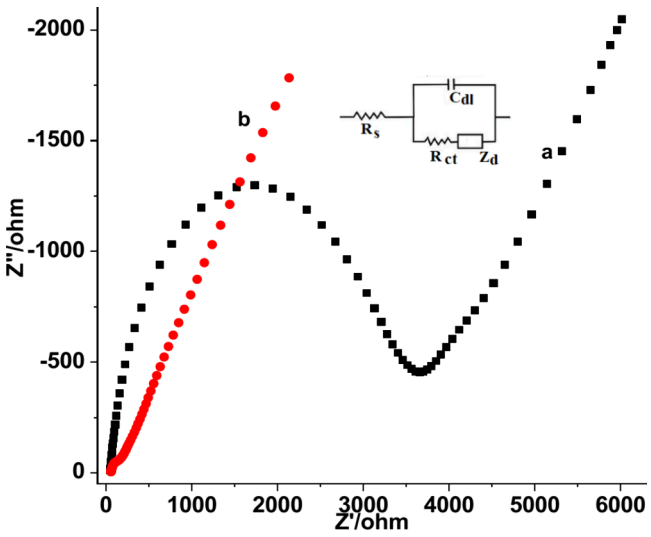

Figure 2. Nyquist plot for (a) bare GCE and (b) poly(DPA)/GCE in pH 7.0 PBS containing 10.0 mM $Fe(CN)_6^{3-/4-}$ and 0.1 M KCl recorded at a frequency range of 0.01−100 000 Hz, potential of 0.23 V, and amplitude of 0.01 V. Inset: proposed equivalent circuit.

poly(DPA)/GCE (curve b) in pH 7.0 PBS containing $Fe(CN)_6^{3-/4-}$ as a probe. The poly(DPA)/GCE (curve b) showed a relatively small diameter semicircle in comparison to the bare electrode (curve a of Figure 2), suggesting that the electrode surface was changed with an electroactive polymer coating to increase the surface conductivity.

Table SM1 presents values for selected circuit elements (R_{ct}, R_s, and C_{dl}) for the two electrodes calculated using eq 1.[18,27] The lower R_{ct} value (112.8 Ω) of poly(DPA)/GCE than the unmodified electrode (4068.7 Ω), which confirms modification of the electrode surface by a more conductive material, might be explained by a reduced overpotential or improved reversibility of the probe, hence a faster electron transfer rate between CLN and the substrate, which could be speculated to be due to the conductive nature of the polymer film.

$$C_{dl} = \frac{1}{2\pi R_{ct} f} \tag{1}$$

where C_{dl} is double layer capacitance, f is frequency corresponding to the maximum imaginary impedance value on the Nyquist plot, and R_{ct} is charge transfer resistance, given by the diameter of the semicircle in the high-frequency region.

The apparent heterogeneous electron transfer rate constant (k^0) and electrode surface roughness (R_F) of poly(DPA) could be calculated using eqs 2 and 3.[14]

$$k^0 = \frac{RT}{F^2 A C R_{ct}} \tag{2}$$

$$R_F = \frac{C_{dl}}{C_S} \tag{3}$$

where A is the surface area, R_{ct} is charge transfer resistance of the electrode, C is the concentration of $[Fe(CN)_6]^{3-/4-}$ (10.0 mM), R is the molar gas constant, T is temperature (298 K), F is the Faraday constant, and C_{dl}, and C_S are the electrochemical double-layer capacitance measured under comparable con-

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ditions from a planar and smooth electrode surface of the same material, respectively.

The k^0 values obtained for the poly(DPA)/GCE are 11 times higher than the bare GCE (Figure 2), clearly indicating that the electrochemical activity of the redox probe is improved on the poly(DPA)/GCE (Table S1). From the slopes of unmodified and poly(DPA)/GCE (Figure S2), a roughness factor of 3.3 is found for the poly(DPA)/GCE compared with the bare GCE. Therefore, it is clear that the DPA-modified electrode has the potential to be used as a very sensitive electrochemical sensor, particularly for CLN.

