

Interactions Study of Co-enzyme-Q₀ with Aniline and Pyrrole Using Square Wave Voltammetric Technique

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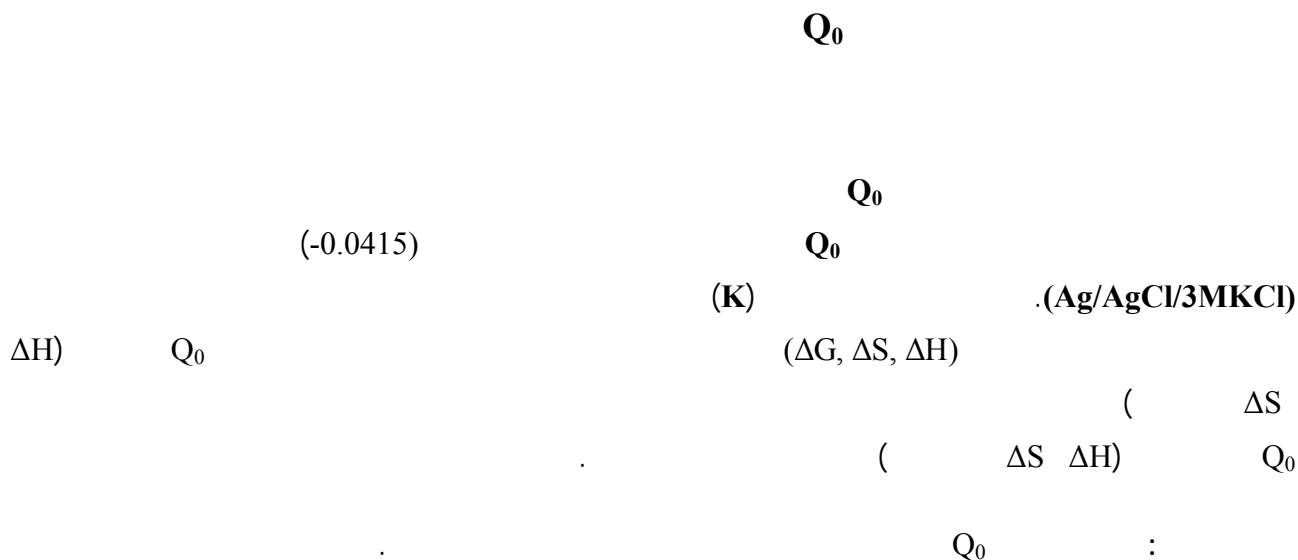
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(Received 20/9/2018 ; Accepted 25/10/2018)

ABSTRACT

The interactions of Co-enzyme Q₀ with Aniline and Pyrrole were studied in aqueous phosphate buffer solution at (pH=7.0) as supporting electrolyte using square wave voltammetry, Co-enzyme Q₀ gives a well-defined square wave voltammetric peak at (-0.0415) volt against the reference electrode (Ag/AgCl/3M KCl). The binding constants (K) were calculated at different temperatures. Vant's hoff equation is applied to calculate the thermodynamic parametrs (ΔH enthalpy changes, ΔS entropy changes and ΔG free energy changes), and then the type of interaction was estimated. The results indicated that the interaction between Co-enzyme -Q₀ and Aniline (ΔH and ΔS negative values) was probably due to Vander Waals forces or hydrogen bonding interaction, and the second interaction between Co-enzyme-Q₀ with Pyrrole (ΔH and ΔS positive values) might be due to the hydrophobic interaction.

Keywords: Co-enzyme-Q₀, Aniline, Pyrrole, Square Wave Voltammetry, Interaction.



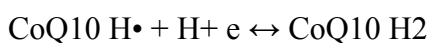
INTRODUCTION

The chemical structure of coenzyme Q₀ (CoQ₀) is C₁₀H₁₂O₄, 2,3-dimethoxy-5-methyl -1,4-benzoquinone, (Al-Nuri *et al.*,2011). Fig. (1) Coenzyme Q (CoQ) also known as Ubiquinone (UQ), is a redox-active lipophilic molecule that is found in all cells and in the membranes of many

organelles where it participates in a variety of cellular processes (Turunen *et al.*, 2004). In the inner membrane of mitochondria, (UQ) diffuses freely and is necessary for the function of the electron transport chain (ETC), as it enables the transfer of electrons from mitochondrial complexes I and II to mitochondrial complex III. Thus, (UQ) is necessary for appropriate mitochondrial adenosine triphosphate (ATP) generation. UQ is also believed to be critically involved in mitochondrial reactive oxygen species (ROS) generation, like semi ubiquinone generated during electrons transport which can react with oxygen molecule to form the superoxide anion. In its reduced form, UQ has been suggested to be an effective antioxidant, protecting cellular membranes from lipid peroxidation. UQ has an aromatic head group and a polyisoprenoid side chain varying in length between species. The side chain comprises 10 subunits in humans (UQ10) and 9 in mice (UQ9), where UQ10 is also present as a minor species (Wang *et al.*, 2015).

