AN ELECTROCHEMICAL SENSOR FOR THE DETECTION OF CLINDAMYCIN HYDROCHLORIDE

A PROJECT REPORT

Submitted by

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to

the APJ Abdul Kalam Technological University

in partial fulfillment of the requirements for the award of the Degree

of

Bachelor of Technology
in
Biotechnology

Department of Biotechnology
SAHRDAYA COLLEGE OF ENGINEERING AND TECHNOLOGY
KODAKARA, THRISSUR - 680684

MAY 2024

DECLARATION

I, undersigned, hereby declare that the project report "AN ELECTROCHEMICAL SENSOR FOR THE DETECTION OF CLINDAMYCIN HYDROCHLORIDE", submitted for the partial fulfillment of the requirements for the award of the degree of Bachelor of Technology of the APJ Abdul Kalam Technological University, Kerala is bonafide work done by me under the guidance of Dr. Dhanya Gangadharan, Department of Biotechnology. This submission represents my ideas in my own words and where ideas or words of others have been included; I have adequately and accurately cited and referenced the original sources. I also declare that I have adhered to the ethics of academic honesty and integrity and have not misrepresented or fabricated any data or idea or fact or source in my submission. I understand that any violation of the above will be a cause for disciplinary action by the institute and/or the University and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been obtained. This report has not been previously formed the basis for the award of any degree, diploma or similar title of any other University.

VIVINA PUTHUR

Kodakara
07-05-2024

DEPARTMENT OF BIOTECHNOLOGY
SAHRDAYA COLLEGE OF ENGINEERING AND TECHNOLOGY, KODAKARA, THRISSUR

BONAFIDE CERTIFICATE

This is to certify that the project report entitled **AN ELECTROCHEMICAL SENSOR FOR THE DETECTION OF CLINDAMYCIN HYDROCHLORIDE** submitted by **VIVINA PUTHUR (SHR20BT060)** to the APJ Abdul Kalam Technological University in partial fulfillment of the requirements for the award of the Degree of Bachelor of Technology in Biotechnology is a bonafide record of the project work carried out by her under our guidance and supervision. This report in any form has not been submitted to any other University or Institute for any other purpose.

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It was their encouragement that helped me to complete the project. I can hardly find words to express my deep appreciation for the help and warm encouragement that I received from my parents. I am extremely thankful and indebted to my friends who supported me in all aspects of the project.

Vivina Puthur

INSTITUTIONAL VISION

Evolve as a leading technology institute to create high caliber leaders and innovators of global standing with strong ethical values to serve the industry and society.

INSTITUTIONAL MISSION

Provide quality technical education that transforms students to be knowledgeable, skilled, innovative and entrepreneurial professionals. Collaborate with academia and industry around the globe, to strengthen the education and research ecosystem. Practice and promote high standards of professional ethics, good discipline, high integrity and social accountability with a passion for holistic excellence.

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We at Sahrdaya are committed to provide Quality Technical Education through continual improvement and by inculcating Moral & Ethical values to mould into Vibrant Engineers with high Professional Standards.

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DEPARTMENT MISSION

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- Encourage and facilitate research that would enable students to acquire new knowledge that is industrially and socially relevant.
- Train students to be leaders who value professional ethics and realize the need for societal contribution and environmental sustainability in their work.

PROGRAMME EDUCATIONAL OBJECTIVES (PEOS)

PEO1	To be equipped with the engineering skills and technical concepts enabling them to take up careers in Biotechnology and allied fields globally.
PEO2	To instill the passion for lifelong learning and scientific discovery that will allow them to conduct research and innovate technologies keeping them motivated to take up research, entrepreneurial or industrial careers.

PEO3	To inculcate entrepreneurial and techno management skills along with professional and ethical responsibility empowering them to be responsible and socially aware citizens.

PROGRAMME SPECIFIC OBJECTIVES (PSOS)

PSO1	To measure, model and manipulate properties of biological systems at the cellular and molecular level so as to produce sustainable products that address environmental and ethical standards.
PSO2	To formulate and execute quantitative and design- oriented analysis of biological systems with modern tools and techniques that generate new knowledge at the interface of engineering and biology.
PSO3	To transform as socially relevant biological engineers having a professional outlook that would enable them to work as a part of a team in an industrial, research or entrepreneurial set up and sustain a desire for higher learning and research

PROGRAMME OUTCOMES (POS)

PO1	Engineering knowledge: Apply the knowledge of mathematics, science, engineering fundamentals, and an engineering specialization to the solution of complex engineering problems.
PO2	Problem analysis: Identify, formulate, review research literature, and analyze complex engineering problems reaching substantiated conclusions using first principles of mathematics, natural sciences, and engineering sciences.
PO3	Design/development of solutions: Design solutions for complex engineering problems and design system components or processes that meet the specified needs with appropriate consideration for the public health and safety, and the cultural, societal, and environmental considerations.

PO4	Conduct investigations of complex problems: Use research-based knowledge and research methods including design of experiments, analysis and interpretation of data, and synthesis of the information to provide valid conclusions.
PO5	Modern tool usage: Create, select, and apply appropriate techniques, resources, and modern engineering and IT tools including prediction and modeling to complex engineering activities with an understanding of the limitations.
PO6	The engineer and society: Apply reasoning informed by the contextual knowledge to assess societal, health, safety, legal and cultural issues and the consequent responsibilities relevant to the professional engineering practice.
PO7	Environment and sustainability: Understand the impact of the professional engineering solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
PO8	Ethics: Apply ethical principles and commit to professional ethics and responsibilities and norms of the engineering practice.
PO9	Individual and team work: Function effectively as an individual, and as a member or leader in diverse teams, and in multidisciplinary settings.
PO10	Communication: Communicate effectively on complex engineering activities with the engineering community and with society at large, such as, being able to comprehend and write effective reports and design documentation, make effective presentations, and give and receive clear instructions.
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PO12	Life-long learning: Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change.

COURSE OBJECTIVES

- To apply engineering knowledge in practical problem solving.
- To foster innovation in design of products, processes or systems.
- To develop creative thinking in finding viable solutions to engineering problems.

COURSE OUTCOMES

The student will be able to

CO1	Model and solve real world problems by applying knowledge across domains.
CO2	Develop products, processes or technologies for sustainable and socially relevant applications.
CO3	Function effectively as an individual and as a leader in diverse teams and to comprehend and execute designated tasks.
CO4	Plan and execute tasks utilizing available resources within timelines, following ethical and professional norms.
CO5	Identify technology/research gaps and propose innovative/creative solutions.
CO6	Organize and communicate technical and scientific findings effectively in written and oral forms

ABSTRACT

A novel electroanalytical sensor has been developed using a cost-effective Screen-Printed Electrode (SPE) modified with Multi-walled 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 electrodes; Clindamycin Hydrochloride; Multi-walled carbon nano tubes; Differential Pulse Voltammetry; Electrochemical sensor; Nanomaterials*

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LIST OF ABBREVIATIONS

ABBREVIATION	DESCRIPTION
AMR	Antimicrobial Resistance
CB	Citrate Buffer
CLD	Clindamycin Hydrochloride
CPE	Carbon Paste Electrodes
CV	Cyclic Voltammetry
DPV	Differential Pulse Voltammetry
GCE	Glassy Carbon electrodes
MWCNT	Multi Walled Carbon Nano Tubes
PB	Phosphate Buffer
SPE	Screen Printed Electrodes

CHAPTER 1

INTRODUCTION

1.1 GENERAL

Antimicrobial resistance (AMR) poses a growing threat to global health as microorganisms evolve to resist the drugs designed to combat them. This phenomenon, encompassing bacteria, viruses, fungi, and parasites, renders standard treatments ineffective. As a result, common infectious diseases become more challenging to manage, leading to persistent infections, increased healthcare costs, and a heightened risk of disease spread [1]. AMR is driven by the overuse and misuse of antimicrobial drugs in humans, animals, and agriculture. Factors such as incomplete antibiotic courses, inappropriate self-medication, and the use of antibiotics as growth promoters in livestock contribute to the development of resistance [2].

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 beta-lactams. 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 [3]. 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.

Clindamycin Hydrochloride (CLD) is a salt form of clindamycin, which is an antibiotic medication used to treat various bacterial infections. The hydrochloride form enhances the stability and solubility of clindamycin in certain formulations [4]. Clindamycin is effective against a range of bacteria and is commonly used in the treatment of skin and soft tissue infections, respiratory tract infections, and other bacterial infections [2].

