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Optimization of Captopril Electrochemical Measurement with Box-Behnken and Taguchi Tests

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Abstract

In this research, an optimization of captopril electrochemical measurement with Box-Behnken and Taguchi experiments was performed using cyclic voltammetric method. Voltammograms were recorded using potentiostat-galvanostat Type III AUTOLABμ model (FRA2). In this study, a three-electrode system, a platinum electrode as the work electrode, a carbon electrode as auxiliary electrode and Ag / AgCl as a reference electrode were used. All experiments were carried out at room temperature. The effect of pH, scanning speed and electrolyte concentration were investigated and the pH = (1, 2, 3, 7) and scanning speed (0.3 - -0.1) V/s yielded the best results. Designing experiments and analyzing data was done using Minitab16 and Design-Expert 8.0.7.1 software based on the results of initial studies. The Box-Behnken design in this experiment could not provide optimal captopril measurements due to the lack of proper and reliable model, and Taguchi method, in addition to designing the experiment, provides optimal conditions for captopril measurement, which is also effective both in measuring concentration of electroactive material in a standard sample and in measuring the actual sample. The observed anodic peak current is equal to the captopril concentration in the ranges of 1 x 10^{-5} to 1 x 10^{-4} M in optimal linear conditions. The detection limit in this experiment is 1 x 10^{-8} M. Samples of captopril 25 mg tablets prepared from SOHA pharmaceuticals and were analyzed by Taguchi's proposed method, which yield favorable results.

Keywords: Captopril, Cyclic Voltammetry, Box-Behnken, Taguchi.

Introduction[

Chemists carry out electrochemical measurements on chemical systems for a variety of reasons. They may obtain a thermodynamic data of a reaction, or they may want to create an unstable interface such as a radical ion, or to study the rate of its destruction and its spectroscopic properties, or they may study a solution to measure its small amounts of metal ions or organic compounds (Mofidi, 2007)

The advantages of electrochemical methods in comparison to other methods include: simplicity, non-degradation analytical, high accuracy, high sensitivity and simple sample preparation. Therefore, the production of voltammetry sensors is important because of its significant advantages. Measuring the potential difference between two floating electrodes in a solution is called potentiometric. The distinction between various electrochemical techniques is related to the type of electrical signal used for measurement. The two main types of electrochemical measurements include potentiostatic and potentiometric methods, both of which require at least two electrodes (conductors) and one sample in contact with the electrode (electrolyte) that forms a chemical cell. Potentiostatic techniques can measure chemical species that are electroactive (can be oxidized or reduced) (Dick, 1973).

A cyclic voltammetry consists of a linear potential sweep method using a fixed and immobile work electrode in a stationary solution with the use of a triangular potential program (Yoshida , 1998). In addition to potential sweep, potentiostatic measures the current through the application of potentials. The resulting current curve is referred to as a cyclic voltammogram in terms of potentiality. A cyclic voltammogram has been used more than any other method for obtaining information on the properties of electrode reactions. The value of this method is due to the considerable information that immediately provides on the nature and the condition of the electrode reactions (whether cathodic or anodic), that is, with the kinetics of non-uniform electron exchange reactions, or the chemical reactions associated with the Electrode reactions, and finally absorption processes and their effects on electrode processes. A cyclic voltammetry in electrochemical decomposition is often the first method to be used to obtain qualitative information about the electroactive range or the electrochemical potential of the electroactivity of matters in the solutions. In cyclic voltammetry, the potential applied to a work electrode, which is a static solution (the solution is not interrupted), changes according to a “triangle” relative to time. In cyclic voltammetry, a variety of solid electrodes or hollow mercury droplets are used as work electrode (Mofidi, 2009).

Captopril (1-[3-mercapto-2-(S)-methyl-1-oxopropyl] (S)-proline) is one of the most famous and most used antihypertensive drugs that most people with hypertension are familiar with. Captopril is an inhibitor of renin enzymes, angiotensin, resulting in dilatation

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of arterial and venous arteries. Captopril is also effective in treating heart failure in addition to hypertension. Captopril is also distributed by Bristol-Myers Squibb as Capoten brand name (Akif et al., 2010). Captopril is obtained from the reaction of proline with 2-s-3-acetyltio-2-methylpropanoyl chloride in the presence of soda during the process of amminolysis of the acetyl group (Shimazaki et al., 1982).

