Iraqi Journal of Science
ISSN: 0067-2904
GIF: 0.851

Electrochemical Study for the Effect of Simvastatin and Atorvastatin on Coenzyme Q_{10} by Differential Pulse Polarography Using Mercury Drop Electrode

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Abstracts

Statin drugs act by inhibiting the enzyme HMG-CoA reductase which is responsible for manufacture of cholesterol and the biosynthesis essential energy production Co-factor (Coenzyme Q_{10}). The aim from this research includes study the effect of statin drugs (Simvastatin and Atorvastatin) on the Coenzyme Q_{10} using differential pulse polarographic technique at a dropping mercury electrode (DME) and in a mixture of [4:1] methanol-phosphate buffer of pH7.0 as supporting an electrolyte. Prior to this, the behaviors of Simvastatin, Atorvastatin and Coenzyme Q_{10} were studied separately in their solvents. The half-wave potential ($E_{1/2}$) of Co Q_{10} were -0.31volt and -1.37Volt, -1.33 Volt for simvastatin and atorvastatin respectively. A mixture of Coenzyme Q_{10} with Simvastatin and Atorvastatin in the same solvent shows a shift in their peak potential (E_p) toward more negative potentials values by (-0.06volt) for Simvastatin and by (-0.09volt) by Atorvastatin.

Keywords: Statin Drugs, Coenzyme Q_{10}, differential pulse polarography.

دراسة كهروكيميائية لتآثرالسيمفاستاتين والأتورفاستاتين مع مساعد الانزيم Q_{10} بواسطة البولاروغرافيا النبضية المشتقة بأستخدام قطب الزئبق المتقاطر

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الخلاصة

تعمل ادوية الستاتينات على تثبيط انزيم HMG−CoA reductase وهو الأنزيم المسؤول عن تصنيع الكوليسترول والتركيب الحيوي لأنتاج الطاقة Coenzyme Q_{10}. ويتضمن هذا البحث دراسة تآثر ادوية الستاتينات (السيمفاستاتين و الأتروفاستاتين) على مساعد الأنزيم Q_{10} باستخدام تقنية البولاروغرافيا النبضي المشتق عند قطب الزئبق المتقاطروالمذاب كل منهما في مزيج من الميثانول والمحلول المنظم الفوسفاتي pH7.0 كألكتروليت ساند بنسبة [1:4]. وقبل دراسة التآثر، درس سلوك كل من ادوية الستاتينات ومساعد الأنزيم Q_{10} بصورة منفصلة في محاليلهما وتم تعيين جهد القمة لمساعد الأنزيم وكانت بقيمة 0.31Volt− و بقيمة 1.37Volt, −1.33Volt− لكل من السيمفاستاتين والأتروفاستاتين. وتبين ان خليط ادوية الستاتينات مع مساعد الأنزيم Q_{10} في نفس المذيب ادى الى ازاحة في قيم جهد القمة لمساعد الأنزيم Q_{10} نحو قيم اكثر سالبية وكانت بمقدار 0.06Volt− بالنسبة للسيمفاستاتين و بمقدار 0.09Volt− بالنسبة للاتروفاستاتين.

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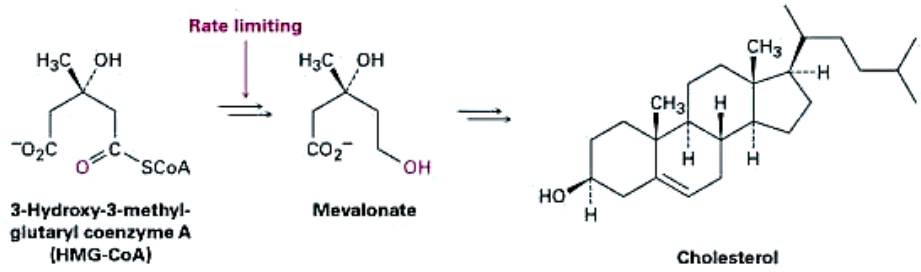
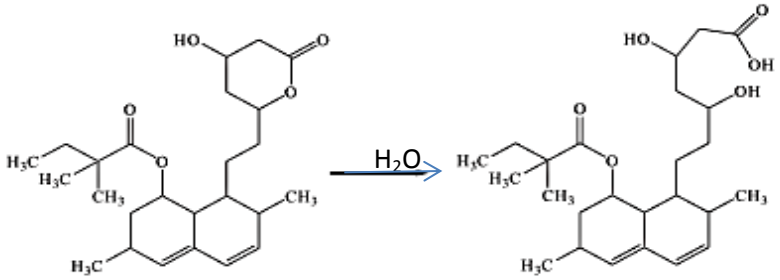
Introduction

