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DETERMINATION THE BINDING CONSTANT OF INTERACTION OF MOBIC WITH GLUTATHIONE USING SQUARE WAVE VOLTAMMETRY

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Abstract

Energy in living system can produced through the electron transfer, which necessary to achieve physiological functions of the cells, as a result free radicals of oxygen as well nitrogen species released and caused cell damage due to oxidation stress. Hence, these reactive species involve in the inflammation. This contradictory effect of oxygen necessitated the development of an antioxidant such as Glutathione (Glu.) to protect cell against oxidation by scavenging and inhibiting the action of free radicals. This research studied the electrochemical behavior of Glu. as antioxidant, Mobic as non-sterodial anti-inflammatory drugs(NSAIDs) and their interaction using square wave voltammetry technique (SWV) on the hanging mercury dropping electrode (HMDE) as working electrode, Pt-wire as an auxiliary electrode and Ag/AgCl in Sat. KCl as a reference electrode. Glutathione showed an reduction peak (at -0.305 V) in pH 4 of acetate buffer solution. While, Mobic showed Under the same default Condition a reduction peak at (-1.15 V). Optimum condition were investigated such as pH, start and end potential, deposition potential, deposition time, equilibrium time, voltage step, amplitude, frequency, the size of the mercury drop and sweep rate. As well, the interaction between the Glu. and Mobic was evaluated by utilizing the calculation of binding constant.

Keywords: Mobic, Glutathione, Square wave voltammetry, Electrochemical behavior, Binding Constant, Non-Steroidal Anti-inflammatory Drugs.

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Introduction

Mobic based on IUPAC name is 4-hydroxy-N-(5-methyl-1,3-thiazol-2-yl)-1,1-dioxo-2-(tridenteriomethyl)-1-ʎ-6,2- benzothiazine-3-carboxamide, figure 1[1,2]. It is a kind of non-steroidal anti-inflammatory drugs (NSAIDs) which possess pharmacological action as analgesic, anti-pyretic agents, osteoarthritis and rheumatoid arthritis of mild to severe pain. It is act as cyclo-oxygenase blocker to inhibit prostaglandin biosynthesis[3-5]. The most common side effects of Mobic include peptic ulcer, diarrhea, vomiting, nausea, and dizziness [6-8].

Mobic is converted in the main site of metabolism, in liver, into four biological inactive metabolites then excreted in urine and faces with an elimination half life time of about 20 hours. This is reflected in a total plasma clearance of 7 to 8 ml/min. Its steady state is reached within 3 to 5 days [7].

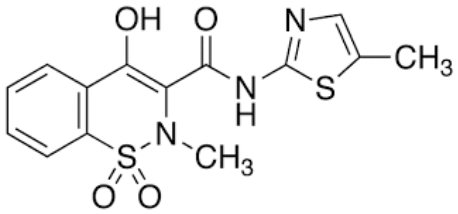

Figure 1: Chemical structure of Mobic

Several analytical methods estimated the Mobic by using mass spectroscopy, high performance liquid chromatography(HPLC) [9,10]. Electrochemical behavior of Mobic evaluated in tablet formulation and in plasma sample by using differential pulse polarography(DPP), and cyclic voltammetry(CV) techniques [11-14]. The oxidation-reduction behavior of Mobic demonstrated that DPP method as a rapid, low cost, possess accuracy and sensitive to determine Mobic in tablet preparation [11].

Glutathione (GSH) , a substance made of three amino acids include cysteine-glutamic acid-glycine, figure 2, is one of endogenous hydrophilic compound (thiol compound) found in every mammalian cells [15-19]. It's the most important antioxidant due to its availability in the cell and its potency to attack, then breakdown, free radicals within the cells. Hence, it known as mother of antioxidants. GSH acts as diverse cellular functions such as intracellular reduction-oxidation reaction and amino homeostasis, gene regulation and intracellular signal transduction. Therefore, GSH is valued sign for several human illness such as occlusive vascular, leukemia, diabetes, and certain sorts of cancers [19].