Test design is a method to systematically performed a series of experiments. The purpose of this method is to obtain reliable and appropriate results based on a limited number of observations. The design of experiments uses statistical tools for this purpose. The main tools in this field are divided into two groups, which include test design and analysis tools; in the design of experiments, there are methods such as complete factorial method, Latin square-based methods, Box-Behnken method, and Taguchi method, etc., and in the analysis of tests, analysis of variance and its derivatives, and regression analysis are among the most important methods (Yadolah, 2008).

Box-Behnken design is a type of response level design. This design has a number of design points and a repetitive central point (Borkowski et al., 1995). In response level designs, two optimization methods are usually used, numerical optimization and graphical optimization. Numerical optimization, optimizes any combination of targets. Myers and Montgomery (1995) presented a multi-response method called the utility function. In graphical optimization, it is necessary to select a region of the testing space that simultaneously meets the conditions of all responses; for this purpose, a combined meter map can be used. Box-Behnken (Box et al., 1960). has proposed a three-level design to match the response level. This design is composed of a combination of 2k factorial design with incomplete block designs. The results of this design are usually efficient in terms of the number of experiments (Montgomer, 2008). The box-Behnken design is a spherical design which all of its points are located on a sphere of $\sqrt{2}$ radius. There are also no points on the corner of the cube in this design. In other words, there are no combinations of levels of controlled factors that are made from the combination of upper and lower levels.

Taguchi's methodology focuses on designing quality when designing products and processes. The Taguchi experimental design method is widely used in various industries, but this method can usually be used for off-line quality control. Taguchi's method for testing in this research is based on "parameter design".

Taguchi suggests two different ways to complete and implement the analysis. The first is the standard method in which the results of a test position or the average value of the result is obtained from the repetition of a test position by the major effect and ANOVA process. The second method, which he strongly recommends for tests with repetition, is the use of signal-to-noise ratio (S/N) for the same steps in the analysis. S/N analysis determines the best and most robust working conditions with the use of changing results.

In this research, an optimization of electrochemical measurement of captopril with Box-Behnken and Taguchi experiment designed was performed using cyclic voltammetric method.

Experimental section

Voltammetric measurements were performed the potentiostat / galvanostat device, Type III μAUTOLAB (FRA2) III. In voltammetric measurements, the Ag/AgCl reference electrode, the work electrode was a platinum electrode type and a glassy carbon auxiliary electrode.

The chemicals used in the tests were all prepared from the Aldrich and Bristol-Myers Squibb companies and they were used without any purification steps. The main solution of captopril with a concentration of 0.1 M was obtained by dissolving 2.18 g of captopril (molecular mass of 217.29) in 100 ml of distilled water. This material was well dissolved in distilled water. The captopril's solubility in the bass water, depending on its structure, is strongly dependent on pH. In order to understand the pH effect on peak current, solutions of captopril with a pH of 1 to 13 were made with 0.1 M of hydrochloric acid solution and 0.1 M solution of soda and their voltammograms were investigated. In voltammetric measurements, a solution of static captopril was prepared in order to determine the effects of the potential traveling velocity on the current and peak shapes, as well as the reversibility or irreversibility of the electrode process; and in a situation that other parameters affecting the Faraday reaction was kept constant, with a change in the potential traveling velocity, the Voltammogram remained fixed.

In the design of the test with Box-Behnken method using Design Expert8 software, 29 experiments were designed to investigate the effect of four factors: captopril concentration, pH, electrolyte concentration, and scanning speed at each of 3 levels. For all experiments, their voltammograms were recorded and analyzed by software; the obtained data were analyzed and optimized and the ability of this method was evaluated. In Taguchi experimental design using minitab16 software, 9 experiments with 3 repeat trials that is a total of 27 experiments were designed in 4 factors including captopril concentration, pH, electrolyte concentration and scanning speed, and 3 levels have been designed; all voltammograms of the experiments have been recorded and analyzed and optimized by the software; moreover, the ability of this method was evaluated. With the use of captopril, solutions of 0.1, 0.01 and 0.001 molar and also with the use of 1, 0.1 and 0.01 molar of chloride acid solutions, and from potassium chloride solid solutions of 0.1 and 0.01 0 and 0.001 molar, electrolytes were prepared and voltammograms of each experiment were analyzed by Taguchi method.