3.2.2. Cyclic Voltammetry. Figure 3 shows the cyclic voltammetric response of $Fe(CN)_6^{3-/4-}$ at unmodified and

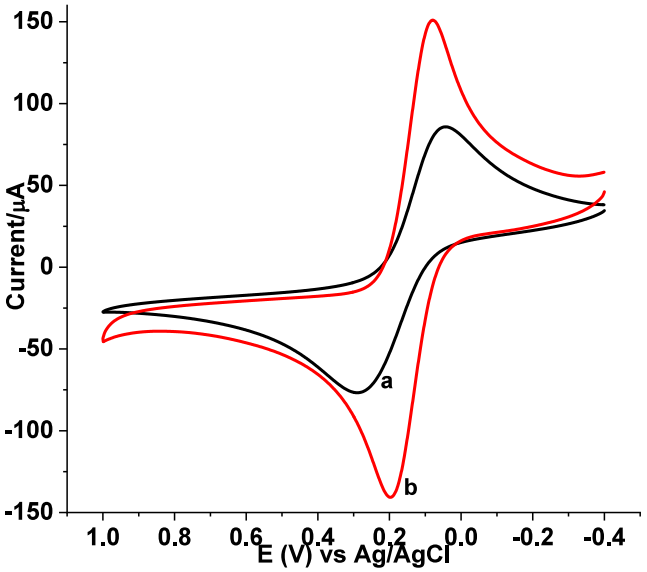

Figure 3. CVs of (a) GCE and (b) poly(DPA)/GCE in pH 7.0 PBS containing 10.0 mM $(Fe(CN)_6)^{3-/4-}$ and 0.1 M KCl at a scan rate of 100 mV s^{-1}.

modified GCEs. The poly(DPA)-modified GCE showed improved peak current intensity and lower peak−peak potential separation than bare GCE, indicating a catalytic property of the modifier toward the probe.

Whereas the conductivity results from the EIS investigation (Figure 2) are consistent with the lowering of peak-to-peak potential separation and improvement of I_{pc}/I_{pa} ratio, the observed peak current enhancement could be the consequence of an increase in the modified electrode's effective surface area (Figure S2). To investigate the effect of surface modification on the electrode surface area, cyclic voltammograms of $Fe(CN)_6^{3-/4-}$ at both the unmodified and poly(DPA)-modified GCEs (Figure S2) were recorded at various scan rates. The active surface area of unmodified and poly(DPA)-modified GCEs was calculated from the slope value of the plot of the peak current versus the square root of the scan rate in the Randles−Sevcik equation (eq 4).[27]

$$I_{pa} = 2.69 \times 10^5 n^{3/2} A D^{1/2} \nu^{1/2} C_o \quad (4)$$

where I_{pa} is the anodic peak current, n is the number of electrons transferred, A is the active surface area of the electrode, D is the diffusion coefficient of $Fe(CN)_6^{3-/4-}$, ν is the scan rate, and C_o is the bulk concentration of the probe.

A 3.3-fold increase in the effective surface area of poly(DPA)/GCE (0.280 cm^2) over that of the unmodified GCE (0.0854 cm^2) indicated that the electrode surface had been changed by the conductive polymer coating, increasing the effective surface area. Thus, it can be deduced that the enhanced active surface area of the electrode is responsible for the peak current enhancement seen for the probe in Figure S2 at the poly(DPA)/GCE.

3.3. Cyclic Voltammetric Investigation of CLN. *3.3.1. Electrochemical Behavior of CLN at a Poly(DPA)-Modified GCE.* Figure 4 illustrates cyclic voltammograms of 1.0

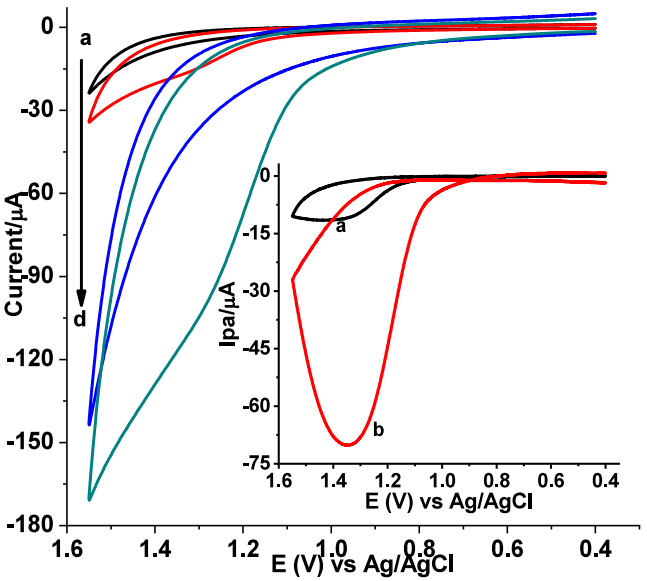

Figure 4. CVs of bare GCE (a and c) and poly(DPA)/GCE (b and d) in pH 7.0 PBS containing no (a and b) and 1.0 mM CLN (c and d) at a scan rate of 100 mV s^{-1}. Inset: blank-subtracted CVs of (a) bare and (b) poly(DPA)/GCE.

mM CLN at a bare GCE and poly(DPA)/GCE in pH 7.0 PBS. The appearance of a well-defined irreversible oxidative peak with more than 6-fold current enhancement at much reduced potential (curve b of inset) at poly(DPA)/GCE confirmed the catalytic performance of the poly(DPA) film toward oxidation of CLN. Moreover, the observed current and potential advantages verified the suitability of the poly(DPA)/GCE for determination of CLN in real samples.

