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ELECTROCHEMICAL BEHAVIOR OF CHLORAMPHENICOL AND ITS DETERMINATION USING CYCLIC VOLTAMETRY

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ABSTRACT: This work uses a method that enhances the performance of glassy carbon electrode (GCE) for the determination of chloramphenicol (CAP). The electrochemical properties of chloramphenicol at electrochemically pretreated glassy carbon electrode were studied using cyclic voltammeter (CV). Electrochemical pretreatment of the electrode greatly enhanced the reduction peak current (Ip) of CAP. CAP shows an irreversible reduction peak at -0.674 V *vs.* Ag/AgCl at the GCE in 0.05 M acetate buffer of pH 5. Detailed experiments were carried out to establish the electrochemical property, the optimal buffer and its pH, electrode pretreatment potential, scan rate dependence study and effect of concentration. Following optimization pH of buffer solution, the peak current response for the reduction of CAP shows an enhanced response, 2.0 times greater than the bare GCE. A series of four CAP determination in 1.6×10^{-6} to 2×10^{-4} M concentration range show a linear calibration curve with r = 0.99997. At this range the limit of detection was LOD = 2.45 µM with a standard deviation of SD = 0.03814. The method was successfully applied to three CAP containing pharmaceutical samples: CAP eye drop, CAP palmitat oral suspension and CAP as sodium succinate and the level of CAP in these samples was verified.

KEYWORDS: glassy carbon electrode (GCE), chloramphenicol (CAP), cyclic voltammetric (CV), pharmaceutical samples.

1 INTRODUCTION

Drug control has been on the global agenda for more than a century.[1] From environmental engineering point of view, pharmaceuticals including antibiotics are a new group of manmade chemicals of concern entering the environment at concentrations such that their health effects are unknown. 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.[2-4]

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 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 improved performance in growth and feed efficiency, to synchronize or control of reproductive cycle and breeding performance also often lead to harmful residual effects.[5]

Chloramphenicol (CAP) {2, 2-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl] acetamide}, is an effective broad-spectrum antibiotic that has widely been used since the 1950s to treat food-producing animals (Fig. 1.1).[7] It has also been used in veterinary medicine as a highly effective and well-tolerated broad-spectrum antibiotic for domestic animals, poultry, as well as aqua-agriculture farming.[6]

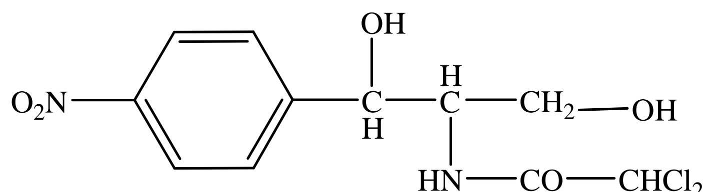


Figure 1.1.Structure of chloramphenicol (CAP) [6]

Chloramphenicol is produced naturally by Streptomyces venezuelae. It is now produced by chemical synthesis followed by a step to isolate stereoisomers. The first commercial production of chloramphenicol in the United States was reported in 1948. It may be released to the environment and may be found in various waste streams because of its use as a medicinal and research antimicrobial agent. If it is released into the atmosphere, chloramphenicol will exist primarily in the particulate phase. Removal of atmospheric chloramphenicol would occur mainly through dry deposition. The atmospheric half-life of chloramphenicol is 12 hours, as it will react with photochemically produced hydroxyl radicals. If released to water, chloramphenicol will be essentially nonvolatile. Adsorption to sediment or, bioconcentration in aquatic organisms is not expected to be important process. If released to soil, chloramphenicol is expected to have high soil mobility. Volatilization of chloramphenicol is not expected from either dry or wet soils. Exposure to chloramphenicol may occur through inhalation, dermal contact, ingestion, or contact with contaminated water or soil. The solubility of chloramphenicol in water at 25 ^{0}C is 2.5 g/l over a wide range of pH. Various biodegradation studies indicate that chloramphenicol may biodegrade in soil and water. It is degraded by biological, chemical, and photolytic means and undergoes oxidation, reduction and condensation reactions upon exposure to light in aqueous solution.[7, 8]

Chloramphenicol is an antimicrobial agent with restricted use. It is used to combat serious infections where other antibiotics are ineffective. It can be used against gram-positive cocci and bacilli and gram-negative aerobic and anaerobic bacteria. Because of its well-known risk to cause cancer, aplastic anemia and carcinogenic properties, its use in human and veterinary medicine is limited by its toxicity. Currently it is used in eye ointments to treat superficial ocular infections involving the conjunctiva or cornea, in topical ointments to treat the external ear or skin, in various tablets for oral administration, and in intravenous suspensions to treat internal infections.[8]

Accordingly, the determination of CAP has to be screening; identifying and quantifying with complex and strict protocol to be obeyed in edible tissues, pharmaceutical formulations and milk and milk products. Thus, a sensitive and reliable method for the determination of CAP at residual levels is urgently needed.[9, 10]

2 OBJECTIVES AND SIGNIFICANCE OF THE STUDY

The objective of this work relies on studying the electrochemical behavior of chloramphenicol and determining its level in pharmaceutical formulations using cyclic voltammeter.

