PART - B
CYCLIC VOLTAMMETRIC STUDIES
Electrochemical methods have proved to be very sensitive for the determination of organic molecules, including drugs and related molecules in pharmaceutical dosage forms and biological fluids. The advance in experimental electrochemical techniques in the field of analysis of drugs is due to their simplicity, low cost and relatively short analysis time when compared with the other techniques. The use of carbon based electrodes, especially glassy carbon electrode, for electroanalytical measurements has increased in recent years because of their applicability to the determination of substances that undergo oxidation reactions, a matter of great importance in the field of clinical and pharmaceutical analysis. Redox properties of drugs can give insights into its metabolic fate or their in vivo redox processes or their pharmaceutical activity.

The importance of pentoxifylline (PTX) is given in Chapter II (p.32). The widespread use of this compound and the need for clinical and pharmacological study require fast and sensitive analytical techniques to determine the presence of the drug in pharmaceutical formulations. Up till now the most common techniques for the determination of the drug in commercial dosage form and biological fluids have been based on HPLC and GC.
Literature survey revealed that no information about the electrochemical redox properties of PTX and no electroanalytical methods for the determination of PTX were reported. The aims of study in this chapter are to establish the experimental conditions, to investigate the oxidation mechanism of PTX and to optimize the conditions for the determination of this compound in pharmaceutical dosage forms using cyclic and differential pulse voltammetric techniques.

**EXPERIMENTAL**

**Materials and reagents**

Pure PTX in powdered form was obtained as a gift sample from Ms. S.S.Antibiotics Pvt. Ltd, Aurangabad, India and was used without further purification. A stock solution (1.0 mM) of PTX was prepared by direct dissolution in doubly distilled water. PTX containing tablets marketed by different medical companies were purchased from the local pharmacy. Phosphate buffer solutions (Ionic strength = 0.2 M) were prepared according to the method of Christian and Purdy\(^{17}\). All other reagents used were of analytical grade. All solutions were prepared in double distilled water.

**Apparatus**

The electrochemical experiments were performed with CH Instruments, USA (Model 1110A,Version 4.01) Electrochemical Analyzer and were carried out in a 10 ml single compartment three-electrode glass cell with a 3 mm diameter glassy carbon electrode (GCE) as the working electrode (Part No.CHI104), a platinum wire as counter electrode and Ag/AgCl (3 M KCl)
electrode as reference electrode. All experiments were carried out at an ambient
temperature of 25 ± 0.1°C. The area of the electrode was obtained by cyclic
voltammetric method using 1.0 mM K₃Fe(CN)₆ as a probe at different scan
rates. For a reversible process, the following Randles-Sevcik formula can be
used:

\[ i_{pa} = (2.69 \times 10^5) n^{3/2} A D_0^{1/2} C_0 v^{1/2} \]  

where \( i_{pa} \) refers to the anodic peak current, \( n \) is the number of electrons
transferred, \( A \) is the surface area of the electrode, \( D_0 \) is diffusion coefficient, \( v \)
is the scan rate and \( C_0 \) is the concentration of K₃Fe(CN)₆. For 1.0 mM
K₃Fe(CN)₆ in 0.1 M KCl electrolyte, \( n = 1 \), \( D_0 = 7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1} \), then from
the slope of the plot of \( i_{pa} \) versus \( v^{1/2} \), the surface area of the electrode can be
calculated. In our experiment the slope was \( 3.44 \times 10^{-6} \text{ pA (Vs}^{-1})^{1/2} \) and the area
of electrode was calculated to be 0.0464 cm².

The experimental conditions for differential pulse voltammetry (DPV)
were: initial \( E: 1.1 \text{ V} \), final: 1.45 V, sensitivity: 5 \( \mu \text{A/V} \), pulse amplitude: 50
mV, sample width: 20 ms, pulse width: 30 ms, pulse period: 600 ms.