Different methods such as spectrophotometric methods (Kathim *et al.*, 2008), derivative spectrophotometry (Al-Nuri *et al.*, 2011), High Performance Liquid Chromatography (Rousseau and Varin, 1998; Tang *et al.*, 2001) and voltammetry (AL-Wahab *et al.*, 2006; Al-Taei *et al.*, 2006; Li *et al.*, 2011; Petrova *et al.*, 2014; Alasaddy, 2015; Gulaboski *et al.*, 2016; Li *et al.*, 2016) had been reported for studies and determination of ubiquinone homologs.

The electrochemical properties of CoQ10 have attracted attention because the CoQ10 participates in a variety of antioxidant reaction. The overall redox process of CoQ10 can be regarded as consisting of a series of consecutive electron-transfers and chemical steps (Michalkiewicz, 2011). It is noteworthy that the pH values have significant effect on the mechanism of the cathodic reduction of CoQ10 (Schrebler *et al.*, 1990; Li *et al.*, 2016)



Moreover, the present redox process that occurs in two one electron, one-proton stages is irreversible. However, the detailed electrochemical mechanism of CoQ10 has remained elusive due to the different electrodes and complicated medium. It is known to us that the metal electrodes can be used to explore the mechanism of biological redox cycling of bioactive molecules (Slawomir, 2007).

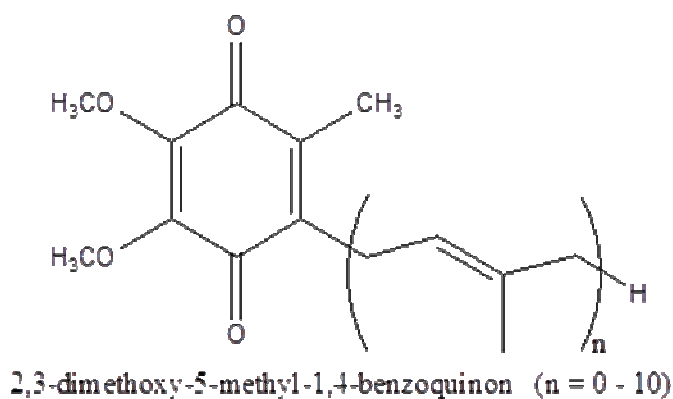


Fig. 1: Structure of coenzyme Q₀, (n = 0)

EXPERIMENTAL

Apparatus

All experiments were performed using 797- VA Computerize stand (Metrohm AG, CH-9101 Herisav, Switzerland). Reference electrode (RE) was Ag/AgCl, with 3M KCl and Pt wire was used as an 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. HAAKE G water bath was used for controlling temperature during experiments.

Reagents Chemicals

Pure Coenzyme Q₀ Solution (10⁻³ M):

A stock solution of co-enzyme- Q₀ was prepared by dissolving 0.0018 gm of pure Coenzyme - Q₀ (supplied by Fluka) in 10 ml absolute ethanol.

Aniline and Pyrrole:

Aniline and Pyrrole monomers were distilled twice under vacuum pressure and stored in the dark bottle before use.

Phosphate buffer solution (P.B.S.):

Phosphate buffer was prepared by mixing certain amounts of 0.2 M of each of Na₂HPO₄ and NaH₂PO₄ solutions.

Procedure:

Voltammetric technique measurements were used to study the interaction of co-enzyme- Q₀ with aniline and to calculate the binding constant (K), the sample cell contained (10ml) of phosphate buffer at (pH=7.0) with a final concentration (9.9x10⁻⁷) M of co-enzyme -Q₀. The square wave voltammogram was recorded for co-enzyme-Q₀ under the measured optimum conditions (Table 1).The appropriate amount of (1.1x10⁻⁴M) of aniline was added to the cell and the square wave voltammogram was recorded at different temperatures in the range (289-308)⁰K in order to calculate the thermodynamic parameters ΔH, ΔS and ΔG. The same procedure was used to calculate the binding constant (K) and thermodynamic parameters of the co-enzyme-Q₀ with (1.44x10⁻⁴ M) of pyrrole.

RESULTS AND DISCUSSION

Typical square wave voltammogram of (9.9x10⁻⁷) M Co-enzyme-Q₀ was recorded in phosphate buffer (pH=7.0) under the previous measured optimum conditions (Table 1) and as shown in Fig. (2).