According to the Andels-Soyek equation (Equation 1), which indicates a direct relationship between peak current and electroactive concentration, and in order to study the effect of captopril concentration on voltammetric peak current, the experiments were implemented. According to the optimal method provided by the model, solutions of captopril from a

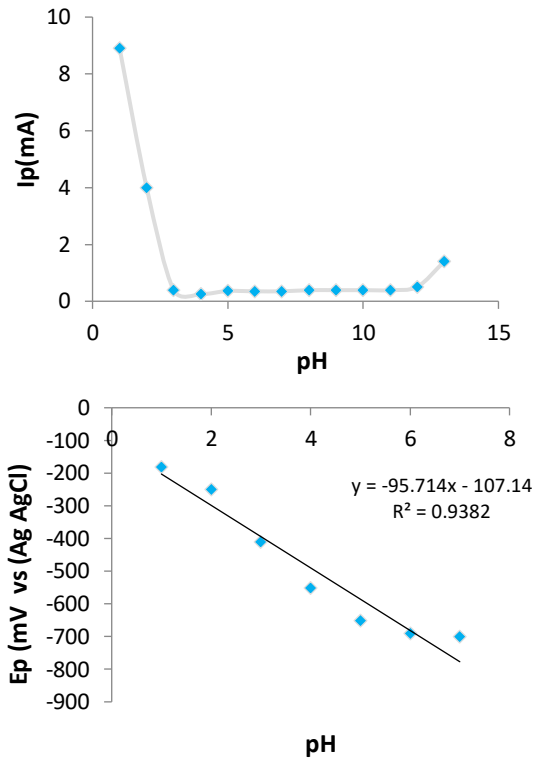
concentration of 1 x 10^{-4} M to 1 x 10^{-8} M was prepared and in a condition which all other parameters were in optimal conditions, the current-potential curves at each of these concentrations were recorded. In the following equation, I_p of the nose of the current (peak) in terms of Acm^{-2}, D is the penetration coefficient in $\alpha^{-1,}$ s^2mc is transmission coefficient, n_α is the number of electrons involved in the determining velocity, v is scanning speed according to Vs^{-1} and ax * ^{C} is the concentration of OX species in the soluble mass in molar values.

$$I_p = (2/99 \times 10^5)\, n(\alpha n_\alpha)^{1/2} AC_s D^{1/2} V^{1/2} \qquad \text{(Equation 1)}$$

Discussion and Conclusion

Results for both electrodes were studies when they used as working electrodes, and it was found that when the platinum electrode is used as a work electrode, the results are better, so the platinum working electrodes were considered. To all experimental solutions, a 10 ml of KCl solution of 0.1 molar was added to remove the current migration of the electro-active species. In this study, due to the lack of observance of the current flow in the operating conditions, captopril measurements on the glassy carbon electrode surface are not possible. However, according to the Voltammogram from the Platinum Electrode, this electrode is an appropriate option for this research.

Since the most redox process of the organic compounds depend on the pH of the solution, therefore, the voltammograms of captopril solution was recorded at constant concentrations at various pHs and the potential range mentioned above have been recorded relative to the Ag/AgCl reference electrode. A general review of the results showed that captopril at pHs of 1, 2, 3, 7 and 13 responded best. Figure 1 shows this important matter.



Graph 1: The effect of the pH of the solution on (a) peak current and (b) the captopril peak potential 0.1 M

The peak potential of captopril oxidation changes linearly by varying the pH of the solution, which resulted line yields a slope of -95 milli Volt per unit of pH change. This slope suggests that the number of electrons and protons involved in the captopril oxidation reaction are equal. In other words, the electrode's mechanism of action does not change in this pH range. On the other hand, it is observed that the anodic current in acidic and neutral pHs are higher than 3,7,2,1 respectively, and the sensitivity of the measurements were higher in these pHs. Therefore, the design of the experiments was carried out at these pHs. Based on the results, it can be said that captopril oxidation is performed according to the following electrode reaction:

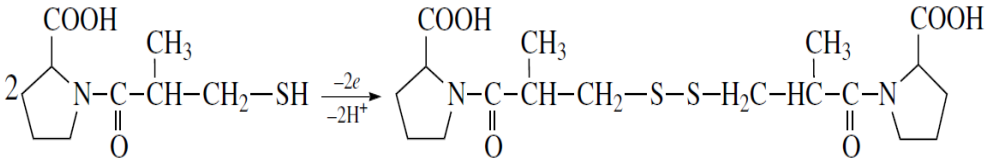
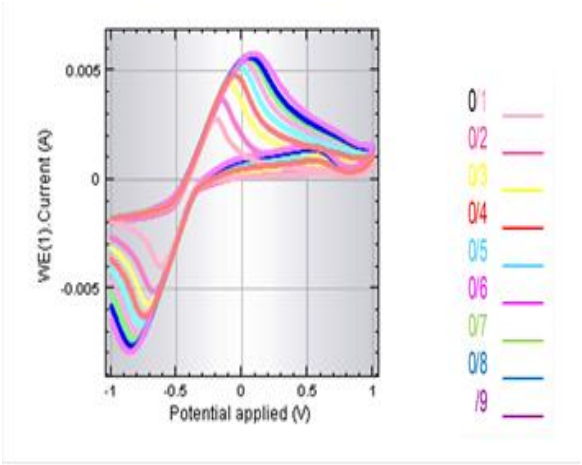


