7.1. INTRODUCTION

Carbenicillin disodium salt (CDS) is a bacteriolytic antibiotic belonging to the carboxypenicillin subgroup of the penicillins. Relative to benzylpenicillin, carbenicillin has a relatively greater spectrum of activity against Gram-negative bacteria such as Pseudomonas aeruginosa, but a lower activity against Gram-positive bacteria\(^1\). Its bactericidal mode of action is analogous to that of benzylpenicillin, which acts on bacteria by inhibiting bacterial cell wall synthesis. The carboxypenicillins are susceptible to degradation by beta-lactamase enzymes, although they are more resistant than ampicillin to degradation. Carbenicillin is also more stable at lower pH than ampicillin. The antibiotic is highly soluble in water and is acid-labile. It is a semi-synthetic analogue of the naturally occurring benzylpenicillin. Carbenicillin at high doses can cause bleeding\(^2\). Use of carbenicillin can cause hypokalemia by promoting potassium loss at the distal convoluted tubule of the kidney. Drug analysis is one of the important tools for drug quality control. Therefore, the development of simple, sensitive, rapid and reliable method for the determination of drug is of great importance. The chemical structure of carbenicillin disodium salt is as follows:
Various analytical methods for the therapeutic monitoring have been reported in the literature for the determination of carbenicillin in commercial dosage form and biological fluids such as high-performance liquid chromatography (HPLC), LC method, electrochemical and electrophoretic method. The main problems encountered in using such methods are either the need for derivatization or the need for time-consuming extraction procedures.

Voltammetric methods satisfy many of the requirements for such tasks particularly owing to their inherent specificity, rapid response, high sensitivity, low cost, simplicity and relatively short analysis time for the determination of organic molecules, including drugs and related molecules in pharmaceutical dosage forms and biological fluids.

Electrochemical methods, especially differential pulse voltammetry (DPV) make it possible to decrease the analysis time as compared to the time exhausted chromatographic methods. The advantages of DPV over other electroanalytical techniques are greater speed of analysis, lower consumption of electroactive species in relation to the other electroanalytical techniques and less problems with blocking of the electrode surface. The gold electrode has been widely used in electrochemical studies and electro analysis for various substrates for a long time because of its stability, wide potential window and fast electron transfer rate.

To the best of our knowledge, till date there is no report on the voltammetric method for the determination of CDS in literature. The aim of
this study is to establish the suitable experimental conditions. Hence, we report the voltammetric behavior of CDS by cyclic, linear and DPV method at gold electrode in this chapter.

7.2. EXPERIMENTAL

7.2.1 Reagents and chemicals

Carbenicillin disodium salt was purchased from Sigma-Aldrich and used as such. A stock solution of CDS (10 mM) was prepared in millipore water. The phosphate buffers from pH 3.0–11.2 were prepared according to the method of Christian and Purdy\textsuperscript{10}. Other reagents used were of analytical or chemical grade. All solutions were prepared with double distilled water.

7.2.2. Instrumentation

Electrochemical measurements were carried out on a CHI 630D electrochemical analyzer (CH Instruments Inc., USA). The voltammetric measurements were carried out in a 10 ml single compartment three-electrode glass cell with Ag/AgCl as a reference electrode, a platinum wire as counter electrode and a 2 mm diameter gold electrode as a working electrode (Part No. CHI101). All the potentials are given against the Ag/AgCl (3 M KCl). pH measurements were performed with Elico LI120 pH meter (Elico Ltd., India). All experiments were carried out at an ambient temperature of 25 ± 0.1 °C.
7.2.3. Area of the electrode