Simvastatin and atorvastatin are examples of statin drugs; statins are the most commonly used and the most effective class of drugs for lowering of plasma cholesterol [1]. Statins reduce cholesterol in the blood by block an enzyme in the liver that synthesizes cholesterol and prevent the liver from producing and releasing cholesterol in the blood stream [2]. The principle action of statins is to inhibit cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductes, an enzyme that controls the rate of cholesterol synthesis in the liver via the mevalonate pathway, it is an enzyme that catalyzes the conversion of HMG-CoA to mevalonate, which through several further biochemical steps is metabolized to cholesterol which takes place in the liver as shown in scheme -1 [1, 3].



Scheme 1- Mavalonat pathway [4].

Simvastatin (1S,3R,7S,8S,8Ar8-{2-[92R,4R)-4-hydroxy-6-oxooxan-2yl]etheyl}-3,7-dimethyl 1,2,3,7,8a-hexahydronaphalen-1-yl 2,2-dimethylbutanoate, belongs to the first generation of statins (Type I statins). They are all derived directly or from fungal metabolites. And share a similar structure which contains a (polar head group) and a hydrophobic moiety which includes a decalin ring [5, 6].The chemical structure of simvastatin contains a β-hydroxy-lacton.physiologically active form of the drug is the β-hydroxy acid, which is formed by a ring–opening reaction of lactone ring. In vivo prodrug lactone form is enzymatically hydrolyzed to its hydroxyl-acid pharmacophore scheme-2 [7].



Scheme 2-Molecular structures of simvastatin and physiologically simvastatin β-hydroxy acid form.

Atorvastatin is a (bR,dR)-2-(r-fluorophenyl)-b,d-dihydroxy-5-isopropyl-3-phenyl-4(phenylcarbamoyl) - pyrrole-1-hepatanoicacid (1:2) trihydrate, belongs to the type II statin. There are synthetic structures contain a different and large hydrophobic moiety. The synthetic statins have in common a fluorinated phenyl group, and a base structure made up of a five or six member ring wione atom substituted with a nitrogen atom Figure- [6, 8].

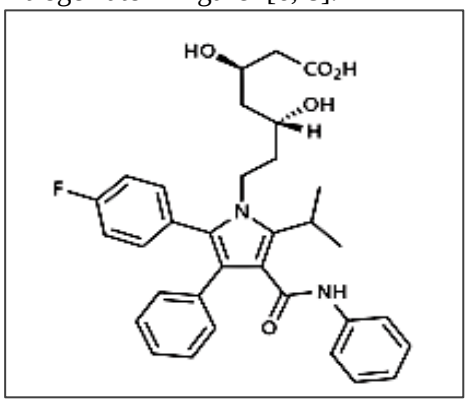


Figure 1-Chemical structure of atorvastatin

Statins produce both therapeutic effects and side effects through the same mechanism, HMG-CoA reductase is at the base of mevalonate pathway, the mevalonate pathway is involved not only in the biosynthesis of cholesterol but also in the biosynthesis of essential co-factor required for energy production Coenzyme Q_{10}, thus statins block the endogenous biosynthesis of both cholesterol and Coenzyme Q_{10} by inhibiting the enzyme HMG-CoA reductase [9, 10].