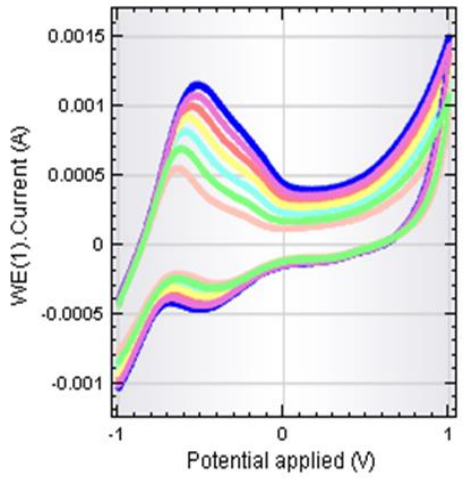
Figure 1: Captopril oxidation, platinum working electrodes

The relative consistency of stagnation of the current function with the velocity change indicates that captopril oxidation mechanism is an electrochemical reaction and there is no initial or final reaction in the oxidation process of this compound.

In order to evaluate the scanning speed on response of the system, and also to determine the mechanism of the transfer of material, captopril voltammogram at pH = 7 and pH =2 was study with a scanning speed of 0.1 to 0.9 v/s; the results are presented in Graphs 2 and 3.



Graph 2: Voltammograph resulted from captopril solution, 0.01 M pH = 2 and 10 ml KCl 0.1 molar on the surface of the platinum electrode, potential scanning ranges from -1 to 1 volt, and scanning speed from 0.1 to 0.9 V/s



Graph 3: Voltammograph resulted from captopril solution 0.01 M at pH = 7 and 10 ml KCl 0.1 molar on the platinum electrode surface, potential scanning ranges from -1 to 1 volt and scanning speed ranges from 0.1 to 0.9 V/s

In the design of the experiment, using the Box-Behnken method, 4 factors including captopril at concentrations of 0.1, 0.01, 0.001 molar, and pH 1, 2, 3, and 0.10 at 0.01, 0.001 M and speed of scanning at speeds of 0.3, 0.2, 0.1 V/s had been designed (Table 1).

Table 1: Includes 4 factors in 3 effective levels in the measurement with Box-Behnken Method

P value (Scan rate)	P value (Electrolyte)	P value (pH)	P value (Captopril)	Level
0.3	1	3	1	+
0.2	2	2	2	0
0.1	3	1	3	-

By setting the number of parameters and the number of levels in the design expert program, 29 experiments were performed to optimize this measurement by the software in accordance with Box-Behnken design. The use of Box-Behnken statistical method is due to the reduction of the number of tests and the simultaneous examination of the experiments. The voltammogram of these experiments was recorded and the maximum current of each test was presented in Table 2.

Table 2: Box-Behnken Design Includes 29 Random Tests for Measuring Optimization

Run	The Experiment Number	P value (Captopril)	P value (pH)	P value (Electrolyte)	P value (Scan rate)	Current peak (A)
1	23	-	-	0	0	0.0023053
2	28	+	-	0	0	0.00029568
3	19	-	+	0	0	0.00490994
4	21	+	+	0	0	0.006799
5	9	0	0	-	-	0.000298888
6	5	0	0	-	+	0.00040475
7	7	0	0	+	-	0.0049823
8	10	0	0	+	+	0.002283
9	4	-	0	-	0	0.002888
10	6	+	0	-	0	0.00037155
11	17	-	0	+	0	0.00013345
12	2	+	0	+	0	0.0023205
13	3	0	-	0	-	0.0022469
14	13	0	+	0	-	0.0020944
15	1	0	-	0	+	0.0025385
16	8	0	+	0	+	0.00036209
17	22	+	0	0	-	0.001485
18	12	-	0	0	+	0.0023056
19	24	+	0	0	+	0.0027921
20	20	0	-	-	0	0.0017038
21	16	0	+	-	0	0.00206545
22	27	0	-	+	0	0.0034219
23	15	0	+	+	0	0.0033783
24	25	0	0	0	0	0.00041113
25	18	0	0	0	0	0.0040475
26	14	0	0	0	0	0.0020416
27	26	0	0	0	0	0.0024659
28	29	0	0	0	0	0.0060345
29	11	0	0	0	0	0.0025668