3.3.2. Scan Rate Effect. The scan rate of CLN was studied to investigate the type of reaction kinetics during the oxidation of CLN and the reversibility of the oxidation reaction. Figure 5A illustrates voltammograms of poly(DPA)/GCE in PBS of pH 6.5 containing 1.0 mM CLN in the scan rate range 10 to 300 mV s^{-1}. The resulting peak potential shift of CLN in the positive direction with increasing scan rate proved the irreversibility of the oxidation of CLN at poly(DPA)/GCE (Figure 5A). Moreover, a better correlation coefficient for the peak current dependence on the square root of the scan rate (R^2 = 0.99688) (Figure 5C) than on the scan rate (R^2 = 0.97041) (Figure 5B) indicated that the oxidation of CLN at poly(DPA)/GCE is predominantly governed by a diffusion-controlled process.[10,28] The slope value of 0.53 for the plot of $\log(I_p)$ versus $\log(\nu)$ further confirmed the diffusion mass transport kinetics of the oxidation of CLN at the poly(DPA)/GCE (Figure 5D), which is close to the ideal value of 0.50 for a completely diffusion-controlled reaction.[27]

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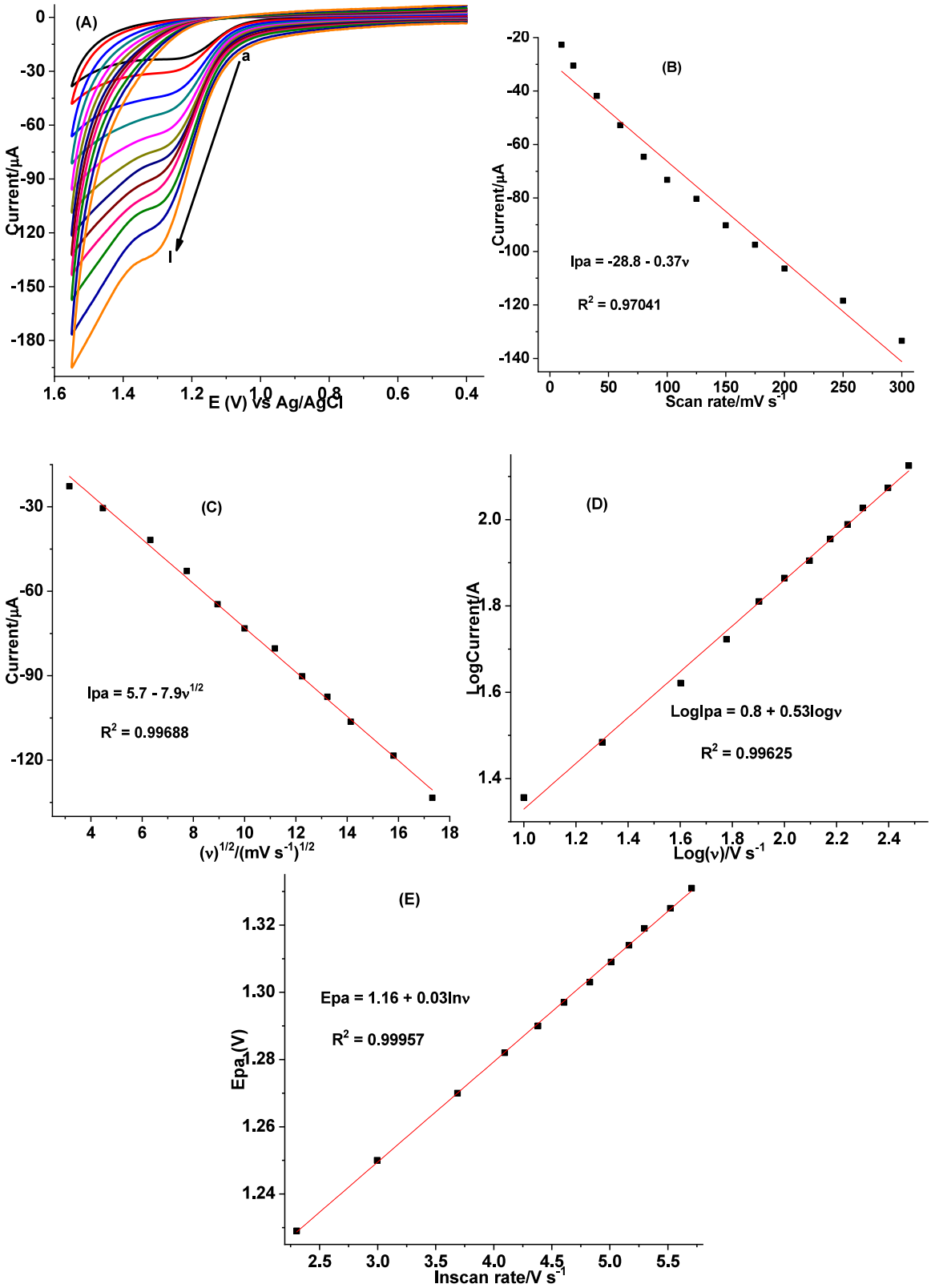

