Square Wave Voltammetric and Computational Study of the Thyroxine-Uracil Interaction

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ABSTRACT
The voltammetric behavior of Thyroxine (T 4) was studied using square wave voltammetry in phosphate buffer solution at (pH 7.0) as supporting electrolyte. Thyroxine gives two well-defined reduction peaks at Ep 1 (-0.359) volt and Ep 2 (-1.01) volt verses (Ag/AgCl/Sat.KCl) as reference electrode. The Gibb’s free energy (∆G), enthalpy (∆H) and entropy (∆S) changes of temperature dependent on (K) were calculated using Van’t Hoff equation for Thyroxine and Uracil binding. The molecular docking between Thyroxine and Uracil has been studied, and the results indicate that the interaction between T4 and Ur was mainly hydrogen bonding and van der Waals interaction.

Keywords: Thyroxine, Uracil, Interaction, Molecular docking.

INTRODUCTION
L-Thyroxine (L-T 4) (+)-3,5,3',5'-tetraiodo-L-thyronine (1) is an important biological compound derived from tyrosine and produced in the thyroid gland (Voet et al., 2002). Also T 4 is the main hormone secreted into the bloodstream by the thyroid gland. It is inactive and most of it is converted to an active form called triiodothyronine (T 3) by organs such as the liver and kidneys. Thyroid hormones play vital roles in regulating the body’s metabolic rate, heart, digestive functions, muscle control, brain development and maintenance of bones, among many other effects not fully studied. The thyroid hormones T 3 and T 4 are unique in that iodine (as iodide) as an essential component of both (Abdul-Fattah et al., 2018).

The usual methods for the determination of T 4 were UV– absorption (Gregorini et al., 2013), Time resolved fluorescence (Wu et al., 1999), Enzyme immunoassays (Tsoncheva, 1988), HPLC (Sawabe et al., 2011), Radioimmunoassay(RIA) (Ping-Jun , 1983), and Chemiluminescence (CL) (Gok and Ates, 2004).
However, these methods have some disadvantages such as expensive instrumentation, time consuming and complicated operations. Cathodic reduction of T₄ on silver electrode was studied by Iwamoto & Co-workers (Iwamoto et al., 1984) in comparison with its multi-step reduction at HMDE. Cathodic stripping square wave voltammetry was applied to determine T₄ in urine, showed that T₄ in Britton Robinson buffer has two reduction peaks in the pH range(2-9) and which involve two steps reduction, at pH 10 has one reduction peak, refer toC-I bonds which is reduced in a single step (Hernandez et al., 1994). Chemically modified carbon paste electrodes are used by Hu’s group in the presence of CTAB (Hu et al., 2004) (Chitravathi et al., 2009) and Chitravathis used phenyl hydrazine as mediator to determine T₄ and the methods are applied for the determination of T₄ in commercial tablets (Chitravathi et al., 2010).

Aboul-Enein and Stefan construct an amperometric biosensor to determine thyroxine based on the immobilization of L-amino acid oxidize (LAAO) on carbon paste electrodes and the two methods were applied to determine thyroxine tablets (Stefan and Aboul-Enein, 2002) (Aboul-Enein et al., 2002). And for the determination of thyroxine by potentiometric sensor and applied method to determine T₄ in levothyroxine tablets and whole blood (Alimadadi et al., 2014) (Moldoveanu et al., 2014).

In the present study, the electrochemical behavior of T₄ and its interaction with Uracil (Ur) were studied as related simple compound to the antithyroid drugs (2). In addition, the binding constant and thermodynamic parameters were evaluated.

![Structure of L-thyroxine](image)

![Antithyroid drugs](image)

**EXPERIMENTAL**

**Reagents and Chemicals:**

A stock solution (10⁻³ M) of L-T₄ was prepared by dissolving T₄(obtained from Alfa company, Germany) in (0.1 M NaOH in 70% ethanol solution); they were kept in darkness at 4°C, 0.2M K₂HPO₄ & 0.2M KH₂PO₄ (obtained from Alfa company, Germany) to prepare 0.1M phosphate buffer solution (PBS) at pH 7.0. The buffer was adjusted to the required pH with the same solutions. Uracil was obtained from BDH laboratory reagent, and all solutions were prepared using deionized water and with no further purification.

**Apparatus:**

All voltammetric measurements were performed using 797- VA Computrace stand (Metrohm AG,CH-9101 Herisav, Switzerland). Reference electrode (RE) was Ag/AgCl/ Sat.KCl and Pt wire was used as auxiliary electrode (AE) and Hanging Mercury Drop Electrode (HMDE) was used as working electrode (WE). pH measurements were performed by using a digital pH meter (HAVANNA) calibrated with standard buffers; for temperature control, a HAAKE G water bath was used.

