

Square Wave Voltammetric and Computational Study of Thyroxine-Thiourea Interaction

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ABSTRACT

The voltammetric behavior of Thyroxine (T₄) 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 Ep₁ (-0.359) volt and Ep₂ (-1.01) volt versus the reference electrode (Ag/AgCl/Sat.KCl). The calibration curve is linear within a two range of concentration, first is (1.996x10⁻⁷ - 19.61x10⁻⁷)M with the R² equal to (0.999) and (0.9963) for Ep₁ and Ep₂ respectively, and second (0.996x10⁻⁶ - 11.857x10⁻⁶) M with the R² equal to (0.9819) and (0.9848) for Ep₁ and Ep₂ respectively. The Gibb's free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) changes of temperature dependent on (K) were calculated using Van't Hoff equation for Thyroxine and Thiourea binding. The molecular docking between Thyroxine and Thiourea has been studied, and the results indicates that the interaction between T₄ and TU was mainly hydrogen bonding and van der Waal's interaction.

Keywords : Thyroxine, Thiourea, Interaction, Molecular docking.

دراسة فولتامترية الموجة المربعة وحاسوبية للتداخل ما بين الثايروكسين والثايويوريا

الملخص

تمت دراسة الخواص الفولتامترية للثايروكسين باستخدام تقنية فولتامترية الموجة المربعة في محلول الفوسفات المنظم عند الدالة الحامضية 7.0 كالكلتروليت مساعد. وقد اظهر الثايروكسين موجتي اختزال واضحة وكانت الموجة الاولى عند الجهد (-0.359) فولت والموجة الثانية عند الجهد (-1.01) فولت ضد قطب المرجع (Ag/AgCl/Sat.KCl). وكما تمت دراسة المنحني القياسي للثايروكسين وكانت العلاقة خطية ضمن مديين من التركيز (1.996x10⁻⁷ - 19.61x10⁻⁷) مولاري وكانت قيمة معامل الارتباط (0.999) و(0.9963) للموجة الاختزال الاولى والثانية على التوالي، والمديين (0.996x10⁻⁶ - 11.857x10⁻⁶) مولاري وكانت قيمة معامل الارتباط (0.9819) و(0.9848) لموجة الاختزال الاولى والثانية على التوالي. كذلك تمت دراسة الترابط ما بين الثايروكسين والثايويوريا وتم حساب ثابت الترابط عند درجات حرارية مختلفة. طبقت معادلة فانن - هوف لحساب القيم الترموداينمكية (ΔG, ΔS, ΔH). درس الاقتران الجزيئي بين الثايروكسين والثايويوريا وتم اثبات ان الارتباط ما بين الثايروكسين والثايويوريا هو ترابط باواصر هيدروجينية وتداخلات فاندرفالز.

الكلمات الدالة : الثايروكسين، الثايويوريا، التداخلات، الاقتران الجزيئي.

INTRODUCTION

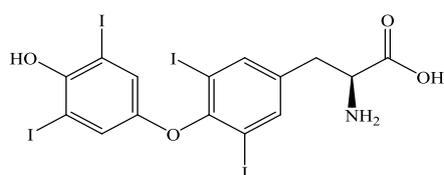
L-Thyroxine (L-T₄) (+)-3,5,3',5'-tetraiodo-L-thyronine (Schem-1) is an important biological compound derived from tyrosine and produced by the thyroid gland (Voet *et al.*, 2002). Also T₄ is the main hormone secreted into the bloodstream by the thyroid gland. It is inactive and most of it is converted to an active form called triiodothyronine (T₃) 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 others effects not fully studied. The thyroid hormones T₃ and T₄ are unique in that iodine (as iodide) is an essential component of both (Murray *et al.*, 2009).

The usual methods for the determination of T₄ were UV- absorption (Gregorini, 2013), Radioimmunoassay (RIA) (Ping-Jun *et al.*, 1983), HPLC (Sawabe *et al.*, 2011), and Chemiluminescence (CL) (Gok and Ates, 2004).