The area of the electrode was obtained by the cyclic voltammetric method using 1.0 mM K₃[Fe(CN)]₆ as a probe at different scan rates. For a reversible process, the following Randles-Sevcik formula can be used:

\[ I_{pa} = 0.4463 \left( \frac{F^3}{RT} \right)^{1/2} n^{3/2} A_0 D_0^{1/2} C_0 \nu^{1/2} \]  

where, \( I_{pa} \) refers to the anodic peak current, \( n \) is the number of electrons transferred, \( A_0 \) is the surface area of the electrode, \( D_0 \) is diffusion coefficient, \( \nu \) is the scan rate and \( C_0 \) is the concentration of K₃[Fe(CN)]₆. For 1.0 mM K₃[Fe(CN)]₆ in 0.1 M KCl electrolyte, \( T = 298 \, \text{K} \), \( R = 8.314 \, \text{J K}^{-1} \, \text{mol}^{-1} \), \( F = 96,480 \, \text{C mol}^{-1} \), \( n = 1 \), \( D_0 = 7.6 \times 10^{-6} \, \text{cm}^2 \, \text{s}^{-1} \), then from the slope of the plot of \( I_{pa} \) versus \( \nu^{1/2} \) relation, the electroactive area was calculated. In our experiment the slope was \( 2 \times 10^{-5} \, \mu\text{A (V s}^{-1})^{1/2} \) and the area of electrode was calculated to be 0.2696 cm².

7.2.4 Analytical procedure

For reproducible results, improved sensitivity, and good resolution of voltammetric peaks, the working electrode polishing was done on micro cloths (Buehler) glued to flat mirrors. A different micro cloth was used for each size of alumina. The particle size used was 0.3, 0.1 and 0.05 µm. The final particle size was 0.05 µm. After initial cleaning of the electrode, it was necessary to polish with 0.05 µm particle size during the time of experiments. Before transferring the electrode to the solution, it was washed with double distilled water.
water. Cyclic voltammograms were recorded in 0.2 M H₂SO₄ at 50 mV s⁻¹ between 0 and 1.6 V, until obtaining the reproducible current-potential curves.

The parameters for differential-pulse voltammetry (DPV) were as follows: initial potential 0.8 V; final potential, 1.4 V; increase in potential, 0.004 V; amplitude, 0.05 V; quiet time, 2 s; sensitivity, 1.0 × 10⁻⁴ A/V.

7.3. RESULTS AND DISCUSSION

7.3.1. Cyclic voltammetric behavior of CDS

The electrochemical behavior of CDS at gold electrode was investigated using cyclic voltammetry (CV) at pH = 3.0. The cyclic voltammograms obtained for 1.0 mM CDS solution at a scan rate of 50 mV s⁻¹ exhibits a well-defined irreversible anodic peak at about 0.966 V at gold electrode. The results are shown in Fig.VII (i) (p. 224). The cathodic peak that appeared corresponds to the reduction of gold oxides¹².

7.3.2. Influence of pH

The electrode reaction might be affected by pH of the medium. The electro-oxidation of 1.0 × 10⁻⁴ M CDS was studied over the pH range of 3.0 – 11.2 in phosphate buffer solution by cyclic voltammetry which is shown in Fig.VII (ii a) (p. 226). With the increase in pH of the solution, peak potential shifted to less positive values, (Fig.VII (ii b) (p. 227)), and obeys the following equation:

\[ E_p(V) = 1.355 - 0.054 \text{pH}; \quad r = 0.992 \]
Figure VII (i)

Cyclic voltammograms at the gold electrode in phosphate buffer solution (pH = 3.0): (a) blank run (b) in the presence of CDS; scan rate 50 mVs$^{-1}$; CDS: 1.0 mM
The slope of this equation is found to be 54 mV/ pH. This closeness of the slope to the expected theoretical value of 59 mV/pH suggests that the number of electrons transferred is equal to that of the hydrogen ions taking part in the electrode reaction.

From the plot of \( I_p \) versus pH (Fig.VII (ii c) (p. 227)) it is clear that, peak current is affected by the pH value. However, the best result with respect to sensitivity accompanied with sharper response was obtained with pH = 3.0. So pH = 3.0 was selected for further experiments.