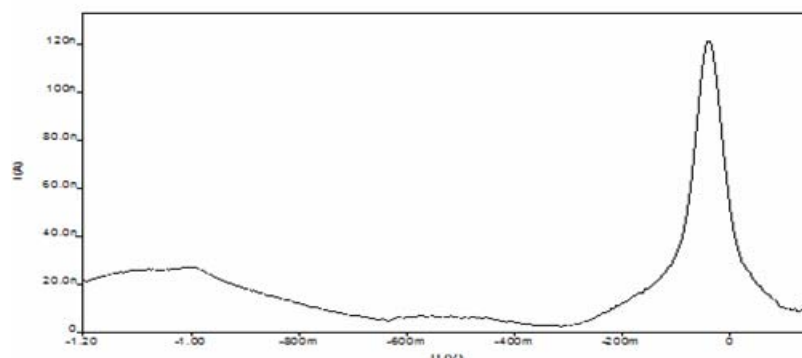


Fig. 2: Square wave voltammogram of (9.9x10⁻⁷) M Co-enzyme-Q₀ at (pH=7.0) under the optimum conditions

It can be seen from Fig. (2), that a well-defined reduction peak appeared at E_{pv} (-0.0415 V) versus (Ag/AgCl, 3M KCl) electrode.

Table 1: The measured optimum condition of Co-enzyme-Q₀ using SWV technique

Variables	Default Conditions Values	Optimum Conditions Values
Deposition Potential (V)	-0.9	-0.2
Deposition Time (Sec.)	60	10
Equilibrium Time (Sec)	5	5.0
Voltage Step (V)	0.006	0.004
Pulse Amplitude (V)	0.02	0.02
Frequency (Hz)	50	50
Drop size (mm)	4	4
pH	---	7.0

The calibration curve of Co-enzyme-Q₀ was constructed using SWV under the optimum condition+ns in Table (1). The square wave voltammograms were recorded for serial additions of (10⁻⁴) M co-enzyme-Q₀ in (10 ml) P.B.S. (pH 7.0), and the peak current at E_{pv} (-0.0415 V) plotted against the co-enzyme-Q₀ concentration is shown in Fig. (3).

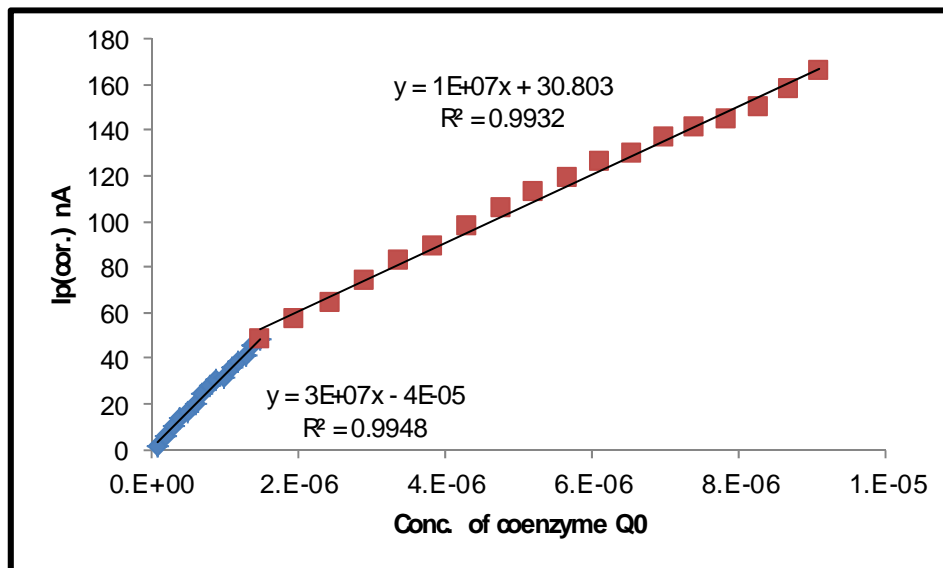


Fig. 3: The relation between peak current (*I_p*) and concentration of Co-enzyme-Q₀ in phosphate buffer (pH7.0) using SWV.

The calibration gives two straight lines depending on concentration rang ,the first one in the concentration range (9.99x10⁻⁸ -1.38x10⁻⁶) M with correlation coefficient (R= 0.9948), and the second in the concentration range (1.96x10⁻⁶ – 9.5x10⁻⁶) M with correlation coefficient (R= 0.9931), this maybe due to the molecular association at high concentrations .

For voltammetric behavior of co-enzyme-Q₀ in the presence of aniline, the square wave voltammogram of (9.9 × 10⁻⁷) M of co-enzyme-Q₀ in phosphate buffer at (pH = 7.0) was recorded at (289⁰ K). Successive amounts of aniline were then added and the square wave voltammograms was recorded after each addition., The results are shown in Fig. (4).

