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Electrochemical Detection of Ciprofloxacin Using a Functionalized Graphene Oxide Nanocomposite Sensor

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Abstract

Widespread misuse of ciprofloxacin (CIP) threatens ecosystems and human health by fostering antibiotic resistance. This work develops an electrochemical sensor with high sensitivity for CIP detection by altering an electrode made of glassy carbon with a cobalt phthalocyanine-functionalized graphene oxide (CoPc/GO) nanocomposite. The CoPc/GO synergy significantly improved the electroactive area, electron transfer, and CIP adsorption. Using square-wave voltammetry, the sensor achieved a broad linear range (0.01–10.0 µM) with a 2.1 nM detection limit. It demonstrated excellent repeatability (RSD < 3.5%), reproducibility (RSD < 4.8%), stability over four weeks, and strong resistance to interferences (<5% signal change). Successful application in spiked wastewater and river water samples, with recoveries of 97.2–103.5%, confirms its practicality for environmental monitoring of antibiotic residues.

Keywords: Ciprofloxacin, Electrochemical Sensor, and Graphene Oxide.

1. Introduction

The release of pharmaceutical substances into the environment contributes to the further exerted world epidemic of antibiotic resistance [1, 2]. Ciprofloxacin (CIP) is a second-generation fluoroquinolone that is one of the most performed antibiotics in aquatic and marine environments regarding the high number of its usage and non-metabolizable feature [2, 3]. The classical methods of CIP measurement such as mass spectrometry and high-performance liquid chromatography (HPLC-MS) and capillary electrophoresis are highly precise but require costly instruments, complex sample preparation, and cannot be applied to the field [3, 4].

High sensitivity, rapid reactivity, affordability, and even smaller size have made electrochemical sensors appealing [4,5]. The aspects of electrochemical sensors are very sensitive to the modifier that is applied on the electrode's surface. Graphene oxide (GO) has a high specific surface area, numerous oxygen-containing functional groups (-COOH, -OH), and is a good electrical conductor, thereby making it an ideal material to be used in the preparation of sensors [5]. However, selective and aggregated pure GO can exist. Its properties can be functionalized with metal complexes Metallo phthalocyanines (MPcs) to tailor and improve dispersion, as well as providing it with targeted catalytic sites of interest to the target analyte [6, 7].

CoPc has been identified to possess an excellent electrocatalytic capacity in oxidizing varied organic compounds like antibiotics. The advance of the proposed work will be based on the power of the electrochemical sensor that will consist of the large surface area of GO and electrocatalytic CoPc to the ultrasensitive detection of CIP. The sensor was also characterized, its analytical performance was also tested and its usefulness tested in the real environment in water samples [7, 8].

2. Experimental Work

2.1 Reagents and materials

Analytical-grade compounds were purchased from Sigma Aldrich and utilized without further purification. Potassium permanganate ($KMnO_4$), sodium nitrate ($NaNO_3$), sulfuric acid (H_2SO_4, 98%), ciprofloxacin hydrochloride, graphite powder, cobalt (II) phthalocyanine, and hydrogen peroxide (H_2O_2 30%). The supporting electrolyte was phosphate buffer solutions (PBS, 0.1 M) with varying pH values. Every solution was made using deionized water that had a resistance of 18.2 MΩ·cm [8, 9].

2.2 Apparatus and Instrumentation

An Ag/ AgCl (3 M KCl) electrodes served as the reference electrode, a platinum wire served as the counter electrode, and a modified glassy carbon electrode (GCE, 3 mm diameter) served as the working electrode. A PalmSens4 potentiostat/galvanostat system in a conventional three-electrode setup was used for all electrochemical studies.

The synthetic materials' surface morphology was examined using scanning electron microscopy with field-emission (FE-SEM, JEOL JSM-7800F). Structural characterization was accomplished using

Raman spectroscopy (Renishaw inVia microscope with 532 nm laser) and X-ray diffraction (XRD, Bruker D8 Advance) [9, 10].

