Trazodone, (2- {3-[4-(3-chlorophenyl)-1-piperazinyl}propyl]-1,2,4-
triazole[4,3-a]pyridine-3(2H)-one hydrochloride) (TRZ), is a weak inhibitor of
monoamine reuptake and its major mechanism of action seems to be the
antagonism at serotonin 5-HT₂/5-HT₁₀ receptors¹. TRZ is used for the treatment
of major depression, sometimes in conjunction with selective serotonin
reuptake inhibitors, like fluoxetine². Unlike the tricyclic antidepressants, TRZ
does not inhibit the peripheral reuptake of noradrenaline, although it may
indirectly facilitate neuronal release. TRZ blocks central α₁-adrenoceptors and
appears to have no effect on the central reuptake of dopamine³. Also TRZ is
used to control sleep disturbance symptoms when using serotonin and
norepinephrine reuptake inhibitors⁴. TRZ is mainly metabolized in the liver by
the cytochrome isoform CYP3A4. The most important metabolite thus formed
is 3-(1-chlorophenyl)piperazine⁵, which was a serotonergic agonist with a long
half-life⁶. The main side effects associated with TRZ administration are:
nausea, insomnia, agitation, dry mouth, constipation, headache, hypotension,
blurred vision and confusion⁷. For these reasons, it was important to analyze
TRZ in real samples.

Some methods have been reported for the determination of TRZ in
pharmaceutical formulations or biological samples including
spectrophotometry, gas chromatography and high performance liquid chromatography (HPLC). Certain electrochemical studies have also been performed by polarography, cyclic voltammetric, coulometric and exhaustive electrolysis on the carbon paste electrode, voltammetry by comparison with chromatography, voltammetry using platinum electrode in rotating condition and by using direct current, differential pulse and alternating current polarography. Although HPLC has been widely applied because of its high sensitivity and selectivity and the ability to minimize interferences, it is time consuming, solvent-usage intensive and requires expensive devices and maintenance. Electrochemical detection of analyte is a very elegant method in analytical chemistry.

Carbon nanotubes (CNTs) continue to receive remarkable attention in electrochemistry. Since their discovery by Iijima in 1991 using transmission electron microscopy, CNTs have been the subject of numerous investigations in chemical, physical and material areas due to their novel structural, mechanical, electronic and chemical properties. The subtle electronic properties suggest that CNTs have the ability to promote charge transfer reactions when used as an electrode. The modification of electrode substrates with multi-walled carbon nanotubes (MWCNTs) for use in analytical sensing has been documented to result in low detection limits, high sensitivities, reduction of over potentials and resistance to surface fouling. MWCNTs have been introduced as electrocatalysts and CNTs modified...
electrodes have been reported to give super performance in the study of a number of biological species\textsuperscript{26}.

To our knowledge, voltammetric determination of TRZ using a MWCNTs modified glassy carbon electrode (GCE) has not been reported yet. The objective of the present chapter is to develop a convenient and sensitive method for the determination of TRZ based on the unusual properties of MWCNTs modified electrode. Here we report the electrochemical oxidation of TRZ on MWCNTs modified glassy carbon electrode. The ability of the modified electrode for voltammetric response of selected compound was evaluated. Finally, this modified electrode was used for the analysis of TRZ in pharmaceutical and urine samples. The resulted biosensor exhibits high sensitivity, rapid response, good reproducibility and freedom of other potentially interfering species.

\textbf{EXPERIMENTAL}

\textbf{Reagents}

TRZ was purchased from Sigma-Aldrich and used without further purification. A 10 mM stock solution was made in doubly distilled water. Multi-walled carbon nanotubes were from Sigma-Aldrich (> 90%, O.D: 10-15 nm, I.D: 2-6 nm, length: 0.1-10 \textmu m). The phosphate buffers from pH 3.0-11.2 (I = 0.2 M) were prepared in doubly distilled water as described by Christian and Purdy\textsuperscript{27}. Other reagents used were of analytical or chemical grade, and their solutions were prepared with doubly distilled water.
**Apparatus**

Electrochemical measurements were carried out on a CHI1110A electrochemical analyzer (CH Instrument Company, USA) 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 bare glassy carbon electrode with a diameter of 3 mm (modified and unmodified) were used as working electrodes, respectively. All of the used electrodes were from CHI Co. and all the potentials in this chapter are given against the Ag/AgCl (3M KCl). Solution pH was measured with an Elico LI120 pH meter (Elico Ltd, India).

