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

***Marwa N. Abdul-Fattah   Saddalah T. Sulaiman   Haitham A. AL-Wahab**
Department of Chemistry/ Collage of Science /University of Mosul
E-mail:marwa_nizar@yahoo.com *

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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 (ΔG), enthalpy (ΔH) and entropy (ΔS) 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 T_4 and Ur was mainly hydrogen bonding and van der Waals interaction.

Keywords: Thyroxine, Uracil, Interaction, Molecular docking.

دراسة فولتامترية الموجة المربعة وحاسوبية للتداخل ما بين الثايروكسين واليوراسيل

الملخص

تمت دراسة الخواص الفولتامترية للثايروكسين باستخدام تقنية فولتامترية الموجة المربعة في محلول الفوسفات المـنظم عند الدالة الحامضية 7.0 كالكتروليت مساعد. وقد اظهر الثايروكسين موجتي اختزال واضحة وكانت الموجة الاولـى عنـد الجهد (-0.359) فولت والموجة الثانية عند الجهد (-1.01) فولت ضد قطب المرجع (Ag/AgCl/Sat.KCl). كـذلك تمـت دراسة الترابط ما بين الثايروكسين واليوراسيل وتم حساب ثابت الترابط عند درجات حرارية مختلفة. طبقت معادلـة فانـت – هوف لحساب القيم الثرموداينمكية(ΔG,ΔS,ΔH). درس الاقتران الجزيئي بين الثايروكسين واليوراسيل وتم اثبات ان الارتباط ما بين الثايروكسين واليوراسيل هو ترابط باواصر هيدروجينية وقوة فاندرفالز.

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

INTRODUCTION

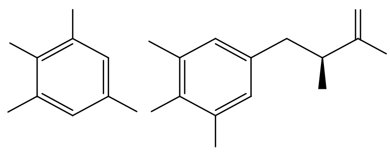
L-Thyroxine (L-T_4) (+)-3,5,3`,5`-tetraiodo-L-thyronine (1) is an important biological compound derived from tyrosine and produced in the thyroid gland (Voet *et al*., 2002). Also T_4 is the main hormone secreted into the bloodstream by the thyroid gland. It is inactive and most of it is converted to 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).

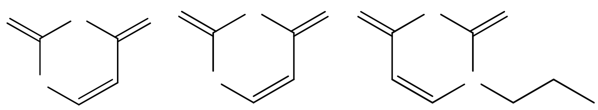
However, these methods have some disadvantages such as expensive instrumentation, time consuming and complicated operations. Cathodic reduction of T_4 on silver electrode was studied by Iwamoto & Co-workers (Iwamoto *et al.*, 1984) in comparison with its multi-step reduction at HMDE. Cathodic stripping square wave voltammetry was applied to determine T_4 in urine, showed that T_4 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 Chitravathiis used phenyl hydrazine as mediator to determine T_4 and the methods are applied for the determination of T_4 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 T_4 in levothyroxine tablets and whole blood (Alimadadi *et al.*, 2014) (Moldoveanu *et al.*, 2014).

In the present study, the electrochemical behavior of T_4 anditsinteractionwith Uracil (Ur) were studied as related simple compound to the antithyroid drugs (2). In addition, the binding constant and thermodynamic parameters were evaluated.



(1) Structure of L-thyroxine



(2) antithyroid drugs)

EXPERIMENTAL

Reagents and Chemicals :

A stock solution (10^{-3} M) of L-T_4 was prepared by dissolving T_4(obtained from Alfa company, Germany) in (0.1 M NaOH in 70% ethanol solution); they were kept in darkness at 4°C , 0.2M K_2HPO_4& 0.2M KH_2PO_4 (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:

HO I

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.

I O

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/Aº). Initial geometry optimization of molecule was carried out using molecular mechanics by the force field method (MMFF94x).

RESULT AND DISCUSSION

Electrochemical behavior of L-T_4 :

Preliminary measurements of T_4 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) Vversus Ag/AgCl/Sat.KCl. The Fig. (1) using optimum instrument conditions.