[5].

Detection of antibiotics plays a pivotal role in managing resistance. Spectrophotometry techniques, such as UV-Visible spectrophotometry, colorimetric assays, ELISA, fluorescence spectrophotometry, and chromatographic methods like HPLC

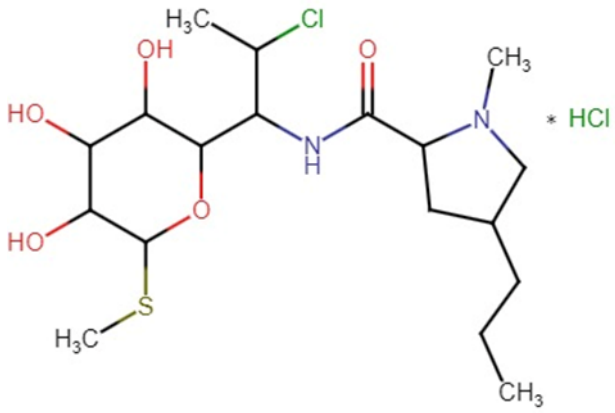

Fig. 1.1: Chemical structure of Clindamycin Hydrochloride

and LC-MS, are essential in monitoring antibiotic concentrations [6]. Despite their versatility, these methods have limitations, including issues of specificity, interference, and sensitivity [7]. 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. 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. Electrochemical sensors, utilizing carbon-based electrodes like Glassy Carbon Electrode (GCE), Carbon Paste Electrode (CPE), and Screen-Printed Electrodes (SPE), offer a promising role in antibiotic detection.

In recent times, there has been a significant rise in using electrochemical biosensors to create portable, affordable, and highly sensitive analytical devices [8]. Voltammetric detection stands out as a favourable option for sensing Clindamycin (CLD) due to its economic viability, biocompatibility, environmental friendliness, and user-friendly nature. Nevertheless, there is a restricted amount of prior literature on electrochemical studies for determining the antibiotic medication Clindamycin Hydrochloride (CLD). SPEs, or screen-printed electrodes, are becoming more and more popular as handheld biosensing devices for in-person and point-of-care detection. These sensors are constructed from a non-conductive, chemically inert substrate over which the required electrodes are printed using screen printing technique [7]. Typically, measurements made for research in fields including healthcare, pharmaceuticals, the food industries, farming, and environment samples employ SPEs [9]. According to sources, biosensors based on screen-printed electrodes deliver precise results that have excellent sensitivity, selectivity. The detection of Clindamycin in SPE based sensors has not been reported, which is a promising area to be explored to construct a simple and sensitive detection device [10]. Furthermore, nanomaterials, such as nanoparticles or nanotubes, promote rapid electron transfer, resulting in quick and reliable

electrochemical responses. By employing nanotechnology in electrochemical sensors, we can develop highly sensitive and selective platforms for antibiotic detection [2]. Multi-walled carbon nanotubes (MWCNTs) have emerged as promising candidates in biosensor technology due to their unique physical, chemical, and electrical properties. These cylindrical nanostructures, composed of rolled-up graphene sheets, possess a high aspect ratio, large surface area, excellent mechanical strength, and remarkable electrical conductivity. These attributes make them ideal for enhancing the performance of biosensors in various applications, including healthcare, environmental monitoring, food safety, and biodefense.

One of the key advantages of incorporating MWCNTs into biosensors is their ability to enhance sensitivity and selectivity. Their large surface area provides ample space for biomolecule immobilization, facilitating efficient capture and detection of target analytes [11]. Additionally, MWCNTs exhibit excellent electrical conductivity, enabling rapid electron transfer during the detection process. This feature improves the signal-to-noise ratio, resulting in enhanced sensitivity and lower detection limits.

CHAPTER 2

LITERATURE REVIEW

2.1 ANTIMICROBIAL RESISTANCE

Antimicrobial resistance (AMR) is a growing global health concern that occurs when microorganisms, such as bacteria, viruses, fungi, and parasites, develop resistance to the drugs designed to kill them. This phenomenon renders standard treatments ineffective, leading to persistent infections, increased healthcare costs, and a higher risk of spread of diseases. Overuse and misuse of antimicrobial drugs in humans, animals, and agriculture contribute to the development of resistance [12]. Incomplete courses of antibiotics, inappropriate self-medication, and the use of antibiotics as growth promoters in livestock are some of the factors driving AMR. Antimicrobial resistance jeopardizes our ability to treat common infectious diseases, making routine medical procedures riskier. Procedures such as surgeries, chemotherapy, and organ transplants become more hazardous when effective antibiotics are unavailable. Antibiotic resistance is a specific type of AMR that occurs when bacteria evolve mechanisms to withstand the effects of antibiotics, rendering these drugs less effective or completely ineffective in treating bacterial infections. This phenomenon poses a significant threat to public health and has far-reaching implications for medical treatments.

2.2 CONVENTIONAL METHODS FOR ANTIBIOTIC DETECTION

Spectrophotometry is a widely used technique in the detection and quantification of antibiotics. This method relies on the measurement of the absorption or transmission of light by a substance, allowing for the analysis of the concentration of antibiotics in a sample [12].

UV-Visible spectrophotometry: It measures the absorbance of ultraviolet or visible light by a sample. Many antibiotics absorb light in the UV-Visible range, and the degree of absorption is directly proportional to their concentration. It is

frequently used to determine the concentration of antibiotics in liquid samples. A calibration curve relating absorbance to concentration is often constructed for accurate quantification [13].

Colorimetric Assays: Some antibiotics can react with specific reagents to produce coloured compounds. The intensity of the colour can be measured spectrophotometrically and correlated with the antibiotic concentration. Colorimetric assays are often used for rapid and sensitive detection of antibiotics, especially in situations where direct measurement of absorbance may be challenging [14].

Enzyme-Linked Immunosorbent Assay (ELISA): ELISA involves the use of antibodies that specifically bind to the antibiotic of interest. Enzymes linked to these antibodies produce a colour change when they react with a substrate, and the intensity of the colour is measured spectrophotometrically. Fluorescence Spectrophotometry: It measures the fluorescence emitted by a substance when exposed to light of a specific wavelength. Some antibiotics exhibit fluorescence, and changes in fluorescence intensity can be used for detection [15].

High-Performance Liquid Chromatography (HPLC) with UV Detection: HPLC is a chromatographic technique that separates components in a mixture. Coupling HPLC with UV detection allows for the identification and quantification of antibiotics based on their absorbance properties [16].

Liquid Chromatography-Mass Spectrometry (LC-MS): LC-MS combines the separation capabilities of liquid chromatography with mass spectrometry for the identification and quantification of compounds, including antibiotics [17]. These spectrophotometric methods are essential in pharmaceutical analysis, clinical diagnostics, and research for monitoring antibiotic concentrations, ensuring quality control, and studying the pharmacokinetics of these drugs. The choice of method depends on factors such as the type of antibiotic, the matrix of the sample, and the required sensitivity and specificity [18].

While spectrophotometry is a powerful and widely used technique, it has some drawbacks and limitations. Here are several factors to consider: limited specificity, sample interference, requires homogeneous samples, limited sensitivity, solubility issues, destructive to samples, limited information on sample composition, limited to absorbing species, instrument sensitivity, cost and complexity. All of these techniques suffer from the shortcomings of limited sensitivity and require laborious procedures like extraction and the use of very costly reagents [7].

2.3 ELECTROCHEMICAL SENSORS

Electrochemical sensors are analytical devices that utilize the principles of electrochemistry to detect and quantify the concentration of a target analyte in a

sample [19]. These sensors typically consist of an electrode or an array of electrodes that facilitate a chemical reaction with the analyte, producing a measurable electrical signal. Electrochemical sensors offer several advantages, including high sensitivity, rapid response times, and the potential for miniaturization. Various types of electrodes are employed as sensors in different applications, each with unique properties and advantages [19]. Carbon-based electrodes are a category of electrodes that are primarily composed of carbon or carbon-containing materials. These electrodes are widely used in electrochemical applications due to their versatility, good electrical conductivity, and ability to be easily modified for specific purposes. Here are some common types of carbon-based electrodes:

1. Glassy Carbon Electrode (GCE): Glassy carbon electrodes are made from a high-temperature heat-treated form of vitreous carbon. GCEs exhibit low background current, good electrical conductivity, and a wide potential window, making them suitable for various electrochemical applications. They are commonly used in electroanalytical chemistry, sensors, and biosensors due to their inert nature and stability [20].
2. Carbon Paste Electrode (CPE): Carbon paste electrodes consist of a mixture of graphite powder and a liquid or solid binder. Highly versatile and easily modifiable, making them suitable for various applications. The paste nature allows for easy shaping to fit specific electrode geometries. They are used in environmental monitoring, pharmaceutical analysis, and biological sensing. Often modified with different materials to enhance selectivity and sensitivity [20].
3. Screen-Printed Electrodes (SPE): Screen-printed electrodes are manufactured by depositing layers of conductive and insulating inks on a substrate, typically made of ceramic or polyester. SPEs are cost-effective, easily mass-produced, and can be customized for specific applications by modifying the ink composition. They are often disposable. They are widely used in point-of-care diagnostics, environmental monitoring, and portable electrochemical devices [21].