**Preparation of MWCNTs modified electrode**

Multi-walled carbon nanotubes (i.e. MWCNTs) was refluxed in the mixture of concentrated H₂SO₄ and HNO₃ for 4-5 hours, then washed with doubly distilled water and dried in vacuum at room temperature. The MWCNTs suspension was prepared by dispersing 2 mg MWCNTs in 10 ml acetonitrile using ultrasonic agitation to obtain a relative stable suspension. The GCE was carefully polished with 0.30 and 0.05 μm α-alumina slurry on a polishing cloth, and then washed in an ultrasonic bath of methanol and water, respectively. The cleaned GCE was coated by casting 15 μl of the black suspension of MWCNTs and dried in air. The electroactive areas of the MWCNT-modified GCE and the bare GCE were obtained by cyclic voltammetry (CV) using 1.0 mM K₃Fe(CN)₆ as a probe at different scan rates as given in Chapter IV (p.104). In bare GCE, the electrode surface was found
to be 0.04638 cm² and for MWCNT-modified GCE, the surface was nearly 4.0-4.5 times greater.

**Analytical procedure**

The MWCNT-modified GCE was first activated in phosphate buffer (I = 0.2 M, pH 7.0) by cyclic voltammetric sweeps between 0 and 1.4 V until stable cyclic voltammograms were obtained. Then electrodes were transferred into another 10 ml of phosphate buffer containing proper amount of TRZ. After accumulating for 180 s at open circuit under stirring and following quiet for 10 s, potential scan was initiated and cyclic voltammograms were recorded between +0.2 and +1.3, with a scan rate of 50 mVs⁻¹. All measurements were carried out at room temperature of 25 ± 0.1°C.

**Sample preparation**

Ten pieces of TRZ tablets were powdered in a mortar. A portion equivalent to a stock solution of a concentration of about 1.0 mM was accurately weighed and transferred into a 100 ml calibrated flask and completed to the volume with double distilled 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 liquid and diluting them with the phosphate buffer solutions. Each solution was transferred to the voltammetric cell and analyzed by standard addition method. The differential pulse voltammograms were recorded between 0.40 and 0.90 V after open-circuit accumulation for 180 s with stirring. The oxidation peak current of TRZ was measured. The parameters for differential pulse
voltammetry (DPV) were pulse width of 0.06 s, pulse increment of 4 mV, pulse period of 0.2 s, pulse amplitude of 50 mV and scan rate of 20 mVs⁻¹. 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 TRZ was calculated using standard addition method.

RESULTS AND DISCUSSION

Cyclic voltammetric behavior of TRZ

The cyclic voltammograms of TRZ at a bare GCE and at MWCNT-modified GCE were shown in Fig. VI (i) (p.161). It can be seen that the TRZ oxidation peaks at the bare GCE was weak and broad due to slow electron transfer, while the response was considerably improved at the MWCNT-modified GCE. At the bare GCE, the peaks were at about 0.79V (peak A) and 1.10V (peak B), but on the MWCNT-modified GCE, the peaks appeared at 0.73V and 1.00V with considerable enhancement in the peak current. This was attributed to the electro catalytic effect caused by MWCNTs.

It also showed that no reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction was a totally irreversible process. Nevertheless, it was found that the oxidation peak current of TRZ showed a remarkable decrease during the successive cyclic voltammetric sweeps (Fig. VI (ii) (p.162)). After the second sweep, the peak current decreased greatly and finally remained unchanged. This phenomenon may be due to the fact that the adsorption of TRZ or its oxidative product occurs at the film electrode surface.
Figure VI (i)

Cyclic voltammograms of 10 μM TRZ on MWCNT-modified GCE (a) and bare GCE (c). Blank CVs of MWCNT-modified GCE (b) and bare GCE (d). Scan rate: 50 mVs⁻¹; supporting electrolyte: phosphate buffer with pH 7.0 (I = 0.2 M); accumulation time: 180 s (at open circuit); volume of MWCNTs suspension: 15 μl
Figure VI (ii)

Successive cyclic voltammograms of 10 μM TRZ on MWCNT-modified GCE
Therefore, the voltammograms corresponding to the first cycle and peak A was generally recorded, since peak A was intense than B.