Significance of the study

The use of CAP in human and veterinary medicine is limited by its toxicity and its use in developed countries is limited to topical application for the treatment of eye infections. However, human use of CAP is found primarily in developing countries due to its low cost.[29] Thus, evaluating and monitoring the level of CAP is necessary to ascertain that the drug should not be misused and does not cause a danger to human and animal health. This research can therefore signify to; create awareness in users about the impacts of CAP and it can be used as a starting material for others who want to search further about CAP as well as other similar antibiotic drugs.

3 EXPERIMENTAL PART

3.1 APPARATUS

Voltammetric measurements were performed using a BAS 100B electrochemical analyzer and a one compartment glass cell vial with a three-electrode configuration (BAS Cell Stand C3). The electrodes used were a glassy carbon disk working electrode with a diameter of 3 mm, a platinum wire auxiliary electrode, and an Ag/AgCl (3 M NaCl) reference electrode. The pH of the buffer solution was measured with Jenway instruments digital pH meter with a glass combination electrode. Mass of solid reagents was measured using Denver instrument balance. All potentials are reported with respect to Ag/AgCl (3M NaCl) reference electrode.

3.2 REAGENTS

All chemicals were analytical grade and organic solvents were HPLC grade. CAP capsule (Sigma-Aldrich USA) was obtained from local pharmacy. Acetic acid, acetone, sodium acetate, sodium dihydrogen phosphate, disodium hydrogen orthophosphate decahydrated, sodium hydroxide, ethyl acetate, and hydrochloric acid were used as received. All the chemicals were in “Blulux” brand form. Distilled water was used throughout the experiment.

3.3 PREPARATION OF SOLUTIONS

A 0.05 M acetate buffer (pH 5) was prepared by dissolving the required amount of sodium acetate in distilled water and the pH of the solution was adjusted by addition of drops of acetic acid and sodium hydroxide. Fresh stock solutions of CAP of concentration 1×10^{-4} M were prepared in distilled water. The working solutions for the voltammetric investigations were prepared by serial dilution of the stock solution with aqueous buffer solutions.

3.4 ELECTROCHEMICAL PRETREATMENT OF GLASSY CARBON ELECTRODE

The glassy carbon electrode was polished with Al_2O_3 powder on a micro-cloth pad and thoroughly rinsed with distilled water. Electrochemical pretreatment of glassy carbon electrode was performed by anodic oxidation at +1.000 V in acetate buffer (pH 5). The electrode was then cycled between –1.000 V and +1.000 V at a scan rate of 100 mV s^{-1} until a stable voltammogram was obtained.

3.5 PREPARATION OF ANALYZED SAMPLES SOLUTIONS

Three samples of CAP were analyzed in this work namely; CAP eye drop, CAP oral suspension and CAP sodium succinate.

3.5.1 CHLORAMPHENICOL EYE DROPS.

10 mL of chloramphenicol eye drop was transferred to five 50 ml volumetric flask and diluted with 10 mL of 0.05 M acetate buffer (pH 5). Standard solution of 1×10^{-4} M CAP was added at 0, 1, 2, and 3 mL volumes to each of the flasks. Then, each flask was made 50 mL with 0.05 M acetate buffer (pH 5).

3.5.2 CHLORAMPHENICOL PALMITATE ORAL SUSPENSION

5 mL of the sample was diluted in 20 mL acetone in four different 50 mL flasks. Addition of standard solution of 1×10^{-4} M CAP was then applied at 0, 1, 2, and 3 mL volumes to each of the flasks. The solution was made ready for voltammetric analysis by filling each flask with 0.05 M acetate buffer (pH 5). Measurements were carried out sequentially from low to high concentration and the vice versa.

3.5.3 CHLORAMPHENICOL SODIUM SUCCINATE

The powder sample per vial was dissolved in 20 mL distilled water. This procedure was repeated for another 3 powder samples followed by addition of 1×10^{-4} M CAP standard solution with 0, 1, 2 and 3 mL volumes. The solutions were transferred to a 50 mL flask and filled with 0.05 M acetate buffer (pH5).

4 RESULT AND DISSCUSION

4.1 ELECTRODE PRETREATMENT

In studying the electrochemical behavior of CAP at electrochemically pretreated GCE cyclic voltammetry was applied. As described in the experimental section, a +1.000 V potential was applied to the freshly polished and cleaned glassy carbon electrode in a solution of acetate buffer for two seconds, followed by cycles between +1.000 V and -1.000 V in the same solution until a stable background voltammogram was obtained.

Figure 5.1 compares the cyclic voltammogram of 1 x 10^{-4} M capsule CAP obtained at a bare and electrochemically pretreated GCE. As it is seen, the peak current of CAP at about -0.674 V obtained at pretreated GCE is 2.0 times greater than that of the bare electrode. This indicates that surface pretreatment improves the poor detection limit of normal carbon electrodes. Thus, there is a substantial enhancement in the peak currents when the glassy carbon electrode is electrochemically pretreated.

