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# Electrocatalytic oxidation of sodium levothyroxine with phenyl hydrazine as a mediator at carbon paste electrode: A cyclic voltammetric study

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ABSTRACT

The electrochemical oxidation of sodium levothyroxine ($T_4$) has been studied on carbon paste electrode (CPE) with phenyl hydrazine homogenous as mediator, using cyclic voltammetric technique in presence of 0.1 M HCl as supporting electrolyte. The charge transfer coefficient ($\alpha n_\alpha$) for $T_4$ in the presence and absence of phenyl hydrazine was determined. The oxidation peak currents represented a linear dependence on $T_4$ concentration from 0.025 mM to 0.1 mM with correlation coefficient 0.997. The effect of concentration and scan rate of sodium levothyroxine in presence of trace phenyl hydrazine concentration was studied. The scan rate effect showed the electrode process is adsorption controlled. The practical application of the phenyl hydrazine mediated CPE in the determination of $T_4$ in a commercial tablet sample demonstrated that it has good selectivity and high sensitivity.



## 1. Introduction

Hydrazine's are nitrogen-containing compounds and constitute an important class of xenobiotic agents occurring in natural organisms. Agents with hydrazine functionality can be metabolized to radical intermediates which have toxic effects, such as carcinogenesis and haemolysis [1,2]. Phenyl hydrazine was the first hydrazine derivative characterized, reported by Emil-Fischer in 1875. Phenyl hydrazine is one of the most widely distributed organic pollutants, which may irritate the eyes, the skin and the trachea, and may produce a rapid haemolysis, resulting in kidney impairment and total anemia. It is produced and used in the manufacture of rocket propellant, dyes, pesticides and pharmaceuticals. It is also found in the environment in various waste streams and also in food products like mushrooms and tobacco [3].

Thyroxine ($T_4$) [Scheme 1] is an important biological component produced in the thyroid glands. The practical significances of thyroxine measurements for the diagnosis of hyperthyroidism and hypothyroidism have been known for many years.

The usual methods for the determination of $T_4$ were immunoassays [4–9] and high performance liquid chromatography (HPLC) [10,11]. However these methods have some disadvantages such as expensive instrumentation and time consuming complicated operations. The detection of $T_4$ has also been achieved by electrochemical techniques at silver and mercury electrodes [12–14]. Chemically modified electrodes were also initially used by Orata and Segor and also Hu's group for the determination of thyroxine [15–19].

Literature survey revealed that no attempt has been made to study the voltammetric behavior of $T_4$ with phenyl hydrazine as a mediator at CPE. In the present work a simple and sensitive voltammetric method is presented for the detection of $T_4$ in presence of phenyl hydrazine at CPE. As a method for the detection of $T_4$, the oxidation peak ($O_1$) was studied since it was more stable than the reduction peak and the selectivity of the oxidation peak was much higher. The application of the phenyl hydrazine mediated CPE in the determination of $T_4$ in a commercial tablet sample without any pretreated steps shows that it was a reliable method in the electroanalytical area.

## 2. Experimental

### 2.1. Apparatus and reagents

Electrochemical measurements were carried out with a model-201 electrochemical analyzer (EA-201 Chemilink system) in a conventional three-electrode system. The working electrode was a carbon paste electrode, having cavity of 3 mm diameter. The counter electrode was a bright platinum wire with a saturated calomel electrode (SCE) completing the circuit.

$T_4$ (obtained from Sigma, >99.0%) was dissolved in methanol with 2% of dilute orthophosphoric acid to prepare 0.5 mM standard stock solutions and stored at 4 °C. Phenyl hydrazine (>97%) was prepared by using double distilled water. Other chemicals used

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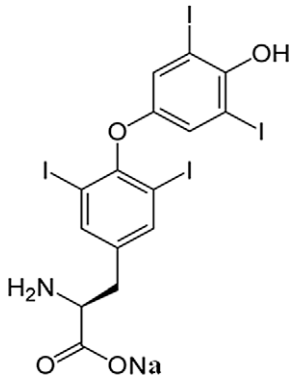



**Scheme 1.** Structure of sodium levothyroxine.

were of analytical grade except for spectroscopically pure graphite powder. All solutions were prepared with double distilled water.