The results were evaluated using Design Expert8 software. Table 3 to Table 5 shows the results of statistical analysis of the data obtained from the implementation of the Box-Behnken design. A statistical survey of Tables 3 to 5 shows that two-factor interaction model has a relatively higher quality for describing the response procedure in the experimental space. As it can be seen, the F statistic for the two-factor interaction model has the highest value of 2.49. The magnitude of this statistic and the smallness of the p value for the two-factor interaction model show that the model with a confidence level of 3.7 percent cannot describe the quantitative relation between the experimental factors and the

maximum peak current. Also, the two-factor interaction model has the maximum value of R^2 statistics and predicted R^2 and not a minimum PRESS and standard deviation. Table 4 shows the results of variance analysis for the selected response procedure model.

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Table 3: Specifications of different models of response procedures

Sum of squares of successive models					
p-value	**F**	**Square of Means**	**Degree of Freedom**	**Sum of Squares**	**Source**
		1148.38	1	1148.38	Average
0.1233	0.02	1.98	4	7.90	Linear
0.0621	2.49	1.77	6	10.64	Two-factor interaction
0.4961	0.89	0.65	4	2.59	Second order
0.3296	1.47	0.84	8	6.76	Cubical
		0.58	6	3.46	Remainder
		40.68	29	1179.72	Total

Sum of squares of successive models						
Comment	**p-value**	**F**	**Square of Means**	**Degree of Freedom**	**Sum of Squares**	**Source**
	0.0501	5.80	1.13	20	22.66	Linear
	0.0816	4.39	0.86	14	12.20	Two-factor interaction
	0.0714	4.83	0.64	10	9.43	Second order
	0.0512	6.84	1.34	2	2.67	Cubical
			0.20	4	0.78	Overall error
Significant	0.0370	2.61	1.85	1	18.54	The response procedure model based on two-factor interaction
	0.1023	2.96	2.11	1	2.11	A-A
	0.0419	4.80	3.41	1	3.41	B-B
	0.0908	3.19	2.27	1	2.27	C-C
	0.6984	0.16	0.11	1	0.11	D-D
	0.4365	0.63	0.45	1	0.45	AB
	0.5866	0.31	0.22	1	0.22	AC
	0.0094	8.46	6.02	1	6.02	AD
	0.2804	1.24	0.88	1	0.88	BC
	0.1411	2.37	1.69	1	1.69	BD
	0.1779	1.59	1.39	1	1.39	CD
			0.71	18	12.80	Remainder
Not significant	0.0816	4.39	0.86	14	12.02	Lack of Fitness

Table 5: Summary of the Status of Model Statistics

PRESS	**Predicted R^2**	**Adjusted R^2**	**R^2**	**Standard Deviation**	**Source**
35.64	-0.1372	0.1274	0.2521	0.99	Linear
37.54	-0.1980	0.3646	0.5915	0.84	Two-factor interaction
55.53	-0.7719	0.3484	0.6742	0.85	Second order
386.20	-11.3230	0.4855	0.8898	0.76	Cubical

The results of ANOVA also show that the equation of two-factor interaction, well describes quantitative relationship between peak current and experimental factors. As it can be seen, the main factor of pH, interaction effect of AD, with a 95% confidence level is significant. The results also show that although the quantity of disparity for the model is not significant for two-factors interaction, only 8.16% of this chance exists, which is very weak and disturbing probability.

The equation coefficients for selected two-factor interaction model which were obtained by implementing the Box-Behnken design for measuring captopril, are obtained and they are presented in Equations 2 and 3, respectively, based on the coded values as well as the actual values of the experimental factors.

Ln(R1) = - 6/29 + 0/42A -0/53B -0/43C -0/096D +0/34AB -0/23AC +1/23AD -0/47BC -0/65BD +0/59CD

(Equation 2)

Ln(R1) = -15/32284 - 3/17193A +0/49774B -1/46076C +48/32773D +0/33560AB -0/23346AC +12/26439AD -0/46930BC -6/49167BD +5/88713CD

(Equation 3)

The results of variance analysis (Table 4) showed that the disparity of two-factor interaction model is not significant and the model is well-suited, but only 8.16% of this chance exists, which is very weak and disturbing probability.

In Taguchi experimental design, experiments were designed from 4 factors in 3 levels in accordance with Table 6.