**Analytical procedure**

The GCE was polished using 0.3 micron Al₂O₃ before each experiment.
After polishing, the electrode was rinsed thoroughly with methanol and doubly
distilled water. After this mechanical treatment, the GCE was placed in 0.2 M
phosphate buffer solution and various voltammograms were recorded until a
steady state baseline voltammogram was obtained. An amount of 10 tablets
were weighed and ground to a homogeneous fine powder in a mortar. Portion
equivalent to a stock solution of a concentration about $4 \times 10^{-3}$ M was accurately weighed and transferred into a 100 ml calibrated flask and completed to the volume with water. The contents of the flask were sonicated for 10 min to affect complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with the doubly distilled water. Each solution was transferred to a voltammetric cell and analyzed under same conditions as used to obtain calibration graph. Voltammograms were recorded as described for pure PTX.

To study the accuracy of the proposed method, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of PTX to known concentration of the tablets. The resulting mixture was analyzed as in pure PTX.

**RESULTS AND DISCUSSION**

**Cyclic voltammetry**

Successive cyclic voltammograms of 0.50 mM PTX obtained in phosphate buffer of pH 3.0 at scan rate of 50 mVs$^{-1}$ are shown in Figure IV (i) (p.106). In cyclic voltammograms one well-defined anodic peak was observed. The fact that no peak was observed in the reverse scan suggests that the oxidation process is an irreversible one. Continued scanning resulted in a negative shift of the oxidation potential and a decrease in the peak current suggesting an adsorbed species formation on the electrode surface.
Figure IV (i)

Successive cyclic voltammograms for 0.50 mM PTX on glassy carbon electrode in pH 3.0 using phosphate buffer solution (0.2 M ionic strength): (1) first, (2) second, (3) third, (4) fourth and (5) blank run. Scan rate: $v = 50 \text{ mVs}^{-1}$
Effect of the scan rate

The effect of potential scan rate (v) on the peak current (Ip) and the peak potential (Ep) of PTX was evaluated (Fig. IV (ii) (p.108)). The influence of the square root of the scan rate on the peak current showed a linear relationship between 10 and 200 mVs\(^{-1}\) (Fig. IV (iii a) (p.109)) \((r \geq 0.989, S \leq 0.014)\), which is typical of diffusion-controlled currents\(^{19}\). As the scan rate was increased from 200 to 500 mVs\(^{-1}\) the currents were adsorption controlled. A linear relationship was observed between log Ip and log v (Fig. IV (iii b) (p.109)) \((r \geq 0.998, S \leq 0.017)\) corresponding to the equation:

\[
\log \text{Ip} (\mu\text{A}) = 0.499 \log v (\text{Vs}^{-1}) + 0.5991.
\]

The slope of 0.49 is close to the theoretically expected value of 0.5 for a purely diffusion-controlled current\(^{19}\).

The peak potential shifted to more positive values with increasing the scan rates. The linear relation between peak potential and logarithm of scan rate \((r \geq 0.9927, S \leq 0.012)\) (Fig. IV (iv) (p.110)) can be expressed as

\[
\text{Ep (V)} = 1.4310 + 0.0568 \log v (\text{Vs}^{-1}).
\]

As for an irreversible electrode process, according to Laviron, Ep is defined by the following equation\(^{20}\).

\[
\text{Ep} = E^{\circ} + \left(\frac{2.303RT}{\alpha nF}\right) \log \left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) \log v \tag{2}
\]

where \(\alpha\) is the transfer coefficient, \(k^0\) the standard heterogeneous rate constant of the reaction, \(n\) the number of electrons transferred, \(v\) the scan rate and \(E^{\circ}\) is the formal redox potential. Other symbols have their usual meanings. Thus the
Cyclic voltammograms in the presence of 0.50 mM PTX in pH 3.0 using phosphate buffer solution (0.2 M ionic strength) on glassy carbon electrode at different scan rates, (1)-(6): 200, 100, 75, 50, 20, 10 mVs⁻¹.
Figure IV (iii)

a) The linear relationship between the peak currents and the square root of scan rates

b) The linear relationship between the logarithm of peak currents and logarithm of scan rates
Figure IV (iv)

