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DEVELOPMENT OF A NOVEL SQUARE WAVE VOLTAMMETRIC METHOD FOR DETERMINATION OF PHENOXYMETHYLPENICILLIN AND BENZYLPENICILLIN IN ANIMAL PRODUCTS AND PHARMACEUTICAL SAMPLES

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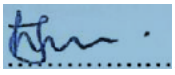
DECLARATION

"I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my own work has been used, this has been properly acknowledged and referenced in accordance with the University of Nairobi's requirements".

Signature… ……  ……. Date…26/11/2021.

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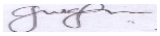
DR. IMMACULATE MICHIRA Signature  Date 26/11/2021
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DEDICATION

I dedicate this work to my lovely wife, Irene Kateve Kiio and daughter, Theodora Mutheu for their steadfast support during the course of this work.

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ABSTRACT

Penicillins have been used widely in humans and animal husbandry to control and manage bacterial infections due to their strong antibacterial activity. However, pressence of penicillin residues in animal products has raised health concerns. The existing detection methods, such as HPLC and enzymatic methods are labour intensive and expensive. There is therefore, a need to develop a simple, more sensitive and field deployable diagnostic tool to determine penicillins levels in animal products.

This research work reports development of a simple square voltammetric method for detection of phenoxymethylpenicillin (penicillin V) and benzylpenicillin (penicillin G) using a surfactant modified support electrolyte, ABS-SDS (acetate buffer solution-sodium dodecyl sulfate) on glassy carbon electrode (GCE). The surfactant enhanced the voltammetric current response for penicillin V and penicillin G for about ten and five times respectively with no significant change in the oxidation potential of these two drugs. The optimal pH and SDS concentration were found to be pH 4.5 and 0.347M respectively.

The diffusion coefficients for penicillin G were estimated to be 6.01×10^{-7} cm^2/sec and 1.39×10^{-6} cm^2/sec in absence and in presence of SDS respectively at optimum conditions in acetate buffer solution. The limit of detection for penicillin G in this method was 2.5×10^3ng/L against a maximum residue limit (MRL) of 4×10^3ng/L set by the European Union. Foreign substance did not interfere with detection of both penicillin V and penicillin G. This implies that the developed method is sensitive enough for use in the analysis of penicillin G and V in diverse samples.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABS	Acetate buffer
ABS/SDS	Sodium dodecyl sulfate in acetate buffer
AMR	Antimicrobial resistance
CE	Counter electrodes
CTAB	Cetyl Trimethyl Ammonium Bromide
D_o	Diffusion coefficient
DPV	Differential pulse voltammetry
Ep_a	Anodic peak potential
Ep_c	Cathodic peak potential
Gaba	Gamma-Amino butyric acid
GCE	Glassy Carbon Electrode
I_d	Net current
I_f	Forward current
I_r	Reverse current
LC	Liquid chromatography
LC-MS-MS	Liquid chromatographic-tandem mass spectrophotometer

LOD	Limit of Detection
LOQ	Limit of quantitation
HPLC	High performance liquid chromatography
PBP	Penicillin binding protein
PEDOT	Poly(3,4-ethylenedioxythiophene)
PLE	Pressurized liquid extraction
RE	Reference Electrode
SDS	Sodium dodecyl sulfate
SWV	Square wave voltammetry
UV	Ultraviolet
WE	Working electrode
WHO	World health organization

CHAPTER ONE

INTRODUCTION

1.1: Background information

Penicillins are antibacterial agents belonging to beta-lactam group of antibiotics. This group of antibiotics is characterized by the presence of a highly reactive four-member ring, comprising of three carbons and nitrogen atom (Fernades *et al*.,2013). Beta-lactam drugs use their reactive four-member ring to bind to transpeptidase. Transpeptidase, also commonly referred as penicillin binding proteins (PBP), is an enzyme responsible for cross linking peptide units during synthesis of peptidoglycan, a major component of cell envelope of almost all bacteria. Cell wall acts as a selective sieve for molecules entering bacteria from its immediate outer environment (Yao and Eefjan, 2016). When cross-linking of the peptide units is compromised, bacterial cell wall synthesis is impaired resulting in lysis and eventual cell death (Etebu and Arikekpar, 2016; Odonkor and Addo, 2011). Common penicillins include phenoxymethylpenicillin (penicillin V), benzylpenicillin (penicillin G), amoxicillin and oxacillin among others. Penicillins are also combined with other antibiotics to form a stronger antibiotic product, through a process called augmentation (Nakazawa *et al*., 2003)

Due to their strong antibacterial activity, penicillins are widely used in humans and in animal husbandry to control and manage bacterial infections (Zervosen *et al.,* 2012). In clinical medicine, penicillin G is the drug of choice against *Neisseria meningitides;* a bacterium which causes meningitis (Jean , *et al*., 2013). In animals, antibiotics are used for disease control especially in intensive animal production and for non-therapeutic purpose such as growth promotion and also as cheaper substitutes for hygiene measures to prevent infections in livestock (Hampton, 2013; Van Boeckel, *et al*., 2017) Antimicrobials are added into animal

feeds to suppress the growth of microbiota in the small intestine (Jensen, 1998). This is done to ensure maximum absorption of digested food and promote faster growth especially for food-producing animals and also protect the animal from toxins produced by the microbiota (Jensen, 1998).

Antibiotics are administered to animals via three routes; injection, oral (added to food and water) and topical application (applied on the skin or by intramammary infusions). All these routes of administration may lead to presence of antibiotic residues in human and animal food (Mitchell *et al*., 1998). Numerous studies report that animals excrete between 17- 75% of antibiotics administered in urine and feaces either unchanged or as active metabolites as reviewed by Massé *et al*., (2014). Other studies have reported presence of antibiotic residues in milk and meat products meant for human consumption (Brown *et al*, 2020; Gustavsson *et al*., 2004; Kang'ethe 2004; Shitandi and Sternesjo, 2004). Surveys from the Food and Drug Administration (FDA) indicate that incorrect use of antibiotics in the management of mastitis is the key source of antibiotic contamination in milk. Additionlly, the presence of drug residues in beef is attributed to beef carcasses of culled dairy cows (Jones, 2009). Higher residue contamination was reported in milk samples from rural households and small traders since there is minimal bulking (Kang'ethe, 2004).

Presence of active pharmaceutical ingredients (APIs) in foods from animal sources exposes human to small doses of antibiotics consequently selecting for antibiotic resistant bacteria. Findings from an invitro study by Inga *et al*., (2003) show that exposure to sub optimal dosages of benzylpenicillin could select s*treptococcus pneumoniae* for resistance to penicillin. In clinical settings, an increase in the risk for carriage of penicillin-resistant *S pneumonia* was reported in children treated daily for more than 5 days with lower than clinically recommended

doses of a β-lactam (Guillermot, *et al*., 1998) Hencewith, antimicrobial residue presence in food products could carry a similar risk. Shitandi and Sternesjo (2004) posit that the risk is higher for growing children due to consumption of large quantities of milk and milk product on the basis of body to weight ratio.

The emergence of antimicrobial resistance (AMR) is major threat to public health. In 2017, WHO released a list of 12 families of resistant bacteria that present a significant threat to human health. The aim of the list was to guide research and development (R&D) of new antibiotics (Willyard, 2017). This is in spite of the numerous classes of antibiotics that have reached the clinic since the first clinical use of salvarsan, a synthetic antibiotic in 1910 (Hutching *et al*., 2019). Flemming, a Nobel laureate for the discovery of penicillin warned that misuse of the drug would lead to its resistance in his acceptance speech. Less than ten years later, resistance to penicillin was reported following its widescale use (Rosenblatt-Farrell, 2009). In 1953, an epidemic of penicillin resistant *Stapyhloccus aureus* spread around the world (Finland, 1955; Hillier, 2006)

Most of the effort to combat antibiotic resistance was only carried out at 'local' level since WHO had refrained from assuming coordinating international roles on surveillance and appropriate use of antibiotics throughout the 1950s and 1960s. (Gradmann, 2013; Podolsky, 2015). At the time, there was optimism that the drug industry would keep up with the antimicrobial resistance race due to the discovery of other antibiotics such as chloramphenicol, tetracyclines, macrolides and others (Podolsky, 2018). However, there has been a gradual decline in the discovery and development of new classes of antibiotic for various reasons. These include a shortage of new metabolic targets and high costs of developing new drugs among other varying reasons (Coates *et al*., 2011)

Penicillins and other antibiotics can cause allergic reactions. It is approximated that 5-10 % of the population is hypersensitive to antibiotics. Symptoms of exposure range from asthsma, skin rash, hives and anaphylatic shock (Dayan, 1993; Jones, 2009). Concomitant administration of antibiotics with oral contraceptives may reduce the efficacy of some oral contraceptive pills (Dickinson *et al*., 2001; Osborne, 2002). Other undesirable effects of antibiotics include the adverse interactions with anticoagulants (warfarin), anticonvulsants (phenobarbitone and phenytoin) and tolbutamide, and oral antidiabetic drug (Bint and Burtt, 1980). So far, only allergic reactions have been conclusively linked to presence of antibiotic residues in food sources from animals and in some cases from plants (Dayan, 1993; Graham *et al*., 2014; Raison-Peyron *et al*., 2001;). But this does not underscore the contribution of consuming these residues in food to AMR and other toxicological effects linked to antibiotics.

The presence of anti-microbial residues in milk has additional marked effects notably from an industrial viewpoint; they interfere with the manufacture of several dairy products. Antibiotic concentrations as low as 1 ppb delay starter activity for butter, cheese and yogurt. Additionally, these residues reduce curdling of milk, production of acid and flavor associated with the manufacture of butter and cause improper ripening of cheeses (Jones, 2009).

Several methods have been applied in detection of penicillins in samples of biological and environmental origin. The most commonly used method has been High-performance liquid chromatography coupled with other methods of detection. This method has been considered to be the most successful due to its high sensitivity and selectivity. However it is expensive and time consuming because it involve derivatization, extraction and purification procedures (Švorc *et al.,* 2012). Various electrochemical techniques have also been used for analysis of

penicillins. They include differential pulse voltammetry (L'ubomir *et al*., 2012; Bergamini *et al*., 2006)) and pulsed amperometry (Koprowski *et al*., 1993) . Electrochemical methods are known to be superior because they are simple, low-cost, fast and they can easily be miniaturized to obtain portable sensors for on-site analysis (Švorc *et al.*, 2012). These properties eliminate limitations found in other analytical techniques (Baranowska *et al.*, 2008). However, few studies has been done on penicillins using electro-analytical methods probably because the electrode must be driven to higher oxidative potential and the resulting voltammograms are not well defined (Švorc *et al*., 2012). There is therefore, a need to improve the currently existing methods for optimum detection of penicillins.

Surfactants have been utilized in numerous applications in chemistry including electrocatalysis and electroanalysis among others (Vittal *et al.*, 2006). Surfactants are known to alter and control properties of electrode surfaces, therefore they have been used to enhance or alter reaction rates (Atta *et al*., 2007).

This work demonstrates application of SDS containing support electrolyte to enhance oxidation signal for detection of penicillin G & V without any chemical modification on glassy carbon electrode surface. The Voltammetric method developed does not need any chemical alteration on the electrode surface or electrochemical pretreatment of the GC electrode; hence this method is simple, straight forward and highly reproducible.

1.2: Statement of the Problem

Global trends show a marked increase in the use of antimicrobials due to an increase in disease burdens for both animals and humans, and intensive livestock production (Muloi, *et al*., 2019; Van Boeckel, *et al*., 2017). It is estimated that the quantities of antibiotics used in livestock

production is almost double that used in human medicine (Aarestrup, 2012). A significant proportion (upto 75 %) of antibiotics consumed by animals are released as active pharmaceutical ingredients (Massé *et al*., 2014). These contaminants end up in the environment where they are likely to be consumed in drinking water (K¨ummerer, 2010) and also in animal milk and meat products (Brown *et al*., 2020; Sitandi & Sternesjo 2004; Rahman *et al*., 2021).

Lack of adequate monitoring systems and detection facilities for antimicrobial residues in foods put the developing countries at a greater risk of consuming residues in animal products than developed countries (Sachi *et al*., 2019). This inadvertent consumption of antimicrobial residues in food is associated with negative effects on consumers' health. These include, selection for drug resistant microbes, hypersensitivity reactions, disruption of the normal gut microbes among others (Falowo and Akimoladun, 2020).

1.3: Objectives

1.3.1: General objective

The general objective of this study was to develop a simple square wave voltammetric method that can be used to determine the presence of penicillins in food samples.

1.3.2: Specific objectives

The specific objectives of the study were;

i. To optimize the electrolyte composition (pH and SDS concentration) for optimum voltammetric detection of the analyte (Penicillin V and Penicillin G)

ii. To validate the voltammetric method by determining its useful linear range (LR), limit of detection (LOD) and limit of quantitation (LOQ), precision and accuracy.

iii. To assess the effects of interferents on detection of the analytes (penicillin G and penicillin V) by the voltammetric method.

iv. To determine the applicability of the method in detecting and quantifying penicillins(V and G) in milk samples.

1.4: Justification and Significance of the Study

Milk is considered nature's most complete food for infants, growing children and adult humans. Some of its most valuable constituents include proteins (casein and whey) and calcium which plays a crucial role in bone development in children and prevention of osteoporosis in the elderly (Park and Haenlein, 2015; Wolfe, 2015). Injudicious usage of antibiotics in livestock production has been attributed to the presence of antibiotic residues in milk and milk products. One of the greatest public health concerns, is that exposure to these antimicrobials will contribute to the selection of antibiotic resistant bacteria rendering particular classes of antibiotics to lose efficacy (Kebede *et al*., 2014; Sachi *et al*., 2019).

To minimize the risk of consuming these antibiotic residues in food, most countries came up with maximum residue limits (MRLs). For instance, the European Union (EU), set MRLs for ß-lactams group i.e. ampicillin, amoxicillin and penicillin G at 4 µg/kg, dicloxacillin and oxacillin at 30 µg/kg, cephalexin at 100 µg/kg and cephapirin 60 µg/kg (The Commission of the European Communities, 1999). Regulatory bodies in most developing countries like Kenya have not set MRLs but rather specify zero tolerance meaning detectable residues are not permissible in foodstuff (Kebede *et al.*, 2014). Additionally, these countries lack elaborate monitoring systems and detection facilities hence consumption of these residues in milk is rife (Brown *et al*., 2020; Kang'ethe *et al.*, 2005; Kosgey *et al*., 2018; Orwa *et al*., 2017; Sachi *et al*., 2019; Shitandi and Sternesjö, 2001, 2004). One of the recommendations after these findings

was that research on cost-effective screening methods needed to be carried out (Kosgey *et al*., 2018.

So far, no work on detection of benzylpenicillin (penicillin G) and phenoxymethylpenicillin (penicillin V) on glassy carbon electrode in sodium dodecyl sulfate (SDS)-acetate buffer media (ABS) has been reported. The developed method has the potential to allow for onsite analysis of samples unlike HPLC and enzymatic methods where samples are analysed in a centralized laboratory. Additionally, this method is simple and cheap compared to the above methods (HPLC and Enzymatic) which are tedious, involving and expensive.

Use of a surface-active agent, sodium dodecyl sulfate (SDS), prevents electrode fouling (Yang *et al*., 2009) and improves the electrochemical properties of glassy carbon electrode surface (Atta *et al*., 2014). Consequently, the voltammograms were well defined. This is in contrast with the common electrochemical methods where the voltammograms are poorly formed.

The use of highly polished glassy carbon electrode makes analysis less tedious and reproducible unlike the use of surface modified electrodes which are tedious and irreproducible (Guo *et al*., 2017). This work therefore, proposes a simple, fast, low cost and field deployable alternative method for detection of penicillin (V and G) in milk samples.

CHAPTER TWO

LITERATURE REVIEW

2.1: Electrochemistry

Electrochemistry is an active field of modern research which involves the study of chemical changes that result from application of electrical energy on electrolytes and how chemical reactions can also be used to produce electrical energy (Magnussen, 2006). Consequently, electrochemistry has been used both in qualitative and quantitative determination of electro-active analytes. Additionally, this branch of science has been applied in development electroanalytical sensors, fuel cells, batteries among others (Bard and Faulkner , 2001).

In research, electrochemical measurements are made using different electroanalytical methods for different reasons. These include obtaining thermodynamic data for a reaction, generating unstable intermediate to study its decay and analyzing solutions for trace elements (Bard and Faulkner , 2001). Electro-analytical methods are important tools in understanding and analyzing chemical systems especially those involving electro-active species. Electrochemical methods can also be used in synthesis of a product or modification of an electrode to improve its properties e.g. Electrodeposition of polyaniline on glassy carbon electrode surface (Wang *et al*, 2011).

The interface between the metal (electrode) and the electrolyte solution is the region where electrochemical reactions take place (Crow, 2017). The interface is usually charged; the metal surface carries excess charge which is balanced by a charge equal but opposite in charge from the solution side. The resulting charge distribution, two narrow regions of equal but opposite

charge form a region known as the electrical double layer. The region extending from the interface to around 3 nm is different from the bulk solution (Zoski, 2007). Reactions that involve charge transfer across the interface are said to be electrochemical reactions. As the current passes on, the electrode surface develops charge due to movement of electrons. The interaction of electro-active materials diffusing from the bulk solution to the charged surface results in a redox reaction, where both oxidation and reduction take place simultaneously.

There are two types of charge transfer reactions occurring at the interface, namely; heterogeneous and homogenous. Heterogeneous electron transfer occurs when an electroactive material in solution moves to the surface of electrode, different phase, where electron exchange occurs at the solution-electrode interface (Zoski, 2007). A reaction is said to be homogenous if the electron transfer reaction takes place between species in the same phase, i.e. solution. In electrode processes, the most commonly used electrode materials are solid metals (silver and gold), liquid metals (mercury), graphite and semi-conductors e.g. indium-tin oxide (Bard and Faulkner , 2001).

Most of the electrode processes take place in an electrochemical cell, containing mainly an electrolyte and electrodes. Aqueous solutions containing soluble salts such as K^+, Cl^- and H^+ in water or other non-aqueous solvents are commonly used as electrolytes. Charge transfer in these aqueous solutions is possible due to presence of large number of mobile ions. A good electrolyte should be sufficiently conductive to be useful in electroanalysis (Andreev and Bruce, 2000).

2.2: Basic Principles of Electrochemistry

2.2.1: Potentiostat

Potentiostat is a device that applies an exact potential and monitors the changes in current in the

electrochemical system (Dryden and Wheeler, 2015). The modern day Potentiostat comes as a package containing electrometer circuits, converters, amplifiers and microprocessor loaded with internal memory (Rubin and Carlos , 2008). Modern Potentiostat operate in digital form, where a 'staircase' modulated potential is commonly used with 'steps' having constant potential increment. Thanks to development in technology, different pulsed methods have been developed. These methods have increased sensitivity and shortened the time taken to analyze a sample. The most common waveforms in the current Potentiostat are linear scan, differential pulse and square wave. Various types of Potentiostat are available in the market and they vary in size, power and sophistication ranging from simple to large research grade Potentiostat (Rubin and Carlos , 2008).

2.2.2: Electrochemical Cell

Electrochemical analysis takes place in an electrochemical cell, which is basically made of three-electrodes suspended in a support electrolyte carrying the substance being analysed as shown in Figure 2.1. The three electrode systems consist of the working/indicator electrode, reference electrode (RE) and counter (auxiliary) electrode (CE). The tips of the three electrodes in a cell should be kept in close proximity as much as possible to reduce solution resistance. The close approach is especially important for the working and reference electrode. Solution resistance between these two electrodes leads to iR drop that manifest as an error in the measured potential between them (Joseph, 2006). A potentiostat is able to electronically compensate for this resistance as in the case between the reference and auxiliary electrode.

The auxiliary electrode should at least have same surface area as the working electrode and positioned symmetrically with respect to working electrode so that the current density and potential experienced on its entire length is constant (Tian, *et al*., 2016).

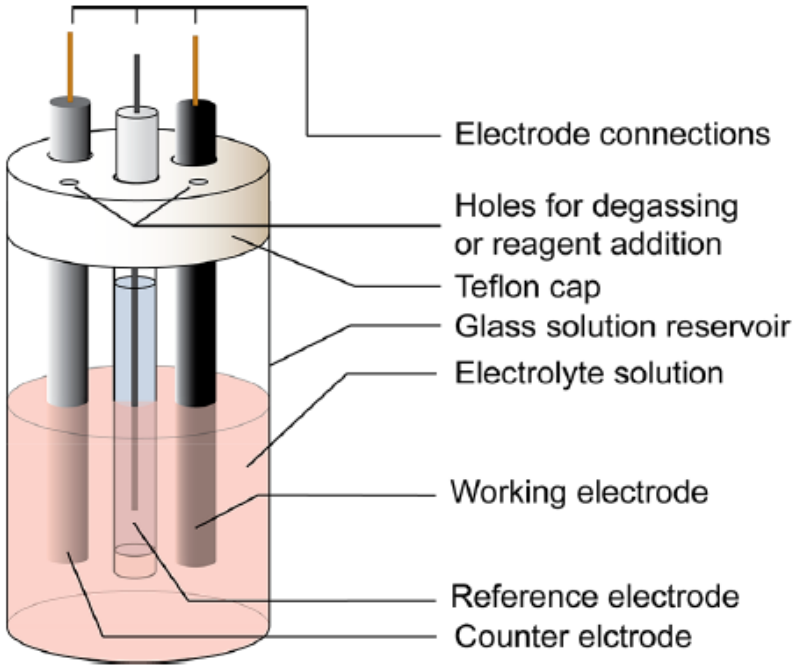


Figure 2.1: A simple electrochemical cell showing working electrode (WE), silver/silver chloride (RE) and platinum wire (CE) (Source: (Elgrishi, *et al*., 2018).

In most electrochemical reactions, the dissolved oxygen in the electrolyte should be removed by purging with inert gas. Oxygen is electro-active in a wide cathodic potential range and its therefore likely to interfere with electrochemical analysis (Joseph, 2006) . Additionally, its presence in a sample solution may lead to oxidation of products formed during electrolysis. Oxygen is removed by purging the electrolyte solution for five to ten minutes with inert gas prior to the experiment. This removes oxygen from solution and maintains a blanket of inert gas above solution during the experiment. The most commonly used inert gases are nitrogen and argon (Elgrishi, *et al*., 2018)

Various cell volumes exist ranging from microlitres to millitres depending on the goal of the researcher. Cells designs are also varied from simple to complex systems integrating build-in gas control, magnetic stirrer, connection to electrochemical analyzer and proper cover. The cell design and material used for the cell construction depends on the experiment at hand and the

nature of the sample (Joseph, 2006). Additionally, cell designs may also differ in size, shape and temperature control capabilities among others.

Glass is the most commonly used material for making electrochemical cells since it is cheaper, transparent, chemically inert and impermeable (Zoski, 2007). Other possible cell materials include Teflon and quartz. Cell cover material should not react with the sample under study. In a quiescent solution a simple design as shown in Figure 2.1 may be used.

2.2.3: Solvent

The choice of the most convenient solvent for electrochemical work is governed by a number of physicochemical properties (Noel and Vasu, 1990). The solvent be in liquid form at room temperature and capable of dissolving ionic substances that form the electrolyte and electro-active species. Additionally, it must possess a sufficiently broad potential window for study of the electro-active species of interest without it being oxidized or reduced within the potential window (Sharma and Zaidi, 2020). Moreover, it must possess a good dielectric constant and the required acid-base properties over the chosen potential window (Sharma and Zaidi, 2020).

Water possesses most of these physico-chemical properties and it's the most commonly used solvent in preparations of highly conducting electrolytes (Guduru and Icaza, 2016). However, water has its limitations. Some analytes are insoluble in water, it can easily be oxidized or reduced and it also forms films on electrodes which compromise electrode reaction and its reproducibility. Despite its limitations, water is the most commonly used solvent (Guduru and Icaza, 2016). Other solvents used include; Acetonitrile, which has poor solubility for ionic salts, dimethyl formamide (DMF), with good dissolving power for ionic species but self decomposes when a potential above +1.0 V is applied, and dimethyl sulphoxide (DMSO) with similar

properties to DMF but with better cathodic potential limits (Noel and Vasu, 1990).

2.2.4: Supporting Electrolytes

A Support or swamping electrolyte is a solution containing ionizable salts or compounds that are not redox-active in a given potential window and whose ionic strength is much greater than the dissolved electro-active substance (Creager, 2007). The choice of support electrolyte primarily depends on whether the analyte can dissolve in it and the electrochemical properties of the solvent (Creager, 2007). Support electrolyte is used to increase conductivity by reducing solution resistance, to reduce transport of redox-active species through migration (electromigration) and maintain constant ionic strength and pH (Joseph, 2006). A good support electrolyte should be (Elgrishi, *et al*., 2018);

i. Electrochemically inert in the potential window of interest.
ii. Very high in concentration to avoid influencing charge transfer kinetics.
iii. Non-absorbent on the electrode surface. This ensures that they don't interfere with electrode reactions by either inhibiting or catalyzing them.
iv. Unreactive with analyte or products forming on the electrode surface.

Some of the inert electrolytes that are used include inorganic salts, buffers like acetate and phosphate; especially if pH control is essential, strong acids like sulphuric acid, hydrochloric acid among others or strong bases like sodium or potassium hydroxide. Tetraalkylammonium salts are also often used especially in organic media (Creager, 2007).

2.2.5: Electrodes

Electrochemical reactions take place at the electrode-solution interface. There are three types of electrodes in an electrochemical cell each playing out a different role during electrochemical reaction.

2.2.5.1: Counter/Auxiliary Electrode

The counter electrode (CE), also referred to as auxiliary electrode is used to close the current circuit in an electrochemical cell. Counter electrode does not participate in cell reaction and it's made from inert materials such as platinum, gold, graphite and glassy carbon. Platinum is the material of choice due to its chemical inertness and fast electrode kinetics (Bard and Faulkner , 2001). As such, platinum wire is commonly used as counter electrode. Since the current flows between the counter electrode and the working electrode, the total surface area of the auxiliary electrode should be higher than that of working electrode to ensure that CE does not impede half reactions of interest (Bard and Faulkner , 2001).

2.2.5.2: Working/Indicator Electrode

It is the surface where the analyte is reduced or oxidized depending on the potential applied on it (Bott, 1997). Electrochemical reaction takes place at a region between the indicator electrode and the sample solution. The choice of the indicator electrode is a critical factor for success in analytical work (Bott, 1997). The exposed surface of this electrode is small to limit the area in which the reaction takes place (Bard and Faulkner , 2001). Consequently, this allows the experimenter to monitor a controlled population of electro-active species as they undergo redox reaction. The material used for working electrode should allow a quick and reproducible electron transfer reaction without allowing accumulation of material on its surface (Grieshaber *et al*., 2008). Ideally the WE surface should be conductive and inert under the experimental

conditions (Bott, 1997). Additionally, the potential window should be large enough to allow greater degree of analyte characterization (Grieshaber *et al*., 2008). The electrode surface should also allow easy renewal after a measurement (Bott, 1997).

Working electrodes are made from different materials and come in variety of shapes and forms (Kounaves, 1997). The most common forms are solid and liquid forms. The solid electrodes are mainly carbon based and metal-based (Bott, 1997). The metal based working electrodes are mainly made of silver, platinum and gold. Platinum's chemical inertness, good electrochemical conductivity and ability to be fabricated in many forms makes it one of the best materials for making working electrodes (Bott, 1997). However, platinum is expensive and easily liberates hydrogen at relatively lower negative potentials in presence of little quantities of water or acid. Gold working electrodes have features similar to platinum. However, it is less preferred especially if potential window for analysis lies in the positive region because of formation of surface oxide. Such films significantly alter the rate of surface reaction which may result in unreproducible data (Joseph, 2006). However, gold useful in preparation of modified surfaces. Most common liquid working electrodes include dropping mercury electrode, hanging mercury drop electrode and static mercury drop electrode (Bott, 1997).

Carbon allows greater negative and positive potential window scan than both platinum and gold. Carbon electrodes come in different forms but the most common types are carbon paste electrode and glassy carbon electrode (GCE) (Kounaves, 1997). Glassy carbon electrodes are the most preferred working electrodes because of their exceptional mechanical and electrical properties, chemical inertness, high reproducibility and broad potential window (Qureshi *et al*., 2009). Moreover, GCE surface can easily be regenerated through polishing using alumina slurries on a polishing cloth (Bott, 1997). Carbon working electrode can be modified using

various materials or chemicals such as polymers to make chemically modified electrodes (Wang *et al*., 2012).

Electrode modification is a deliberate alteration of the electrode surface to meet the electro-analytical need of the researcher. Electrode surfaces are modified to accelerate electron transfer reactions, for preferential accumulation of a species of interest or selective membrane permeation among other reasons (Madkour, 2000). Electrode modification occurs through attachment of various functional groups such as surfactants, hydroxyl and carbonyl among others (Wang *et al*., 2012).

A solid electrode requires careful pre-treatment unlike mercury working electrode. Solid electrodes are polished clean on a wet polishing pad. This can be done using alumina slurries of different particle sizes (Bott, 1997). Electrochemical activation is important for solid electrodes to obtain reproducible voltammograms. However, if the electrodes are modified with other substances such as polymers or composites, their surfaces are not polished.

2.2.5.3: Reference Electrode

The individual potential of a cell cannot be directly established but rather its relative potential is measured relative to an electrode with a fixed or/and known electrode potential. The potential of reference electrode is not affected by chemical species in the support electrolyte and should therefore remain stable on passing a little current (Smith and Stevenson, 2007). Owing to these features, it is used as the standard against which the potential of the working electrodes is established (Inzelt *et al*., 2013).

Since no absolute standard for measurement of electrochemical potential exists, the standard hydrogen electrode (SHE) has been adopted as a primary reference electrode. SHE has several inherent limitations such as, difficulty in maintenance and reproducibility and the ease with which the platinum is poisoned. Due to such limitations secondary reference electrodes are used e.g. calomel electrode (Hg/Hg_2Cl_2) and Ag/AgCl. Ag/AgCl electrode consists of silver wire immersed in saturated solution containing silver chloride and potassium chloride (Inzelt *et al*., 2013).

2.2.6: Polarizable and Non-Polarizable Interfaces

Some electrodes do not allow transfer of electron across the metal-solution interface regardless of the potential imposed on the electrode by an external source of voltage. Such kind of electrodes are said to be ideal polarizable electrodes (IPE) (Bard and Faulkner , 2001). Application of charge from an external source causes a substantial build-up of excess charge at the interface. Consequently, there is an enormous change in potential upon application of an infinitesimal current. IPEs are represented by a horizontal region in current-potential curve as shown in Figure 2.2 a.

Conversely, some electrodes allow electrons to quickly pass across the electrode/solution interface. Such electrodes are said to be non-polarizable (Bard and Faulkner , 2001). Change in applied external potential may cause more electrons to pass swiftly across the electrode/solution interface. Consequently, less charge accumulates at the electrode surface, i.e., the electrode- solution interface is not polarized. An ideal non-polarizable electrode can therefore be described as an electrode of fixed potential as shown in Figure 2.2 b.

