AN ELECTROCHEMICAL SENSOR FOR THE DETECTION OF CLINDAMYCIN HYDROCHLORIDE

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ABSTRACT A novel electroanalytical method has been developed using a cost-effective modified Screen-Printed Electrode (SPE) using Multi-Wall Carbon nano tubes (MWCNTs) that can detect Clindamycin Hydrochloride (CLD). The optimization was conducted by Differential Pulse Voltammetry (DPV), a method based on electrochemical oxidation, encompassing a range linearly spanning 1.4 μM till 3 mM. The oxidation peak was found at +0.6 V. Despite the presence of common molecules such as glucose, sucrose, calcium carbonate, magnesium sulfate, ammonium sulfate, sodium nitrate, urea, dopamine and other antibiotics, the sensor demonstrated high selectivity and did not interfere with them. The study extended the applicability of the sensor to diverse samples, including food, water, and environmental samples, showcasing excellent recovery of Clindamycin. The sensor demonstrated commendable repeatability and reproducibility, establishing its utility for both environmental sample analysis and diagnostic purposes. Enhanced sensitivity was achieved through the incorporation of nanomaterials (MWCNTs) onto the screen-printed electrodes, enabling specific detection of the antibiotic even in the presence of interfering molecules. This advancement opens avenues for real-time monitoring of antibiotics in chemical, pharmaceutical, animal, and wastewater samples, showcasing the potential of electrochemical sensors in diverse fields.

KEYWORDS Screen printed electrode; Clindamycin Hydrochloride; Multi-walled carbon nano tubes; Differential Pulse Voltammetry; Electrochemical sensor; Nanomaterials

I. INTRODUCTION

Antibiotics, utilized for treating human ailments and extensively employed in veterinary medicine for prevention and enhancing animal growth, fall into diverse antibiotic classes, including nitroimidazoles, sulfonamides, tetracyclines, macrolides, fluoroquinolones and β-lactams [1]. Inadequate usage and improper disposal of these medications lead to the buildup of residues in the environment, giving rise to significant health issues, including cardiac arrhythmia, nausea, organ toxicity and allergic reactions [2]. Global antibiotic use in animals exceeds that in human medicine, presenting potential health risks to consumers because of the existence of antimicrobial residues in animal-derived food products [3]. Pharmaceutical residues, primarily entering the aquatic environment through post-use excretions, improper disposal of expired drugs, and waste from production processes, have raised concerns regarding their adverse effects [4]. The indiscriminate use and mismanagement of antibiotics in the 21st century has fueled the rise of antimicrobial resistance posing a significant global health and economic challenge [5]. The poor metabolism and non-biodegradable nature of antibiotics further contribute to the widespread activation and transmission of bacterial resistance genes from the environment to humans [6]. World Health Organization officially recognized antibiotic resistance, a worldwide issue. Addressing these issues requires innovative detection techniques and appropriate surveillance of antibiotic residue accumulation in clinical, livestock, and environmental settings. Clindamycin Hydrochloride, a salt derivative of clindamycin, serves as an antibiotic medication extensively employed in the treatment of diverse bacterial infections. Its hydrochloride form enhances clindamycin's stability and solubility in specific formulations [7]. Effective against a broad spectrum of bacteria, clindamycin finds common use in dealing with respiratory tract infections, infections of the skin and soft tissues, and other bacterial ailments. Various techniques

employed for its detection include spectrophotometry , potentiometry , liquid chromatographic-mass spectroscopy , spectrofluorimetry ,HPLC, and fluorescence quenching [8]. However, these techniques share limitations such as limited sensitivity and involve labor-intensive procedures, including extraction and the use of expensive reagents. For instance, the limit of quantification for the HPLC technique determining CLD concentration in human serum is 0.2 ug/ml [9]. Similarly, the detection and quantification limits for the spectrophotometric technique, measuring CLD at 195 nm, are 80 and 60 nanograms/ml, respectively, within the range of values from 80-6000 nanograms/ml [4]. In recent times, there has been a significant rise in using electrochemical biosensors to create portable, affordable, and highly sensitive analytical devices. Voltammetric detection stands out as a favourable option for sensing Clindamycin (CLD) due to its economic viability, biocompatibility, environmental friendliness, and user-friendly nature [10]. Nevertheless, there is a restricted amount of prior literature on electrochemical studies for determining the antibiotic medication Clindamycin Hydrochloride (CLD).