**Influence of amount of MWCNTs**

Figure VI (iii a) (p.164) shows that the amount of MWCNTs has influence on the peak current. When the amount is from 12 to 18 μl, the peak current is more stable and higher. After that amount, it decreases. This is related to the thickness of the film. If the film was too thin, the TRZ amount adsorbed was small, resulting in the small peak current. When it was too thick, the film conductivity reduced and the film became not so stable as MWCNTs could leave off the electrode surface. Thus it blocks the electrode surface and hence the peak current decreases. At 15 μl of MWCNTs, the peak current was highest. Therefore, 15 μl MWCNTs suspension solution was used in the remaining studies.

**Influence of accumulation potential and time**

It was important to fix the accumulation potential and time when adsorption studies were undertaken. Both conditions could affect the amount of adsorption of TRZ at the electrode. Bearing this in mind, the effect of accumulation potential and time on peak current response was studied by CV. The concentration of TRZ used was 10 μM.

When accumulation potential was varied from +0.4 to -0.4V, the peak current changed a little. Hence, accumulation at open circuit was adopted. The peak current increased very rapidly with increasing accumulation time, which induced rapid adsorption of TRZ on the surface of the modified electrode.
Figure VI (iii)

a) Influence of MWCNTs suspension (0.2 mg ml\(^{-1}\)) volume used on the anodic peak current

![Graph a: Influence of MWCNTs suspension volume on anodic peak current](image)

b) Variation of the anodic peak current with accumulation time

![Graph b: Variation of anodic peak current with accumulation time](image)
The peak current reached the maximum after 180 s and then being unchanged (Fig. VI (iii b) (p.164)). This indicates the saturation accumulation. As too long accumulation time might reduce the stability of MWCNTs film, 180 s was generally chosen as accumulation time.

**Influence of pH**

Within the range of pH 3.0-11.2, the peak potential shifted to less positive values for both the peaks with increasing the pH of the buffer solution (Fig. VI (iv) (p.166)). However, by increasing the pH, the potential of the peak A is shifted to less positive values till pH 7.0, then becomes almost pH independent (Fig. VI (v a) (p.167)). Basically, two linear regions are obtained, one between pH 3.0 and 7.0 with a slope of 53 mV/pH and another between pH 7.0 and 11.2 with a slope of 14 mV/pH. The intersection of the curves is located around pH 7.5, which was close to the pK_a of the piperazine moiety. The first trend corresponds to the conditions where the molecule is oxidized in its mono-protonated form. The intensity was increased to a high value at pH 7.0 (Fig. VI (v b) (p.167)), then the peak intensity decreases continuously. By increasing the pH of the solution, the potential of the peak B changes very little and is almost pH independent. The highest intensity for the peak B was obtained around pH 6.0. Above pH 8.0, the peak B was no longer present. As compared to peak A, peak B was less intense and moreover it can be observed within pH 8.0.
Figure VI (iv)

Influence of pH on the shape of anodic peak. pH: 3.0 (a), 5.0 (b), 6.0 (c), 7.0 (d) and 10.0 (e)
Figure VI (v)

a) Influence of pH on the peak potential of TRZ for peaks A and B

b) Variation of peak currents of peaks A and B with pH
Influence of scan rate

Useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behavior of TRZ at different scan rates from 10 to 300 mV s\(^{-1}\) (Fig. VI (vi) (p.169)) was also studied. There is a good linear relationship between peak current and scan rate. The equations are

\[
Ip (\mu A) = 556.43 \, v \, (Vs^{-1}) + 4.46 \text{ and } \\
Ip (\mu A) = 71.43 \, v \, (Vs^{-1}) - 0.408,
\]

for peaks A and B respectively as shown in Figure VI (vii a) (p.170) \((r \geq 0.9943, S \leq 0.014; r \geq 0.996, S \leq 0.017)\). This indicates that the electrode process was controlled by adsorption rather than diffusion. In addition, there was a linear relation between log Ip and log v, corresponding to the following equation:

\[
\log Ip (\mu A) = 0.968 \log v \, (Vs^{-1}) + 2.75 \text{ and } \\
\log Ip (\mu A) = 0.9827 \log v \, (Vs^{-1}) + 1.933,
\]

for peaks A and B respectively (Fig. VI (vii b) (p.170)) \((r \geq 0.9985, S \leq 0.011; r \geq 0.9962, S \leq 0.015)\). The slope of 0.968 and 0.9827 are close to the theoretically expected value of 1.0 for an adsorption controlled process\(^{28}\).

