Voltammetric oxidation and determination of cinnarizine at glassy carbon electrode modified with multi-walled carbon nanotubes

Cinnarizine (CNR), 1-(diphenylmethyl)-4-(3-phenyl-2-propenyl)piperazine is a piperazine derivative with histamine H₁-receptor and calcium channel blocker. It improves the cerebral blood flow. It is widely used orally for the treatment of cerebral apoplexy, cerebral arteriosclerosis and post-traumatic cerebral symptoms. It is also used for the control of nausea and vomiting. Many studies showed that CNR is highly effective against motion sickness and in contrast with other drugs, it should have fewer side effects. It is also used in the treatment of cerebral and peripheral vascular disorders.

CNR was determined spectrophotometrically in pure form and in the binary mixtures. It was also determined by using gas chromatography, high-performance liquid chromatography, fluorescence spectroscopy and ion selective electrode. A chemiluminesence method for flow injection analysis of CNR and a capillary electrophoresis method were also reported. The only one method based on voltammetric reduction of CNR at glassy carbon electrode (GCE) was reported recently. Although spectroscopic and chromatographic methods were widely used for the analysis of various pharmaceutical drugs, most of these methods require separation and/or pretreatment steps. These methods are time consuming, solvent-usage intensive and requires expensive devices and maintenance. Electrochemical detection of
analyte is a very elegant method in analytical chemistry. The interest in developing electrochemical-sensing devices for use in environmental monitoring, clinical assays or process control is growing rapidly. Electrochemical sensors satisfy many of the requirements for such tasks particularly owing to their inherent specificity, rapid response, sensitivity, environment friendly and simplicity of preparation.

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.

To the best of our knowledge, voltammetric determination of CNR 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 CNR based on the unusual
properties of MWCNTs modified electrode. Here we report the electrochemical oxidation of CNR 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 CNR in pharmaceutical and urine samples. The resulted biosensor exhibits high sensitivity, rapid response, good reproducibility and freedom of other potentially interfering species.

**EXPERIMENTAL**

**Reagents**

CNR was purchased from Sigma-Aldrich and used without further purification. A 10 mM stock solution was made in methanol. Multi-walled carbon nanotubes were from Sigma-Aldrich (> 90%, O.D: 10-15 nm, I.D: 2-6 nm, length: 0.1-10 μm). The universal Britton-Robinson (BR) buffers (boric acid, phosphoric acid, acetic acid 0.04 M each and sodium hydroxide, 0.2 M) from pH 2.5–5.5, acetate buffer from pH 3.0-5.0 and phosphate buffer from pH 3.0-5.0 (I = 0.2 M) were prepared in doubly distilled water. Neutral and alkaline media were avoided as CNR undergoes a precipitation in such media. 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 h, then washed with doubly distilled water and dried in vacuum at room temperature. The MWCNTs suspension was prepared by dispersing 2 mg of 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 5 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 2.5-3.0 times greater.

**Analytical procedure**

The MWCNT-modified GCE was first activated in BR buffer (pH 2.5) in the presence of 20% (v/v) methanol by cyclic voltammetric sweeps between
0 and 1.6 V until stable cyclic voltammograms were obtained. Then electrodes were transferred into another cell of BR buffer (pH 2.5) containing proper amount of CNR, keeping the final concentration of methanol constant at 20% (v/v). 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 +0.6 and +1.6, 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 CNR 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 methanol. 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 BR buffer solutions. Each solution was transferred to the voltammetric cell and analyzed by standard addition method. The differential pulse voltammograms were recorded between 0.60 and 1.40 V after open-circuit accumulation for 180 s with stirring. The oxidation peak current of CNR 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 CNR was calculated using standard addition method.

RESULTS AND DISCUSSION

Cyclic voltammetric behavior of CNR

The cyclic voltammograms of CNR at a bare GCE and at MWCNT-modified GCE were shown in Fig. VII (i) (p.194). It can be seen that the CNR oxidation peak 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 peak was at about 1.31V, but on the MWCNT-modified GCE, the peak appeared at about 1.20, with considerable enhancement in the peak current. This was attributed to the electro catalytic effect caused by MWCNTs. The reason for the better performance of the MWCNT-modified GCE may be due to the nanometer dimensions of the MWCNTs, the electronic structure and the topological defects present on the MWCNTs surfaces. Meanwhile the MWCNTs increase the effective area of the electrode. The modified electrode has no electrochemical activity in phosphate buffer solution, but the background current becomes larger, which is attributed to the fact that MWCNTs can increase the surface activity remarkably.