2.4 ELECTROANALYTICAL TECHNIQUES

Voltammetry is an electroanalytical technique that measures the current as a function of an applied potential. This method is widely used to investigate the electrochemical behaviour of species in solution. Voltammetry provides valuable information about the redox processes, kinetics, and concentration of electroactive species. There are several variations of voltammetry, each with its own specific advantages and applications [22]. The major types of voltammetry are:

- Cyclic Voltammetry (CV): In cyclic voltammetry, the potential is swept linearly with time, and the resulting current is measured. The potential waveform typically forms a triangular or sawtooth shape. CV is commonly used to study redox processes, determine formal potentials, and investigate the reversibility of electrochemical reactions.
- Linear Sweep Voltammetry (LSV): Similar to cyclic voltammetry, linear sweep voltammetry involves sweeping the potential linearly with time. However, in LSV, the potential is not reversed after reaching a certain point. LSV is often used for rapid determination of analyte concentrations and studying irreversible electrochemical reactions [23].
- Differential Pulse Voltammetry (DPV): DPV involves applying a series of potential pulses to the working electrode with a short interval between pulses. The resulting current response is measured. DPV is widely used for the determination of trace analytes due to its high sensitivity and ability to resolve overlapping peaks in complex mixtures.

Chronoamperometry is a technique used in electrochemistry to study the current as a function of time at a constant applied potential. In this method, a constant potential is applied to an electrochemical cell, and the resulting current is measured over time. This technique is particularly useful for studying electrochemical reactions, determining reaction kinetics, and characterizing electroactive species [22]. The time-dependent current response can provide insights into reaction mechanisms, reaction rates, and the behaviour of species involved in the electrochemical process [14].

2.5 CLINDAMYCIN HYDROCHLORIDE

Clindamycin hydrochloride (HCl) is an antibiotic medication used to treat various bacterial infections. It belongs to the lincosamide class of antibiotics and is effective against a range of gram-positive bacteria. Clindamycin is prescribed to treat various bacterial infections, including skin and soft tissue infections, respiratory tract infections, bone and joint infections, intra-abdominal infections, and certain dental infections. Clindamycin is available in different formulations, including oral capsules, tablets, and topical solutions [23].

Clindamycin interferes with bacterial protein synthesis by binding to the 50S subunit of the bacterial ribosome. This inhibits the growth and multiplication of susceptible bacteria. In some regions, clindamycin is used in animal husbandry for promoting growth in animals. The use of antibiotics in livestock for non-therapeutic purposes can contribute to the development of antibiotic-resistant strains of bacteria. The misuse of clindamycin and other antibiotics contributes to the development of antibiotic-resistant strains of bacteria [23]. This occurs when bacteria evolve to survive exposure

to the antibiotic, making future treatments less effective. Clindamycin, like any medication, can cause side effects. Misuse, such as taking the antibiotic when it is not necessary, increases the risk of side effects. Moreover, allergic reactions can occur, and these may be severe in some cases [5].
[15].

2.6 NANOTECHNOLOGY

Nanotechnology has revolutionized electrochemical sensors, significantly enhancing their capabilities for detecting various analytes. The miniature scale of nanomaterials provides a high surface area, allowing for precise immobilization of biorecognition elements like antibodies or aptamers. This increased surface area enhances the sensor's sensitivity, enabling the detection of even trace amounts of analytes. Furthermore, nanomaterials, such as nanoparticles or nanotubes, promote rapid electron transfer, resulting in quick and reliable electrochemical responses [24]. Detecting antibiotics is of paramount importance due to their widespread use in medicine and agriculture. Their overuse has led to antibiotic residues in the environment, posing a risk to ecosystems and human health. By employing nanotechnology in electrochemical sensors, we can develop highly sensitive and selective platforms for antibiotic detection. This is crucial in monitoring and mitigating the environmental impact of antibiotic contamination, as well as in preventing antibiotic-resistant bacterial strains from emerging, thus safeguarding both human health and ecological balance. The integration of nanotechnology with electrochemical sensors holds the key to addressing these contemporary challenges [19].
Multi-walled carbon nanotubes (MWCNTs) have garnered significant interest in biosensor applications due to their unique properties, including high surface area, excellent electrical conductivity, and biocompatibility [25]. In biosensing, MWCNTs serve as platforms for immobilizing biomolecules, enhancing electron transfer, and improving sensitivity and selectivity of detection. MWCNTs possess a high surface area-to-volume ratio and a large aspect ratio, providing ample sites for biomolecule immobilization. This property allows for enhanced sensitivity by increasing the density of capture molecules on the sensor surface, facilitating efficient recognition of target analytes [26].

CHAPTER 3

MATERIALS AND METHODS

3.1 REAGENTS USED

3.1.1 DRUGS

The drug used is:

- Clindamycin Hydrochloride (CLD)
  Clindamycin hydrochloride of the brand Bioclan is used. Each tablet is 300mg by weight.

Fig. 3.1: Clindamycin Hydrochloride - 300mg Tablet

3.1.2 SOLVENTS

The solvents used are:

- Deionised water
  Deionized water, also known as demineralized water or DI water, is water that has undergone a process to remove ions and minerals. Deionized water is essentially free of ions, including minerals like calcium, magnesium, sodium, and chloride. This absence of ions makes it an excellent solvent for certain applications where the presence of ions could interfere and it has low electrical conductivity. DI water is produced to a high level of purity and it has a neutral pH around 7. Its lack of acidic or basic ions makes it useful in applications

where a neutral environment is necessary. Clindamycin Hydrochloride is dissolved in DI water [27].

3.1.3 ELECTROLYTES

The electrolytes used are:

- Citrate buffer
  Citrate buffer refers to a buffer solution containing citric acid and its conjugate base, sodium citrate. Citrate buffer was prepared by mixing citric acid (usually in the form of citric acid monohydrate) and sodium citrate in a specific ratio. The exact formulation varied depending on the desired pH. The pH of citrate buffer can be adjusted based on the specific requirements of the experiment. Citrate buffers are often used in the pH range of 3.0 to 6.2 [17]. Citrate buffer is known for its buffering capacity, which helps maintain a relatively constant pH even when an acidic or basic substance is added. Prepared citrate buffer solutions are often stored at room temperature, and the pH should be checked before use to ensure its stability [28].

- Phosphate buffer
  Phosphate buffer was prepared by mixing a salt of dihydrogen phosphate (NaH_2PO_4) and a salt of hydrogen phosphate (Na_2HPO_4) in the appropriate ratio to achieve the desired pH. The pH of a phosphate buffer can be adjusted based on the ratio of the two phosphate forms. Phosphate buffers are effective in a pH range of around 6.0 to 8.0. Phosphate buffers exhibit good buffering capacity within their effective pH range, helping to resist changes in pH when acids or bases are added [22].

3.2 INSTRUMENTATION

3.2.1 SCREEN PRINTED ELECTRODES

Screen-printed electrodes (SPEs) are widely used in cyclic voltammetry (CV) due to their versatility, ease of use, and cost-effectiveness. SPEs are often disposable and can be mass-produced using screen-printing technology. This makes them suitable for field applications, rapid analysis, and situations where the convenience of disposable electrodes is beneficial [29].
SPEs can be easily modified with various materials and nanomaterials to enhance their electrochemical properties. SPEs are compact and can be manufactured in small sizes, enabling miniaturized electrochemical devices. This is advantageous

for applications where space is limited, such as in microfluidic systems. The screen-printing process allows for the simultaneous deposition of multiple layers, facilitating the incorporation of working, reference, and counter electrodes on a single substrate. This simplifies electrode fabrication and reduces the need for intricate assembly [30].