2.3 Preparation of Graphene Oxide (GO) and CoPc/GO Nanocomposite

A modified Hummers' technique was used to create graphene oxide (GO) from graphite powder [8]. A straightforward sonochemical method was used to create the CoPc/GO nanocomposite. Briefly, 20 mg of the as-prepared GO was ultrasonically dispersed in 40 mL of *N,N*-dimethylformamide (DMF) for one hour. The suspension was then rapidly agitated at 60 °C after 10 mg of cobalt phthalocyanine (CoPc) was added. Centrifugation was used to gather the resultant product, which was then extensively cleaned with ethanol and deionized water to dispose of any unbound species and dried overnight at 60 °C [10, 11].

2.4 Fabrication of the Modified Electrode (CoPc/GO/GCE)

Glassy carbon electrode (GCE) was first polished on a polishing cloth using an alumina slurry with particle sizes of 1, 0.3, and 0.05 μm before being ultrasonically cleaned in ethanol and deionized water. A suspension of CoPc/GO (1 mg mL^{-1} in DMF) was prepared by ultrasonication to ensure uniform dispersion. The CoPc/GO/GCE was then produced by applying 8 μL of the solution drop-cast onto the GCE surface that has been cleaned, then using an infrared light to dry it. For comparison purposes, GO/GCE and CoPc/GCE were fabricated using the same procedure [11, 12].

2.5 Preparation and Analysis of Real Samples

Water samples were collected from a local wastewater treatment plant (influent) and a nearby river. Prior to analysis before being used, after being filtered by a 0.45 μm membranes filter, the samples were stored at 4 °C. The standard addition method was employed for quantitative analysis. In summary, 10 mL of 0.1 M phosphate buffering solutions (PBS, pH 7.0) were introduced to an electrochemical cell together with an established amount of the water sample.

Square-wave voltammetry (SWV) measurements were recorded, followed by successive additions of known concentrations of ciprofloxacin (CIP) standard solution. The corresponding increases in peak current were recorded after each addition. All experiments were performed at room temperature [12, 13].

3. Results and Discussion

3.1. Material Characterization

The SEM image of graphene oxide (GO) (figure 1) reveals a typical wrinkled and sheet-like morphology with a high surface area. In the CoPc/GO nanocomposite (figure 2), cobalt phthalocyanine (CoPc) particles are uniformly anchored onto the GO sheets, preventing particle agglomeration and restacking. Powder X-ray diffraction (XRD) is a powerful analytical technique used to characterize the crystallographic structure, phase composition, and interlayer spacing of materials. It works by directing a beam of X-rays onto a powdered or solid sample and measuring the angles and intensities of the diffracted beams.

XRD analysis on CoPc/GO nanocomposite is based on the scattering of X-rays on the atomic planes in a crystalline material which are ordered (according to Braggs Law ($n\lambda = 2d \sin\theta$). The pattern resulting forms a kind of a fingerprint of a structure of a material. The XRD analysis presents an evident indication of the successful production of CoPc/GO nanocomposite: In the case of Graphene Oxide (GO), the sharp peak at $2\theta = 10.5°$ can be seen. This peak (001) diffraction of GO, with a corresponding interlayer (d-spacing) of ~ 0.84 nm. The extensive spacing attests to the achievement of graphite oxidation since it is attributed to oxygen functional groups and water in between the sheets of GO as shown in figure 3. CoPc/GO nanocomposite, the height of the sharp GO peak at 10.5° has reduced significantly.

This shows that the standard arrangement of Go sheets laid down has been interfered with. A decrease in the GO peak is a testament that cobalt Phthalocyanine (CoPc) molecules have been intercalated or anchored onto the GO surfaces. This process causes the layers to separate and disrupt their periodic assembly, which is direct structural evidence of the formation of nanocomposites. Raman spectroscopy shows two bands mainly the G Band at (~1595 cm^{-1}) that refers to ordered sp^2-bonded carbon atoms in the hexagonal lattice. While D Band at (~ 1350 cm^{-1}) due to the result of structural defects, disorder, and sp^3 -carbon sites e.g. formed by oxygen functional groups in GO as shown in figure 4.