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 CNR showed a
Figure VII (i)
Cyclic voltammograms of 0.10 mM CNR 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: BR buffer with pH 2.5; accumulation time: 180 s (at open circuit); volume of MWCNTs suspension: 5 μl
remarkable decrease during the successive cyclic voltammetric sweeps as shown in Figure VII (ii) (p.196)).

After the successive scan, the peak current decreased greatly and finally remained unchanged. This phenomenon may be attributed to the adsorption of oxidative product of CNR at the modified electrode surface. To know whether the CNR itself adsorbs on the electrode surface or not, a simple experiment was carried out. The MWCNT-modified GCE was placed in the 0.10 mM CNR solution for 180 s with stirring. Afterwards modified electrode was taken out, placed in buffer solution of pH 2.5 and voltammogram was recorded and is shown in Fig. VII (iii a) (p.197). The peak obtained is mainly due to the CNR already adsorbed on the electrode surface. The Fig. VII (iii b) (p.197) shows the cyclic voltammogram of MWCNT-modified GCE in buffer solution of pH 2.5. This experiment clearly indicated the adsorption of CNR on the electrode surface before electrochemical oxidation. Therefore, the voltammograms corresponding to the first anodic cycle and peak was generally recorded.

**Influence of amount of MWCNTs**

Figure VIII (iv a) (p.198) shows that the amount of MWCNTs has influence on the peak current. At 5 µl of MWCNTs, the peak current was highest. After that amount, it decreases. This is related to the thickness of the film. If the film was too thin, the CNR 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
Figure VII (ii)

Successive cyclic voltammograms of 0.10 mM CNR on MWCNT-modified GCE
Figure VII (iii)

(a) Cyclic voltammogram of MWCNT-modified GCE taken out from 0.10 mM CNR solution with 180 s stirring in buffer solution of pH 2.5 and (b) Cyclic voltammogram of MWCNT-modified GCE in buffer solution of pH 2.5 (blank)
Figure VII (iv)

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

![Graph showing the influence of MWCNTs suspension volume on the anodic peak current.]

b) Variation of the anodic peak current with accumulation time

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

b) Variation of the anodic peak current with accumulation time
decreases. Therefore, 5 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 CNR 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 CNR used was 0.10 mM.

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 CNR on the surface of the modified electrode. The peak current reached the maximum after 180s and then being unchanged (Fig. VII (iv b) (p.198)). 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**

The electrode reaction might be affected by the buffer solution and pH of the medium. The effect of different supporting electrolytes on the current response was investigated by CV, by taking acetate, phosphate and BR buffers. The results showed that high peak current was obtained in BR buffer with pH 2.5 (Fig. VII (v a) (p.200)). Within the range of pH 2.5-5.5, dramatically decreased peak current response was found with a peak broadening. The peak
Figure VII (v)

a) Variation of peak currents of CNR with pH

![Graph showing variation of peak currents of CNR with pH]

b) Influence of pH on the peak potential of CNR

![Graph showing influence of pH on the peak potential of CNR]
potential was almost pH independent as shown in Figure VII (v b) (p.200). From pH 6.0, CNR undergoes precipitation; hence the study was restricted to pH 2.5-5.5.

**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 CNR at different scan rates from 10 to 400 mV s\(^{-1}\) was also studied (Fig. VII (vi) (p.202)). There is a good linear relationship between peak current and scan rate. The equation representing this was

\[
I_p (\mu A) = 56.45 \nu (V s^{-1}) + 10.68
\]

as shown in Fig. VII (vii a) (p.203) \((r \geq 0.991, S \leq 0.013)\). This indicates that the electrode process was controlled by adsorption rather than diffusion. In addition, there was a linear relation between \(\log I_p\) and \(\log \nu\) (Fig. VII (vii b) (p.203)) \((r \geq 0.992, S \leq 0.019)\), corresponding to the following equation:

\[
\log I_p (\mu A) = 0.9824 \log \nu (V s^{-1}) + 1.66.
\]

The slope of 0.9824 was very close to the theoretically expected value of 1.0 for an adsorption-controlled process\(^{27}\). 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

\[
E_p (V) = 1.4036 + 0.1227 \log \nu (V s^{-1})
\]

