6.1. INTRODUCTION

Atorvastatin, an antihyperlipoproteinemic drug, \((3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, \((ATOR)\) as shown in Scheme 1, works by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme found in liver tissue that plays a key role in the biosynthesis of cholesterol\(^1\). It is used to reduce total cholesterol, low-density lipoprotein cholesterol\(^2\), apo-B\(^3\), triglycerides\(^4\) levels and CRP\(^5\) as well as to increase high-density lipoprotein levels. It is also used in the treatment of heterozygous familial hypercholesterolemia in pediatric patients and homozygous familial hypercholesterolemia\(^6\), primary dysbetalipoproteinemia (Fredrickson Type III) and also combined hyperlipidemia. It also stabilizes plaque and prevents risk of strokes, heart attack or other heart complications through anti-inflammatory and other mechanisms.

Scheme 1

A review of the literature reveals that a few analytical methods for
the determination of ATOR in pharmaceutical preparations and biological matrices relying on the use of chromatographic techniques such as liquid chromatography mass spectrometry (LC/MS/MS), liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS), high-performance liquid chromatography (HPLC) with electrospray tandem mass spectrometry and spectrophotometry have been described. The main problems encountered in using such methods are either the need for derivatization or the need for time-consuming extraction procedures. Electrochemical sensors satisfy many of the requirements for such tasks particularly owing to their inherent specificity, rapid response, sensitivity and simplicity of preparation for the determination of organic molecules, including drugs and related molecules in pharmaceutical dosage forms and biological fluids. Till date there is only one report on electro-oxidation of ATOR with glassy carbon electrode (GCE) and two reports on electro-oxidation of atorvastatin calcium using boron-doped diamond electrode and GCE.

Carbon electrodes, especially glassy and paste electrodes are widely used in electrochemical investigations because of their low background current, wide potential windows, chemical inertness, low cost, and suitability for detection of various organic and biological compounds. Among these, carbon paste electrodes (CPEs), due to unique characteristics such as versatility of chemical modification, renewability of the electrode surface, and compatibility with various electron mediators, have been extensively used in these studies.
Surfactants are a kind of amphiphilic ion or molecule with a hydrophilic head compatible with water on one side and long hydrophobic tail compatible with oil on the other side. They have been widely used in the field of electrochemical and electroanalytical chemistry. The surfactant can change the electrochemical process through adsorption at interfaces or aggregation into supramolecular structure. Many groups have successfully employed surfactants for the analysis of some biomolecules in their works. In the present chapter, the experimental results showed that cationic surfactant-CTAB had a distinct enhancement effect on the electrochemical responses of ATOR at the carbon paste electrode.

To the best of our knowledge, there is no report on the electro-oxidation and determination of ATOR at CPE in the presence of CTAB. The main objective of the present work in this chapter is to develop a convenient and sensitive electroanalytical method for the determination of ATOR at CPE in the presence of CTAB. The electrode was also tested for the determination of ATOR in pharmaceutical and urine samples. Although the electrochemical behavior of ATOR on the surface of boron-doped diamond and an unmodified GCE is reported, the voltammetric oxidation of ATOR at CPE in the presence of CTAB is better with respect to low cost for graphite powder based electrode and low detection limit, which is investigated in this chapter.
6.2. EXPERIMENTAL

6.2.1. Instrumentation

Electrochemical measurements were carried out on a CHI110A electrochemical analyzer coupled with a conventional three-electrode cell. A three-electrode cell was used with a Ag/AgCl as reference electrode, a Pt wire as counter electrode and a self-made carbon paste electrode as a working electrode. All the potentials are given against the Ag/AgCl (3 M KCl). pH measurements were performed with Elico LI120 pH meter (Elico Ltd., India).

6.2.2. Reagents and chemicals

ATOR was obtained from Cipla Ltd. India, and used without further purification. A stock solution of ATOR (1.0 mM) was prepared in methanol. The phosphate buffers from pH 3.0–11.2 were prepared according to the method of Christian and Purdy\textsuperscript{21}. The ATOR containing tablets i.e. Atorva 20 (Zydus Healthcare: Batch No. ZHK 3802) were purchased from a local pharmacy. All surfactants obtained from Hi-Media Pvt. Ltd., were dissolved in doubly distilled water to form $1.0 \times 10^{-2}$ M solutions. Other reagents used were of analytical or chemical grade. All solutions were prepared with double distilled water.

