VOLTAMMETRIC METHODS FOR DETERMINATION OF ANTI BIOTICS

Tassew Alemayehu*

College of Natural and Computational Sciences, Department of Chemistry, Adigrat University, Adigrat, P.O.Box 50, Ethiopia.

ABSTRACT

This paper justifies the application of voltammetric methods like cyclic voltammeter and square wave voltammeter for the determination of some common antibiotic drugs. Antibiotics are drugs that fight infections caused by bacteria or other microbes. Some of the natural antibiotics are benzyl penicillin, streptomycin, chloramphenicol, tetracyclines and macrolides. Electrochemical methods are widely used in many applications because they are simple, fast; involve no more reagents and low cost. Several methods have been developed for the determination of antibiotics using electrochemical detection, such as; voltammeter at electrochemically activated carbon fiber microelectrodes and capillary-zone electrophoresis with amperometric detection at a carbon disk electrode and a carbon fiber micro-disk array electrode. Electrochemical analysis can be thought of in terms of two broad classes of measurement, potentiometry (potential measurement) and amperometry (current measurement). The voltammeter is an electro analytical method that depends on the measurement of current as a function of applied potential. It is a versatile technique for research purpose, it allows searching into several aspects of the electrochemical reactions, namely those reactions in which electrons exchanges are involved between reactants and products. The use of antibiotics to bring about improved performance in growth and feed efficiency and breeding performance also often lead to harmful residual effects. Accordingly, the determination of antibiotics has to be identified and quantified with complex and strict protocol to be obeyed in pharmaceutical formulations. Thus, a sensitive and reliable method for the determination of antibiotics at residual levels is urgently needed. Therefore, this paper focuses on the two voltammeter methods, namely cyclic and square wave voltammeters.

KEY WORDS: Voltammeter, Electrochemical Reactions.

INTRODUCTION

Antibiotics or antimicrobial drugs are the drugs that fight infections caused by bacteria or other microbes. They are small molecules that at low concentrations inhibit the growth of microorganisms or kill them. Some of the natural antibiotics are benzyl penicillin, streptomycin, chloramphenicol, tetracyclines and macrolides. Semi-synthetic antibiotics are derivatives of natural antibiotics, obtained by small alterations in structural formulas of natural antibiotics e.g. naficillin and cloxacillin.

Currently, the term antibiotic is also used for synthetic substances such as sulfonamides, nitrofurans and quinolones [1-4]. Use of Antibiotic that might result in deposition of residues in meat, milk and eggs must not be permitted in food intended for human consumption. If use of antibiotics is necessary as in the prevention and treatment of animal diseases, a withholding period must be observed until the residues are negligible or no longer detected. The use of antibiotics to bring about improving performance in growth and feed efficiency, to synchronize or control of the reproductive cycle and breeding performance also often lead to harmful residual effects [5].

Accordingly, the determination of antibiotics has to be identified and quantified with complex and strict protocol to be obeyed in edible tissues, pharmaceutical formulations and milk and milk products.

Thus, sensitive and reliable methods for the determination of some common antibiotics at residual levels are urgently needed [6,7].

Corresponding Author:- **Tassew Alemayehu** Email:-tass.alex21@gmail.com

MATERIALS AND METHODS

Several analytical methods have been reported for the determination of antibiotics in various samples, such as shrimp, seafood, meat, eggs, milk, honey, animal feeds, urine, serum and pharmaceutical formulations based on liquid chromatography (LC), liquid chromatography–mass spectrometry (LC-MS), gas chromatography (GC), gas chromatography–mass spectrometry(GC-MS), capillary zone electrophoresis, enzyme-linked immunosorbent assay (ELISA), spectrophotometry, and chemiluminescence. LC-MS is a common method that is used to determine CAP, because of its high sensitivity, and low limit of detection. However, it needs an expensive apparatus and reagents, and is time-consuming. A sensitive, rapid and cheap method for analysis is still needed. Electrochemical methods are widely used in many applications because they are simple, fast; involve no more reagents and low cost. Several methods have been developed for the determination of CAP using electrochemical detection, such as voltammetry at electrochemically activated carbon fiber microelectrodes and capillary-zone electrophoresis with amperometric detection at a carbon disk electrode and a carbon fiber micro-disk array electrode [8].