The peak potential shifted to more positive values with increasing the scan rates. The linear relation between peak potential and logarithm of scan rate can be expressed as

\[
Ep (V) = 0.8588 + 0.0817 \log v \, (Vs^{-1}) \text{ and } \\
Ep (V) = 1.0979 + 0.0729 \log v \, (Vs^{-1}),
\]
Figure VI (vi)

Cyclic voltammograms of 10 μM TRZ on MWCNT-modified GCE with different scan rates. (a) to (f) were 10, 50, 100, 150, 200 and 300 mVs⁻¹, respectively.
Figure VI (vii)

a) Dependence of the oxidation peak current of peaks A and B on the scan rate

![Graph showing the dependence of peak current on scan rate for peaks A and B.]

b) Dependence of the logarithm of peak current on logarithm of scan rate for peaks A and B

![Graph showing the logarithmic relationship between peak current and scan rate for peaks A and B.]

170
Figure VI (viii)

Relationship between peak potential and logarithm of scan rates for the peaks A and B.

![Graph showing the relationship between peak potential and logarithm of scan rates for peaks A and B. The x-axis represents log v (Vs⁻¹) and the y-axis represents Ep (V). Peaks A and B are plotted with different markers and lines.}]
for the peaks A and B respectively (Fig. VI (viii) (p.171)) (r \geq 0.991, S \leq 0.019; r \geq 0.986, S \leq 0.012).

As for an irreversible electrode process, Laviron equation is used as given in Chapter IV (p.107). In this system, for peak A, the slope was 0.0817, taking T = 298 K, R = 8.314 J K^{-1} mol^{-1} and F = 96480 C, αn was calculated to be 0.7238. Generally, for an irreversible process α was assumed to be 0.5^{29}. Further, the number of electron (n) transferred in the electro oxidation of TRZ was calculated to be 1.45. 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 can be obtained from the intercept of Ep versus v curve by extrapolating to the vertical axis at v = 0^{30}. For peak A, the intercept for Ep versus log v plot was 0.8588 and E^0 was obtained to be 0.7207 V, the k^0 was calculated to be 1.39 \times 10^3 s^{-1}. Similarly for the peak B, n was found to be 1.62 and k^0 was 1.57 \times 10^3 s^{-1}.

So, we may assume that oxidation steps of TRZ were located on the piperazine moiety, which represents a typical redox system with two electron oxidation processes in acidic and basic media. The mechanism of oxidation of TRZ for the peak A follows as given below. We may postulate that when the aliphatic nitrogen of the piperazine moiety, distal to the benzene ring of the molecule is protonated, oxidation occurs with the removal of a proton (Scheme 1). Above pH 8.0, oxidation exclusively occurs at the most basic piperazine nitrogen (distal) following the well-established aliphatic tertiary amine oxidation pathway^{31}.
TRZ looses an electron to form a cation radical, which on loosing a proton and an electron in subsequent steps to form a quaternary Schiff base. Thus resulted quaternary Schiff base was rapidly hydrolysed to the secondary amine, 1-(3-Chloro-phenyl)-piperazine and an aldehyde, 3-(3-Oxo-[1,2,4]triazolo[4,3-a]pyridin-2-yl)-propionaldehyde. Such a mechanism of oxidation for peak A is shown in Scheme 1.
The oxidation mechanism for peak B is entirely different, as it was present up to pH 8.0 only. This oxidation step of TRZ occurs at the triazolopyridine moiety of the molecule. The mechanism for peak B is shown in Scheme 2, in which TRZ loses an electron from nitrogen attached to the
aliphatic chain to form a cation radical. This cation radical on rapid hydrolysis gives 2-((3-[4-(3-Chloro-phenyl)-piperazin-1-yl]-propyl)-hydrazono)-2H-pyridine-1-carboxylic acid, which on decarboxylation gives rise to N-{3-[4-(3-Chloro-phenyl)-piperazin-1-yl]-propyl}-N-(1H-pyridin-2-ylidene)-hydrazine. Such proposed mechanisms and all the results obtained are in agreement with the report of Kauffmann et al\textsuperscript{13}.
Calibration curve