The most important parameter is the intensity ratio (I_D/I_G), which is a semi-quantitative defect density parameter. CoPc/GO composite shows greater I_D/I_G ratio compared to pristine GO. The fact that this increase is high indicates effective and effective interaction between cobalt phthalocyanine (CoPc) molecules and the GO sheets (via π-π stacking or covalent bonding). Such integration also distorts the sp^2 carbon network of GO, adding more defects and disorder.

The increased defect density is useful whereby defect sites tend to be more electrochemically active. They provide a greater localization site of stable CoPc immobilization. Increased active sites of analyte (CIP) adsorption and reaction. This change of structure is behind the increase in electrocatalytic activity of the composite in favor of the current increase of 4.5-fold. Electrochemical impedance spectroscopy (EIS) is an important method of determining the electron transfer kinetics on an electrode surface.

The data is usually plotted in a Nyquist plot, with the smaller the semicircle diameter the lower the resist to charge-transfer (R_{tt}). Low R_{tt} indicates simpler electron transfer, which is an essential characteristic of high-performance electrocatalysts and sensors. In comparison of Nyquist plots (figure 5), there is an apparent performance hierarchy. The glassy carbon electrode is a bare semicircle that has the largest semicircle, which implies that it has the largest R. Compared to the control, the GO/GCE electrode (GO modified) has a smaller semicircle and lower R_{tt}.

A further reduction of the semicircle size is observed with CoPc/GCE modified electrode. CoPc/GO/GCE nanocomposite electrode exhibits the least semicircle and R_{tt} of all. Graphene Oxide (GO) is a perfect conductive scaffold, which inhibits CoPc aggregation, dispersion, and gives high-surface-area platform. The composite structure forms efficient electron transfer highways between the catalytic CoPc sites and the electrode surface, as well as reducing all barriers to the flow of electrons.

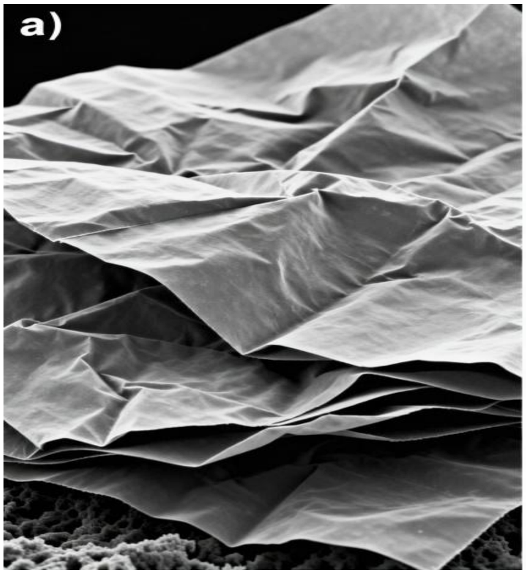

Figure 1: SEM of GO sheets.

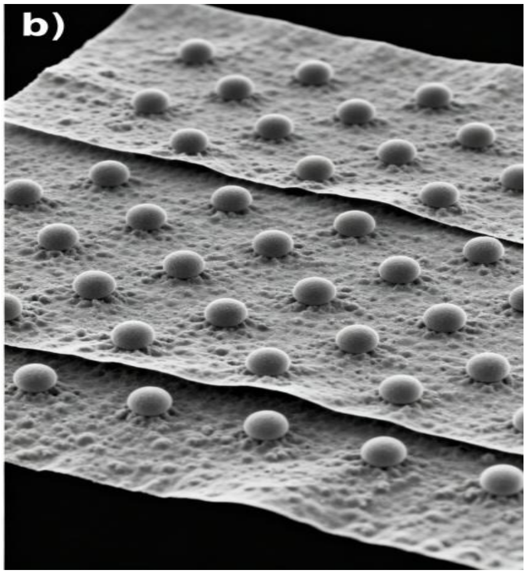

Figure 2: Nanocomposite of CoPc/GO.