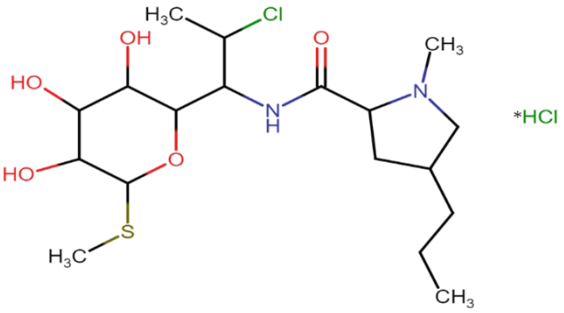

FIGURE 1. Chemical structure of Clindamycin Hydrochloride

II. MATERIALS AND METHODS

A. REAGENTS AND INSTRUMENTATION

The analytical reagent grade was met by all reagents used for the experiments. CLINDAMYCIN HYDROCHLORIDE (CLD) tablets with the brand name BIOCLAN were purchased. Deionized water(DI) was used for making the CLD stock solution. Working standards have been made on a daily basis in supporting electrolyte (0.1 M phosphate buffer). Deionized water (DI) was used for the reagent preparation. The creation of screen-printed electrodes (SPE) involved the utilization of conductive inks comprised of carbon (Code No. 050, Sun Chemical C2030519P4, Siltech Corporation Inc., Bangalore), silver (Code No. 060, Siltech Corporation Inc., Bangalore), and Ag/AgCl paste (CAS No. 7783-90-06, Product Code: NCZ-SPI-104, Nanochemazone, Chemazone Inc., Canada). It was modified using multi-walled carbon nano tubes to enhance its sensitivity. Field-emission Scanning Electron Microscopes (FE-SEM) is the method employed to evaluate the modified SPE's characteristics. Electrochemical characterization of CLD was performed using a DY2300 series Bi-potentiostat from Digi-IVY (Germany).

B. FABRICATION OF SCREEN PRINTED ELECTRODES

Polyethylene terephthalate (PET) sheets underwent a screen-printing process to create electrodes using the standard three-electrode setup, which has electrodes for reference, counter electrodes and working electrodes. The PET sheets were preheated at 100°C for one hour. A silver ink base coating was then screen printed onto the sheets, followed by air-drying for an hour. Subsequently, a carbon layer was printed and dried for an hour at 60°C. Finally, Ag/AgCl ink was applied to the reference carbon track tip and let it to dry at 60°C for an hour. On the working electrode, MWCNTs were printed, and they were dried at 100°C for one hour. These electrodes, termed screen-printed electrodes (SPEs), were successfully fabricated through this process.

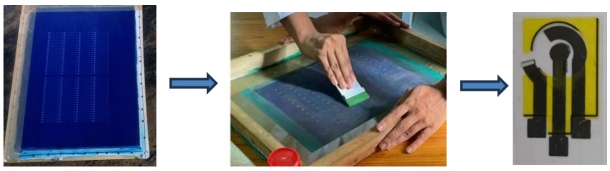

FIGURE 2. Screen Printing Procedure

III. ELECTROCHEMICAL CHARACTERIZATION OF CLD

Phosphate buffer (PB) with a molarity of 0.1 and a pH of 7.4 was utilized for electrochemical characterization of CLD employing electrochemical methods such as Cyclic Voltammetry (CV) as well as DPV. Using a scan rate of 50 mV/s and voltage ranging from 0 to 1 V, CV was performed. DPV was employed to analyze sensitivity, linearity, interference, and real sample data within a voltage window ranging from 0 to 1V. The electrochemical behavior of various interfering molecules, including glucose, sucrose, maltose, ascorbic acid, uric acid, urea, dopamine, and ions present in water samples, as well as commonly administered drugs, were investigated using DPV. Field-Emission Scanning Electron Microscopy (FE-SEM) experiment was conducted at National Institute of Technology, Calicut, Kerala, India. The FE-SEM image (Fig. 3) of the Multi-Walled Carbon Nanotube (MWCNTs) modified Screen Printed Electrode highlights the enhanced surface morphology and increased electroactive surface area.

IV. RESULT AND DISCUSSION

The optimization of experimental conditions was done.