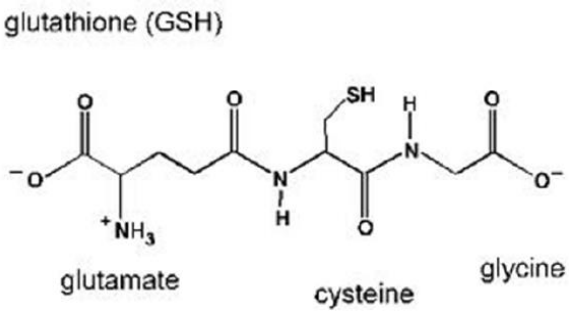

Figure 2: Chemical structure of Glutathione

Many analytical methods established the evaluation of GSH in biological fluid and pharmaceutical formulation using spectrophotometer, fluorescent, HPLC, electrophoresis type of capillary and by using electron chemical methods that based on direct detection or electrocatalytic approach have attracted greater consideration due to their accuracy and lower in cost [20-26]. The objective of this work is to evaluate the electrochemical behavior

of Mobic using square wave voltammetry, in addition to determine the binding constant of Mobic interaction with GSH.

Experimental:

Materials and Methods

All chemicals and solvents used were obtained from Sigma-Aldrich Germany & Fluka Switzerland company. Mobic and GSH were supplied by the state enterprise for drug industries and medical appliances in Samarra, Iraq. The pH of the buffer solutions was evaluated using pH-meter supplied by HANNA company. Electrochemical measurements were achieved, using 797 VA Computrace Model which supplied by Switzerland Metrohm company [27], applying technique of square wave voltammetry technique (SWV) on the hanging mercury dropping electrode (HMDE) as working electrode, Pt-wire as an auxiliary electrode and Ag/AgCl in Sat. KCl as a reference electrode.

Procedure

The electrochemical behavior of Mobic and GSH were carried out in acetate buffer solutions (pH 3,4,5,6, and 7) which prepared from a mixture of (0.2 M) of sodium acetate and (0.2 M) of acetic acid then adjusting by 0.1 M of HCl or 0.1M of NaOH to obtain a certain pH. Stock solution of Mobic was prepared of 10^{-2}M in acetonitrile. Also, stock solution of GSH was prepared of 10^{-3}M in distilled water.

Standardization carried out using solution of (250 ppm) of acetate salts of the Cu^{+2}, Cd^{+2}, Zn^{+2}, and Pb^{+2} were prepared in distilled water to confirm the calibration curve of the polarographic analyzer, of 797 VA Computrace Metrohm by using SWV technique. The experimental conditions were investigated at -1.5V of start potential, +0.25V of end of potential, 0.002V voltage step, 0.02V of amplitude, 50Hz of frequency, 5mmof drop size, and sweep rate of 0.1V/s. The results of polarogram of standardization solution showed in fig.3 [27,28].

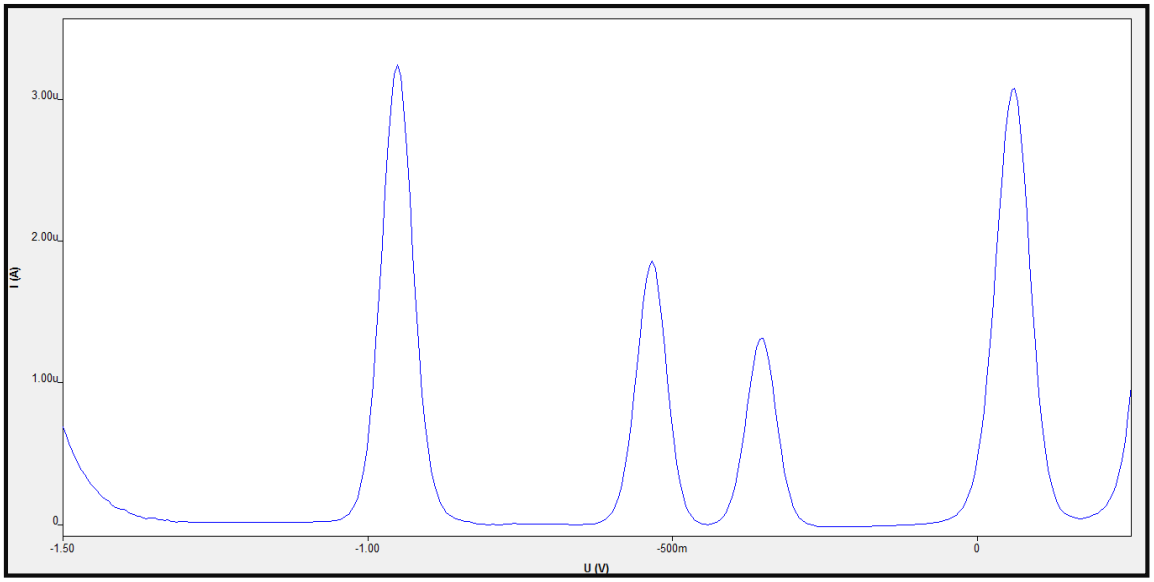

Figure 3: SWV Polarogram of Standardization solution consists of Cu^{+2},Cd^{+2},Zn^{+2}, and Pb^{+2}