Coenzyme Q_{10} is a fat soluble, synthesized by human body. It was first is isolated from beef heart mitochondria in 1957 by Frederick Crane. It is a Quinone with an isoprenoid side chain [11]. The primary function of Coenzyme Q_{10} is an indispensable cofactor in the electron transport of mitochondria, function as an electron carrier between the enzyme complexes of the respiratory chain It is also a lipid-soluble antioxidant that scavenges lipid radicals within biological membranes [12].The aim of this work is the study the effect of statin drugs on the peak potintial of Coenzyme Q_{10} by the application of differential pulse polarographic techniqe.

Experimental

Chemicals and Reagents

- Simvastatin and Atorvastatin in pure form were supplied by Drug Control Department for Iraq Ministry of Health.
- Phosphate buffer solution pH7.0 ($K_2HPO_4.2H_2O$ and $NaHPO_4.H_2O$) was supplied from Radiometer Copenhagen.
- Ubiquinonem (Coenzyme Q_{10}) from Fluka product of USA.
- Absolute Methanol with purity 99% from the Merck Chemical Company.

Because of the low solubility of statin drugs and CoQ_{10} in water, polarographic measurements were done in a mixture of Methanol and phosphate buffer.

Simvastatin (5×10^{-4}M) was prepared by dissolved (0.0209gm) in (80 ml) methanol and (20 ml) phosphate buffer pH7.0 Atorvastatin (5×10^{-4}M) was prepared by dissolved (0.0279gm) in (80 ml) methanol and (20 ml) phosphate buffer pH7.0

A stock standard solution of Coenzyme Q_{10} (3×10^{-5} M) was prepared by dissolved (0.0259gm) in methanol. A working solution of Coenzyme Q_{10} (10^{-5}M) was prepare by dissolving (33.3ml) from stock solution of Coenzyme Q_{10} and complete to the 100ml by adding methanol and 20 ml of phosphate buffer pH7.0 .All solution were prepare freshly just before use.

Instrumentation

Polarographic Analyzer model 174A was used for polarographic measurement. It was coupled to an (Kompenson X-Y Recorder model C1924) the supplier of the company Siemens. The Polarographic cell composed of three electrodes, the mercury drop electrode, which is normally a cathode of the polarographic cell flows from a glass capillary tube, a saturated calomel reference electrode and the counter electrode (Mercury pool) into which the drop falls, acts as an anode.

Procedure

A mixture of (1:1) proportion of statin drugs with Coenzyme Q_{10} were placed in the polarographic cell. Then recording the polarographic curve, the solution was deoxygenated by purging with purified nitrogen through the solution for several minutes. The instrumental setting for the DDP was as follows: Potential scane rate 10 mv/SEC, Drop time 0.5, Sec, Modulation Amplitude 50 mV, current Range 0.5 mA.All measurements were carried out at room temperature.

Results and Discussion:

Prior to the study of the interaction of statin drugs with CoQ_{10}, has been studied the electrochemistry reduction and behavior of both statin drugs and Coenzyme Q_{10} separately, each dissolved in a mixture of methanol and the solution of phosphate buffer pH7 as a supporting electrolyte with ratio 4:1.These values measured against the references calomel electrode.

Simvastatin gave two reduction waves, the first wave at (-0.4volt) is an adsorption wave, and the second wave at (-1.37 volt) refers to the reduction wave as shown in Figure-2.That is in good agreement with previous polarographic investigation [13].

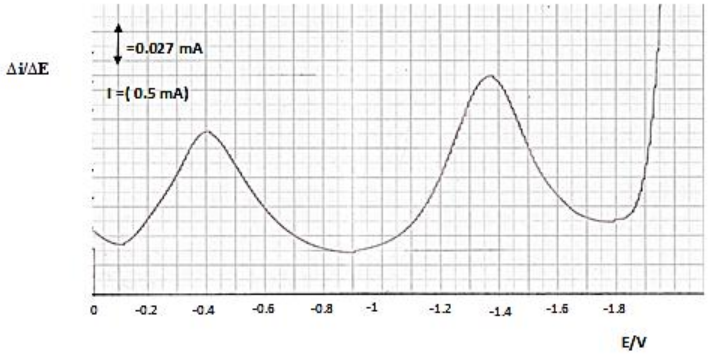


Figure 2-Polargram of (5×10^{-4}M) simvastatin at drop mercury electrode in a mixture of (4:1) methanol and phosphate buffer pH 7.