### 2.2. Preparation of carbon paste electrode

The carbon paste electrode was prepared by hand mixing 70% graphite powder and 30% silicon oil in an agate mortar for about 30 min to get homogeneous carbon paste. This carbon paste was then packed into the cavity of a Teflon tube electrode (3 mm in diameter). Before measurement the electrode was smoothened on a piece of transparent paper to get a uniform, smooth and fresh surface.

## 3. Results and discussions

### 3.1. Electrocatalytic oxidation of sodium levothyroxine at carbon paste electrode

Experimental results show that phenyl hydrazine acts as a suitable intermediate for electron transfer in the oxidation of $T_4$ at the surface of carbon paste electrode. Fig. 1a shows the cyclic voltammetric responses of $T_4$ at carbon paste electrode with phenyl hydrazine blank (curve a), in the absence (curve b) and in the presence (curve c) of 0.1 mM phenyl hydrazine in 0.1 M HCl as supporting electrolyte. $T_4$ oxidation peak current ($O_1$) increases sharply in the presence of phenyl hydrazine.

In the absence of phenyl hydrazine, a well-defined oxidation peak appears at 780 mV ($O_1$) in the positive scan when the potential initially sweeps from 0.0 mV to 1000 mV and two indiscernible reduction peaks ($R_1$ and $R_2$) at 520 mV and 330 mV are obtained on the reversal scan (Fig. 1b). However the peak currents of $O_1$ decrease greatly and another oxidation peak ($O_2$) at about 420 mV appears on the second scan. During following successive cyclic scans, the peak current of $O_1$ decrease all the same with the increasing of scan number, resulting from the fact that electrode surface is blocked by the strong adsorption of the reaction products. When the electrode potential was scanned over the range of 500–1000 mV, the $O_1$ signal is unchanged but no peaks were observed in the reverse scan. All results show that electrochemical oxidation of $T_4$ is a totally irreversible process, which can be explained by the strong adsorption of reduction products of $T_4$ at the electrode surface. The peaks $O_2$ and $R_2$ are ascribed to the electrochemical responses of the product of $T_4$ [20]. According to Iwamoto et al.'s report [13] $R_2$ and $O_2$ are attributed to the reduction and the oxidation of the iodine atoms on $T_4$ respectively, and $O_2$ always appears following the $R_2$. As for the oxidation peak $O_1$ is considered it may be caused by the oxidation of the phenolic hydroxyl group on the $T_4$ molecule and the $R_1$ is the reduction response of the products of $T_4$ such as the hydroquinone–benzoquinone redox system produced from the oxidation of phenolic hydroxyl group on $T_4$ [21].

Fig. 1a shows the electrochemical responses of $T_4$ with phenyl hydrazine blank (curve a), in the absence (curve b) and in the presence (curve c) of phenyl hydrazine at CPE. It is clear from Fig. 1a that, the anodic peak current ($O_1$) of $T_4$ in the presence of phenyl hydrazine is much enhanced than at the bare CPE. Also the oxidation peak potential of $T_4$ in the presence of phenyl hydrazine shifts slightly from 780 mV to 800 mV. The anodic peak current difference ($I_{pa}$) in the presence and absence of phenyl hydrazine shows that phenyl hydrazine acts as a suitable intermediate for electron transfer in the oxidation of $T_4$.

The main difficulty in determining the exact mechanism is identification of the intermediate in the oxidation process [22]. The hydrazine's are easier to oxidize so their oxidation behaviors were studied, and the result shows that the oxidation process is based on the hydrazine moiety and not on their derivative groups [23–27].

