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Determination of Chloramphenicol in Pharmaceutical Samples at Electrochemically Pretreated Glassy Carbon Electrode

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Abstract

This study focuses on the importance of electrochemical pretreatment of glassy carbon electrode (GCE) for the determination of chloramphenicol (CAP) in pharmaceutical formulations using square wave voltammeter. Electrochemical pretreatment of the electrode greatly enhanced the reduction peak current (Ip) of CAP that it shows a reduction peak current response at -0.032 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, effect of concentration and square wave voltammetric parameters. Following optimization of the instrumental parameters and pH of buffer solution, the peak current response for the reduction of CAP shows an enhanced response, 5.82 times greater than the bare GCE. 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: square wave voltammeter (SWV); pharmaceutical samples; glassy carbon electrode (GCE); chloramphenicol (CAP).

1. Introduction

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 drugs are the drugs that struggle infections caused by bacteria or other microbes. They are small molecules that at low concentrations inhibit the growth of microorganisms or kill them. The first use of antimicrobials for treatment of infections in

veterinary medicine was in the late 1940s, shortly after their development. One of the natural antibiotics is chloramphenicol [1-3].

Utilize of antibiotics must not be permitted in food intended for human consumption since their harmful residual effects might result in deposition of residues in meat, milk and eggs. 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 [4].

Chloramphenicol (CAP) {2, 2-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl] acetamide}, which can be represented in a chemical formula of $C_{11}H_{12}Cl_2N_2O_5$ is an effective broad-spectrum antibiotic that has widely been used since the 1950s to treat food-producing animals.[7] 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. It is degraded by biological, chemical, and photolytic means and undergoes oxidation, reduction and condensation reactions upon exposure to light in aqueous solution [5,6].

The of use CAP in human and veterinary medicine is limited by its toxicity because of its well-known risk to cause cancer, aplastic anemia and carcinogenic properties [6]. However, human use of CAP is found primarily in developing countries due to its low cost. Therefore, the level of CAP should be quantified at its residual levels in pharmaceutical formulations and milk and milk products using a sensitive and reliable method to find out that the drug should not be misused and does not cause a danger to human and animal health [7,8].

This research can therefore signify to; create awareness in users about the impacts of CAP and as a starting material for others who want to search further about CAP as well as other similar antibiotic drugs.

2. Objective of the study

The objective of this study is to determine the level of chloramphenicol in pharmaceutical formulations using square wave voltammeter.

3. Methodology (Experimental part)

3.1 Apparatus and Reagents

BAS 100B electrochemical analyzer of one compartment glass cell vial with a three-electrode configuration (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) was used for voltammetric measurements. The electrodes used were a. The pH of the buffer solution was measured with Jenway instruments digital pH meter. Denver instrument balance was used for mass measurement of solid reagents.

All chemicals were analytical grade and organic solvents were HPLC grade. Chloramphenicol (CAP) capsule was obtained from local pharmacy. The chemicals used for the study were; Acetic acid, acetone, sodium acetate, sodium dihydrogen phosphate, disodium hydrogen orthophosphate decahydrated, sodium hydroxide, ethyl

acetate, and hydrochloric acid all with "Blulux" brand form. Distilled water was used throughout the experiment.

3.2 Preparation of solutions

The required amount of sodium acetate was dissolved in distilled water to prepare 0.05 M acetate buffer (pH 5) by adjusting the pH of the solution upon addition of drops of acetic acid and sodium hydroxide. Fresh stock solutions of CAP of concentration 1 x 10^{-4} M were prepared in distilled water and the working solutions were prepared by serial dilution of the stock solution with aqueous buffer solutions.

3.3 Preparation of Analyzed Samples solutions

3.3.1 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 x 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.

3.3.2 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 x 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 (pH 5) up to the mark.

3.3.3 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 x 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).