### 7.3.3. Influence of scan rate

Useful information involving electrochemical mechanism generally can be acquired from the relationship between peak current and scan rate. Therefore, the voltammetric behavior of CDS at different scan rates was also studied using cyclic voltammetry (Fig.VII (iii a) (p.228)) and linear sweep voltammetry (Fig.VII (iv) (p.230)). Scan rate studies were carried out to assess whether the processes on gold electrode were under diffusion or adsorption-controlled. The influence of the scan rate on the peak current showed a linear relationship in the range of 0.01 to 0.25 mV s\(^{-1}\) for CV (Fig.VII (iii b) (p. 229)) and in the range of 0.01 to 0.25 mV s\(^{-1}\) for LSV which is of a typical adsorption controlled process, and the equations can be expressed as,

\[
I_p (\mu A) = 181.1 \ u (V s^{-1}) - 3.174, \ r = 0.991 \text{ for CV}
\]
\[
I_p (\mu A) = 166.5 \ u (V s^{-1}) - 2.431, \ r = 0.995 \text{ for LSV}
\]
Figure VII (ii)

(a) Influence of pH on the shape of anodic peak; pH: 3.0 (a), 4.2 (b), 5.0 (c), 6.0 (d), 7.0 (e), 8.0 (f), 10.4 (g), 11.2 (h).
(b) Influence of pH on the peak potential of CDS on gold electrode at scan rate of 50mV/s in phosphate buffer.
(c) Variation of peak currents of CDS with pH. Other conditions are as in Fig.VII (i) (p. 224)
Figure VII (iii)

(a) Cyclic voltammograms for the oxidation of CDS at different scan rates

(a) 0.01  (b) 0.025  (c) 0.05  (d) 0.075  (e) 0.1  (f) 0.15  (g) 0.20  (h) 0.25/Vs$^{-1}$
(b) Dependence of oxidation peak current on the scan rate

![Graph showing the dependence of oxidation peak current on the scan rate.]

(c) Linear relation between logarithm of peak current and logarithm of scan rate

![Graph showing the linear relation between logarithm of peak current and logarithm of scan rate.]

Chapter VII
(d) Dependence of oxidation peak potential on the logarithm of scan rate

**Figure VII (iv)**

Linear sweep voltammograms of 1.0 mM CDS at gold electrode with different scan rates Curves (a) 0.01 (b) 0.025 (c) 0.05 (d) 0.075 (e) 0.1 (f) 0.15 (g) 0.20 (h) 0.25/Vs⁻¹, respectively. Other conditions are as in Fig.VII (i) (p. 224)
A plot of logarithm of anodic peak current versus logarithm of scan rate gave a straight line with a slope of 0.864 for CV (Fig.VII (iii b) (p. 229)) and 0.853 for LSV, which are close to the theoretical value of 1.0, which is expected for an ideal reaction for the adsorption-controlled electrode process. The equations obtained were:

\[
\log I_p (\mu A) = 0.864 \log \nu (V s^{-1}) + 2.211, r = 0.997 \text{ for CV}
\]

\[
\log I_p (\mu A) = 0.853 \log \nu (V s^{-1}) + 2.153, r = 0.998 \text{ for LSV}
\]

The \(E_p\) of the oxidation peak was also dependent on scan rate. The peak potential shifted to more positive values on increasing the scan rate, which confirms the irreversibility of the oxidation process and a linear relationship between peak potential and logarithm of scan rate for CV (Fig.VII (iii d) (p. 230)) and for LSV can be expressed by the following equations,

\[
E_p (V) = 0.064 + 1.267 \log \nu (V s^{-1}); r = 0.991 \text{ for CV}
\]

\[
E_p (V) = 0.073 + 1.284 \log \nu (V s^{-1}); r = 0.989 \text{ for LSV}
\]