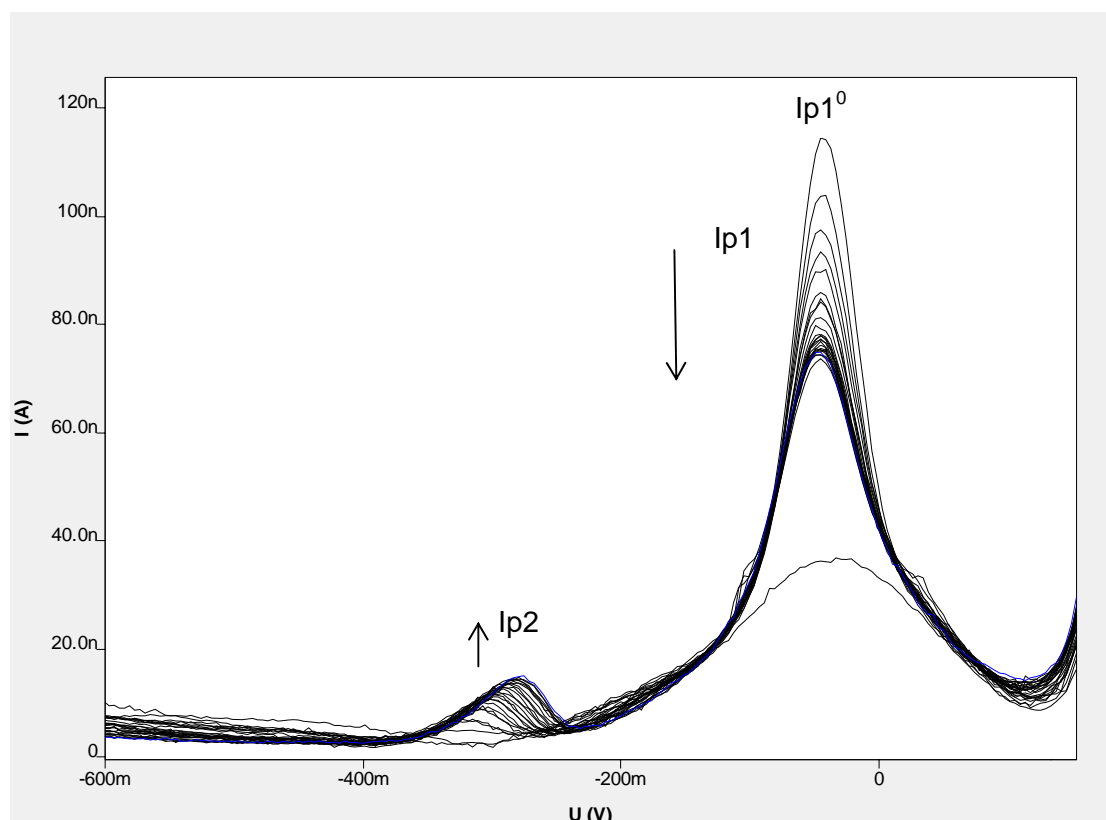


Fig. 4: Square wave voltammogram of (9.9×10^{-7}) M Co-enzyme-Q₀ in the presence of the successive additions of Aniline

Whereas:

I_{p1^0} = peak current for Co-enzyme-Q₀ in the absence of Aniline.

I_{p1} = peak current for Co-enzyme-Q₀ in the presence of Aniline.

I_{p2} = A small peak current appeared after addition of Aniline to the Co-enzyme-Q₀.

The peak current I_{p1^0} of co-enzyme-Q₀ at E_{pv} (-0.0415 V) decreased gradually with the addition of aniline I_{p1} until it reached constant value. Weak peak current I_{p2} appeared as a reduction wave at (-0.284V) increased marginally to a certain extent (until reaches constant value) with a gradually added of few amounts aniline. This behavior may be due to the interaction of co-enzyme-Q₀ with aniline. The same procedure was applied to the interaction of co-enzyme-Q₀ with Pyrrole. The results are shown in Fig. (5).