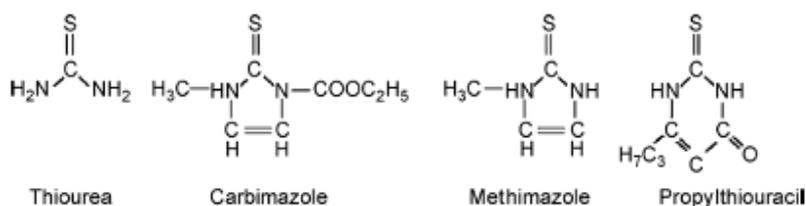
However, these methods have some disadvantages such as expensive instrumentation, and time consuming and complicated operations. Electrochemical techniques have also been used for the detection of T₄. Holak and Shostak (Holak and Shostak, 1979) determined T₄ by differential pulse polarography (DPP), with the use of ion-exchange resins to isolate the amino acids from the matrix, and the application of these methods in pharmaceuticals. Jacobsen and Fonahn (Jacobsen and Fonahn, 1980) carried out the most comprehensive study to date on the electrochemical behavior of T₄ by DPP at a dropping mercury electrode (DME); they proposed a possible mechanism of reaction on the electrode surface. Both reports (Holak and Shostak, 1979) (Jacobsen and Fonahn, 1980) showed that the reduction of T₄ involves the substitution of iodine by hydrogen. With the exchange of eight electrons and eight protons. Jacobsen and Fonahn found T₄ to exhibit adsorptive properties at the surface of the DME on analyzing capillarity curves for solutions containing the amino acid. Also Hamdoon used DPP and differential pulse stripping voltammetry (DPS) methods for the determination of thyroxine (Hamdoon, 1989).

The glassy carbon electrode modified with Multi-Wall Carbon Nano Tubes (MWCNTs) was reported by K. Wu *et al.* and applied the method to determine T₄ in human serum (Wu *et al.*, 2004).

In the present study, the electrochemical behavior of T₄ and its interaction with thiourea (TU) were studied as related simple compound to the antithyroid drugs (Schem-2). In addition, the binding constant and thermodynamic parameters were also calculated.



Schem-1 Structure of L-thyroxine



Schem-2 Thioureylene drugs are related to thiourea (the thiocarbamide group is essential for their antithyroid activities)

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. Thiourea was obtained from BDH laboratory reagent, and all solutions were prepared using deionized water and used without further purification.

Apparatus :

All voltammetric measurements were performed using 797- VA Computrace stand (Metrohm AG, CH-9101 Herisau, 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 version (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 and 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/Å°). 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 give two well-defined peaks at (-0.359 and -1.01) V versus Ag/AgCl/Sat.KCl. As shown in Fig. (1) using optimum instrument conditions.

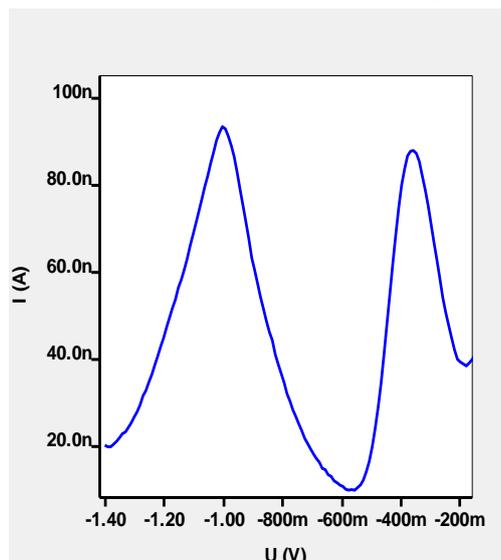


Fig.1: The S.W.Voltammogram of (4.98×10^{-6} M) T₄ in PBS at pH 7.0

Optimum Condition for T₄ using SWV :

A set of S.W. experiments were carried out using a solution containing (4.98×10^{-6}) M T₄ in PBS pH 7.0.