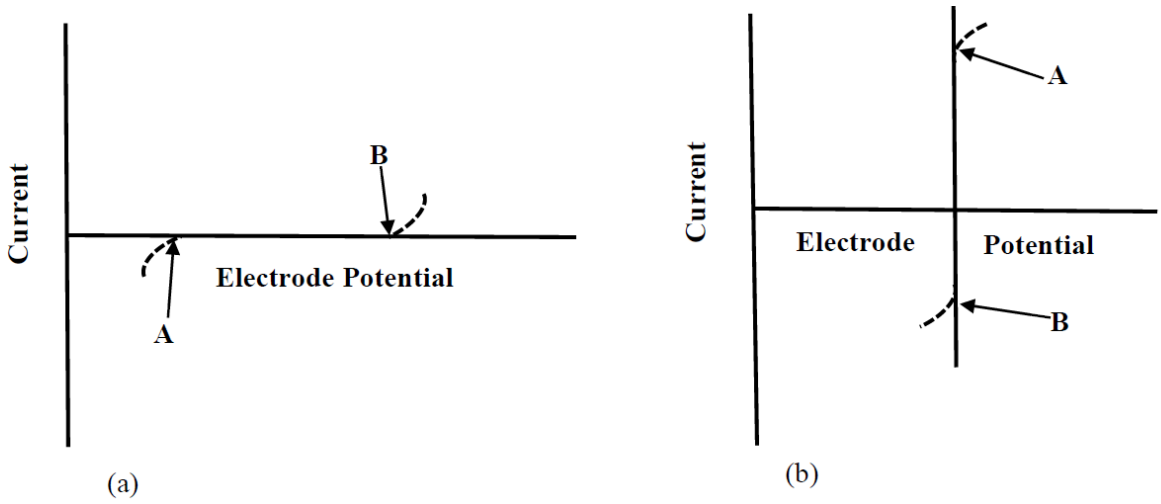


Figure 2.2: Current –Potential curves for a) ideal polarizable electrode b) ideal non-polarizable electrode. The broken lines show deviation from ideal behavior by real electrodes. Source:(Bard and Faulkner , 2001)

2.3: Electrode Processes

Electrode processes take place between the electrode surface and solution, in a region commonly reffered as the interface. Characteristics at the interface differ remarkably from those of the bulk (Bard and Faulkner , 2001). This region includes the electrode surface and part of solution influenced by it. In a three electrode system, refence electrode is characterised by a constant potential while the potential of the indicator electrode is controlled with respect to that of the reference electrode (Inzelt *et al*., 2013). In a solid working electrode, there are many electronic energy levels forming a band. The highest energy of this band is called a fermi level (Bard and Faulkner , 2001). The solution side consists of single molecules which have discrete energy levels. The energy of electrons at the electrode surface can be controled by scanning the potential negatively or positively (Bard and Faulkner , 2001). Pushing the electrode to more negative potential causes the energy of the electrons to be increased therefore raising the fermi level. Consequently, the electrons attain enough energy to jump into the vacant energy level of redox-active species in the sample solution as shown in Figue 2.3

(Bockris and Khan, 1983). As electrons jump from the electrode into solution, a reduction current is produced.

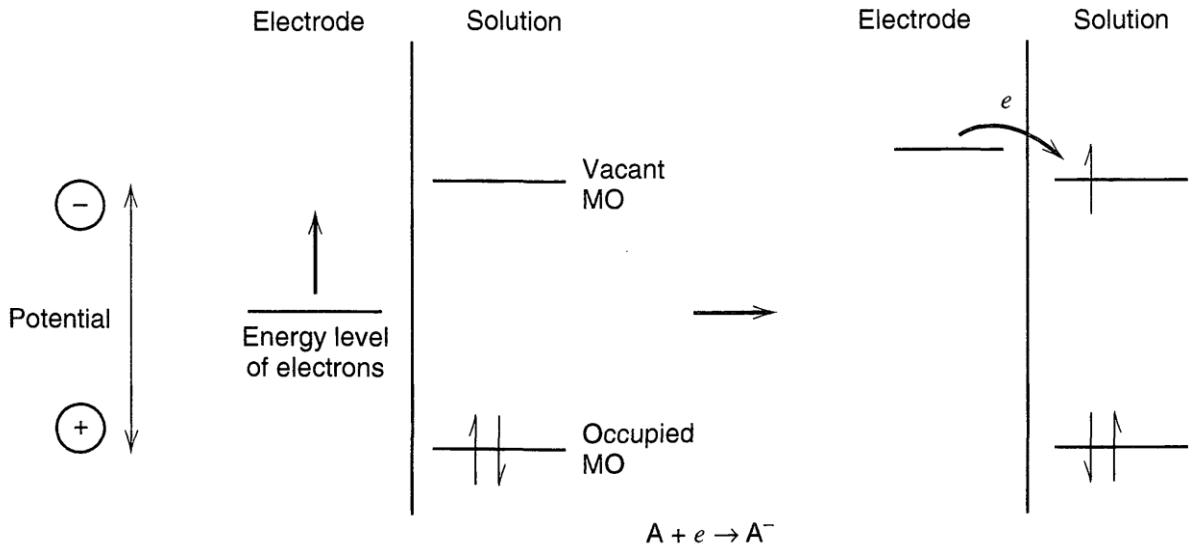


Figure 2.3: Reduction of species A source: (Bard and Faulkner , 2001)

Similarly, when the potential of the working electrode is scanned in the positive direction, the build-up of positive charge lowers the electron energy at the electrode. Consequently, the fermi level drops and the electrons from electro-active analytes jump from a less energy favorable solution to a more energy favorable energy state at the electrode surface (Bockris and Khan, 1983). As a result, the analyte flow from the electrolyte to the electrode resulting in oxidation of solvated species and flow of current as shown in Figure 2.4. The potentials at which oxidation and reduction of the analyte species take place across the interface are related to standard potentials, E^o which is unique to a particular chemical specie (Bard and Faulkner , 2001).

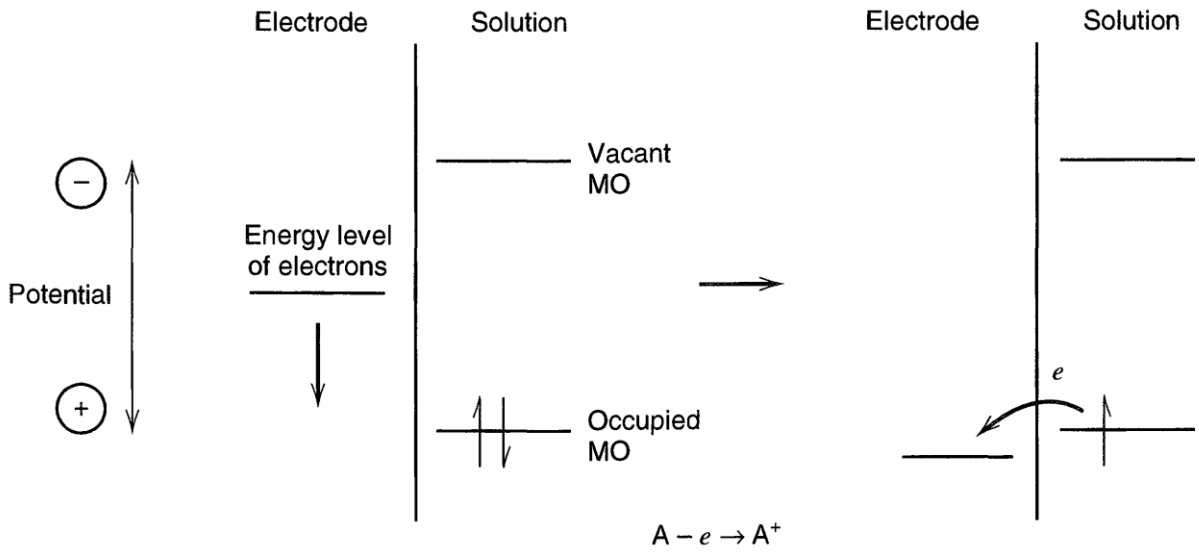


Figure 2.4: Oxidation of species A Source: (Bard and Faulkner , 2001)

2.3.1: Electrical Double Layer

When potential is applied to a working electrode dipped in a solution or electrolyte, charge accumulates at the electrode surface. The excess charge at the electrode surface causes an accumulation of excess ions/molecules of opposite charge in the solution/electrolyte (Bockris and Khan, 1983). The interaction between the ions in solution and the charged electrode surface is assumed to be electrostatic (Bard and Faulkner , 2001). The attracted ions approach the electrode surface and form a layer to balance the excess charge at the electrode surface. The resulting charge separation of two regions of opposite but equal charges give rise to a region called electrical double layer (Bockris and Khan, 1983). The solution side of the double layer is believed to be made of a number of "layers" (Joseph, 2006). Since these layers form in the solution side in response to the excess charge forming on the electrode (q_e), a negative charge at the electrode would cause an array of positively charged ions to form on the solution side(q_s) and vice versa. This is so because the electrode/solution interface must be neutral ($q_e + q_s = 0$) (Joseph, 2006).

According to the Helmholtz model, the solution side consists of inner and outer Helmholtz planes. The inner Helmholtz layer is the closest to the electrode surface and it consists of all species that are specifically adsorbed. If only one type of molecule or ion is adsorbed, and they all sit in same positions then their centres define the inner Helmholtz plane (IHP). The outer Helmholtz plane (OHP) consists of ions/molecules closest to the electrode but not specifically adsorbed. Their solvation sphere is intact and they are bound to the electrode by electrostatic forces (Joseph, 2006).

Both the inner and outer Helmholtz plane constitutes the stern layer or compact layer. This layer is strongly attached to electrode surface to such a degree that the layer will remain intact even when the electrode is withdrawn from the solution (Bard and Faulkner , 2001).

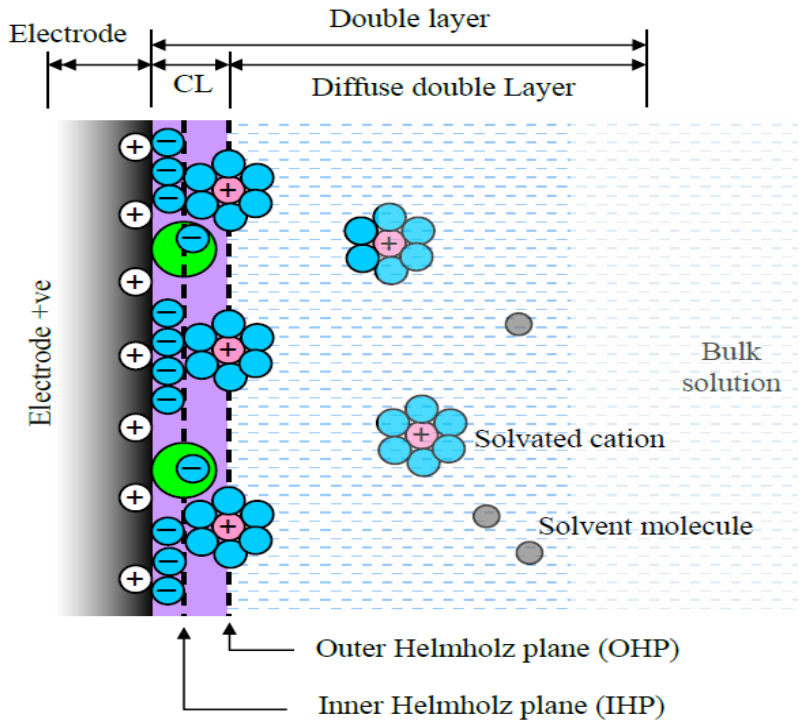


Figure 2.5: Schematic representation of electrical double layer. Source: (Mithu *et al.*, 2017)

The outer layer that extends beyond the compact layer is referred as the diffuse layer. This region consists of free ions scattered into bulk solution and extends from the OHP into the bulk

solution where the total charge of the compact layer and diffuse layer equals to but opposite to the net charge on the electrode side (Bockris and Khan, 1983).

2.3.2: Charge Transfer Across the Interface

Electrode processes are heterogeneous reactions in which charge transfer occurs on the electrode-solution interface. The electrons pass through the electrode to the interface where charged species in the solution pick them in a reduction process. This may lead to deposition as shown by the Equation 2.1 (Bockris and Khan, 1983).

$$M^{n+} + ne \rightarrow M_{s.} \; . \qquad (2.1)$$

Conversely during oxidation, an electro-active species present in solution moves to the interface where it loses electron, in an oxidation process according to the Equation 2.2. The oxidized species goes back to the bulk solution after oxidation at the interface.

$$M_s \rightarrow M^{n+} + ne. \qquad (2.2)$$

These redox-reactions occurring at the interface may not be the only reactions taking place. Sometimes the redox process is followed by other chemical reactions which may result in formation of products that are not electro-active. Additionally, the products of electrode process may further undergo oxidation or reduction processes in the sample solution to form other oxidized or reduced chemical species as shown in Figure 2.6 below (Joseph, 2006).

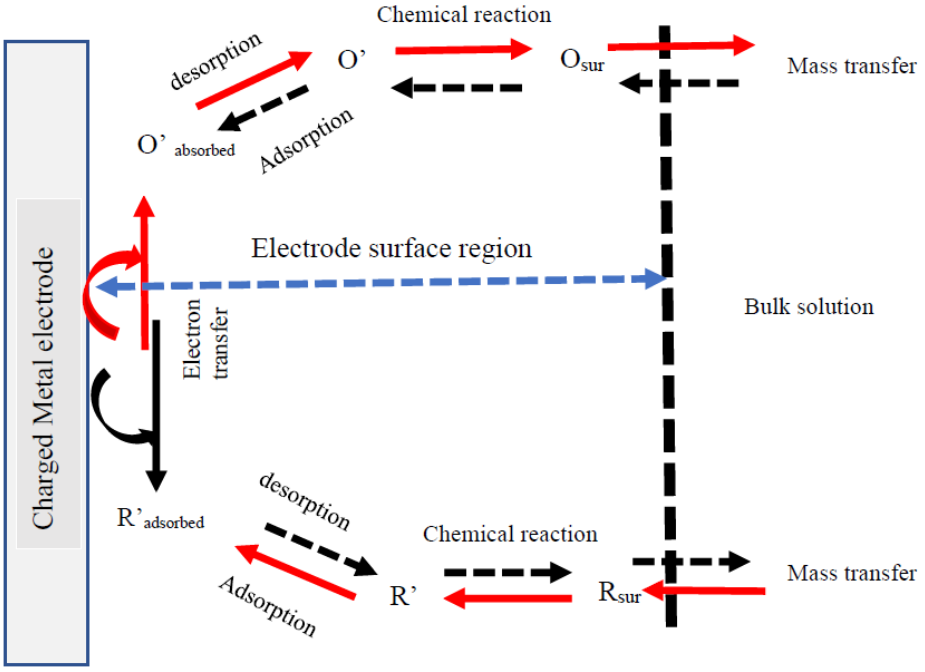


Figure 2.6: General steps in an electrode reaction (source: Bockris and Khan, 1983)

2.3.2.1: Rate Determining Steps

The rate at which the oxidized species are converted to its reduced form and vice versa is determined by the slowest step (Bard and Faulkner , 2001), amongst the steps discussed below;

i. Electrons transfer through the electrode

Electron movement through the electrode is an extremely fast process since the choice of electrode material is based on superiority of its electronic conductivity. Electrode materials commonly used include silver, platinum, stainless steel and mercury. It is therefore unlikely that charge transfer across the electrode could be the slowest electrode step.

ii. Charge transfer across electrode-solution interface

Electrons transfer rate across the solution-electrode surface can be expressed using rate constant, k_{et} which can be very fast at extreme potential. Since k_{et} can be made to be fast, then

the rate at which electrons are transferred across the interface cannot be a slow step, i.e. rate-limiting step.

iii. Mass transfer

If the first two steps are fast, i.e. movement of electron through electrode and its transfer across the interface, then the rate at which the oxidized species are reduced or vice versa will be limited by the movement of redox-active analyte from the bulk of solution to the electrode surface (Monk, 2001). The concentration of the electrogenerated species decreases near the electrode/solution interface as electrons are transferred between the charged electrode and the electro-active analyte in solution. Consequently, a concentration gradient develops which causes the movement of electro-active analyte to the electrode surface and diffusion of the electrogenerated materials away from the electrode surface. This movement of reactants and products to and away from the interface respectively is called mass transport (Bockris and Khan, 1983).

2.3.3: Mass Transport

Mass transport takes place in three distinct ways namely; diffusion, convection and migration.

2.3.3.1: Migration

This is the movement of ions through solution due to electrical gradient where ions are attracted by the electrode of opposite charge. The rate of migration depends on a number of factors, which includes; the size and charge on the ion and the force of attraction between the ion and the charged surface (Monk, 2001). It is desirable to remove the effects of migration in water-based solution. This is done by addition of excess unreactive ionic salt, called swamping electrolyte. The presence of the swamping electrolyte leads to accumulation of opposite

charged ions at the interface which reduces all the migration effects. Common swamping electrolytes used include potassium chloride and potassium nitrate (Creager, 2007).

2.3.3.2: Convection

Convection is a process in which the whole solution is moved to or away from the interface. The electro-active species are transported to or away from the electrode surface by the moving solution. There are two types of convection, natural and forced convection (Zoski, 2007). Forced convection is a deliberate motion introduced by researcher probably to increase the movement of materials of interest to or away from the interface. This may be done by stirring the solution, heating the solution, using rotating disc electrode or pumping gas among other methods. Forced convection methods yields irreproducible results unless attention is given to the geometry of the electrode and uniformity of the convection motion (Zoski, 2007) Natural convection occurs when electrolysis creates a region near electrode interface where concentration is depleted or enhanced than the bulk solution. Consequently, the solution species move by influence of gravity resulting from density difference. The extent to which the electrolyte solution responds to the gravitational pull occasioned by density difference depends on the geometry of the cell (Monk, 2001).

However, natural convection driving force is too small and it takes a lot of time for this force to overcome inertia of solution mass. Therefore, natural convection is insignificant especially in rapid experiments. In most electrochemical experiments the effects of convection are minimized by working in quiet solution (Bard and Faulkner , 2001).

2.3.3.3: Diffusion Mode

Diffusion occurs in response to inhomogeneous solution seeking to maximize its entropy (Zoski, 2007). Therefore, solute particles enrich regions of low concentration at expense of their neighboring regions of high concentration. Diffusion affects both charged and non-charged particles alike (Zoski, 2007). If the potential applied at the electrode surface oxidizes or reduces the analyte, its concentration at the electrode surface is reduced. Consequently, more analyte moves from the bulk solution to the electrode surface where they are reduced or oxidized. A concentration gradient develops in the vicinity of the electrode resulting to diffusion of analyte material to or away from the interface. The amount of substance being transported across a unit area of a surface in a unit time is called flux. This rate is defined by Fick's law which states that the rate at which electrogenerated material strike the electrode surface is directly proportional to the concentration gradient away from the electrode surface (Bard and Faulkner, 2001).

The concentration gradient results from exhaustion of electrogenerated material near the interface and build-up of product at the same time as the current flows. Fick's first law applies only in a steady state, where there is no change in concentration profile with time. Since systems may develop non-steady state conditions, the second law which introduces time-dependence is adopted for such systems (Bard and Faulkner, 2001).

Mass transport is a collective contribution of the three modes of transport, and it determines the rate at which the analyte impinges on the electrode surface, flux, which is expressed as;

$$j_i = function\ of \begin{pmatrix} component\ due\ to\ diffusion \\ component\ due\ to\ migration \\ component\ due\ to\ convection \end{pmatrix}$$

However, in a quiet solution containing a support electrolyte, diffusion provides the only means by which an electro-analyte species moves to the electrode (Monk, 2001).

2.3.4: Faradaic and Non-Faradaic Processes

Two different types of current may flow during an electrode process in an electrochemical cell. One kind of current involves transfer of electrons across the electrode/solution interface. This electron transfer process results into a chemical reaction. The resulting chemical reaction may either be an oxidation or a reduction process and it is governed by Faraday's law (Oldham and Zoski, 1983). If the electron transfer process is such that the amount of substance reduced or oxidized at the electrified surface is proportional to amount of current passed, then the process is said to be Faradaic.

Potential regions exist where faradaic processes cannot take place because such processes are considered thermodynamically or kinetically unfavorable. At such potentials no charge passes through the electrode/solution interface but other processes such as adsorption and desorption of both electroactive and electro-inert species occurs. Changes in potential can result in structural changes in the electrical double layer and consequently alter solution composition. These events may lead to flow of short-lived external current even when there is no flow of electrons across the interface (Oldham and Zoski, 1983). The portion of current in an electrochemical system which cannot be related to redox processes is said to be non-faradaic current. The resulting current is said to be non-faradaic current since it is not governed the faraday's law while the processes which bring about such current are said to be non-faradaic processes (Monk, 2001).

During an electrode process, both faradaic and non-faradaic current is produced. In most cases faradaic current is more useful than non-faradaic current and therefore many electrochemical processes are designed to minimize or eliminate the contribution of non-faradaic current. The concentration of electro-active substance can be estimated by measuring the faradaic current produced when the substance is oxidized or reduced at the electrode surface (Bard and Faulkner, 2001).

2.4: Electro-Analytical Methods

This is a group of analytical methods used to study analytes that are electro-responsive. They choice method for electro-active samples because of their ability to detect analytes at very low concentration using relatively inexpensive equipments (Özel *et al*., 2014). They often target a specific oxidation state of an element unlike other methods which may be interested on the concentrations of the element. They therefore provide information about activities as opposed to concentration (Alcalde *et al*., 2019). The analyte is studied in a support electrolyte by measuring current at the potential at which the analyte is reduced or oxidized. Electro-analytical methods are mainly classified into three groups; potentiometry, coulometry and voltammetry.

The basic concept of analytical methods is similar and involves the following stages (Bard and Faulkner , 2001 as demonstrated in Figure 2.7.

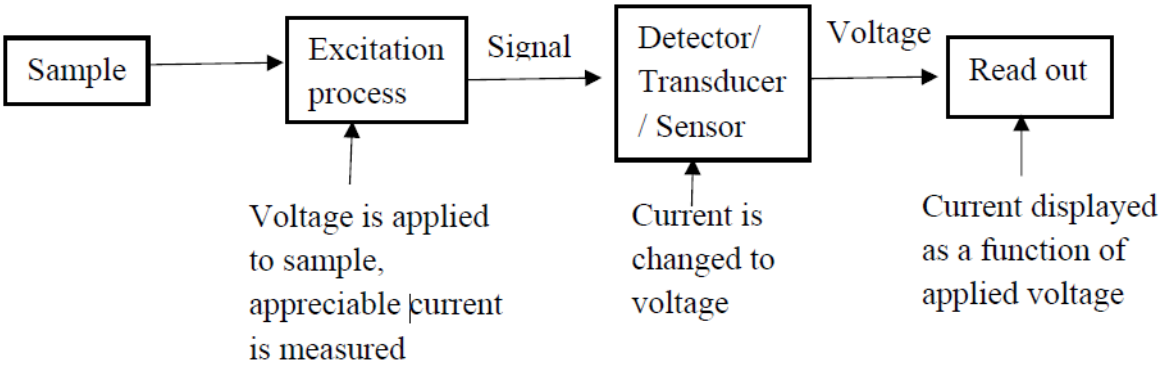


Figure 2.7: Basic functionality of electrochemical methods (Source: Bard and Faulkner , 2001)

2.4.1: Voltammetry

Voltammetry is a group of electro-analytical microanalysis techniques in which the chemical behavior of an electro-active substance is gathered from the current obtained when potential is applied on the indicator electrode (Pandit, *et al*., 2017). Potential is scanned at varying scan rate (*v)*, cathodically or anodically resulting into a current-potential plot called a voltammogram. The analyte material undergoes reduction or oxidation by varying the applied potential on the electrode. Change in potential forces electro-active species in solution to either gain or lose electron. This occurs because the electrode is polarized. Most often, a potentiostat provides the power required in voltammetry (Kounaves, 1997).

There are various techniques used in voltammetry. Voltammetric methods are classified depending on;

i) The type of electrode used

ii) How the potential is applied to the indicator electrode to drive an electrochemical reaction.

iii) How the current resulting from the electrochemical reaction is measured.

Based on these factors, some of the common methods used to analyze electro-active samples are discussed in the following subsections.

2.4.1.1: Linear Sweep Voltammetry

It's a voltammetric technique which involves scanning the potential of the indicator electrode from an initial point, E_i to final point E_f at a certain sweep rate (v) while recording the resulting current. Sweep rate refers to the rate at which the potential is scanned per unit time. The scan rate is constant in a particular scan but can be varied to provide more information depending on the need of the analyst. The potential is scanned from a point where there is no response from the analyte, E_i to a point where the current is the limiting factor, E_f. Linear scan voltammograms have a sigmoidal shape as demonstrated below.

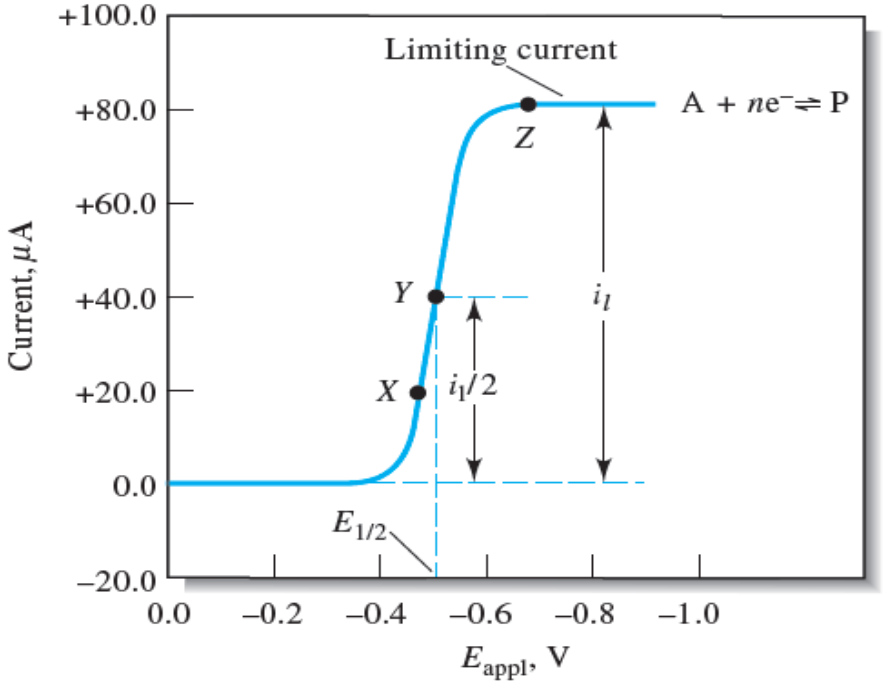


Figure 2.8: Typical linear sweep voltammetry for reduction of species A to P. Source: **(**Skoog *et al.*, 2014**)**

Limiting current is the limiting value of a faradaic current, usually illustrated on a voltammogram as a plateau after a steep rise in current. Limiting current results from a decreasing movement of electrogenerated species to the electrode surface (Skoog *et al.*, 2014). Since the magnitude of faradaic current is proportionate to the rate of transfer of analyte to the electrode surface, limiting current is an indication of constant thickness of diffusion layer. However, this will only happen when the support electrolyte is stirred. In absence of stirring, diffusion layer increases resulting into a peak current instead of limiting current (Skoog *et al.*, 2014). Therefore, at the potential where the analyte is oxidized or reduced, a peak or trough is formed.

2.4.1.2: Cyclic Voltammetry

Cyclic voltammetry is a fundamental method used to provide qualitative information on electrochemical properties of an analyte dissolved in a support electrolyte or at the surface of an electrode (Elgrishi, *et al*., 2018). Besides providing key information on kinetics of electrode reactions, cyclic voltammetry provides an easy and fast method to locate redox potentials of redox-active analytes (Kounaves, 1997). This is done by scanning the potential of the indicator electrode linearly. The corresponding trace of current versus potential is referred as a cyclic voltammogram as shown in the Figure 2.9.

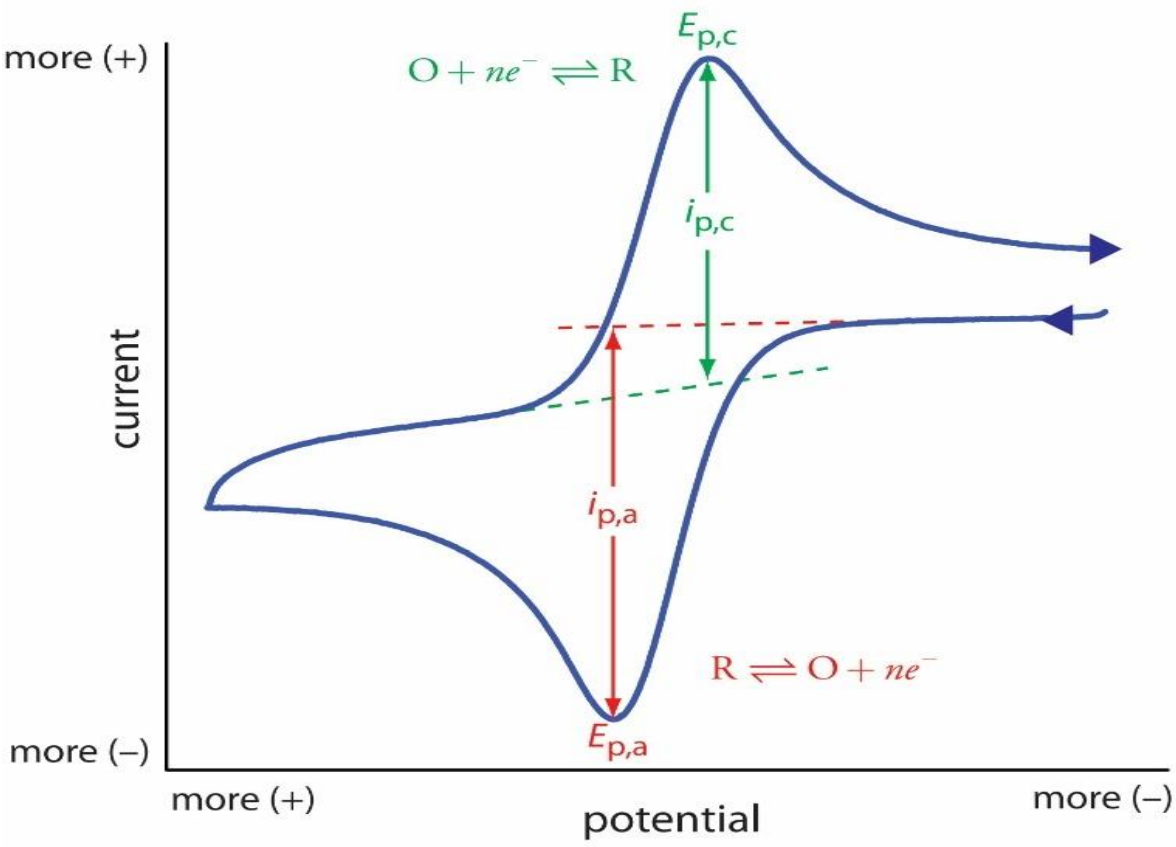


Figure 2.9: A cyclic voltammogram of a reversible redox reaction. Source (Elgrishi, *et al*., 2018)

Cyclic voltammogram uses cyclic potential waveform which consists of a forward and a reverse scan (Elgrishi, *et al*., 2018). If the forward scan is anodic (increasing potential) then

the reverse scan will be cathodic (decreasing potential) and vice versa. However, most of time it is assumed that the solution contains oxidized species at the beginning; therefore, the first scan is done in increasing negative potentials. As the characteristic formal potential (E^o) of the analyte is approached, cathodic current grows exponentially until a peak is formed (Elgrishi, *et al*., 2018). The scan is reversed after traversing the reduction potential window. The reduced species that were formed during the forward scan are re-oxidized back resulting into anodic peak. When the scan is done in quiescent solution, the diffusion layer increases leading to formation of a peak (Leonat *et al*, 2013; El-Maali *et al*., 2005)

In cases where electrochemical study of polymer films of the analyte is required, multiple scans are done to electrodeposit the polymer film on the electrode surface (Sun *et al*., 1990). Electrodeposition modifies electrode surface thus improving the electrochemical properties of some electrodes. Cyclic voltammetry provides very useful information, for example, in reversible redox systems a plot of peak current versus square-root of scan rate produces Randles-Sevcik plot which is used to calculate diffusion coefficient of the redox species (Elgrishi, *et al*., 2018).

2.4.1.2.1: Circuit

The potential of the working electrode is scanned linearly in a quiescent support electrolyte using a polarizing circuit while simultaneously monitoring the current produced using a measuring circuit (Elgrishi, *et al*., 2018).

2.4.1.2.2: Scan rate

The rate at which potential changes with time is called scan rate. Basically, the scan rate controls the time spent on an electrochemical reaction. At fast a scan rate, the diffusion layer formed is

small, consequently high currents are recorded. At slow scan rate, the diffusion layer is large and the diffusion of electro-active material to the electrode surface is slow, hence low currents are recorded (Elgrishi, *et al*., 2018). Square-root of scan rate is correlated with peak current i_p for reversible systems i.e (Bard and Faulkner, 2001).

$$i_p \propto \nu^{1/2} \qquad (2.3)$$

2.4.1.2.3: Switching potentials and excitation Signal

Cyclic voltammetry involves scanning the potential of the working electrode immersed in an unstirred solution linearly. The varying potential impressed on the working electrode, produces a characteristic measurable current response called the excitation signal (Kissinger and Heineman, 1983).