6.2.3. Preparation of electrode

The CPE was prepared by mixing 1.0 g graphite powder and 0.5 ml paraffin oil in an agate mortar, and this mixture was then homogenized. After
that, the paste was pressed manually into the cavity of the electrode body, and the surface was smoothed against weighing paper. Unless otherwise stated, the paste was carefully removed prior to pressing a new portion into the electrode after every measurement.

The area of the electrode was calculated using Randles-Sevcik\textsuperscript{22} formula as given in chapter V (p. 169). In our experiment the slope was found to be $1 \times 10^{-5} \mu A (V s^{-1})^{1/2}$ and the area of electrode was calculated to be $0.1348 \text{ cm}^2$.

6.2.4. Analytical procedure

The CPE was first activated in phosphate buffer (pH=3.0, Ionic strength = 0.2 M) by cyclic voltammetric sweeps between 0.0 to 2.0 V until a stable cyclic voltammogram was obtained. Then electrodes were transferred into another 10 ml of phosphate buffer (0.2 M, pH=3.0) containing proper amount of ATOR and CTAB. After accumulating for 20 s at open circuit under stirring and following quiet for 10 s, potential scan was initiated and cyclic voltammograms were recorded between +0.4 and +1.4 V, with a scan rate of 50 mV s\textsuperscript{-1}. All measurements were carried out at room temperature of $25 \pm 0.1 ^\circ C$.

6.2.5. Sample preparation

ATOR containing tablet i.e. Atorva 20 was used. The solution was prepared as given in Chapter V (p. 170). The differential pulse voltammograms were recorded between 0.6 and 1.2 V after open-circuit accumulation for 20 s with stirring. The oxidation peak current of ATOR was measured. The
parameters for differential pulse voltammetry (DPV) were as given in Chapter V (p. 171). To study the accuracy of the proposed method and to check the interferences from excipients used in the dosage form, recovery experiments were carried out. The concentration of ATOR was calculated using standard addition method.

6.3. RESULTS and DISCUSSION

6.3.1. Cyclic voltammetric behavior of ATOR

The electrochemical behavior of ATOR at CPE and in the presence of CTAB was investigated using cyclic voltammetry (CV). The results are shown in Figure VI (i) (p. 200). No apparent cyclic voltammetric signals were observed in the phosphate buffer solution in the presence (curve c) and absence (curve d) of CTAB, which indicates that CTAB is an electrochemically inactive material in the working potential range. The ATOR exhibits an anodic peak (at about 1.07 V curve b) at the CPE in the absence of CTAB. After the addition of 10 µM CTAB, the oxidation peak current of ATOR increases greatly (curve a). This indicates that CTAB can make the electron transfer of ATOR more easily and shows obvious enhancement effect to the oxidation of ATOR. The peak current enhancement was undoubtedly attributed to the interaction of CTAB with ATOR and CPE. It is well known that surfactants can be adsorbed on a hydrophilic surface to form surfactant film, which may alter the over voltage of the electrode and influence the rate of electron transfer. In the presence of CTAB, the electrode surface may form a hydrophilic film with positive charge.
Figure VI (i)
Cyclic voltammograms at the carbon paste electrode in 0.2M phosphate buffer solution (pH=3.0): (a) in the presence of ATOR and CTAB; (b) in the presence of ATOR; (c) in the presence of CTAB; and (d) in the absence of CTAB, Scan rate: 50mVs$^{-1}$; t(acc): 20 s (at open circuit), ATOR: 5.0 \times 10^{-5} \text{ M}, \text{CTAB: 10 \mu M}$
This hydrophilic layer increases the concentration of ATOR at the electrode surface.

On the reverse scan, no corresponding reduction peak was observed, indicating that the electrode process of ATOR is an irreversible one. It was found that the oxidation peak current of ATOR showed a remarkable decrease during the successive cyclic voltammetric sweeps (Fig. VI (ii) (p. 202)). A decrease in the oxidation peak current occurs with the number of successive sweeps. This phenomenon may be due to the fact that the adsorption of ATOR or its oxidative product occurs at the electrode surface. Therefore, the voltammograms corresponding to the first cycle was generally recorded.