Table 6: Includes 4 factors in 3 effective levels in Taguchi measurement

P value (Scan rate)	P value (Electrolyte)	P value (pH)	P value (Captopril)	Level
0.1	0.1	1	0.1	1
0.2	0.01	2	0.01	2
0.3	0.001	7	0.001	3

By setting the number of parameters and the number of levels in Minitab program by software, the following experiments were designed by Taguchi method. According to Table 7, the proposed experiments were performed and their voltammogram were recorded. The results of these experiments were included in the peak current. Also the signal-to-noise ratio was also deviation from the mean and average of each experiment.

Table 7: Taguchi design includes 27 experiments to optimize measurement with results

Rank	P value (Captopril)	P value (pH)	P value (Electrolyte)	P value (Electrolyte)	Peak Current	SNR	STD	Mean
1	1	1	1	1	0.0035455	-48.1691	0.0004238	0.0039490
2	1	1	1	1	0.0039109	*	*	*
3	1	1	1	1	0.0043906	*	*	*
4	1	2	2	2	0.0034460	-48.3773	0.0003462	0.0038452
5	1	2	2	2	0.0040265	*	*	*
6	1	2	2	2	0.0040361	*	*	*
7	1	3	3	3	0.0010764	-59.7403	0.0000455	0.0010324
8	1	3	3	3	0.0010352	*	*	*
9	1	3	3	3	0.0098550	*	*	*
10	2	1	2	3	0.0037860	-48.2932	0.0000702	0.0038502
11	2	1	2	3	0.0039252	*	*	*
12	2	1	2	3	0.0038364	*	*	*
13	2	2	3	1	0.0019647	-54.2431	0.0000524	0.0019416
14	2	2	3	1	0.0019785	*	*	*
15	2	2	3	1	0.0018817	*	*	*
16	2	3	1	2	0.0003959	-66.8406	0.0000585	0.0004630
17	2	3	1	2	0.0004897	*	*	*
18	2	3	1	2	0.0005035	*	*	*
19	3	1	3	2	0.0088492	-41.5813	0.0004373	0.0083578
20	3	1	3	2	0.0080115	*	*	*
21	3	1	3	2	0.0082126	*	*	*
22	3	2	1	3	0.00476229	-46.8604	0.00019	0.0045469
23	3	2	1	3	0.0044720	*	*	*
24	3	2	1	3	0.0044058	*	*	*
25	3	3	2	1	0.0000735	-79.6003	0.0000567	0.0001298
26	3	3	2	1	0.0001289	*	*	*
27	3	3	2	1	0.000187	*	*	*

The results of the designed experiments by Taguchi method were statistically evaluated. In the Taguchi method, for statistical analysis and more precise results, a converted response function is used which is defined as the ratio of the sign of each effect (s) to the effects of the error (n). The advantage of using this new response in statistical analysis, relative to the initial form of response is to compare the magnitude of the effects of each major factor with the effects of more precise error and distortion factors than the actual effects of factors on the system.

How to calculate S/N ratio depending on which type of optimization, is different. Since the response in this study is considered to be the peak current, and the goal is to maximize the response, the S/N ratio is calculated based on Equation (4).

$$\frac{S}{N} = -10 \log \frac{(1/y_1^2 + 1/y_2^2 + \Lambda + 1/y_n^2)}{n}$$

(Equation 4)

y in this equation id the measured response value for each experiment in each test, and n is the number of replicates of the experiments (here it is equal to 3). The new goal is to maximize this answer. Analysis and presentation of the charts was done by Minitab.

According to Fig. 4, in the captopril level 1 concentration factor, pH factor 1, electrolyte concentration level 3 and sweeping velocity factor level 3 are optimal.

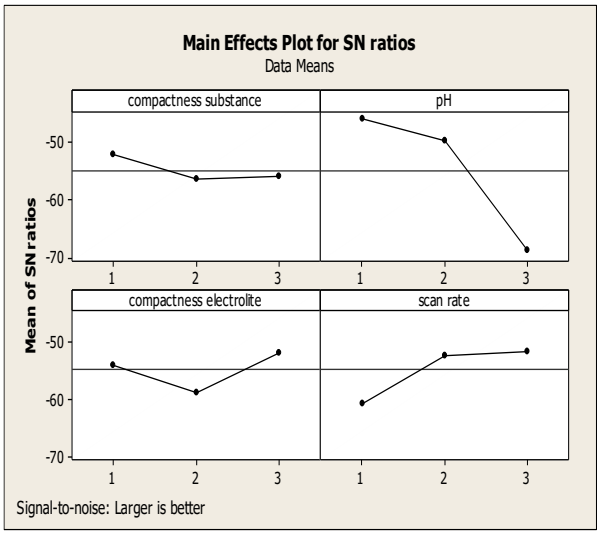

Figure 2: Signal-to-noise ratio at each factor level

Taguchi predicts the optimal performance in Table 8 with the referred results.