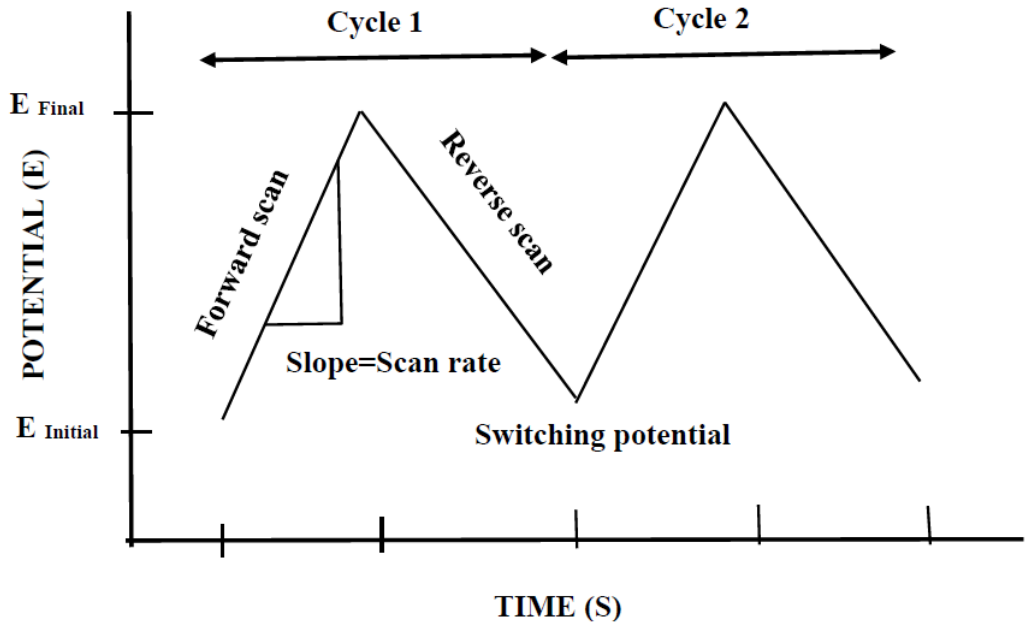


Figure 2.10: Potential-time excitation signal for cyclic voltammetry. Source: (Kissinger and Heineman, 1983).

The excitation signal for cyclic voltammetry is a linear potential cycle with a triangular waveform. The potential at which the scan is reversed is referred as the switching potential and it corresponds with the final potential of the linear scan (Elgrishi, *et al*., 2018).

2.4.1.2.4: Nature of a Cyclic Voltammograms

As the potential of working electrode is cycled to lower potential values, current begins to flow when voltage reaches a value that allows reduction to take place. The current keeps increasing while all the electro-active species at the vicinity of working electrode are reduced till a peak current is attained. This peak current is called cathodic peak current (I/p_a) and the potential at which it occurs is referred as cathodic peak potential *(E_{Pc})* (Elgrishi, *et al*., 2018). Current decreases as the electro-active species near the electrode surface are depleted. When the potential scan is reversed, the reduced species at electrode surface are oxidized. The current begins to flow until a peak it attained. The current at the peak is known as anodic peak current (I/p_a) while the corresponding potential is called anodic peak potential *(E_{Pc})*. Current drops after the peak is reached as the reduced species gets depleted at the electrode surface (Elgrishi, *et al*., 2018).. The position of peaks on potential axis is related to formal potential which is obtained from the average of the cathodic and anodic peak potentials as shown in Equation 2.4 below (Elgrishi, *et al*., 2018).

$$E^O = \frac{E_{p,a}+E_{p,c}}{2} \qquad (2.4)$$

2.4.1.2.4.1: Reversible Cyclic Voltammetric processes

A cyclic process is said to be reversible if electron transfer is quick enough to ensure that the concentration of the species being reduced and oxidized at the electrode surface remains

constant during electrode potential sweeping (Bard and Faulkner, 2001). The concentrations of the species undergoing oxidation and reduction can be related using the Nernst equation (Bard and Faulkner, 2001) as expressed below.

$$E = E_O + \frac{RT}{nF} ln \frac{C_{ox}}{C_{red}} \quad (2.5)$$

where, n is number of electrons, R is the universal gas constant ($8.314JK^{-1}mol^{-1}$), T is temperature in kelvins and F is Faraday constant ($1F=96485.33C\ mol^{-1}$)

The magnitude of peak currents for a reversible electrode process can be estimated using the Randles-Sevcik equation (Elgrishi, *et al*., 2018). given below;

$$i_{pa} = 0.446nFAc\sqrt{\frac{nFvD}{RT}} \approx (2.69 \times 10^5)n^{3/2}AD^{1/2}cV^{1/2} \quad (2.6)$$

Where,

n = Number of electrons,

D = Diffusion coefficient of electro-active analyte in square centimeter per second (cm^2s^{-1}),

V = Potential scan rate in volts per second (Vs^{-}),

A = Electrode area in square centimeters (cm^2),

C = Concentration of the electro-active species in moles per cubic centimeter (mol/cm^3).

At 25°C, all reversible systems are characterized by the following features.

i) Peak current is directly proportional to square root of scan rate (*Equation* 2.3)

ii) The ratio of peak currents, i.e. anodic and cathodic current is one.

$$i_{pa}/i_{pc} = 1 \quad (2.7)$$

iii) The position of the formal potential is related to anodic *(E_{Pa})* and cathodic peak potential *(E_{Pc})*, i.e. E^o centered between E_{Pa} and E_{Pc} (*Equation* 2.4)

iv) Peak separation for reversible one electron system is 59mV as given in Equation 2.8 below where n is the number of electrons. Equation 2.8 (Kissinger and Heineman, 1983) can therefore be used to calculate number of electrons in a fast electron transfer process.

$$\Delta E_p = E_{pa} - E_{pc} = {}^{59}/_{n}\, mV \qquad (2.8)$$

A system that fulfills all the above conditions is said to be electro-reversible. If a system does not fulfill any of the above conditions, then, it's not fully reversible. Such systems could either be quasi-reversible or irreversible. For reversible multi-electron transfer systems, the voltammogram will have several distinct peaks if the formal potentials of each redox-couple are well separated out (Elgrishi, *et al*., 2018).

2.4.1.2.4.2: Irreversible Cyclic Voltammetric processes

They are marked by a sluggish charge transfer across electrode/solution interface. Consequently, only the forward peak is observed sometimes with a suppressed reverse peak (Nicholson, 1965). The peaks are also smaller in size and widely separated as shown in Figure 2.11. Peak potential will shift with increasing scan rate. Irreversible reactions give smaller currents compared to reversible systems and therefore a bigger overpotential is required for reduction. Therefore, the peak potentials appear at higher values beyond the E^o. Current peak for systems that are irreversible is given by Equation 2.9 (Bard and Faulkner, 2001)

$$i_p = 2.99 \times 10^5 n(\propto n)^{1/2} A D_o^{1/2} v^{1/2} C_o \qquad (2.9)$$

Where $(\propto n_a) = \frac{47.7}{E_p - Ep_{/2}}$ (2.10)

where α is the electron transfer coefficient and n is the number of electrons involved in the electrode process.

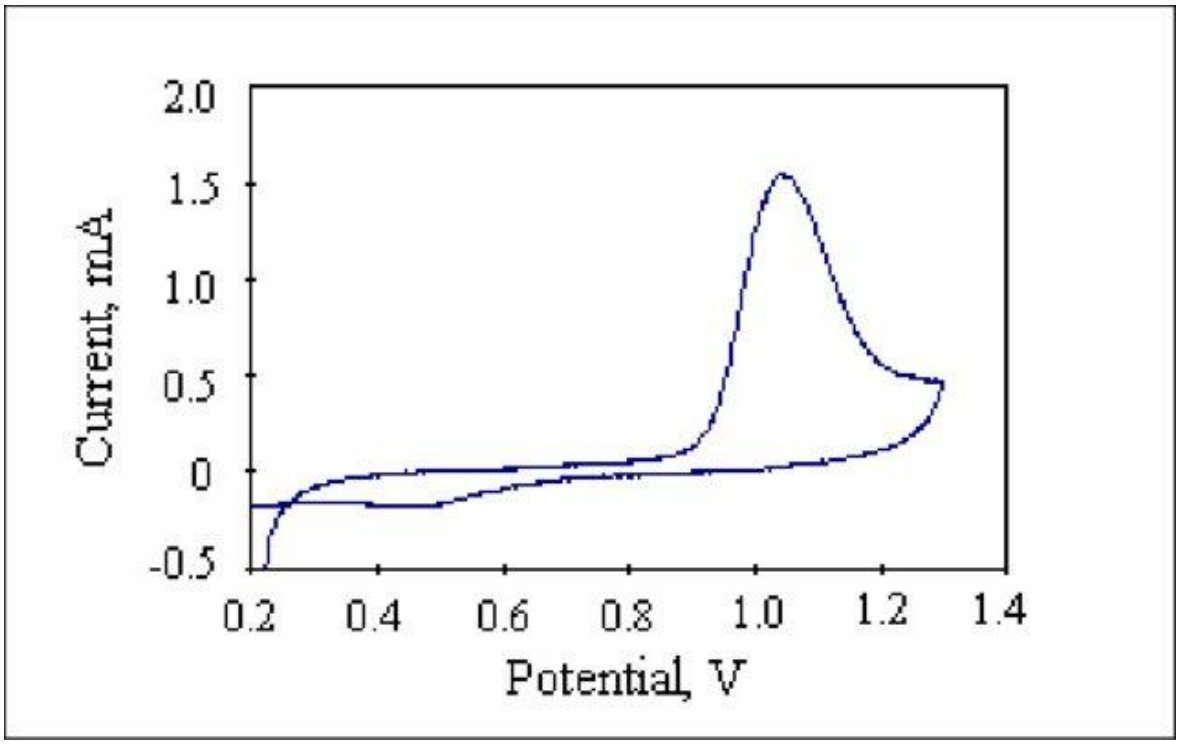


Figure 2.11: An irreversible cyclic voltammogram of 8mM phenol in sulfuric acid 0.5M on platinum electrode. Source: (Pirvu *et al*., 2008)

The peak separation, E_p which determines whether electrode process is reversible or not and is given by the Equation 2.11 (Bard and Faulkner, 2001)

$$E_p = E^o - \frac{RT}{\propto n_a F}\left(0.78 - ln\frac{K^o}{D^{1/2}} + \ln\left(\frac{\propto n_a F v}{RT}\right)^{1/2}\right) \quad (2.11)$$

Where $\propto$ is transfer coefficient, k^o is the standard electrode reaction constant, n_a is the number of electrons involved in charge-transfer step while the rest have their usual meaning.

Irreversible cyclic System at 25^{o}C is characterized by:

i. Absence of reverse peak

ii. E_p shift $= \frac{30}{\propto n_a} mV$ (2.12)

where $\propto$ is transfer coefficient

iii. $E_p - Ep_{/2} = \frac{47.7}{\propto na} m$ (2.13)

2.4.1.2.4.3: Characteristics of quasi reversible systems

Quasi reversible cyclic voltammograms result from slow charge transfer across the electrode-solution interface. As a result of slow electron transfer, equal concentration of the oxidized and reduced species cannot be maintained. Consequently, the peaks are more widely separated than in reversible systems and peak separation differs with the scan rate (Monk, 2001). The shape of the voltammograms depends on the heterogeneous rate constant and scan rate. With increasing scan rate, the quasi-reversible systems exhibit irreversible behavior while at lower scanning rate, they show near reversible tendency. Acording to Monk (2001), quasi-reversible system at 25 ^{o}C are characterize by:

i. Peak current (i_p) increases with increasing scan rate though the increase is not proportional to scan rate.

ii. The ratio of peak currents, i.e., anodic and cathodic current is one (*Equation* 2.7) assuming $\propto_c = \propto_a = {}^{1}/_{2}$

iii. $\Delta E_p > {}^{59}/_{n}$ mV (2.14)
which increases with increase in scan rate

iv. E_{pc} shift negatively with increasing scan rate (v)

2.4.1.3: Stripping Voltammetry

Stripping voltammetry is one of the most sensitive methods of electro-analysis. It involves pre-concentration of the electro-active material from dilute solutions to indicator electrode surface. The electro-active material is then stripped off from the working electrode surface by changing

the direction of the current (Brainina *et al*., 2000). Usually mercury drop/film electrode is used in a stirred solution. Striping methods involve three key steps, deposition, quiet time and stripping. Some of the advantages of stripping voltammetry include very low limit detection and multi-element and speciation capabilities (Achterberg and Braungardt, 1999).Commonly used stripping methods include; anodic and cathodic stripping voltammetry.

2.4.1.4: Anodic Stripping Voltammetry

It involves two main steps, one, electrodeposition of electro-active material at the electrode surface with the aim of pre-concentrating the species of interest. This step is carried out in stirred solution to enhance mass transfer (Brainina *et al*., 2000). The second step involves a linear potential scan in anodic direction to strip out the electroplated material while recording the resulting current. This step is done in unstirred solution. ASV is mostly used in trace metal analysis (Dragoe, *et al*., 2006). The electrodeposited metal is a couple of hundred times more than what is in the solution. As the potential is scanned anodically, electro-active species deposited at the electrode surface are oxidized resulting to a peak current signal at the potential where oxidation takes place (Brainina *et al*., 2000).

2.4.1.5: Cathodic Stripping Voltammetry

It is a sensitive electroanalytical technique used for analysis of low levels of organic and inorganic compounds in solution (Barra and Dos Santos, 2001). Like anodic stripping voltammetry, it is a two-step method involving deposition and stripping. Deposition is the first step and it occurs at oxidizing potential. The anodic deposition is followed by sweeping the potential cathodically which strips the electrodeposited analyte from the electrode into solution (Brainina *et al*., 2000)..

$$M(Hg) \rightarrow M^{n+} + ne \quad (2.15)$$

This technique is suitable for a wide range of compounds forming insoluble salts with mercury, e.g. penicillins (Achterberg and Braungardt, 1999).

2.4.2: Pulse Voltammetry

In voltammetry the potential is gradually changed with time. However, in pulse Voltammetry the initial potential i.e., where no reaction takes place, is directly driven to a final potential where reaction is at its maximum (Molina and González, 2016). Since pulse methods involve only the initial and final potential, current signal is plotted against time. In spite of voltammetric methods being good analytical tools, at lower concentration the currents cause non-faradaic effects which lower the accuracy to unacceptable limits (Molina and González, 2016). However, pulse voltammetry methods can attain a lower detection limit, down to 10^{-8}M by reducing the effects of non-faradaic currents. When the indicator electrode is dropping mercury electrode (DME), highest currents are attained at the tail end of each drop's life-time. To increase sensitivity and discriminate against non-faradaic current, current is monitored during the end of each drop's life time (Molina and González, 2016). In pulse methods, current is measured during the last 15% of its lifetime; this significantly increases the sensitivity of these methods. The most common used polarographic methods are discussed below.

2.4.2.1: Features of Normal Pulse Voltammetry (NPV)

It involves application of a series of potential pulses of increasing amplitude at the dropping mercury electrode (DME) (Molina and González, 2016). To ensure that the analyte is not

involved in any reaction, the potential of the working electrode is kept at constant potential between pulses. Measurement of current is done when the effect of non-faradaic current is almost zero. The pulses are short-lived to ensure a thinner diffusion layer, higher flux of analyte, hence increased faradaic current (Molina and González, 2016).

2.4.2.2: Features of Differential Pulse Voltammetry (DPV)

This method is useful in trace analysis for both organic and inorganic substances. The potential pulse is fixed and superimposed on linear potential ramp and applied to hanging mercury working electrode(HMDE) just before the end of the drop's life time (Madkour, 2000). The current is recorded before and after pulse application and the difference is graphed against the applied potential as shown in Figure 2.12.

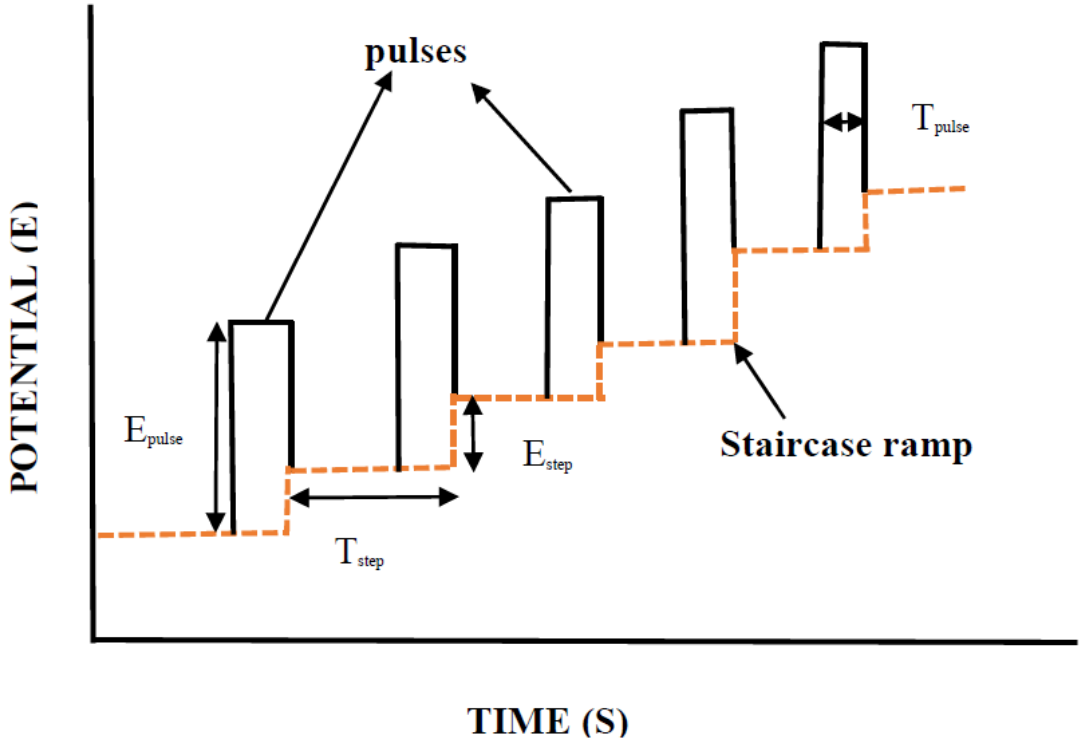


Figure 2.12: Potential-time function resulting from superimposition of potential pulses on staircase ramp (Osteryoung and Osteryoung 1985)

The height of peak current obtained from the resulting voltammogram is dependent on the concentration of the analyte present. The height of the peak current may be expressed using the Equation 2.16.

$$\Delta i_p = \frac{nFAD^{1/2}C}{\sqrt{\pi}t_m}\left(\frac{1-\sigma}{1+\sigma}\right) \quad (2.16)$$

Where $\sigma = \exp\left(\left(\frac{nF}{RT}\right)\left(\frac{\Delta E}{2}\right)\right)$ (2.17)

ΔE is pulse amplitude,

t_m is the time after application of pulse when current is sampled,

A is the electrode surface area

D is diffusion coefficient for the species

C is the concentration of the analyte

N is the number of electrons

F is the faraday constant

R is gas constant and T is temperature.

The highest value for $\frac{1-\sigma}{1+\sigma}$ obtained from large impulse is one. The peak potential E_p occurs near the half-potential and it can be used for species identification (*Equation 2.18*)

$$E_p = E_{1/2} - \frac{\Delta E}{2} \quad (2.18)$$

2.4.2.3: Square Wave Voltammetry (SWV)

It is one of the most powerful analytical tools which offers a great deal of speed, high sensitivity, reduced fouling of electrode and better discrimination of charging and background currents (Molina *et al*., 2011; Batchelor-McAuley, *et al*., 2015). Square wave voltammetry consists of two waveforms; symmetrical square wave and staircase waveform. The

symmetrical square wave is superimposed on a base staircase potential and applied on the working electrode as shown in Figure 2.13. One of the square wave pulses coincides with the staircase step while the other one takes place mid-way through the staircase step and continues until the end of the step. The first pulse occurs in the scan direction and it is referred as forward pulse. In reverse pulse, the amplitude is applied in direction opposite to the scan (Molina *et al*., 2011).

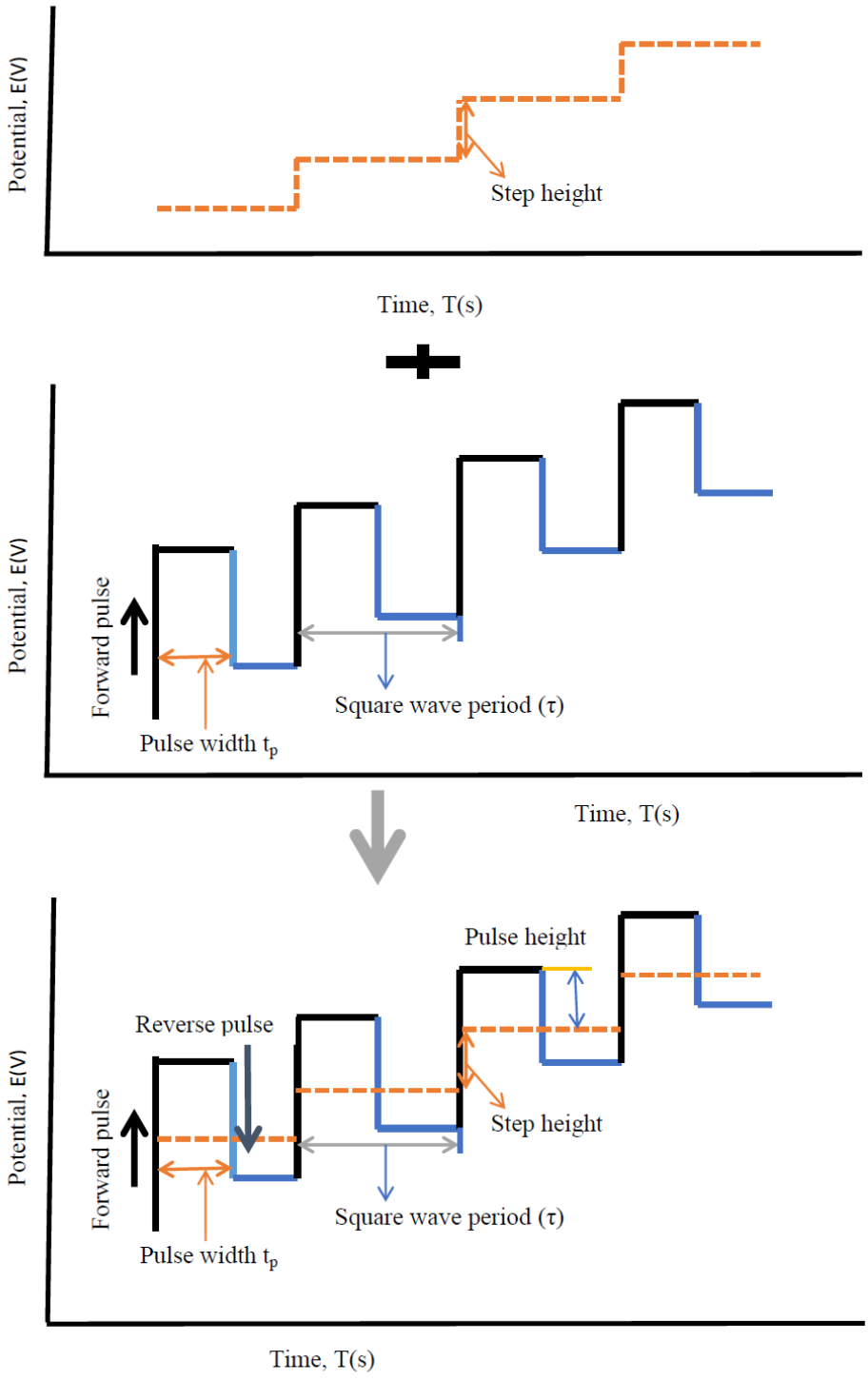


Figure 2.13: A wave form showing square wave superimposed on a base staircase (Osteryoung and Ousteryoung, 1985)

During a square wave procedure, the potential of the indicator electrode is stepped up through a series of forward and reverse pulses from initial to final potential (Skoog *et al*., 2014**).** Square wave is characterized by a number of parameters namely; square wave frequency *(f)*, staircase period or square wave period (τ), pulse width (t_p= τ/2), step height (ΔE_s) and pulse height (ΔE_{sw}) (Skoog *et al*., 2014**)**. Square wave period describes the sum total of time taken by both the forward and reverse pulse. Square wave frequency is the inverse of square wave period (*f* =1/ τ) and it is expressed in hertz (Hz). The duration of each pulse, i.e. pulse width is half the square wave period; t_p= τ/2. (Skoog *et al*., 2014**)**

The reverse pulse re-oxidizes the reduced product formed during the forward pulse at the electrode surface. Current measurements are sampled twice in every wave cycle, one at the end of the forward pulse (i_1) and the other one at the end of the reverse pulse(i_2) (Wang, 2000). Current measurement near the end of the pulse effectively discriminates against capacitative current. The net current $\Delta i(i_2 - i_1)$ is plotted against base staircase potential to give a peak shaped voltammogram which is symmetrical about half-wave potential as shown in Figure 2.14. The peak current is proportional to the concentration of the analyte (Wang, 2000).

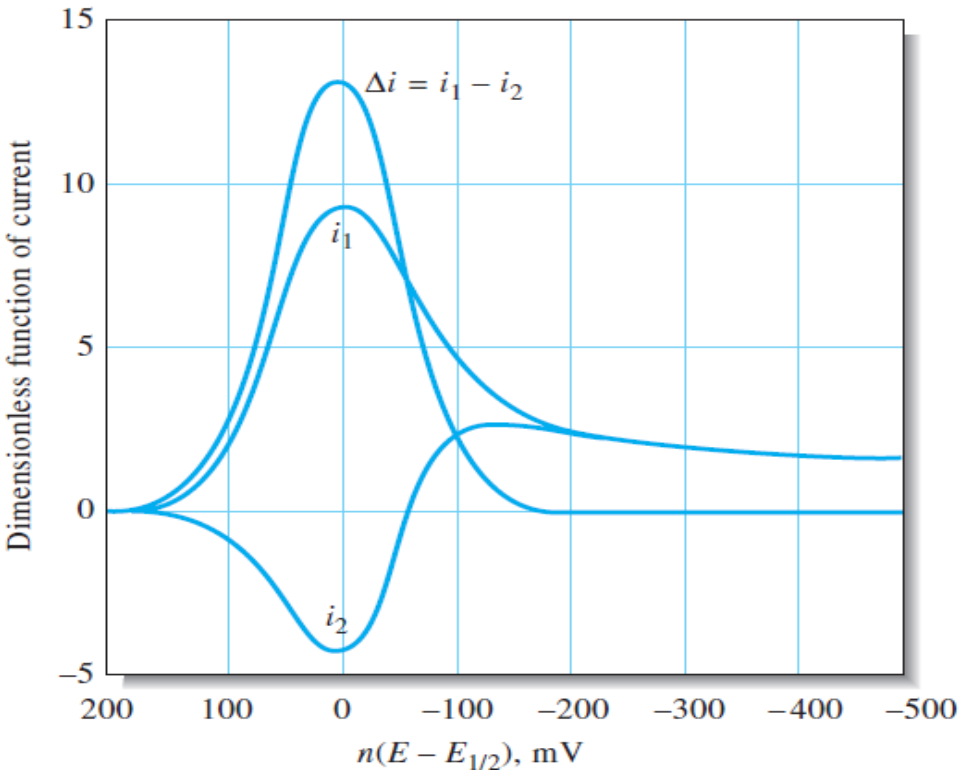


Figure 2.14: A typical SWV containing the forward, reverse and net currents. Source: (Skoog *et al*., 2014**)**

The net current is greater than both the reverse and forward current since it's a difference between them (Williams-Dottin, 2001). The net current is used in most analytical work because it dicriminates against charging current. SWV has higher sensitivity than differential pulse voltammetry and it's able to achieve detention limits as low as 10^{-8}M. It also has an additional advantage of faster analysis time due to the frequency range used, i.e. 1Hz to 120Hz. Due to the its speed, square wave analysis is up to one hundred times faster than other pulse techniques (Wengenack, 1987). Sometimes static working electrodes such as hanging mercury drop electrode are used. In such cases, mercury drop is deposited on the tip of electrode and remains there throughout analysis. This significantly reduces the amount of mercury used.

Square wave voltammetry can also provide kinetic information on the processes taking place at the electrode surface. Analyzing the forward and reverse currents provides useful

information regarding the nature of the electrode process, i.e., whether reversible, quasi reversible or irreversible. When the forward and the reverse currents are symmetrical, the process is reversible. In such cases the electron transfer is fast enough to maintain the oxidized and reduced species concentration at equilibrium at the interface. However, if the forward and reverse currents are asymmetrical, then the rate of charge transfer is not fast enough hence the process may be quasi reversible or completely irreversible.

Compared to cyclic voltammetry, SWV offers many advantages over cyclic voltammetry such as high scanning speed, excellent sensitivity, ability to reject the background current and high signal to noise ratio (Kounaves, 2007). It therefore used to characterize electro-active species whose cyclic voltammograms are poorly formed by producing clear peaks and also analyzing species at very low concentrations (Williams-Dottin A., 2001). Generally, SWV is a better analytical tools than the other pulse methods, because of its ability to suppress non-faradaic currents, greater sensitivity than differential pulse voltammetry, faster scan times, and wider area of applicability (Bard and Faulkner , 2001). Additionally, this method is free from oxygen interference because it is very fast and the limiting current plateau of oxygen is automatically cancelled out. Additionally, square wave signals are insensitive to currents arising from convective mass transport if the convective time is longer than the pulse width (Wengenack, 1987).

2.5: Surfactants

Surfactants or Surface active agents are a group of amphiphilic compounds possessing hydrophilic polar head and hydrophobic tail (Farn, 2006). The polar head group contains functional groups such as thiols, alcohols, ethers, acids, sulfates, amines, amide, sulfonate

phosphate among others. The non-polar tail group consists of long alkyl or alkylbenzene hydrocarbon chain. Hydrophobic groups reduce their contact with water by aggregating with their hydrophilic groups oriented towards the aqueous phase (Lawrence, 1994). Aggregates of surfactants are known as *micelles.* Micelles are formed from soluble surfactants solution above their critical micelle concentration (CMC) (Andersen, *et al*., 2009). These aggregates formed possess regions of hydrophobic and hydrophilic character. Surfactants possess two important properties that are crucial in electrochemistry

I. Tendency to migrate to interfaces or surface and aggregate into supramolecular structures (Vittal *et al.,* 2006).
II. Ability of surfactant molecules to orient themselves to minimize the contact of hydrophobic end with water, consequently changing the properties at the interface (Farn, 2006).This has been used to alter or enhance reaction kinetics and control reaction pathway.

Owing to the above properties, surfactants have been proven to be effective in electrochemical analysis of pharmaceuticals and compounds of biological origin. They have been shown to improve accumulation of electro-active species of some compounds such as ethopropazine and protect the electrode surface from fouling (Atta *et al.,* 2011; Huang *et al.,* 2004). Surfactants have also been shown to facilitate simultaneous determination of some compounds such as, ascorbic acid and dopamine (Dos-Reis *et al*., 2005) and catechol and hydroquinone (Peng and Gao, 2006). Surfactants have also been used to increase solubility of some organic compounds during electro-analysis (Vittal *et al.,* 2006). Moreover, surfactants have also been found to control electrochemical catalysis through their micro-structures.