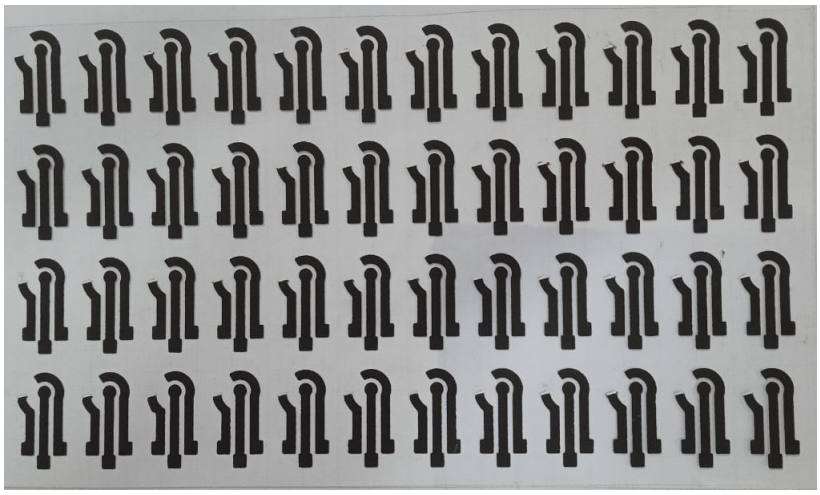

Fig. 3.2: A sheet of Screen Printed Electrodes

3.2.2 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 [31]. 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 allowed to dry for an hour at 60°C. MWCNTs was printed on the working electrode and dried for one hour at 100°C. These electrodes, termed screen-printed electrodes (SPEs), were successfully fabricated through this process [32].

3.2.3 FABRICATION OF NANO MODIFIED SCREEN PRINTED ELECTRODES

A significant advancement in electrode fabrication has been achieved through the incorporation of nanostructures, particularly using multi-walled carbon nanotubes (MWCNTs), into screen-printed electrodes (SPEs). This modification process involves preparing a paste with a ratio of 1:2 MWCNTs to carbon, which is then coated onto the working electrode of the pre-existing SPE. Subsequently, the coated electrode undergoes a drying process in a hot air oven set at 100°C for one hour.

This meticulous procedure enhances the performance characteristics of the electrode, offering improved conductivity, surface area, and electron transfer kinetics [33]. The resulting nano-modified screen-printed electrodes exhibit enhanced sensitivity and selectivity, making them invaluable tools in various electrochemical applications, ranging from environmental monitoring to biomedical diagnostics [34].

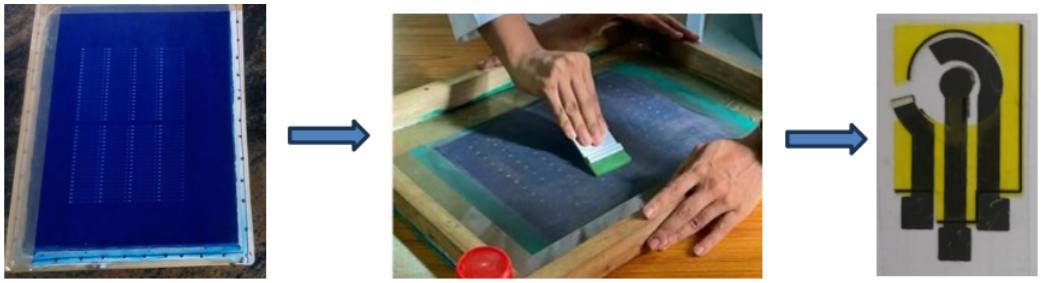

Fig. 3.3: Screen Printing Procedure

3.2.4 POTENTIOSTAT

Using a DY2300 series Bi-potentiostat from Digi-IVY (Germany), electrochemical characterization of CLD was carried out. A potentiostat, such as the DY2300 series, is an instrument in electrochemical analysis, specifically designed to control the potential of an electrode to measure and manipulate electrochemical reactions. It ensures a constant potential between a working electrode and a reference electrode while measuring the resulting current. This precise control allows researchers to investigate the kinetics, mechanisms, and thermodynamics of various electrochemical processes [35]. Potentiostats are widely used in corrosion studies, battery research, sensor development, and other applications where understanding and manipulating electrochemical reactions are essential. They play a key role in advancing scientific research and technological innovations in diverse fields. [7].

3.2.5 SOFTWARES USED

- DY2300:
  The DY2300 is an advanced, portable bi-potentiostat designed for high-performance electrochemical analysis. With two integrated channels, it features low-noise analog components, selectable gain stages, and a 16-bit bias DAC for precise measurements. The user-friendly LabVIEW interface allows for easy experimental setup, data analysis, and file management [36]. Equipped with innovative software and hardware, the DY2300 is suitable for various applications, including rotating ring disk electrode experiments, sub-picoampere

current measurement, and sensor conditioning in scientific research, education, and industry [14].

Fig. 3.4: DY2300 Potentiostat

- OriginPro:
  OriginPro is a scientific graphing and data analysis software developed by OriginLab Corporation. It is commonly used in various scientific and engineering fields for tasks such as data analysis, graphing, and visualization. OriginPro offers a wide range of tools for data analysis, statistics, and curve fitting. Users can perform various mathematical and statistical operations on their data to derive meaningful insights [37]. The software provides powerful graphing tools for creating 2D and 3D graphs, including scatter plots, line graphs, bar charts, contour plots, and more. Users can customize the appearance of graphs and add annotations for clarity [38].

3.3 METHODS

3.3.1 CYCLIC VOLTAMMETRY

Cyclic Voltammetry (CV) is an electrochemical technique used to study the redox behavior of a chemical species. CV involves applying a potential (voltage) to an electrochemical cell and measuring the resulting current. The potential is varied linearly with time, creating a cyclic voltammogram that provides information about the electrochemical reactions occurring at the working electrode. The components of the Electrochemical Cell include

- Working Electrode: The electrode where the electrochemical reactions take place.
- Reference Electrode: A stable electrode with a known and constant potential used as a reference point.
- Counter Electrode: A conductive electrode that completes the electrical circuit.

The potential is swept linearly or stepped between two limits. The direction of

the potential sweep is reversed to complete a cycle [39]. The resulting current is recorded at each potential. The output of a cyclic voltammetry experiment is a cyclic voltammogram, which is a plot of current (y-axis) versus potential (x-axis). Peaks or waves in the voltammogram represent redox processes [7]. The peak Characteristics include

- Peak Potential (Ep): The potential at which the peak current occurs.
- Peak Current (Ip): The maximum current at the peak potential.
- Anodic and Cathodic Peaks: CV typically exhibits both anodic (oxidation) and cathodic (reduction) peaks.

The separation between anodic and cathodic peaks provides information about the reversibility of the redox process. The shape of the voltammogram can yield insights into the kinetics of the electrochemical reaction. CV assumes that the electrode processes are reversible, and deviations can occur in the presence of irreversible reactions or mass transport limitations. The interpretation of complex voltammograms may require additional techniques or theoretical modeling [22].

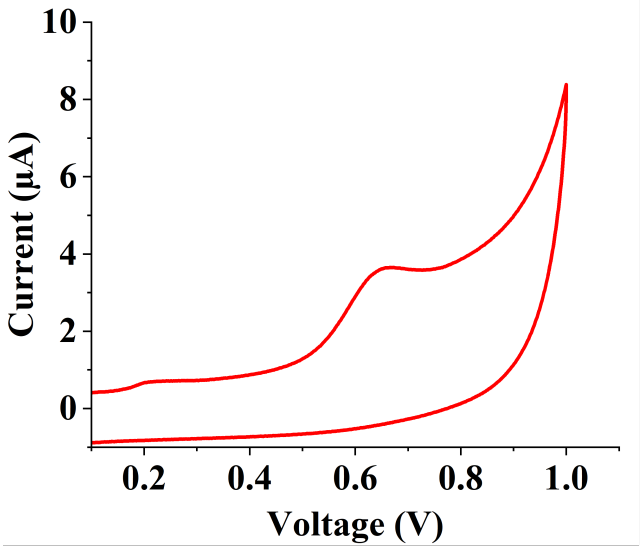

Fig. 3.5: A Cyclic Voltammogram

3.3.2 DIFFERENTIAL PULSE VOLTAMMETRY

Differential Pulse Voltammetry (DPV) is an electrochemical technique used for the quantitative analysis of substances based on their electrochemical behavior. It involves the application of a series of potential pulses to an electrochemical cell, where the current response is measured differentially at the end of each pulse. DPV utilizes discrete voltage pulses superimposed on a linear potential ramp. This allows for enhanced sensitivity and selectivity in detecting electroactive species.