The optimum conditions were obtained by changing the operating condition continuously and the best results obtained either give the highest peak current or the best shape voltammogram, as showed in Fig. (1). The results obtained are shown in (Table 1) and the obtained optimum condition was used in all the subsequent experiments.

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

Effect of L-thyroxine concentration :

The calibration curve of T₄ was constructed using SWV under the optimum conditions (Table 1) and potential between (-1.4 - -0.1)V. The S.W. Voltammograms were recorded for the sequence additions of (10^{-4}) M and (10^{-3}) M respectively as stock solution of T₄ in (10 ml) PBS (pH 7.0). Fig. (2) shows the result of these measurements, and the peak current plotted against the T₄ concentration is shown in Fig. (3).

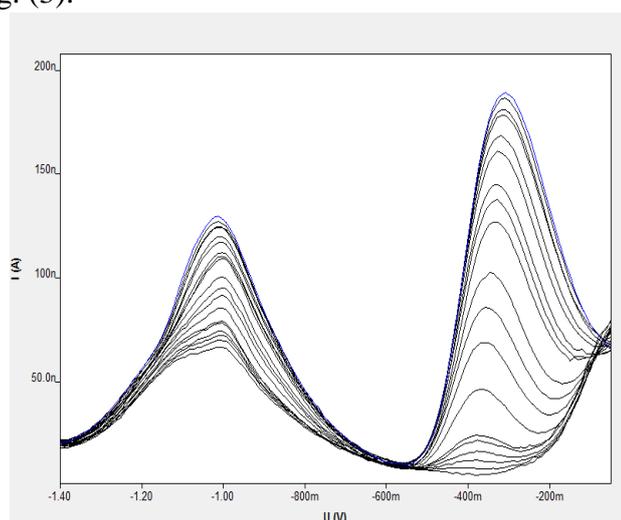


Fig. 2: Variation of Ip with T₄ concentration (Calibration Curve)

A plot of peak current versus concentrations gives a two straight lines, the first at $(1.996 \times 10^{-7} - 19.61 \times 10^{-7})$ M with the R^2 equal to (0.999) and (0.9963) for Ep₁ and Ep₂ respectively, the second at $(0.996 \times 10^{-6} - 11.857 \times 10^{-6})$ M range, with the R^2 equal to (0.9819) and (0.9848) for Ep₁ and Ep₂ respectively.

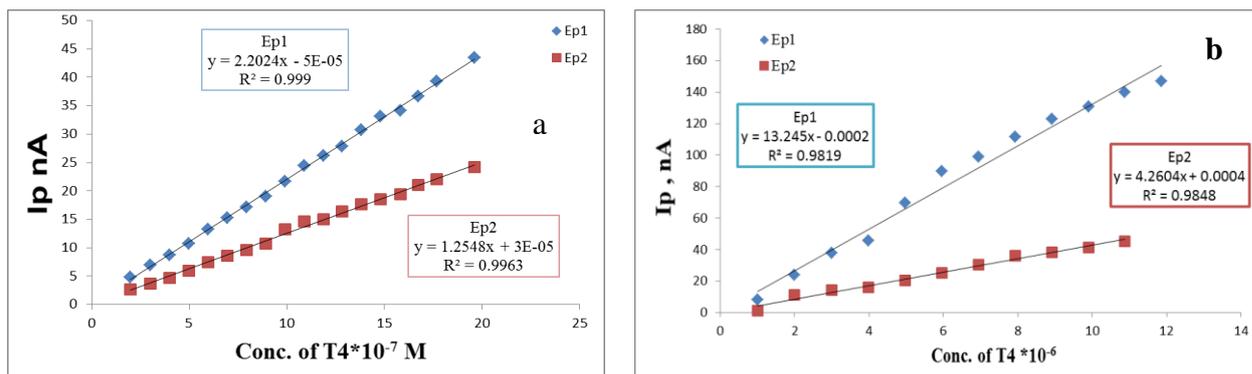


Fig. 3 : Plot of concentration of L-T₄ vs. peak current. (a) at 10⁻⁴M and (b) at 10⁻³M stock solution