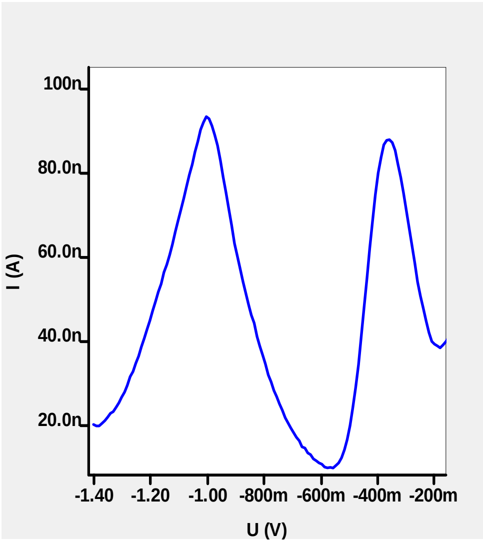


Fig.1: The S.W.Voltammogram of (4.98×10^{-6} M) T_4 in PBS at pH 7.0

Also the optimum condition has been studied and the results obtained are shown in (Table 1) and all subsequent experiments usedthese 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 T_4 was constructed using SWV under the optimum conditions (Table 1) and potential between (-1.4_-0.1)Vand gives a two straight lines; the first, at (1.996×10^{-7} - 19.61×10^{-7})M with the R^2 equal to (0.999) and (0.9963) for Ep_1 and Ep_2 respectively, the second at (0.996×10^{-6} - 11.857×10^{-6})M range, with the R^2 equal to (0.9819) and (0.9848) for Ep_1 and Ep_2 respectively (Abdul-Fattah *et al*., 2018).

Effect of Temperature on T_4 :

The S.W.Voltammogram of (9.9×10^{-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) :

Table 2: The value of Ip of T_4 at different temperature

Temp. (K)	288	293	298	303	308
Ep_1 (V)	-0.339	-0.329	-0.319	-0.309	-0.299
Ip^o_1 (nA)	167	188	197	209	237

The result shows that the diffusion current (Ip^o) 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 T_4-Ur Interaction:

To study the interaction betweenThyroxine andUracil, a successive amount of Uracil (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 (288,293,295,303,308) ºK and the voltammogram was recorded for each addition. The peak current was measured at Ep_1 = (-0.365 V) because it was more sensitive than Ep_2 , which belongs to reduction peak of L-T_4 ; denoted as Ip^o 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).

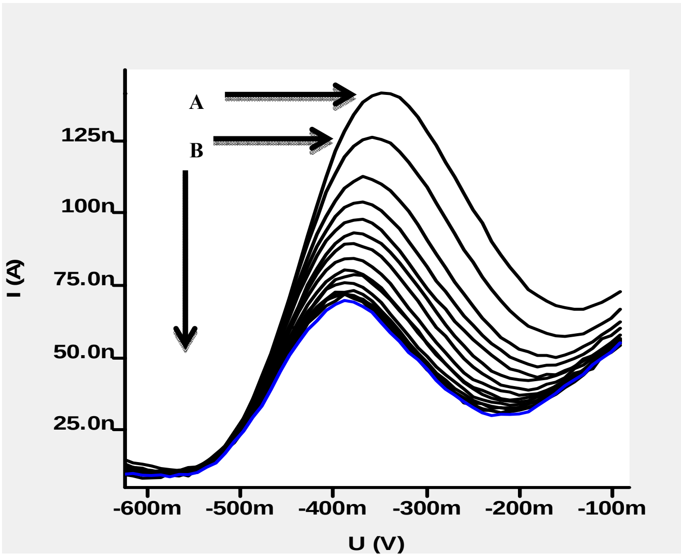


Fig.2: SW Voltammogram [9.9 x10^{-6}] molar (L-Thyroxine) in the A)absence of UracilB) with the successive additionalof Uracil

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

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

$$T4 + Ur \rightleftharpoons T4 - Ur$$

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

$\ln (Ip/(Ip^o - Ip)) = \ln (1/[Conc.(M)]) - \ln (K)$ ……….. [1]

Where K is apparent binding constant, Ip^o and Ip the peak current of the free (T_4) and the complex (T_4-Ur), respectively. then the plot of ln (1/[Conc.Uracil (M)]) versus ln ($Ip/(Ip^o–Ip)$) give linear relation with intercept of ln (K) Equation (1), the results shown in Fig. (3) and (Table 3):