Figure 5. (A) CVs of poly(DPA)/GCE in pH 6.5 PBS containing 1.0 mM CLN at various scan rates (a–l: 10, 20, 40, 60, 80, 100, 125, 150, 175, 200, 250, and 300 mV s^{-1}, respectively); plot of (B) I_{pa} vs ν, (C) I_{pa} vs $\nu^{1/2}$, (D) log I_{pa} vs log ν, and (E) E_p vs ln ν.

The number of electrons involved (n) and the electron transfer coefficient (α) for oxidation of CLN at the poly(DPA)/GCE were determined from the data obtained from cyclic voltammograms of CLN at a scan rate of 100 mV s^{-1} (Figure 5A) and calculated using eqs 5 and 6).[27]

$$E_p - E_{p1/2} = \frac{47.7}{\alpha n} \quad (5)$$

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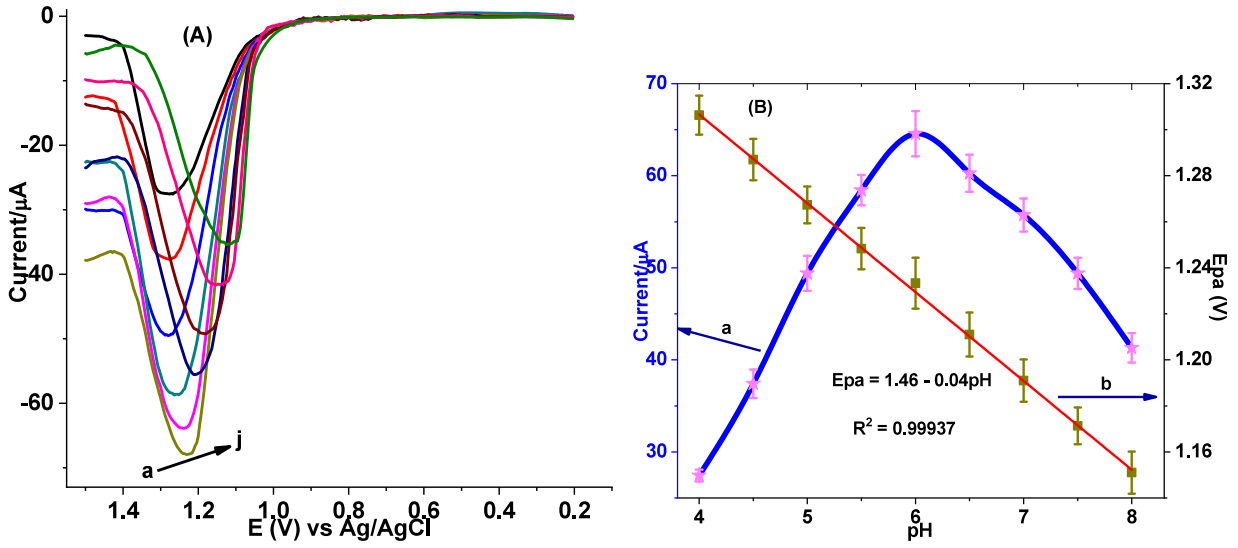
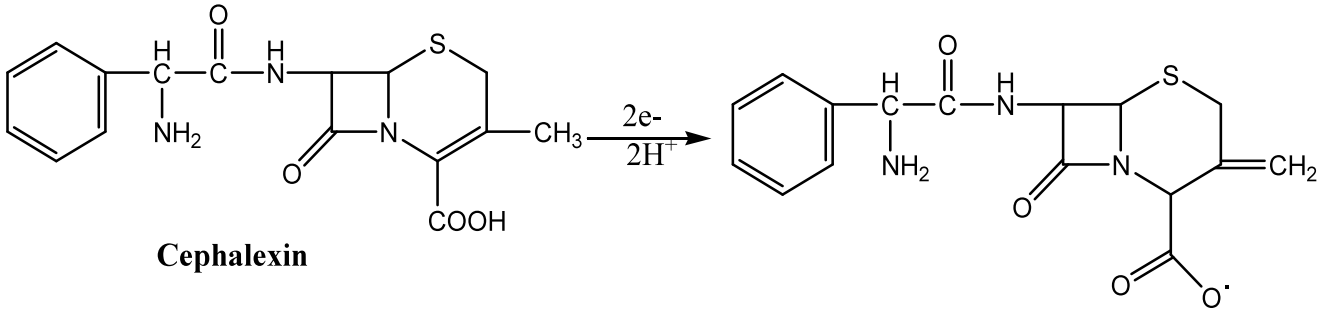