4. Result and Discussion

4.1 Electrode Pretreatment

The electrochemical behavior of CAP was studied at electrochemically pretreated glassy carbon electrode using square wave voltammeter. Electrochemical pretreatment of the glassy carbon disk electrode was conducted first to illustrate the dependence of the net peak current response of CAP on the sensitivity of the working electrode. A potential of +1.000 V was applied to the glassy carbon electrode, which was freshly polished and cleaned with Al2O3 powder, in a solution of acetate buffer for two seconds, followed by potential cycles between +1.000 V and -1.000 V in the same solution.

Figure 4.1Compares the square wave voltammogram of 1 x 10^{-4} M capsule CAP obtained at a bare and electrochemically pretreated glassy carbon electrode. As it is seen, the peak current of CAP at about -0.032 V obtained at pretreated GCE is 5.82 times greater than that of the bare electrode. This indicates that surface pretreatment improves the poor detection limit of normal carbon electrodes.

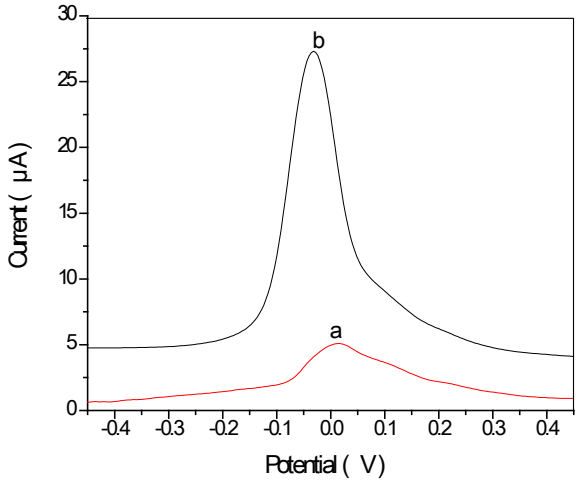

Figure 4.1 Square wave voltammogram of 1 x 10^{-4} M CAP: (a) at bare GCE; (b) at treated GCE; in 5 x10^{-2} acetate buffer (pH 5) at a scan rate of 100 mV s^{-1}

4.2 Electrochemical behavior of CAP at treated GCE

The Square Wave Voltammogram

4.2.1 Optimization of Voltammetric Parameters

The instrumental parameters in square wave voltammetry were interrelated and have a combined influence on the peak current. Hence, in order to establish the optimum conditions in the determination of CAP, the influence of instrumental parameters on the current response was studied. The influence of the pulse amplitude (ΔE) on the peak current was studied in the range 20 to 70mV. The peak current increased sharply up to 50 mV then reached a steady state value. The effect of the potential step (ΔEs) on the peak current was also investigated in the range 4 to 18 mV. The plot of the peak current as a function of potential step increased sharply at the beginning and continued increasing gently and 14 mV was chosen as the optimum value. The square wave frequency (f) was varied from 10 to 70Hz and 40 Hz was chosen to be the optimum value. Finally the instrumental parameters selected were: ΔE = 50 mV, ΔEs = 14 mV and f = 40 Hz. (Figure 5.10).

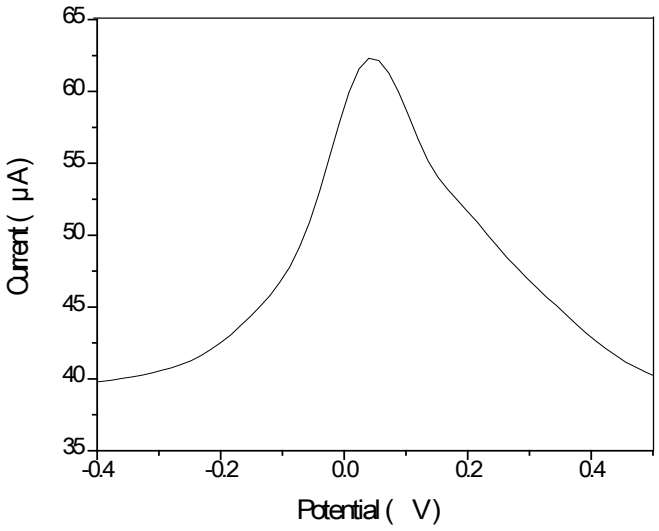

Figure 4.2 Square wave voltammogram of 2 x 10^{-4} M standard solution of CAP using the