(Fig. VII (viii) (p.204)) \((r \geq 0.991, S \leq 0.011)\). As for an irreversible electrode process, Laviron equation is given as in Chapter IV (p.107). In this system, the slope was 0.1227, taking \(T = 298 K\), \(R = 8.314 J K^{-1} mol^{-1}\) and
Figure VII (vi)

Cyclic voltammograms of 0.10 mM CNR on MWCNT-modified GCE with different scan rates. (a) to (g) were 10, 50, 100, 150, 200, 300 and 400 mVs\(^{-1}\), respectively.
Figure VII (vii)

a) Dependence of the oxidation peak current on the scan rate

\[ \text{log } v \ (\text{Vs}^{-1}) \]

b) Dependence of the logarithm of peak current on logarithm of scan rate

\[ \text{log } I_p \ (\mu\text{A}) \]
Figure VII (viii)

Relationship between peak potential and logarithm of scan rate
$F = 96480 \text{ C}$, $\alpha n$ was calculated to be $0.4819$. Generally for an irreversible process, $\alpha$ was assumed to be $0.5$. Further, the number of electron ($n$) transferred in the electro oxidation of CNR was calculated to be $0.96 \sim 1.0$. 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 $E^0$ can be obtained from the intercept of $E_p$ versus $v$ curve by extrapolating to the vertical axis at $v = 0^{28}$. The intercept for $E_p$ versus $\log v$ plot was $1.4036$ and $E^0$ was obtained to be $1.2033$, the $k^0$ was calculated to be $721.42 \text{ s}^{-1}$.

Taking into account that CNR contains an aliphatic tertiary amine in its molecular structure, it presents a basic center with the availability of non-bonding electron as donor. So, we may assume that the oxidation step of CNR is located on the piperazine ring. CNR loses an electron from nitrogen on the piperazine ring to form a cation radical, which on losing 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-Benzhydryl-piperazine and an aldehyde, 3-Phenyl-propenal. The mechanism is shown in Scheme 1.

![Chemical Structure](attachment:image.png)
Scheme 1
This observation was in accordance with the earlier report on the oxidation of flunarizine, a fluorinated derivative of CNR\textsuperscript{25}.

**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 CNR 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 CNR. The BR buffer solution of pH 2.5 was selected as the supporting electrolyte for the quantification as CNR gave maximum peak current at pH 2.5. The peak at about 1.12 V was considered for the analysis. Differential pulse voltammograms obtained with increasing amounts of CNR showed that the peak current increased linearly with increasing concentration, as shown in Fig. VII (ix) (p.208).

Using the optimum conditions described above (p.192), linear calibration curves were obtained for CNR in the range of 0.09 to 6.0 \( \mu \text{M} \) (\( r \geq 0.9963, S \leq 0.014 \)). The linear equation was

\[
Ip (\mu\text{A}) = 0.1593 + 1.4567 \ C (\mu\text{M}).
\]

Deviation from linearity was observed for more concentrated solutions, due to the adsorption of CNR 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 2.58 nM and 8.59 nM respectively. The LOD and LOQ were...
Figure VII (ix)

Differential-pulse voltammograms of MWCNT-modified GCE in CNR solution at different concentrations (μM): 0.09 (1), 0.3 (2), 0.6 (3), 1.0 (4), 3.0 (5) and 6.0 (6). Inset: Plot of the peak current against the concentration of CNR.
calculated using the equations given in Chapter IV (p.118). This method was better as compared with other reported electrochemical method\textsuperscript{14}.

In order to study the reproducibility of the electrode preparation procedure, a 1.0 \( \mu \text{M} \) CNR solution was measured with the same electrode (renewed every time) for every several hours within a day, the R.S.D. of the peak current was 2.96\% (number of measurements = 8). As to the between day reproducibility, it was similar to that of within a day if the temperature was kept almost unchanged. Owing to the adsorption of oxidative product of CNR 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 CNR viz. Vertigil (Cipla Co. India) and Vergo (Alkem Co. India) were studied. The procedures for the tablet analysis were followed as described in earlier section (p.192). The results are in good agreement with the content marked in the label (Table VII (i) (p.210)).