Figure 6. (A) Blank-corrected SWVs of poly(DPA)/GCE in PBS of various pHs (a−i: 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5, respectively) containing 1.0 mM CLN and (B) plot of (mean ± %RSD) CLN (a) I_{pa} vs pH and (b) E_{pa} vs pH in the entire pH range.

Scheme 2. Proposed Oxidative Reaction Mechanism of CLN



$$E_p = E^\circ + \frac{RT}{(1-\alpha)nF}\left\{0.780 + \ln\left(\frac{D_R^{1/2}}{k^\circ}\right) + \ln\left[\frac{(1-\alpha)nF\nu}{RT}\right]^{1/2}\right\} \quad (6)$$

where E_p is the peak potential, E° is the formal potential, α is the electron transfer coefficient, k° is electrochemical rate constant, T is the experimental temperature (298 K), F is Faraday's constant, R is the gas constant, ν is the scan rate, and D is the diffusion coefficient.

E_p and $E_{p1/2}$ for the CV taken at a scan rate of 100 mV s^{-1} were 1272 and 1218 mV, respectively. Accordingly, the value of αn was calculated from eq 5 as 0.88. Considering α for a totally irreversible electrode process to be 0.50,[29] the number of electrons involved during the electrochemical oxidation of CLN at the poly(DPA)/GCE surface was estimated to be 1.76 (~2.0), which is in line with the reported literature.[10,15]

Moreover, from the slope of the plot of E_p versus $\ln(\nu)$ for CLN (Figure 5E) (slope = $0.013/n(1-\alpha) = 0.03$), the value of $n(1-\alpha)$ at the experimental temperature of 25 °C calculated using eq 6 was 0.43. Taking the two electrons for oxidation of CLN calculated using eq 5, the electron transfer coefficient (α) was estimated to be 0.785, confirming the irreversibility of the oxidation of CLN at the surface of poly(DPA)/GCE.[10,18,27]

3.3.3. pH Study. The influence of the pH of PBS on the oxidation peak current and peak potential response of poly(DPA)/GCE over the pH range 4.0−8.5 helps to evaluate the proton:electron ratio in the reaction and involvement of a proton in the oxidation of CLN. Figure 6A presents cyclic voltammograms of poly(DPA)/GCE in various pH values of PBS containing 1.0 mM CLN. The observed peak potential shift following the pH variation from 4.0 to 8.5 in the negative direction indicated proton participation during the oxidation of CLN at the poly(DPA)/GCE. The CLN oxidative peak current at the poly(DPA)/GCE surface was observed to increase following a pH change from 4.0 to 6.5, which then decreased at pH values beyond 6.5 (curve a of Figure 6B), making pH 6.5 the optimum.

This trend might be ascribed to the Coulombic forces of interaction exhibited between the modifier DPA (pK_a 2.81)[30] and CLN (pK_a 2.56 and 6.88).[31−33] The lower current at low pH values was attributed to the strong repulsive forces between protonated CLN and the poly(DPA) film. The current increment at pH 6.5 was due to strong electrostatic attraction of the cationic charged CLN with an increasing negatively charged poly(DPA). However, above pH 6.5 the current decreased rapidly, indicating a strong electrostatic repulsion between deprotonated CLN with the negatively charged poly(DPA) film.

Furthermore, the peak potential dependence on the pH for CLN oxidation at poly(DPA)/GCE was investigated. The slope value of 0.04 V, which is near the Nernstian value of 0.059 V/pH (at 25 °C), for a plot of oxidative peak potential versus pH of the

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PBS (curve b of Figure 6B) showed involvement of an equal number of electrons and protons in CLN oxidation.[34]

Based on the calculated kinetic parameters (n and α) and proton:electron ratio (1:1), a reaction mechanism was proposed (Scheme 2), which is in agreement with the literature.[10,15]

3.4. SWV Determination of CLN at Poly(DPA)/GCE. Square wave voltammetry was used for CLN quantification in tablet formulation and human urine samples due to its superior sensitivity, low detection limits, and capacity to differentiate across background currents.[13,14,28] The electrocatalytic effect of the poly(DPA) polymer film toward CLN oxidation is indicated by the peak current obtained at the poly(DPA)/GCE, as shown in Figure 7, being more than six times higher than the peak current recorded at the bare GCE with an overpotential decrease ($\Delta E \sim 113$ mV).