For an irreversible electrode process, according to Laviron, \(E_p\) is defined by the following equation,

\[
E_p = E^{0'} + \left( \frac{2.303RT}{\alpha n F} \right) \log \left( \frac{Rk^0}{\alpha n F} \right) + \left( \frac{2.303RT}{\alpha n F} \right) \log \nu \tag{2}
\]

where \(\alpha\) (alpha) is the transfer coefficient, \(k^0\) the standard heterogeneous rate constant of the reaction, \(n\) the number of electrons transferred, \(\nu\) (nu) the scan rate and \(E^{0'}\) is the formal redox potential. Other symbols have their usual meanings. Thus the value of \(\alpha n\) can be easily calculated from the slope of \(E_p\) versus \(\log \nu\). In this system, the slope is 0.064 for CV and 0.073 for LSV,
taking \( T = 298 \) K, and substituting the values of \( R \) and \( F \), \( \alpha n \) was calculated. According to Bard and Faulkner \(^{16} \), \( \alpha \) can be given as, \( \beta \)

\[
\alpha = \frac{47.7}{E_p - E_{p/2}} \text{mV} \tag{3}
\]

where \( E_{p/2} \) is the potential where the current is at half the peak value. So, from this we obtained the value of \( \alpha \). Further, the number of electron (n) transferred in the electrooxidation of CDS was also calculated using cyclic and linear sweep voltammetry. The value of \( k^0 \) can be determined from the intercept of the above plot if the value of \( E^{0'} \) is known. The value of \( E^{0'} \) in equation (2) can be obtained from the intercept of \( E_p \) versus \( \nu \) curve by extrapolating to the vertical axis at \( \nu = 0 \) \(^{17} \). In our system the intercept for \( E_p \) versus \( \log \nu \) plot was 1.267 for CV and 1.284 for LSV methods. All the values of \( \alpha n, \alpha, n, E^{0'} \) and \( k^0 \) obtained from cyclic and linear sweep voltammetry are tabulated in Table VII (i) (p. 233).

### 7.3.4. Calibration curve and detection limit

To develop a voltammetry method for determining the drug, we selected the differential-pulse voltammetric mode, because the peaks are sharper and better defined at lower concentrations of CDS than those obtained by cyclic voltammetry. According to the obtained results, it was possible to apply this technique to the quantitative analysis of CDS. A phosphate buffer solution of pH 3.0 was selected as the supporting electrolyte for the quantification of CDS because it gave the maximum peak current at pH 3.0.
Table VII (i)

Calculated values of $\alpha n$, $\alpha$, $n$, $E^{0'}$, and $k^0$ for the electro-oxidation of CDS by cyclic voltammetry (CV) and linear sweep voltammetry (LSV)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CV</th>
<th>LSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha n$</td>
<td>0.924</td>
<td>0.810</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.675</td>
<td>0.676</td>
</tr>
<tr>
<td>$n$</td>
<td>1.368</td>
<td>1.197</td>
</tr>
<tr>
<td>$E^{0'}$</td>
<td>1.267</td>
<td>1.284</td>
</tr>
<tr>
<td>$k^0$</td>
<td>461.5</td>
<td>727.7</td>
</tr>
</tbody>
</table>

$\alpha$ is the transfer coefficient, $n$ is the number of electrons transferred, $E^{0'}$ is the formal redox potential in V, and $k^0$ is the standard heterogeneous rate constant of the reaction in cm s$^{-1}$. 
Chapter – VII

Differential-pulse voltammograms obtained with increasing amounts of CDS showed that the peak current increased linearly with increasing concentration, as shown in Fig. VII (v) (p. 236). Using the optimum conditions described previously, a linear calibration curve was obtained for CDS in the range from $1.0 \times 10^{-4}$ to $5.0 \times 10^{-6}$ M (Inset: (Fig. VII (v) (p. 236)). The linear equation was,

$$I_p (\mu A) = 0.037 C (\mu M) + 2.221; r = 0.998$$

Deviation from linearity was observed for more concentrated solutions, due to the adsorption of CDS or its oxidation product on the electrode surface. Related statistical data of the calibration curves were obtained from the nine different determinations. The limit of detection (LOD) and quantification (LOQ) were $6.859 \times 10^{-7}$ M and $2.286 \times 10^{-6}$ M, respectively. The LOD and LOQ were calculated using the following equations:

$$\text{LOD} = \frac{3s}{m} \quad \text{LOQ} = \frac{10s}{m}$$

where, $s$ is the standard deviation of the peak currents of the blank (four runs), and $m$ is the slope of the calibration curve.