A. IMPACT OF ELECTROLYTE

The electrochemical behaviour of 706 μM CLD with several supporting electrolytes, namely acetate buffer (AB), phosphate buffer (PB) and citrate buffer (CB) were tested using cyclic voltammetry (CV) [11]. Fig. 4 presents the

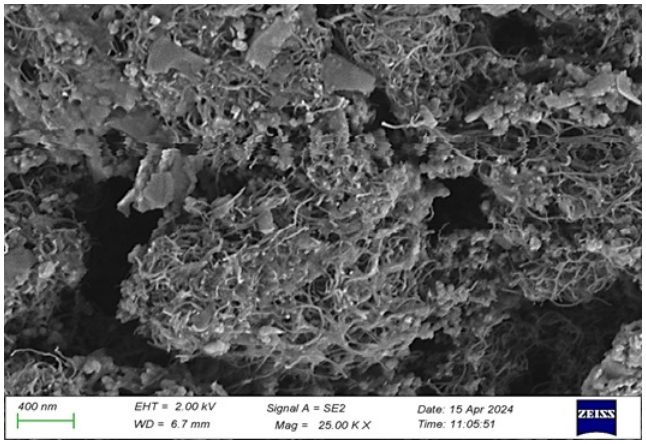

FIGURE 3. FE-SEM image of modified SPE at 25 KX magnification

findings of the CV responses. The data clearly show that phosphate buffer (PB) is the optimal environment for CLD oxidation where the peak anodic potential is observed at 0.6 V. Consequently, all subsequent investigations of CLD were conducted in the optimized supporting electrolyte of phosphate buffer (PB).

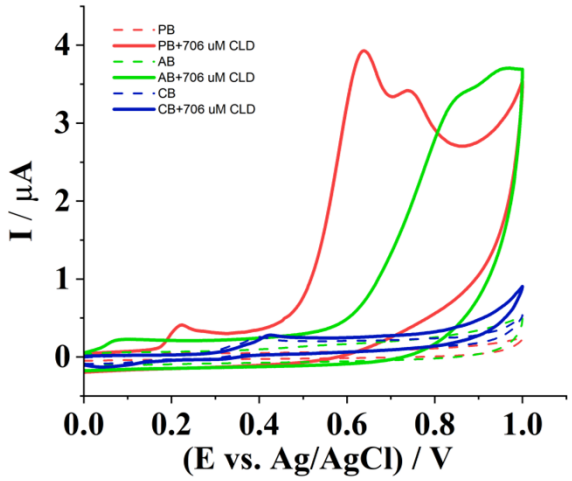

FIGURE 4. Cyclic Voltammograms for 706 μM CLD in supporting electrolytes (CB, AB, and PB)

B. OPTIMISATION OF pH TOWARDS CLD

The electrochemical oxidation behaviour of 706 μM CLD was experimented across a range of pH values in phosphate buffer (PB) spanning from pH 5.4 to 8.4. Fig.5 illustrates the increasing anodic current responses of CLD with increasing pH. As the pH rose, the oxidation potential shifted towards more negative values, and at pH 7.4, a distinct and sharp oxidation peak was observed, accompanied by higher current. Consequently, subsequent investigations of CLD were carried out in PB with a pH of 7.4. The noticeable shift towards a more negative potential for the anodic peak with increasing pH suggests protons' significance in the oxidation process reaction of CLD in PB [12]. The decrease in anodic current response with the rise in pH could be attributed to a scarcity of protons. Optimizing the electrolyte's pH is crucial for precise electrochemical measurements, as pH significantly influences redox potential [13]. Maintaining a specific pH is essential for consistent and reliable results, impacting the surface charge of the electrode, which, in turn, influences biomolecule adsorption and interaction [14]. In our experimentation, various pH buffers (5.4, 6.4, 7.4, 8.4) were tested. The peak currents for the respective pH are 0 μA, 25.8 μA, 39.1 μA and 38.6 μA. This study revealed that a phosphate buffer with a pH of 7.4 provided the optimal conditions, yielding a distinct oxidation peak and increased current for enhanced electrochemical performance.