The current is measured differentially, meaning the difference in current between the beginning and the end of each potential pulse is recorded. This helps to

minimize background noise and enhances the detection of analytes. DPV generates characteristic peaks in the resulting voltammogram. The position, height, and width of these peaks provide valuable information about the electrochemical properties of the analyte. DPV is known for its high sensitivity, making it suitable for the detection of trace amounts of substances in a variety of applications, including environmental monitoring, pharmaceutical analysis, and biochemical research [2].

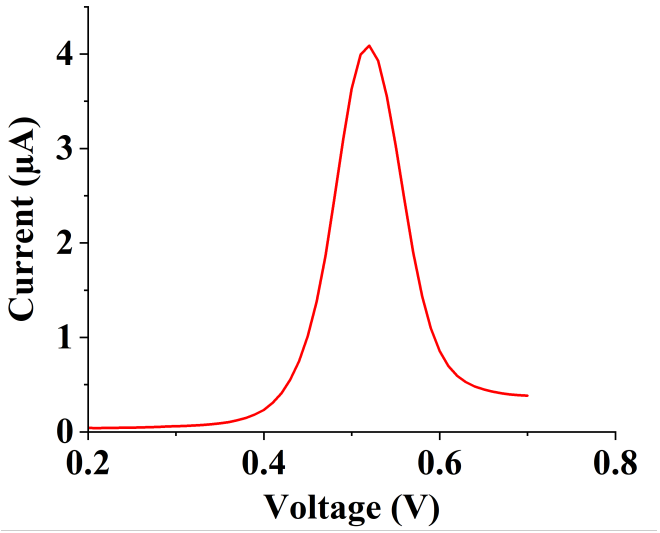

Fig. 3.6: A Differential Pulse Voltammogram

CHAPTER 4

RESULTS AND DISCUSSION

4.1 WORK ON BARE SCREEN PRINTED ELECRTODES

4.1.1 OPTIMIZATION OF EXPERIMENTAL CONDITIONS - EFFECT OF ELECTROLYTE

CV was performed to evaluate the electrochemical behavior of 706 μM CLD in various supporting electrolytes including phosphate buffer (PB), citrate buffer (CB) and acetate buffer (AB). The plot of obtained CV responses is shown in Fig. 4.1. The responses evidently show that the oxidation of CLD is most favorable in PB with the peak anodic potential at 0.65 V. Henceforth all the further investigations of CLD were carried out in the optimal supporting electrolyte of PB. Electrolytes provide ions that carry electric current within the solution. In CV and DPV, the movement of ions is essential for the redox reactions occurring at the electrode surface.

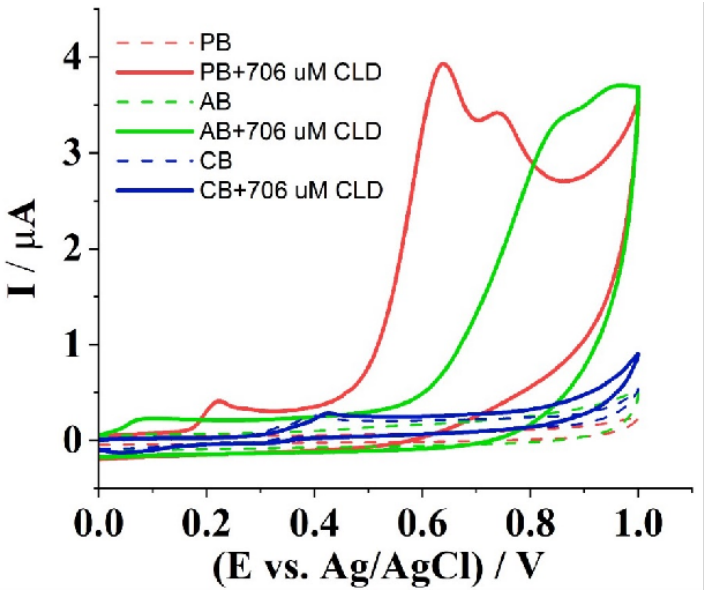

Fig. 4.1: Cyclic Voltammograms for 706 μM CLD in supporting electrolytes (CB, AB,and PB)

4.1.2 OPTIMISATION OF pH TOWARDS CLD

Electrochemical oxidation behavior of 706 μM CLD was investigated in varying pH of PB (pH 5.4 - 8.4). Fig. 4.2 shows the increased anodic current responses of CLD as the pH increased. As pH increased the oxidation potential shifted to more negative values and at pH 7.4 a narrow and sharp oxidation peak was observed with higher current. Therefore, subsequent investigations of CLD were carried out in PB of pH 7.4. An evident shift observed for the cathodic potential to a more negative potential on increasing pH suggests the involvement of protons in the oxidation reaction of CLD in PB [40]. Shortage of protons on increased pH can be the reason for decreasing anodic current response with increase in pH.

Optimizing the pH of the electrolyte is important for precise electrochemical measurements, as pH significantly influences redox potential. Maintaining a specific pH is vital for consistent and reliable results, as it affects the surface charge of the electrode, impacting biomolecule adsorption and interaction [10]. In our experimentation, varying pH buffers of 5.4, 6.4, 7.4, 8.4 were tested. Through this study, it was determined that a Phosphate Buffer with a pH of 7.4 proved to be the optimal condition, yielding a well-defined oxidation peak for enhanced electrochemical performance [4].

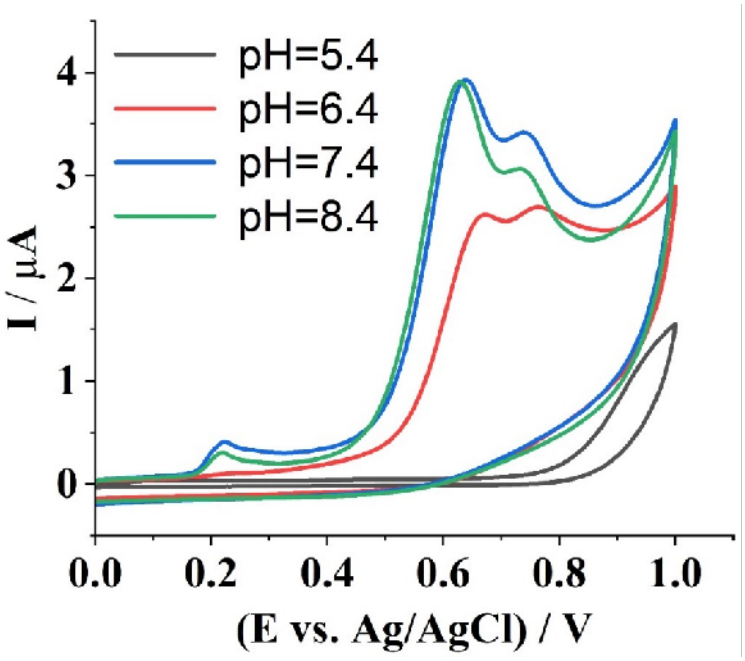

Fig. 4.2: Cyclic Voltammograms with 706 μM CLD at different pH of PB

4.1.3 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 [17]. Through experimentation with different molarities (0.05 M, 0.1 M, 0.2 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.