6.3.2. Influence of accumulation potential and time

The influences of accumulation potential and accumulation time have been studied by cyclic voltammetric method as these could affect the amount of adsorption of ATOR at the electrode. Usually open circuit accumulation is widely used in electroanalytical chemistry to accumulate analyte and improve the sensitivity. The influence of accumulation time ranging from 0 to 60 s on the oxidation of ATOR at CPE was as shown in Figure VI (iii) (p. 203). The oxidation peak current increases greatly at the first 20 s and then slowly levels off. Therefore, the optimal accumulation time of 20 s was employed in further experiments.

With the change of accumulation potential, the peak current of ATOR varied slightly. So, the accumulation potential has no such effect on the peak
Figure VI (ii)

Successive cyclic voltammograms of $5.0 \times 10^{-5}$ M ATOR at CPE in the presence of CTAB. Scan rate: 50mVs$^{-1}$; t(acc): 20 s (at open circuit), ATOR: $5.0 \times 10^{-5}$ M, CTAB: 10 µM
Figure VI (iii)
Variation of the cyclic voltammetric anodic peak current with accumulation time Scan rate: 50mVs$^{-1}$, ATOR: $5.0 \times 10^{-5}$ M, CTAB: 10 µM.

![Graph showing variation of cyclic voltammetric anodic peak current with accumulation time.]

Figure VI (iv)
Dependence of the oxidation peak current on [CTAB]

![Graph showing dependence of oxidation peak current on [CTAB].]
current of ATOR, which in turn indicates that adsorption of CTAB is independent of the charge on the electrode surface. It is consistent with the fact that CTAB is adsorbed on the electrode surface through hydrophobic interaction with paraffin oil. Therefore the accumulation was carried out at open-circuit conditions.

6.3.3. Influence of concentration of CTAB

Amongst different surfactants used, such as sodium dodecyl sulfate (SDS), sodium dodecylbenzene sulfonate (SDBS) and cetyl trimethyl ammonium bromide (CTAB), CTAB could only promote the oxidation of ATOR at carbon paste electrode effectively. The effect of CTAB concentration on the oxidation of ATOR was as shown in Figure VI (iv) (p. 203). When the concentration of CTAB was increased from 0 to 10 µM, the peak current increased to a maximum. However, when CTAB concentration was increased beyond 10 µM, the peak current began to decrease. It may be due to the formation of CTAB layer on the electrode surface, which blocks the electron transfer between ATOR and the electrode.

6.3.4. Influence of pH

The electrode reaction might be affected by pH of the medium. The electro-oxidation of 50 µM ATOR was studied over the pH range of 3.0-11.2 in phosphate buffer solution by cyclic voltammetry. The oxidation peak appeared between pH 3.0 to 9.2 and there after sharp oxidation peak gradually
disappeared (Fig. VI (v) (p. 206)).

With the increase in solution pH, the peak potential linearly shifts to less positive values and the linear relation between \( E_p \) and pH (Fig. VI (vi A) (p. 207)) can be expressed as,

\[
E_p/V = 1.1182 - 0.0234 \text{ pH}; \quad r = 0.9908.
\]

The slope of this equation is found to be 23.4 mV / pH, close to the theoretical value of 30 mV / pH that involve two electrons and a proton transfer in the rate-determining step\(^{14,23-25}\). From the plot of \( I_p \) versus pH (Fig. VI (vi B) (p. 207)) it is clear that, peak current is affected by the pH value. The peak current decreased linearly with the increase in pH of solution. So, the buffer solution with pH=3.0 was selected for further experiments.

### 6.3.5. Influence of scan rate

Useful information involving electrochemical mechanism generally can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behavior of ATOR at different scan rates from 10 to 250 mV s\(^{-1}\) (Fig. VI (vii) (p. 208)) was also studied. The graph (Fig. VI (vii) (Inset) (p. 208)) shows that there is a good linear relationship between current and scan rate and the equation can be expressed as follows,

\[
I_p/\mu \text{A} = 30.28 \text{ u/V s}^{-1} + 0.4764; \quad r = 0.9964
\]

This indicates that the electrode process was controlled by adsorption rather than diffusion.
Figure VI (v)

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), 9.2 (g) Scan rate: 50mVs$^{-1}$; t(acc): 20 s (at open circuit), ATOR: 5.0 x10$^{-5}$ M, CTAB: 10 µM.
Figure VI (vi)