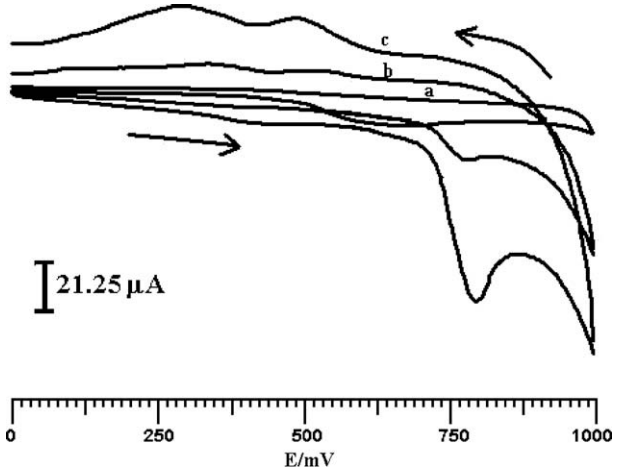



**Fig. 1a.** Cyclic voltammograms of 0.1 mM phenyl hydrazine (curve a) and 0.1 mM sodium levothyroxine in the absence (curve b) and presence (curve c) of 0.1 mM phenyl hydrazine at a carbon paste electrode in 0.1 M HCl with a scan rate of 100 mV/s, in the potential range 0.0 –1000 mV.

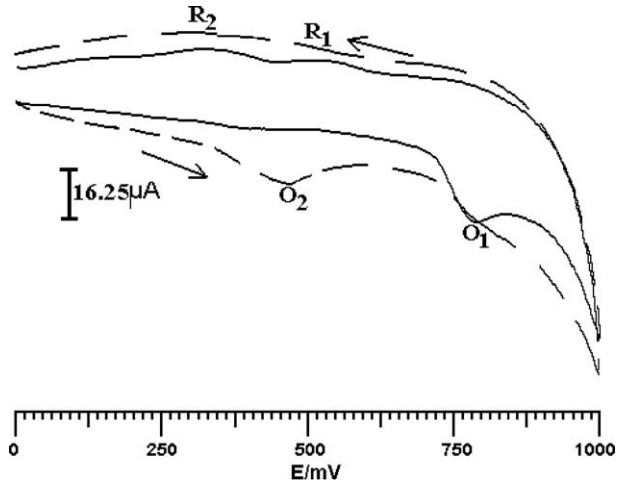



**Fig. 1b.** Appearance of new peak at around 480 mV (dashed line) after the first scan in the electrochemical response of 0.1 mM sodium levothyroxine in 0.1 M HCl; scan rate, 100 mV/s.

The proposed sequence of reactions that occur between phenyl hydrazine and $T_4$ at carbon paste electrode is shown below.

$$\text{Phenylhydrazine}_{(aq)} \rightarrow (\text{Phenylhydrazine})_{ox(aq)}$$

$$(\text{Phenylhydrazine})_{ox(aq)} + \text{Sodium levothyroxine}_{(aq)} \rightarrow \text{Phenylhydrazine}_{(aq)} + (\text{Sodium levothyroxine})_{ox(aq)}$$

The above sequence of reactions between phenyl hydrazine and $T_4$ can be explained as follows. First phenyl hydrazine undergoes oxidation to diazenyl benzene as shown in the step 1 of Scheme 2 and attach to the electrode surface as shown in step 1 of Scheme 3 followed by two electron transfer to the electrode and the oxidized phenyl hydrazine then helps the $T_4$ to undergo oxidation probably either one of the way (2a or 2b) as shown in Scheme 3.

But when the system contains methanol (since the dilution media for sodium levothyroxine is methanol) then there is a chance of formation of methoxy benzene, hydrazine with dinitrogen as the leaving group in small amounts giving the overall reaction as shown in Scheme 2 [28–31].

### 3.2. Effect of phenyl hydrazine concentration

The effect of phenyl hydrazine concentration on the anodic peak current was studied for the range of 0.025–0.2 mM phenyl hydrazine concentration, in the solutions containing 0.1 mM $T_4$ in 0.1 M HCl was shown in Figs. 2a and 2b. The results showed that by increasing phenyl hydrazine concentration up to 0.1 mM the anodic peak current increased, whereas higher concentration of phenyl hydrazine caused a slight decrease in the peak current and almost keeps unchangeable. This may be due to the fact that the adsorption of phenyl hydrazine at the carbon paste electrode surface tends to saturation due to the formation of phenyl hydrazine aggregations. Therefore 0.1 mM was selected as the optimal mediator concentration. The peak potential for the system shifts slightly to a more positive potential.