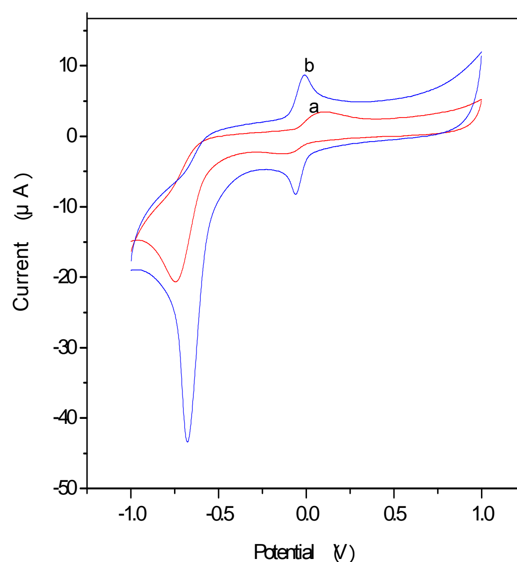


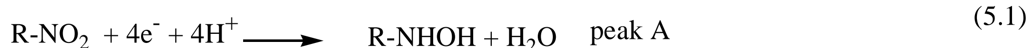
Figure 4.1 Cyclic voltammogram of 1 x 10^{-4} M CAP at: (a) bare GCE; (b) treated GCE; in 5 x 10^{-2} M acetate buffer (pH 5) at a scan rate of 100 mV s^{-1}

4.2 ELECTROCHEMICAL BEHAVIOR OF CAP AT TREATED GCE

The Cyclic Voltammogram

The electrochemical behavior of CAP was characterized by cyclic voltammetric technique as shown in Figure 4.2 below. During the first cycle, in the cathodic direction, a reduction peak (peak A) was observed at -0.674 V. On the reverse anodic scan no oxidation peak was observed corresponding to peak A, indicating that the reduction peak is irreversible while an oxidation peak (peak B_1) appeared at 0.005 V. During the second cathodic sweep, a new reduction peak (peak B_2) that is chemically reversible with peak B_1 was observed at -0.068 V.

Hence the observed peaks of CAP in Figure 5.3 can be described by the following electrochemical reactions.[7]



$$R\text{-}NO_2 + 4e^- + 4H^+ \longrightarrow R\text{-}NHOH + H_2O \quad \text{peak A} \qquad (5.1)$$

$$R\text{-}NHOH \rightleftharpoons R\text{-}NO + 2H^+ + 2e^- \quad \text{peak} B_1/B_2 \qquad (5.2)$$

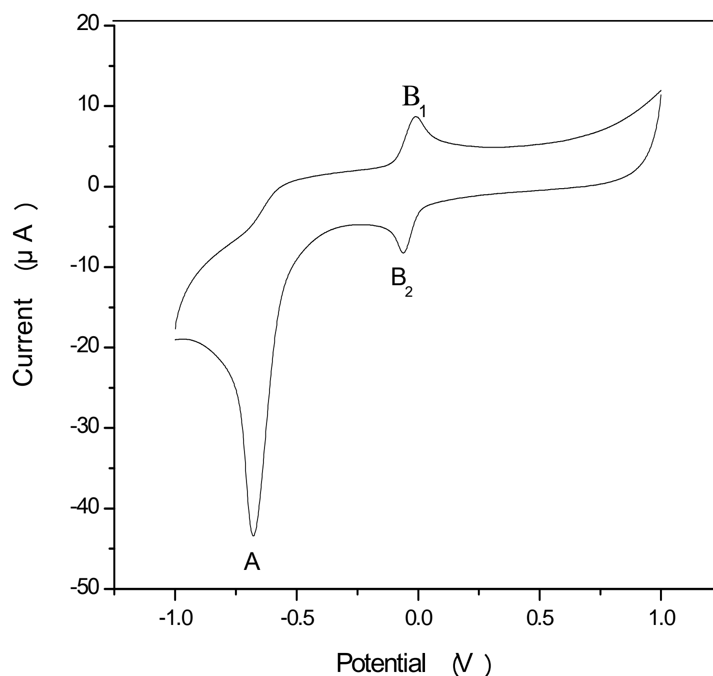


Figure 4.2 Cyclic voltammogram of 1 x 10^{-4} M CAP at treated GCE; in 5 x 10^{-2} M acetate buffer (pH 5) at a scan rate 100 mV s^{-1}

According to some literatures, the electrochemical reduction of nitro- aromatic compounds has been claimed to be a complex process that depends on; the number of nitro groups, their relative positions on the ring, and the nature of the substituent on the aromatic system.[30] CAP-NO_2 type nitro compounds display two reduction peaks (Fig. 4.2); the first one (peak B_2) is similar to those appearing in the reduction of the nitro compounds lacking a proton donor group and the second peak (peak A) is a new wave appearing at less negative potentials, associated with nitro to hydroxylamine reduction through a self-protonation reaction. The cathodic peak potentials of nitro CAP, change to less negative potentials when the position of this group in the main molecule is changed from ortho-,meta- to para- position.[31] The electrolytic reduction of nitro group (-NO_2) to hydroxylamine group (-NHOH) involves four electron irreversible reduction, whereas reduction of (–NO) to (–NHOH) involves two electron reversible reduction. [11-15]

As the number of cycles is increased the peak currents of the redox couple (B_1 and B_2) peaks increased while the peak current of peak A decreased for the first four repetitive cycles (Fig.4.3), showing that the product of the irreversible reduction of CAP remained on or near the electrode surface and was oxidized on the anodic sweep. This indicates that the irreversible reduction of CAP (peak A) is responsible for the formation of B_1 and B_2.