**Computational study:**

The Molecular Operating Environment MOE v.(2009) software developed by (Chemical Computing Group, Montreal, Canada) was used for the graphical illustrations and molecular interaction study.
Molecular mechanics and quantum chemical calculations were performed to study the geometries, electronic structures. The 3D structures were drawn and used as the starting point for energy minimization. The energy minimizations were performed until the gradient was below (Minimum RMS Gradient 0.0001 Kcal/mol/A^3). Initial geometry optimization of molecule was carried out using molecular mechanics by the force field method (MMFF94x).

RESULT AND DISCUSSION

Electrochemical behavior of L-T4:

Preliminary measurements of T4 using SWV and the three-electrode system with HMDE as working electrode in PBS at pH 7.0 as supporting electrolyte gives two well-defined peaks at (-0.359 and -1.01) V versus Ag/AgCl/Sat.KCl. The Fig. (1) using optimum instrument conditions.

Also the optimum condition has been studied and the results obtained are shown in (Table 1) and all subsequent experiments used these conditions (Abdul-Fattah et al., 2018).

Table 1: The optimum condition values of thyroxine by using SWV technique

| Conditions          | Optimum Condition Values |
|---------------------|--------------------------|
| Deposition Potential (V) | -0.4                     |
| Deposition Time (Sec.)     | 70                       |
| Equilibrium Time (Sec)     | 5.0                      |
| Voltage Step (V)          | 0.010                    |
| Amplitude (V)             | 0.04                     |
| Frequency (Hz)            | 50                       |
| Drop size (mm)            | 7                        |
| pH                           | 7.0                      |

The calibration curve of T4 was constructed using SWV under the optimum conditions (Table 1) and potential between (-1.4 -0.1)V and gives a two straight lines; the first, at (1.996x10^-7 - 19.61x10^-7)M with the R^2 equal to (0.999) and (0.9963) for Ep1 and Ep2 respectively, the second at (0.996x10^-6 - 11.857x10^-6)M range, with the R^2 equal to (0.9819) and (0.9848) for Ep1 and Ep2 respectively (Abdul-Fattah et al., 2018).
Effect of Temperature on T4:

The S.W.Voltammogram of (9.9 x 10^{-6} M) (L-Thyroxine) in phosphate buffer solution at (pH=7), using the optimal conditions, were recorded at different temperatures (288, 293, 298, 303, 308) K. The peak potential Ep and the diffusion current (Ip) for the reduction of L-Thyroxine were measured and the results are shown in (Table 2):

| Temp. (K) | 288 | 293 | 298 | 303 | 308 |
|---------|-----|-----|-----|-----|-----|
| Ep1 (V) | -0.339 | -0.329 | -0.319 | -0.309 | -0.299 |
| Ipº1 (nA) | 167 | 188 | 197 | 209 | 237 |

The result shows that the diffusion current (Ipº) was found here to be increased with increasing temperature. This in fact is due to that the diffusion rate increased with temperature.

Voltammetric study of T4-Ur Interaction:

To study the interaction between Thyroxine and Uracil, a successive amount of Uracil (1 x 10^{-4} M as a stock solution) was added to voltammetric cell containing (9.9 x 10^{-6} M) (L-Thyroxine) in phosphate buffer solution at (pH 7.0) at different temperatures (288, 293, 295, 303, 308) K and the voltammogram was recorded for each addition. The peak current was measured at Ep1 = (-0.365 V) because it was more sensitive than Ep2, which belongs to reduction peak of L-T4; denoted as Ipº Fig (2 A). It is very clear from Fig. (2), the peak current Ip decreased gradually with the sequence additions of Uracil until reaches constant value (saturation).

![SW Voltammogram](image)

**Fig.2:** SW Voltammogram [9.9 x 10^{-6}] molar (L-Thyroxine) in the A) absence of Uracil B) with the successive additional of Uracil

Determination of Binding Constant (K) for (L-Thyroxine – Uracil):

The interaction of (L-Thyroxine) with Uracil can be described using the following equation:

\[ T4 + Ur \rightleftharpoons T4 - Ur \]

An equation for voltammetric determination can be deduced according to (Jalali and Dorraji, 2012) the current diffusion equation was described as follows:

\[ \ln \left( \frac{Ipº}{Ipº - Ip} \right) = \ln \left( \frac{1}{[\text{Conc.(M)}]} \right) - \ln (K) \]  

[1]