Electrochemical analysis can be thought of in terms of two broad classes of measurement, one in which the potential that develops between two electrodes is measured (potentiometry) and another in which the current that flows between two electrodes is measured (amperometry). In potentiometry, it is often helpful to arrange things such that the current is very low (e.g., by placing a high-resistance voltmeter in series between two electrodes). The electrochemical potential of one electrode (the reference electrode) is usually fixed, so the measured cell potential can be interpreted in terms of an equilibrium half-cell reaction involving an analyte species in contact with the other electrode (the working electrode). In favorable cases, one can use data from potentiometric measurements to calculate analyte concentrations directly from the Nernst equation. The potentiometry is a simple and straightforward analytical method, and is routinely used to solve many problems in the analysis of electrochemically active or charged analytes [9].

Voltammeter

The voltammeter is an electro analytical method that depends on the measurement of current as a function of applied potential. It is a versatile technique for research purpose, it allows searching into several aspects of the electrochemical reactions, namely those reactions in which electrons exchanges are involved between reactants and products. For such reactions, it is possible to investigate the laws governing the dependence of the current on the potential imposed on an electrode doped into the reaction environment. Voltammeter employs conditions that encourage polarization of the working electrode and is widely used by analytical, inorganic, physical and biological
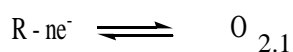
chemists for fundamental studies of; redox processes in various media, adsorption process on surfaces, and electron transfer mechanisms at chemically modified electrode surfaces. Voltammetric assay for chloramphenicol on carbon electrodes just gains poor detection limit because normal carbon electrodes are not sensitive. In order to improve this, surface pretreatment seem to be the most essential matter. A simple method of surface pretreatment is electrochemical pretreatment, which requires treating the carbon electrode at high potential in a given solvent and electrolyte to generate surface functional groups like phenol, carboxyl, carboxylic etc... These functional groups are believed to be the mediators to speed up electron transfer rate so enhance the sensitivity and reproducibility of the electrode [10-12].

Cyclic Voltammetry

Cyclic Voltammeter (CV) is perhaps the most effective electro analytical technique available for the mechanistic study of redox systems [13]. It is the most widely used technique for acquiring qualitative information about electrochemical reactions and offers a rapid location of redox potentials of the electro active species. It enables the electrode potential to be rapidly scanned in search of redox couples. Once located, a couple can then be characterized from the potentials of peaks on the cyclic voltammogram and from changes caused by variation of the scan rate. CV is often the first experiment performed in an electrochemical study [14]. It is very frequently used because it offers a wealth of experimental information and insights into both the kinetic and thermodynamic details of many chemical systems. Because of significant advances in the theoretical understanding of the technique today, even complex chemical systems such as electrodes modified with film or particulate deposits may be studied quantitatively by cyclic voltammetry. At early electrochemical work, measurements were usually undertaken under equilibrium conditions (potentiometry) where extremely accurate measurements of thermodynamic properties are possible. However, it was soon realized that the time dependence of signals can provide useful kinetic data. Many early voltammetric studies were conducted on solid electrodes made from metals such as gold or platinum. However, the complexity of the chemical processes at the interface between solid metals and aqueous electrolytes inhibited the rapid development of novel transient methods [15]. The potential sweep rate (v) used in conventional experiments ranges from a few mV s^{-1} up to a few hundred V s^{-1}. The current response over a range of potentials is measured, starting at an initial value and varying the potential in a linear manner up to a predefined limit value. At this potential (switching potential), the direction of the potential scan is reversed, and the same potential window is scanned in the opposite direction (Fig. 2.1). It follows that the potential limits and the potential sweep rate are the basic adjustable parameters [16, 17].

The four important observable parameters that characterize the cyclic voltammogram are: the two peak potentials (E_p) and the two peak currents (I_p) (Fig.2.2). The potential peak is the analytical parameter that allows making a qualitative characterization of a redox couple in a solution and the peak current height is proportional to the concentration of the electro active compound in the solution: and then correspond to the analytical parameter useful for a quantitative analysis [10].

The shape of a cyclic voltammogram is representative of the behavior of the studied system. Generally two limiting cases of study systems do exist: reversible electrode process and irreversible electrode process.

$$R - ne^- \rightleftharpoons 0 \quad 2.1$$

Two competitive effects; an increase in the oxidation rate of R which causes an increase in current and decreases in the concentration of R from its bulk value in order to satisfy the Nernst equation and to set up a concentration gradient has occurred during the oxidation process. As a result, a current proportional to the value of this new concentration at the electrode surface flows in the external circuit. Once this gradient exists, it does not remain constant, but starts to relax owing to the diffusion. At the same moment the electrode potential still changes and therefore the surface concentration of R further decreases until it effectively reaches zero. Once the concentration of R reaches zero at the electrode surface, the concentration gradient starts to decrease, due to the relaxation effect and hence the current flowing must decrease. Quantitative information is obtained by relating current to the concentration of analyte in the bulk solution and qualitative information is obtained from the voltammogram by extracting the standard-state potential for redox reaction. The concentration of the electroactive species can be quantitatively determined by the measurement of limiting current which is linear function of the concentration of electro active species in bulk solution. This quantitative information is given by the Randles-Sevcik equation shown in (Eq. 2.2) [10].