Optimized instrumental parameters: ΔE = 50 mV, ΔEs = 14 mV and f = 40 Hz

4.2.2 Effect of pH

In the square wave voltammetry (SWV), when the pH of the supporting electrolyte is increased, the peak current of the voltammogram is shifted to a more negative potential. The peak current obtained in a buffer of pH 6 is much less than that obtained for the buffer solutions of pH 5. Similar peak current dependence on pH was obtained as can be seen in Figure 4.3 below. The optimum pH chosen, pH with higher peak current, was pH 5 in both techniques.

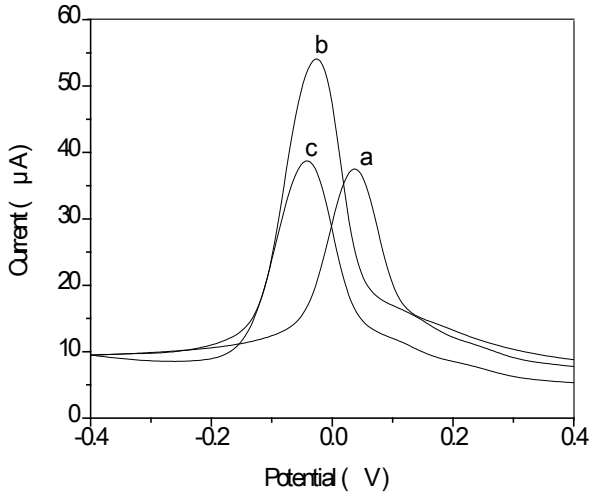

Figure 4.3 Square wave voltammogram of 2x 10^{-4} M CAP in pH range of (a) pH 4, (b) pH 5 and (c) pH 6 in 5 x

10^{-2} M acetate buffers at treated GCE and a scan rate of 100 mV s^{-1}

The shift in the SWV peak potential which is corresponding to the peak current, as a function of pH obtained when the pH was varied using the square wave voltammeter mode (Fig.4.4). A linear range which is described by the following equation was obtained.

$$Ep/V = -62.7\ pH + 302.9; \qquad r = -0.99379 \qquad (4.1)$$

The dependence of the peak potential on the pH has slope of -62.7 mV per unit pH. 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].

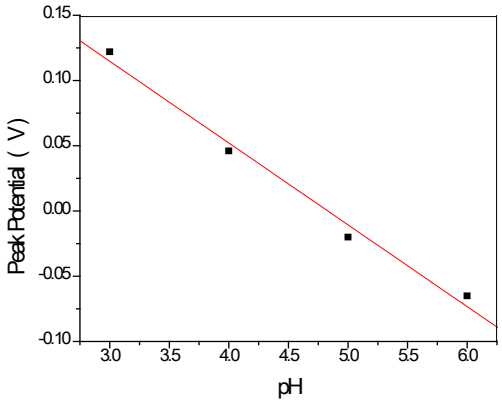

Figure 4.4 Shift in the SWV peak potential of 2 x 10^{-4} M CAP as a function of pH

4.2.3 Effect CAP Concentration on Peak Current

Figure 4.5 shows the Square wave voltammetry responses of chloramphenicol solutions in the concentration range of 1.6 x 10^{-6} to 2 x 10^{-4} M acetate buffer (pH 5) at treated GCE with a scan rate of 100 mV s^{-1}. In this range, the net peak current was found to be directly proportional to the bulk concentration of CAP. The linear range for SWV (inset of Fig. 4.5) was r = 0.9999 with slope of B = 0.1864, y-intercept of A = 14.41 µA and standard deviation of SD = 0.14322. The value of LOD in this technique was also as low as 2.305 µM at the given concentration range.