Where K is apparent binding constant, Ipº and Ip the peak current of the free (T4) and the complex (T4-Ur), respectively. then the plot of \( \ln \left( \frac{1}{[\text{Conc. Uracil (M)}]} \right) \) versus \( \ln \left( \frac{Ipº}{Ipº - Ip} \right) \) give linear relation with intercept of \( \ln (K) \) Equation (1), the results shown in Fig. (3) and (Table 3):
Fig. 3: (a-e) plot ln (1/Conc. of Uracil (M)) versus ln (Ip/(Ip°−Ip)) of Thyroxine and Uracil interaction at (a=288, b=293, c=295, d=303, e=308) °K
Table 3: The binding constant at different temperatures (288, 293, 298, 303, 308)°K

| Temp. °K | 288 | 293 | 298 | 303 | 308 |
|----------|-----|-----|-----|-----|-----|
| ln K (Ep1) | 12.039 | 11.303 | 10.357 | 9.597 | 8.6496 |
| K x 10^4, molar^-1 | 16.92 | 8.11 | 3.15 | 1.47 | 0.571 |

The result indicates that the value of K was found decreasing with increasing temperature in Ep1.

Calculation of Thermodynamic Parameters:

The plotting of ln K against 1/T using Van’t Hoff equation (equation 2), gives a linear relationship Fig. (4). The enthalpy change (∆H) was obtained from the slope, ∆S from intercept and Gibb’s free energy (∆G) was calculated from Equation (3):

\[
\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad \ldots \ldots \ [2]
\]

\[
\Delta G = -RT \ln K \quad \ldots \ldots \ [3]
\]

![Graph showing the relation between ln K and 1/T](image)

Fig.4: The relation between ln K and 1/T K^-1 for interaction between L-Thyroxine and Uracil

Table 4: The thermodynamic parameters at different temperatures (288, 293, 298, 303, 308)°K

| Temp °K | 1/T | ln K | K x 10^4, molar^-1 | ∆H(KJ/mole) | ∆G(KJ/mole) | ∆S(J/mole.K) |
|----------|-----|-----|-------------------|-------------|-------------|-------------|
| 288      | 0.003472 | 12.039 | 16.92         | -122.997    | -28.83      | -326.491    |
| 293      | 0.003413 | 11.303 | 8.11           | -122.997    | -27.53      |             |
| 298      | 0.003356 | 10.357 | 3.15           | -122.997    | -25.66      |             |
| 303      | 0.003300 | 9.597  | 1.47           | -122.997    | -24.18      |             |
| 308      | 0.0032468 | 8.6496 | 0.571          | -122.997    | -22.33      |             |

From (Table 4), one can see that the negative value of ∆H indicates the exothermicity of the binding interaction while a negative Gibb’s free energy change (∆G) represents a spontaneous occurrence of the interaction and the negative energy change (∆S) shows that the system becomes more order.

From thermodynamics parameters (∆H<0, ∆S<0), it is clear that the van der Waals and hydrogen bonding is the main force in the interaction (Zhao, 2010).

Molecular Docking:

To predict the structure of molecular complex between two or more molecules (Ferreira et al., 2015), the molecular docking technique was performed to get the best orientation and conformation of complex. As shown in Fig. (5a, 5b, 5c).
Fig. 5 : (a,b,c) Molecular Docking between Thyroxine and Uracil
From Fig. (5a, 5b, 5c), we observe that Thyroxine interacts with Uracil by H-bonding and electrostatic forces.

The oxygen of carboxylic group of T4 was very closed with hydrogen of Ur with distance (1.35°A) and oxygen of carbonyl group (C=O) of Ur also was very closed with hydrogen of amine group in T4 with distance (1.64°A) as hydrogen bonding between them (as shown in figure 7a and 7b with white dashed line) and that agrees with thermodynamic result about Ep1 ($\Delta H<0$ and $\Delta S<0$).

On the other hand, the phenolic ring ($\pi$ electron) of T4 was also interacted with two nitrogen's atoms of Urring making a cation–$\pi$ interaction and phenolic ring ($\pi$ electron) of T4 with Ur aromatic ring’s as $\pi-\pi$ stacking (as shown in figure 5c with a yellow dashed line) that suggest an electrostatic forces, as shown in Fig. (5c). The result of molecular docking between T4 and Ur is shown in (Table 5):

**Table 5: Molecular Docking result between T4 and Ur**

| $E_{min}$ Of ligand(Ur) Kcal/mol | T4, Kcal/mol | T4-Lig aft. Docking, Kcal/mol |
|---------------------------------|-------------|-----------------------------|
| -29.1091                        | 89.1110     | 8.7169                      |

**CONCLUSION**

In this paper, the interaction of thyroxine hormone with uracil has been studied by electrochemical method. The experimental results indicate that uracil can interact with thyroxine through hydrogen bond and van der Waals forces. The binding constant (K) between thyroxine and uracil was determined (16.92x10^4 – 0.571x10^4) at temperature range (288-308)°K, thermodynamic parameters also were calculated. The molecular docking also has been studied between thyroxine and uracil.

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