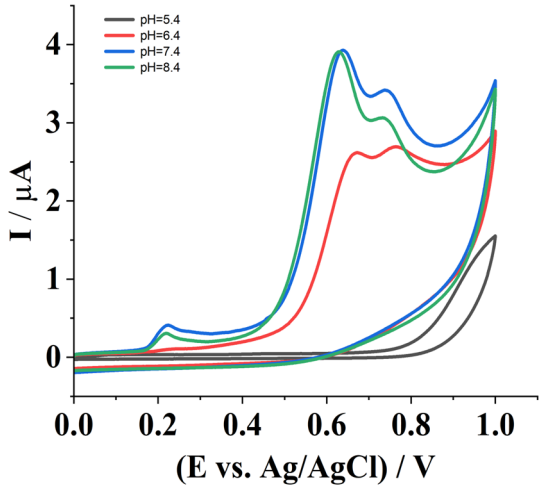

FIGURE 5. Cyclic Voltammograms with 706 μM CLD at different pH of PB

C. OPTIMISATION OF MOLARITY TOWARDS CLD

Optimizing the molarity of the electrolyte is crucial for achieving optimal sensitivity in the electrochemical biosensor. The sensor's response may vary linearly within a specific concentration range, with potential saturation effects at higher concentrations [15]. Through experimentation with varying molarities (0.2, 0.1, and 0.05 M) of phosphate buffer, it was determined that a 0.1 M phosphate buffer provided the optimal conditions for electrochemical characterization, ensuring a balanced and reliable performance of the biosensor [16]. The peak currents obtained for the respective molarities are 64.5 μA, 83.6 μA and 80.5 μA. It is clear from the experimental data and Fig. 6 that PB of 0.1 M is apt for further electrochemical characterization of CLD as it has the highest current intensity and also depicts a defined peak that decreases the chances of interferences.

D. EVALUATION OF RESPONSE TOWARDS SCAN RATE

A solution containing 706 μM CLD in 0.1 M PB was used to investigate the relation between anodic current and scan rate,

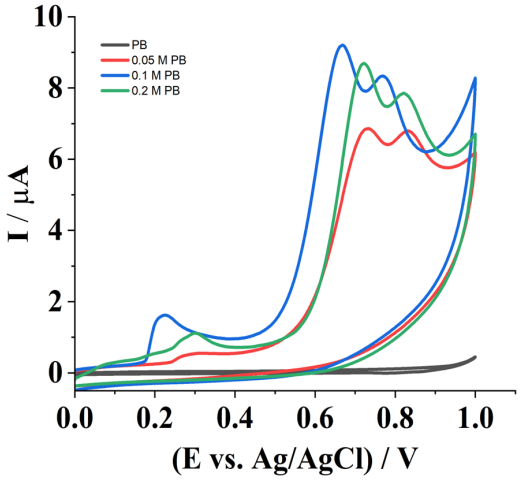

FIGURE 6. Cyclic Voltammograms with 706 μM CLD at different molarities of PB

ν (Vs^{-1}) ranging from 1 to 500 mVs^{-1}. The cyclic voltammogram produced for various scan rates is displayed in Fig. 7. The linear relationship of anodic current was studied with scan rate, Fig. 8, and taking the square root of the rate of scan Fig. 9. High regression coefficient (R^2) obtained from Fig. 8 ($R^2 = 0.998$) evidently proves that the oxidation of CLD on SPE is an adsorption-controlled phenomenon. Fig. 10 demonstrates the anodic peak potential's linear logarithmic dependence on scan rate. One way to determine if diffusion is controlling the process is to examine the fit between $\log[I_p]$ and $\log$[scan rate] slope. An electrode process that is diffusion-controlled has a slope value that is almost equal to 0.5. On the other hand, an adsorption-controlled process's slope is around 1.0 [17]. Using the Laviron equation (1), how many electrons were involved in the oxidation reaction was computed [18].

$$E_{\text{pa}} = E_0 + \frac{2.303RT}{((1-\alpha)nF)} logv \qquad \text{eqn (1)}$$

where
E_0 represents the formal potential in V,
R is the universal gas constant with a value of 8.314 $JK^{-1}mol^{-1}$,
T is the working temperature (298 K),
F is the Faraday's constant having a value of 96485 $Cmol^{-1}$,
v is the scan rate in V/s and α is the transfer coefficient, whose value is 0.5.
E_{pa} was calculated using the calibration equation of the plot peak potential versus scan rate. The calibration equation was observed as:

$$E_{\text{pa}}(V) = 0.06084 logv(V/s) + 0.7664, R^2 = 0.995 \qquad \text{eqn (2)}$$

Equating both the slopes obtained in above equations (1) and (2) shows that 1.51 electrons are involved.