Electrochemical procedure

The electrochemical behavior of 9.9x10^{-5}M of Mobic at HMDE, effect of scan rate in the range between -0.25V to +0.18V , and the influence of pH in the range of 3,4,5,6, and 7 on the peak of potential current of Mobic were investigated by applying SWV technique. The effects of voltage step, pulse amplitude, frequency, and drop size were employed to achieve the optimum condition.

The electrochemical behavior of 1.96x10^{-5}M of Glu. at HMDE, effect of scan rate in the range between (-0.05V to +0.25V) , and the influence of pH in the range of (3 – 7) on the peak of potential current of Glu. were investigated by applying SWV technique. Also, the effects of voltage step, pulse amplitude, frequency, and drop size were employed to investigate the optimum condition of Glu. polarogram.

Calculate the Binding Constant of Interaction

By considering the frequent addition of 10^{-7}M of Mobic into 4.98x10^{-5}M of Glu. solution using acetate buffer solution of (pH7) was conducting to calculate the binding constant (K) based on the following equation [28-30]:

$$\ln\left(\frac{Ip}{Ip°-Ip}\right) = \ln\left(\frac{1}{[Drug]}\right) - \ln K \quad \text{.....(1)}$$

Where, Ip° refers to the current of an oxidation peak of Glu., and Ip refers to the current of a reduction peak of Mobic to calculate the binding constant (K).

Results and Discussion

Electrochemical Behavior of Mobic at HMDE

SWV polarogram of 9.9x10^{-5}M of Mobic at HMDE in acetate buffer of pH4 with scan rate ranged between -1.45V to -0.9V, at default condition of the polarography apparatus, Table 1.

The influence of pH on the reduction wave of Mobic was shown in Table 2. It was observed an increasing in diffusion current by increasing the value of pH(3,4, and 5), then the current decreased at pH 6 and disappeared at pH 7. Hence, the optimum pH was at the value of 4.

Condition	Default Condition Values
Start Potential(V)	-1.5
End Potential(V)	-0.9
Voltage Step(V)	0.002
Amplitude(V)	0.02
Frequency(Hz)	50
Drop size(mm)	5
Sweep rate(V/s)	0.1

Table 1: The values of default condition for 9.9x10^{-5} M Mobic in pH4 using SWV

pH	Ep(V)	Ip(A)
3	-1.08	$10^{-7}\times1.07$
4	-1.15	$10^{-7}\times1.39$
5	-1.21	$10^{-7}\times1.54$
6	-1.28	$10^{-7}\times1.5$
7	-1.44	$10^{-8}\times8.25$

Table 2: The influence of pH on the potential voltage (Ep) and diffusion current (Ip) for Mobic

Table 3 and Fig.4 showed the optimum conditions of SWV polarogram of Mobic at pH 4.

Conditions	Optimum Condition Values
Start Potential(V)	-1.45
End Potential(V)	-0.9
Voltage Step(V)	0.005
Amplitude(V)	0.02
Frequency(Hz)	50
Drop size(mm)	5
Sweep rate(V/s)	0.25