### 3.3. Effect of sodium levothyroxine concentration

The cyclic voltammogram showed successive enhancement of anodic peak current with increase in concentration of $T_4$. The variation of peak current ($I_{pa}$) with $T_4$ concentration (Fig. 3) in presence of 0.1 mM phenyl hydrazine was linear in the range of 0.025–0.1 mM with a correlation coefficient 0.997. It was also observed that the anodic peak potential ($E_{pa}$) was shifted towards positive potential with increasing concentration showing adsorption of the oxidized product over the electrode surface. The detection limit of $T_4$ in the presence of 0.1 mM phenyl hydrazine was found to be 2.5 μM by cyclic voltammetric method.

### 3.4. Effect of scan rate

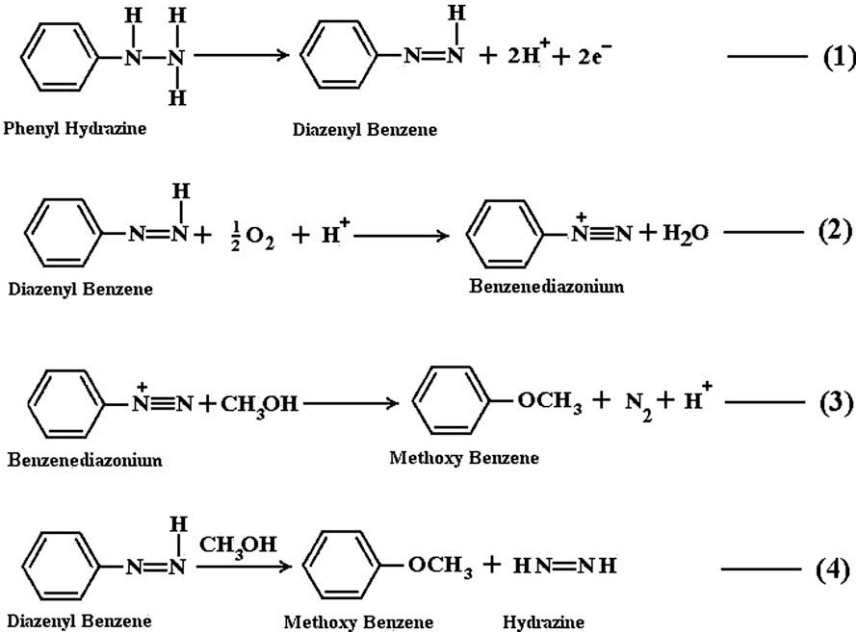
The effect of the potential scan rate on the electrocatalytic properties of phenyl hydrazine in a 0.1 M HCl supporting electrolyte containing 0.1 mM $T_4$ was studied. The obtained results showed that the anodic peak current increased linearly with the increase of scan rate in the range of 100–350 mV/s, it seems that the electrode process is controlled by adsorption. This is consistent with the discussion above, i.e. the decrease of the peak current of $O_1$ with increase of scan numbers.

The dependence of the oxidation peak current ($I_{pa}$) as well as peak current function ($I_{pa}/v^{-1/2}$) and also peak potential on the scan rate ($v$) were studied in the range 100–350 mV/s as shown in Figs. 4a–4c. A linear relationship was observed between log $I_{pa}$ and log $v$ with a correlation coefficient of 0.990 (Fig. 4a). The plot of $I_{pa}/v^{-1/2}$ vs. log $v$ indicated an increase in peak current with an increase in sweep rate (Fig. 4b) confirming that the electrode process at the electrode surface has some adsorption. Also, the plot of peak potential $E_{pa}$ vs. log $v$ (Fig. 4c) was linear with a correlation coefficient of 0.998. Fig. 4c shows the relationship between the oxidation peak potential $E_{pa}$ and the log $v$ and can be expressed by the following equation:

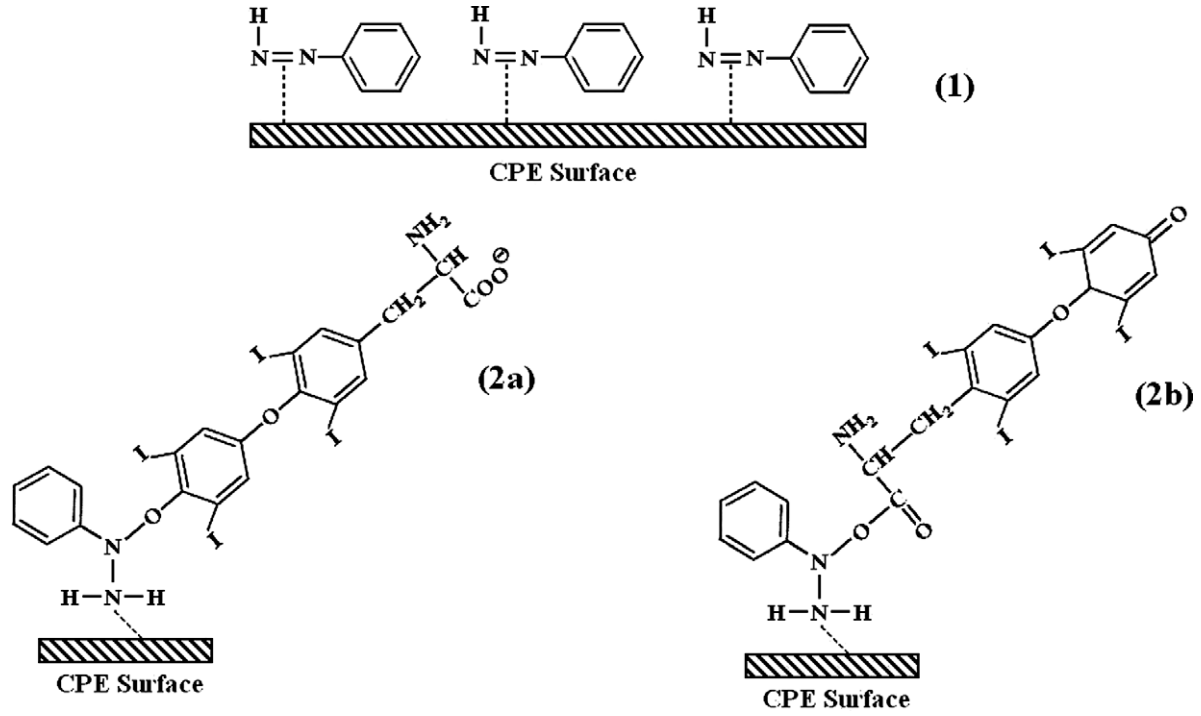
$$E_{pa} = 0.1329 \log v + 0.535 \quad (R = 0.998) \tag{1}$$

It can be noted from Fig. 4c that, along with an increase in the scan rate, the peak potential for the catalytic oxidation of $T_4$ shifts to the more positive potentials, suggesting a kinetic limitation to the reaction between the phenyl hydrazine and $T_4$.

The values of $\alpha n_\alpha$ (where $\alpha n_\alpha$ is the charge transfer coefficient) were calculated for the oxidation peak of $T_4$ in 0.1 M HCl in the presence and absence of phenyl hydrazine mediator at CPE, according to the following equation [32].




**Scheme 2.** Proposed mechanism of phenyl hydrazine.




**Scheme 3.** Probable mechanism of phenyl hydrazine with sodium levothyroxine.

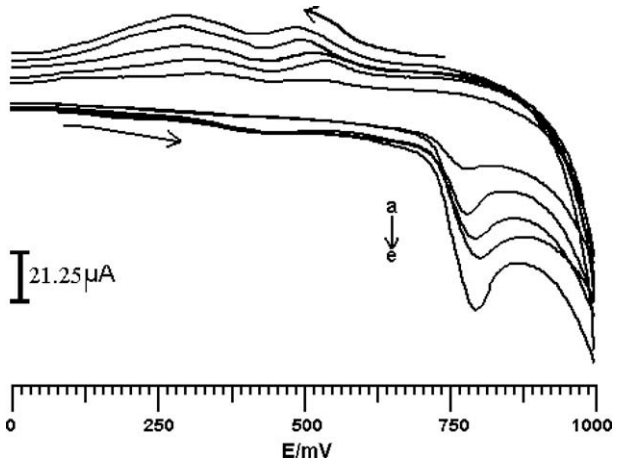



**Fig. 2a.** Cyclic voltammograms for 0.1 mM sodium levothyroxine at different concentrations of phenyl hydrazine (a–e): (a) bare CPE, (b) 0.025,(c) 0.05, (d) 0.075 and (e) 0.1 mM phenyl hydrazine.