The recovery test of CNR ranging from 0.10 to 1.0 \( \mu \text{M} \) 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 CNR. The recoveries in different samples were found to lie in the range from 98.47 \% to 103.1 \%, with R.S.D. of 3.74 \%.
Table VII (i)
Comparative studies for CNR in Vertigil and Vergo tablets by proposed and literature methods and mean recoveries in spiked tablets

<table>
<thead>
<tr>
<th></th>
<th>AdSV&lt;sup&gt;a14&lt;/sup&gt;</th>
<th>Vertigil&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Vergo&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labelled claim (mg)</td>
<td>25.0</td>
<td>20.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Amount found (mg)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>24.82</td>
<td>19.57</td>
<td>24.89</td>
</tr>
<tr>
<td>R.S.D. (%)</td>
<td>1.65</td>
<td>2.17</td>
<td>0.82</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>9.89</td>
<td>9.85</td>
</tr>
<tr>
<td>Recovered (%)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-----</td>
<td>98.9</td>
<td>98.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adsorptive stripping voltammetry.

<sup>b</sup>Cipla Co.

<sup>c</sup>Alkem Pentacare Co.

<sup>d</sup>Each value is the mean of five experiments.

<sup>e</sup>Recovery value is the mean of five experiments.
Interference

The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than ± 5% for determination of CNR. Under the optimum experimental conditions, the effects of potential interferents on the voltammetric response of 1.0 μM CNR as a standard were evaluated. The experimental results (Table VII (ii) (p.212)) show that thousand-fold excess concentration of glucose, starch, sucrose, dextrose, talk, gum acacia, magnesium stearate and ascorbic acid did not interfere; however, citric acid, lactic acid and tartaric acid interfered with the voltammetric signal of CNR.

Detection of CNR in urine samples

The developed differential-pulse voltammetric method for the CNR determination was applied to urine samples. The recoveries from urine were measured by spiking drug free urine with known amounts of CNR. 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 CNR into the detect system of urine sample. The calibration graph was used for the determination of spiked CNR in urine samples. The detection results of four urine samples obtained are listed in Table VII (iii) (p.213). The recovery determined was in the range from 99.0 % to 100.4 % and the standard deviation and relative standard deviation are listed in Table VII (iii) (p.213).
### Table VII (ii)

Influence of potential interferents on the voltammetric response of 1.0 μM CNR

<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>-0.71</td>
</tr>
<tr>
<td>Starch</td>
<td>1.0</td>
<td>-2.42</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.0</td>
<td>-0.12</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.0</td>
<td>+4.12</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1.0</td>
<td>-10.23</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>1.0</td>
<td>+1.65</td>
</tr>
<tr>
<td>Talc</td>
<td>1.0</td>
<td>-0.36</td>
</tr>
<tr>
<td>Gum acacia</td>
<td>1.0</td>
<td>-0.16</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1.0</td>
<td>-3.46</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>1.0</td>
<td>+8.25</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>1.0</td>
<td>-9.12</td>
</tr>
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</table>
Table VII (iii)

Determination of CNR in urine samples

<table>
<thead>
<tr>
<th>Urine</th>
<th>Spiked (μM)</th>
<th>Found (μM)a</th>
<th>Recovery (%)</th>
<th>S.D.± R.S.D.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.3</td>
<td>0.297</td>
<td>99.0</td>
<td>0.0042 ± 1.52</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.5</td>
<td>0.502</td>
<td>100.4</td>
<td>0.0034 ± 0.67</td>
</tr>
<tr>
<td>Sample 3</td>
<td>3.0</td>
<td>2.98</td>
<td>99.3</td>
<td>0.0335 ± 1.12</td>
</tr>
<tr>
<td>Sample 4</td>
<td>5.0</td>
<td>4.96</td>
<td>99.2</td>
<td>0.0394 ± 0.79</td>
</tr>
</tbody>
</table>

aAverage of five determinations
Importance of Chapter VII

In this work, a multi-walled carbon nanotubes modified glassy carbon electrode has been successfully developed for electrocatalytic oxidation of CNR in BR buffer solution. MWCNTs showed electrocatalytic action for the oxidation of CNR, characterizing by the enhancement of the peak current, which was probably due to the larger surface area of MWCNTs. A suitable electrochemical oxidation mechanism for CNR was proposed. The peak at about 1.12 V was suitable for analysis and the peak current was linear to CNR concentrations over a certain range under the selected conditions. This sensor can be used for voltammetric determination of selected analyte as low as 2.58 nM with good reproducibility. The modified electrode has been used to determine CNR 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 CNR in spiked urine samples demonstrated the applicability of the method for real sample analysis.
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