Atorvastatin also gave two reduction waves, the first wave at (-0.43Volt) is an adsorption wave, the second wave at (-1.33Volt) refers to the reduction wave as shown in Figure 3.That is in good agreement with previous polarographic investigation [8].

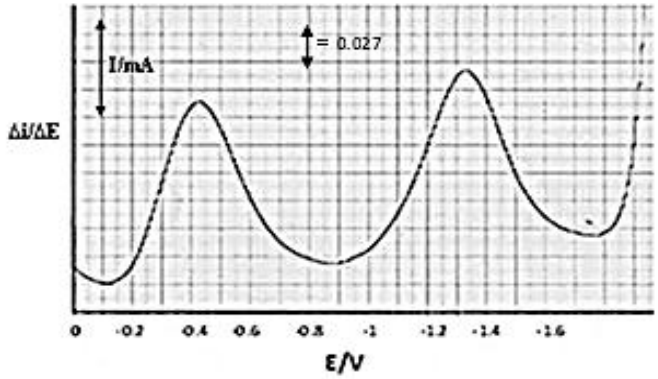


Figure 3-Polargram of (5×10^{-4}M) Atorvastatin at drop mercury electrode in a mixture of (4:1) methanol and phosphate buffer pH 7.0

The dropping mercury electrode may adsorbs the electroactive species or the product of the electrode reaction, this adsorption is due to surface forces, the particles of the dissolved substance may be bound to the surfacec by physical,in this case a separate wave, the adsorption wave is formed [14]. In figure (-4and -5) these polarogograms shows two waves. An adsorption prewave is observer if the electrolysis product is adsorbed and causes the electrode reaction to occur more easily[15]. And been provable, it was found that when reducing the high of mercury head, decreased the value of the limiting adsorption current. I_a = const h. The limiting adsorption current dependence on the height of the mercury head [14]. Electrochemistry reduction of Coenzyme Q_{10} were done in (4:1) methanol and in the solution of phosphate buffer pH 7.0 as a supporting electrolyte, it is reduced in two successive one-electron reduction steps which show two waves at a potential value in the range of (-0.31-1.27 volt) as shown in Figure-4. That is in good agreement with previous polarographic investigation [16].

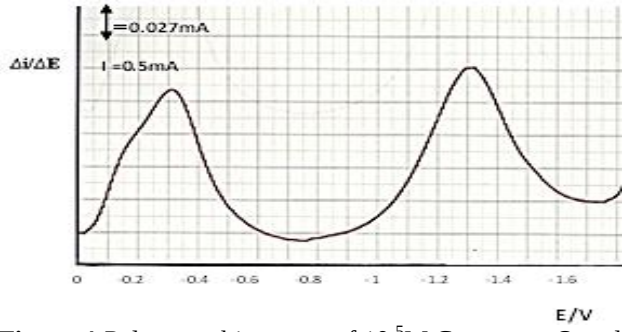
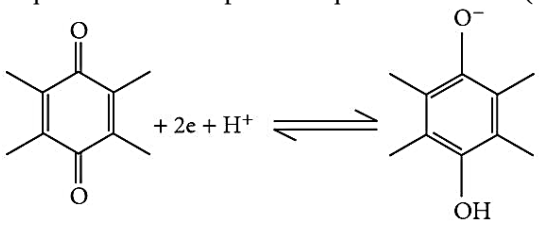


Figure 4-Polarographic waves of 10^{-5}M Coenzyme Q at drop mercury electrode in (4:1) methanol and phosphate buffer pH 7.0

The first wave at-0.31V is completely reversible one proton one electron leads to produce semiquinone Q^{-}(quinone anion radical) and the second step at -1.3V is irreversible, the reoxidation of semiquinone leads to produce quinone dianion (Q^{2-}) as shown in scheme-3.