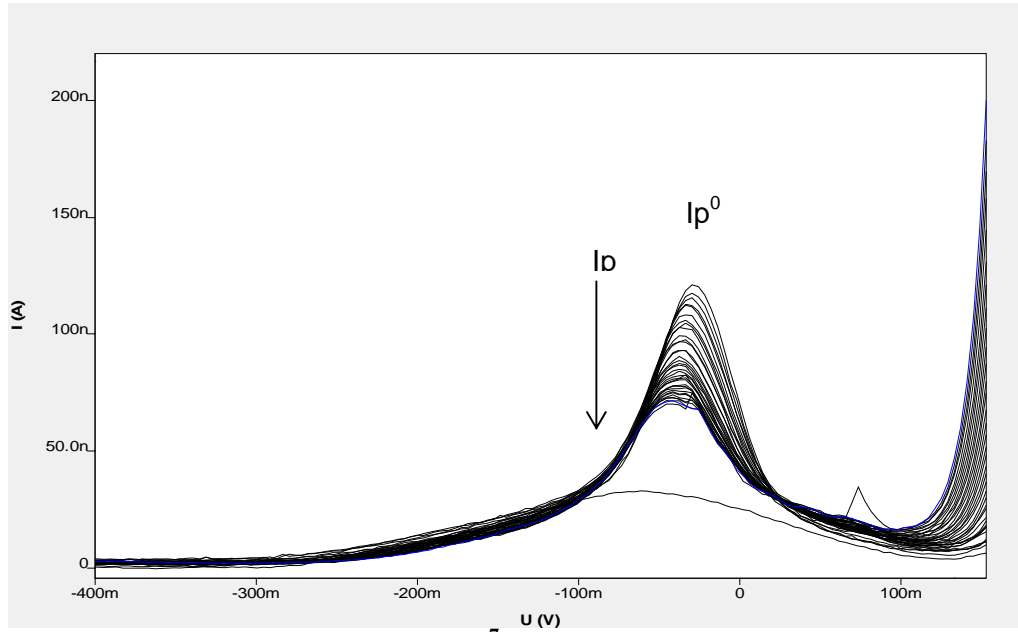


Fig. 5: Square wave voltammogram of (9.9×10^{-7}) M Co-enzyme- Q_0 in the presence of the successive additions of Pyrrole.

Where as:

I_p^0 = peak current for Co-enzyme- Q_0 in the absence of Pyrrole.

I_p = peak current for Co-enzyme- Q_0 in the presence of Pyrrole.

The peak current I_p^0 of co-enzyme- Q_0 at E_{pv} (-0.0415 V) was found to be decreased gradually with the additions of pyrrole I_p . This behavior is due to the interaction of co-enzyme- Q_0 with pyrrole until it reaches constant value.

To calculate the binding constant (K) for the interaction of co-enzyme- Q_0 with aniline and pyrrole the equation (1) was used (Feng *et al.*,1997):

$$\ln (I_p / (I_p^0 - I_p)) = \ln (1 / [\text{Conc. (M)}]) - \ln (K) \dots\dots\dots 1$$

Where K is apparent binding constant, then the plot of $\ln (I_p / (I_p^0 - I_p))$ versus $\ln (1 / [\text{Conc. Aniline (or Pyrrole) (M)}])$ that gives linear relation with intercept of $\ln (K)$.

The square wave voltammograms of (9.9×10^{-7}) M of co-enzyme- Q_0 in the presence of aniline (or Pyrrole), (successive additions) were recorded at different temperatures (289, 293, 298, 303, 308) $^{\circ}\text{K}$, under the optimum conditions shown in (Table 1). The binding constants K for the interaction of co-enzyme Q_0 with aniline (or Pyrrole) were calculated using the equation 1 by plotting $\ln (I_{p1} / (I_{p1}^0 - I_{p1}))$ versus $\ln (1 / [\text{Conc. Aniline (M)}])$ for aniline, (or pyrrole) . A linear relations with intercept of $\ln (K)$ equation (1) at different temperatures (289,293,298,303,308) $^{\circ}\text{K}$ for each aniline and pyrrole were obtained. The results are shown in Figs. (6), (7).