(A) Influence of pH on the peak potential of ATOR

(B) Influence of pH on the peak current of ATOR
Figure VI (vii)

Cyclic voltammograms of $5.0 \times 10^{-5}$ M ATOR at CPE in the presence of CTAB with different scan rates. (a)–(h) were 10, 25, 50, 75, 100, 150, 200 and 250 mVs$^{-1}$, respectively. Scan rate: 50 mVs$^{-1}$; t(acc): 20 s (at open circuit), ATOR: $5.0 \times 10^{-5}$ M, CTAB: 10 μM. Inset: Dependence of the oxidation peak current on the scan rate
A plot of logarithm of anodic peak current vs. logarithm of scan rate gave a straight line with a slope of 0.8001 (Fig. VI (viii A) (p. 210)) close to the theoretical value of 1.0, which is the expected for an ideal reaction of surface species. So, it confirms that the process appears to have an important adsorptive component and the equation can be expressed as,

$$\log I_p/\mu A = 0.8001 \log \upsilon/V s^{-1} + 1.3653, \ r = 0.9927$$

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 (Fig. VI (viii B) (p. 210)) can be expressed by the following equation,

$$E_p/V = 1.1242 + 0.0482 \log \upsilon/V s^{-1}; \ r = 0.9806.$$

For an adsorption-controlled and irreversible electrode process, according to Laviron equation (Chapter V (p. 180) the value of $\alpha n$ can be easily calculated from the slope of $E_p$ vs. log $\upsilon$. In this system, the slope was 0.0482, taking $T = 298 \text{ K}$, and substituting the values of $R$ and $F$, $\alpha n$ is calculated to be 1.2503. Generally $\alpha$ is assumed to be 0.5 in total irreversible electrode process. Further, the number of electron ($n$) transferred in the electro-oxidation of ATOR is calculated to be $\sim 2$.

**6.3.6. Mechanism**

We may assume that the oxidation steps of ATOR were located on the heterocyclic amine (pyrrole ring), which represents a typical redox system with
(A) Dependence of the logarithm of peak current on logarithm of scan rate

(B) Relationship between peak potential and logarithm of scan rate
two electron oxidation process as shown in Scheme 2. The mechanism of oxidation of ATOR can be proposed as follows: We may postulate that, ATOR initially loses an electron to form a cation radical which on losing a proton and an electron in subsequent steps forms a quaternary Schiff base. Thus, resulted quaternary Schiff base was rapidly hydrolysed to form 3, 5-Dihydroxy-7-oxo-

\[ \text{Scheme 2} \]
heptanoic acid and 5-(4-Fluoro-phenyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid phenylamide. Such types of mechanisms have been proposed in earlier reports\textsuperscript{14, 26, 27}.

**6.3.7. Calibration curve and detection limit**

According to the obtained results, it was possible to apply differential pulse voltammetric technique to the quantitative determination of ATOR. The phosphate buffer solution of pH=3.0 was selected as the supporting electrolyte for the quantification of ATOR as it gave a maximum peak current at pH=3.0. Differential pulse voltammograms obtained with increasing amounts of ATOR showed that the peak current increased linearly with increasing concentration, as shown in Figure VI (ix) (p. 213). Using the optimum conditions described above, linear calibration curves were obtained for ATOR in the range of 0.05 to 10 μM (Fig. VI (ix) (Inset) (p. 213)). The linear equation was $I_p/\mu A = 0.12 [\text{ATOR}]/\mu M + 0.1825$; $r = 0.9909$. Deviation from linearity was observed for more concentrated solutions, due to the adsorption of ATOR or its oxidation product on the electrode surface. Related statistical data of the calibration curves were obtained from the five different determinations. The limit of detection (LOD) and quantification (LOQ) were calculated as given in Chapter V (p. 185) and were found to be $4.08 \times 10^{-9}$ M and $1.36 \times 10^{-8}$ M, respectively. The detection limits reported for different classical methods and electrodes are tabulated in Table VI (i) (p. 214). This method was better as compared with other reported classical/electrochemical methods\textsuperscript{7, 10, 13, 14}. 


Figure VI (ix)

Differential-pulse voltammograms of CTAB modified CPE in ATOR solution at different concentrations: 0.05 (1), 0.1 (2), 0.3 (3), 0.75 (4), 2.25 (5), 3.0 (6), 8.0 (7) and 10.0 (8) µM. Inset: Plot of the peak current against the concentration of ATOR.
Comparison of linear range and detection limits for ATOR to different classical methods and electrodes.