Precision of the method was investigated by intra- and inter-day determination of CDS at two different concentrations ($n = 3$) within the linear range. Accuracy of the methods expressed as bias% and RSD% for intra and inter days are as shown in Table VII (ii) (p. 237), which indicated high precision of the proposed method.
Figure VII (v)

Differential pulse voltammograms for increasing concentration of CDS (mM):
(a) 5.0 (b) 10.0 (c) 25.0 (d) 45.0 (e) 65.0 (f) 80.0 (g) 100.0 × µM; other conditions are same as in Fig.VII (i) (p. 224). Inset: Plot of peak current against the concentration of CDS.
In order to ascertain the repeatability of the analysis, 6 measurements of \(1 \times 10^{-4}\) M CDS solution were carried out using gold electrode at intervals of 30 min. The RSD value of peak current was found to be 2.160%, which indicated that electrode has good repeatability. As to the reproducibility between days, it was similar to that of within a day repeatability if, the temperature was kept almost unchanged.

7.3.5. Effect of excipients

For the possible analytical application of the proposed method, the effect of some common excipients used in pharmaceutical preparation was examined. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than ± 5 % for determination of CDS. The effects of these excipients on the voltammetric response was carried by analyzing sample solutions containing a fixed amount of carbenicillin \((1.0 \times 10^{-4} \text{ M})\) spiked with various excess amount of each excipient under the same experimental conditions. The experimental result (Table VII (iii) (p. 239)) showed that ten–fold excess of D-glucose, sucrose, ascorbic acid, citric acid, tartaric acid, dextrose, KCl, MnSO\(_4\), FeSO\(_4\) and CaCl\(_2\) did not interfere with the voltammetric signal of CDS. Thus, the procedures were able to assay CDS in the presence of excipients, and hence it can be considered specific.
Chapter – VII

Table VII (ii)

Analytical precision and accuracy of CDS determination by differential pulse voltammetry

<table>
<thead>
<tr>
<th>Added (M)</th>
<th>Found(^a) (M)</th>
<th>SD</th>
<th>Accuracy bias (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intraday</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 × 10(^{-6})</td>
<td>0.987</td>
<td>0.010</td>
<td>−1.247</td>
<td>1.075</td>
</tr>
<tr>
<td>1.0 × 10(^{-4})</td>
<td>1.010</td>
<td>0.029</td>
<td>−1.020</td>
<td>2.927</td>
</tr>
<tr>
<td><strong>Interday</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 × 10(^{-6})</td>
<td>0.997</td>
<td>0.021</td>
<td>−0.26</td>
<td>2.156</td>
</tr>
<tr>
<td>1.0 × 10(^{-4})</td>
<td>0.980</td>
<td>0.012</td>
<td>−1.98</td>
<td>1.275</td>
</tr>
</tbody>
</table>

\(^a\)Average of three determinations
7.3.6. Detection of CDS in urine samples

The applicability of the DPV to the determination of carbenicillin in spiked urine was investigated. The recoveries from urine were measured by spiking drug free urine with known amounts of CDS. The urine samples were diluted 100 times with the phosphate buffer solution before analysis without further pretreatments. A quantitative determination can be carried out by adding the standard solution of CDS into the detect system of urine sample. The calibration graph was used for the determination of spiked CDS in urine samples. The detection results of five urine samples obtained are listed in Table VII (v) (p. 239). The recovery determined was in the range from 97.82% to 101.04% and the R.S.D. was 1.326%. Thus, satisfactory recoveries of the analyte from the real samples and a good agreement between the concentration ranges studied and the real ranges encountered in the urine samples when treated with the drug, make the developed method applicable in clinical analysis.
Table VII (iii)