Table 3: The optimum condition of $9.9\text{x}10^{-5}$M of Mobic in pH4

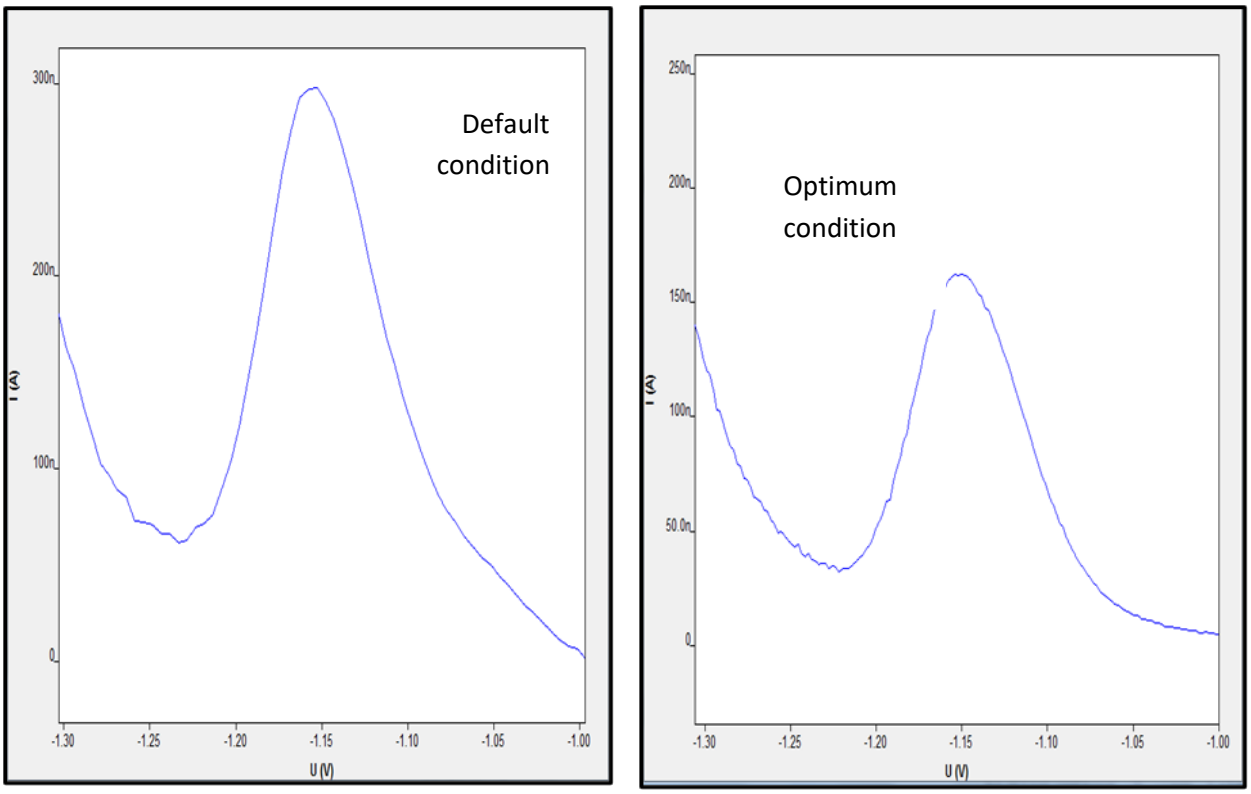

Figure 4: SWV Polarogram of 9.9x10^{-5} M of Mobic before and after optimum conditions

Conditions	Optimum Condition Values
Start Potential(V)	-0.65
End Potential(V)	+0.1
Voltage Step(V)	0.004
Amplitude(V)	0.05
Frequency(Hz)	50
Drop size(mm)	9
Sweep rate(V/s)	0.2

Table 4: Optimum condition of 9.9x10^{-9} M Glu. in pH4

Moreover, the optimum conditions of 9.9x10^{-9} M Glu. in pH4 Glu. presented in Table4, and its SWV polarogram investigated in fig.5.