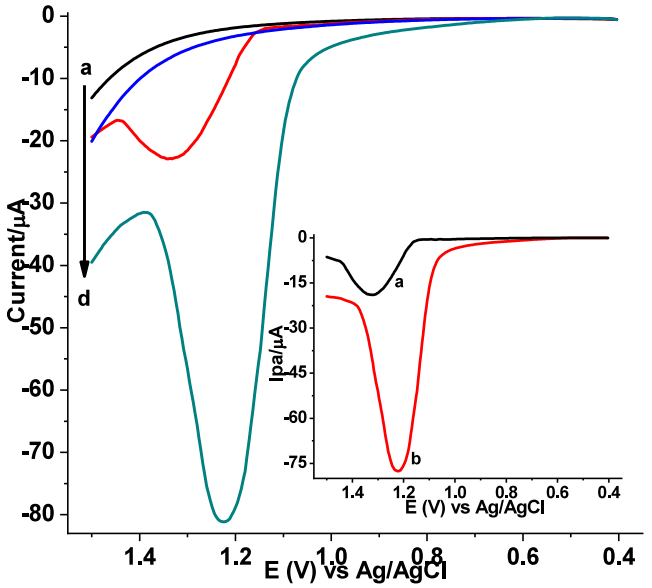

Figure 7. SWVs of unmodified GCE (a and c) and poly(DPA)/GCE (b and d) in pH 6.5 PBS containing no CLN (a and b) and 1.0 mM CLN (c and d).

SWV parameters such as step potential, frequency and square wave amplitude were optimized maintaining the others unchanged. The SWVs of poly(DPA)/GCE in pH 6.5 PBS with 1.0 mM CLN are shown in Figure S3(A,C) at different step potentials, amplitudes, and frequency, respectively. The ideal step potential, amplitude, and square wave frequency were determined to be 8 mV, 35 mV, and 20 Hz, respectively, by surrendering both peak current augmentation and peak shape broadening.

3.5. Calibration Curve. SWV was used to record the oxidative peak current dependence on various CLN concentrations under optimum solution and technique conditions. Background-adjusted SWVs at different CLN doses in pH 6.5 PBS at the poly(DPA)/GCE are displayed in Figure 8. As shown from the inset of the figure, the oxidative peak current showed a linear relationship with CLN concentration in the range 0.05−300.0 μM with correlation coefficient, limit of detection (LoD = $3s/m$), and limit of quantification (LoQ = $10s/m$) of 0.99948, 2.1 nM, and 7.1 nM, respectively, where s is standard deviation of the blank ($n = 7$) and m is the slope of the regression equation. RSD values below 3.6%, associated with triplicate current

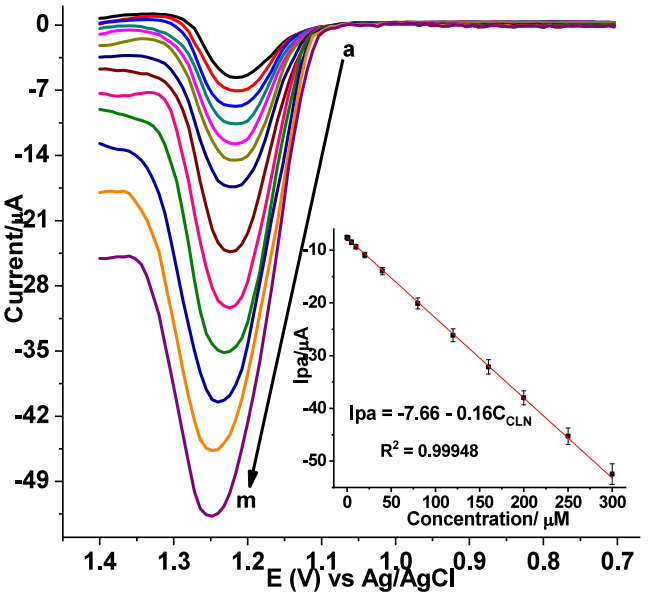

Figure 8. Blank-corrected SWVs of poly(DPA)/GCE in pH 6.5 PBS for different concentrations of CLN (a−m: 0.05, 0.5, 1.0, 5.0, 10.0, 20.0, 40.0, 80.0, 120.0, 160.0, 200.0, 250.0, and 300.0 μM, respectively) at step potential: 8 mV, amplitude: 35 mV, and frequency: 20 Hz. Inset: plot of I_{pa} (mean ± %RSD as error bar) vs concentration of CLN.

measurement for the standard CLN solutions, showed the precision of the method.