The linear relationship between the peak potential and logarithm of scan rate
value of $\alpha n$ can be easily calculated from the slope of $E_p$ versus $\log v$. In this system, the slope was 0.0568, taking $T = 298$ K, $R = 8.314$ J K$^{-1}$ mol$^{-1}$ and $F = 96480$ C, $\alpha n$ was calculated to be 1.0412. Generally, $\alpha$ is assumed$^{21}$ to be 0.5 in totally irreversible electrode process. Further, the number of electron ($n$) transferred in the electro oxidation of PTX was calculated to be $2.08 \sim 2.0$. 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 eqn.(2) can be obtained from the intercept of $E_p$ versus $v$ curve by extrapolating to the vertical axis at $v = 0$.$^{22}$ The intercept for $E_p$ versus $\log v$ plot was 1.4310 and $E^{0'}$ was obtained to be 1.3547 V, the $k^0$ was calculated to be 892.69 s$^{-1}$.

**Effect of [PTX]**

The effect of concentration for the electrochemical oxidation of PTX was evaluated and is as shown in Figure IV (v) (p.112). A plot of $I_p$ versus the concentration of pentoxifylline shows linearity (Fig. IV (vi) p.(113)) ($r \geq 0.995$, $S \leq 0.018$), indicating further that the electrode process is diffusion-controlled, with correlation coefficient of 0.995. The linear relation expressing dependence of $I_p$ on concentration in the range 0.05 to 0.40 mM can be described as:

$$I_p \ (\mu A) = 52.824 \ C + 4.9724,$$

where $C$ is concentration.

**Effect of pH**

The electrochemical oxidation of PTX was also studied over the pH range 3.0-9.0 in phosphate buffer solutions ($I = 0.2$ M). The pH dependence of the peak potential and peak current obtained when cyclic voltammetry was
Figure IV (v)

Cyclic voltammograms in pH 3.0 using phosphate buffer solution (0.2 M ionic strength) on glassy carbon electrode at different concentrations of PTX (mM), (a)-(h): 0.40, 0.35, 0.25, 0.20, 0.15, 0.10, 0.05, 0
Figure IV (vi)

The linear relationship between the peak current and concentration of PTX in the range 0.05 to 0.50 mM
used is as follows. With the increase in pH of the solution, peak potential shifts to less positive potentials (Fig. IV (vii a) (p.115)) \((r \geq 0.997, S \leq 0.008)\), and obey the following equation:

\[ E_p(V) = 1.3573 - 0.0561 \text{pH}. \]

A slope of 56.1 mV/pH suggests that the number of electron transfer is equal with that of hydrogen ions taking part in the electrode reaction. The solution pH influences the peak current considerably. The peak current decreases linearly with the increase of solution pH (Fig. IV (vii b) (p.115)), to which a quantitative explanation is given as follows. The study on theophylline, which has similar structure with PTX, was studied by cyclic voltammetry at the glassy carbon electrode\(^{23}\). Taking into account that the voltammograms of theophylline closely match with the voltammograms of PTX, we may assume that the oxidation steps are similar.

**Oxidation sites of PTX**

UV spectra of 20 \(\mu\)M PTX in 0.2 M phosphate buffer solution at pH 3.0 before and after electrolysis are shown in Fig. IV (viii) (p.116). The electrolysis was carried out for 6 hrs. Two absorption peaks are found at 208 and 272 nm before electrolysis (curve a), but after depleting electrolysis the relative weak absorption peak at 272 nm vanishes, the strong absorption peak at short wavelength remains, but a slight blue shift to 205 nm occurs (curve b). We speculate that electro oxidation might have led to destruction of the \(\pi\) bond conjugate system in PTX. The number of electrons transferred \(n\) is calculated to be 2. Summing up all the above experimental results a possible electrode
Figure IV (vii)

a) The linear relation between $E_p$ and pH

![Graph showing the linear relation between $E_p$ and pH.]

b) The plot of $I_p$ versus pH

![Graph showing the plot of $I_p$ versus pH.]