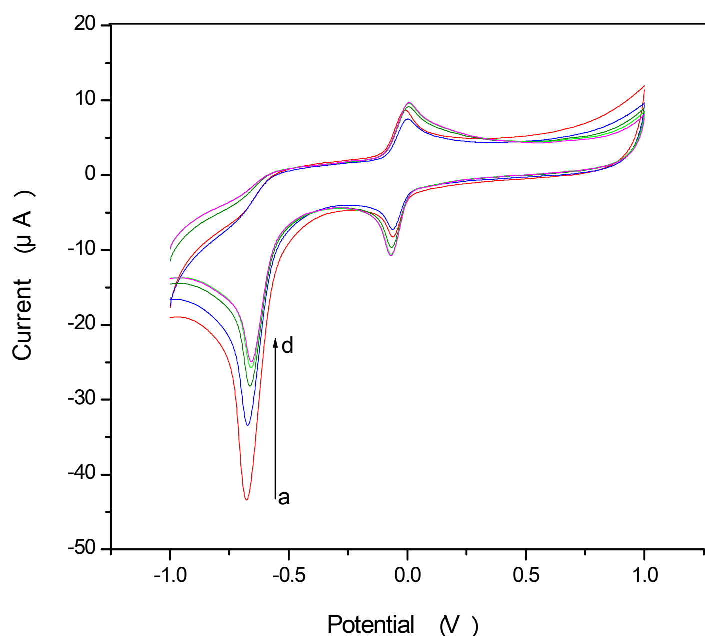


Figure 4.3 Cyclic voltammogram of 1 x 10^{-4} M CAP: for four repetitive cycles, (a) to (d) at treated GCE; in 5 x 10^{-2} M acetate buffer (pH 5) at a scan rate of 100 mV s^{-1}

4.2.1 EFFECT OF BUFFER AND PH OF SUPPORTING ELECTROLYTE

A series of buffer solutions including acidic buffer, KCl/HCl; acetate buffer, CH_3COOH/CH_3COONa and phosphate buffer, KH_2PO_4/Na_2HPO_4 were tested as supporting electrolytes for their suitability in the determination of CAP. The peak height and shape of the voltammograms were considered for the choice of the supporting electrolytes (Figure 4.4 compares their cyclic voltammogram). The optimum buffer solution chosen for subsequent studies was acetate buffer and was used throughout the study.

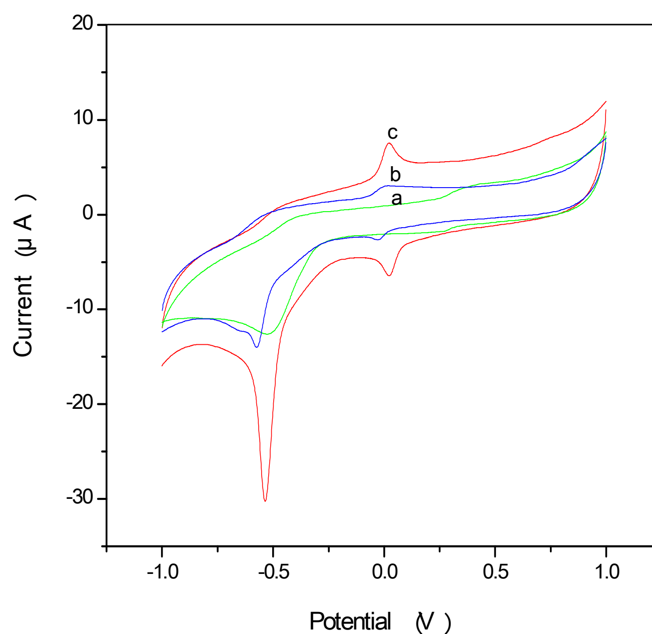


Figure 4.4 Cyclic voltammograms of 2 x 10^{-4} M CAP in: (a) 1M KCl/HCl buffer (pH 0.9), (b) 1M KH_2PO_4/Na_2HPO_4 buffer (pH 6); (c) CH_3COONa/CH_3COOH buffer (pH 5.3); 5 x 10^{-2} M at treated GCE with scan rate of 100 mV s^{-1}.

The influence of pH on the peak current of CAP was investigated over the range of pH 4- 6. There is a variation in current and peak potential with pH; when the pH is increased, the peak current is shifted to a more negative potential (Figure 4.5) The peak current is low at high pH ranges and starts increasing as the pH decreases and reaches maximum at pH 5, then decreases slightly low pH. The high current values in acidic buffer solutions are expected since the reduction of the nitro group of CAP to hydroxylamine involves H^+ ions (eqn. 4.1).