Voltammetric study of T₄-TU Interaction:

To study the interaction between Thyroxine and Thiourea, a successive amount of Thiourea (1 × 10⁻⁴ M as a stock solution) was added to voltammetric cell containing (9.9 × 10⁻⁶ M) (L-Thyroxine) in phosphate buffer solution at (pH 7.0) at different temperatures (288, 293, 295, 303) °K and the voltammogram was recorded for each addition. The peak current was measured at Ep₁ = (-0.365 V) because it is more sensitive than Ep₂, which belongs to the reduction peak of L-T₄; denoted as Ip^o Fig (4 A). Gradually. In each addition, the voltammogram was recorded Fig (4 B) for second addition of Thiourea and the peak currents wave was measured at corresponding Ep and denoted as Ip. It is very clear from Fig. (4), the peak current Ip decreased gradually with the sequence addition of Thiourea until it reached constant value (saturation).

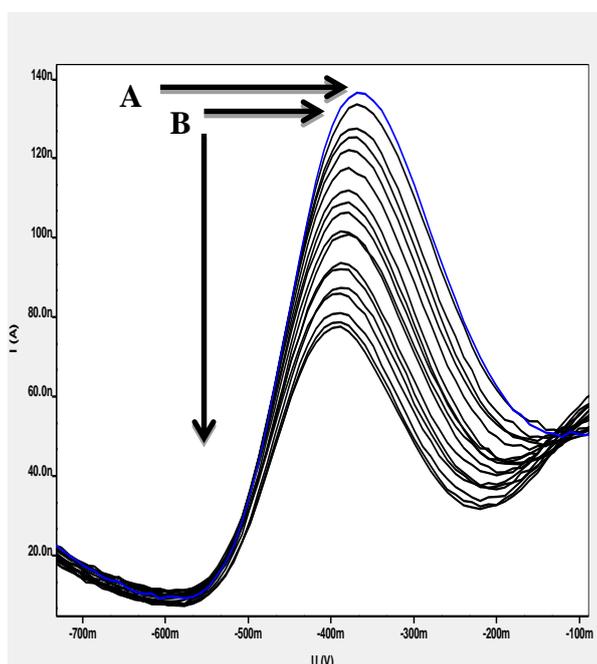


Fig.4 : SW Voltammogram [9.9 × 10⁻⁶] molar (L-Thyroxine) in the A) absence of Thiourea B) with the successive additions of Thiourea

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

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



An equation for voltammetric determination can be deduced according to (Jalali and Dorraji, 2012) the

current diffusion equation was described as follows :-

$$\ln (I_p/(I_p^{\circ} - I_p)) = \ln (1/[Conc.(M)]) - \ln (K) \dots\dots\dots[1]$$

Where K is apparent binding constant, I_p° and I_p , the peak current of the free (T_4) and the complex (T_4 -TU), respectively. Then the plot of $\ln (1/[Conc.Thiourea (M)])$ versus $\ln (I_p/(I_p^{\circ}-I_p))$ gives linear relation with intercept of $-\ln (K)$ Equation (1) .