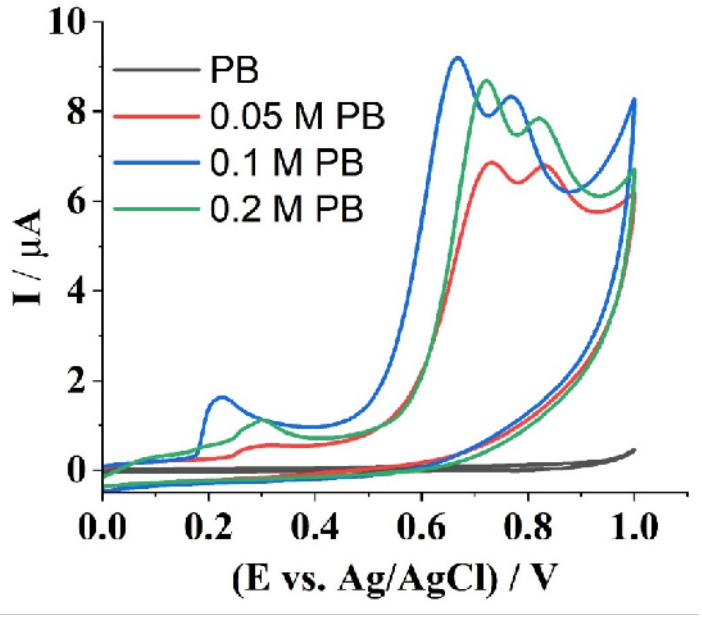

Fig. 4.3: Cyclic Voltammograms with 706 μM CLD at different molarities of PB

4.1.4 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, ranging from 1 to 500 mV s^{-1}. Fig. 4.4 shows the cyclic voltammogram obtained for different scan rates. The linear relationship of anodic current was studied with scan rate (Fig. 4.5) and square root of scan rate (Fig. 4.6). The high regression coefficient (R^2) obtained from Fig. 4.5 (R^2 = 0.989) evidently proves that the oxidation of CLD on SPE is an adsorption controlled phenomenon.

Fig. 4.7 shows the linear logarithmic dependency of anodic peak potential with scan rate. Using the Laviron equation (1) the number of electrons participating in the oxidation reaction was calculated [40].

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

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:

$$E_{pa}(V) = 0.09004 logv(mV/s) + 0.80042, R^2 = 0.998$$

eqn (2)

Equating both the slopes obtained in above equations (1) and (2) shows that 1.51 electrons are involved. This confirms that the electro-oxidation of CLD in 0.1 M PB of pH 7.4 involves two electrons and two protons [41].

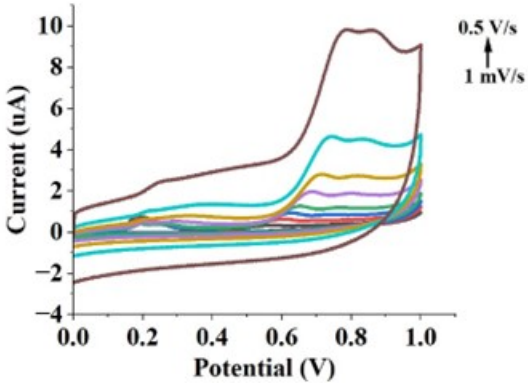

Fig. 4.4: CV at different scan rates

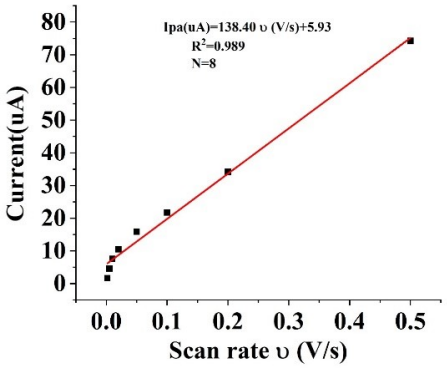

Fig. 4.5: Plot between anodic peak current (Ipa) versus scan rate

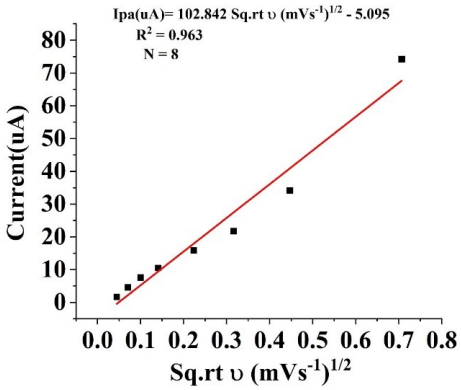

Fig. 4.6: Ipa versus square root of scan rate

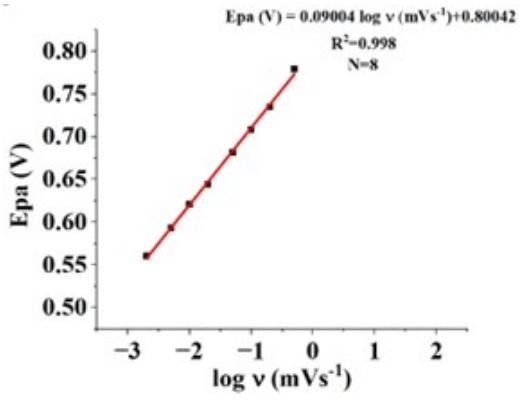

Fig. 4.7: Epa versus log of scan rate

4.1.5 LINEARITY STUDIES

In the linearity analysis, Differential Pulse Voltammetry (DPV) was employed in phosphate buffer (PB). The recorded current exhibited a consistent increase as the CLD concentration elevated, particularly noticeable at a potential of 0.65V. This systematic investigation serves to establish the correlation between CLD concentration and resulting current, providing insights into the sensor's sensitivity and response across a spectrum of concentrations [42]. The linear concentration range detected by the bare SPE is 7 µM to 700 µM. 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.

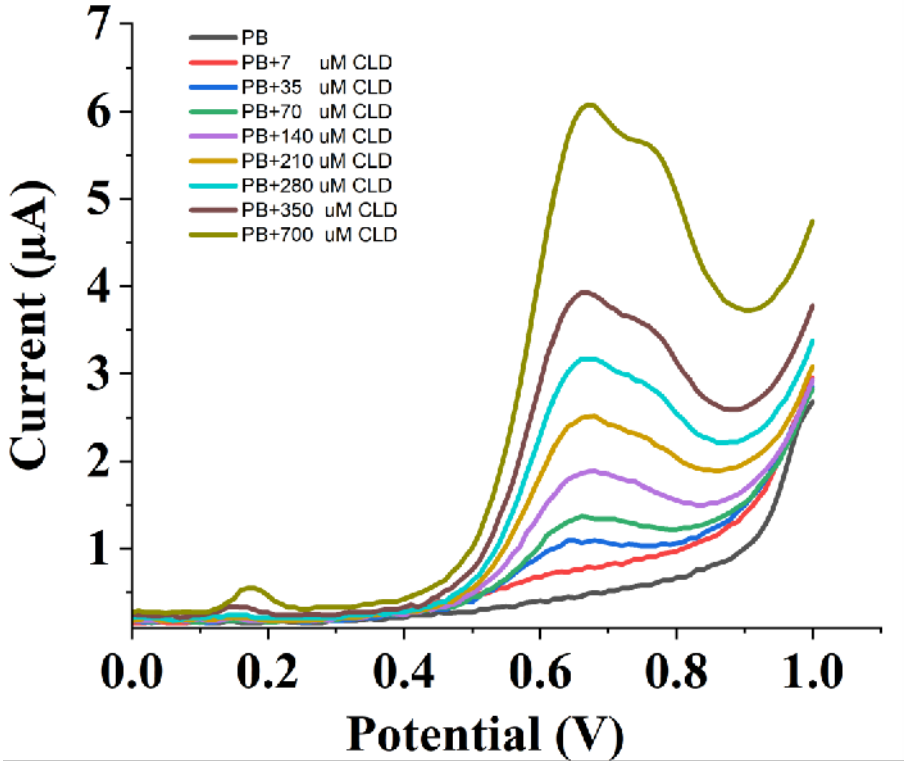

Fig. 4.8: Cyclic Voltammograms obtained with CLD at different concentrations

4.2 WORK ON MWCNTS MODIFIED SCREEN PRINTED ELECRTODE

4.2.1 MORPHOLOGICAL CHARACTERIZATION OF MODIFIED SPE

Field-Emission Scanning Electron Microscopy (FE-SEM) experiment was conducted at National Institute of Technology, Calicut, Kerala, India. The FE-SEM image of the Multi-Walled Carbon Nanotube (MWCNTs) modified Screen Printed Electrode highlights the enhanced surface morphology and increased electroactive

surface area. The microscopic images are shown in Fig. 4.9 and 4.10 at various magnifications. Microscopic images demonstrated the homogeneous dispersion of 40 nm-diameter granular carbon nanoparticles on the sensor surface. A comparative analysis with the bare electrode reveals significant improvements in structural uniformity and electron transfer kinetics, promising enhanced electrochemical performance [43]. FE-SEM is widely used in materials science to characterize the morphology, structure, and composition of materials at the micro and nano-scale. It is valuable for studying nanoparticles, thin films, coatings, and composite materials.