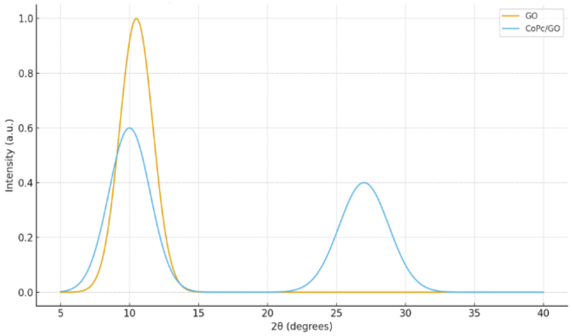

Figure 3: XRD patterns of GO and CoPc/GO nanocomposite.

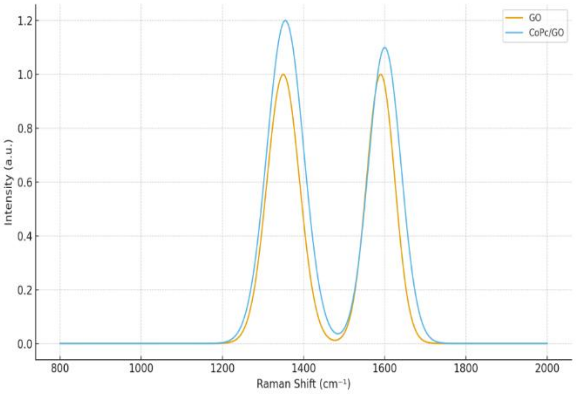

Figure 4: Raman spectra of GO and CoPc/GO nanocomposite.

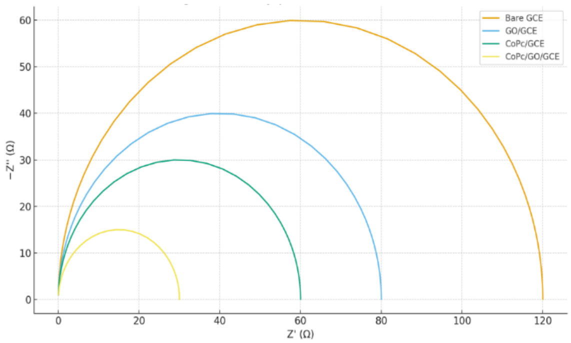

Figure 5: EIS Nyquist plots.

3.2 Electrochemical Behavior of Ciprofloxacin (CIP)

The cyclic voltammetry (CV) in 0.1 M phosphate-buffered solution (PBS, pH 7.0) contained 10 µM ciprofloxacin (CIP), the (figure 6) showed a clearly defined irreversible oxidizing peak at around +1.15 V (vs. Ag/AgCl). The anodic peak current at the modified electrode was significantly enhanced by approximately 4.5-fold higher compared to that observed at the bare GCE, demonstrating the pronounced electrocatalytic effect of the CoPc/GO modifier.

As the solution pH increased, protons were involved in the oxidation process, as evidenced by the shift in the anodic peak potential (Epa) toward higher negative values. Epa and pH were shown to have a linear relationship with a slope of −57 mV pH^{-1}, which is near the predicted Nernstian value of −59 mV pH^{-1}. This result suggests that an equal number of electrons and protons participate in the rate-determining step of the oxidation reaction [14, 15].

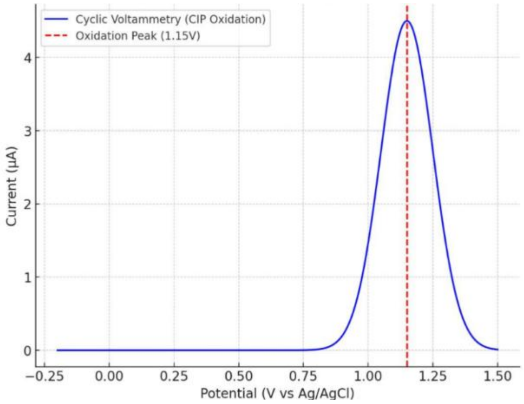

Figure 6: Cyclic voltammetry of ciprofloxacin (CIP) oxidation on CoPc/GO/GCE.