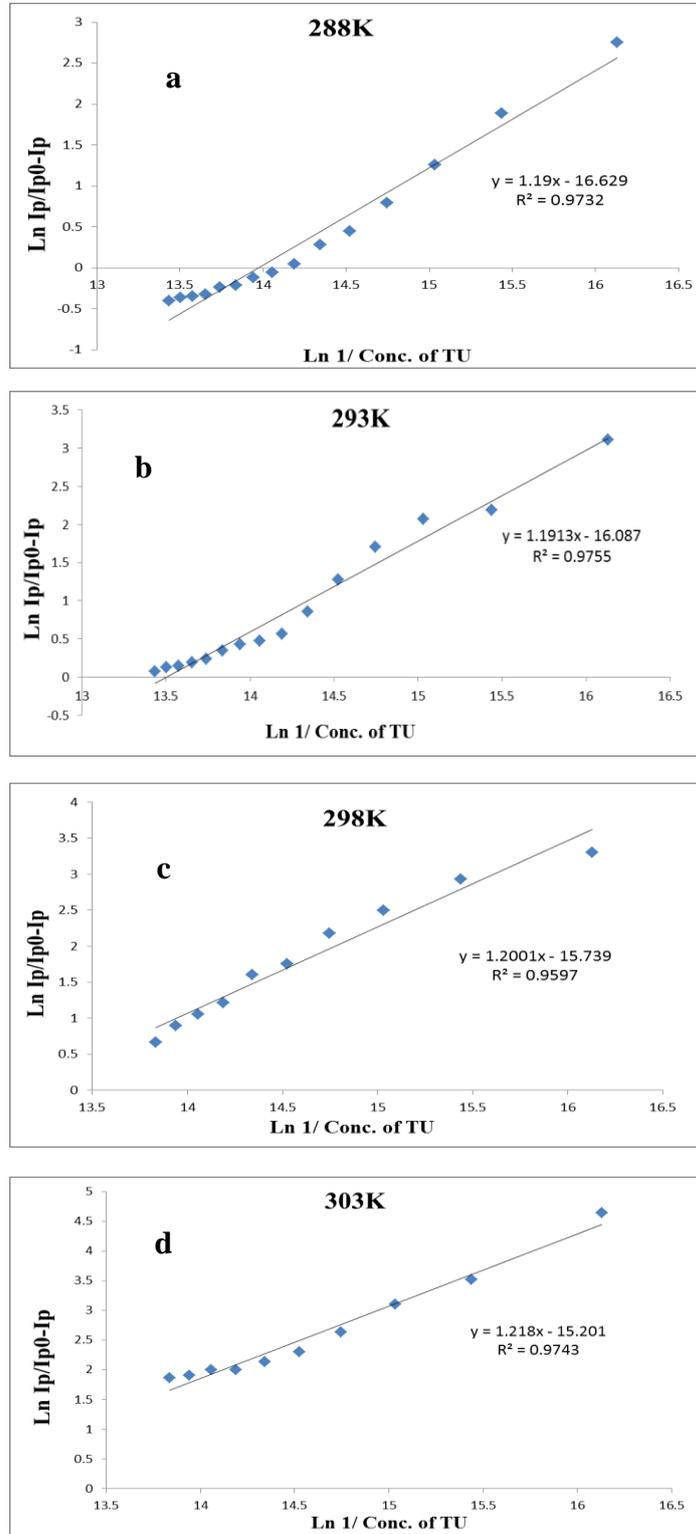


Fig. 5: (a-d) plot $\ln (1/[Conc. of Thiourea (M)])$ versus $\ln (I_p/(I_p^{\circ}-I_p))$ of Thyroxine and Thiourea interaction at (a=288, b=293, c=295, d=303) °K

Table 2: The binding constant at different temperature (288,293,298,303)°K

Temp. °K	288	293	298	303
ln K (Ep ₁)	16.629	16.087	15.739	15.201
K x10 ⁶ , molar ⁻¹	16.67	9.69	6.84	3.40

The result shows that the value of K was decreased with increasing temperature.