4.3 Determination of Concentration and the Detection Limit

Square wave voltammetry were applied in determining 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)) with a linear equation represented (eqn 4.2).

$$y = A + B * x,\ y = h.d \qquad (4.2)$$

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

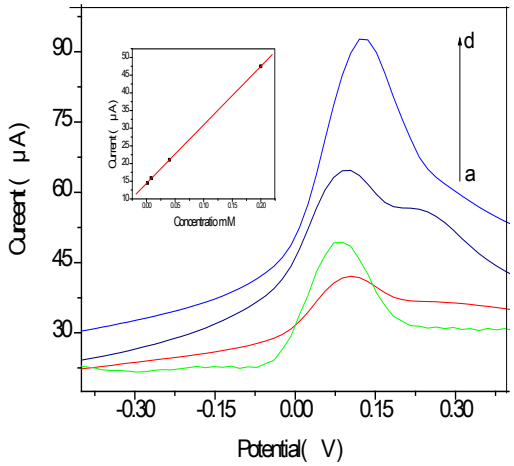

Figure 4.5 Square wave voltammogram of CAP at different concentration range of (a) 0.0016 (b) 0.008, (c) 0.05 and (d) 0.2 mM in 0.05 M acetate buffer (pH 5) at treated GCE with a scan rate of 100 mV s^{-1} with a plot of peak current as a function of concentration in the inset of the figure.

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 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 correlation for the SWV plot was given as r = 0. 99094 and the amount of CAP obtained in the sample from extrapolation of the curve to h = 0 with A= 16.802 µA and B = 0.4166 was x= 26.97 $mgmL^{-1}$. The results are still nearer to the expected values showing that the finding is initiative for further work.

4.3.2 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. 8) gave a linear relationship of r = 0. 9988 was obtained for the SWV response.

The concentration of CAP in the sample was calculated to be x = 114.57 mgmL^{-1}. In the same way to the previous results, good agreement with expected values was obtained.

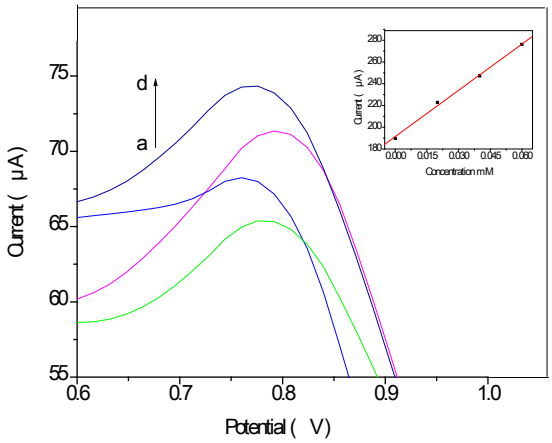

Figure 4.6 Square wave voltammogram of CAP oral suspension at different additions of; (a) 0, (b) 1, (c) 2 and (d) 3 mL of 1 x 10^{-4} M CAP in 0.05 M acetate buffer (pH 5) at treated GCE and a scan rate of 100 mV s^{-1} with a plot of peak current as a function of concentration in the inset of the figure.