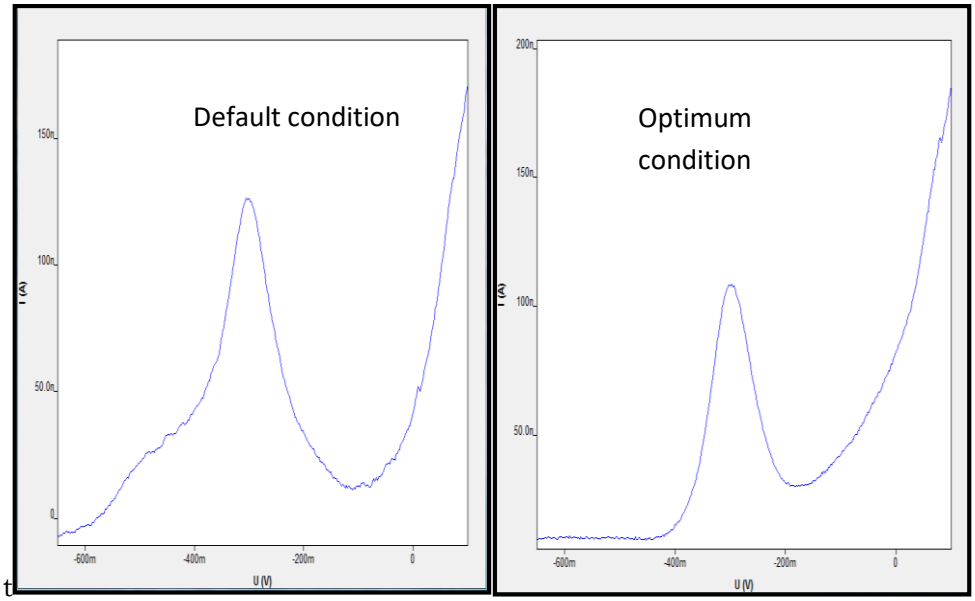

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Figure 5: SWV Polarogram 9.9x10^{-9} M Glu. in pH4 before and after optimum condition

Time (Min.)	Ep(V) of Interaction	Ip(A) x10^{-7} of Interaction
0	-0.38	8.73
5	-0.38	8.63
10	-0.376	8.92
15	-0.376	8.85
20	-0.38	8.77
25	-0.38	8.89
30	-0.38	8.67
35	-0.38	8.40
40	-0.376	8.52
45	-0.38	8.90
50	-0.372	8.91
S.D		**±0.174147**

Table 5: Effect of time on the interaction of Mobic and Glu.

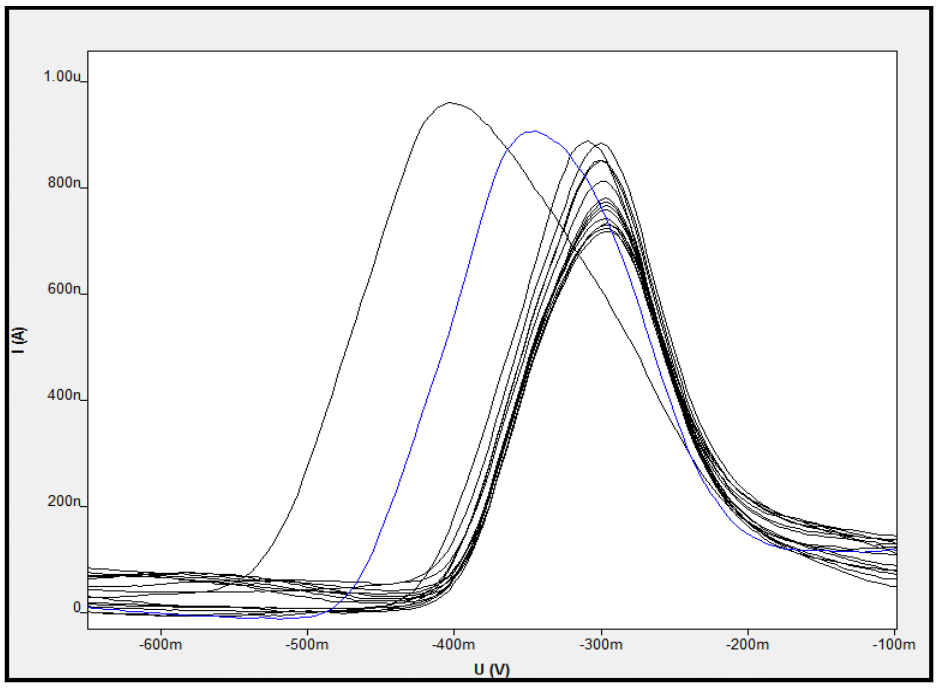

Figure 6: SWV Polarogram Frequent addition of 10^{-3}M of Mobic into $9.9x10^{-5}$M of Glu. in acetate buffer solution of pH4

Influence of time on the Mobic interaction with Glu. presented in Table 5, and its SWV polarogram showed in fig.6.