$$I_p = (2.69 \times 10^5) \, n^{3/2} A C_i D_i^{1/2} v^{1/2} \quad (2.2)$$

Where I_p is the peak current in Ampere, n the number of electrons involved in the electrode reaction, A the surface area in cm^2, C_i the concentration of species i in mol cm^{-3}, D_i the diffusion coefficient of species i in $cm^2 \, s^{-1}$ and v the scan rate in $V \, s^{-1}$.

Accordingly, the current is directly proportional to concentration and increases with the square root of the scan rate.

Square Wave Voltammetry

Square-wave voltammeter (SWV) is one of the four major voltammetric techniques provided by modern computer-controlled electro analytical instruments. In this technique a rapid step scanning of potential is applied to the electrode and moreover on each step a high frequency square wave is imposed. The current is sampled two times at the end of the two half waves. If the amplitude of the wave is very little and the redox system is reversible, during the first half wave the electro active species can be reduced or oxidized, while, in the second half at the contrary, it can be oxidized or reduced. The two currents are then summed up and so, the sensitivity is increased. The other three important techniques are single scan and cyclic staircase, pulse, and differential pulse voltammeter. All the four are either directly applied or after a pre concentration to record the stripping process. Adsorptive stripping SWV is the best electro analytical method for the determination of electro active organic molecules that are adsorbed on the electrode surface. SWV was developed by combining the high-amplitude, high-frequency square wave with the fast staircase waveform and by using computer-controlled instruments instead of analog hardware. SWV has been applied in numerous electrochemical and electro analytical measurements. Apart from the investigation of the charge transfer kinetics of dissolved zinc ions and adsorbed organic species mentioned above, the mechanisms of redox reactions of titanium (III), iron (II) and adsorbed metal complexes were analyzed. Electro analytical application of SWV can be divided into direct and stripping measurements. Analytes measured directly, without accumulation, were Bi(III), Cu(II), Pb(II), Tl(I), In(III), Cd(II), Zn(II), Fe(III/II)oxalate, Ni(II), tert-butyl hydro peroxide and N-acetyl penicillin amine thionitrite. The stripping measurements were based either on the accumulation of amalgams and metal deposits on solid electrodes, or on the adsorptive accumulation of organic substances and metal complexes. Anodic stripping SWV was applied to thin mercury film covered macro electrodes and micro electrodes. The square wave voltammetric method was also used in the determination of chloramphenicol (CAP) using electrochemically pretreated glassy carbon electrode [12, 14].

Electrochemical Cell

An electrochemical cell is the experimental apparatus for generating electricity through the use of a spontaneous redox reaction. A simple electrochemical cell consists of two electronic conductors (electrodes) connected via the external circuit (metallic conductor) and separated by an ionic conductor that is called the electrolyte. An electrode is the interface at which dissolved substrates may pick up or lose electron(s).The electrodes can be either pure metallic conductors, or mixed electronic and ionic conductors, the separator must be an electronic insulator to prevent a short circuit between the electrodes. The most

common experimental configuration for electrochemical cell (Fig.2.3) has three electrodes, i.e., counter or auxiliary electrode (C), reference electrode (R), and working electrode (W), all immersed in a liquid and connected to a potentiostat. The potentiostat allows the potential difference between the reference and working electrode to be controlled with minimal interference from IR (ohmic) drop. In this configuration, the current flowing through the reference electrode also can be minimized thereby avoiding polarization of the reference electrode and hence keeping the applied potential distribution between the working and reference electrode stable. Positioning the reference electrode close to the working electrode, further helps to minimize the IR drop between the references and working electrode due to the resistivity of the solution phase [15-18].