3.6. Implementing the Method to Determine CLN in Actual Samples. The efficacy of the developed method for estimating CLN content using the poly(DPA)/GCE was tested on urine samples and four different brands of pharmaceutical tablets.

3.6.1. Tablet Samples. Tablet samples of four brands (Cephast, Felexin, Salexin, and Cephalex) were prepared in accordance with the protocol provided for the experimental part to determine the CLN content in each tablet sample. Additionally, the amount found was contrasted with the tablet manufacturer's stated CLN level. The SWVs of CLN tablet samples with nominal concentrations of 20.0 and 40.0 μM for each tablet brand are displayed in Figure S4. Table 1 provides an overview of the measured CLN content in the tablet samples based on the calibration regression equation calculation and a comparison with the nominal values. The precision of the established technique and the proximity of the detected levels to the expected quantity were proved by the detected CLN amount, which was within the range of 97.00−100.00% and had a percentage RSD value of less than 3.5%. Consequently, the devised approach could be successfully used in pharmaceutical formulations to determine the CLN.

3.6.2. Human Urine Sample. The developed sensor was also used to measure CLN in human urine samples that were prepared according to the protocol outlined in the Materials and Methods section. The existence of a peak (Figure 9a′) at a potential that is different from CLN's characteristic potential, which was allocated for creatinine, proved that CLN was absent from the urine sample that was analyzed.[14]

3.7. Method Validation. The developed method for CLN determination utilizing poly(DPA)/GCE has been verified in real samples, such as tablet and urine samples, through the use of spiked recovery, interference, and stability experiments.

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Table 1. An Overview of the Percentage of Identified CLN Content across Four Brands of Tablets and a Comparison of the Detected Amounts to the Nominal Values

Tablet brand	Labeled value (mg/tablet)	Nominal amount in sample (μM)	Detected drug in		Detected %
			sample (μM)[a]	tablet (mg/tablet)	
Cephast	500	20.0	19.63 ± 0.025	490.75	98.15
		40.0	39.63 ± 0.028	495.40	99.08
Felexin	500	20.0	20.00 ± 0.017	500.00	100.00
		40.0	40.00 ± 0.020	500.00	100.00
Salexin	500	20.0	19.40 ± 0.030	485.00	97.00
		40.0	39.25 ± 0.035	490.63	98.13
Cephalex	500	20.0	19.87 ± 0.023	496.75	99.35
		40.0	39.75 ± 0.021	496.88	99.40

[a]Detected mean CLN ± %RSD.

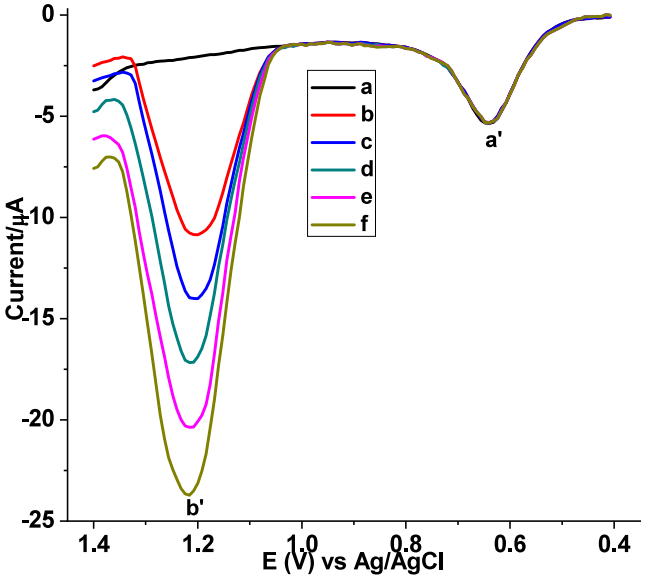

Figure 9. Background-corrected SWVs of poly(DPA)/GCE in pH 6.5 PBS containing (a) unspiked urine sample, (b) a + 20.0 μM standard CLN, (c) a + 40.0 μM standard CLN, (d) a + 60.0 μM standard CLN, (e) a + 80.0 μM standard CLN, and (f) a + 100.0 μM standard CLN.