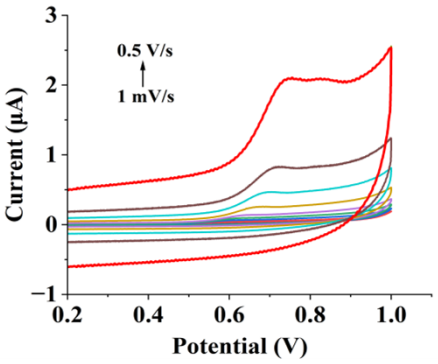

FIGURE 7. Cyclic Voltammograms obtained on SPE with 706 μM CLD in PB, pH 7.4, maintaining a 0.1 molarity at different scan rates

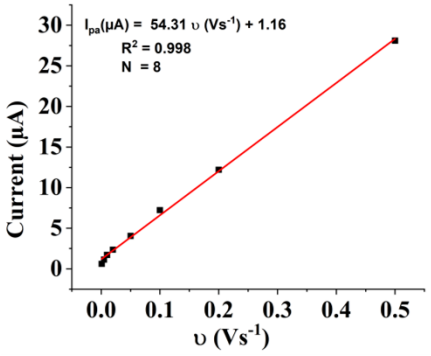

FIGURE 8. Plot of scan rate vs anodic maximum current (Ipa)

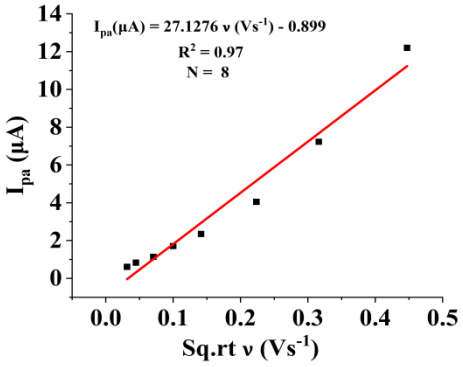

FIGURE 9. Ipa relative to square root of scanning rate

E. LINEARITY STUDIES

The linearity analysis in PB was performed using DPV. The recorded current (Fig. 11 and 12) exhibited a consistent increase as the CLD concentration elevated, particularly noticeable at a potential of 0.6V. This systematic investigation serves to establish the correlation between CLD concentration and resulting current, providing insights into the sensor's

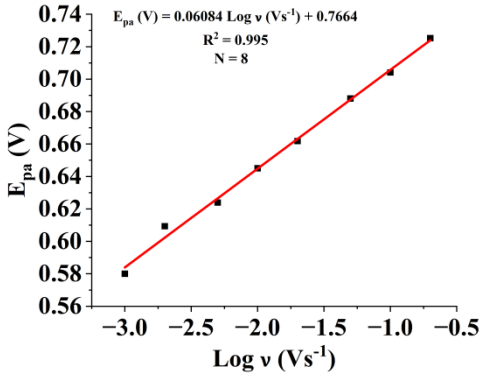

FIGURE 10. anodic maximum potential (Epa) against the logarithm of the rate of scan

sensitivity and response across a spectrum of concentrations [19]. Our SPE modified with MWCNTs exhibited a linear range of 1.4 μM to 3 mM while the bare SPE had a linear range of of 7 μM to 700 μM at a voltage of 0.65 V. The linearity assessment is crucial for gauging the sensor's reliability and accuracy in quantifying CLD concentrations within the specified range, contributing to the overall understanding of its analytical performance.

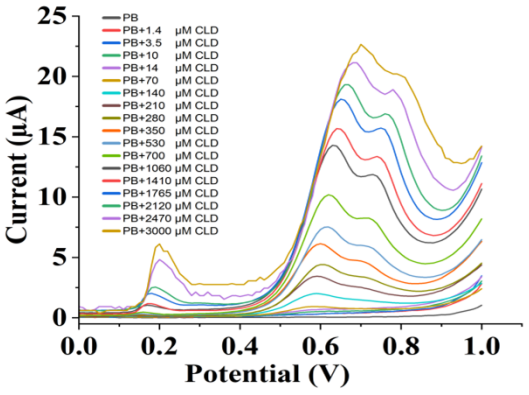

FIGURE 11. Differential voltammograms obtained with CLD at different concentrations on SPE modified with MWCNTs

F. INTERFERENCE STUDIES

The validation of sensor selectivity is of paramount importance. Detecting CLD in real samples may face challenges due to the presence of other electroactive molecules and ions. Therefore, the study assessed the impact of common coexisting species, such as uric acid (0.5 mM), ascorbic acid (0.14 mM), urea (1.31 mM), and common ions like 0.3 mM of Na^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, CO_3^{2-}. Additionally, the study included glucose, maltose, sucrose, and dopamine at the same concentrations, analysed using DPV as demonstrated in Figure 13. To evaluate selectivity, known concentrations of CLD (706 μM) were spiked into solutions containing these biomolecules. In the DPV study, neither the tested molecules and ions nor the common biomolecules exhibited