2.5.1: Classification of Surfactants and Their Chemistry

Two main types of surfactants exist, non-ionic and ionic surfactants. Ionic surfactants are further classified into anionic, cationic and amphoteric or zwitterion (Salager, 2002; Farn, 2008). The classification is based on the charge on the surfactant's head.

i) Non- ionic surfactants - These surfactants do not dissociate into ions and therefore do not have an electrical charge (Salager, 2002). However, some like tertiary amine oxides acquire charge depending on the pH. They don't dissociate in polar solvents because their hydrophilic group is non-dissociating type, e.g., alcohols, ethers phenols amide etc.

ii) Anionic surfactants- are amphiphilic substance that contain anionic group attached directly or through an intermediate to a long hydrocarbon chain (Farn, 2008). They are most commonly used for laundry work because of their superior cleaning properties. They dissociate in water to form surface-active anions and hydrated cations. The hydrocarbon chain can be a straight chain aliphatic radical as in the case of soap or other groups. The most common example of anionic surfactant is sodium dodecyl sulphate or soap.

iii) Cationic surfactants- a surfactant is said to be cationic if it dissociates in a solvent to form a surface-active cation and a normal anion (Farn, 2008). Cationic surfactants are not good cleaning agents and therefore are not used in general purpose detergents. They are mainly used as germicidal or antistatic agents (Azarmi and Ashjaran, 2015).

iv) Amphoteric/ zwitterionic- If the surfactant has the oppositely charged centers in one molecule displaying both cationic and anionic character is said to be amphoteric or

zwitterionic surfactant. Depending on the pH of the solvent media, amphoteric surfactants may be cationic, anionic or non-ionic.

2.5.2: Micelles in Aqueous Media

Solubility of surfactant is dependent on the length of the hydrocarbon tail. Surfactants with long hydrophobic moiety are less soluble while those with short hydrophobic moiety are more soluble (Farn, 2008). Surfactants form true solutions at low concentration (Salager, 2002). In these solutions, the surfactant molecules are dispersed and they don't self-aggregate to form micelles. However, as the concentration increases, due to their amphiphilic nature, they self-associate in solution leading to formation of micelles (Vittal *et al*., 2006). Micelles are therefore clusters of long chain surfactant molecules which form spontaneously from solution at a certain concentration (Salager, 2002). This concentration depends on the size of the hydrocarbon chain, the hydrophilic polar head group, temperature, pH presence of co-solutes, salt concentration and counter-ions (Atta *et al.*, 2014). In water the hydrophilic head is oriented towards water while hydrophobic chain is aligned facing away from water to interior of the micelle (Salager, 2002).

2.5.3: Electrode Modification Using Surfactants

Modification of an electrode surface using surfactants provides an excellent way to control and modify reactions at the electrode surface (Atta *et al.*, 2014). Surfactants can be used to modify electrode surface in two ways:

i) Surface modification which involves changing the properties of the electrode surface through adsorption or self-assembly of the surfactant.

ii) Bulk modification which involves mixing the surfactant intimately with electrode material to make a paste e.g., carbon paste.

Different approaches have been used to adsorb surfactants on electrode surface. These approaches may involve physico-chemisorption of highly ordered self-assembled mono-layers (SAMs)(Angel-Kaifer and Marielle-Gomez 1999). The surfactant self-assembly structures can also form through physio-sorption of the surfactant molecules on the electrode surface. This can be done by drop-casting a dilute surfactant solution on electrode surface or immersing the electrode in surfactant solution (Attwood and Florence, 2012).

2.5.4: Influence of surfactants on the electrochemical kinetics

There are various factors to put into consideration when estimating the influence surfactants have on the electrochemical kinetics of surface reaction. Among these are the dimension, polarities and molecular structures of micelles, orientation of the electro-active species within the surfactant (Kamble, *et al*., 2017). Additionally, distance between the electrode and electro-active analyte and how the surfactant orients itself on electrode should be taken into account (Atta *et al.*, 2014).

Adsorbed surfactant film has two major influencies on electron transfer rate. One, it may deny the analyte access to the electrode surface, therefore blocking charge transfer (Mackay, 1994). Blocking effect may result from the unfavorable orientation of the surfactant and electrogenerated species on the electrode surface (Guidelli and Foresti, 1977). Secondly the

presence of the surfactant may enhance the electrochemical reaction on the electrode surface especially if electro-active species have been accumulated and well oriented on the adsorbed surfactant film (Marino and Brajter-Toth, 1993). The coulombic attraction forces and hydrophobic interaction between electro-active species and adsorbed surfactant film are responsible for pre-concentration process (Guidelli and Foresti, 1977). However, the presence of the adsorbed surfactant increases the distance between the analyte and electrode surface. According to electron transfer theory, charge transfer rate is inversely proportional to the distance between the electro-active material and the electrode (Guidelli and Foresti, 1977).

2.5.5: Surfactant Adsorption

Surfactants are characterized by tendency to migrate to interface and orient themselves in a certain way due to their dual polarity (Hosseinzadeh *et al*, 2009; Paria and Khilar, 2004). Surfactant molecules can adsorb on the electrode surface from bulk solution through various mechanisms. These include:

i) Ion exchange which occurs via substitution of counter ions by surfactant ions bearing the same charge (Farn, 2008).

ii) Through pairing of surfactant ions with oppositely charged counterions. The surfactant ions adsorb on the sites that are not occupied by counterions (Farn, 2008).

iii) Hydrophobic bonding -if hydrophobic group is adsorbed on the electrode surface, it can attract other hydrophobic groups of molecules in solution. Such kind of attractions can lead to attachment of a surfactant on electrode surface (Salager, 2002).

iv) Adsorption due to attraction between pi electrons and positive nucleus of adsorbed material. This occurs especially if the surfactant contains electron rich groups while the adsorbed material has positively charged sites or electron deficient groups. The attraction between these groups would lead to attachment of surfactant on the electrode surface (Salager, 2002).

v) Adsorption through van der waals forces between the surfactant molecules and absorbent molecules from solution (Farn, 2008).

2.5.6: Electron transfer in surfactant containing solutions

Presence of aggregates of surfactants in a support electrolyte can significantly affect the electron transfer kinetics across the solution\electrode interface (Farn, 2008). Surfactants tend to absorb at the electrode surface in different ways beyond the critical micelle concentration (Kamble, *et al*., 2017). On hydrophilic electrode surface, head down orientation of surfactant molecules is preferred (Rusling, 1997). When the surfactant film is attached on the electrode surface, electron transfer begins when an electro-active species draws near a surfactant modified electrode surface (Kamble, *et al*., 2017). There are two possible ways for electron transfer. The first one involves displacement of adsorbed surfactant by electro-active species. The second way involves attachment of the electro-active species together with surfactant moieties within one head group (Atta *et al.*, 2014). This may involve the dissociation of the solubilized analyte, followed by its entry into the surfactant film. The micelles in solution then combine with the aggregates on the electrode surface therefore carrying the analyte close to facilitate electron transfer (Rusling, 1997)

2.5.7: Role of surfactants in electrochemistry

Surfactants are attractive in electrochemistry because of their inherent properties which include:

i) Ability to stabilize radicals or intermediate products in a reaction (Farn, 2008).

ii) Ability to dissolve hydrophobic substances due to their charge duality (Atta *et al.*, 2014)

iii) Ability to change the structure of electrical double layer, potential at which analyte is reduced or oxidized, charge transfer and the velocity at which the analyte disperses to the electrode surface (Hosseinzadeh *et al*., 2009; Love and Dorsey, 1984).

Adsorbed surfactant can modify the properties of the electrode surface in various ways. In case ionic surfactant is adsorbed on the electrode, the electrode surface is charged which will lead to attraction and consequently build-up of oppositely charged analytes and repulsion of similarly charged species (Kamble, *et al*., 2017). Loosely adsorbed hydrophobic layers will significantly increase hydrophobic analytes at the electrodes surface. Moreover, a coating of a surfactant at electrode surface can prevent direct contact of analytes and electrode which prevents electrode fouling (Yang, and Hu, 2007; Yang et al., 2009). Surfactant molecules besides solubilizing organic compounds also provide a distinct way in which molecules can be oriented at the electrode surface.

2.5.7.1: Use of surfactants in heavy metal analysis

Micellar systems have been used in analysis of various heavy metals. Sodium dodecyl sulfate(SDS), has been used as antifouling and homogenizing in extraction of heavy metals (Hoyer and Jensen, 2004). SDS has also been used to suppress absorption of interferences in complex sample matrices (fruit juices, milk powder, and beer among others) in determination of heavy metals (Jia *et al*.,2008). The ability of SDS to suppress adsorption of interfering species is attributed to the fact that SDS is strongly adsorbed on mercury electrode over a wide potential range and can therefore displace adsorbed interferences. Additionally, SDS can scavenge interfering species by forming aggregates especially with other surface-active compounds (Hoyer and Jensen, 2004).

Deng *et al.*, (2011) reported a sensitive procedure for determination of trace Morin (VI) using SDS modified carbon paste electrodes in presence of complexing ligand, Morin. The hydrophobic and electrostatic interaction between morin molecules and SDS caused a higher aggregation of Mo (IV) at the electrode surface hence facilitating electron transfer (Atta *et al.*, 2014)

Cationic surfactants of quaternary ammonium salt on carbon paste electrode have been used to determine three heavy metals using cathodic stripping differential pulse voltammetry (Atta *et al.*, 2014). The three metals were in the form of Platinum (IV), Iridium (III) and Osmium (IV). Additionally, he described a procedure for determination of chromium based on synergistic pre-concentration of the chromate ion on the same electrode.

2.5.7.2: Suitability of surfactant modified electrode for drug analysis

Surfactant's ability to modify properties at the interface of solid and aqueous media improves drug wetting ability and consequently increases the surface area available for the drug to dissolve (Atta *et al.*, 2014). As such, surfactants have been used in fabrication of simple, quick and sensitive systems for diagnosis of drugs (Atta *et al.*, 2011). Surfactants are effective in drug analysis because of their ability to dissolve and pre-concentrate insoluble drugs through coulombic and hydrophobic interactions (Gutiérrez-Fernández *et al.*, 2004; Zhang *et al*., 2002).

Atta *et al*., (2011) used poly(3,4-ethylenedioxythiophene) PEDOT/Pt electrode in presence of SDS to isolate and determine Morphine (Mo) from codeine which has similar structure as Mo and interferes with analysis of Mo in urine or blood. Using similar conditions, he did further work on voltammetric behavior of isoniazid. The electrochemical response was greatly enhanced in presence of SDS, with two clearly defined irreversible anodic peaks of isoniazid at +0.63 V and 0.82 V. The anodic peaks were attributed to irreversible oxidation of isoniazid on the PEDOT/Pt surface. No peaks were reported in absence of SDS due to electrostatic repulsion between the positively charged PEDOT and cationic isoniazid. The anionic SDS greatly enhances anodic peak current of isoniazid, facilitates quick transfer of cationic isoniazid to electron surface hence increasing reaction rate (Atta *et al.*, 2011).

Electrode modification using surfactants increases sensitivity of electrode surface hence improving detection limits of drugs as demonstrated by Brahman *et al.*, 2012. Using polymer carbon paste electrodes modified with polymer in presence centrimonium bromide (CTAB), a surfactant, he developed a simple method for determination of anticancer drug flutamide.

2.6: Detection of penicillin in biological and environmental samples

Various methods have been used to determine penicillins in various matrices including biological, pharmaceutical among others.

2.6.1: Chromatographic Methods

Various chromatographic methods have been used in analysis of beta-lactams, the most common method being high performance liquid chromatogram (Schenck and Callery, 1998). High performance liquid chromatogram has been the most successful in determination of beta-lactams. This is probably because HPLC can be coupled with other methods to increase its detection ability. Sørensen *et al*., (1997) reported a method that simultaneously detected and quantified six antibiotics, penicillin G, amoxicillin, ampicillin, oxacillin, cloxacillin, dicloxacillin in raw cow milk. The limit of quantitation of penicillin G for this method was reported to be 1.9 μg/l. HPLC coupled with tandem mass spectroscopy has also been used to simultaneously determine antibiotics of different families, beta-lactams included (Cazorla-Reyes *et al*., 2014). Cazorla-Reyes *et al*., (2014) successfully separated penicillins, aminoglycosides, macrolides, quinolones, cephalosporins, carbapenems, glycopeptides and polypeptides. Briscoe *et al*.,(2012) developed a method for simultaneous determination of ten unbound beta-lactams in human plasma using HPLC coupled with ultraviolet (UV) spectroscopy . The concentration of benzylpenicillin in human plasma was found to have a low and high concentration of 0.63 µg/ml and 7.76 µg/ml respectively. HPLC is a very sensitive method, however it is expensive and time consuming because it involve derivatization, extraction and purification procedures (Švorc *et al.*, 2012).

2.6.2: Microbial Assays

Microbial assay methods of detecting penicillins comprise majorly, the microbial inhibition test and immunoassays. Microbial inhibition test involves incubating a susceptible organism in the presence of the sample under study i.e beta-lactam drugs (Babington *et al*., 2012). In absence of an antibiotic, the organism grows and can be detected visually either by opacity of the agar growth medium or by a color change due to production of acid. A high number of microbial inhibition tests are commercially available for analysis of penicillins (Babington *et al*., 2012). The limit of detection of many of these commercial microbial inhibition methods for beta lactams range from 2-100 µg/kg (Chen, *et al.*, 2015) and therefore their sensitivity levels met the standards set by European union regulation (Kurittu *et al*., 2000; Chen, *et al*., 2015)

Immunochemical methods are based on the ability of an antibody to bind specifically to a certain analyte (Märtlbauer *et al*., 1994). Few studies have reported analysis of penicillin G and penicillin V using immunochemical methods. Broto *et al*., (2015) reported immunochemical detection of penicillins (V and G) using biohybrid magnetic particles. The limit of detection of penicillin G was reported as 0.1 µg/L way below the maximum residue limit of 4.0 µg/L established by European union. Märtlbauer *et al*., (1994) developed immunochemical method using a specific polyclonal and monoclonal antibodies against beta-lactams and sulfonamides. The limit of detection for penicillin G was below the limit set by European union.

Microbial methods are tedious, some lack specificity and sometimes produce false positive because of the of presence of high somatic cell counts (Schenck and Callery, 1998).

2.6.3: Electrochemical methods

Various electrochemical methods have been used to analyse penicillins in different mediums. Norouzi *et al.*, (2006) coupled fourier transformation with cyclic voltammetry to detect penicillin G using a gold ultraelctrode. The limit of detection of this method was reported as 3.43 ng/mL. Wirzal *et al*., (2020) using differential pulse voltammetric method at boron doped diamond electrode reported presence of penicillin G with a limit of detection and quantitation of 0.23 µm and 1.5 µm respectively. A similar study of penicillin V in pharmaceutical and human urine using differential pulse voltammetry reported limit of detection of 0.25 µm on boron doped diamond electrode (Švorc Ľ *et al*., 2012). Other elctrochemical methods such as cathodic stripping voltammetry (Forsman, 1982), pulsed amperometric method (Koprowski *et al*., 1993) have also been used in analysis of penicillins. It is worth mentioning that other beta-lactams, such as amoxicillin have also been analysed using electrochemical methods (Bergamini *et al*., 2006)

Square wave voltammetry has been the method of choice in determination of beta lactams and other related drugs. This method has been used in simultaneous determination of paracetamol and penicillin V at a bare boron-doped diamond electrode (Švorc *et al*., 2012). Santos *et al*., (2008) reported determination of amoxicillin on a glutaraldehyde cross-linked polyglutamic acid modified glassy carbon electrode. Using pre- heated boron doped diamond electrode, Švorc *et al*., (2015) reported simultaneous determination of paracetamol and codeine in pharmaceuticals and human fluids. Brycht, *et al*., 2015 also determined a novel fungucide fenfuram using square wave voltammetry. No study has reported determination of benzylpenicillin (Penicillin G) using square wave voltammetry.

Biosensors have also been used to analyse beta-lactams. Gustavsson *et al*., (2004) successfully determined penicillin G using surface plasmon resonance based biosensor. Stred'anský *et al*., (2000) also reported presence of penicillin G, urea and oxalacetate using an amperometric sensor. Chen *et al*, 2015 developed an immunochromatic assay for simultaneous detection of 15 beta-lactams. The limit of detection of penicillin V and penicillin G were reported both as 0.5 ng/ mL (Chen, *et al.*, 2015)

Most electrochemical methods produce poorly formed voltammograms because penicillins are not easily oxidized. Moreover, the electrode must be driven to higher oxidative potential. Biosensor fabrication is a tedious and complex process that depends on a number of factors to function optimally (Bizzotto *et al*., 2018). Moreover, its difficult to produce antibodies against beta-lactams due to the instability of beta-lactam ring (Chen, *et al.*, 2015)

2.7: Development and Validation of Analytical Method for Penicillins Analysis

2.7.1: Method development

Analytical chemistry mainly involves separation, characterization and estimation of the components of a sample. Method development may involve adapting an existing method and making minor adjustments to make it suitable for a particular need (Swartz and Krull, 2018). The ultimate goal in method development is to come up with an experimental method of measurement capable of obtaining information from qualitative and quantitative tests of a given a sample.

Method development involves various steps, for electro-analytical procedures it may involve the following (Panchumarthy *et al.* , 2015)

i) Characterization of analyte, which involves collecting all the necessary data on the chemical and physical properties of the analyte such as solubility, optical properties and electrochemical properties of the analyte.

ii) Acquiring a suitable standard analyte for method

iii) Understanding the strength, accuracy, precision and general working of electro-analytical methods

iv) Selection of the electro-analytical method suited for the purpose of the study

v) Optimizing of experimental conditions for the best response

vi) Evaluation of matrix effects and interferences

vii) Applying the proposed procedure on the standard and real samples

viii) Evaluating the validity of the procedure

Analysis of drugs is very vital because it deals with an important aspect of life.

2.7.2: Analytical method validation

Analytical methods are validated to ensure that the method employed for specific tests meets the intended requirements (Swartz and Krull, 2018). The results obtained from method validation is used as a measure of the method's ability to produce quality, reliable and consistent results (Ravisankar *et al.*, 2015). Analytical method needs to be certified before their regular use or when conditions for which it was validated change e.g. change in sample matrix (Kalra, 2011). A method is considered for validation when there is need to confirm that its performance parameters are good enough for use in solving a certain analytical problem (Kalra, 2011). These tests include, Accuracy, precision, stability study, limit of detection, quantitation and linear range.

2.7.2.1: Accuracy

Accuracy is a measure of how close a result obtained from an experiment is from the expected results. It can also be defined as a measure of the degree of agreement of the results obtained relative to a reference value (Patil *et al*., 2014). Therefore, accuracy of a measurement can be expresssed as the difference between the sample mean and the true value shown in equation 2.19 (Panchumarthy *et al*., 2015)

$$E = X - \mu \quad (2.19)$$

Where μ is the actual (reference value) and X is sample mean. Accuracy can also be expressed as percentage relative error, E_r. $E_r = \frac{x-\mu}{\mu} \times 100$ (2.20)

In most cases accuracy is determined and represented using recovery studies (Panchumarthy *et al*., 2015). However, it can also be expressed in other different ways such as;

i) As a comparison to a certified reference
ii) Comparison with other results obtained using a validated or referenced method
iii) By monitoring the results after standard addition of the analyte (Mehmet and Sibel , 2011)

Comparison with a certified reference is preferred for simple analytes with no complex sample matrix. The results obtained results are compared with other results from a certified external source.

A blank matrix can also be spiked with known standard at different concentrations then recovery studies done to ascertain the accuracy of the method (Taverniers *et al*., 2004). Possible effects from potential interferents in the solution matrix can also be monitored. The international council of harmonization of technical requirements for pharmaceuticals for

human use (ICH) document on validation methodology recommends that accuracy be assessed using three concentrations with three triplicates and results reported as percentage recovery of the known analyte (50%-150%). The results obtained from this method can be expressed as percentage recovery with their relative standard deviation (RDS %) (Rewaria and Swamy, 2013).

Standard addition of the analyte is done when no matrix blank is available. Varying but known concentrations of analyte are spiked into the sample matrix under study. The accuracy of this method is determined by percentage recovery of the analyte added in the sample matrix (Taverniers *et al*., 2004).

Comparison with another certified procedure is done by comparing obtained results from those of another certified procedure which is used as a reference (Rewaria and Swamy, 2013)

A measurement system is considered valid if it is accurate and precise and therefore accuracy is viewed as a combination of correctness and precision (Mehmet and Sibel , 2011).

2.7.2.2: Precision

Precision is a measure of agreement for multiple measurements on the same sample. It demonstrates how close a number of measurements collected from the same sample are (Patil *et al.*, 2014). Precision involves analyzing the same specimen multiple times under similar analytical conditions. As such precision is categorized into three levels;

i) Repeatability – it's a form of precision which is expressed as standard deviation the results obtained when analysis is done in the same laboratory by the same analyst using the same device for a relatively short duration. ICH provides that data can be collected in two ways; three replicates of three different concentrations or at least six replicas to be measured at 100% of test concentration (Menditto *et al*., 2007).

ii) Intermediate precision – It is established by comparing results obtained in different days or by different analyst within the same laboratory using different instruments. The aim is to establish if the method will produce the similar results in the same laboratory (Menditto *et al*., 2007).

iii) Reproducibility- it's done by analyzing the same sample in different laboratories. This is done as a confirmation that the adopted technique will produce similar results even when used in different laboratories. It gives more meaningful precision than any other methods (Menditto *et al*., 2007).

2.7.2.3: Estimating the Limit of Detection (LOD)

It refers to the lowest quantity of an analyte that can be detected without being quantified with an acceptable uncertainty (Panchumarthy *et al*., 2015). LOD calculation can be determined in three ways, namely

i) Visual evaluation method- This is done by analyzing the active analyte of known concentration to establish the minimum concentration which can be reliably detected (Panchumarthy *et al*., 2015).

ii) Based on calculation using the standard deviation of response and the slope of the calibration curve. LOD is expressed as;

$$C_{LOD} = \frac{3S}{m} \qquad (2.21)$$

This is the most commonly used approach in electro-analysis. The estimates of the standard deviation "s" can be worked out in different ways. First, the magnitude of the blank response is obtained by analyzing three-six blank samples. Secondly, the lowest amount of calibration standard which is analytically responsive should be measured three-six times and their standard

deviation calculated. Thirdly, the standard deviation can be obtained from the regression line using Equation 2.21 (Mehmet and Sibel , 2011).

2.7.2.4: Estimating the Limit of Quantitation (LOQ)

It is the minimum amount of measurand that can be detected from a specimen and quantitatively determined with acceptable accuracy and precision (Panchumarthy *et al*., 2015). Limit of quantitation is obtained in a similar approach as limit of detection (LOD). This includes; estimation from visual evaluation and calculation from the slope of the calibration curve at low concentration using Equation 2.22.

$$C_{LOQ} = \frac{10S}{m} \tag{2.22}$$

LOQ can also be obtained from LOD using the relationship

$$LOQ = 3.3 \times LOD \tag{2.23}$$

2.7.2.5: Estimating the linearity and range

Method's linearity is the ability of the method to provide test results that are directly proportional to the concentration of the analyte at a specified range (Panchumarthy *et al*., 2015). Linearity of data is often established from the correlation coefficient and the y- intercept. A correlation coefficient of 0.999 is normally considered as acceptable fit of analytical data to regression line (Masato *et al*., 2012). The linear regression line should have a y-intercept value close to zero. However, correlation coefficient of 0.99 may not be a sufficient proof of linearity. ICH guidelines recommend at least five serial dilutions of the standard solution to establish linearity. Range of data measurements of an analyte is defined as the interval between the

highest and lowest levels of analyte which were obtained with acceptable linearity, precision and accuracy (Panchumarthy *et al*., 2015).

2.7.2.6: Estimating the Ruggedness of analytical method

Ruggedness refers to the degree to which the test results can be reproduced under different test conditions (Rewaria and Swamy, 2013), for example when done by a different analyst, in different laboratory or using a different machine at a different time. Ruggedness is therefore used as a measure of constancy of results when external factors such as analyst, laboratory, instrument or time are varied (Rewaria and Swamy, 2013).

2.7.2.7: Estimating robustness of analytical method

It refers to ability of a method to remain unchanged despite little intentional variations in parameters (Rewaria and Swamy, 2013). It can also be defined as the ability to reproduce analytical method in different laboratory or different conditions without significant difference in the results of the two methods. Robustness is assessed using parameters such as sensitivity, selectivity, precision, accuracy, specificity among others (Panchumarthy *et al*., 2015).

2.8: Statistical Treatment of Analytical Data

Data produced during method validation must be analysed statistically to demonstrate the validity of the method applied in the study (Van-Zoonen *et al.*, 1999). When handling results, there are two possible errors that an analyst can make; rejecting hypothesis that two quantities

are the same, when they are statistically similar or accepting two quantities as same when they are statistically different (Ortiz *et al*., 2003). Statistical data treatment tools are used to prevent such errors. These data treatment methods are used to determine confidence level, the number of replicates required for credible results, whether two sets of measurements differ significantly, comparing the means of two sets of data or deciding to reject or retain a result that appears as outlier in a set of data, among others. Statistical methods commonly used for data treatment are mean, standard deviation and confidence interval.

CHAPTER THREE

MATERIALS AND METHODS

3.1: Chemicals and Solutions

All chemicals used in this work were of analytical grade quality and no further purification was carried out before use. The chemicals used included; sodium dodecyl sulfate, sodium acetate, acetic acid (glacial), acetonitrile, Potassium dihydrogen phosphate monobasic, Phosphate dibasic heptahydrate, buffer solutions for calibrating pH meter, Penicillin G sodium, penicillin V potassium salt were all procured from fisher scientific. Other chemicals including Zinc chloride, Iron III chloride, Iron II chloride, Calcium chloride, Magnesium chloride, Copper sulphate, Potassium chloride, Potassium nitrate, Potassium hexacyanoferrate (III), Sodium chloride, Sodium hydroxide, hydrochloric acid were all sourced from sigma Aldrich. Pen-strep, a common penicillin drug used to treat mastitis was bought over the counter. Detergents used were locally obtained. For voltammetry, the solutions used were acetate buffer (ABS) and acetate buffer containing sodium dodecyl sulfate (SDS/ABS). Only de-ionized water was used throughout this work. The rest of the chemicals were of reagent grade.

3.2: Apparatus

All electroanalytical work was done using a CHI 1232B Electrochemical Station (CH Instruments, Inc., USA) composed of a three-electrode system (CH Instrument Inc., USA). The three-electrode system comprised of a 3 mm diameter glassy carbon (working electrode), a platinum wire (auxiliary electrode) and Ag/AgCl (reference electrode). All pH measurements were done on a pH meter Bench – Model CyberScan pH Tutor (Eutech Instruments). A 10.0mL electrochemical cell was used for all analytical work at room temperature. Separation by

centrifugal force was done using MSE 869-Minor centrifuge, while De-ionized water was obtained using B114 Elga-Star wall mounted De-ionizer with disposable cartridges. All data was analyzed using Kaleidagraph software, version 4.1.1. Micro-litre solutions were transferred using adjustable Eppendorf micro-pipette.

3.3: Preparation of working electrode

Glassy carbon surface is very active when polished, so it is gradually deactivated on exposure to the atmosphere or during electrochemical reaction. It is therefore important to polish the surface before measurements are taken or sometimes between measurements. Polishing renews the electrode surface by eliminating the effects of previous use especially if the past analytes adsorbed on the electrode surface.

3.3.1: Polishing the glassy carbon working electrode

The working electrode, glassy carbon electrodes was polished with 0.3 µm then 0.05 µm micro-polish (CH Instruments) slurries on a soft cloth or on a smooth glass plate. Little ultra-pure water was added to the micro-polish to make a slurry. The working electrode was polished by moving it in a figure eight motion on a slurried smooth glass plate/soft cloth for five minutes as shown in Figure 3.1. In cases where scratches would appear on the electrode surface after polishing, the procedure was repeated starting with the larger particle polishing slurry. The electrodes were then rinsed using a stream of de-ionized water to remove adsorbed particles and other possible contaminants. The actual surface area of the working electrode was 0.071 cm^2. This procedure was repeated before the carbon working electrode was used for analysis.

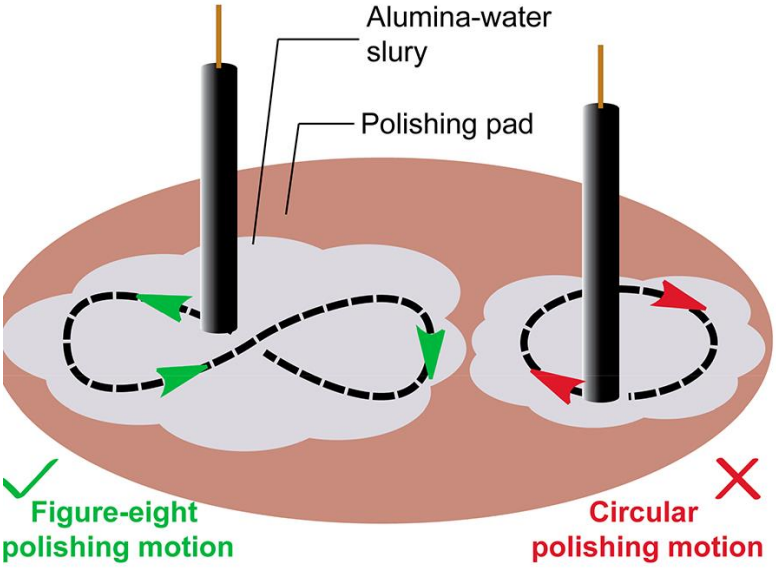


Figure 3.1: Electrode polishing (Elgrishi, *et al*., 2018).

3.4: Preparation of Solutions

All polar solutions were prepared using de-ionized water.

3.4.1: Potassium Chloride Solution (0.1M) Preparation

0.1 M Potassium chloride solution was prepared by dissolving 1.865 g of the salt in de-ionized water and the final volumes made up to 250 ml.

3.4.2: Phosphate buffer saline solution preparation

Phosphate buffer saline solution (pH 7.2) was prepared by dissolving 11.5 g of sodium phosphate dibasic heptahydrate, 2.0 g of potassium dihydrogen phosphate, 2.0 g of potassium chloride and 80.0g of sodium chloride in 800 ml of de-ionized water and the final solution made up to 1 litre using de-ionized water. The solution pH was adjusted using 1.0M sodium hydroxide and 1.0 M hydrochloric acid.

3.4.3: Preparation of 2 mM potassium ferricyanide solution

To make 2 mM potassium ferricyanide solution; 0.165 g of potassium hexacyanoferrate (III), $K_3Fe\ (CN)_6$ was dissolved in potassium chloride (0.1M) solution and made up to 250 ml. similarly a solution of potassium hexacyanoferrate (III) in phosphate buffer was made by dissolving equal amount of $K_3Fe\ (CN)_6$ in phosphate buffer saline and final solution made up to 250 ml.

3.4.4: Preparation of Acetate Buffer Solution (ABS)

Acetate buffer solution was prepared by dissolving 1.5 g of sodium acetate and 1ml of concentrated acetic acid in de-ionized water and made up to 500 ml. The pH of the resulting acetate buffer (ABS) was adjusted accordingly using 1 M hydrochloric acid or concentrated acetic acid. The pH of the final solution was 4.5

3.4.5: Preparation of sodium dodecyl sulfate in acetate buffer solution

After preparing the acetate buffer, pH 4.5 (section 3.4.4), 25 g of sodium dodecyl sulphate was dissolved in it and made up to 250 ml. The resulting mixture, sodium dodecyl sulfate- Acetate buffer solution (SDS-ABS) was stirred over warm water for 30 minutes until a homogenous solution was formed. SDS-ABS solution was left to cool down. The pH of the resulting SDS-Acetate buffer was adjusted accordingly using the acetic acid.

3.4.6: Preparation of interferent solutions

To prepare 0.1M interferent solutions, a given mass of salt was weighed for each salt as indicated in Table 3.1 and dissolved in 10ml de-ionized water by stirring.

Table 3.1: Preparation of interferent solutions from salts with cations or anions of interest

Type of salt	Mass of salt dissolved (grams)
0.1M copper sulphate solution	0.25g
0.1M sodium nitrate solution	0.084g
0.1M potassium chloride solution	0.075g
0.1M potassium dihydrogen orthophosphate solution	0.136
0.1M potassium nitrate solution	0.10g
0.1M magnesium chloride solution	0.20g
0.1M calcium chloride solution	0.11g
0.1M Iron (III) chloride solution	0.16g
0.1M zinc chloride solution	0.136g

3.4.7: Preparation of Standard Solutions

3.4.7.1: Preparation of 0.1M Penicillin G Solution

0.1 M penicillin G solution was prepared by dissolving 0.17 g of penicillin G in 5 ml of de-ionized water.

3.4.7.2: Preparation of penicillin v solution

0.14 M penicillin V solution was prepared by dissolving 0.49 g of penicillin V in a mixture containing 9 ml de-ionized water and 1ml of acetonitrile. Acetonitrile was added to increase solubility of penicillin V.