Calculation of Thermodynamic Parameters :-

The plotting of ln K against 1/T using Van't Hoff equation (Equation 2) gives a linear relationship Fig. (6). The enthalpy change (ΔH) was obtained from the slope, ΔS from intercept and Gibb's free energy (ΔG) was calculated from (Equation 3):

$$\ln K = -\Delta H / RT + \Delta S / R \quad \dots\dots\dots [2]$$

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

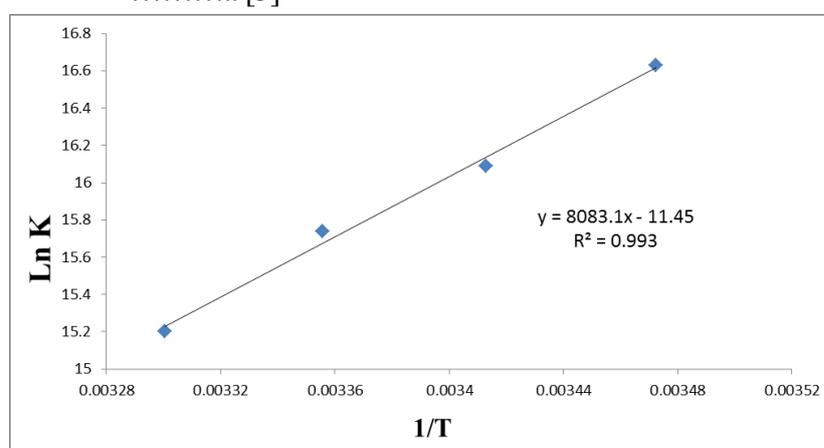


Fig. 6: The relation between ln K and 1/T K⁻¹ for interaction between L-Thyroxine and Thiourea

Table 3: The thermodynamic parameters at different temperatures (288,293,298,303)°K

Temp °K	1/T	ln K	K x10 ⁶ , molar ⁻¹	ΔH (KJ/mole)	ΔG (KJ/mole)	ΔS (J/mole.K)
288	0.003472	16.629	16.67	-67.202	-39.817	-95.1953
293	0.003413	16.087	9.69		-39.187	
298	0.003356	15.739	6.84		-38.994	
303	0.003300	15.201	3.40		-38.293	

From (Table 3), it can be seen that the negative value of ΔG reveals that the interaction process is spontaneous, the negative value of ΔH indicates that the interaction is exothermic and the negative value of ΔS indicates that the interaction is order.

From thermodynamics parameters ($\Delta H < 0$, $\Delta S < 0$), it is clear that the van der Waal's 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 as the best orientation and conformation of complex, as shown in Fig. (7).