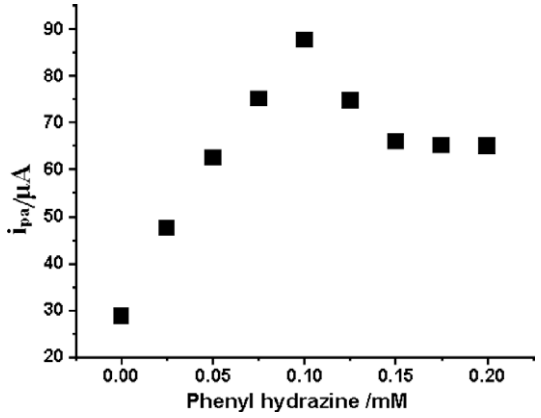



**Fig. 2b.** Plot of different concentrations of phenyl hydrazine vs. anodic peak current in the presence of 0.1 mM sodium levothyroxine.

$$\alpha n_{\alpha} = 0.0477/(E_{pa} - E_{pa}/2) \quad (2)$$

The values for $\alpha n_{\alpha}$ were found to be 0.91 and 0.64 for the oxidation of $T_4$ at CPE in the presence and absence of phenyl hydrazine respectively. These results clearly show that the rate of the electron-transfer process is greatly enhanced in presence of mediator. This phenomenon is thus confirmed by large $I_{pa}$ values recorded at the CPE in presence of phenyl hydrazine.

### 3.5. Analytical application

In order to evaluate the applicability of proposed method, $T_4$ was determined in the commercially available Eltroxine IP tablets (declared content is 100 mcg of $T_4$ in one tablet). The average mass of 10 tablets were weighed accurately and finely powdered and transferred to a 50 ml volumetric flask and dis-

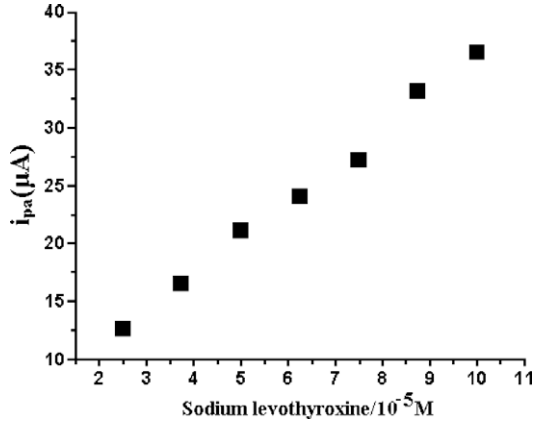



**Fig. 3.** Plot of concentration of sodium levothyroxine vs. anodic peak current in the presence of 0.1 mM phenyl hydrazine.

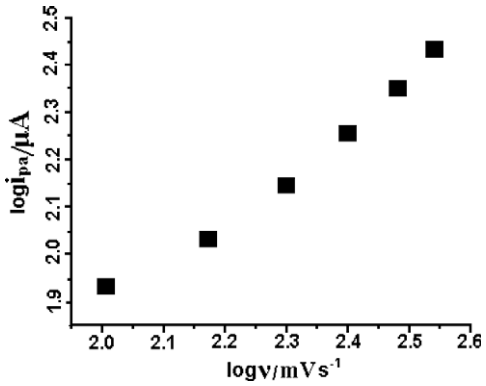



**Fig. 4a.** Dependence of $\log I_{pa}$ on $\log v$ in the presence of 0.1 mM sodium levothyroxine and 0.1 mM phenyl hydrazine.