3.4.7.3: Preparation of penicillin G sample from a drug (pen-strep)

2 mM penicillin G sample was prepared by dissolving 3 ml of pen-strep drug in 200 ml of acetonitrile-water solution (ratio 1:1) and made up to 250 ml using the same acetonitrile-water mixture.

3.5: Sample collection and treatment

10 litres of milk were sourced from a farmer in Yatta, Machakos country. Samples containing 20 ml of the sourced milk were spiked with 0.1 M penicillin G. An aliquot 2 ml of acetonitrile was added under constant stirring for 20 minutes to coagulate and deproteinize the milk. The mixture was filtered using a filter paper (Whatman, 125 mm) and the supernatant recovered. The supernatant was centrifuged for 30 minutes at 10,000 rpm using MSE 869-Minor centrifuge to remove any suspended matter. The resulting supernatant was kept at -20^{o}C when not in use.

3.6: Analytical Techniques

All voltammetric measurements were carried out in the cyclic (CV) and square wave (SWV) voltammetric modes.

3.6.1: Cyclic Voltammetry

Potential in all cyclic voltammogram studies were done between 1.0 V and 2.0 V with a sample interval of 0.001 V and a quiet time of 0.1 seconds.

3.6.2: Square Wave Voltammetry

Square-wave studies were done at the same potential range, 1.0 V to 2.0 V with an amplitude and frequency of 0.025V and 15Hz respectively. All electro-analytical work was done using a three-electrode system in a 10.0ml electrochemical cell.

3.7: Samples Analysis and Optimization

3.7.1: Characterization of Working Electrode

To determine whether the bare glassy carbon electrode was redox-active, a cyclic voltammetry studies of 2 mM potassium ferricyanide was done from an initial potential of -0.2 V to 1.0 V with sample interval of 0.001V and a quiet time of 0.1sec at varying scan rate in both 0.1M potassium chloride solution and in phosphate buffer saline.

3.7.2: Buffer pH

To study the effect of pH, 10 ml aliquots of the sodium acetate buffer- sodium dodecyl sulfate (ABS-SDS) solution pH were adjusted using concentrated acetic acid to make solutions of different pH ranging from 3.0, 4.0, 4.5, 5.0 and 6.0. The effect of change in pH on the voltammetric peak currents of penicillin V at glassy carbon electrode (GCE) was monitored using square wave voltammetry. To determine the optimal buffer pH, a graph of voltammetric current against pH, was plotted.

3.7.3: Optimizing the Concentration of Surfactant (Sodium Dodecyl Sulfate)

To obtain the most suitable concentration of the surfactant (SDS) required for the optimum voltammetric response, various concentrations of SDS were added to the acetate buffer solution, the electrolyte solution. Current measurements were first done in a blank (without penicillin V) and in presence of 0.1M penicillin V by scanning potential positively from 1.0 V to 2.0 V at a scan rate of 0.1 V/s in the acetate buffer solution, pH 4.5 using square wave

voltammetry method. In a 10 ml electrochemical cell, 0.001 g, 0.05 g, 0.1 g, 0.5 g, and 1 g of sodium dodecyl sulphate were separately added to the solution containing 0.1M penicillin and acetate buffer solution (pH 4.5) and the voltammetric current measured using square wave voltammetry. Square wave voltammetry of the analyte (penicillin V) was run by scanning potential positively from 1.0 V to 2.0 V at a scan rate of 0.1V/s in the acetate buffer solution, pH 4.5. A plot of surfactant (SDS) concentration versus voltammetric peak current was done to determine the optimum concentration of SDS.

3.7.4: Cyclic Voltammetry Studies of the Analytes

3.7.4.1: Cyclic voltammetry of penicillin v in presence and absence of the surfactant

To identify the potential at which penicillin V is oxidized, 100 µl of 0.14 M penicillin V were added into 10 ml acetate buffer solution and potential scanned from 1.0 V to 2.0 V at different scan rates ranging from 0.01 V/s to 0.1 V/s using cyclic Voltammetry method. To study the effects of the surfactant on the potential and current response, 1 g of sodium dodecyl sulfate was added into a 10 ml electrochemical cell containing 100 µl of 0.14 M penicillin V in acetate buffer solution. The potential was scanned from 1.0 V to 2.0 V at different scan rates ranging from 0.01 V/s to 0.1 V/s using cyclic Voltammetry method.

3.7.4.1: Cyclic voltammetry of penicillin G in presence and absence of the surfactant

To study the electrochemical behaviour of penicillin G, 200 µl of 0.1 M penicillin G were added into 10 ml acetate buffer solution and potential scanned from 1.0 V to 2.0 V at varied scan rates ranging from 0.01 V/s to 0.1V/s using cyclic Voltammetry method. To understand the effects of the surfactant on the potential and current response, 1 g of sodium dodecyl sulfate was added into a 10 ml electrochemical cell containing 200 µl of 0.1M penicillin G in acetate buffer solution. The potential was scanned from 1.0 V to 2.0 V at different scan rates ranging from 0.01 V/s to 0.1 V/s using cyclic Voltammetry method.

3.8: Validation of Methods

3.8.1: Precision of the Proposed Method

To study precision of the voltammetric method, 100 ml solution of acetate buffer-sodium dodecyl sulfate (ABS-SDS) was aliquoted into 10mL replicates and each replica was spiked with 100 µl of 0.14 M penicillin V and stirred to give homogenous mixture. Voltammetric currents and oxidation potential of penicillin V were obtained by running a square wave voltammetry from initial of 1.0 V to 2.0 V in quiescent solution. The experiment was repeated by spiking a new batch of ten replicates of ABS-SDS solution with 200 µl of 0.1 M penicillin G solution and running a square wave voltammetry for the same potential range in a quiescent solution. The average current, standard deviation and relative standard deviation were calculated from the resulting current.

3.8.2: Accuracy of the proposed method

To test the accuracy of the proposed voltammetric method, 10 ml of ABS-SDS solution was spiked with 100 µl of 0.14 M penicillin V. The voltammetric response was monitored using square wave voltammetry. An additional 50 µl of 0.14 M penicillin V was added to the same solution, which corresponded to 50% of the initial concentration. The current response was recorded. An additional 100 µl and 150 µl of 0.14 M penicillin V were added to the same solution and current measured after every addition. The 100 µl and 150 µl of 0.14 M penicillin V added corresponded to 100% and 150% increase in concentration of the initial solution. This procedure was repeated eight times for each of the three different concentrations (50%, 100% and 150%) and the results recorded.

To test accuracy of the method using penicillin G, 10 ml of ABS-SDS solution was spiked with 200 µl of 0.1 M penicillin G and current monitored using square wave voltammetry. Additional 100 µl, 200 µl and 300 µl of 0.1 M penicillin G were added to the same solution separately and

current monitored using square wave voltammetry. The added concentrations corresponded to 50%, 100% and 150% increase in concentration of the original solution. This procedure was repeated eight times for each of the three different concentrations (50%, 100% and 150%) and the current readings recorded. The average current reading for each concentration was recorded.

3.8.3: Linearity, Limit of Detection and Limit of Quantitation

To study the limit of detection, quantitation and linearity of both penicillin V and G, voltammetric currents of the serial dilutions of both penicillin V and G in ABS-SDS solution were done using square wave voltammetry on glassy carbon electrode. For instance, 10 ml of ABS-SDS was spiked with 100 μl of 0.14 M penicillin V and the voltammetric response recorded using square-wave voltammetry. Serial dilution of the initial solution was done by dividing the solution into two equal portions. To one of the two portions, a similar amount of ABS-SDS solution was added and the solution stirred and allowed to settle. The voltammetric response of the diluted solution was studied using square wave voltammetry and results recorded. This was continued until no peak was observed.

Similarly, 200 μl of 0.1M Penicillin G were added to 10ml of ABS-SDS in an electrochemical cell, stirred and allowed to settle. Voltammetric response of the solution was studied using square wave voltammetry. This was repeated with serial dilutions of the initial solution until no peak was observed. The linearity of the voltammetric method was tested by making a calibration plot to calculate the correlation coefficient, slope, intercept values. The limit of detection (LOD) for penicillin V and G was based on three times the standard deviation of the baseline (Gumustas and A Ozkan, 2011), and were estimated using Equation 2.21. Similarly, the limit of quantitation (LOQ) was obtained following Equation 2.22 (Gumustas and Ozkan, 2011).

3.9: Interference in the analytical method

Natural samples normally contain substances that can be reduced or oxidized at a certain potential. This may seriously interfere with the analytical ability of a method or instrument especially if the interferents are oxidized or reduced at the same potential as the analyte of interest. The most common interferents include; K^+, Fe^{3+}, Mg^{2+}, Na^+, Zn^{2+}, Ca^{2+}, Cu^{2+}, PO_4^{3-}, Cl^-, SO_4^{2-} and NO_3^-. Their effect in voltammetric determination of both penicillin V and G was studied to ensure the developed method was suitable for analysis of the above drugs.

3.9.1: Determination of the effects of interferants

To study the effect of the interferents, 50μl of each interferent solution (0.1M) prepared above were added separately into 10ml ABS-SDS solution. An aliquot (50μl) of penicillin V or G was added separately with stirring into 10ml electrochemical cell and the solution allowed to settle. The resulting voltammetric current and potential were monitored using square wave voltammetry by scanning potential from 1.0V to 2.0V at a scan rate of 0.1V/s.

3.10: Analysis of milk spiked with penicillin G and Pen -Strep Samples

3.10.1: Cyclic voltammetry of penicillin G in milk samples and Pen-strep sample

200 μl of the supernatant solution prepared in section 3.5 were added to 10 ml of ABS-SDS. The potential was scanned from 1.0 V to 2.0 V at different scan rates ranging from 0.01 V/s to 0.11 V/s using cyclic voltammetry method. The experiment was repeated using 200 μl of 2 mM of Penicillin G drug, Pen-strep solution prepared in section 3.4.7.3

3.10.2: Precision of the method in determination of Penicillin G in cow milk

To determine the precision of the method in milk samples, 10 ml of ABS-SDS solution was spiked with 200 µl of supernatant as prepared in procedure 3.5. This was repeated for ten

samples of the supernatant. Similarly, the same procedure was repeated for ten samples containing penicillin G drug, Pen-strep prepared in section 3.4.7.3. Oxidation potential and voltammetric current of the spiked solutions were studied using square wave voltammetry by scanning potential from 1.0 V to 2.0 V at a scan rate of 0.1 V/s.

3.10.3: Accuracy of the method in determination of Penicillin G in cow milk

The supernatant prepared in procedure 3.5 was used to study accuracy in determination of penicillin G in milk sample. This was done by spiking 10 ml ABS-SDS solution with 200 µl of the supernatant. Additional 100 µl, 200 µl and 300 µl were added to the ABS-SDS solution separately and current response monitored using square voltammetry by scanning potential from 1.0 V to 2.0 V at a scan rate of 0.1V/s. This procedure is repeated eight more times and the results recorded. The same procedure was used to study accuracy in determining penicillin G in pen-strep, as prepared in section 3.4.7.3.

3.10.4: Determining Limit of Detection of penicillin G in cow milk

200 μl of supernatant prepared in section 3.5 were added to 10 ml of ABS-SDS in an electrochemical cell, stirred and allowed to settle. Voltammetric response of the solution was studied using square wave voltammetry by scanning potential from 1.0 V to 2.0 V at a scan rate of 0.1V/s. This was repeated with serial dilutions of the initial solution until no peak was observed. The linearity of the voltammetric method was tested by drawing a calibration plot to calculate the correlation coefficient, slope and intercept values.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1: Characterization of Working Electrode

Potassium ferricyanide is known to be well behaved electrochemically, hence it is used as a model system in most electrochemical systems (Rooney *et al*., 2000). Figure 4.1 shows reversible cyclic voltammetry of potassium hexacyanoferrate (III) (Ferricyanide) in 0.1M KCl and PBS (pH 7.4). Current peaks increased as the scan rate was increased.

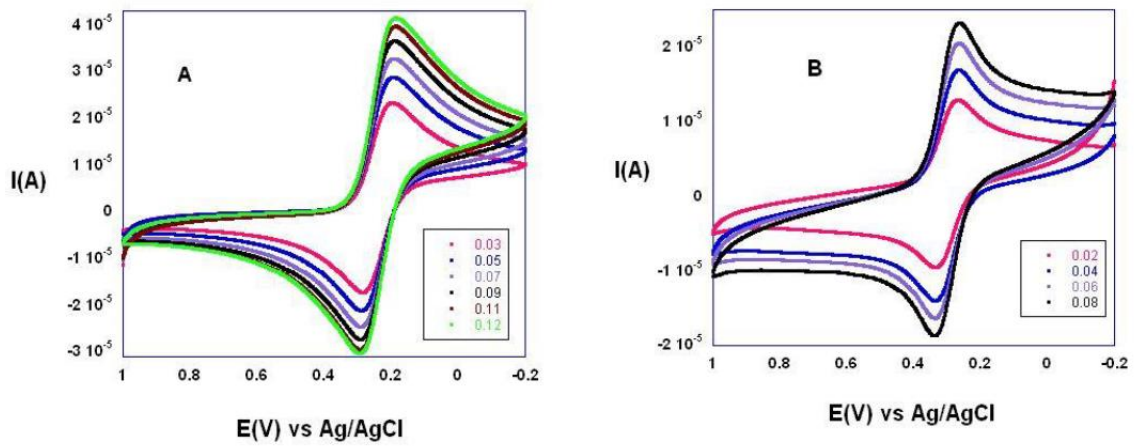


Figure 4.1: A cyclic voltammogram of 0.1M potassium ferricyanide at bare glassy carbon electrode using (a) potassium chloride (b) phosphate buffer solution as support electrolyte. Initial potential: -0. 2.0V; high potential: 1.0V; sample interval: 0.001V, quiet time: 0.1sec.

During the positive potential scan, $Fe(CN)_6^{-4}$ is oxidized and anodic current due to this process is peaked at 0.33V. This oxidation process is represented by the Equation 4.1 shown below

$$Fe(CN)_6^{-4} + e \rightarrow Fe(CN)_6^{-3} \qquad (4.1)$$

Similarly, when the scan is reversed, the oxidized species formed at the interface are quickly reduced resulting to a cathodic peak at 0. 27V (Equation 4.2). The current maximum occurred at the same potential both in the positive and negative going potential at different scan rate i.e.,

it doesn't shift with increase in scan rate. This demonstrates a fast electron transfer process taking place at the indicator electrode surface, hence a reversible process (Craig and Dale, 2014).

$$\mathrm{Fe}(CN)_6^{-4} + e \leftrightarrow \mathrm{Fe}(CN)_6^{-3} \qquad (4.2)$$

When the peak current (I_p) was plotted against square-root of scan rate ($V^{1/2}$), the resulting plot was linear (*Figure 4.2*) and the gradient of the line was used to determine the rate of transfer of ferricyanide ions on to the electrode surface, i.e., diffusion coefficient using Randles-Sevcik equation (Equation 2.6).

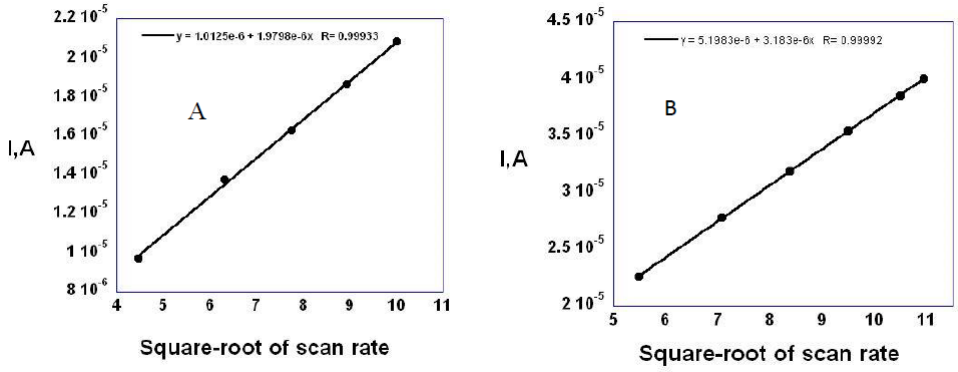


Figure 4.2: A plot of current versus square-root of scan rate for voltammetric determination of ferricyanide ions in potassium chloride (A) and phosphate buffer saline solution (B)

The diffusion coefficient of ferricyanide ions in potassium chloride and phosphate buffer saline solution was estimated to be $1.1\text{x}10^{-6}\text{cm}^2/\text{sec}$ and $2.8\text{x}10^{-6}$ cm^2/sec respectively. The experimental values obtained above are close to the reported value of $6.9\text{x}10^{-6}\text{cm}^2/\text{sec}$ 1.0M $K_3Fe(CN)_6$ in aqueous potassium chloride solutions (Gomathi *et al.*, 1991). The electrode surface was therefore active enough for redox process.

4.1.1: The Effect of pH in Determination of Penicillin V

A plot of the voltammetric currents against buffer pH was expressed in form of a histogram (Figure 4.3). The peak heights of the histogram were used to determine the optimal buffer pH for the proposed method. Figure 4.3 shows that the highest voltammetric peak current responses for 1.4mM penicillin V were at pH 4.5. Below and above this pH, the current responses were lower probably because of instability of penicillins above and below pH of 4.5. The highest voltammetric current corresponds to highest sensitivity. Consequently, a pH 4.5 was selected for all voltammetric measurements.

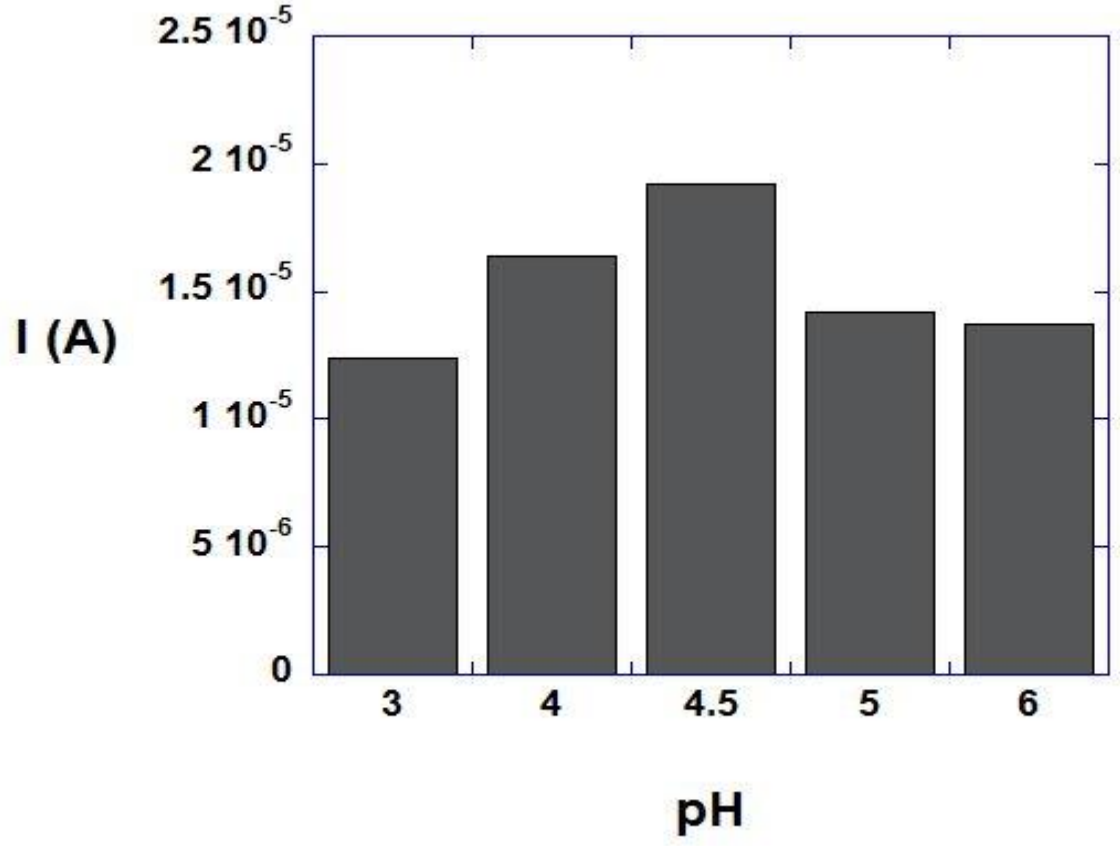


Figure 4.3: Effect of pH on the square wave voltammetric peak currents of 1.4mM Penicillin V at the GC electrode in acetate buffer. The pH values considered were 3, 4, 4.5, 5 and 6.

4.1.2: Optimizing the Concentration of Surfactant Sodium Dodecyl Sulfate (SDS)

The effects of varying SDS concentrations on voltammetric peak currents of 1.4 mM penicillin V are shown by the voltammograms presented in Figure 4.4.

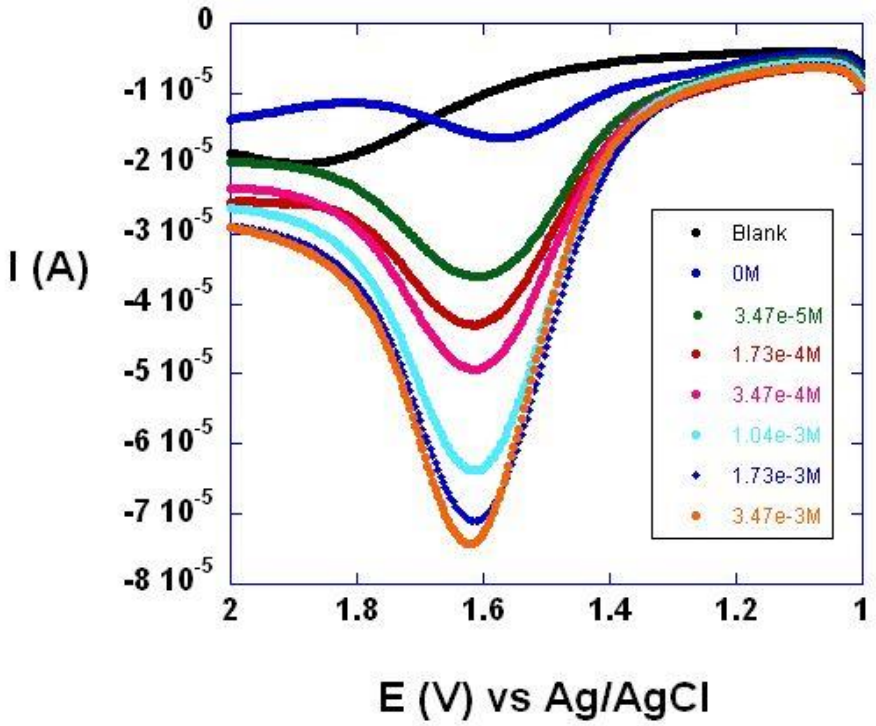


Figure 4.4: Effect of successive addition of sodium dodecyl sulfate (SDS) on the voltammetric response of 1.4mM Penicillin V in ABS, pH 4.5 at GC electrode. The amplitude and frequency were 0.025V and 15Hz respectively

The amount of SDS that enabled the GC to give amaximum voltammetric current was determined by ploting current reading from the voltammograms in Figure 4.4 and plotted against the concentration of SDS (Figure 4.5)

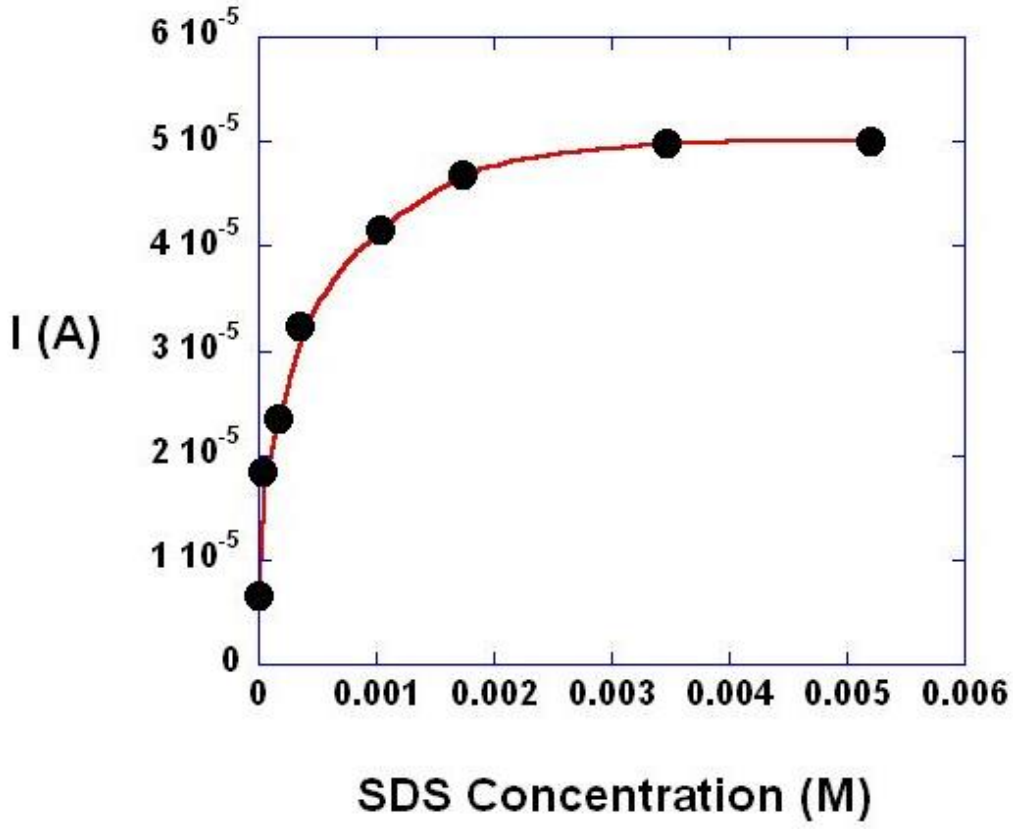


Figure 4.5: Plot of varying SDS concentrations versus voltammetric current responses of 1.4mM Penicillin V in ABS, pH 4.5 at GC electrode. The amplitude and frequency were 0.025V and 15Hz respectively**.**

There was a corresponding increase in voltammetric current as the amount of SDS increased up to $3.47 \times 10^{-3} M$ SDS, whereas beyond this concentration the voltammetric currents remained constant possibly due to saturation. Similar observation was made by Galal *et al*., (2011) in electrolyte containing sodium dodecyl sulfate when they were determining terazosin, an antihypertensive drug on GCE.

Optimum current response was obtained at SDS concentration of $3.47 \times 10^{-3} M$ in 10ml electrochemical cell in ABS at pH 4.5. Therefore, 0.347M SDS concentration was adopted for all subsequent work.

4.1.3: Effects of Surfactant (Sodium Dodecyl Sulfate) on Voltammetric Currents of penicillin (V and G)

Effects of addition of surfactant, sodium dodecyl sulfate, in support electrolyte were observed for both penicillin G and penicillin V using square wave voltammetry.

4.1.4.1: Effects of Surfactant (Sodium Dodecyl Sulfate) on the Voltammetric Current of Penicillin G

In a blank solution (without penicillin G), no voltammogram was observed. A small voltammogram peaked at 1.6V region was observed in absence of surfactant, SDS. In presence of SDS, an enhanced voltammogram with a peak current almost five times compared to the current obtained in absence of SDS was observed as shown in Figure 4.6.

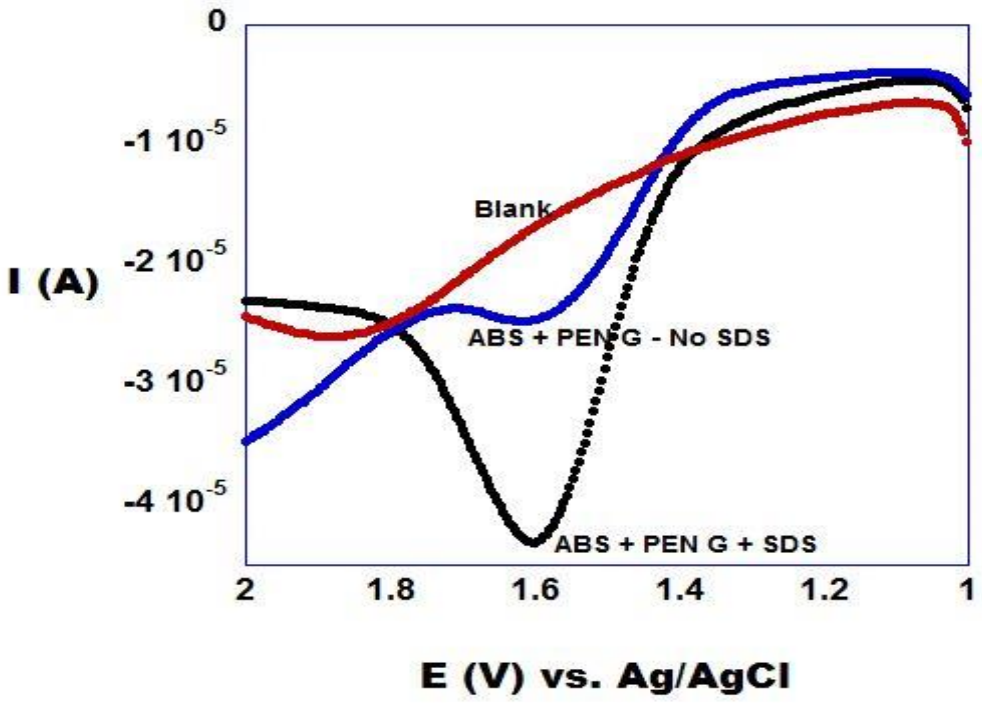
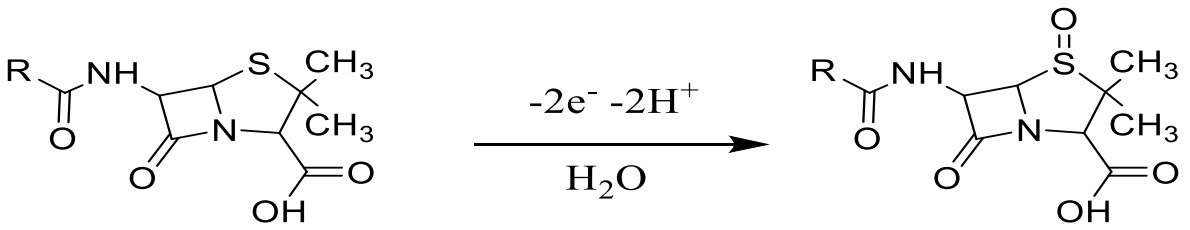


Figure 4.6: Square wave voltammograms of 2.0mM Penicillin G in Acetate Buffer Solution, pH 4.5 (Blue) and 2.0mM Penicillin G in 0.347M SDS/ABS, pH 4.5 (black) at GC electrode. The amplitude and frequency were 0.025V and 15Hz respectively.

There was no significant change in the position of the peak potential in both voltammograms in presence or in absence of SDS. Both voltammograms peaked at 1.6 V. Very little capacitative current with no distinct features was noted in the blank solution (without penicillin G).

In ultra-violet spectroscopy (UV-Vis) occurrence of surface plasmon resonance (SPR) peak at a certain wavelength indicates a spectroscopic signature for the presence of a certain material (Liang *et al*., 2012). Similarly, in voltammetry, appearance of a peak at a particular potential indicates the presence of redox-active substance which is associated with that particular potential. Presence of SDS probably aids in electron transfer process. Absence of a voltammogram in the blank is an indication that the voltammogram peaked at 1.6V was due to addition of penicillin G (Figure 4.6).