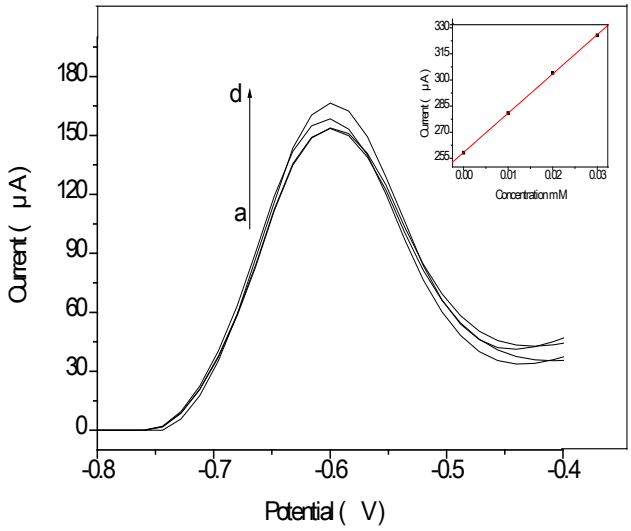

Figure 4.7 Square wave voltammogram of CAP Sodium succinate at different additions of; (a) 0, (b) 1, (c) 2 and (d) 3 mL of 1 x 10^{-4} M CAP in 0.05 M acetate buffer (pH 5) at treated GCE and a scan rate of 100 mV s^{-1} with a plot of peak current as a function of concentration in the inset of the figure.

4.3.3 Analysis of CAP as Eye Drop

The amount of CAP was detected in CAP eye drop sample using square wave voltammetric technique. 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 for the Square wave voltammetric response (inset of Fig.4.6) show a good correlation of r = 0.999 with slope of B = 1.784 and y-intercept of A= 106. 683 µA.

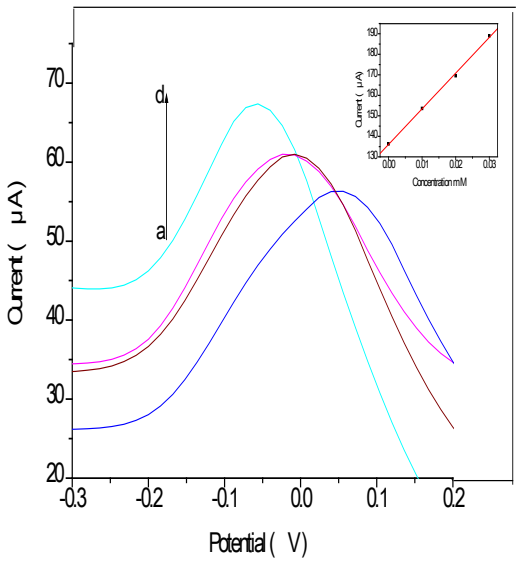

Figure 4.8 Square wave voltammograms of CAP eye drop at different additions of; (a) 0, (b) 1, (c) 2 and (d) 3 mL 1 x 10^{-4} M CAP in 0.05 M acetate buffer (pH 5) at treated GCE and a scan rate of 100 mV s^{-1} with a plot of peak current as a function of concentration in the inset of the figure.

The concentration of CAP found from the ratio of negative y-intercept to slope of the curve for the SWV response was x = 5.98 $mgmL^{-1}$ at zero current response.

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.

5. Conclusion and Recommendation

5.1 Conclusion

The study described in this paper proves that CPA can be determined by square wave voltammeter with electrochemical stability in pH 5 acetate buffer solution using electrochemically pretreated glassy carbon electrode in which the electrode exhibited excellent performance for the reductive detection of chloramphenicol. The electrochemical pretreatment, the buffer system and the optimized instrumental parameters were found to

greatly influence the response of the voltammetric method. This method was successfully applied for the determination of CAP in pharmaceutical formulations in the form of eye drop, oral suspension and sodium succinate. The application of the method used for the determination of chloramphenicol in these samples shows that the method is sensitive and precise as the experimental results are closer to the pharmaceutical values. Since electrochemical pretreatment of GCE solves the poor detection limit of normal GCE for CAP determination successfully therefore electrode pretreatment is more important and reasonable.

Table 1 Comparison between voltammetric 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	5.98 ± 1.07976	0.98
2	CAP Oral suspension	25	26.97 ± 1.57371	1.97
3	CAP Sodium succinate	100	114.57 ± 0.47756	14.57

Key; SD = standard deviation

5.2 Recommendation

Since square wave voltammetry is good for quantitative and qualitative analysis, it is recommended to use this technique for determining the level of chloramphenicol in other sources and also other similar antibiotics.

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