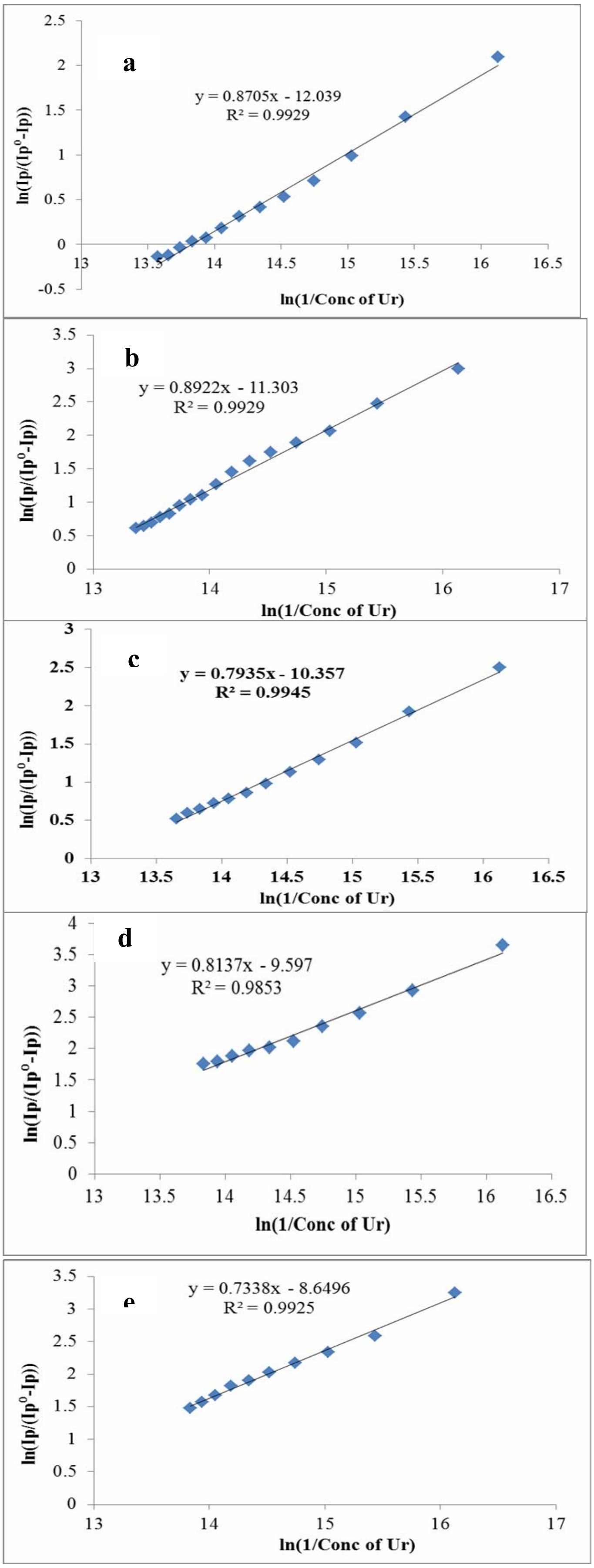


Fig. 3: (a-e) plot ln (1/[Conc. of Uracil (M)]) versus ln (Ip/(Ip°–Ip)) of Thyroxine and Uracilinteraction at (a=288, b=293, c=295, d=303, e=308) °K

Table 3: The binding constant at different temperatures (288,293,298,303,308)°K

Temp. °K	288	293	298	303	308
ln K (Ep_1)	12.039	11.303	10.357	9.597	8.6496
$Kx10^4$, 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 Ep_1.

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 (ΔH) was obtained from the slope, ΔS from intercept and Gibb`s free energy (ΔG) was calculated from Equation (3) :

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

$\Delta G = -RT \ln K$ ……….. [3]

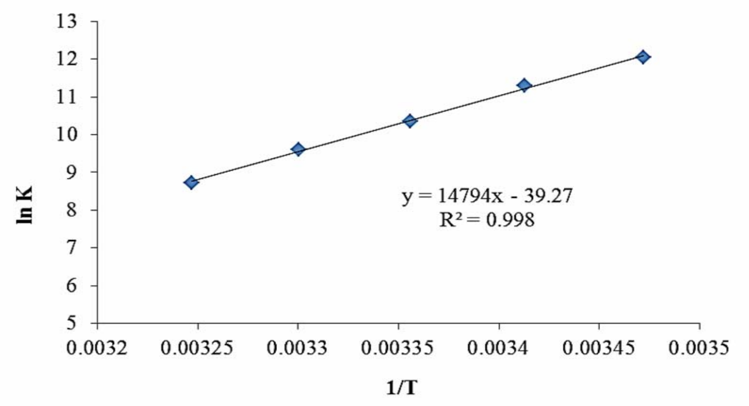


Fig.4: The relation between ln K and 1/T K^{-1} for interaction between L-Thyroxine and Uracil

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

Temp °K	1/T	ln K	K $x10^4$ molar $^{-1}$	ΔH(KJ/mole)	ΔG(KJ/mole)	ΔS(J/mole.K)
288	0.003472	12.039	16.92	-122.997	-28.83	-326.491
293	0.003413	11.303	8.11		-27.53	
298	0.003356	10.357	3.15		-25.66	
303	0.003300	9.597	1.47		-24.18	
308	0.0032468	8.6496	0.571		-22.33	

From (Table 4), one can see that the negative value of ΔH indicates the exothermicity of the binding interaction while a negative Gibb`s free energy change (ΔG) represents a spontaneous occurrence of the interaction and the negative energy change (ΔS) shows that the system becomes more order.