Electrode Materials

(i) Reference electrode: In most electrochemical measurements, it is necessary to keep one of the electrodes in an electrochemical cell at a constant potential. This so-called reference electrode allows control of the potential of a working electrode (e.g. in voltammetry) or the measurement of an indicator electrode (e.g. in potentiometry). The standard hydrogen electrode plays the role of a basic reference element in electrochemical devices; however, in practice, it is difficult to handle. Therefore, secondary reference electrodes are preferred in most experiments. A secondary reference electrode must fulfill the following criteria: (i) it should be chemically and electrochemically reversible, i.e. its potential is governed by the Nernst equation and does not change in time; (ii) the potential must remain almost constant when a small current passes through the electrode and reverse to its original value after such small current flow (i.e. a non-polarisable electrode); and (iii) the thermal coefficient of potential should be small. Whereas there is no reference electrode that offers all these properties to the same extent, some electrodes are very close to that ideal behavior. In general, secondary reference electrodes are electrodes of the second kind, i.e. metal electrodes coupled to solubility equilibrium of a salt of this metal and an electrolyte solution containing a fixed concentration of the anion of the sparingly soluble metal salt. In addition to this kind of electrodes, for some special cases, other reference systems exist. The commonly used are aqueous Ag/AgCl or calomel half cells, which can be obtained commercially or easily prepared in the laboratory [15].

The Silver/Silver Chloride Electrode

This electrode of the second kind is the most frequently used reference electrode in practical measurements, because the construction is very simple, the potential is very well reproducible, and this electrode is free of mercury! Normally, a silver wire is covered with silver chloride, which can be achieved electrochemically or thermally. Electrochemically produced films are thinner than thermally produced films. The construction of a commercially available silver/silver chloride electrode is similar to the calomel electrode (Fig. 2.4). Because reference systems based on silver/silver chloride can be produced in a very small size, they are often used in Microsystems. The electrolyte solution in these reference systems is normally a potassium chloride solution (mostly saturated or 3 M), and only seldom sodium or lithium chloride. The electrode net reaction is [19];

Compared to the calomel electrode, the silver/silver chloride reference system has the great advantage that measurements at elevated temperatures are possible. Special devices have been developed based on the silver/silver chloride reference systems for measurements in high-temperature aqueous solutions and under changing pressure conditions [19].

(ii) Counter electrode: non-reactive high surface area electrode, commonly platinum wire.

(iii) Working electrode: Electronic conductors used as electrodes are metals, rarely metal oxides, various forms of carbon and also rarely some polymers. In aqueous solutions, platinum and gold are used for electrode reactions with positive standard potentials, while mercury is useful for reactions with negative standard potentials. However, in non-aqueous aprotic media, under extremely dry conditions and in the absence of dissolved oxygen, these restrictions do not apply and the working windows are determined by the decomposition of supporting electrolytes or solvents. Carbon electrodes are made of various materials, such as graphite of spectral purity, glassy carbon, graphite powder with liquid or solid binders, carbon fibers, highly oriented pyrolytic graphite (HOPG), carbon nano tubes, boron-doped diamond and titanium carbide. The glassy, or vitreous, carbon is manufactured by very slow carbonization of a premodeled polymeric resin body in an inert atmosphere at temperatures rising from 300 ^{0}C and finishing at 1200 ^{0}C. The material is macroscopically isotropic and seemingly pore less because the existing pores are tightly closed [19].
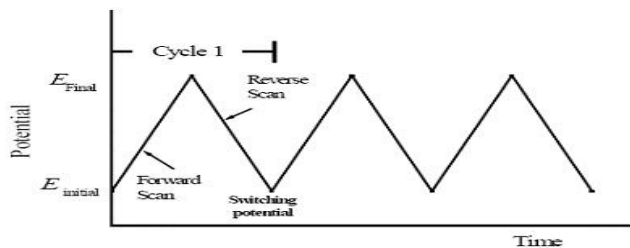
SUPPORTING ELECTROLYTE

Limitation of migration is achieved by screening the electrode using a supporting electrolyte meaning a solution in which ions do not discharge themselves at the electrode in the experimental conditions. This electrolyte is added at high concentration to the sample and could be a simple salt, acid, base, or also a buffer solution or a chelating reagent. The supporting electrolyte surrounds the electrode with ions having the same charge with the depolarizing agent. An electrolyte is needed in order to provide electrical conductivity between the two electrodes. In principle, electrolytes can be used in all three physical states: solid, liquid, and gas. Solid electrolytes are confined to special studies using solid-state electrochemical cells and sensors for gases such as oxygen, hydrogen, sulfur dioxide, and carbon dioxide as well as for ion-selective electrodes.