It has been demonstrated that chemical oxidation of different penicillins in aqueous and organo-aqueous media by different oxidation groups leads to formation of their sulfoxides. This implies that the reactive site for the chemical oxidation is the penicillin backbone. The electrochemical oxidation of penicillin G requires two protons and two electrons (Ľ-Švorc *et al*., 2012). The electroactive site, sulfide moiety in penicillin backbone is oxidized into a sulfoxide derivative of penicillin G in presence of two protons and electrons and a water molecule as shown in Scheme 1 below.



Scheme 1: Proposed mechanism of the electrochemical oxidation of penicillin G

4.1.4.2: Effects of Surfactant (Sodium Dodecyl Sulfate) on the Voltammetric Current of Penicillin V

Voltammograms formed at peak at 1.6V region were formed in acetate buffer solution containing penicillin V and in presence of both penicillin V and sodium dodecyl sulfate. No voltammogram was observed in the blank solution (in absence of penicillin V) as shown in Figure 4.7

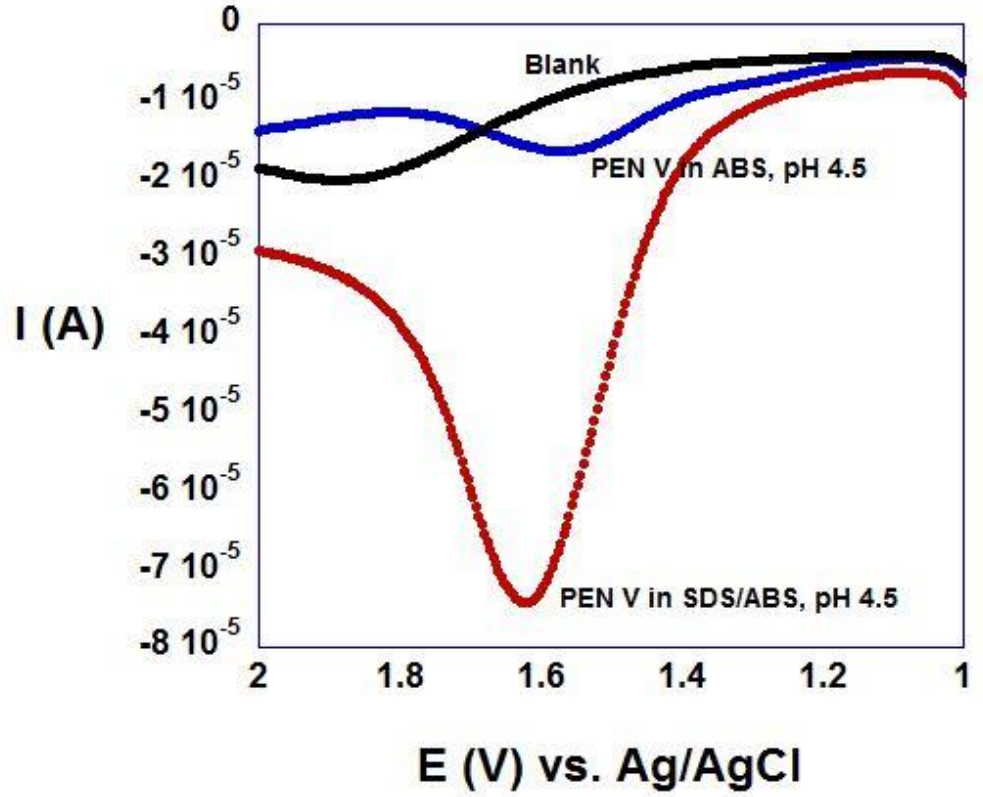


Figure 4.7: Square wave voltammograms of 1.4mM Penicillin V in ABS, pH 4.5 (Blue) and 1.4mM Penicillin V in 0.347M SDS/ABS, pH 4.5 (red) at GC electrode. The amplitude and frequency were 0.025V and 15Hz respectively.

Voltammetric current in presence of penicillin V and SDS was enhanced almost ten times compared to the voltammogram of penicillin V in absence of SDS. The voltammogram of penicillin V also formed at peak at 1.6V with no observable shift in peak potential.

The square wave voltammetric responses of penicillin G and penicillin V at the glassy carbon electrode in ABS, pH 4.5 and SDS/ABS, pH 4.5 had striking similarity in shape and the peak potential, i.e., both voltammograms peaked at 1.6V. The voltammetric current peaks for both penicillin V and Penicillin G are remarkably higher in presence of SDS as compared to in its absence (ABS, pH 4.5) under similar conditions. Besides the similarity in the two voltammograms in terms of shape and peak potential, there is a marked difference in peak current enhancement. The difference in current enhancement may probably have resulted from the manner in which the two drugs interact with the surfactant and/or the swamping solution (acetate buffer solution).

Enhanced voltammetric currents is an indication that addition of 0.00347M SDS to ABS, pH 4.5 probably facilitated quick charge transfer at the electrode surface hence significantly increased the sensitivity for detection of penicillin V & G. This behavior is consistent with earlier observations by Galal *et al.*, (2012) who used sodium dodecyl sulphate to improve current response in self-assembled monolayers of cysteine on gold nanoparticles.

4.1.4: Cyclic Voltammetry

Cyclic voltammetry was used to elucidate the electrochemical of penicillin G and V and examine effects of changes in support electrolyte on the analyte.

4.1.5.1: Cyclic Voltammetry of Penicillin V (Phenoxymethylpenicillin)

Cyclic voltammograms of penicillin V at a polished GC electrode gave a distinct irreversible voltammetric peak at high positive potential of 1.6 V versus Ag/AgCl in ABS at pH 4.5 (Figures 4.8).

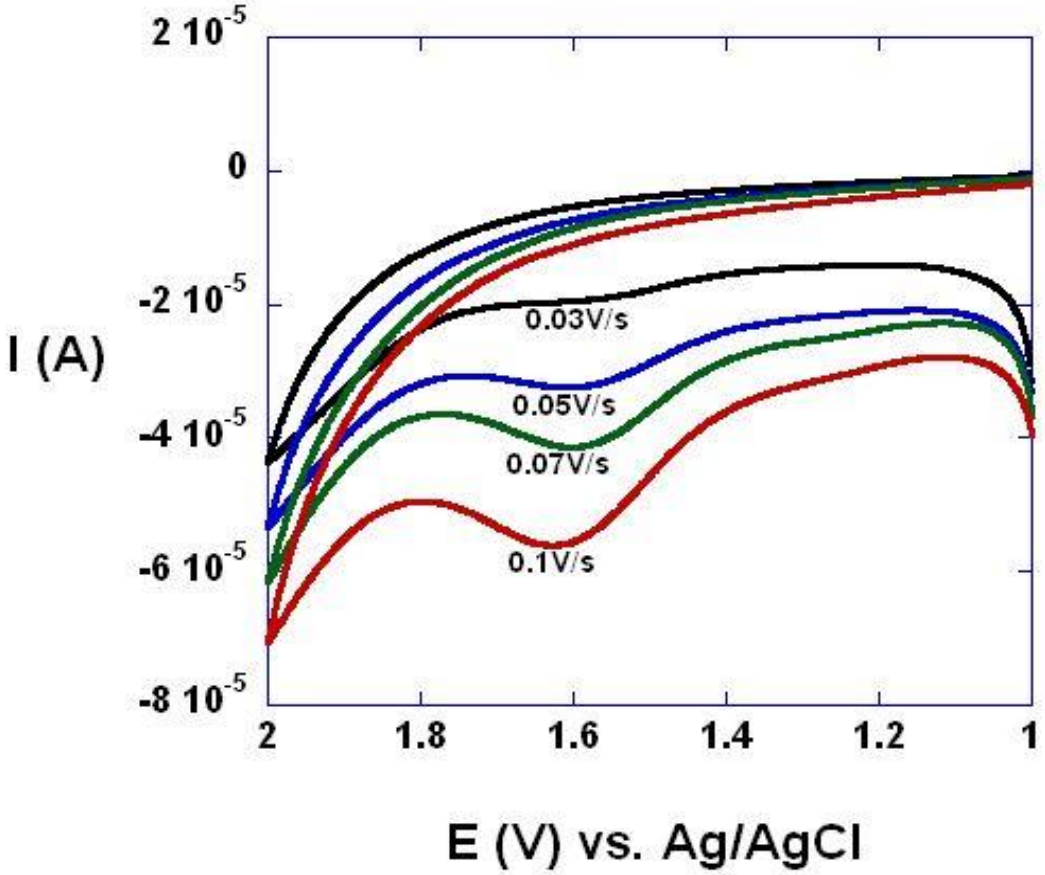


Figure 4.8: (A) Cyclic voltammograms of 1.4mM phenoxymethylpenicillin in ABS, pH 4.5 on glassy carbon electrode. Initial potential: 1.0V; high potential: 2.0V; low potential: 1.0V; sample interval: 0.001V, quiet time: 0.1sec. Scan rates were varied from 30mV/s to 100mV/s.

A plot of oxidative current(A) against square-root of scan rate formed a linear plot according to Randles-Sevcik equation (Figure 4.9).

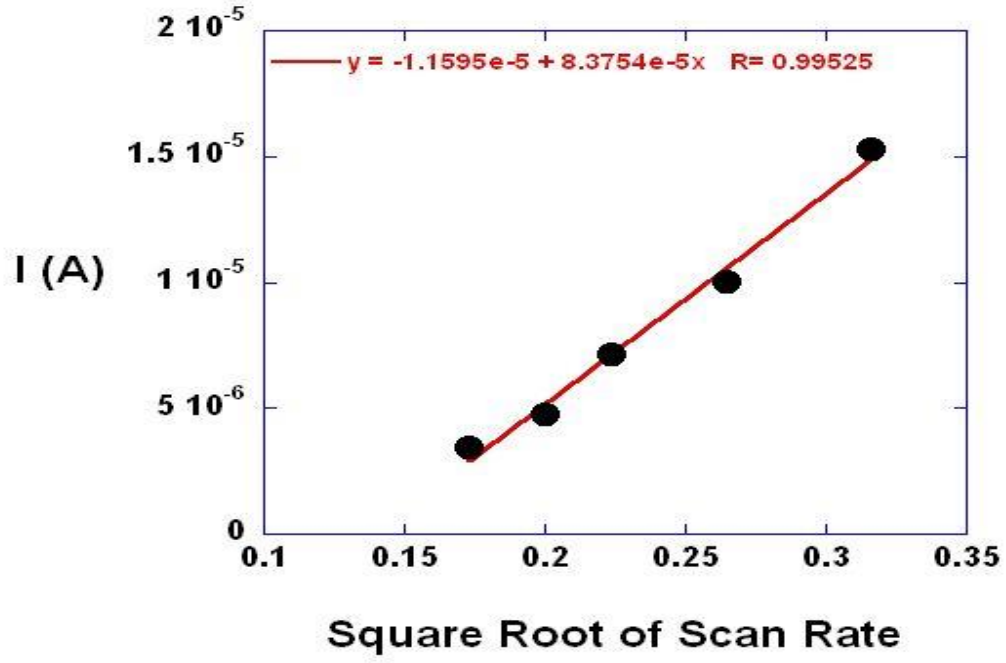


Figure 4.9: Randles-Sevcik plot of anodic currents (i_{pa}) versus the square root of scan rates ($v^{1/2}$) of 1.4mM penicillin V in ABS, pH 4.5.

Cyclic voltammograms of penicillin V at a polished GC electrode in presence of sodium dodecyl sulfate gave a distinct irreversible voltammetric peak at high positive potential of 1.6 V versus Ag/AgCl in ABS at pH 4.5 (Figures 4.10). There was no significant change in potential with varying scan rates.

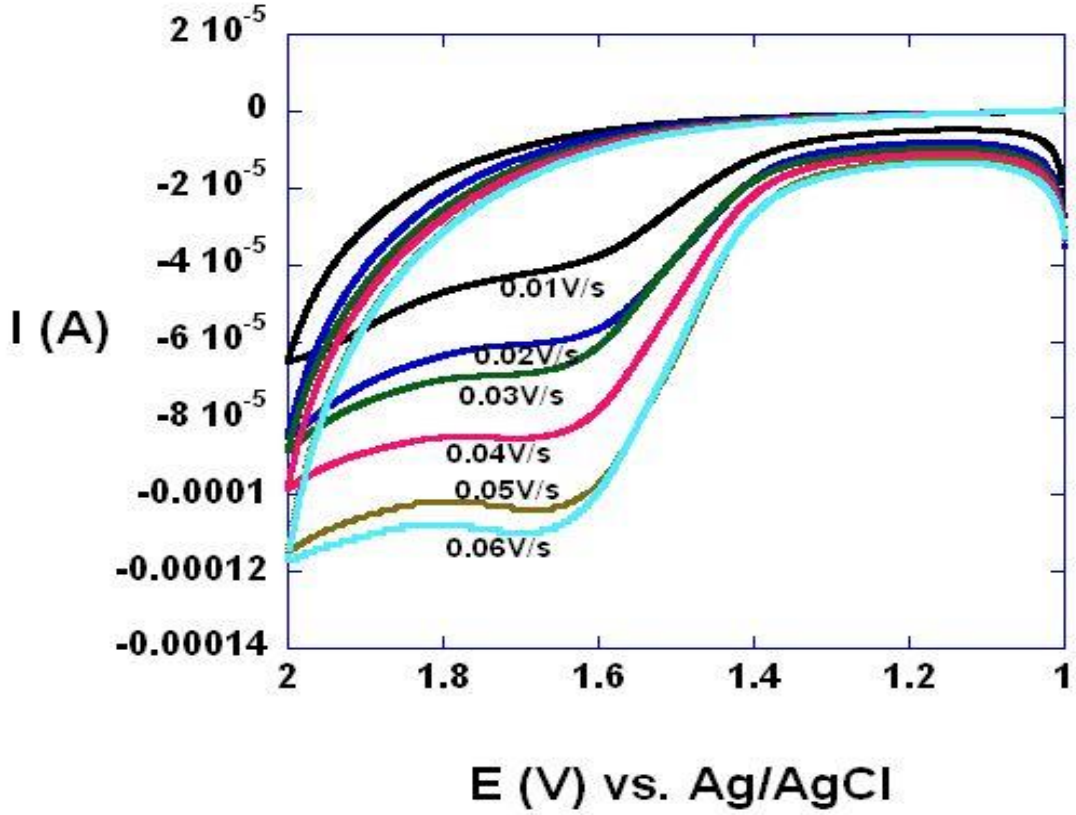


Figure 4.10: Cyclic voltammograms of 1.4mM penicillin V in 0.347M SDS in ABS, pH 4.5 on glassy carbon electrode. Initial potential: 1.0V; high potential: 2.0V; low potential: 1.0V; sample interval: 0.001V, quiet time: 0.1sec. Scan rates were varied from 10mV/s to 60mV/s.

A plot of oxidative current(A) against square-root of scan rate formed a linear plot according to Randles-Sevcik equation (Figure 4.11).

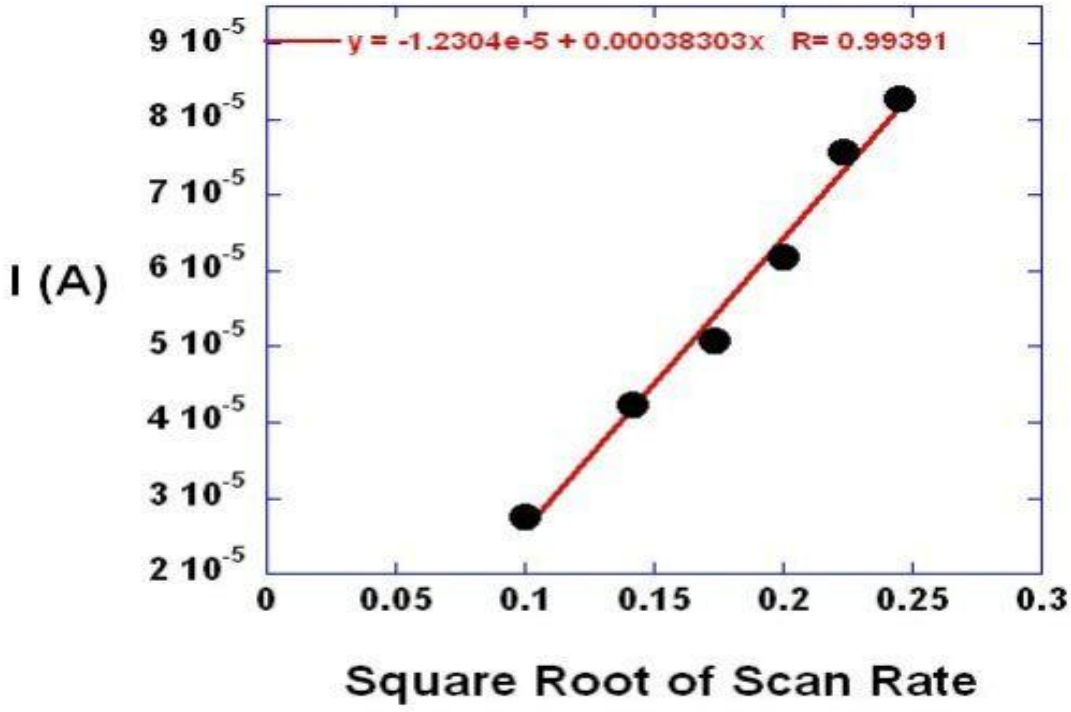


Figure 4.11: Randles-Sevcik plot of anodic currents (i_{pa}) versus the square root of scan rates ($v^{1/2}$) of 1.4mM penicillin V in 0.347M SDS in ABS, pH 4.5.

In both cases (in absence and presence of SDS) current increased with increasing scan rates. This is probably because at higher scan rates the diffusion layer is reduced therefore resulting into high currents. At lower scan rates, the size of diffusion layer increases significantly reducing the current.

The oxidation potentials in ABS, pH 4.5 (in absence of SDS) compares relatively well with those taken in SDS/ABS, pH 4.5 on GC electrodes as shown in Figures 4.8 and 4.10. In presence of SDS the forward scans produced a current peak slightly past 1.6V while there was no reductive current peak in the reverse scan.

A slight difference in potential was observed in presence and in absence of sodium dodecyl sulfate. There was also a slight difference in the shape of the voltammograms in the presence and absence of SDS as shown in Figure 4.8 and 4.10. These slight differences could be attributed to the slight change in the chemistry of the electrolyte. It can be established from the voltammograms that the electrochemical oxidation of penicillin V in absence and in presence of sodium dodecyl sulfate leads to an irreversible process since even at high scan rate, the reverse process does not produce reductive peak. The irreversibility of the system is probably because the oxidative step was followed by a chemical reaction which led to formation of a product which was not redox active as shown by equations 4.3 and 4.4 below.

$$Red \leftrightarrow Ox + e \tag{4.3}$$

$$Ox \rightarrow z \tag{4.4}$$

The peak potentials in both cases (in absence and presence of SDS) were within the potential range observed by Ľubomír *et al.,* (2012) for phenoxymethylpenicillin on bare boron-doped diamond electrode using differential pulse voltammetric method.

Current readings from the voltammograms in Figures 4.8 and 4.10 were plotted against the square root of the scan rate to give Randles-Sevcik plots shown in Figure 4.9 and 4.11. These plots were linear indicating that the electrochemical reaction for the oxidation of penicillin V on glassy carbon electrode is dorminantly diffusion controlled. However, the Randles-sevcik plot had a none-zero y-intercept which implied that other modes of transport were involved in transfer of penicillin V to the electrode surface but to a small extent

The gradient of the plots (4.9 and 4.11) were used in Randles-Sevcik equation (Equation 2.6) to calculate the diffusion coefficient of the analyte, penicillin V. However, this was only possible if the number of electrons involved in the oxidation process was known. The number of electrons (n) used in the oxidation of penicillin V was calculated from the slope of the plots of potential, E, versus log [i/i_d-i] according to the Equation 4.5 below.

$$E = E_{1/2} - \frac{0.0591}{n}\log\left(\frac{i}{i_d - i}\right) \qquad (4.5)$$

Where $E_{1/2}$ =half-wave potential, E=potential at any point on the wave i_d= peak current, i=current at any point on the wave and n =number of electrons involved.

In both cases the number of electrons were approximately two. Applying the Randles-Sevcik equation and the gradient from the plots, the diffusion coefficient of penicillin V in ABS/SDS, pH 4.5 and ABS, pH 4.5 were found to be $1.26 \times 10^{-5} cm^2/sec$ and $6.01 \times 10^{-7} cm^2/sec$.

4.1.5.2: Cyclic Voltammetry of Penicillin G (Benzylpenicillin)

Cyclic voltammograms of penicillin G at a polished glassy carbon electrode gave a clear irreversible peak at high positive potential of 1.6 V versus Ag/AgCl in ABS, pH 4.5 (Figure 4.12).

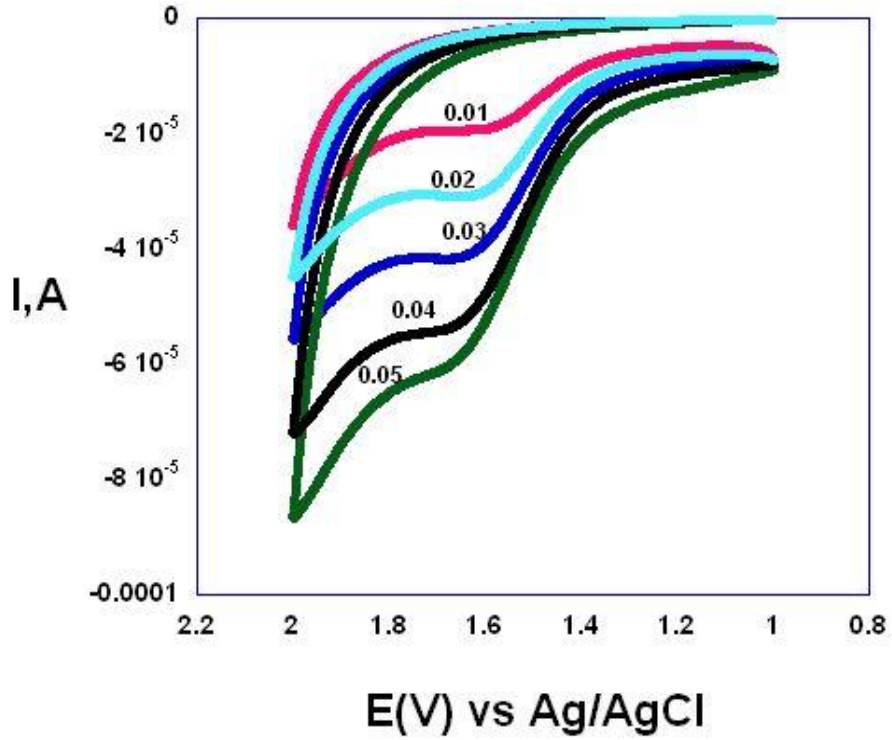


Figure 4.12: Cyclic voltammogram of 2.0mM penicillin G in acetate buffer (ABS), pH 4.5 on glassy carbon electrode. Initial potential: 1.0V; high potential: 2.0V; low potential: 1.0V; sample interval: 0.001V, quiet time: 0.1sec. Scan rates ranging from 10mV/s to 50mV/s

A plot of oxidative current(A) against square-root of scan rate formed a linear plot as shown in

Figure 4.13.

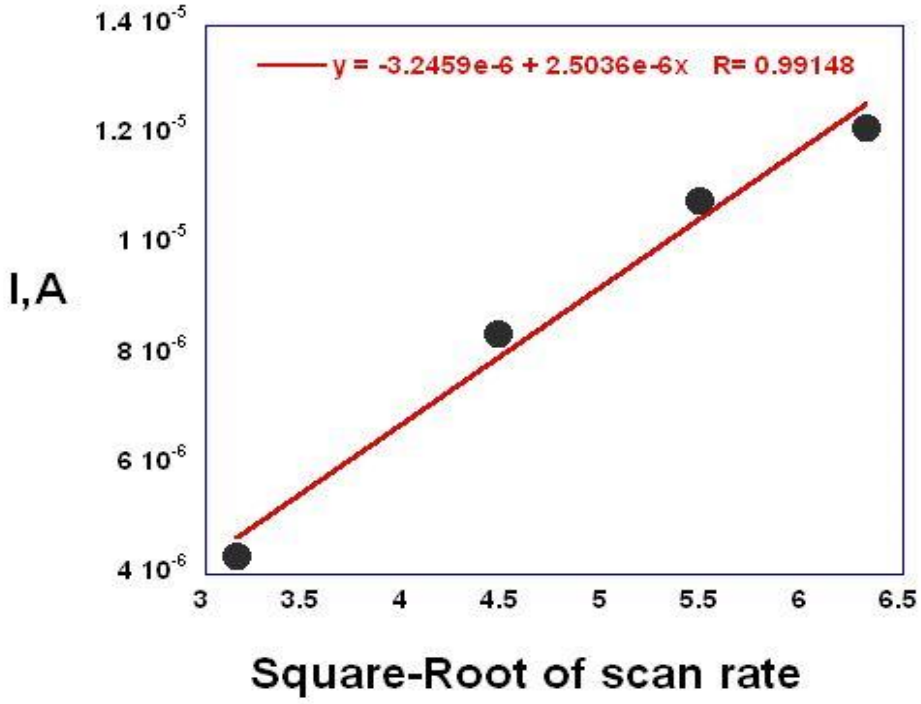


Figure 4.13: Randles-Sevcik plot of oxidation peak currents (i_{pa}) versus the square root of scan rates ($v^{1/2}$) of 2.0mM penicillin G in ABS, pH 4.5 on glassy carbon electrode.

Cyclic voltammograms of penicillin G at a polished GC electrode in presence of sodium dodecyl sulfate gave a distinct irreversible voltammetric peak at high positive potential of 1.6 V versus Ag/AgCl in ABS at pH 4.5 (Figure 4.14).

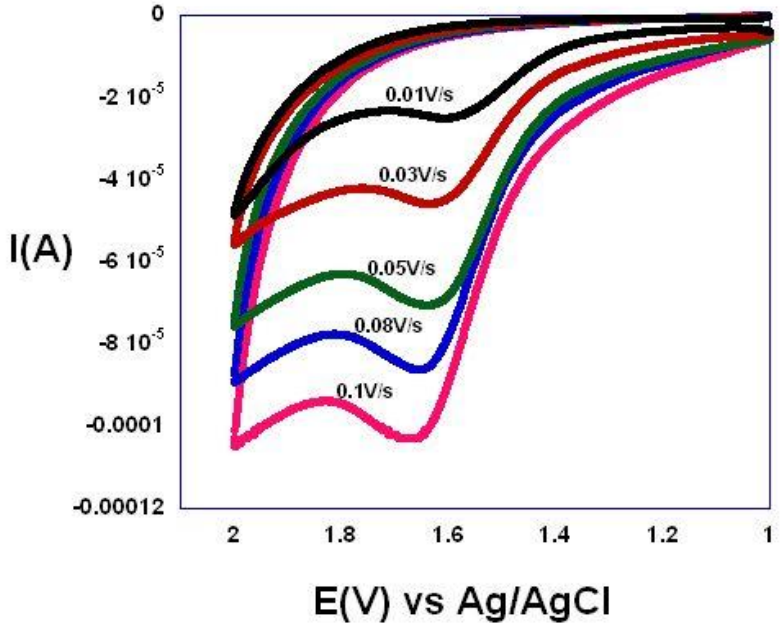


Figure 4.14: Cyclic voltammogram of 2.0mM penicillin G in 0.347M SDS in ABS, pH 4.5 on glassy carbon electrode. Initial potential: 1.0V; high potential: 2.0V; low potential: 1.0V; sample interval: 0.001V, quiet time: 0.1sec at different scan rates.

A plot of oxidation currents (i_{pa}) against the square root of scan rates ($v^{1/2}$) of penicillin G between 0.01 and 0.1 V/s in ABS/SDS media was linear as shown in Figure 4.15.

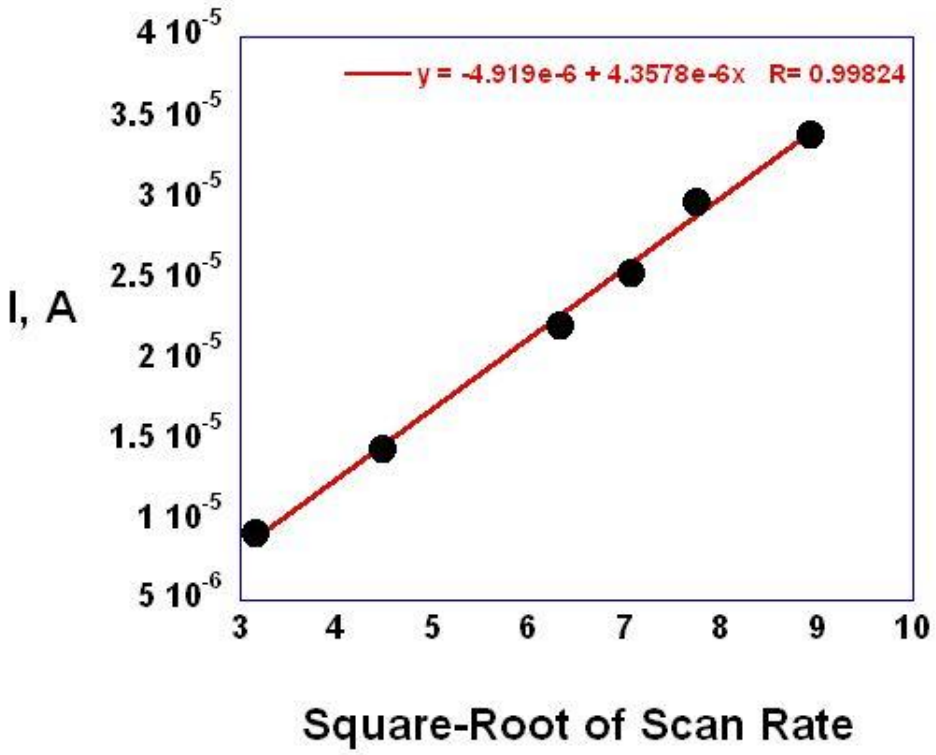


Figure 4.15: Randles-Sevcik plot of oxidation peak currents (i_{pa}) against square root of scan rate ($v^{1/2}$) of 2.0mM penicillin G in 0.347M SDS in ABS, pH 4.5.

The oxidation potential of benzylpenicillin in presence of sodium dodecyl sulfate (SDS/ABS, pH 4.5) compares relatively well with the oxidation potential in absence of SDS (ABS, pH 4.5) on GC electrodes as shown in Figures 4.12 and 4.14. The slight difference particularly with respect to the shape of the voltammogram can be attributed to the slight change in the chemistry of the electrolyte.

Plots of oxidation peak currents against square root of scan rates were linear in both cases (Figures 4.13 and 4.15) indicating that the electrochemical reaction for the oxidation of penicillin G on glassy carbon electrode is dorminantly diffusion controlled (Bard *et al.*, 2004; Guto *et al*., 2017)

The diffusion coefficients of penicillin G in both acetate buffer solutions and SDS/acetate buffer solution were obtained from the slopes of the linear plots as provided by Randles–Sevcik equation, Equation 2.6. Linear plots drawn at low scan rates demonstrate that the electrode process for oxidation of penicillin G is diffusion controlled (Bard *et al.,* 2004; Guto *et al*., 2017). Accordingly, the diffusion coefficients calculated from Randles-Sevcik equation were 1.39×10^{-6} cm^2/sec and 6.01×10^{-7} cm^2/sec for SDS/ABS, pH 4.5 and ABS, pH 4.5 respectively on highly polished glassy carbon electrodes. Similarly, the diffusion coefficient for phenoxymethylpenicillin (penicillin V) was estimated at $1.26 \times 10^{-5} cm^2$/sec and $6.01 \times 10^{-7} cm^2$/sec on SDS/ABS, pH 4.5 and ABS, pH 4.5 respectively on the same glassy carbon electrodes.

In both cases the diffusion coefficient is higher in acetate-buffer sodium dodecyl sulfate solution (ABS/SDS). The change in diffusion coefficient may be attributed to possible interaction between sodium dodecyl sulfate and penicillin G/V. This interaction could possibly lead to pre-concentration of penicillin G/V at the interface (Atta *et al.*, 2011; Huang *et al.*, 2004). A higher concentration of both penicillin G and V near the electrode surface will most likely lead to a higher diffusion rate of the analytes. A summary of the effects of adding SDS in support electrolyte in voltammetric determination of penicillin G and V are shown in Table 4.1.