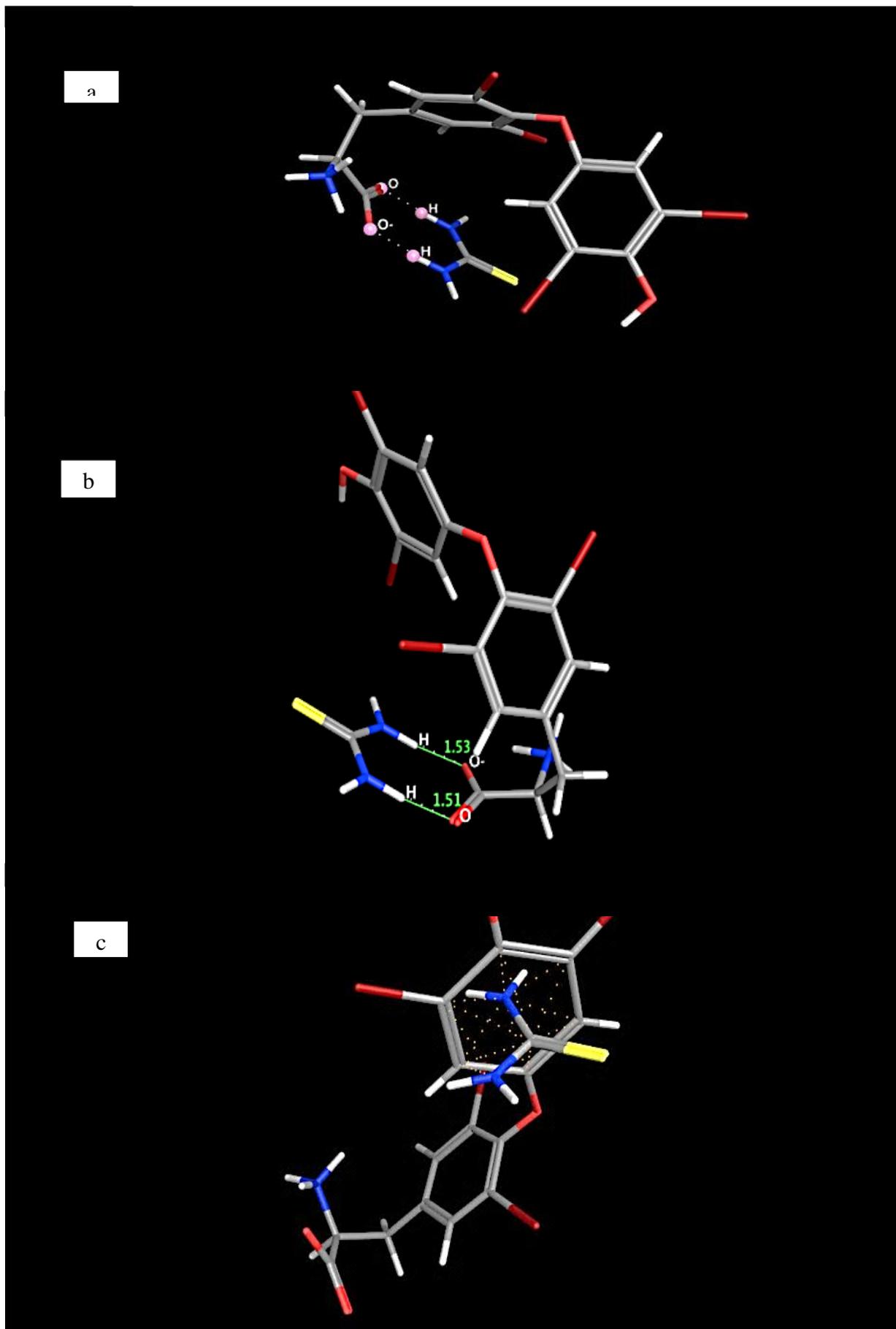


Fig. 7: Molecular Docking between Thyroxine and Thiourea

Fig.(7a,7b, 7c) shows that Thyroxine interacts with Thiourea by H-bonding and electrostatic forces.

It can be seen from Fig. (7a, 7b) that the oxygen of carboxylic group of T₄ was very closed with hydrogen of TU with distance 1.51 and 1.53 Å as hydrogen bonding between them (as shown in Fig.(7a) and 7b with white dashed line) and which was accepted with thermodynamic result about E_{p1} ($\Delta H < 0$ and $\Delta S < 0$).

On the other hand, the phenolic ring (π electrons) of thyroxine, also interacted with nitrogen's of TU ring, makes a cation- π interaction with the phenolic ring between the Nitrogen and the ring π electrons (as shown in Fig.(7c) with a yellow dashed line) suggest electrostatic forces. The result of molecular docking between T₄ and TU shown in (Table 4).

Table 4: Molecular Docking result between T₄ and TU

E _{min} Of ligand(TU),Kcal/mol	E _{min} T ₄ , Kcal/mole	T ₄ -Lig aft. Docking,Kcal/mole
-29.4707	89.1110	14.3433

CONCLUSION

In this paper, the interaction of thyroxine hormone with thiourea has been studied by electrochemical method. The experimental results indicates that thiourea can interact with thyroxine through hydrogen bond and van der Waals force. The binding constant (K) between thyroxine and thiourea was determined to be ($16.67 \times 10^6 - 3.40 \times 10^6$) at temperature range (288-303)°K, thermodynamic parameters also were calculated. The molecular docking also has been studied between thyroxine and thiourea.

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