The most common solid electrolyte sensor is a pH electrode in which a glass membrane is an ionic conductor with sodium ions as charge carriers. Liquid electrolytes are either solutions of ionic salts, acids and bases, or molten ionic salts. Gaseous electrolytes are some ionized vapors, but the gases that exhibit mixed electronic and ionic conduction are not good separators [15]. in analytical electrochemistry the most common electrolytes are solutions. The basis of ionic conduction is the mobility of ions. In liquid electrolytes it is the consequence of a three-dimensional random movement of ions. The characteristic of the random walk is that the mean distance $< x >$ traveled by the ion is zero, but the mean square distance $< x^2 >$ is proportional to time. Because of this movement, the concentration of ions is uniform throughout the volume of the electrolyte in the absence of an electric field. Under the influence of a certain force, e.g., in an electric field, the ions acquire a nonrandom component of velocity in the direction of the force. The velocity developed under unit applied force is called the absolute mobility of the ion. In liquids, all ions are mobile and contribute to the conductivity, provided that no ion pairing occurs. Another type of supporting electrolyte is buffer solution, a solution of weak acid or a weak base and its salt, in which both components must be present. The solution has the ability to resist changes in pH upon the addition of small amounts of either acid or base. A simple buffer solution can be prepared by adding comparable amount of acetic acid (CH_3COOH) and its salt sodium acetate (CH_3COONa) to water. The equilibrium concentration of both the acid and conjugate base is assumed to be the same as the starting concentration [18, 19].
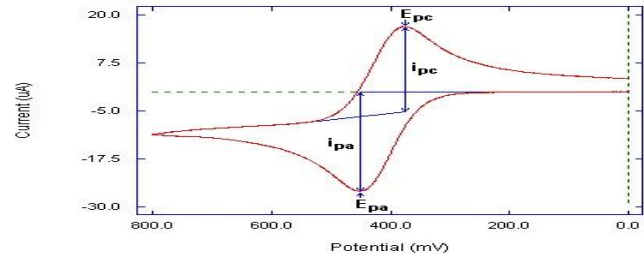
Standard addition method (spiking)

Standard Addition is a test technique for investigating suspicious test results. It is a generally applicable calibration technique, devised to overcome a particular type of matrix effect that would otherwise give rise to a biased result. The method is commonly used to determine the concentration of an analyte that is in a complex matrix such as biological fluids, soil samples, etc [19]. The reason for using the standard additions method is that the matrix may contain other components that interfere with the analyte signal causing inaccuracy in the determined concentration. The idea is to add analyte to the sample ("spike" the sample) and monitor the change in instrument response. The change in instrument response between the sample and the spiked samples is assumed to be due only to changes in analyte concentration. The procedure for standard additions is to split the sample into several even aliquots in separate volumetric flasks of the same volume. A standard containing the analyte is then added in increasing volumes to the subsequent flasks and each flask is then diluted to volume with the selected diluents. The concentration of the standard added to the flasks is then calculated. The instrument response is then measured for all of the diluted solutions and the data is plotted with a concentration of standard added to the x-axis and instrument response (peak current) in the y-axis. Linear regression is performed and the slope (m) and y-intercept (b) of the calibration curve are used to calculate the concentration of analyte in the sample. Measurement is followed by extrapolation of the calibration line to zero response [20-23].
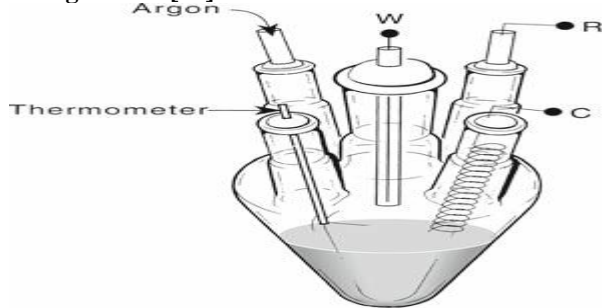
Figure 1 Potential-time signals in cyclic voltammetry experiments [11].	**Figure 2. Typical Cyclic voltammogram showing the important peak parameters [13].**
Figure 3. Electrochemical Cell with three-electrode configuration [15].	**Figure 4. Typical construction of a silver/silver chloride reference electrode [19].**

CONCLUSION

Since drug control is a global agenda now a day's pharmaceuticals including antibiotics are great concerns of the environment. This is because they enter to the environment at unknown concentrations that may cause health effects for humans as well as other living things. Therefore reliable scientific researches should be conducted to quantify their amount in different samples. For such fundamental and imperative scientific investigations voltammetric methods specifically cyclic and square wave voltammeters are advantageous and preferable as they are simple, fast and versatile techniques. That is why this review focuses in describing the functionality and advantages of these techniques.

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