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Research Paper

Factor Effect Estimation in Ciprofloxacin-Iron Interaction

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Abstract- *Interaction of drug molecules with exogenous substances such as metal ions is of significant importance from both physiological and pharmaceutical aspects of view. The effects of different variables on complex formation between ciprofloxacin and Iron (III) ions were evaluated and compared via facile modern statistical techniques such as Response Surface Methodology (RSM), Ruggedness testing, Factorial design and Taguchi method. Results of all methods were in relatively good agreement with each other and revealed that contact time of the reactants and pH may play more important roles in complex formation between ciprofloxacin and ferric ions.*

Keywords: Ciprofloxacin, Ruggedness, Complexation, Box-Behnken design.

Introduction

Ciprofloxacin (CIP) with the IUPAC name of 1-Cyclopropyl-6-Fluoro 4-oxo-7- Piperazin-1-yl- Quinoline-3-Carboxylic acid is an antibacterial agent belonging to the major family of synthetic flouroquinolones which is administrated for various infections of the body (Figure 1). Because of the complexity in the pharmacokinetics of flouroquinolones there is a potential for several types of drug interactions [1]. The adsorption of quinolone drugs is decreased when they are administrated simultaneously with aluminium and magnesium antacids or other metal ions present in pharmaceuticals. The proposed reason for such behavior is the chelate formation of the quinolone with metal ions [2].

Compounds including carboxylate group are among the most ubiquitous compounds and the increasing interest in their complexes can be attributed to significantly important biological properties [3-4]. Ciprofloxacin acts as a bidentate deprotonated ligand bound to the metal ions through the carbonyl oxygen of pyridine ring and one carboxylate oxygen [5-6]. High molecular weight and molar absorptivity of ciprofloxacin provides a supporting evidence of its suitability for spectrophotometric methods. The major application of spectrophotometric methods is in the quantitative analysis of metal ions, anions, complex ions and other organic species. Preparation and investigation of metal complexes with biologically active compounds, in which the drug molecules play a role as ligand, have been regarded as a research domain of increasing interest for inorganic, pharmaceutical and medicinal chemistry. These studies have absorbed much attention as an approach to new drug development.

Complex formation between ciprofloxacin and some metal ions have been reported [5- 8], but no systematic optimization procedures have been performed except one for zirconium, Molybdenium, vanadium and Tungesten complexes with ciprofloxacin [8]. Some complexes of ciprofloxacin were screened for their activity against several bacteria, showing similar to that of the corresponding free ligands [7]. It is well understood that in order to obtain the best qualitative and quantitative metal complex yields, several effective variables need to be carefully optimized. Experimental designs are called into play every day in questions of industrial planning, resource allocation, scheduling, laboratorial processes and etc. classic optimization method can be run by varying any one of the process parameters and keeping the other parameters constant. When multiple variables are involved, it becomes difficult to study the system using the common approach of varying only one factor at a time, while holding the others constant. The statistical new designs consider all factors simultaneously and hence provide the possibility for evaluation of the whole effects at once. Modern experimental designs have been regarded as one of the most favorable techniques in covering a wide area of practical statistics and obtain unambiguous results with the least expense. Designs on the bases of two levels for each factor are called Ruggedness-testing or screening designs [9]. This design is similar to that for 2^K fractional factorial design and provides information about the first order effect of each

factor [10]. The experimental design for Ruggedness testing is balanced in that each factor level is paired on equal number of times with uppercase and lowercase levels for every other factor.

One of the most efficient and common statistical designs is response surface method which have been designed for factors with more than three levels in which quadratic models can be established. The most popular response surface methodologies are Central composite [11], Box-Behnken [11-13] and Doehlert designs [14]. Box-Behnken designs are response surface designs, particularly made to require only 3 levels, coded as-1, 0, and +1. They are formed by combining two-level factorial designs with incomplete block designs. This procedure creates designs with desirable statistical properties but, most importantly, with only a fraction of the experiments required for a three-level factorial. Because there are only three levels, the quadratic model is appropriate.