From thermodynamics parameters (ΔH<0, ΔS<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).

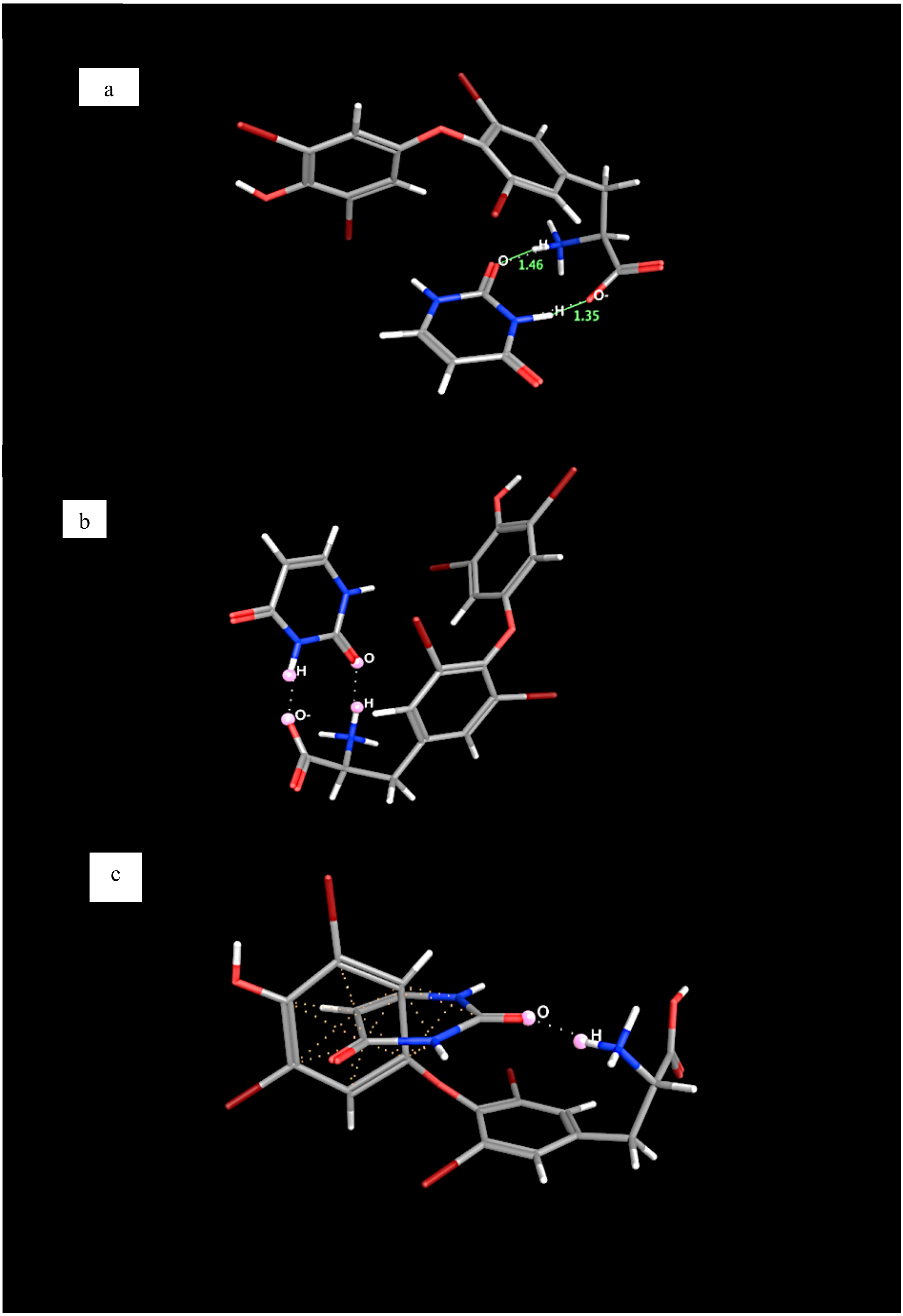


Fig. 5 : (a,b,c) Molecular Docking between Thyroxine and Uracil

From Fig. (5a,5b, 5c), we observe that Thyroxine interacts with Uracil by H-bonding and electrostatic forces.

The oxygen of carboxylic group of T_4 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 T_4 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 Ep_1 ($\Delta H<0$ and $\Delta S<0$).

On the other hand, the phenolic ring (π electrone) of T_4 was also interacted with two nitrogen's atoms of Urring making a cation–pi interaction and phenolic ring (π electrone) of T_4 with Ur aromatic ring`s as π- π stacking (as shown in figure5c with a yellow dashed line) that suggestsan 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 T_4 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)ºK, thermodynamic parameters also were calculated. The molecular docking also has been studied between thyroxine and uracil.

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