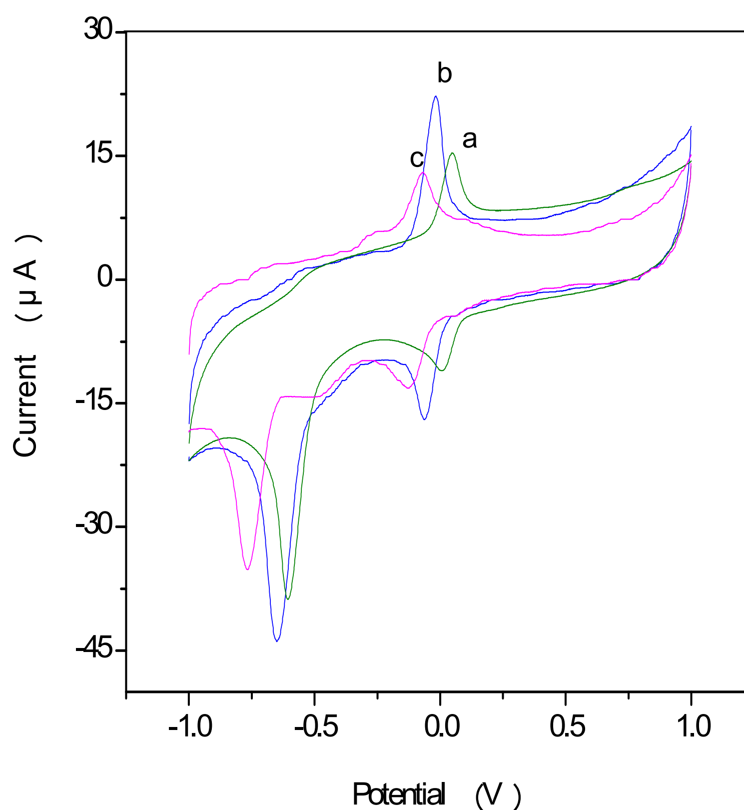


Figure 4.5 Cyclic voltammogram of 2 x 10^{-4} M CAP in pH range of (a) pH 4, (b) pH 5 and (c) pH 6 in 5 x 10^{-2} M acetate buffer at treated GCE and a scan rate of 100 mV s^{-1}

The shift in the cyclic voltammetry peak potential that can be obtained easily corresponding to the peak current, as a function of pH was studied and linear dependence was observed (Fig. 4.6). A linear range which is described by the following equation was obtained.[7]

$$Ep/V = -83.65pH + 397.65; \quad r = -0.99134 \quad (4.3)$$

The dependence of the peak potential on the pH has slope of -83.65 mV per unit pH. This implies that the ratio of the number of protons to the electrons is 1:1 for the step in which the electrode Process is reversible which is in accordance to equation (4.1). Electrode processes involving a weak acid or a weak base have a potential - pH variations which show a change in slope at pH = pKa. [7]

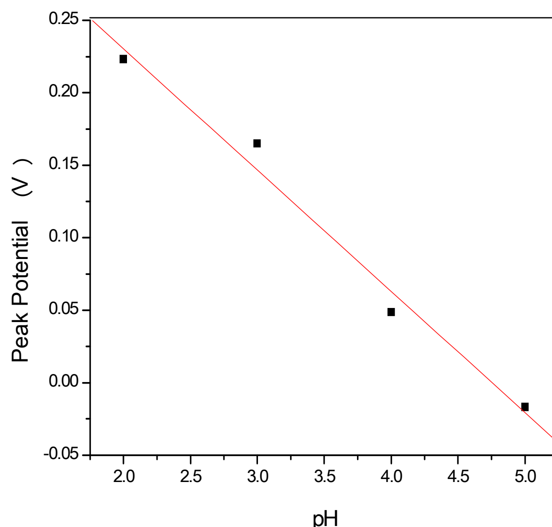


Figure 4.6 Shift in the CV peak potential of 2 x 10^{-4} M CAP as a function of pH

4.2.2 Scan rate dependence study of Peak Current

The net cathodic peak current has a linear relationship with the square root of the scan rate ($v^{1/2}$) with a correlation coefficient (r) of 0.9982 as can be seen in the inset of the graph (Figure 4.7). The results indicated that the electrochemical reaction of chloramphenicol is a diffusion controlled process.

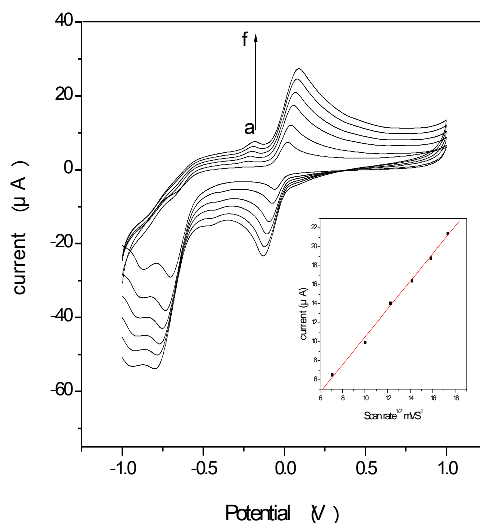


Figure 4.7 Cyclic voltammogram of 2 x 10^{-4} M CAP at different scan rates (a) 50 to (f) 300 mVs^{-1} in 0.05 M acetate buffer (pH 5) at treated GCE.

$$y = A + B * x;\ i_p = 1.46041.v^{1/2} - 4.08729 \quad (4.4)$$

4.2.3 Effect CAP Concentration on Peak Current

Figure 4.8 shows the cyclic voltammetry responses of chloramphenicol solutions in the concentration range of 1.6 x 10^{-6} to 2 x 10^{-4} M. The net peak current was found to be directly proportional to the bulk concentration of CAP in the given concentration range with a linear relationship of (r = 0.999), as shown in the inset of the figure.