<table>
<thead>
<tr>
<th>Classical method/ type of electrode</th>
<th>Linear range (μg/mL)</th>
<th>Detection limits (μg/mL)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>0.5 – 86.0</td>
<td>0.0084</td>
<td>7</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>5.0 – 25.0</td>
<td>1.0700</td>
<td>10</td>
</tr>
<tr>
<td>Boron doped diamond electrode</td>
<td>1.16 – 46.6</td>
<td>0.2745</td>
<td>13</td>
</tr>
<tr>
<td>Glassy carbon electrode</td>
<td>2.41 – 120</td>
<td>0.7195</td>
<td>14</td>
</tr>
<tr>
<td>Carbon paste electrode with CTAB</td>
<td>0.027 – 5.58 (= 0.05-10 μM)</td>
<td>0.0022 (= 4.08 nM)</td>
<td>Present work</td>
</tr>
</tbody>
</table>
Precision of the method was investigated by intra- and inter-day determination of ATOR at two different concentrations (n = 6) within the linear range. Accuracy of the methods expressed as bias% and RSD% for intra and inter days are as shown in Table VI (ii) (p. 216), which indicated high precision of the proposed method.

In order to ascertain the repeatability of the analysis, 6 measurements of 10 μM ATOR solution were carried out using carbon paste electrode in the presence of CTAB at intervals of 30 min. The RSD value of peak current was found to be 2.43%, which indicated that carbon paste electrode in the presence of CTAB 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. Owing to the adsorption of ATOR or its oxidative products on to the electrode surface, the current response of the electrode would decrease after successive use. In this case, the electrode should be modified again.

6.3.8. Effect of excipients

The experimental results (Table VI (iii) (p. 216)) showed that thousand-fold excess of citric acid, dextrose, glucose, lactose, oxalic acid, starch and sucrose did not interfere with the voltammetric signal of ATOR. Thus, the procedures were able to assay ATOR in the presence of excipients, and hence it can be considered specific.
### Table VI (ii)

Analytical precision and accuracy of ATOR determination by differential pulse voltammetry

<table>
<thead>
<tr>
<th>Added (µM)</th>
<th>Found[^a] (µM)</th>
<th>Accuracy bias (%)</th>
<th>R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraday</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.0990</td>
<td>-0.93</td>
<td>3.67</td>
</tr>
<tr>
<td>10</td>
<td>9.8976</td>
<td>-1.12</td>
<td>2.57</td>
</tr>
<tr>
<td>Interday</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.0993</td>
<td>-0.603</td>
<td>2.98</td>
</tr>
<tr>
<td>10</td>
<td>9.8733</td>
<td>-1.26</td>
<td>3.32</td>
</tr>
</tbody>
</table>

[^a] Average of six determinations

### Table VI (iii)

Influence of potential excipients on the voltammetric response of $1.0 \times 10^{-6}$ M ATOR.

<table>
<thead>
<tr>
<th>Excipients</th>
<th>Concentration (mM)</th>
<th>Signal change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>1.0</td>
<td>+1.82</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.0</td>
<td>+2.61</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0</td>
<td>+1.82</td>
</tr>
<tr>
<td>Lactose</td>
<td>1.0</td>
<td>+1.83</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>1.0</td>
<td>+2.62</td>
</tr>
<tr>
<td>Starch</td>
<td>1.0</td>
<td>+1.84</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.0</td>
<td>+3.68</td>
</tr>
</tbody>
</table>
6.3.9. Determination of ATOR in pharmaceutical preparations and recovery test

The proposed method was validated for the determination of ATOR in pharmaceutical preparations in “Atorva 20” tablets (20 mg per tablet) as a real sample by applying DPV using the standard addition method. The procedure for the tablet analysis was followed as described in section 2.5. The results are in good agreement with the content marked in the label (Table VI (iv) (p. 218)).