Scheme 3-Two- electron one proton reduction of Coenzyme Q_{10} in aqueous buffer.

The interaction of drugs with the enzyme assistant Q_{10} were studied using high concentration of drugs much higher than the assistant enzyme Q_{10} concentration to ensure occurrence of interaction between them and deviation of balance towards the formation of molecular complexes. Due to the significant self-association of the Coenzyme $Q_{10,}$ it was found necessary to study their interaction under dilute concentration, ($< 10^{-5}$ mol/L), in order to avoid any interference with the complication process. The interaction between the Coenzyme Q_{10} and statin drugs was followed: through the recording a polarographic curves for the mixture in period of time, it took three to four hours to obtain an equilibrium state which were noted by the fixed values of $E_{1/2}$ and $I_{P.}$ The results of a polarographic measurements obtained were shown in table (-1and -2) and Figure (-5and-6).

Table 1-peak potential (Ep) measured in Volt, diffusion current (id) measured in milliamper for the reduce of mixture CoQ_{10} concentration (10^{-5}M) with Simvastatin ratio (1:1) and values change in peak potential ($\Delta E_{1/2}$) to reduce the CoQ_{10} in Phosphate buffer solution pH7.0 at DME.

Substance	E_PVolt	ΔE_P Volt	Id (mA)	T(min)
Coenzyme Q_{10} (10^{-}M)	-0.31	000	0.12	00
Coenzyme Q10+ Simvastatin in phosphate buffer pH7.	-0.33	-0.02	0.08	30
	-0.34	-0.03	0.09	60
	-0.35	-0.04	0.13	90
	-0.36	-0.05	0.14	120
	-0.37	-0.06	0.20	150

Table 2-peak potential (Ep) measured in Volt diffusion current (id) measured in milliamp and reduce mixture CoQ_{10} concentration (10^{-5}) with Atorvastatin with ratio 1:1and values change in the peak potential (ΔE_P) to reduce the CoQ_{10} in Phosphate buffersolution pH7.0.

Substance	E_PVolt	ΔE_P Volt	Id (mA)	T(min)
Coenzyme Q10 (10^{-}M)	0.31	000	0.12	
Coenzyme Q_{10} + Atorvastatin in phosphate buffer pH7.0	0.35	0.04	0.08	30
	0.36	0.05	0.09	60
	0.37	0.06	0.11	90
	0.38	0.07	0.16	120
	0.39	0.08	0.18	150
	0.4	0.09	0.23	180