Figure IV (viii)

UV spectra of 20 μM PTX in phosphate buffer solution at pH 3.0. (a) before electrolysis and (b) after electrolysis
reaction mechanism of PTX might be expressed as shown in Scheme 1.

Chemical kinetic method

In order to distinguish between the oxidative pathways of electrochemical and chemical processes, kinetics of oxidation of PTX by permanganate was carried. This oxidation mechanism\textsuperscript{24} is as shown in chapter II (p.55). From this, we can conclude that the electrochemical and chemical oxidation pathways are different.

Differential-pulse voltammetry

In order to increase speed and sensitivity, many forms of potential modulation have been developed. In this technique, a pulse of desired amplitude and width over it modulates the excitation signal i.e. linear sweep. This modulated signal is applied to the electrode. The corresponding resultant diffusion current is sampled two times namely, once before the application of the pulse and second before the end of the pulse. The difference of the two sampled currents is taken as the measure of the actual cell current and plotted against applied potential.
Analytical applications

In order to develop a voltammetric method for determining the drug, we selected the differential-pulse voltammetric mode, since the peaks are sharper and better defined at lower concentration of PTX than those obtained by cyclic voltammetry, with a lower background current, resulting in improved resolution. According to the obtained results, it was possible to apply these techniques to the quantitative analysis of PTX. The phosphate buffer solution of pH 3.0 was selected as the supporting electrolyte for the quantification of PTX as it gave maximum peak current at pH 3.0.

Differential-pulse voltammograms obtained with increasing amounts of PTX showed that the peak currents increased linearly with increasing concentration (Fig. IV (ix) (p.119)). Using the optimum conditions as described earlier (p.104), linear calibration curves were obtained for PTX in the range of 0.02 to 6.0 μM (r ≥ 0.998, S ≤ 0.007). Deviation from linearity was observed for more concentrated solutions, due to the adsorption of PTX or its oxidation product on the electrode surface at higher concentration. The linear equation was

\[ I_p (\mu A) = 11.9619 \ C (\mu M) - 0.1861. \]

Related statistical data of the calibration curves were obtained from four different calibration curves. The limit of detection (LOD) and quantification (LOQ) were 0.44 nM and 1.47 nM respectively. The LOD and LOQ were calculated on the peak current using the following equations:

\[ \text{LOD} = 3s/m, \quad \text{LOQ} = 10s/m \]
Figure IV (ix)

Differential-pulse voltammograms for increasing concentrations of PTX in pH 3.0 using phosphate buffer solutions (0.2 M ionic strength) on glassy carbon electrode. PTX concentration (µM): (1) 0.05, (2) 0.10, (3) 0.20, (4) 0.30 and (5) 0.35. Inset: Plot of current versus concentration of PTX.
where $s$ is the standard deviation of the peak currents of the blank (four runs) and $m$ is the slope of the calibration curve\textsuperscript{25}. Sample solutions recorded after 48 hrs did not show any appreciable change in the assay values. Unknown concentrations of PTX can be obtained from calibration curve.

**Determination of PTX in tablets**

In order to evaluate the applicability of the proposed method, two commercial medicinal samples containing PTX viz. Trental\textsuperscript{\textregistered} 400 (Aventis Pharma. Ltd., Ankleshwar: Batch No.026593) and Flexital (Sun Pharmaceutical Industries, Dadra: Batch No.AD50481) are studied. The tablets were grounded to powder, dissolved in water and then further diluted so that PTX concentration falls in the range of calibration plot. Differential-pulse voltammograms were then recorded under exactly identical conditions that were employed while recording differential-pulse voltammograms for plotting calibration plot. It was found that PTX concentration determined for various tablets using this method are in good agreement with the reported values. The results were compared with those obtained by the published HPLC method\textsuperscript{15}. The values of experimentally determined PTX and reported PTX amounts in various tablets are as shown in Table IV (i) (p.121).