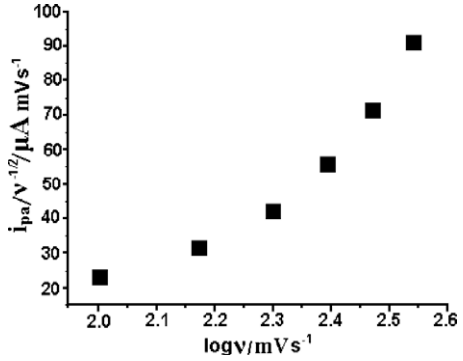



**Fig. 4b.** Dependence of $I_{pa}/v^{-1/2}$ on $\log v$ in the presence of 0.1 mM sodium levothyroxine and 0.1 mM phenyl hydrazine.

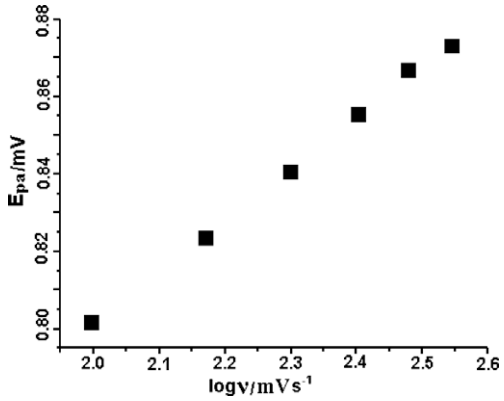



**Fig. 4c.** Dependence of $E_{pa}$ on $\log v$ in the presence of 0.1 mM sodium levothyroxine and 0.1 mM phenyl hydrazine.

solved in methanol. The mixture was sonicated for 30 min and it was then filtered. After that a suitable aliquot of the clear filtrate was quantitatively diluted with 0.1 M HCl solution and the determination of sodium levothyroxine in tablets was carried out by applying a calibration plot. A typical cyclic voltammogram for the determination of $T_4$ in the commercial eltroxine tablets is as shown in the Fig. 5. $T_4$ in commercial Eltroxine IP tablets obtained from cyclic voltammetric determination are presented in Table 1. The results were satisfactory, showing that the proposed method could be efficiently used for the determination of $T_4$ in pharmaceutical preparations.

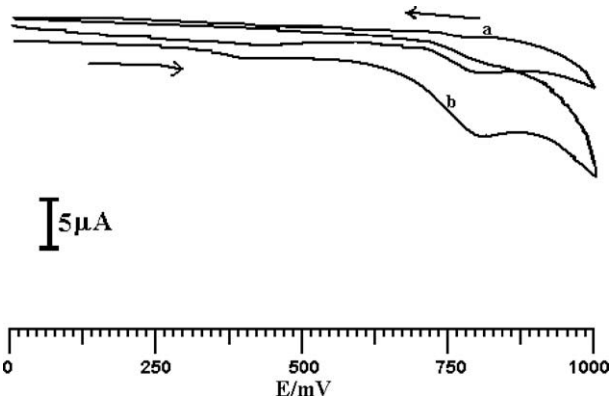



**Fig. 5.** Typical cyclic voltammograms for the determination of sodium levothyroxine in a commercial tablet sample in the absence (curve a) and in the presence (curve b) of 0.1 mM phenyl hydrazine at a carbon paste electrode with a scan rate of 100 mV/s.

**Table 1**
Determination results of sodium levothyroxine in the commercial eltroxine tablets.

| Sample no. | Specified (mcg/tab) | Detected (mcg/tab) | RSD% ($n$ = 3) |
|---|---|---|---|
| 1 | 100 | 98 | 1.94 |
| 2 | 100 | 99 | 1.75 |
| 3 | 100 | 96 | 1.50 |

## 4. Conclusion

This is a new cyclic voltammetric method approach for the determination of $T_4$ using phenyl hydrazine as the mediator. The electrochemical oxidation of $T_4$ at carbon paste electrode showed that the oxidation peak current of $T_4$ was improved in the presence of phenyl hydrazine. Phenyl hydrazine is oxidized at the hydrazine moiety mainly through a two electron process with diazene as intermediate. The electrochemical response is adsorption controlled and irreversible in nature. In the presence of methanol with dilute orthophosphoric acid as the preparation medium for $T_4$, the oxidation peak was more selective for the determination of $T_4$. The oxidation peak current of $T_4$ was linear in range 0.025–0.1 mM, with a detection limit of 2.5 μM. The proposed method has been practically and successfully applied for the determination of $T_4$ in commercial tablets.

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