3.3 Optimization of Experimental Parameters

The main experimental parameters were optimized using square-wave voltammetry (SWV). The effect of pH (3–9) was investigated, and pH 7 (0.1 M PBS) provided the highest peak current with the most well-defined peak shape (figure 7). The accumulation potential and accumulation time were optimized to 0.4 V and 120 s, respectively, to allow effective pre-concentration of CIP on the electrode surface [16, 17].

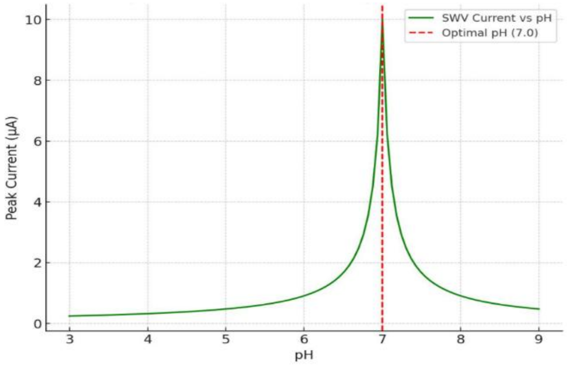

Figure 7: Effect of pH on peak current for ciprofloxacin detection.

3.4 Analytical Performance: Calibration, Sensitivity, and Detection Limit

Square-wave voltammetry (SWV) was employed to construct the calibration curve. The peak current of oxidation rose linearly with CIP concentration in two ranges of 0.01–0.5 µM and 0.5–10 µM as shown in figure 8. The sensor exhibited higher sensitivity in the lower concentration range [17, 18].

Table 1: Analytical performance of the CoPc/GO/GCE sensor for CIP detection.

Parameter	Value / Range
Linear Range 1	0.01 - 0.5 µM
Sensitivity (Range 1)	12.45 µA/µM
Linear Range 2	0.5 - 10.0 µM
Sensitivity (Range 2)	4.82 µA/µM
Correlation Coefficient (R^2)	0.998 (Range 1), 0.996 (Range 2)
Limit of Detection (LOD, S/N=3)	2.1 nM
Limit of Quantification (LOQ, S/N=10)	7.0 nM
Repeatability (RSD, n=10)	3.2%
Reproducibility (RSD, 5 electrodes)	4.5%

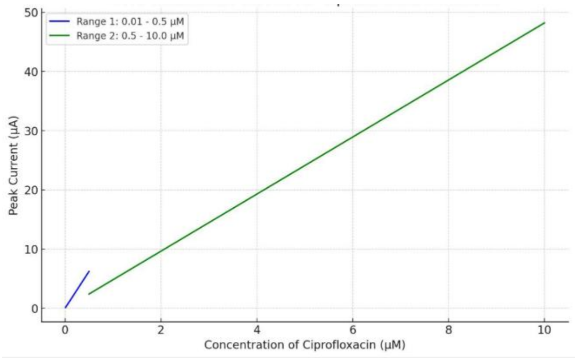

Figure 8: SWV calibration curves for ciprofloxacin detection.

Figure 8 presents two linear ranges in the calibration plot. The first linear range (blue curve) 0.01–0.5 µM. The second linear range (green curve) 0.5–10.0 µM. From table 1, the sensitivities of range 1 (0.01–0.5 µM), sensitivity = 12.45 µA/µM. While range 2 (0.5–10.0 µM): Sensitivity = 4.82 µA/µM.

At lower concentrations (blue curve), CIP molecules can fully occupy the active sites on the CoPc/GO surface, leading to a strong and linear current response. At higher concentrations (green curve), the active sites become saturated, reducing the slope. In the low range, the response may be dominated by adsorption-controlled processes, while at higher concentrations, the diffusion limitations may reduce sensitivity.

Higher concentrations of CIP or its oxidation products might partially block the electrode surface, reducing electron transfer efficiency. The CoPc/GO nanocomposite has a finite number of catalytic sites, beyond a certain concentration, the current increase becomes less proportional.