Influence of potential excipients on the voltammetric response of $1.0 \times 10^{-5}\text{M}$ CDS

<table>
<thead>
<tr>
<th>Excipients</th>
<th>Concentration (mM)</th>
<th>Signal change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-glucose</td>
<td>10</td>
<td>−0.89</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10</td>
<td>−1.28</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>10</td>
<td>−1.28</td>
</tr>
<tr>
<td>Citric acid</td>
<td>10</td>
<td>−1.68</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>10</td>
<td>−0.89</td>
</tr>
<tr>
<td>Dextrose</td>
<td>10</td>
<td>0.38</td>
</tr>
<tr>
<td>KCl</td>
<td>10</td>
<td>3.55</td>
</tr>
<tr>
<td>MnSO₄</td>
<td>10</td>
<td>−2.87</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>10</td>
<td>0.83</td>
</tr>
<tr>
<td>FeSO₄</td>
<td>10</td>
<td>−1.09</td>
</tr>
</tbody>
</table>

Table VII (iv)

Determination of CDS in urine samples

<table>
<thead>
<tr>
<th>Urine</th>
<th>Spiked $(\times 10^{-5}\text{M})$</th>
<th>Detected$^a$ $(\times 10^{-5}\text{M})$</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>8.0</td>
<td>7.96</td>
<td>99.56</td>
<td>2.16</td>
</tr>
<tr>
<td>Sample 2</td>
<td>6.5</td>
<td>6.56</td>
<td>101.04</td>
<td>1.54</td>
</tr>
<tr>
<td>Sample 3</td>
<td>4.5</td>
<td>4.40</td>
<td>97.82</td>
<td>3.31</td>
</tr>
<tr>
<td>Sample 4</td>
<td>1.0</td>
<td>0.99</td>
<td>99.31</td>
<td>1.58</td>
</tr>
</tbody>
</table>

$^a$Average of six determinations
7.4. IMPORTANCE OF CHAPTER

The electrochemical oxidation of CDS at gold electrode in phosphate buffer solution (pH = 3.0) has been investigated. The results indicated that CDS undergoes one electron and one proton transfer and is a adsorption-controlled process. The differential-pulse voltammetric procedure can be used successfully to determine CDS. High percentage recovery and study of excipients showed that the method is free from the interferences of the commonly used excipients and additives in the formulations of drug. In addition, the results obtained in the analysis of CDS in spiked urine samples demonstrated the applicability of the method in real sample clinical analysis. This method can be a good alternative for the analytical determination of CDS, because it is simple, sensitive, fast, accurate, and inexpensive. The proposed methods are suitable for quality control laboratories as well as pharmacokinetic studies where economy and time are essential.
7.5. REFERENCES

1. J. E. F. Reynolds,

2. P. A. Womey,
   J. Pharm. Sci., 70, 824 (1981)

3. W. Naidong,

   Research microbiology, 140, 579 (1989)

5. J. A. Squella and L. J. Núñez-Vergara,

6. J. Zima, I. Svancara, J. Barek and K. Vytras,

7. N. Erk,

8. E. E. Ferapontova,

9. H. Y. Xia and X. Y. Hu,

10. G. D. Christian and W. C. Purdy,

11. B. Rezaei and S. Damiri,

13. I. Martins, F. C. Cristiani and S. C. Larissa,
   *Talanta*. **85**, 1 (2011)

14. D. K. Gosser,
   “*Cyclic Voltammetry: Simulation and Analysis of Reaction Mechanisms*” VCH, New York (1993), p. 43

15. E. Laviron,

16. A. J. Bard and L. R. Faulkner,

17. W. Yunhua, J. Xiaobo and H. Shengshui,