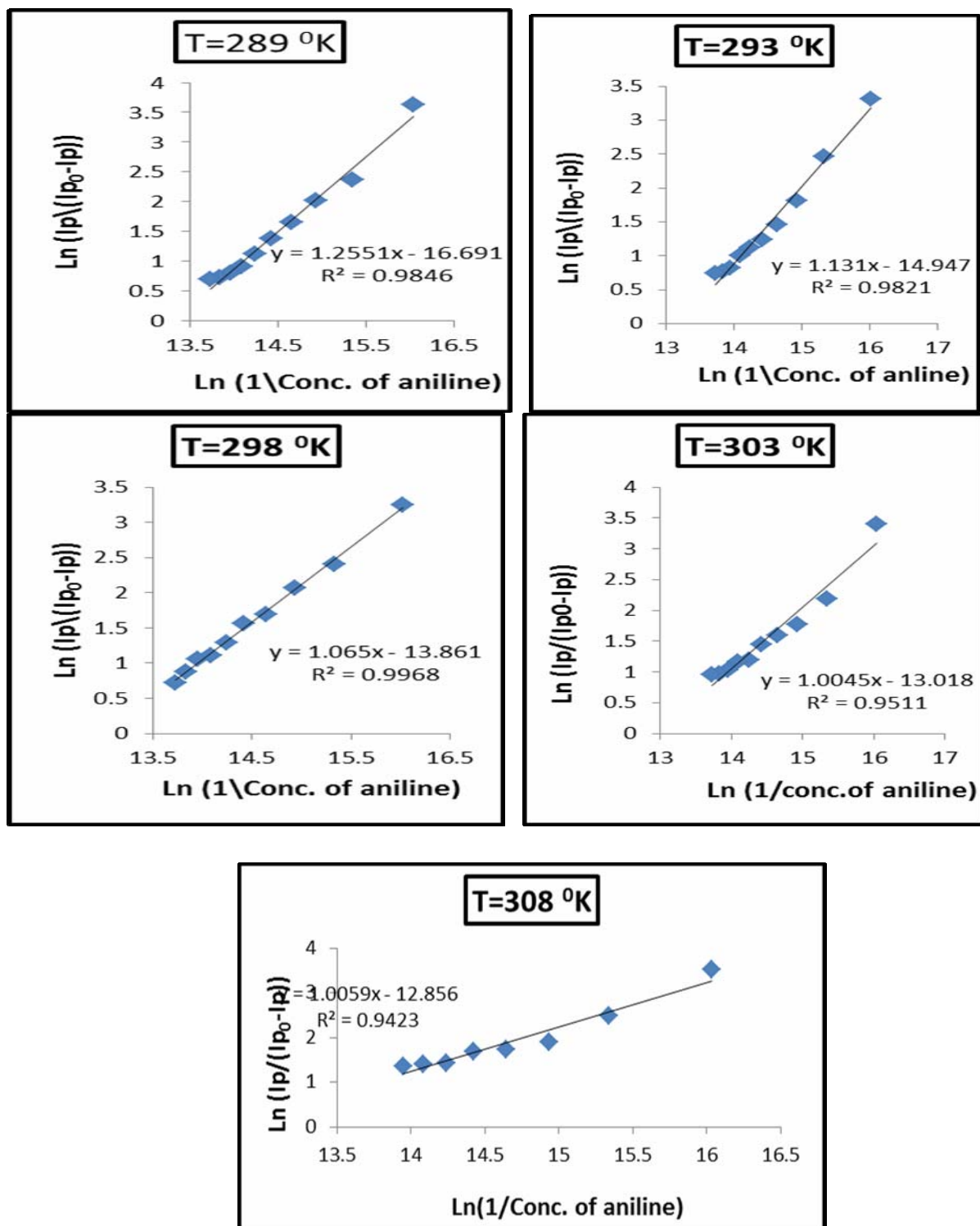


Fig. 6: plot $\ln(I_p/(I_{p0}-I_p))$ versus $\ln(1/[\text{Conc. of Aniline(M)}])$ of Co-enzyme-Q₀ and Aniline interaction at (289, 293, 298, 303, 308) °K.