Table 4.1: Summary of effects of addition of sodium dodecyl sulfate on oxidation potential and diffusion coefficient of penicillin G and V.

	Media	$E^{o'}$ (Oxid. Potential)	R^2 (Correlation coefficient)	D_o (Diffusion Coefficient)
Pen G	ABS, pH 4.5	1.62V versus Ag/AgCl	0.9915	6.01×10^{-7} cm^2/sec
	SDS in ABS, pH 4.5	1.6V versus Ag/AgCl	0.9982	1.39×10^{-6} cm^2/sec
Pen V	ABS, pH 4.5	1.6V vs. Ag/AgCl	0.9952	6.01×10^{-7} cm^2/sec
	SDS in ABS, pH 4.5	1.61V vs. Ag/AgCl	0.9939	1.26×10^{-5} cm^2/sec

4.2: Validation of the Voltammetric Method

Validation tests included, accuracy, precision, limit of detection and quantitation and linear range. These tests were done on the proposed method using penicillin V and penicillin G.

4.2.1: Linearity, Limit of Detection and Limit of Quantitation in Voltammetric Determination of Penicillin V

A comparison of voltammetric currents at varying concentration of phenoxymethylpenicillin gave an overlay as shown Figure 4.16.

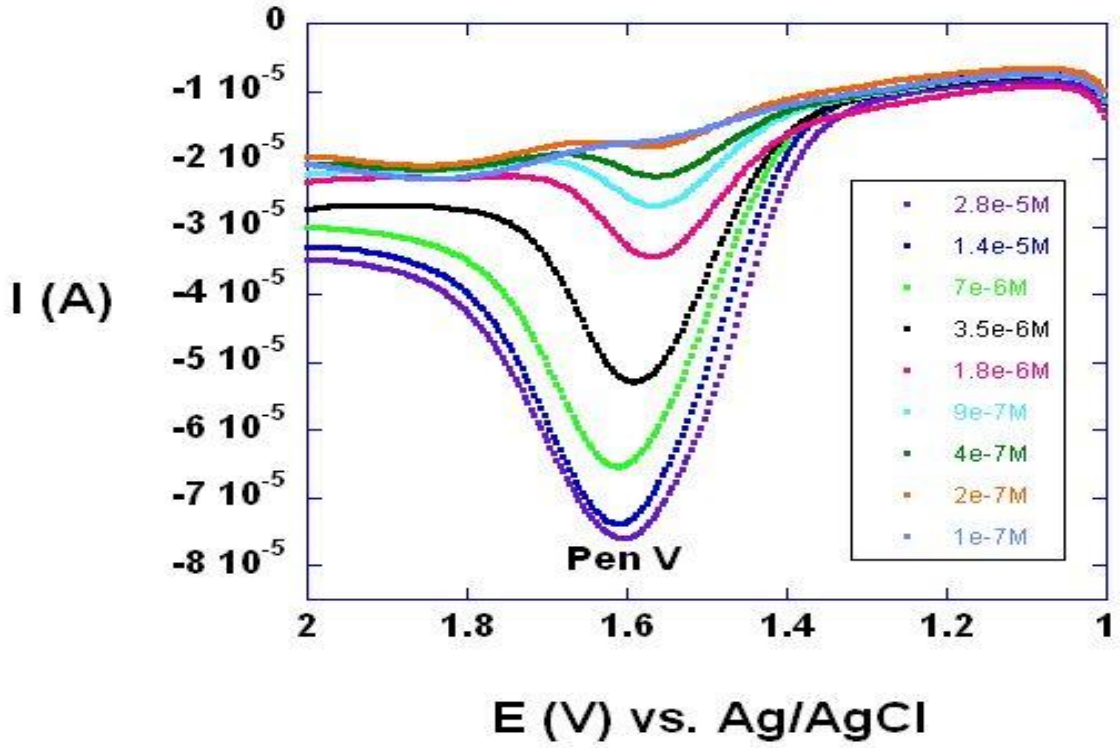


Figure 4.16: Square wave voltammetry of different concentrations of phenoxymethylpenicillin in the SDS/ABS, pH 4.5; frequency: 15Hz and amplitude 0.025V

A plot of voltammetric peak currents against concentration gave a linear regression plot (Figure 4.17).

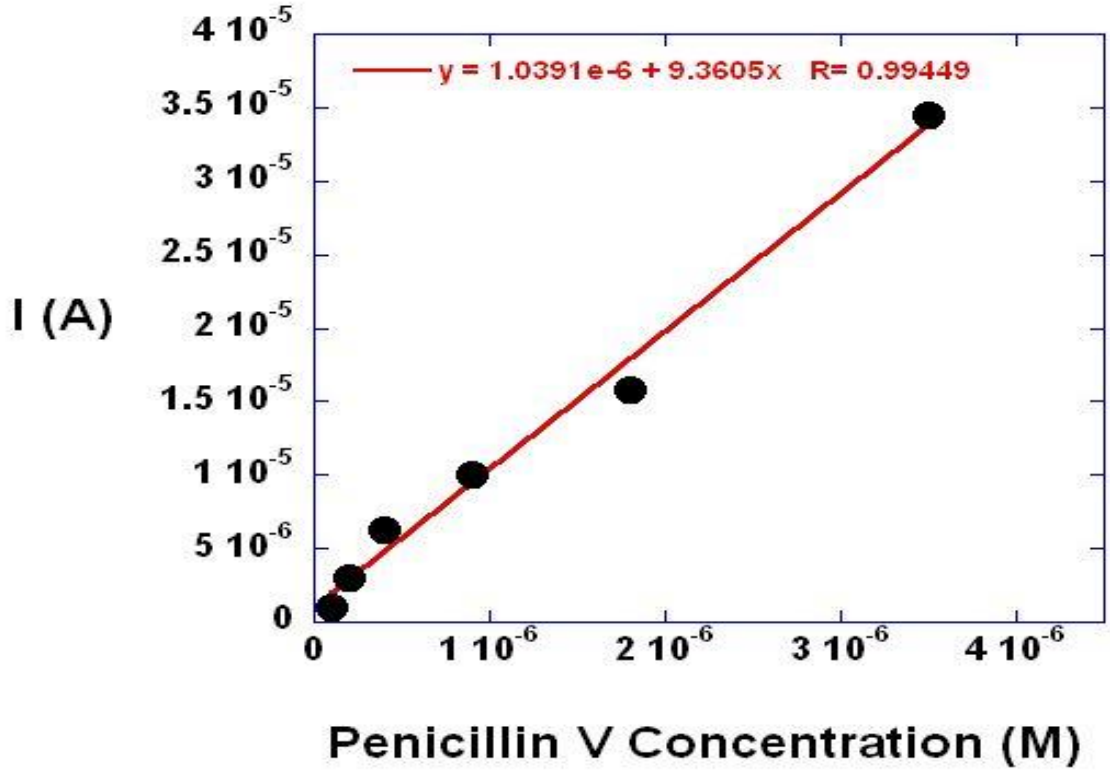


Figure 4.17: Calibration plot of concentration of penicillin V versus voltammetric current.

The linear regression plot gave a correlation coefficient of 0.994 indicating a linear dependence of the voltammetric current intensity on the analyte (penicillin V) concentration in SDS/ABS, pH 4.5 over the range 34.6 µM – 0.04µM penicillin V (Table 4.2).

Table 4.2: The linear concentration range (LCR), limit of detection (LOD), limit of quantitation (LOQ) and the regression equation (RE) of Penicillin V in Acetate buffer, pH 4.5 and SDS/Acetate buffer, pH 4.5 on bare GC electrode.

	Solvent	LCR	LOD	LOQ	RE	R^2
1.	ABS, pH 4.5	28.0 – 3.5µM	3.5µM	14.0µM	y=-1.0e-7+0.804x	0.98771
2.	SDS in ABS, pH 4.5	34.6 –0.04µM	0.04µM	0.12µM	y=1.0391e-5+9.3605x	0.99449

The limit of detection was based on three times the standard deviation of the baseline and were approximated to be 0.04µM in SDS/ABS, pH 4.5 (Table 4.2). The Limit of detection (LOD) of this method was generally established to be in the region where the signal to noise ratio is greater than three (Thompson *et al.*, 2002; De-Bièvre and Günzler, 2005; Guideline, 2005).

The limit of quantification for this method was found to be 0.12µM in SDS/ABS, pH 4.5 and it was based on three times the LOD (Table 4.2).

4.2.2: Linearity, Limit of Detection and Limit of Quantitation in Voltammetric Determination of Penicillin G

A comparison of voltammetric currents at varying concentration of benzylpenicillin gave an overlay as shown Figure 4.18.

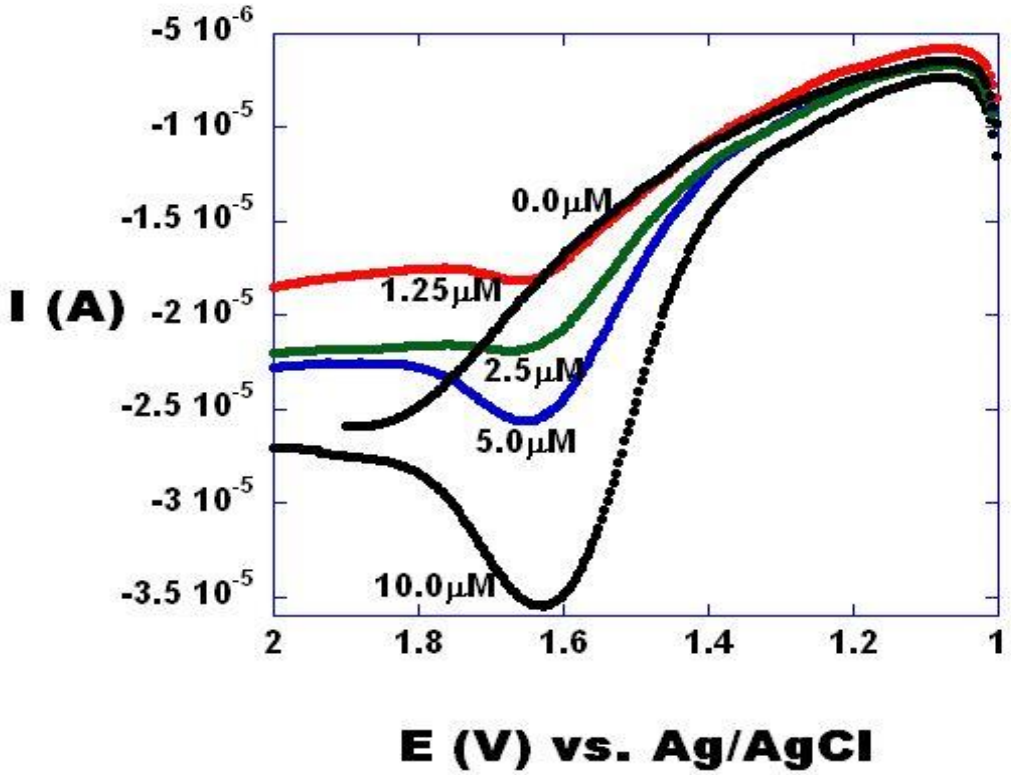


Figure 4.18: Square wave voltammograms of penicillin G at varying benzylpenicillin concentrations in 0.347M SDS in ABS, pH 4.5; frequency: 15Hz and amplitude 0.025V.

A calibration plot of the anodic peak current against penicillin G concentration was linear as shown in Figure 4.19 below

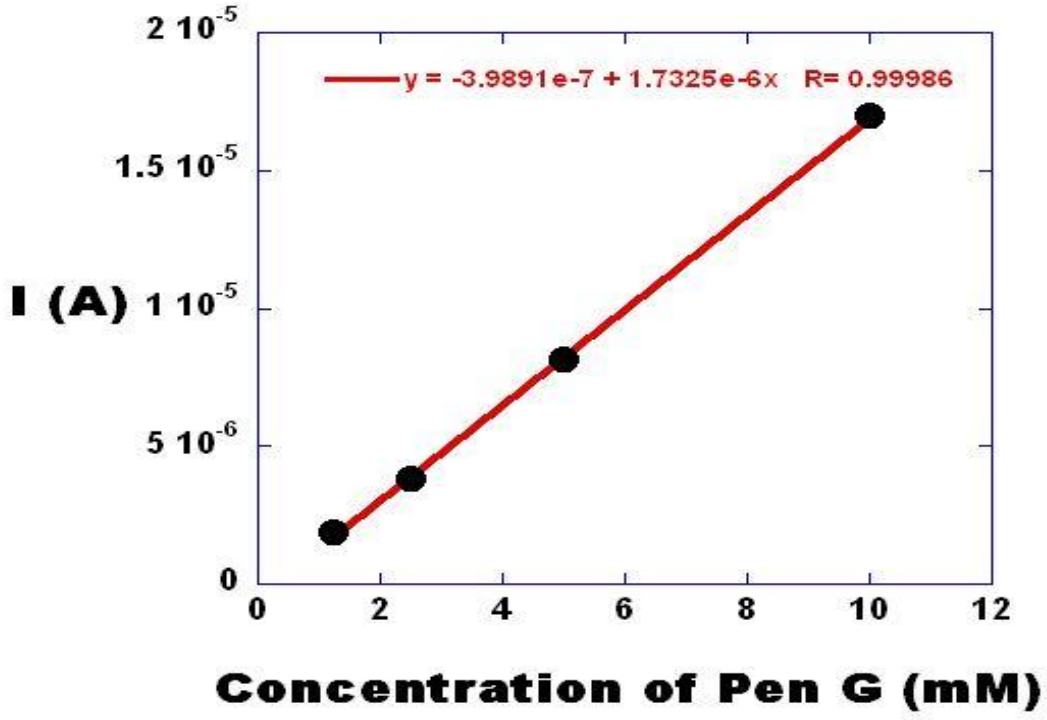


Figure 4.19: A plot of concentration of penicillin G versus voltammetric current.

The calibration plot was found to be linear over the range 15.0–1.25µM in SDS/ABS, pH 4.5 as shown in Table 4.3

Table 4. 3: LCR, LOD, LOQ, R^2 and regression equation for penicillin G in ABS, pH 4.5 and ABS/SDS, pH 4.5 on bare Glassy Carbon electrode

	Solvent	LCR	LOD	LOQ	RE	R^2
1.	ABS, pH 4.5	10.0 – 2.5µM	2.5µM	7.5µM	y=-3.2e-6+2.5e-6x	0.99148
2.	SDS in ABS, pH 4.5	15.0–1.25µM	1.25µM	3.75µM	y=-3.99e-6+1.73e-6x	0.99986

LCR = Linear concentration range, LOD = Limit of detection, LOQ = Limit of quantitation, RE= Regression equation.

The Linearity of the calibration plot implies that there is a clear linear dependence of the current intensity on the benzylpenicillin concentration in SDS/ABS, pH 4.5 over the range 15.0–1.25µM.

The limit of detection was estimated to be 1.25µM in SDS/ABS, pH 4.5 based on three times the standard deviation of the baseline (*Equation 2.21*) while the limit of quantitation (Equation 2.23) was found to be 3.75µM as shown in the Table 4.3. The LOD was established to be within the region where the signal to noise ratio is equal or greater than three (Feier *et al*., 2017; Švorc *et al*., 2012; De-Bièvre and Günzler, 2005).

4.2.3: Precision Studies in Determination of Pen V and G in ABS-SDS

The oxidation potential and voltammetric currents of penicillin V and penicillin G were obtained using square wave voltammetry as shown in the Table 4.4.

Table 4.4: Oxidation potential and voltammetric currents of penicillin V and G obtained using square wave voltammetry

	Penicillin V		Penicillin G	
Trial	Potential (V)	Current (A)	Potential(V)	Current (A)
1	1.580	2.456×10^{-5}	1.60	1.84×10^{-5}
2	1.588	2.478×10^{-5}	1.61	1.81×10^{-5}
3	1.584	2.531×10^{-5}	1.60	1.96×10^{-5}
4	1.580	2.449×10^{-5}	1.60	1.96×10^{-5}
5	1.584	2.449×10^{-5}	1.60	1.94×10^{-5}
6	1.592	2.454×10^{-5}	1.60	2.04×10^{-5}
7	1.588	2.474×10^{-5}	1.60	1.98×10^{-5}
8	1.584	$2.441\times1o^{-5}$	1.60	1.98×10^{-5}
9	1.588	2.454×10^{-5}	1.60	1.86×10^{-5}
10	1.608	2.478×10^{-5}	1.60	1.81×10^{-5}

The voltammetric currents obtained from the above data were analysed to determine how precise the measurements were. The average value, standard deviation and relative standard deviation for both penicillin V and G were calculated and reported as shown in Table 4.5.

Table 4.5: Summary of statistical values obtained from square wave voltammetric currents of penicillin G and V

	Calculated statistical parameter	**Pen G (standard)**	**Pen V**
1.	Number of replicate Sample	10	10
2.	Average Value	$1.92x10^{-5}$	$2.47x10^{-5}$
3.	Standard Deviation (SD)	$8.12x10^{-7}$	$2.62x10^{-7}$
4.	RSD%	4.23%	1.06%

The standard deviation and relative standard deviation were $2.62x10^{-7}$ and 1.06% respectively for penicillin V and $8.12x10^{-7}$ and 4.23% respectively for penicillin G. Since the standard deviation was very small (below 10%) (Guideline, 2005; De-Bièvre and Günzler, 2005; Oyagi *et al*., 2016; Thompson *et al*., 2002) it was concluded that the voltammetric currents were precise enough, an indication that the developed voltammetric method had good precision.

4.2.4: Accuracy Studies in Determination of Pen V and G in ABS-SDS

On standard addition of 50%, 100% and 150% of the original analyte concentration the resulting voltammetric currents were reported as shown in Table 4.6 below.

Table 4.6: Recovery studies after standard addition (50%-150%) of .14M penicillin V and 0.2M penicillin G

	Sample	Original (mM)	Current (A)	Added (mM)	Current expected(A)	Found (A)	Recovery, %
1.	Pen V	1.4	$1.84x10^{-5}$	0.7	$2.76x10^{-5}$	$2.73\ x10^{-5}$	98.9%
		1.4	$1.84x10^{-5}$	1.4	$3.68x10^{-5}$	$3.59x10^{-5}$	97.6%
		1.4	$1.84x10^{-5}$	2.1	$4.60x10^{-5}$	$4.48x10^{-5}$	97.4%
2.	Pen G Standard)	2	$7.69x10^{-6}$	1	$1.153x10^{-5}$	$1.15x10^{-5}$	99.3%
		2	$7.69x10^{-6}$	2	$1.54x10^{-5}$	$1.66x10^{-5}$	108.1%
		2	$7.69x10^{-6}$	3	$1.92x10^{-5}$	$2.03x10^{-5}$	105.6%

Current readings were done in triplicates for the three different concentrations as recommended by ICH and the average of each concentration recorded as shown in the Table 4.6

The percentage recovery was done by comparing the current obtained with the expected current. From the results the percentage recovery was ranging from 97.4 - 108.1% which is within the recommended range of 90% to 110% by international council for harmonization of technical requirements for pharmaceuticals for human use (ICH) document on validation methodology (Guideline, 2005). As such the proposed method is precise and accurate in determination of both analytes, penicillin G and V.

4.3: Effect of interferents

The effects of Na^{+}, Mg^{2+} K^{+}, Fe^{3+}, Zn^{2+}, Ca^{2+}, Cu^{2+}, PO_4^{3-}, Cl^{-}, NO_3^{-}, and SO_4^{2-} on determination of penicillin V and G are provided in Table 4.7

Table 4.7: Effect of addition of interferents on ABS-SDS penicillin G

Interferents	Potential (V)	Current (A)
Control	1.60	1.82×10^{-5}A
Calcium chloride	1.60	2.10×10^{-5}A
Copper sulphate	1.60	1.82×10^{-5}A
Iron sulphate	1.60	2.95×10^{-5}A
Potassium chloride	1.61	2.15×10^{-5}A
Potassium dihydrogen orthophosphate	1.61	1.88×10^{-5}A
Potassium nitrate	1.60	2.2×10^{-5}A
Sodium nitrate	1.62	1.85×10^{-5}A
Zinc chloride	1.61	1.99×10^{-5}A

The above data were used to plot the voltammetric current (I) against potential (E) for the various ions (Figure 4.20).

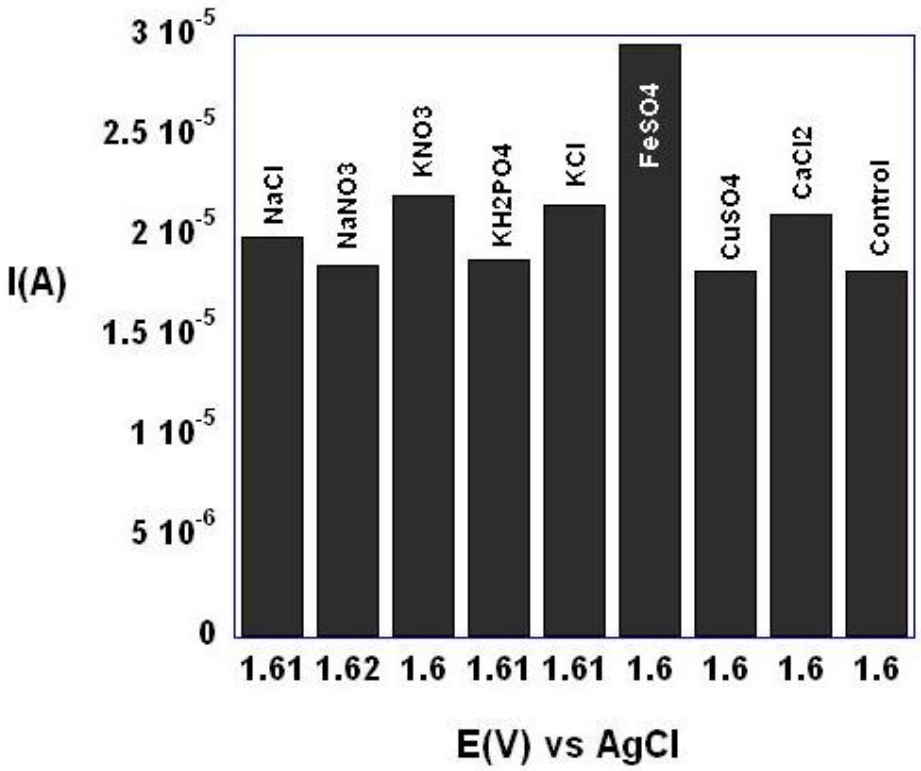


Figure 4.20: Effect of interferents on voltammetric current in determination of penicillin G in acetate buffer-sodium dodecyl sulphate solution.

From the histogram there was little change on voltammetric current except in iron II sulphate where current was significantly increased. The mean potential of the above data points was 1.6055V, with a standard deviation of 0.007 and a relative standard deviation of 0.45%. The variability of these data points from the control was also tested using student t test at a certain confidence interval to check if they lie within the acceptable limits.

$$Confidence\ level = \bar{x} \pm \frac{ts}{\sqrt{n}} \qquad (4.6)$$

At 95% confidence level, the limits of the potential should lie between 1.5994-1.6116. The mean potential (1.6055V) lies within the stated range. There was no significant change in the potential at 95% confidence limit and therefore, these substances did not have significant effect on the oxidation potential of penicillin G.

Similarly, the mean current was found to lie within the expected range, 1.8191-2.4229 x 10^{-5}, therefore there was no significant change in the current in presence of interferents. However, Fe^{3+} significantly increased the current, which actually helped in voltammetric determination of penicillin G. A similar observation was made when the effects of the same interferents were studied using penicillin V in acetate buffer-sodium dodecyl sulfate solution as shown in the Table 4.8.

Table 4.8: Effect of addition of interferents on ABS-SDS penicillin V

Interferents	Potential (V)	Current (A)
Control	1.59	3.18 x 10^{-5} A
Calcium chloride	1.61	3.30×10^{-5}A
Copper sulphate	1.60	3.20×10^{-5}A
Iron (III) Chloride	1.61	4.45×10^{-5}A
Potassium chloride	1.59	3.40×10^{-5}A
Potassium dihydrogen orthophosphate	1.59	3.36×10^{-5}A
Potassium nitrate	1.59	3.32×10^{-5}A
Magnesium chloride	1.59	3.31×10^{-5}A
Sodium nitrate	1.59	3.38×10^{-5}A
Zinc chloride	1.59	3.28×10^{-5}A

A plot of the voltammetric current (I) against potential (E) of the above data was expressed in form of a histogram as shown in Figure 4.21.

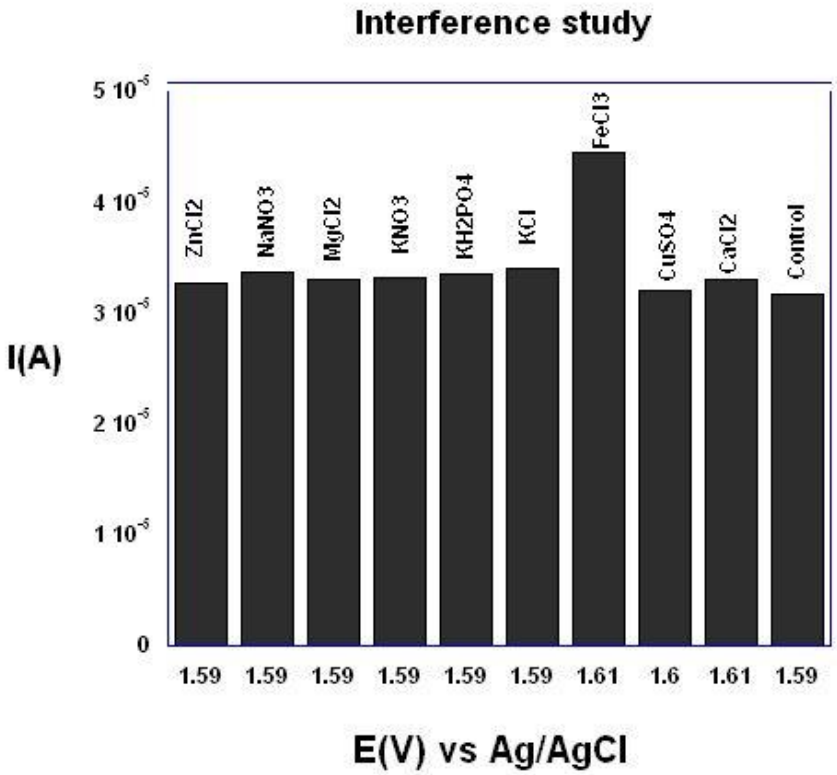


Figure 4.21: Effect of interferents on voltammetric current in determination of penicillin V in acetate buffer-sodium dodecyl sulphate solution

There was no significant change in the oxidation potential of penicillin V, since the standard deviation from mean and relative standard deviation reported were 0.0085 and 0.53% respectively.

Natural samples normally contain enormous number of easily oxidizable/reducible groups. Serious interference in penicillin V and G determination could have occurred if some of the interferences were adsorbed at the electrode surface together with the analyte of interest. This happens when the interferants are oxidized or reduced at potentials close to that of the analyte ions or if the electrode surface cannot discriminate the analyte from other substances dissolved in the support electrolyte (Sila *et al*., 2018).

Most of these substances had no significant effect on both voltammetric current and oxidation potential of both penicillin V and penicillin G. However, salts of iron enhanced the oxidation current significantly with no significant effect on the oxidation potential of both penicillin V and G. It's worth mentioning that for trace levels of these interferants in less contaminated real samples; their interfering effect will not pose any significant effect on their analysis.

4.4: Evaluation of the developed Method

4.4.1: Cyclic Voltammetry of Cow Milk Sample Containing Penicillin G and Penicillin G Containing Capsules

Cyclic voltammetry of penicillin G in cow milk gave only one irreversible oxidation peak around 1.65V versus Ag/AgCl as shown in Figure 4.22.

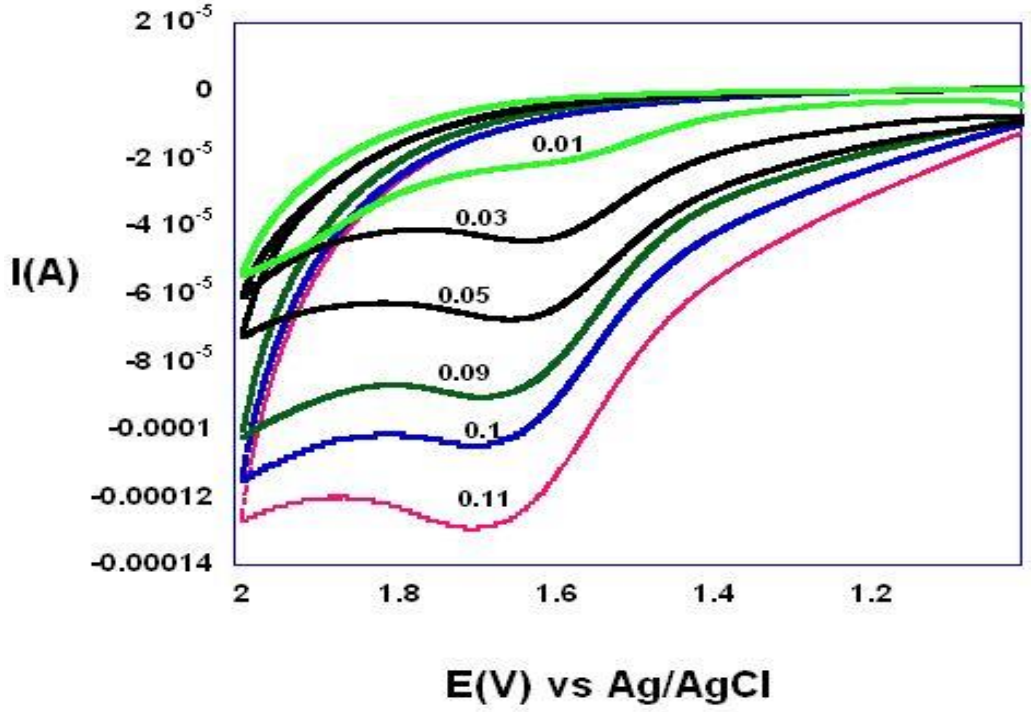


Figure 4.22: An overlay of cyclic voltammograms of 0.1M penicillin G in cow milk, pH 4.5 on glassy carbon electrode at different scan rates. Initial potential: 1.0V; high potential: 2.0V; low potential: 1.0V

A plot of oxidation currents (i_{pa}) against the square root of scan rates ($v^{1/2}$) of penicillin G in ABS/SDS media is linear as shown in Figure 4.23

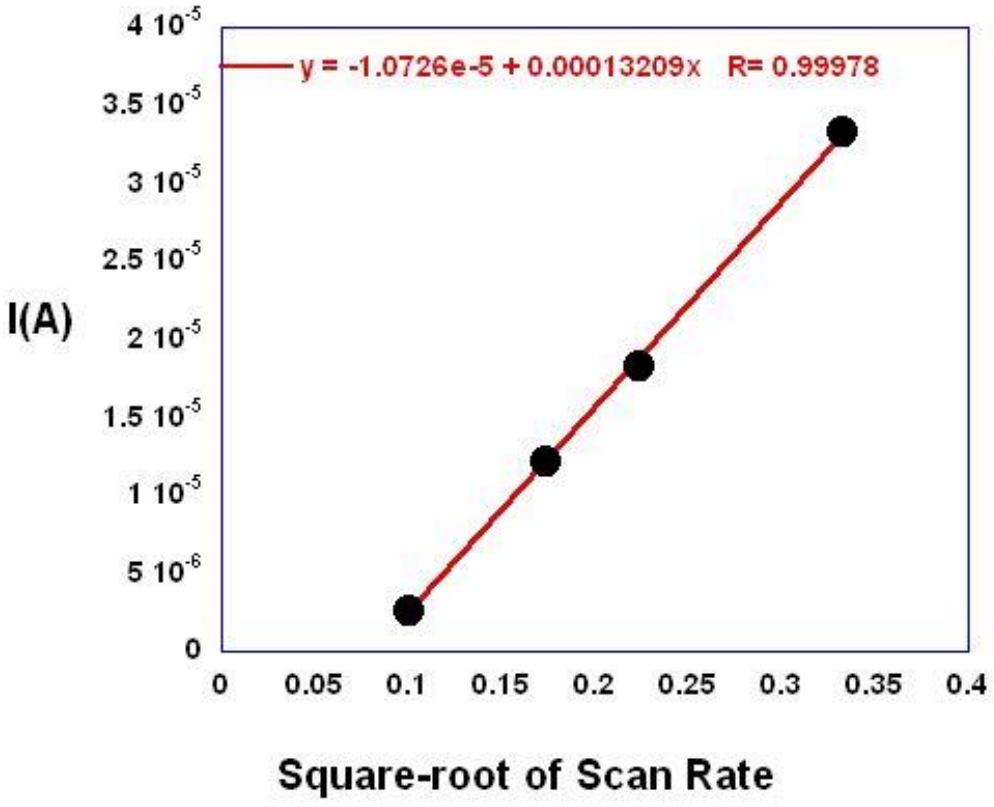


Figure 4.23: A plot of anodic peak currents (i_{pa}) against square root of scan rate ($v^{1/2}$) for penicillin G in ABS/SDS solution.