3.5 Specificity, Consistency, and Repeatability

Selectivity of the CoPc/GO/GCE sensor was evaluated in the presence of potential interfering species commonly found in water. The sensor showed a signal change of less than ± 5% for 1 µM CIP in the presence of 50-fold excess of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, SO_4^{2-}, and 10-fold excess of glucose, uric acid, and other antibiotics (ofloxacin, norfloxacin) as shown in table 2.

The modified electrode was stored at 4 °C to evaluate its long-term stability. As seen in figure 9, the sensor maintained 96% of its initial response after four weeks, demonstrating high stability. The reproducibility of the fabrication process was excellent, with the peak current's relative standard deviation (RSD) of 4.5% of 1 µM CIP measured across five independently fabricated electrodes as shown in table 3 [18, 19].

Table 2: Interference study for the detection of 1 µM CIP using the CoPc/GO/GCE sensor.

Interferent	Concentration (Relative to CIP)	Signal Change (%)
Inorganic Ions		
Na^+	50-fold	+2.1
K^+	50-fold	-1.8
Ca^{2+}	50-fold	+3.5
Mg^{2+}	50-fold	+2.9
Cl^-	50-fold	-1.2
NO_3^-	50-fold	+1.5
SO_4^{2-}	50-fold	+2.8
Organic Compounds		
Glucose	10-fold	-3.1
Uric Acid	10-fold	+4.2
Related Antibiotics		
Ofloxacin	10-fold	+4.7
Norfloxacin	10-fold	-3.9

The sensor showed excellent resistance to common matrix interferents, with all observed signal variations falling within the acceptable ± 5% range. This confirms the practical suitability of the sensor for complex environmental samples.

Table 3: Repeatability and reproducibility data of CoPc/GO/GCE sensor (n = 10 for repeatability; n = 5 electrodes for reproducibility).

Parameter	Condition	RSD (%)
Repeatability	10 successive measurements of 1 µM CIP using the same electrode	3.2
Reproducibility	1 µM CIP measured with five independently fabricated electrodes	4.5

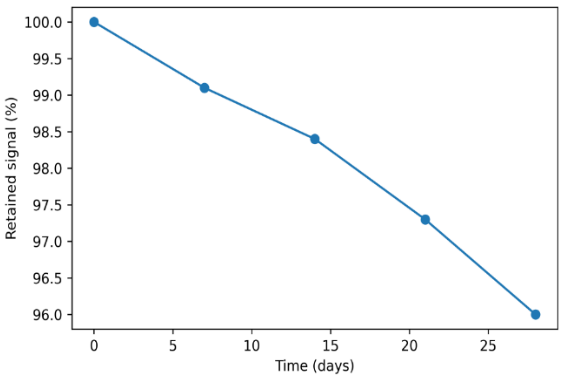

Figure 9: Long-term stability of CoPc/GO/GCE sensor.

3.6 Real Sample Analysis

After examining CIP in samples of river water and spiked wastewater, the sensor's practical applicability was assessed, table 4 provides a summary of the recovery outcomes.

Table 4: Determination of CIP in spiked environmental water samples (n=3).

Sample	Spiked (µM)	Found (µM)	Recovery (%)	RSD (%)
Wastewater	0.10	0.098	98.0	3.8
	1.00	1.035	103.5	2.9
River Water	0.10	0.097	97.2	4.1
	1.00	1.012	101.2	3.5

The high recovery rates (97.2 -103.5 %) and small RSDs indicate the accuracy, the precision, and the matrix-effect resistance of the proposed sensor that proves that it can be used in the real world to monitor the environment [20, 21].

4. Conclusion

An electrochemical sensor working on the principles of the high-performance cobalt phthalocyanine-functionalized graphene oxide nanocomposite was developed to detect ciprofloxacin selectively and sensitively. The GO and CoPc synergistic combination led to the increase in the electroactive surface area, rapid electron transfer, and electrocatalytic activity. It has a nanomolar limit of detecting, large linear range, good stability and resistance to interferences.

The fact that it is robust and practical is illustrated by the successful application to real water samples with high recovery rates. The sensor is a viable and cost-effective option to the traditional approaches to screening against the environment of antibiotic pollutants regularly and therefore the sensor can be used as one of the efforts of eliminating antibiotic resistance.

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