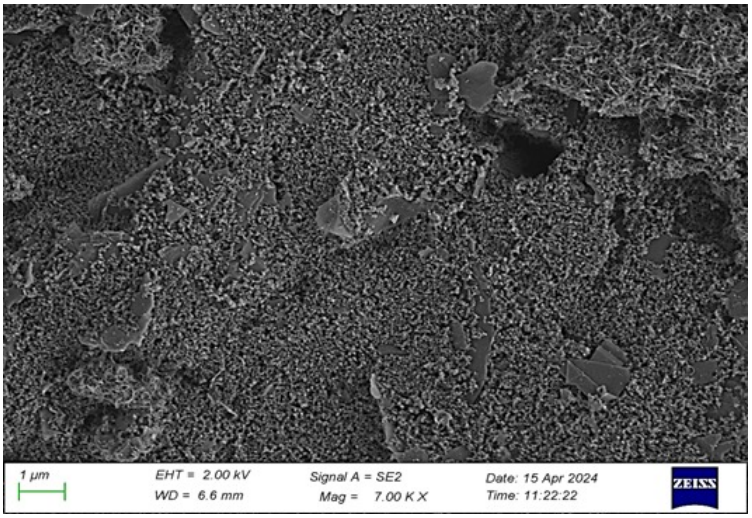

Fig. 4.9: FE-SEM image of modified SPE at 7 KX magnification

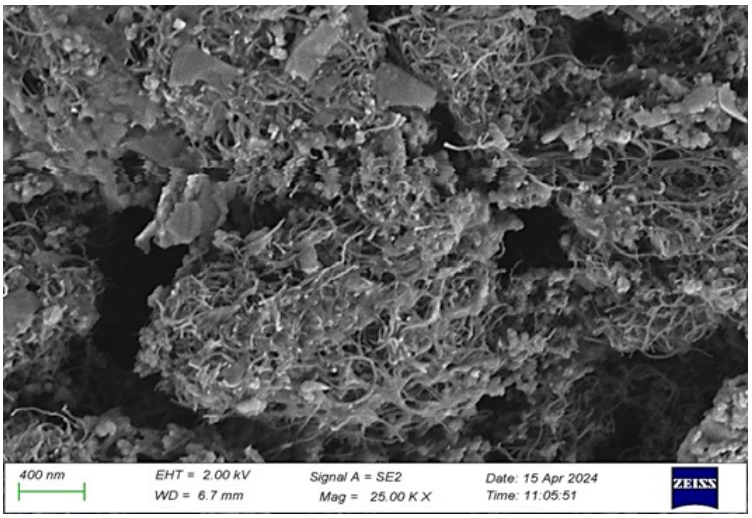

Fig. 4.10: FE-SEM image of modified SPE at 25 KX magnification

4.2.2 ELECTROCHEMICAL OPTIMIZATION

The electrochemical active area plays a pivotal role in the real-time monitoring of samples in electrochemical sensors. To assess the electrochemical active area of a developed sensor, 5mM $[Fe(CN)_6]^{3/4}$ solution containing 0.1 M KCl was employed

at varying scan rates. The obtained voltammogram, spanning from 5 mV s^{-1} to 1 V s^{-1}, is depicted in Fig. 4.11 and Fig. 4.12 illustrates the calibration plots correlating anodic and cathodic peak currents with the scan rate. The calibration equation for the anodic peak current and the square root of the scan rate was derived as follows:

I_{pa} (µA) = 0.00611 $v^{1/2}$ $(mVs^{-1})^{1/2}$ - 2.605 (R^2 = 0.996) eqn (3)

The linear relationship between current and the square root of the scan rate confirms the diffusion-controlled nature of $[Fe(CN)_6]^{3/4}$ mass transfer [25]. Moreover, the electrochemical active area was determined using the Randles-Sevcik relation :

$Ip = 2.69 \times 10^5\ n^{3/2}AD^{1/2}Cv^{1/2}$ eqn (4)

where C, Ip, D, n, v, and A represent the concentration of $[Fe(CN)_6]^{3/4}$, peak current (µA), diffusion coefficient ($7.6x10^{-6}$ cm^2 s^{-1}), number of electrons (n = 1), scan rate (V s^{-1}), and electrode surface area (cm^2), respectively. By comparing equations 3 and 4, the effective electrochemical active area was calculated to be 0.924 cm^2.

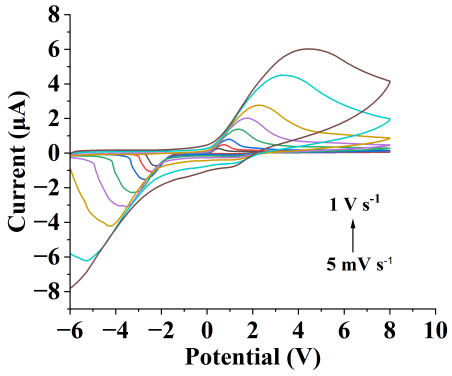

Fig. 4.11: CV of 5 mM $[Fe(CN)_6]^{3/4}$ in 0.1 M KCl at scan rates

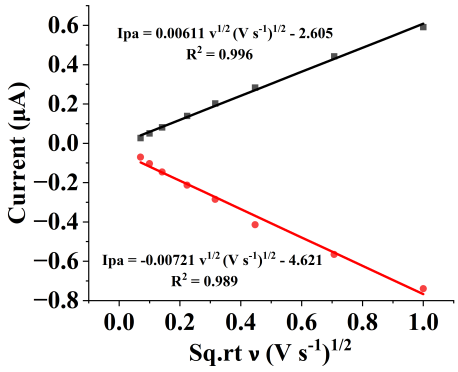

Fig. 4.12: Plot between peak currents (Ip) versus square root of scan rate

4.2.3 EVALUATION OF RESPONSE TOWARDS SCAN RATE

A solution comprising 706 µM CLD in 0.1 M PB was utilized to explore the correlation between anodic current and scan rate, spanning from 1 to 500 mVs^{-1}. Fig. 4.13 depicts the cyclic voltammogram acquired for various scan rates. The linear association of anodic current was examined with both scan rate (Fig. 4.14) and the square root of the scan rate (Fig. 4.15). The notably high regression coefficient (R2) obtained from Fig. 4.14 (R^2 = 0.998) convincingly demonstrates that the oxidation of CLD on SPE is governed by adsorption.

Using the Laviron equation (1) the number of electrons participating in the oxidation reaction was found to be 1.5.

E_{pa} was calculated using the calibration equation of the plot peak potential versus scan rate. The calibration equation was observed as:

$$E_{\mathrm{pa}}(V) = 0.06084 log v(mV/s) + 0.7664, R^2 = 0.995$$

eqn (5)