These oxidation peaks of milk sample containing penicillin G were well defined and the oxidation currents for the analyte (penicillin G) were found to increase with increasing scan rate as demonstrated in Figure 4.22. This behaviour is associated with fast electron transfer systems (Bard and Faulkner , 2001) It was also observed that the oxidation potentials for the penicillin G in cow milk (Figure 4.22) compared relatively well with that obtained for penicillin G in SDS/ABS media (Figure 4.14). The slight difference, particularly with respect to the shape of the voltammogram, can be attributed to the additional components from cow milk that were not separated during sample treatment.

The plot of voltametric peak currents against square root of scan rate gave a linear plot indicating a diffusion-controlled process (Bard and Faulkner , 2001). Moreover, the slope in Figure 4.23 was used to calculate the rate of diffusion of the analyte using Randles-Sevcik equation (Equation 2.6). The number of electrons (n) used in the oxidation of penicillin G were previously found to be approximately two. The calculated value for diffusion coefficient (D_o) for penicillin G in cow milk was found to be $1.494x10^{-6}$ cm^2/s.

Oxidation potentials for the procaine-penicillin drug, a common antibiotic used in animal husbandly showed similar results to those of penicillin G in ABS-SDS as shown in Figure 4.24.

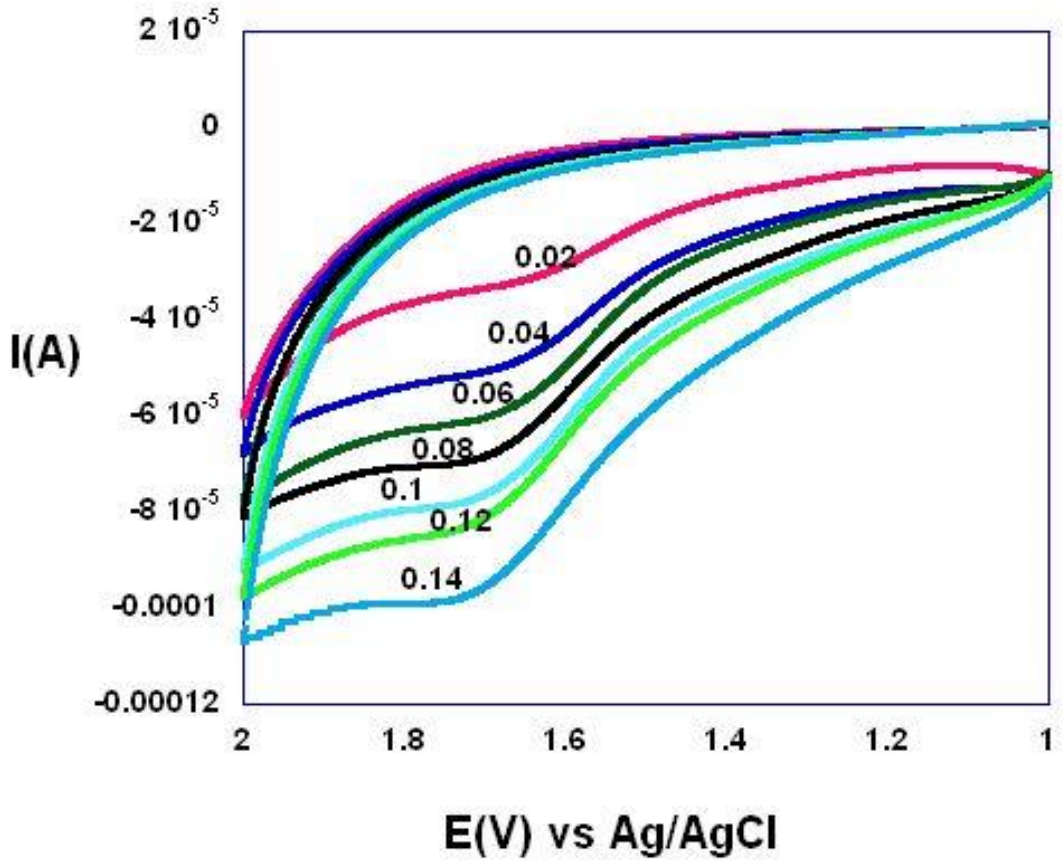


Figure 4.24: An overlay of cyclic voltammograms of 0.1M penicillin G in procaine penicillin drug, pH 4.5 on glassy carbon electrode at different scan rates. Initial potential: 1.0V; high potential: 2.0V; low potential: 1.0V

A plot of oxidation currents (i_{pa}) against the square root of scan rates ($v^{1/2}$) of penicillin G in procaine penicillin drug in ABS/SDS media was linear, with a coefficient correlation of 0.99 as shown in the Figure 4.25.

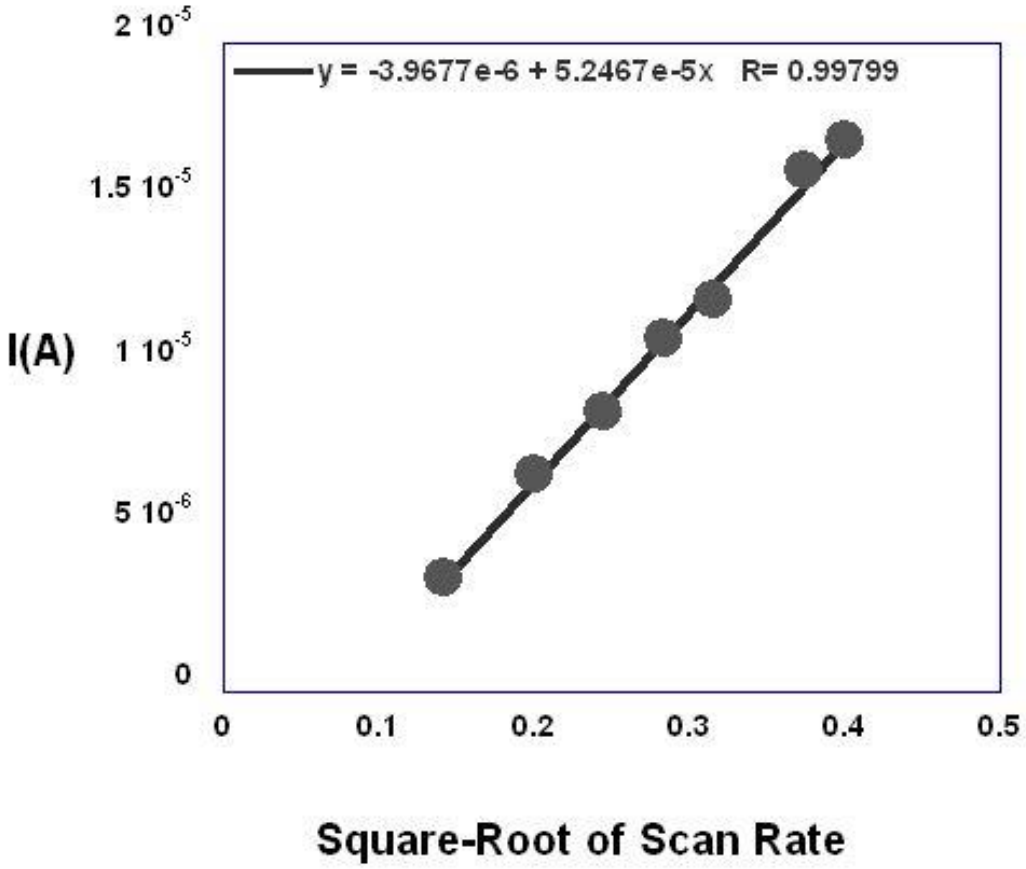


Figure 4.25: A plot of anodic peak currents (i_{pa}) against square root of scan rate ($v^{1/2}$) in procaine penicillin drug.

The oxidation currents for the penicillin G in procaine penicillin drug were found to increase with increasing scan rate as demonstrated in Figure 4.24. The peak potential in procaine-penicillin G shifted to higher potential which may be attributed to other components of the drug.

The linearity of the plot in Figure 4.25 is an indication that rate of transfer of penicillin G in the drug to the electrode surface was mainly diffusion controlled. However, the linear plot did not pass through the origin implying that other mass transfer processes might have influenced the electrode process but to a small extent. Since the number of electrons involved in the oxidation process was estimated to be two, diffusion coefficient of penicillin G in procaine

drug was estimated using Randles-Sevcik equation (Equation 2.6). This was done by replacing $I_p/v^{1/2}$ term in Randles-Sevcik equation with the slope of the plot in Figure 4.25. Using Randles-Sevcik equation, the diffusion coefficient of penicillin G in penicillin drug was approximated to be 2.358×10^{-7} cm^2/s.

As shown in Table 4.9, the D_o for penicillin G in cow milk and in procaine penicillin drug were found to be 1.494×10^{-6} cm^2/s and 2.358×10^{-7} cm^2/s, respectively. This difference could be ascribed to the differences in sample components which possibly interacted with penicillin G differently.

Table 4.9: Electrochemical properties of penicillin G in cow milk and pharmaceutical samples

No	Media	D_o, cm^2/sec	$E_{oxidation}$, V	n
1.	Pen G in cow milk	1.494×10^{-6}	1.65	2
2.	Pen G in Pen-strep	2.358×10^{-7}	1.65	2
3.	Pen G in ABS-SDS	1.392×10^{-6}	1.65	2

D_o: diffusion coefficient obtained from the Randles-Sevcik equation, n=number of electrons.

The oxidation of penicillin G in both samples compares favorably well with those obtained when pure penicillin G was studied in ABS-SDS solution and those of Freier *et al*., (2017) who found out that penicillin G on boron doped diamond electrode oxidized at 1.6V versus Ag/AgCl. The slight change in potential is probably attributed to the sample matrix both in the milk and in the procaine penicillin drug. Freier *et al*., (2017) attributed the oxidation potential response at 1.6V to the presence of penicillin G.

4.4.2: Precision in Determination of Pen G in Cow Milk and in Procaine Penicillin Drug

The potentials and peak currents of samples of milk spiked with penicillin G and procaine penicillin drug were obtained as shown in Table 4.10.

Table 4.10: Potential and current response of penicillin G (cow milk and drug) using ABS-SDS solution

	Penicillin G (cow milk)		Penicillin G (drug)	
Trial	Potential (V)	Current (A)	Potential(V)	Current (A)
1	1.65	8.65×10^{-6}	1.64	8.95×10^{-6}
2	1.64	8.78×10^{-6}	1.66	8.94×10^{-6}
3	1.64	9.25×10^{-6}	1.64	8.27×10^{-6}
4	1.66	9.38×10^{-6}	1.64	8.43×10^{-6}
5	1.64	9.43×10^{-6}	1.63	7.93×10^{-6}
6	1.65	8.67×10^{-6}	1.64	8.02×10^{-6}
7	1.66	8.48×10^{-6}	1.66	8.04×10^{-6}
8	1.65	9.41×10^{-6}	1.65	9.05×10^{-6}
9	1.66	8.69×10^{-6}	1.66	9.12×10^{-6}
10	1.64	9.45×10^{-6}	1.66	8.86×10^{-6}

The average potential of penicillin G (in milk and drug) were calculated and the standard deviations of individual potentials from the average potential were found to be 0.0087 and 0.011 for penicillin G in milk and penicillin G in the drug respectively. This implies that there was no significant change in the position of the oxidation potential.

The relative standard deviation of voltammetric currents for penicillin G in cow milk was found to be 4.38% while in pharmaceuticals was found to be 5.51% as shown in the Table 4.11.

Table 4.11: Experimental results showing mean, standard deviation and relative standard deviation of current response of penicillin G in cow milk and pharmaceutical samples

	Calculated statistical parameter	Pen G in cow milk	Pen G (pen-strep)
1.	Number of replicate Samples	10	10
2.	Average Value	9.02×10^{-6}	8.56×10^{-6}
3.	Standard Deviation (SD)	3.95×10^{-7}	4.7142×10^{-7}
4.	RSD%	4.38%	5.51%

The recommended maximum relative standard deviation (RSD %) is 10% (Guideline, 2005; De-Bièvre and Günzler, 2005; Oyagi *et al*., 2016; Thompson *et al*., 2002). Therefore, the obtained RSD% values for this method were within the acceptable range

4.4.3: Accuracy in Determination of Pen G in Cow Milk and in Procaine Penicillin Drug

An overlay of square wave voltammograms of cow milk samples spiked with penicillin G (50%, 100% and 150%) is shown in Figure 4.26

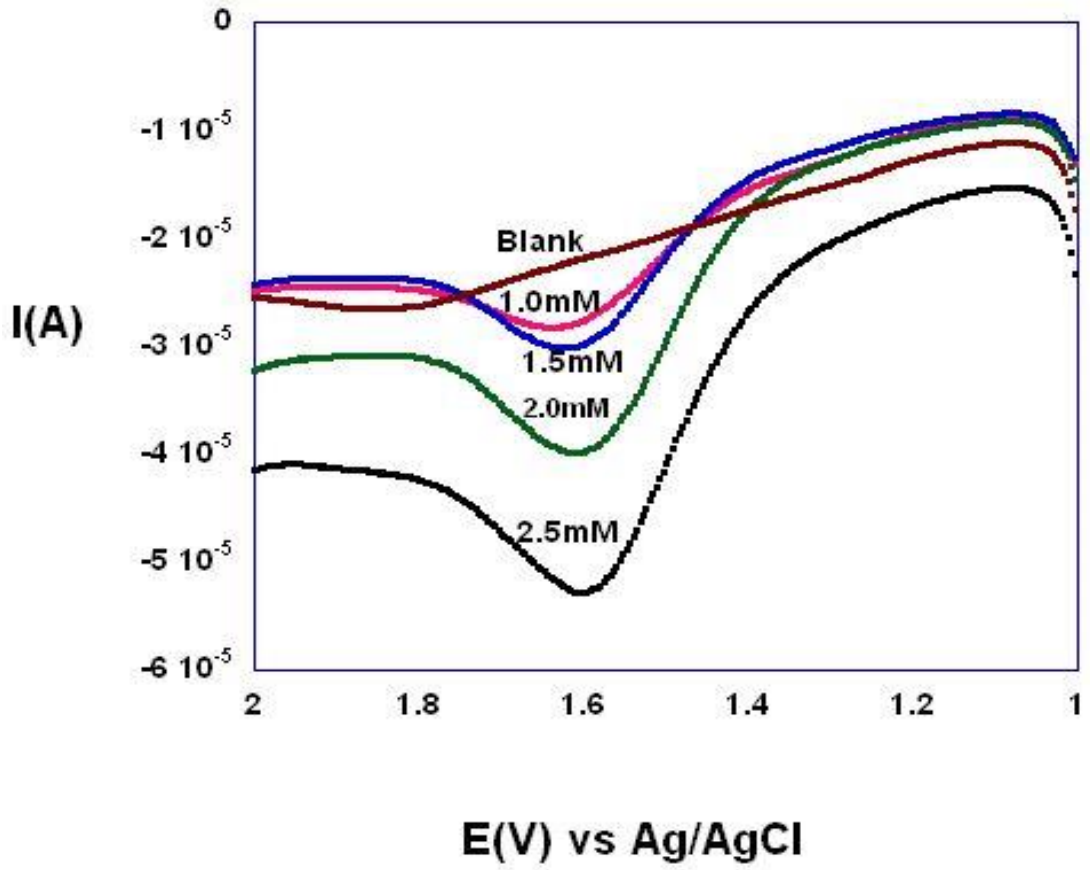


Figure 4.26: An overlay of square wave voltammograms of penicillin G in cow milk spiked with 50%, 100% and 150% concentrations of penicillin G at pH 4.5 on glassy carbon electrode at different scan rates. Initial potential: 1.0V; high potential: 2.0V; low potential: 1.0V.

A plot of the resulting voltammetric current against concentration was linear as shown Figure 4.27.

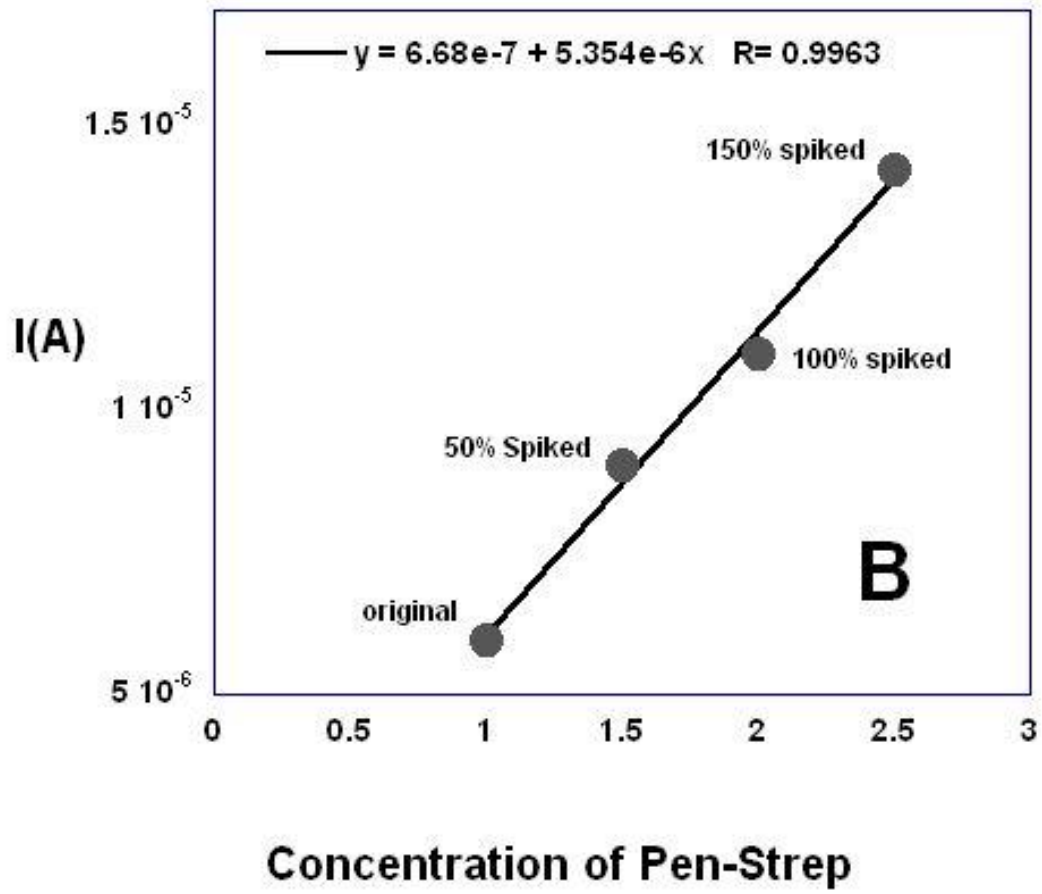


Figure 4.27: Plot of voltammetric current versus concentration (mM) of Penicillin G in drug.

Peak currents were noted to decrease with decrease in penicillin concentration and the potentials peaked at 1.6V. There was no current peak in the blank possibly because the analyte (penicillin G) was absent. The current peak at 1.6V were attributed to presence of penicillin G.

The linearity of the peak currents against penicillin G concentration implies that there was proportional relationship between current and analyte concentration. This is an indication that the proposed voltammetric method is accurate in detection and quantitative determination of benzylpenicillin in acetate buffer-sodium dodecyl sulfate solution (Guideline, 2005).

The percentage recoveries of milk sample spiked with penicillin G and penicillin G in procaine penicillin drug (pharmaceutical sample) were obtained as shown in Table 4.12.

Table 4.12: The percent recovery values of analyte from spiked standard solutions of penicillin G in cow milk and pharmaceutical samples

	Sample	Original (mM)	Current (A)	Added (mM)	Current (A)	Found (A)	Recovery, %
1.	Pen G (Cow milk)	1×10^{-2}	8.01×10^{-6}	5.0×10^{-3}	1.20×10^{-5}	1.15×10^{-5}	98.5%
		1×10^{-2}	8.01×10^{-6}	1×10^{-2}	1.60×10^{-5}	1.66×10^{-5}	103.0%
		1×10^{-2}	8.01×10^{-6}	1.5×10^{-2}	2.00×10^{-5}	2.03×10^{-5}	101.0%
2.	Pen G (Pharm)	4×10^{-3}	5.83×10^{-6}	2×10^{-3}	8.7×10^{-6}	8.4×10^{-6}	96.0%
		4×10^{-3}	5.83×10^{-6}	4×10^{-3}	1.17×10^{-5}	1.08×10^{-5}	92.0%
		4×10^{-3}	5.83×10^{-6}	6×10^{-3}	1.46×10^{-5}	1.38×10^{-5}	94.0%

The Percentage recovery of the penicillin G from the sample was obtained as shown in Equation 4.7

$$\%\, recovery = \frac{obtained\ current}{expected\ current} \times 100 \qquad (4.7)$$

The percent recoveries for penicillin G were found to lie between 98.5% - 103.0% for the cow milk and 92.0% - 96.0% for the procaine penicillin drug sample (Table 4.12). These recovery percentages were within the recommended 90.0% - 110.0% range (Guideline, 2005).

4.4.4: Detection Limit of Penicillin G in Cow Milk

An overlay of voltammograms of penicillin G at different concentration were obtained as shown in figure in Figure 4.28.

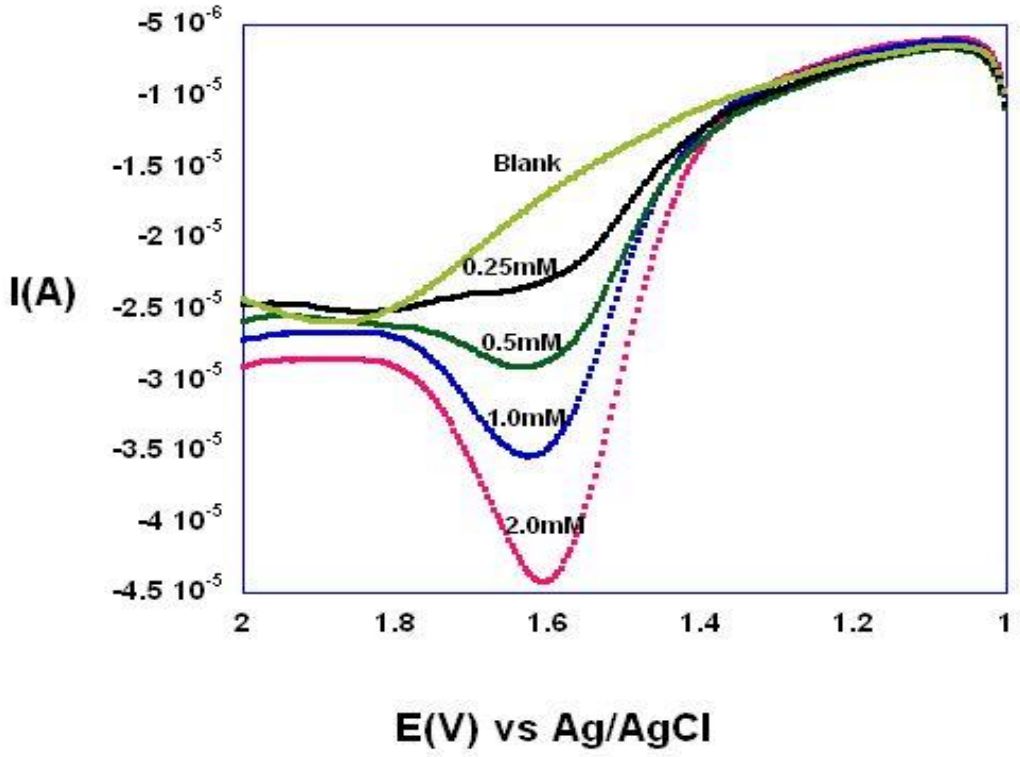


Figure 4.28: Square wave voltammetry of different concentration of Penicillin G in cow milk on glassy carbon electrode. Initial potential: 1.0V; high potential: 2.0V; low potential: 1.0V.

A plot of peak currents against concentration of penicillin G was linear as shown in Figure 4.29

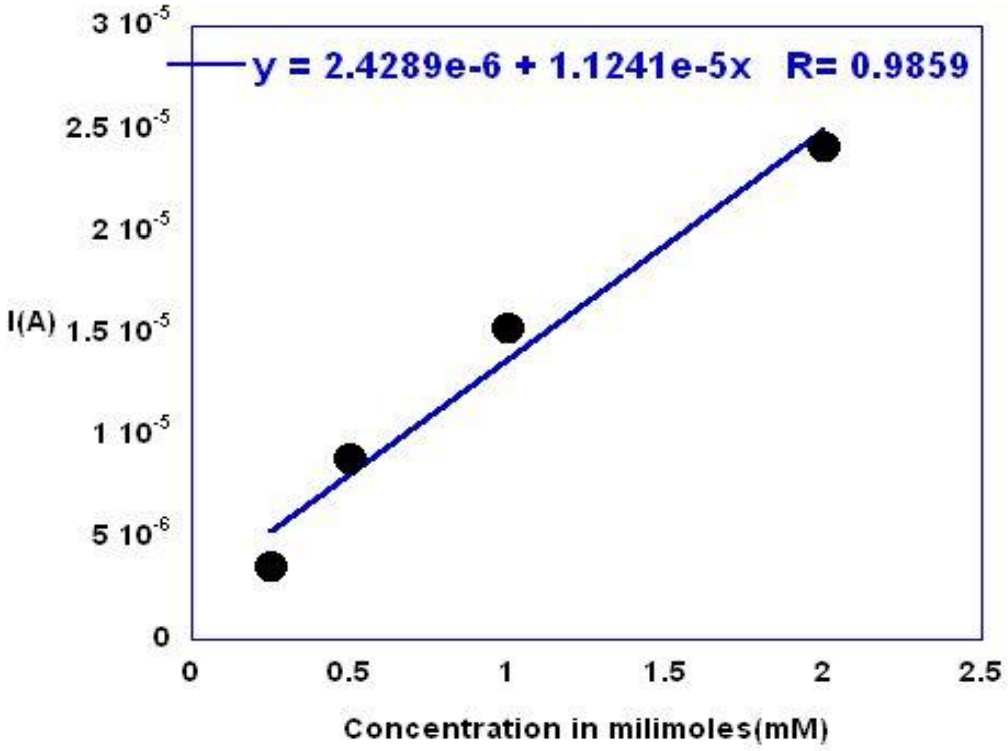


Figure 4.29: Linear plot of current (A) against concentration (mM) of penicillin G in cow milk.

The voltammetric currents reduced linearly as the concentration of penicillin G solution was diluted. Using the developed voltammetric method linear range was established from $2.5x10^3$ng/L to $2.0x10^4$ng/L with correlation coefficient (R) of 0.9859 for penicillin G (Figure 4.29).

Additionally, the limit of detection of penicillin G using voltammetric method was estimated to be 2.5×10^3 ng/L where the signal-to-noise ratio was three. The maximum residue limit (MRL) of penicillin G set by the European Union is 4.0×10^3 ng/L in milk fit for human consumption (Li *et al*., 2015).

Therefore, the developed method is suitable for qualitative and quantitative analysis of penicillin G in milk because it achieves a lower detection limit than the minimum residue limit

set by European union. Moreover, the method is sufficiently precise and accurate and it is not significantly affected by interferents that are common in milk and other environmental samples.

4.4.5: Developed Method Versus Other Methods

The suitability of the developed voltammetric technique was compared to other electro-analytical techniques which have been used to analyze penicillins as provided in Table 4.13.

Table 4.13: Detection limits of some of the voltammetric methods used in analysis of penicillins.

No.	Electrode	Support Electrolyte	Technique	LR (µM)	LOD (µM)	Ref.
1.	BDD	Acetate buffer solution, pH 4.0	Differential Pulse Voltammetry	0.5 - 40	0.25	L'ubomir *et al*., 2012
2.	Au	Acetate buffer solution, pH 4.7	Pulsed Amperometric Detection	-	0.4	Koprowski *et al*., 1993
3.	HMT probe Pt	Phosphate buffer saline with sodium chloride	Amperometric Detection	4 - 200	4	Stred'ansky *et al*., 2000
4.	[VO(salen)]CPE	-	Differential Pulse Voltammetry	-	16.6	Bergamini *et al*., 2006
5.	Pt DEN-modified SPCEs	Penicillinase enzyme in 100 mM NaCl	-	0.1–500	0.1	Ju and Kim, 2016
6.	GCE	Sodium dodecyl sulfate/Acetate buffer solution, pH 4.5	Square wave voltammetry	0.04–34.6	0.04	Sila *et al.*, 2018

The limit of detection of the developed method is much lower than those reported for determination of penicillin V and G as shown in Table 4.13. Moreover, the use of highly polished GCE and sodium dodecyl sulfate is simple compared to electrode modification processes used in some electrochemical methods

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1: Conclusions

A simple square wave voltammetric method based on SDS/ABS, pH 4.5 using bare glassy carbon electrode has been developed for electrochemical detection of penicillin V and penicillin G and for determination of penicillin G in ABS/SDS/bovine milk. Presence of sodium dodecyl sulfate in acetate buffer solution, was found to amplify the oxidation current signal of penicillin V and penicillin G by almost ten and five times respectively with no significant shifts on the oxidation potentials. A pH of 4.5 and SDS concentration of 0.347 M were found to be the optimum conditions for voltammetric determination of the analytes since they induced the highest current response.

The useful linear range for penicillin V in this method was found to be 0.04 µM – 34.6 µM, while detection limit and limit of quantitation was 0.04 µM and 0.12 µM respectively in SDS/ABS, at pH 4.5. In absence of SDS, the linear range, detection limit and limit of quantitation for penicillin V was 3.5 µM – 14.0µM, 3.5µM, 14µM respectively in acetate buffer solution (ABS) at pH 4.5. Similarly, linear range for penicillin G was found to be between 1.25 µM – 15.0µM, while the detection limit was 1.25 µM in SDS/ABS solution. The limit of quantitation was estimated to be 3.75 µM in SDS/ABS solution. In absence of SDS, the linear concentration range, detection limit and limit of quantitation for penicillin G was 2.5 µM – 10 µM, 2.5 µM and 7.5 µM respectively in ABS at pH 4.5

Foreign ions such as Na^{+}, K^{+}, Mg^{2+}, Zn^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, Cl^{-}, NO_3^{-}, PO_4^{3-} and SO_4^{2-} were found to have insignificant effect on the current signals and oxidation potentials of the

penicillin V and G. This electrochemical method is sensitive enough for penicillin V and penicillin G analysis. Therefore, presence of sodium dodecyl sulfate in acetate buffer solution (ABS), was used to modify and control properties of GCE surface and pre-concentrate the penicillins on electrode surface. Consequently, this would improve electron transfer across the electrode/solution interface and also prevent against fouling.

The presence of sodium dodecyl sulfate (SDS) in this method was made to increase the selectivity and sensitivity of the bare glassy carbon electrode (GCE) surface, and facilitate detection of penicillin V and penicillin G. No pre-treatment of the glassy carbon electrode was required in this method, hence the method is simple and reproducible .

5.2: Recommendations

This study recommends the following:

- Further work be done to determine the morphology of the surfactant modified electrode.
- Further work be carried out to determine the best orientation of surfactant molecules to maximize electron transfer process
- More work be done to check suitability of the developed method on other classes of antibiotics.

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