In order to develop a voltammetric method for determining the drug, we selected the differential-pulse voltammetric mode, because the peaks are sharper and better defined at lower concentration of TRZ 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 this technique to the quantitative analysis of TRZ. The phosphate buffer solution of pH 7.0 was selected as the supporting electrolyte for the quantification of TRZ as it gave maximum peak current at pH 7.0. The peak at about 0.68V was considered for the analysis. Differential pulse voltammograms obtained with increasing amounts of TRZ showed that the peak current increased linearly with increasing concentration, as shown in Fig. VI (ix) (p.177) ($r \geq 0.9984$, $S \leq 0.018$). Using the optimum conditions described above, linear calibration curves were obtained for TRZ in the range of 0.2 to 10 µM. The linear equation was

$$I_p \, (\mu A) = 1.8714 + 0.4931 \, C \, (\mu M).$$
Differential-pulse voltammograms of MWCNT-modified GCE in TRZ solution at different concentrations: 0.2 (1), 1.0 (2), 2.0 (3), 4.0 (4), 6.0 (5) and 8.0 (6) μM. Inset: Plot of the peak current against the concentration of TRZ
Deviation from linearity was observed for more concentrated solutions, due to the adsorption of TRZ or its oxidation product on the electrode surface. Related statistical data of the calibration curves were obtained from five different calibration curves. The limit of detection (LOD) and quantification (LOQ) were 24 and 81 nM respectively. The LOD and LOQ were calculated using the following equations given in Chapter IV (p.118). The detection limits reported at different electrodes are tabulated in Table VI (i) (p.179). This method was better as compared with other reported electrochemical methods\textsuperscript{13,15,16}.

In order to study the reproducibility of the electrode preparation procedure, a 1.0 μM TRZ solution was measured with the same electrode (renewed every time) for every several hours within day, the R.S.D. of the peak current was 3.26 % (number of measurements = 6). As to the between day reproducibility, it was similar to that of within day if the temperature was kept almost unchanged. Owing to the adsorption of TRZ or its oxidative products on to the electrode surface, the current response of the modified electrode would decrease after successive use. In this case, the electrode should be modified again.

**Tablet analysis**

In order to evaluate the applicability of the proposed method in the pharmaceutical sample analysis, two commercial medicinal samples containing TRZ viz. Depryl (Cipla Co. India) and Tazodac (Zy-Alidac Co. India) were studied. The procedures for the tablet analysis were followed as described in
Table VI (i)

Comparison of detection limits for TRZ at different electrodes

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>Detection limits (nM)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon paste electrode</td>
<td>100</td>
<td>[13]</td>
</tr>
<tr>
<td>Platinum electrode (stationary)</td>
<td>2500</td>
<td>[15]</td>
</tr>
<tr>
<td>Platinum electrode (rotating)</td>
<td>1700</td>
<td>[15]</td>
</tr>
<tr>
<td>Dropping mercury electrode</td>
<td>7397</td>
<td>[16]</td>
</tr>
<tr>
<td>MWCNT-modified GCE</td>
<td>24</td>
<td>Present work</td>
</tr>
</tbody>
</table>
earlier section (p.159). The results are in good agreement with the content marked in the label (Table VI (ii) (p.181)).

The recovery test of TRZ ranging from 0.5 to 5.0 μM was performed using differential-pulse voltammetry. Recovery studies were carried out after the addition of known amounts of the drug to various pre-analyzed formulations of TRZ. The recoveries in different samples were found to lie in the range from 98.7 % to 102.2 %, with R.S.D. of 2.94 %.

Interference

Under the optimum experimental conditions, the effects of potential interferents on the voltammetric response of 1.0 μM TRZ were evaluated. The experimental results (Table VI (iii) (p.182)) show that thousand-fold of glucose, starch, sucrose, dextrose, talk, gum acacia, lactic acid and tartaric acid, and hundred-fold of citric acid and oxalic acid did not interfere with the voltammetric signal of TRZ. However, ten-fold of ascorbic acid had apparent influence on the voltammetric signal of TRZ.