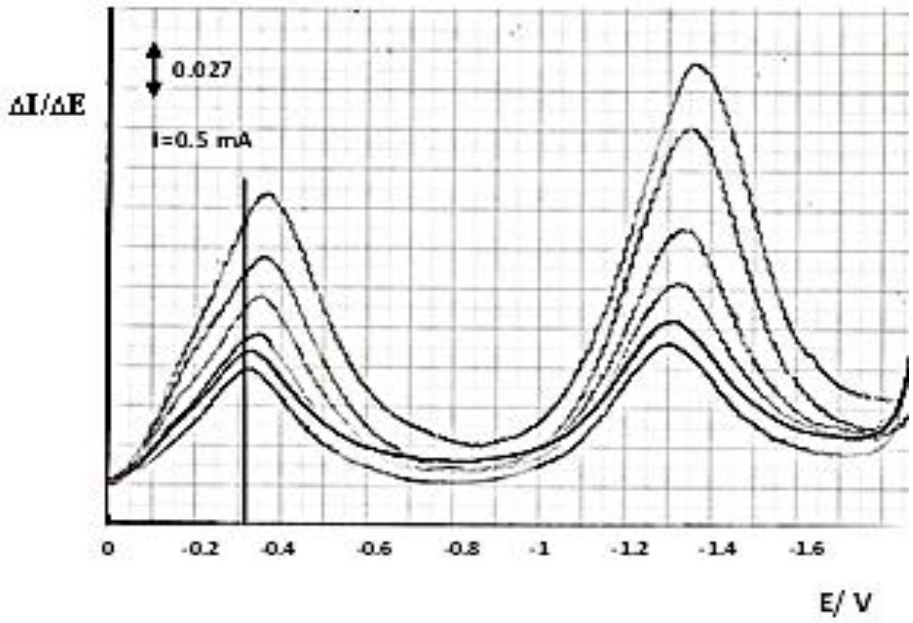


Figure 5-Polarogram for mixture Coenzyme Q_{10} with simvastatin illustrating the change values peak potential (E_p) and diffusion current for Coenzyme Q_{10} by adding simvastatin at different time.

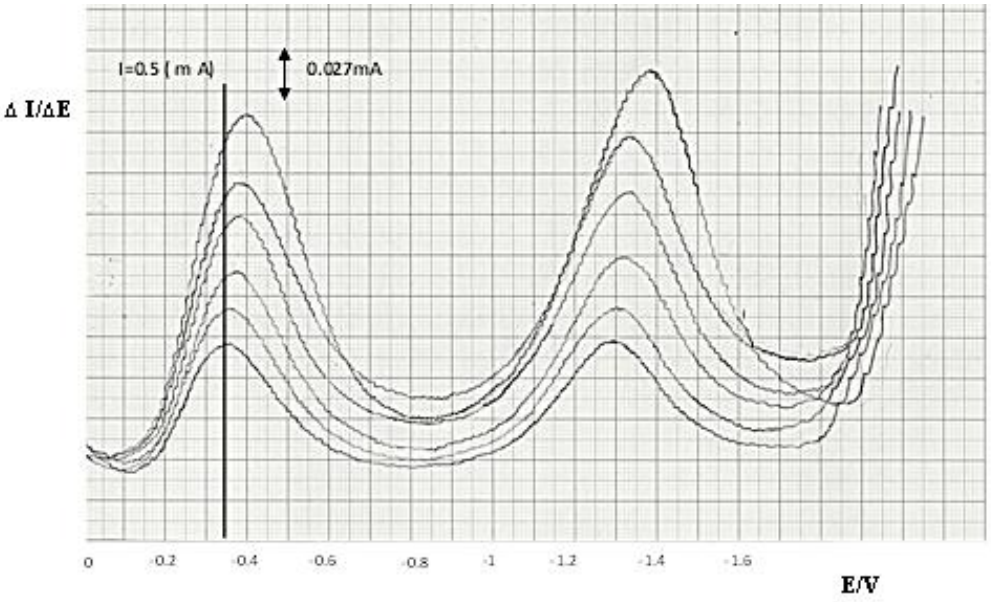


Figure 6-Polarogram for mixture Coenzyme Q_{10} with atorvastatin illustrating the change values peak potential (E_p) and diffusion current for Coenzyme Q_{10} by adding simvastatin at different time.

From results a polarographic measurement in table 1and 2and figure the following observation were obtained.

1. The addition of statin drugs to Coenzyme Q_{10} cause a shift in E_P value to a more negative potential due to the formation of molecular complex, this will block the role of Coenzyme Q_{10} as an electron carrier in the respiratory chain.
2. This interact between the statin drugs and Coenzyme Q_{10} raise the energy level of the lowest unoccupied molecular orbital of Coenzyme Q_{10} (LUMO) which lead to an increase in its redact potential of this results agreed with other previous work of Coenzyme Q_{10} with antimalarial drugs [17].

   A number of papers have been published, in which the relation between half-wave potential and the extent of conjugation in the system is treated in terms of quantum mechanics. Thus, it was

found that there is an excellent correlation between the half-wave potentials of hydrocarbons and the energy of their *lowest molecular orbitals* LUMO [14].