3.7.1. Spike Recovery Test. 3.7.1.1. Human Urine Sample. By spiking the previously analyzed human urine sample with several CLN standard solutions, it was possible to recover CLN from the human urine sample (Figure 9). Regardless of the amount of spiked CLN, the urine sample showed a peak centered at a potential that was away from the characteristic potential for CLN (peak a′) with precisely constant current intensity, proving that the peak is not for CLN. The peak in the unspiked urine is not for CLN, as demonstrated by the appearance of new peaks (peak b′) at the typical potential of CLN, whose current intensity rose with the amount of spiked CLN to the urine samples (curves b−f). The obtained percent recoveries in the range 99.05−100.00% (Table S2) confirmed the reliability of the developed sensor.

3.7.1.2. Tablet Samples. To assess the accuracy of the new poly(DPA)/GCE technique, recovery studies were conducted for spiked standard CLN in tablet sample solutions. Standard CLN solutions containing 0.0, 20.0, 40.0, and 60.0 μM were added to the previously examined Felexin and Salexin tablet sample solutions in order to perform the recovery investigation (Figure S5). Substantial recovery values were obtained in the range of 98.00% to 101.42% (Table S3), indicating that the devised method can be used successfully for the determination of CLN in pharmaceutical tablet formulations.

3.7.2. Interference Study. The choice of poly(DPA)/GCE for determining CLN in the presence of interfering chemicals, such as ampicillin (AMP), amoxicillin (AMX), cefadroxil (CDL), and cloxacillin (CLOX), was investigated using SWV measurements. In the interference studies, drugs that could be present in the CLN tablet or have structural similarities to CLN and commonly administered together with CLN were selected.

At different concentrations (0−80.0 μM), the effects of each interferent were investigated (Figure S6). The selectivity and accuracy of the suggested approach for the specific determination of CLN were verified by the detection of CLN with an associated error of less than 4.05%, even in the presence of potential interferents at their various levels (Table S4).

3.7.3. Repeatability and Stability Studies. The stability of the modifier and the reproducibility of the results were demonstrated by the current response of poly(DPA)/GCE for 1.0 mM CLN in pH 6.5 PBS, which showed an error of only 1.86% (%RSD) for five consecutive SWV data taken at regular intervals in 2 h in a day (Figure S7A) and an error of under 3.10% for six SWV measurements in 30 days taken at durations of 5 days (Figure S7B). The established technique for determining CLN in actual samples was generally justified by the precision, accuracy, selectivity, reproducibility of the results, and stability of the innovative electrode modification.

3.8. Evaluating the Created Sensor against Published Methods. The developed sensor's performance was compared

Table 2. Efficiency of the New Approach Compared to Recently Published, Specified Works for CLN Detection

Electrode	Technique	Dynamic range (μM)	LoD (μM)	Ref
poly(reso)/GCE	SWV	0.1−300.0	0.0031	10
HgE	Polarographic	0.1−25.0	0.05	15
MPTS-MWCNT/GCE	DPV	0.5−50.0	0.12	35
NiONPs/CPE	Amperometric	2.5−35.0 and 65.0−1230.0	1.3	36
poly(DPA)/GCE	SWV	0.05−300	0.0021	This work

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to previously published ones in order to determine the CLN while taking the substrate's nature, linear dynamic range, limit of detection, and modifier type into account.

Compared with the other reported approaches, the current method, which uses a chip modifier and a straightforward modification process, demonstrated the smallest detection limits and the widest linear dynamic range (Table 2).

4. CONCLUSIONS

The poly(DPA)-modified GCE's synthesis was confirmed by the use of CV and electrochemical impedance spectroscopy. Superior electrocatalytic performance of poly(DPA)/GCE in the oxidation of CLN was possibly because of the improved effective surface area, electrical conductivity, and surface roughness of the electrode. For the purpose of determining CLN in pharmaceutical formulations and urine samples, the poly(DPA)/GCE-based SWV approach was employed. In accordance with the optimum solution and SWV parameters, the tablet samples under examination had a CLN content ranging from 97.0% to 100.00% of their allocated values, demonstrating the precision and accuracy of the devised method. The method reliability was demonstrated by the excellent spike and interference recovery outcomes in both types of real samples. The current approach employing poly(DPA)/GCE demonstrated impressive electrochemical benefits, proving the viability of the developed approach. These benefits included a broader linear concentration range, a lower limit of detection, outstanding spike and interference recovery results, and excellent stability and reproducibility of the newly prepared sensor. Given this, the current approach could make a good candidate to quantify CLN in a variety of real samples with complex matrices.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c04730.

> Cyclic voltammograms of CLN at various polymerization scan cycles, cyclic voltammograms of $Fe(CN)_6^{3-/4-}$, tables of calculated circuit elements for the studied electrodes; optimization of square-wave voltammetry parameters; SWV of CLN in tablets; spike recovery study in real samples (urine and tablets); percent interference study; and stability of the sensor (PDF)

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