Table 8: Prediction of results for optimal performance

Parameter	P value (Captopril)	P value (pH)	P value (Electrolyte)	P value (Scan rate)	SNR	STD	Mean
Level	1	1	3	3	-37.0278	0.00030240	0.00587690

Then, at the optimal levels of the factors, that is the captopril concentration level 1, pH factor level 1, electrolyte concentration factor level 3, sweeping velocity factor level 3 were confirmed (Table 9 and Figure. 3). Which according to the experimental results graph is close to the theoretical results presented by Taguchi.

Table 9: Optimal conditions for measuring captopril

Studied Parameters	Calculated optimum amount	S/N rates at optimum conditions
Captopril concentration (molar)	0.1	-52.0956
pH	1	-46.0145
Electrolyte concentration (molar)	0.001	-51.8549
Scan speed V/s	0.3	-51.6313
Average current responses in experiments	---	-54.640
The predicted response in optimal conditions (S/N)	---	-37.0278

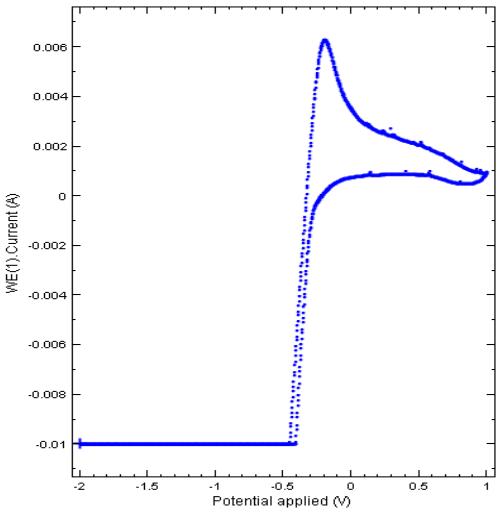

Figure 3: Resulted voltammograms from captopril solution, 0.001M at pH = 1 and 10 ml of 0.001M KCl, on platinum electrode, potential sweep range from -1 to 1 volt and scan speed 0.3 V/s

Table 10 shows the results of standard ANOVA analysis for factors and their levels. From the results of this table, it can be concluded that all 4 parameters are involved in this analysis, but the share of the contribution of pH is relatively more effective.

Table 10: Results from ANOVA analysis

Row	Parameter	Degree of Freedom		Sum of Squares	Variance	Contribution share
1	Captopril concentration	2		2.29298×10^{-5}	1.14649×10^{-5}	14.333%
2	pH	2		0.000106475	5.32376×10^{-5}	66.5562%
3	Electrolyte concentration	2		5.90587×10^{-6}	2.95294×10^{-6}	3.691%
4	Scanning speed	2		2.15886×10^{-6}	1.07943×10^{-5}	13.49497%
5	Error	18		3.07819×10^{-6}	1.71011×10^{-7}	1.92%
6	Total	26		0.000159978	7.86208×10^{-6}	100%

In order to determine the linear boundary of the system response, the response of the platinum electrode current was recorded at pH = 1 and KCl = 0.001 and the scan speed was 0.3 at different concentrations of captopril, and the results are presented in the calibration curve shown in Figures 4 and 5.

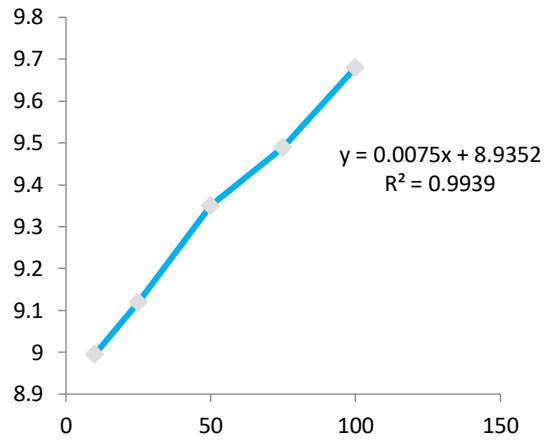

Figure 4: Calibration curve obtained for different concentrations of captopril at pH = 1 and 0.001 molar KCl 0 and scanning speed 0.3 V/s