3. The difference in the ability of Simvastatin and Atorvastatin to form complex with Coenzyme Q_{10} were note also from the difference in ΔE_P cause by their interactions which illustrated in table 1and 2. This is due to the difference in their functional groups which may participate in the formation of their molecular complexes with Coenzyme Q_{10}.
4. diffusion current of the mixture of Coenzyme Q_{10} and statin drugs decrease at first due to their interact and the formation of a molecular complex which illustrating that this formation is very fast then id increase again with time may be due to a rearrangement of two molecules to form complex which have a higher diffusion coefficient than the parent molecular.
5. theoratical calculation: the second order perturbation theory and Huckel molecular orbitals were used to calculate the interaction energy of statin drugs and Coenzyme Q_{10}.

This interaction increase the energy level of the lowest unoccupied molecular orbital of Coenzyme Q_{10}.

Kinetic Calculation

Calculated rate constant from first and second order for interaction of Coenzyme Q_{10} with atorvastatin and simvastatin by tracking the increase in a diffusion current of Coenzyme Q_{10} with statin drugs with time.

Interaction equation from first order:

I = diffusion current at t.

I_0 = diffusion current at t = 0

k= Rate constant

Rate constant (k) has been calculated from mile straight-line equal to $\left(\frac{-k}{2.303}\right)$ the output of the draw (Log i) against time shown in Figure -7, -8.

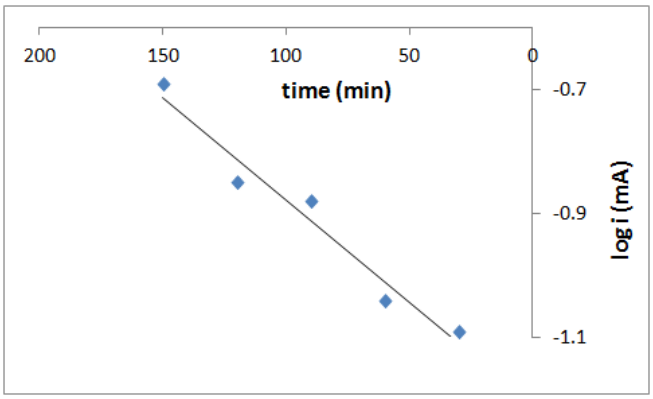


Figure 7-Determenation of rate constant from first order for interaction of Coenzyme Q_{10} with simvastatin in (1:1) proportion using differential pulse polarography.

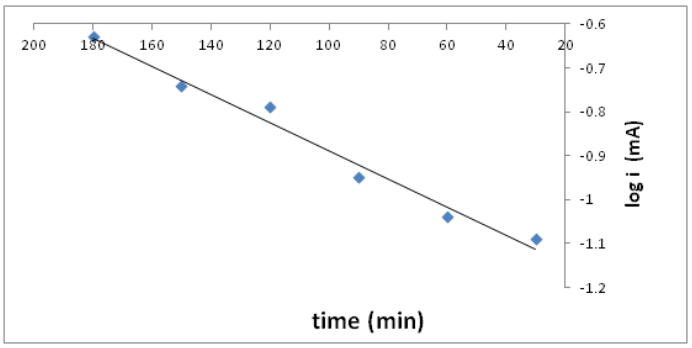


Figure 8-Determination of rate constant from first order for interaction of Coenzyme Q_{10} with atorvastatin in (1:1) proportion.

Illustrates Table-3 values constant rate interaction from first order for Coenzyme Q_{10} with simvastatin and atorvastatin in 1:1.

Table 3-Values rate constant from first order for interaction of Coenzyme Q_{10} (10^{-5}) with simvastatin and atorvastatin using differential pulse polarography.

Coenzyme Q_{10} + …	**K(min^{-1})**
Simvastatin	7.59×10^{-3}
Atorvastatin	7.36×10^{-3}

The straighter line graph and values of rate constant prove the interaction of coenzyme Q_{10} with simvastatin and atorvastatin corresponds to conditions kinetics of first order interaction.
Calculated rate constant interaction from second order for Coenzyme Q_{10} with atorvastatin and simvastatin from equation as followed:

$$t = \frac{x}{ta(a-x)}$$

The relationship between time (min) and $\left(\frac{x}{a-x}\right)$ equivalent to $\left(\frac{id-i}{i}\right)$ was plotted as shown in Figure -9,-10.