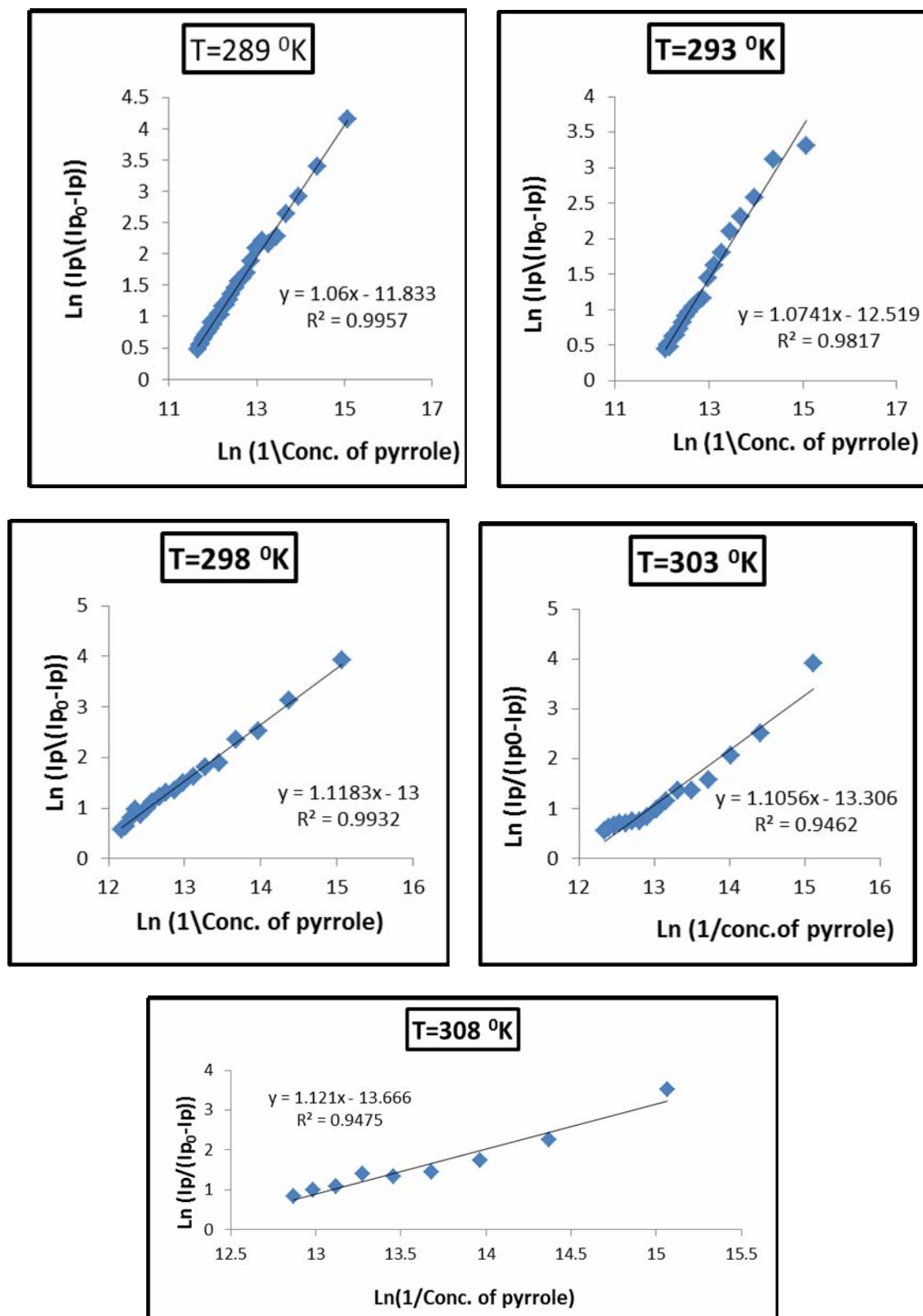


Fig. 7: plot $\ln(Ip/(Ip^0-Ip))$ versus $\ln(1/[\text{Conc. of Pyrrole}(\text{M})])$ of Co-enzyme- Q_0 and Pyrrole interaction at (289, 293, 298, 303, 308) °K.

The values of binding constant (K) were calculated from the intercept of the plot of Figs. (6,7) and tabulated in (Tables 2 and 3).

To calculate the thermodynamic parameters by plotting $\ln K$ against $1/T$ using Vant Hoffe equation gives a linear relationship as shown in Figs. (8) and (9).

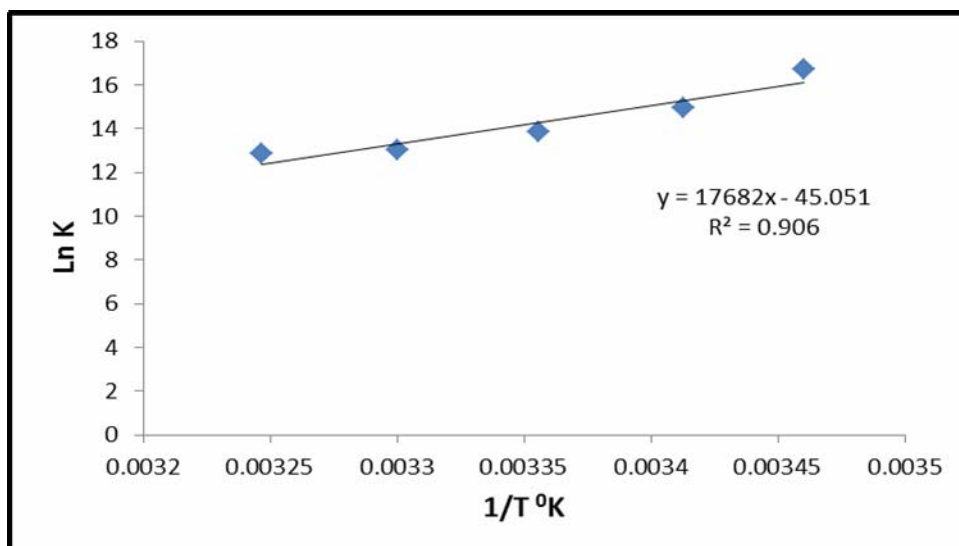


Fig. 8: The relation between $\ln K$ and $1/T$ °K for interaction between Co-enzyme-Q₀ and Aniline