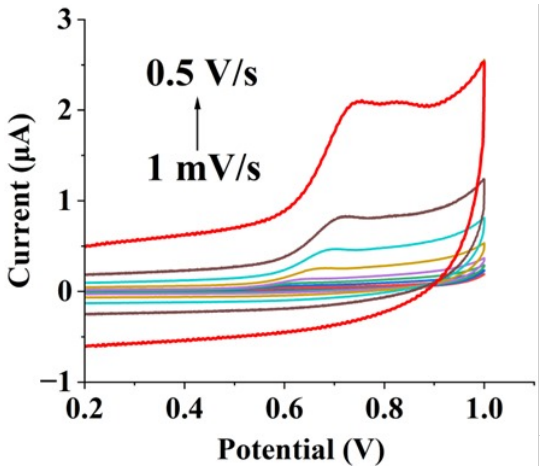

Fig. 4.13: CV at different scan rates

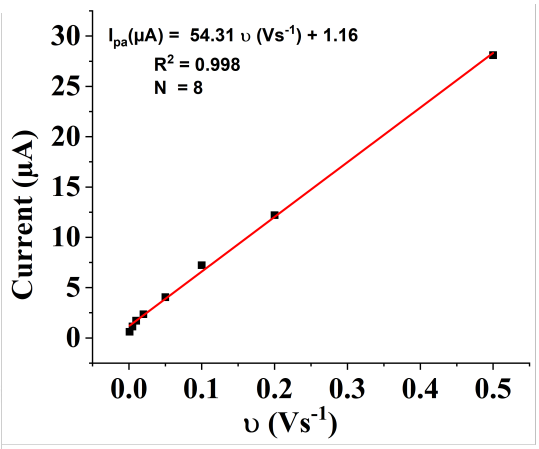

Fig. 4.14: Plot between anodic peak current (Ipa) versus scan rate

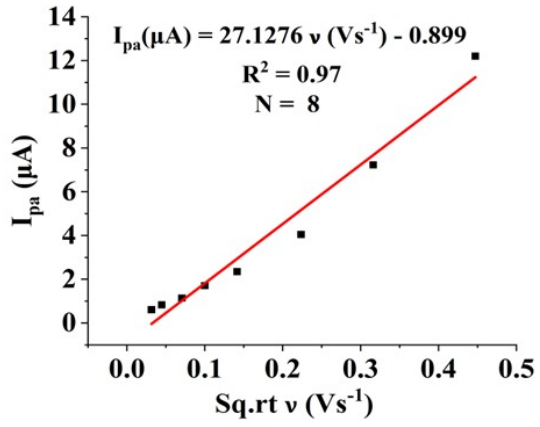

Fig. 4.15: Ipa versus square root of scan rate

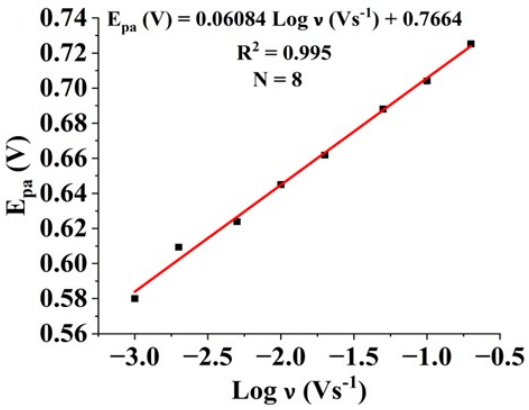

Fig. 4.16: Epa versus log of scan rate

4.2.4 LINEARITY STUDIES

In the analysis of linearity, Differential Pulse Voltammetry (DPV) was utilized within a phosphate buffer (PB) solution. The recorded current demonstrated a consistent rise with increasing CLD concentration, particularly notable at a potential of 0.6V. This systematic exploration aims to establish the relationship between CLD concentration and resultant current, offering insights into the sensor's sensitivity and response across various concentration levels. The linear concentration span detected

by the MWCNTs modified SPE ranges from 1.4 µM to 3000 µM. This evaluation of linearity is pivotal for assessing the sensor's dependability and precision in quantifying CLD concentrations within the specified range, thereby enhancing the overall comprehension of its analytical capability[38].

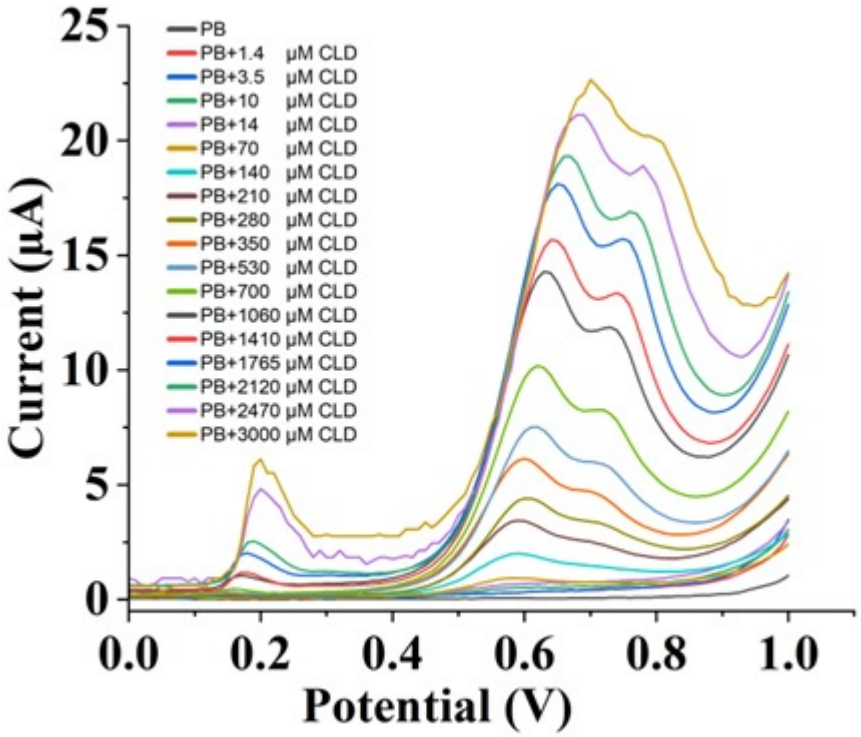

Fig. 4.17: Cyclic Voltammograms obtained with CLD at different concentrations

4.2.5 REAL SAMPLE ANALYSIS

Water samples from taps, ponds, and wells were used for the actual sample analysis. Also tested was drinking water that had been taken from a water filter. The efficiency of the sensor was also tested with blood serum samples. Percentage of recovery of the sensor was studied by spiking known concentrations (706 µM) of CLD into the real samples. The DPV results obtained by analyzing the real samples are shown. Table. 4.1 shows the recoveries obtained in the real samples.

Table 4.1 Real-time sensing of CLD in water and serum samples

Sample	Volume of sample added (µl)	Spiked (µM)	Found (µM)	Recovery (%)
Drinking Water	0	-	-	-
	5	706	697	98.72
	10	706	703	99.57
	15	706	705.6	99.94
Tap Water	0	-	-	-
	5	706	702	99.43
	10	706	700.6	99.2
	15	706	705	99.85
Pond Water	0	-	-	-
	5	706	699	99
	10	706	697	98.72
	15	706	704.1	99.73
Blood Serum	0	-	-	-
	5	706	705	99.85

4.2.6 REPEATABILITY AND REPRODUCIBILITY

To assess repeatability and reproducibility, CLD was subjected to a series of tests. Using DPV, the electrolytic oxidation of CLD was examined on ten different electrodes. The sensor that was created had a 4.57% relative standard deviation (RSD). After cleaning the electrode with deionized water, repeatability was assessed using ten successive runs of CLD, yielding an RSD of 2.38%. According to the investigation, the SPE that was made showed a high degree of reproducibility and repeatability, especially when it was kept sealed.

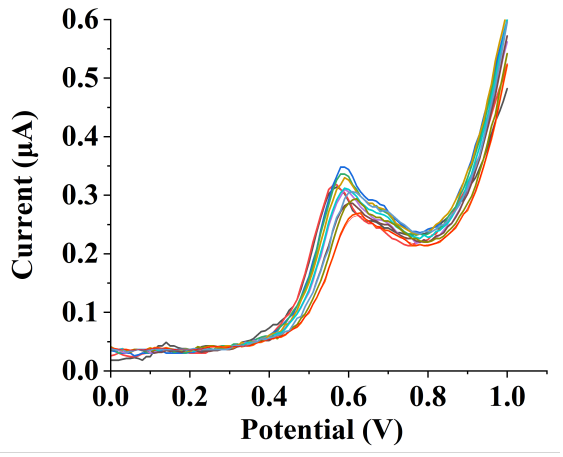

Fig. 4.18: DPV obtained on different runs on the same electrode in 0.1 M PB containing 706 uM CLD

CHAPTER 5

CONCLUSION

The goal of this study was to accurately and precisely detect CLD using a low-cost, highly sensitive sensor that was built on screen-printed electrodes (SPE). The sensor's wide dynamic range, high sensitivity, and low detection limit all demonstrate its excellent performance. The sensor is particularly noteworthy for its strong selectivity for CLD due presence of Multi-walled carbon nano tubes (MWCNTs), as seen by its capacity to identify the molecule in the presence of common interfering substances and other antibiotics. The proposed sensor has a wide range of practical applications, such as medicines, food and water safety, and diagnostic drug analysis. The sensor's efficacy in real sample analysis is clear because it produces good recovery results without requiring laborious pre-treatment procedures. The implications of these findings are significant, as they suggest that the proposed sensor has vast potential across various fields, including pharmaceuticals, food and water safety, and diagnostic drug analysis. Its ability to accurately detect CLD without extensive sample pre-treatment procedures underscores its practical utility in real-world applications. Furthermore, the sensor's performance in real sample analysis, as evidenced by its good recovery results, highlights its efficacy and reliability in practical settings.

In conclusion, the developed sensor offers a promising solution for the sensitive and selective detection of CLD, addressing critical needs in various industries. Its wide-ranging applications and ease of use make it a valuable tool for ensuring product safety, quality control, and regulatory compliance in diverse sectors.

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