Conc. of Glu.(M)	Ep°(V)		Ip°(A)x10^{-7}	
9.9 x10^{-5}	-0.404		8.92	
Conc. of Mel.x10^{-7} (M)	**Ep(V)**	**10^{-7}×Ip(A)**	**ln(1/[drug])**	**ln(Ip/(Ip°-Ip))**
9.99	-0.344	8.67	13.8165110583	3.5461631519
19.96	-0.309	8.36	13.1243653801	2.7032769224
29.91	-0.305	7.98	12.7199027783	2.1388138152
39.84	-0.301	7.89	12.4331991182	2.0360373326
49.75	-0.301	7.81	12.2110650871	1.9510449485
59.64	-0.301	7.48	12.0297356271	1.6475896784
69.51	-0.297	7.22	11.8765818655	1.4462267018
79.37	-0.297	7.19	11.7440381859	1.4245697632
89.197	-0.297	7.10	11.6272482442	1.3612582830
99.01	-0.297	7.08	11.5228747958	1.3475083361
108.80	-0.297	7.00	11.4285567434	1.2935849630
118.58	-0.297	6.92	11.3425331124	1.2412685891
128.33	-0.297	6.82	11.2634827873	1.1779221271
138.07	-0.297	6.64	11.1903565764	1.0689365205
147.78	-0.297	6.48	11.1223506694	0.9767224711

Table 6: The effect of frequent addition of Mobic on the current of oxidation peak of Glu.

The results showed steady current of Mobic interaction with Glu. and obtained standard deviation of 0.174147.

According to equation1 the relationship of ln (Ip/ Ip° – Ip) against ln(1/ [Drug]), represents in Table 6 , was obtained a straight line with correlation coefficient of (R^2= 0.9731) and an intercept equal to (-ln K) with negative value of 8.8322 as shown in Fig.7.

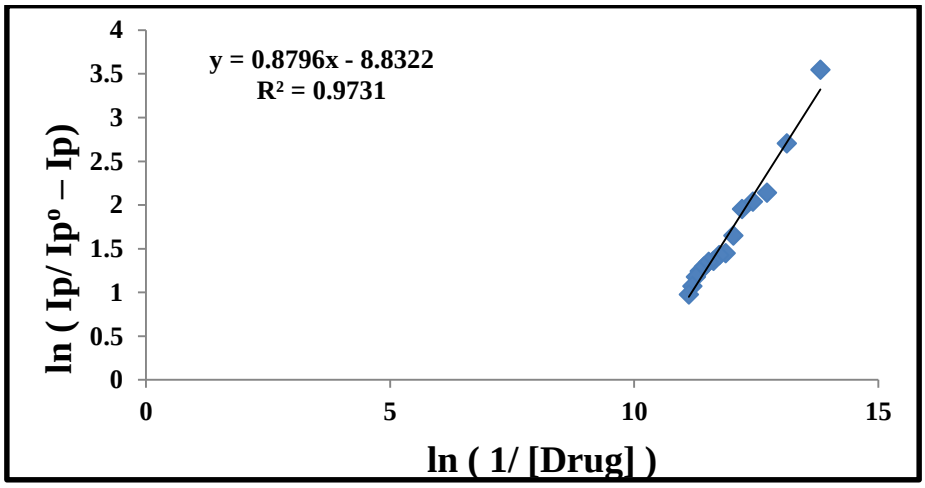

Figure 7: Relationship of ln (Ip/ Ipº – Ip) against ln(1/ [Drug]) of interaction of Glu. and Mobic

Therefore, the binding constant, K, obtained to be 6.85x103 which interpreted high interaction of Mobic with Glu.. In addition, SWV polarogram of Glu. was shifted the potential (Ep of 100Mv) into wave of Ep of 300mV.

Conclusion

SWV of Glu. at HMDE against Ag/AgCl in Sat. KCl showed that the reduction peak potential of 0.305 V with an appropriate peak in pH4 at over the range of scan rates , While Mobic recorded a reduction peak potential of -1.15V with an appropriate peak in pH4. From the interaction study, the peak of potential shifted to the negative value with the scan rate confirmed the irreversibility of the reaction. Furthermore, that shift of potential peak after optimum condition (from -115mV into the -404mV) indicated the contribution of protons in the oxidation process. Also, the binding constant of Glu. interaction with Mobic illustrated the positive value of K equal to $6.851x10^3$ with correlation coefficient of 0.9731. Hence, drugs in potential of anti-inflammatory activity which exhibiting antioxidant properties need to be done before recommending their use in clinical practice. As well, advised for taking antioxidant before administrating NSAIDs to reduce the oxidative stress.

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