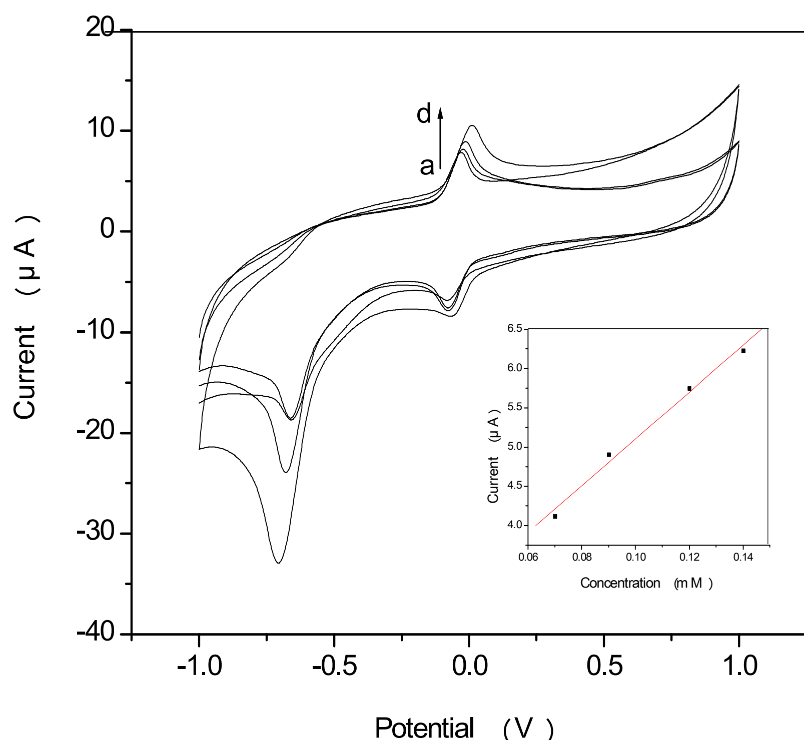


Figure 4.8 Cyclic voltammograms of CAP at different concentration range of (a) 0.0016, to (d) 0.2 mM in 0.05 M acetate buffer (pH 5) at treated GCE and a scan rate of 100 mV s^{-1} with a plot of peak current as a function of concentration in the inset of the figure.

4.3 DETERMINATION OF CONCENTRATION AND THE DETECTION LIMIT

Cyclic voltammeter was applied to determine the concentration of CAP in the three samples: CAP eye drop, CAP oral suspension and sodium succinate. The concentration of CAP in these samples was determined from the calibration curve plotted as standard concentration added *Vs* corrected peak current (the product of the peak height (h) and dilution factor (d)) represented in (eqn 4.5).

$$y = A + B * x, \; y = h.d \quad (4.5)$$

Where y = corrected peak current in μA. A = y-intercept in μA, B = slope of the curve and *x* = concentration in $mgmL^{-1}$

The unknown concentration "x" was then calculated at zero current response (h = 0) by extrapolating the linear curve to the left of the origin. At this point the value of "x" becomes the ratio of negative of y-intercept to the slop of the graph (x = -A/B). The magnitude of the ratio was taken as the required concentration of CAP in the given sample.

4.3.1 ANALYSIS OF CAP AS EYE DROP

The amount of CAP was detected in CAP eye drop sample using CV. The analysis was applied in 10 mL of the sample followed by standard addition of 1 x 10^{-4} M CAP solution. The net peak current increases with increase in the volume of standard added. The plot of peak current versus concentration added (inset of Fig.4.9) show good relationship of r = 0.998.

The concentration of CAP in the sample was then calculated to be x = 5.79 $mgmL^{-1}$ from the calibration curve at zero current response (h = 0) and is the ratio of –A = 101.375μA to B = 1.75.