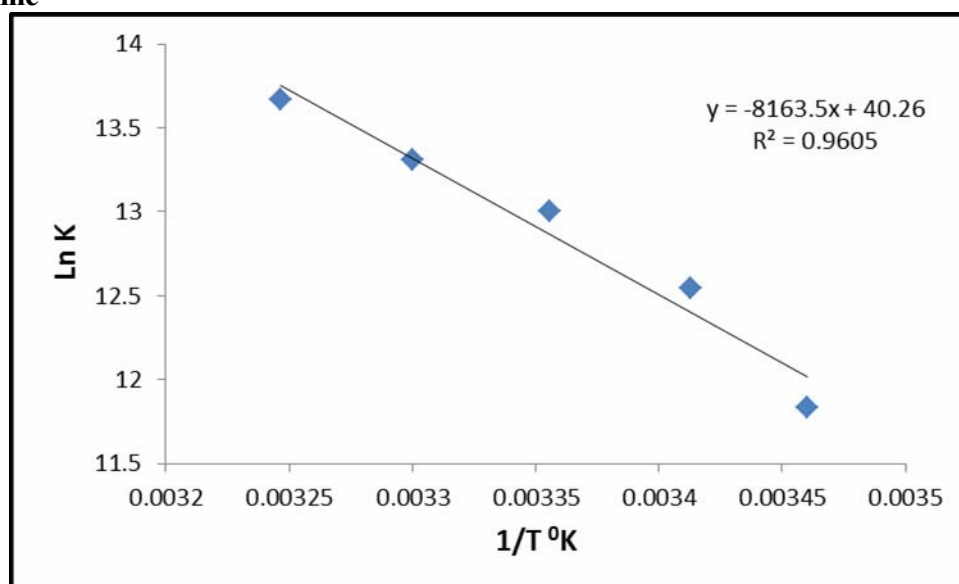


Fig. 9 : The relation between $\ln K$ and $1/T$ °K for interaction between Co-enzyme-Q₀ and Pyrrole

The change in enthalpy (ΔH) was obtained from the slope $\Delta H = -\text{Slope } R$, and other thermodynamic parameters (ΔG and ΔS) were calculated as follows:

$$\Delta G = -RT \ln K$$

$$\ln K = -\Delta H / RT + \Delta S / R$$

From which the thermodynamic quantities of the interaction of Co-enzyme-Q₀ with aniline (and pyrrole) can be calculated as shown in (Table 2 and 3).

Table 2: The thermodynamic parameters at different temperature (289, 293, 298, 303, 308) °K for interaction between Co-enzyme-Q₀ and Aniline.

Temp °K	Ln K	$K \times 10^6 \text{ M}^{-1}$	$\Delta H(\text{KJ/mole})$	$\Delta G(\text{KJ/mole})$	$\Delta S(\text{J/mole.K})$
289	16.691	17.73410181	-147.008148	-40.10423349	-374.554014
293	14.947	3.100270736		-36.41092189	
298	13.861	1.046539955		-34.34162549	
303	13.018	0.4504489360		-32.79419056	
308	12.856	0.3830803357		-32.92051347	

Table 3: The thermodynamic parameters at different temperature (289, 293, 298, 303, 308) °K for interaction between Co-enzyme-Q₀ and Pyrrole

Temp °K	Ln K	K x10 ⁶ M ⁻¹	ΔH(KJ/mole)	Δ G(KJ/mole)	Δ S(J/mole.K)
289	11.833	0.137723041	76.771476	-28.43169342	364.095002
293	12.545	0.280688277		-30.55964509	
298	13	0.442413392		-32.208436	
303	13.306	0.600789558		-33.51970345	
308	13.666	0.861129346		-34.994690192	

According to the estimated K-values in (Table 2), the calculation of the enthalpy change (ΔH) shows negative value (exothermic), and the temperature rise causes as expected a decrease in the K-values of the interaction of co-enzyme-Q₀ with aniline. The calculated free energy changes (ΔG) of this interaction were negative values indicating the possible spontaneous process at the mentioned experimental conditions. The negative values of entropy change (ΔS) indicates that the complex formed after the interaction of co-enzyme-Q₀ with aniline is more ordered. From the (Table 3) it is clear that the K-values of the interaction of co-enzyme-Q₀ with pyrrole was found to increase with increasing temperature and the enthalpy change (ΔH) showed positive value (endothermic). The positive value of entropy change (ΔS) indicates that geometrical configuration after the interaction of co-enzyme-Q₀ with pyrrole is more in disorder. The calculated free energy changes (ΔG) of this interaction were negative values indicating the possible spontaneous process.

CONCLUSION

From the viewpoint of thermodynamics, $\Delta H > 0$ and $\Delta S > 0$ imply that a hydrophobic interaction is the main force; $\Delta H < 0$ and $\Delta S < 0$ reflecting vander Waals forces or hydrogen bonding $\Delta H < 0$ and $\Delta S > 0$ suggests electrostatic forces that play a key role (Zhao *et al.*, 2010).

Hence from the results in (Table 2), the interaction of co-enzyme-Q₀ with aniline is vander Waals forces or hydrogen bonding while the interaction of co-enzyme-Q₀ with pyrrole is a hydrophobic interaction according to the results obtained in (Table 3).

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