A comparison with an official reference determination method has not been possible in any pharmacopoeias, because so far no other procedure for the quantitation of ATOR from pharmaceutical formulations has been reported. For this reason, proposed method was compared with the literature method\textsuperscript{10}. (Table VI (iv) (p. 218)) compares the results of the analysis of ATOR between proposed and literature method. However, the proposed method is sensitive, selective and more precise than the spectrophotometric assay. The F and student t-tests were carried out on the data and statistically examined the validity of the obtained results by spectrophotometric and voltammetric methods. According to the students t and variance ratio F-test, the calculated t and F values were less than the theoretical values in either test at the 95% confidence level. This indicates that there is no significant difference between the performances of the proposed and spectrophotometric methods with regards to accuracy and precision.

The recovery test of ATOR was also carried out in the range of 0.5 to
## Table VI (iv)

Results of the assay and the recovery test of ATOR in pharmaceutical preparations using differential pulse voltammetry.

<table>
<thead>
<tr>
<th></th>
<th>DPV</th>
<th>Spectrophotometric method&lt;sup&gt;10&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labelled claim (mg)</td>
<td>20.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Amount found (mg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.84</td>
<td>20.18</td>
</tr>
<tr>
<td>Recovery %</td>
<td>99.2</td>
<td>100.9</td>
</tr>
<tr>
<td>RSD %</td>
<td>0.75</td>
<td>0.29</td>
</tr>
<tr>
<td>Bias %</td>
<td>-0.8</td>
<td>-</td>
</tr>
<tr>
<td>Calculated t</td>
<td>2.38</td>
<td>t&lt;sub&gt;th&lt;/sub&gt;: 2.776</td>
</tr>
<tr>
<td>Calculated F</td>
<td>6.25</td>
<td>F&lt;sub&gt;th&lt;/sub&gt;: 19.2</td>
</tr>
<tr>
<td>Amount of pure drug added (mg)</td>
<td>3.00</td>
<td>-</td>
</tr>
<tr>
<td>Amount found (mg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.98</td>
<td>-</td>
</tr>
<tr>
<td>Recovery %</td>
<td>99.33</td>
<td>-</td>
</tr>
<tr>
<td>RSD %</td>
<td>0.89</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean value of five determinations

th : theoretical
8.0 µM using differential pulse voltammetry. The recoveries in different samples were found to lie in the range from 98.14% to 102.65%, with R.S.D. of 1.71%.

6.3.10. Detection of ATOR in urine samples

The applicability of the DPV to the determination of ATOR in spiked urine was investigated. The recoveries from urine were measured similarly as given in Chapter V (p. 188). The detection results of five urine samples obtained are listed in Table VI (v) (p. 220). The recovery determined was in the range from 98.7% to 103.5% and the R.S.D. was 2.04%. Thus, satisfactory recoveries of the analyte from the real samples make the developed method applicable in clinical analysis.

6.4. IMPORTANCE OF CHAPTER VI

In this work, voltammetric oxidation of ATOR in the presence of cetyltrimethyl ammonium bromide at carbon paste electrode in phosphate buffer solution (pH=3.0) has been investigated. The oxidation mechanism involves transfer of two electrons. The peak current was linear to ATOR concentrations over a certain range, under the selected conditions. This helps in voltammetric determination of selected analyte as low as $4.08 \times 10^{-9}$ M with good reproducibility. The electrode has been used to determine ATOR in pharmaceutical samples and in spiked urine samples which demonstrated the applicability of the method for real sample analysis.
**Table VI (v)**

Determination of ATOR in urine samples using differential pulse voltammetry.

<table>
<thead>
<tr>
<th>Urine</th>
<th>Spiked (µM)</th>
<th>Detected[^a] (µM)</th>
<th>Recovery (%)</th>
<th>R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>8.0</td>
<td>8.016</td>
<td>100.2</td>
<td>0.27</td>
</tr>
<tr>
<td>Sample 2</td>
<td>5.0</td>
<td>5.069</td>
<td>101.4</td>
<td>0.52</td>
</tr>
<tr>
<td>Sample 3</td>
<td>3.0</td>
<td>3.106</td>
<td>103.5</td>
<td>0.59</td>
</tr>
<tr>
<td>Sample 4</td>
<td>2.0</td>
<td>1.973</td>
<td>98.7</td>
<td>0.77</td>
</tr>
<tr>
<td>Sample 5</td>
<td>0.5</td>
<td>0.4964</td>
<td>99.3</td>
<td>0.58</td>
</tr>
</tbody>
</table>

[^a] Average of five determinations
6.5. REFERENCES


13. B. Dogan-Topal, B. Uslu and S. A. Ozkan,