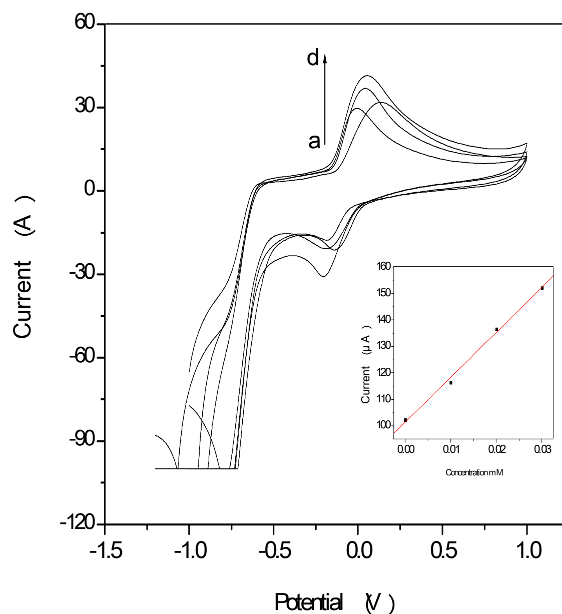


Figure 4.9 Cyclic voltammogram of CAP eye drop at additions; (a) 0, (b) 1, (c) 2 and (d) 3 mL 1 x 10^{-4} M CAP in 0.05 M acetate buffer (pH 5) at treated GCE and a scan rate of 100 mV s^{-1} with a plot of peak current as a function of concentration in the inset of the figure.

4.3.2 ANALYSIS OF CAP AS ORAL SUSPENSION

The analysis was applied in the same manner with the analysis of CAP in eye drop samples and the net peak current increases linearly with concentration added. The plot of peak current versus concentration added show good linear range of r = 0. 99927. (Inset of Figure 4.10).

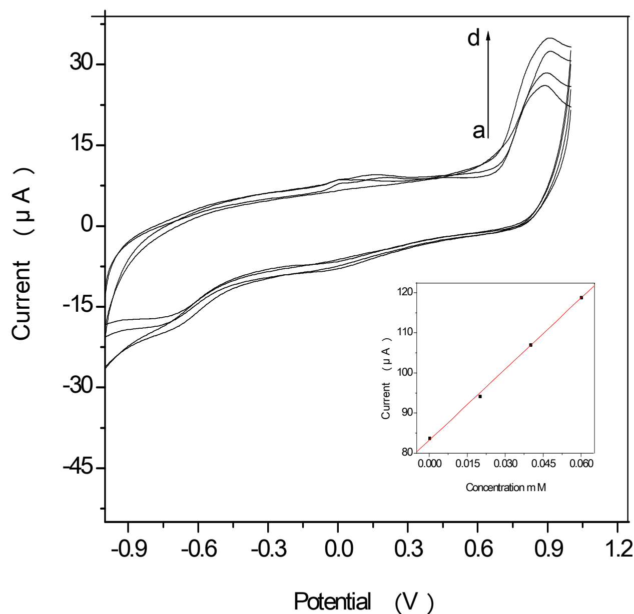


Figure 4.10 Cyclic voltammograms of CAP oral suspension at additions; (a) 0, to (d) 3 mL of 1 x 10^{-4} M CAP in 0.05 M acetate buffer (pH 5) at treated GCE and a scan rate of 100 mV s^{-1}.

The concentration of CAP with A = 80.245 µA, B = 0.5988 and extrapolating the curve to zero current was x = 26.802 mgmL^{-1}.

4.3.3 ANALYSIS OF CAP AS SODIUM SUCCINATE

In this case the analysis was carried out in the powder sample per vial by dissolving the powder in distilled water followed by addition of different volumes of 1 x 10^{-4} M CAP solution. As usual, an increase in peak current with increasing concentration was observed. The plot of peak current as a function of concentration added (inset of fig.4.11) gave a linear relationship of r = 0. 99999, slope of B =2.409 and y-intercept of A = 321.27µA.

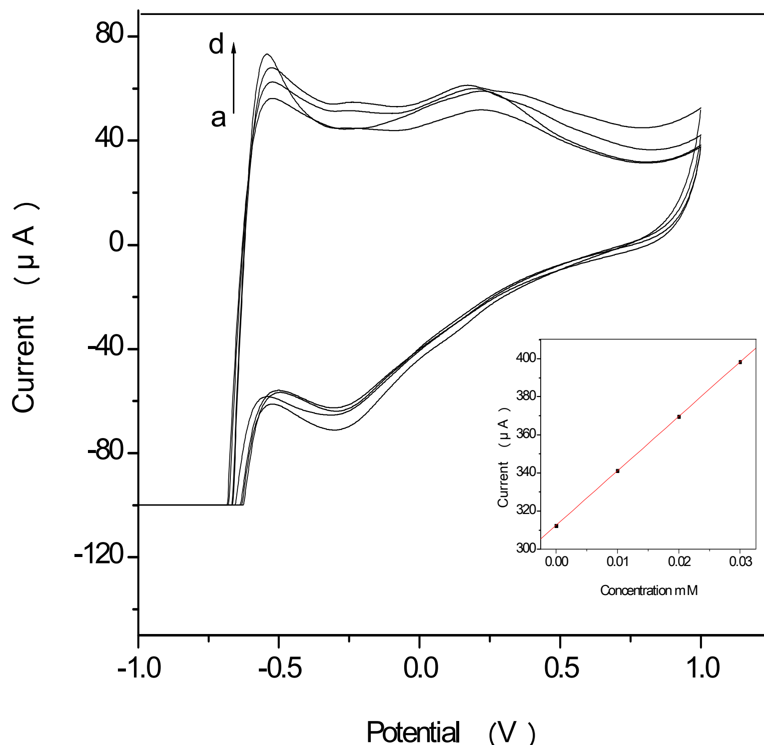


Figure 4.11 Cyclic voltammograms of CAP Sodium Succinate at additions; (a) 0, to (d) 3 mL of 1 x 10^{-4} M CAP in 0.05 M acetate buffer (pH 5) at treated GCE and a scan rate of 100 mV s^{-1}.