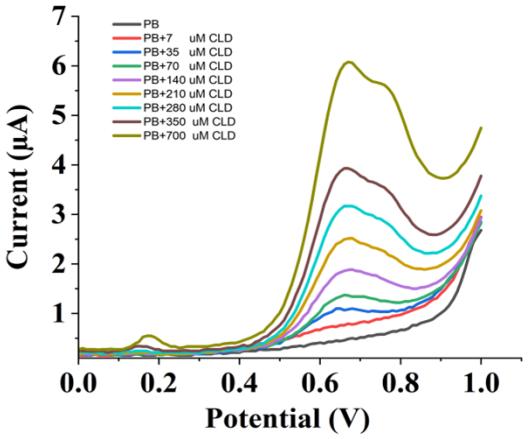

FIGURE 12. Differential voltammograms obtained with CLD at different concentrations on bare SPE

distinct peaks, confirming the high selectivity of the screen-printed electrode (SPE) towards CLD. This high selectivity enhances the accuracy of monitoring CLD in real samples [20]. Additionally, interference from commonly used drugs like Amoxicillin (AMN), Tinidazole (TNZ) and Paracetamol (PRCM) was studied as shown in Fig 14. There was no interference at the 0.6 V point of CLD. This again proved the highly selective nature of our SPE modified with MWCNTs.

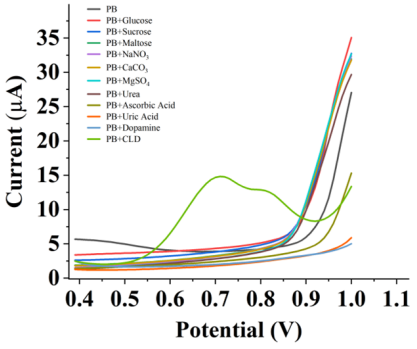

FIGURE 13. Differential pulse voltammograms obtained on SPE in the presence of interfering biomolecules

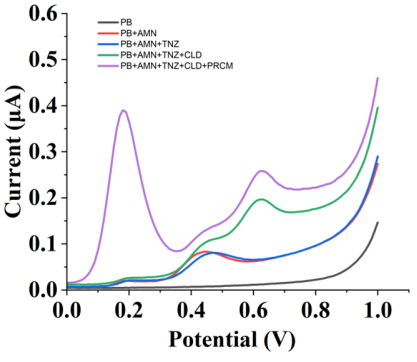

FIGURE 14. Differential pulse voltammograms obtained onSPE when drug molecules are present to cause interference

G. REPEATABILITY AND REPRODUCIBILITY STUDIES

Reproducibility and repeatability were examined by testing a triplicate set of CLD. The electro-oxidation of CLD on ten distinct electrodes was investigated using DPV [21]. The developed sensor exhibited 4.57% as the relative standard deviation (RSD). Replicability was studied on ten runs of CLD on the same electrode after washing the electrode with deionized water (Fig. 15). Results indicated an RSD of 2.38%. Investigation showed that the domestically fabricated SPE offers great reproducibility and repeatability when stored in a sealed condition [22].

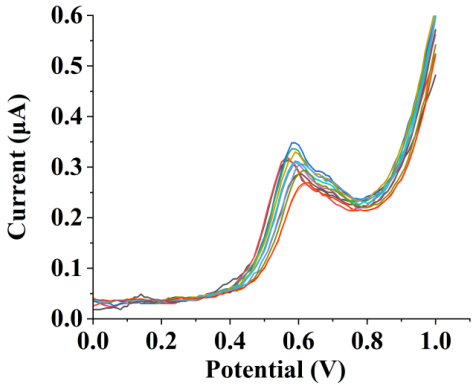

FIGURE 15. Differential pulse voltammograms obtained on SPE on different runs on the same electrode in 0.1 M PB containing 706 μM CLD

V. CONCLUSION

This study successfully attained accurate and precise detection of CLD utilizing an ultrasensitive and cost-effective sensor based on screen-printed electrodes (SPE). The MWCNTs modified sensor's superior performance is evident through its low detection limit, high sensitivity, and expansive dynamic range. Notably, the sensor exhibits high selectivity for CLD, as demonstrated by its ability to detect the compound even when additional antibiotics and common interfering compounds are present. The practical utility of the developed sensor extends to diverse applications, including water and food safety, pharmaceuticals, and diagnostic drug analysis. The sensor's effectiveness in real sample analysis is evident, as it does not necessitate complex pre-treatment processes, and it yields satisfactory recovery results.

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