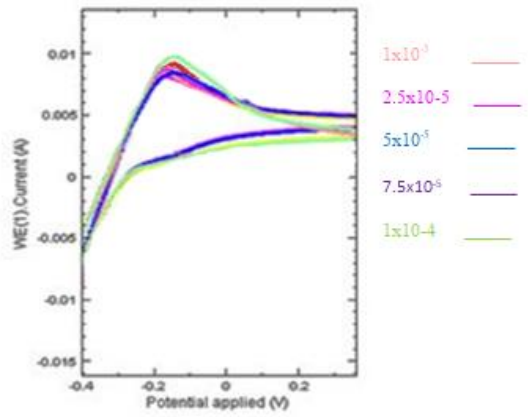

Figure 5: The obtained voltammogram for different concentrations of captopril in pH = 1and 0.001 molar KCl and scanning speed of 0.3 V/s

The results indicate a continuous increase in peak current with increasing captopril concentration in accordance with the Rendal-Soyek equation. The anodic peak current curve according to the concentration shown in Figure. 4 shows that in the concentration range from 1×10^{-4} to 1×10^{-5} molar, there is a linear relationship with the coefficient correlation of 0.99 between the concentration and the anodic peak current that this dependence can be shown in the form of the following equation:

$$I_p(mA) = 7/5 \times 10^{-3} \ [Captopril] + 8/9352$$

(Equation 5)

Accordingly, we can consider the range of concentrations from $1x10^{-4}$ to $1x10^{-5}$ molar as the working range of the electrode.

With the use of 25 milligram captopril from Iran Soha Pharmaceuticals, 22.9 µM solution have been prepared. 5 pills had been grounded into 250 ml of distilled water and then 10 ml of this solution was poured into 1,000 ml of distilled water and the voltammograms of prepared solution have been recorded and presented in optimum conditions. This experiment has been repeated many times. The average I_p obtained is 9.102 mA. By putting this value in Equation 5, the following results are obtained (Table 11) and (Fig. 8).

Table 11: Experimental and theoretical Results of Captopril Pills

Error rate (µM)	Obtained concentration of Captopril (µM)	Theoretical concentration of Captopril (µM)	Peak current (mA)
-0.66	22.24	22.9	9.102

Figure 6: Obtained voltammograms of the actual solution of captopril, at pH = 1 and 10 ml of KCl 0.001 molar on the surface of the platinum electrode, the potential scanning range from -1 to 1 volt and the scan speed of 0.3 V/s

Therefore, in the current study, platinum electrode was introduced in this measurement as the appropriate electrode. The design of the experiment by the Box-Behnken method in the two-factor interaction model although did not have a significant disproportion, but the predicted responses by the model did not agree well with the actual values. Therefore, Box-Behnken cannot be efficient in the optimization measurements. In contrast, optimization with the use of Taguchi experimental design provided a well-optimized model that is closer to actual response values. Also, according to Taguchi method, the best conditions for the analysis of captopril concentrations are pH = 1 and KCl= 0.001 M and the scanning speed of 0.3 V/s. That this optimal design in the experimental was predicted correctly.

References

Akif, M.; Georgiadis, D.; Mahajan, A.; Dive, V.; Sturrock, E. D.; Isaac, R. E.; Acharya, K. R. 2010, Journal of Molecular Biology , p: 502

Borkowski ,John J. 1995, Spherical Prediction-Variance Properties of Central Composite and Box—Behnken Designs, Technometrics, 374, pp: 399-410

Box, G. E. P., Behnken, D. W., 1960, "Some New Three Level Designs for the Study of Quantitative Variables." Technometrics, Vol. 2, pp: 455-476.

Dick, J. D.,1973, Analytical Chemistry, McGraw-Hill, London, p:647.

Mofidi, J, 2007, Electrochemical Principles, Tehran University Press, Eighth Edition

Mofidi, J, 2009, Analytical Electrochemistry, Seventh Edition, Tehran University Press

Montgomery, D.C., 2008, .Design and Analysis of Experiments, John Wiley&Sons Inc.

Shimazaki, M.; Hasegawa, J.; Kan, K.; Nomura, K.; Nose, Y.; Kondo, H.; Ohashi, T.; Watanabe, K., 1982. Chem. Pharm. Bull. 30, pp: 3139–3146.

Yadolah Dodge, 2008, “The Concise Encyclopedia of Statistics”, Springer, pp: 162-163

Yoshida, K., 1998, "Electro oxidation in Organic Chemistry", john Wiley and Sovs, New York.