Calculation of concentration from the calibration curve at extrapolated zero current value gave a concentration of x = 109.3 mgmL^{-1}.

4.4 COMPARISON ON CONCENTRATION DETERMINED WITH PHARMACEUTICAL VALUES

The concentrations of CAP obtained in these three tablet samples were compared with the given pharmaceutical values. The values obtained from the voltammetric analysis show very good agreement with the pharmaceutical ones. As it is seen (Table 1), all the voltammetric results are greater than the pharmaceutical values.

From the results obtained, we can understand that cyclic voltammeter has good detection limit and give stable voltammogram.

Table 1 Comparison between cyclic voltammeter analysis results and pharmaceutical values.

No.	Sample type	Concentration Expected (mgmL-1)	Concentration found (mgmL-1) ± SD	Concentration difference (mgmL-1)
1	CAP Eye drop	5	5.79 ± 1.73241	0.79 (13.6%)
2	CAP Oral suspension	25	26.802 ± 0.713	1.802 (6.72%)
3	CAP Sodium succinate	100	109.3 ±0.14911	9.3 (8.5%)

Key; SD = standard deviation

5 CONCLUSION

The method described in this work has shown that CPA can be determined by cyclic voltammetry using electrochemically pretreated glassy carbon electrode with electrochemical stability in pH 5 acetate buffer solution. This electrode exhibited excellent performance for the reductive detection of chloramphenicol. Well-defined voltammograms were obtained at the GCE, which exhibited higher sensitivity. The electrochemical pretreatment, the buffer system and the optimized instrumental parameters were found to greatly influence the response of the voltammeter method. This method was successfully applied for the determination of CAP in pharmaceutical formulations (eye drop, oral suspension and sodium succinate), showing that the method is sensitive and precise as the experimental results are closer to the pharmaceutical values. Thus, electrochemical pretreatment of GCE solves the poor detection limit of normal GCE for CAP determination successfully and this pretreatment is more important and reasonable.

6 RECOMMENDATION

Since cyclic voltammeter can be used in both quantitative analysis and for displaying well-defined voltammograms (qualitative analysis), it is recommended to use this technique for determining the level of chloramphenicol in other sources and also other similar antibiotics.

REFERENCES

1. United Nations Office on Drugs and Crime (UNODC), World Drug Report, New York, **2009**
2. Elmolla, E. S.; Chaudhuri, M. Photocatalytic Degradation of Some Antibiotics in Aqueous Solutio, Universiti Teknologi Petronas, **2009,** p 1-9.
3. Zhou, Y. New Insights In To the Structure, Function and Evolution of Tetr Family Transcriptional Regulator, University of Toronto, **2009,** p 1-16
4. Kaya, S. E.; Filazi, A. Determination of Antibiotic Residues in Milk Samples Ankara University, Ankara - Turkey, **2010**, p 1-5
5. Nisha, A.R. Antibiotic Residues: a Global Health Hazard, College of Veterinary and Animal Sciences, Veterinary World, **2008**, p 375-377
6. Hailemichael, A. Voltammetric Determination of Chloramphenicol at Electrochemically Pretreated Glassy Carbon Electrode, National University of Lesotho, *Southern Africa Chemical Society*, **2007**, p 1-12
7. Fuller, D.G. Antibiotic Treatment for Bacterial Meningitis in Children in Developing Countries, Annals of Tropical Paediatrics, **2003**, p 233-253.
8. Wongtavatchai, J. Chloramphenicol First Draft, Chulalongkorn University, **2002**, p 8-31.
9. Tamošinas, V. Chloramphenicol Determination in Milk by Liquid Chromatography – Tandem Mass Spectrometry, Vilnius University, **2006**, p 25–29.
10. Chuanuwatanukul, S. Electrochemical Analysis of Chloramphenicol Using Boron-Doped Diamond Electrode, *the Japan Society for Analytical Chemistry*, Kayama University, **2008**, P1-6
11. Larsen, J. W. Mechanism of the Carbon Catalyzed Reduction of Nitrobenzene by Hydrazine, Lehigh University, **1999**, p 1-7
12. Polati, K. Electroreduction of Nitrobenzene to p-aminophenol Using Voltammetric. *Journal of Applied Electrochemistry*, Ankara University, Turkey, **2002,** p 1-7
13. Maggini, M. Ion-pairing Effects on the Reduction of Nitroarenes in Propan-2-01Solutions, an Electrochemical Investigation. Padova, Italy, **1986**, p 131
14. Tocher, J. the Interaction of Nitro- aromatic Drugs with Aminothiols: Chemotherapy Research Unit, University of East London, **1995,** p 1-5
15. Moreno, N. J. Mechanism of Toxicity of Nitro-compounds, Environmental Health Perspectives, **1985**, p 199-208.