Detection of TRZ in urine samples

The developed differential-pulse voltammetric method for the TRZ determination was applied to urine samples. The recoveries from urine were measured by spiking drug free urine with known amounts of TRZ. The urine samples were diluted 100 times with the phosphate buffer solution before analysis without further pretreatments. A quantitative analysis can be carried out by adding the standard solution of TRZ into the detect system of urine sample. The calibration graph was used for the determination of spiked TRZ in
Table VI (ii)

Comparative studies for TRZ in Depryl and Tazodac tablets by proposed and literature methods and mean recoveries in spiked tablets

<table>
<thead>
<tr>
<th></th>
<th>DPP(^{16})</th>
<th>Depryl(^a)</th>
<th>Tazodac(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labelled claim (mg)</td>
<td>100.0</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Amount found (mg)(^b)</td>
<td>101.0</td>
<td>24.94</td>
<td>24.86</td>
</tr>
<tr>
<td>R.S.D. (%)</td>
<td>-----</td>
<td>0.29</td>
<td>1.09</td>
</tr>
<tr>
<td>Added (mg)</td>
<td>-----</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Found (mg)</td>
<td>-----</td>
<td>9.97</td>
<td>9.65</td>
</tr>
<tr>
<td>Recovered (%)(^c)</td>
<td>99.7</td>
<td>96.5</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 VI (iii)

Influence of potential interferents on the voltammetric response of 1.0 \( \mu \text{M} \) TRZ

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Concentration (mM)</th>
<th>Signal change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1.0</td>
<td>-1.21</td>
</tr>
<tr>
<td>Starch</td>
<td>1.0</td>
<td>-3.13</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.0</td>
<td>-0.89</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.0</td>
<td>-1.32</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1.0</td>
<td>-7.23</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>1.0</td>
<td>+1.61</td>
</tr>
<tr>
<td>Talc</td>
<td>1.0</td>
<td>+0.92</td>
</tr>
<tr>
<td>Gum acacia</td>
<td>1.0</td>
<td>+2.25</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1.0</td>
<td>+180.65</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>1.0</td>
<td>+2.52</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>1.0</td>
<td>-2.65</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>1.0</td>
<td>+10.24</td>
</tr>
</tbody>
</table>
Table VI (iv)

Determination of TRZ in urine samples

<table>
<thead>
<tr>
<th>Urine</th>
<th>Spiked (μM)</th>
<th>Found (μM)</th>
<th>Recovery (%)</th>
<th>S.D. ± R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.3</td>
<td>0.3015</td>
<td>100.50</td>
<td>0.0115 ± 3.81</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.7</td>
<td>0.7009</td>
<td>100.13</td>
<td>0.0087 ± 1.25</td>
</tr>
<tr>
<td>Sample 3</td>
<td>3.0</td>
<td>3.096</td>
<td>103.20</td>
<td>0.0183 ± 0.59</td>
</tr>
<tr>
<td>Sample 4</td>
<td>7.0</td>
<td>6.941</td>
<td>99.15</td>
<td>0.0796 ± 1.15</td>
</tr>
</tbody>
</table>

*Average of five determinations*
urine samples. The detection results of four urine samples obtained are listed in Table VI (iv) (p.183). The recovery determined was in the range from 99.15 % to 103.20 % and the standard deviation and relative standard deviations are listed in Table VI (iv) (p.183).

**Importance of Chapter VI**

In this chapter, a multi-walled carbon nanotube modified glassy carbon electrode has been successfully developed for electrocatalytic oxidation of TRZ in phosphate buffer solution. When the potential was made to move, TRZ produced two anodic peaks at about 0.73 and 1.00 V in pH 7.0 phosphate buffer. MWCNTs showed electrocatalytic action for the oxidation of TRZ, characterizing by the enhancement of the peak current, which was probably due to the larger surface area of MWCNTs. A suitable oxidation mechanism was proposed. The peak at about 0.68 V was suitable for analysis and the peak current was linear to TRZ concentrations over a certain range under the selected conditions. This sensor can be used for voltammetric determination of selected analyte as low as 24 nM with good reproducibility. The modified electrode has been used to determine TRZ in pharmaceutical samples. The proposed method offered the advantages of accuracy and time saving as well as simplicity of reagents and apparatus. In addition, the results obtained in the analysis of TRZ in spiked urine samples demonstrated the applicability of the method for real sample analysis.
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