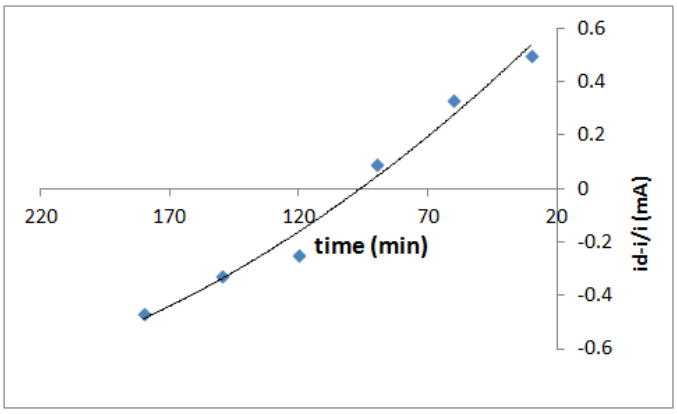


Figure 9-The application of the equation interaction from second order for interaction Coenzyme Q_{10} with simvastatin.

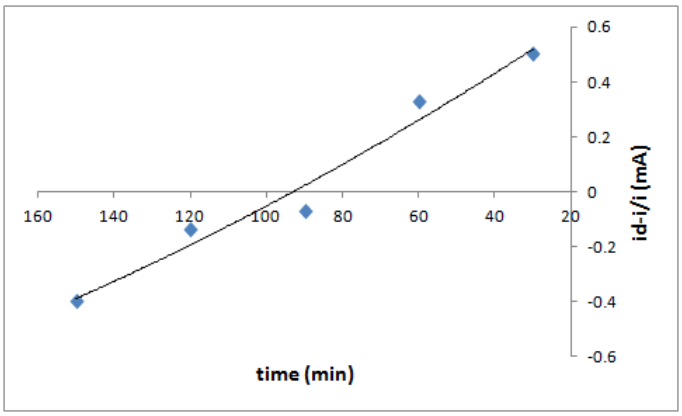


Figure 10-The application of the equation Interaction from second order for interaction Coenzyme Q_{10} with atorvastatin.

Figure -9 and -10 were shown the relationship non-line and no compatible with kinetics second order.
Was calculated free energy of activation from the following the relationship

$$K_1 = \frac{kT}{h} e^{-\Delta G^{\neq}/RT}$$

K= rate constant (min^{-1})
k= Boltzmann's constant $1.38\times10^{-23}J.k^{-1}$
h= Planck's constant $1.38\times10^{-34}JS$.
R= Constant gas 8.3 $mol^{-1}k^{-1}J$
Δ**G** = free energy of activation mol^{-1}.KJ.
Results have been installed in Table-4**.**

Table 4-Values free energy of activation for interaction of Coenzyme Q_{10} with simvastatin and atorvastatin.

Coenzyme Q10 +….	**ΔG (KJ.mol^{-1})**
Simvastatin	41.23
Atorvastatin	41.67

Conclusions

The addition of statin drugs to Coenzyme Q_{10} cause a shift in *peak potential (E_p)* of Coenzyme Q_{10} toward more negative value as a result of interaction. The shift was attributed to the formation of *molecular complexes,* thus cut off the flow of electrons and led to disabling to work within the respiratory chain as a carrier electrons. Simvastatin and Atorvastatin differ in their ability to form complexes with $CoQ_{10,}$ it was found that Atorvastatin show a higher shift in E_P values than Simvastatin because of their structure characteristics, Atorvastatin have additional hydrogen binding and polar interactions. Also observed an increase in diffusion current of Coenzyme Q_{10} due to high diffusion of the interacting molecules.

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