**Recovery test of PTX**

The recovery tests of PTX ranging from 0.09 to 3.2 \( \mu \text{M} \) were performed using differential-pulse voltammetry. Recovery studies were carried out after the addition of known amounts of the drug to various pre-analyzed formulation of PTX. The results are listed in Table IV (ii) (p.122). The recoveries in
Table IV (i)
Comparative studies for PTX tablets (in Trental®400 and Flexital) by proposed
and literature methods and mean recoveries in spiked tablets

<table>
<thead>
<tr>
<th></th>
<th>HPLC(^{15})</th>
<th>Trental(^{400})(^{a})</th>
<th>Flexital(^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labelled claim (mg)</td>
<td>600.0</td>
<td>400.0</td>
<td>400.0</td>
</tr>
<tr>
<td>Amount found (mg)(^{b})</td>
<td>613.3</td>
<td>398.9</td>
<td>395.4</td>
</tr>
<tr>
<td>R.S.D. (%)</td>
<td>1.41</td>
<td>0.198</td>
<td>0.41</td>
</tr>
<tr>
<td>Added (mg)</td>
<td>----</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Found (mg)</td>
<td>----</td>
<td>24.97</td>
<td>24.65</td>
</tr>
<tr>
<td>Recovered (%)(^{c})</td>
<td>99.87</td>
<td>98.41</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)Names of the tablets.

\(^{b}\)Each value is the mean of five experiments.

\(^{c}\)Recovery value is the mean of five experiments.
<table>
<thead>
<tr>
<th>Added (µM)</th>
<th>Found (µM)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.09</td>
<td>0.089</td>
<td>99.4</td>
</tr>
<tr>
<td>0.24</td>
<td>0.244</td>
<td>101.6</td>
</tr>
<tr>
<td>0.30</td>
<td>0.299</td>
<td>99.7</td>
</tr>
<tr>
<td>0.40</td>
<td>0.390</td>
<td>97.6</td>
</tr>
<tr>
<td>0.70</td>
<td>0.701</td>
<td>100.2</td>
</tr>
<tr>
<td>0.80</td>
<td>0.793</td>
<td>99.1</td>
</tr>
<tr>
<td>1.60</td>
<td>1.650</td>
<td>103.2</td>
</tr>
<tr>
<td>3.20</td>
<td>3.190</td>
<td>99.9</td>
</tr>
</tbody>
</table>
Table IV (iii)
Influence of interferents on the voltammetric response of 0.10 μM PTX at the glassy carbon electrode

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Concentration of interferents (μM)</th>
<th>Change in current response for PTX (μA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>0.01</td>
<td>0.1482</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.1933</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.2152</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.2639</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.01</td>
<td>0.0231</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.0653</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.1913</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.2340</td>
</tr>
<tr>
<td>Starch</td>
<td>0.01</td>
<td>0.2172</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.1998</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.0344</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.0025</td>
</tr>
</tbody>
</table>
different samples were found to lie in the range from 97.60% to 103.19%. The relative standard deviation was ±1.575%.

**Effect of interferents**

PTX is formulated in single as well as multi-component tablets. Interference studies were carried out in order to investigate the effect of co-formulated substances such as glucose, starch and sucrose on the voltammetric response of PTX. Differential-pulse voltammetric experiments were carried out for 0.10 μM PTX in the presence of 0.01 to 0.10 μM of each of the interferents. The results are listed in Table IV (iii) (p.123).

**Importance of Chapter IV**

The electrochemical behavior of PTX on glassy carbon electrode was established and studied for the first time. PTX is irreversibly oxidized at a high potentials on glassy carbon electrode. The proposed differential-pulse voltammetric procedure can be used successfully to determine PTX in tablet dosage form. It compares reasonably with the reported HPLC method and can be a good alternative for the analytical determination of PTX because it is simple, fast and low cost. Furthermore, the present method could possibly be adopted for the pharmacokinetic studies as well as clinical and quality control laboratories.
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