In continuation to our work in factor effect estimations on drug-metal interactions [15-16], we aimed at determining the effect of different variables such as pH, temperature, ciprofloxacin concentration, stirring rate, contact time, solvent type and ionic strength on complex formation via different statistical and experimental design techniques.

Material and Methods

Ciprofloxacin powder was purchased from RAZAC pharmaceutical Co. (Iran) with a high purity (> 99.8%). Standard solution of Fe (III) was prepared via dissolving of pure Iron in 1:3 nitric acid solutions. Distilled deionized water was used for preparation of all solutions. All Spectrophotometric measurements were performed using Backman DU60 model with quartz cells. Acidity measurements were done by digital 523 pH meter. The interaction between ciprofloxacin and metal ions were studied in the presence of 10^{-1} M tartarate ion in aqueous / ethanolic solution to avoid hydrolysis.

All the experimental designs were organized using seven factors at assigned levels except for Box-Behnken design which was performed by 4 factors. All statistical analysis, modeling and numerical optimization was performed using Minitab 15 statistical software and Design-Expert software-v.6 (State-Ease, Corp., Minnesota). For all experiments, 2 ml of 5×10^{-3} M aqueous solution of ferric ion and 2 ml of acetate buffer were mixed and shaken with 4 ml of ethanolic or aqueous ciprofloxacin reagent in desired conditions.

Results and Discussion

Effects of various parameters have been evaluated in the Iron-ciprofloxacin complex formation, pH (factor A), temperature (factor B), ligand (ciprofloxacin) concentration (factor C), contact time (factor D), ionic strength (factor E), solvent type (factor F) and stirring rate (factor G). Solvent type is an example of categorical factors which can not be indicated by numerical levels. For this qualitative variable, two assigned levels were considered as water and ethanol. (See Table 1)

The percentage ferric ion complexation with ciprofloxacin reagent was considered as response. The average of three replicate measurements was used for each trial in different testing methods. The percentage of removed metal ion for each treatment can be calculated using equation (1).

$$\%\text{Metal binding ability} = \text{Metal ion removal } (\%) = \left(\frac{A_0 - A}{A_0}\right)\times 100 \quad ..(1)$$

Where A_0 is the initial metal ion absorbance and A represents the final absorbance of metal ion solution.

For performing Ruggedness testing, all the experiments were performed according to the protocols introduced in Table 3. In the factors under study, the upper limit for each factor is shown by a capital letter while a small letter indicates the lower limit of each factor.

In Ruggedness method, the effect of changing the level for each factor, E_i, is determined via subtracting the average response when the factor is at its uppercase level from the average value when it is at its lowercase level,

$$E_i = \frac{(\sum R_i)_{uppercase}}{4} - \frac{(\sum R_i)_{lowercase}}{4}$$

The ordered calculated effects by their absolute levels for each variable are listed in Table 4.

In factorial design, all the experiments were performed according to the protocols introduced by a two-level factorial design in Table 5. The upper and lower limits for each factor are coded. Number of center points per block and number of replicates for corner points were set to zero and 1, respectively.

For Taguchi design, the whole experiments were performed according to the protocols introduced by a L8 Taguchi design in Table 6. The upper and lower limits for each factor are coded.

Main effect plots for factorial and Taguchi designs are depicted in Figure 3 and 4, respectively.

It was revealed by Ruggedness testing that in ciprofloxacin-Fe complex formation , pH (factor A) posed the greatest effect on the response while ligand concentration (factor C) had the least significant effect on response. The least effect of ligand concentration may be explained by this fact that ciprofloxacin is present in sufficient amounts to form a 1:2 complex with ferric ions and variation of its concentration wouldn't have a sensible effect on response. Acidic medium was required due to the lack of metal hydroxide precipitation but the results showed preference of slightly acidic media to strongly acidic media. The reason may be attributed to the better metal binding because of the higher hydrolysis in carboxylic group and producing negative charged carboxylate site which tends to chelate with positive ferric ions.

Type of solvent used for dissolving ciprofloxacin reagent possesses the second great effect on response which demonstrates that solvent selection is of significance importance. Ethyl alcohol may be prefered since it provides

a medium in which ciprofloxacin molecules would have a more opportunity to collide efficiently with ferric ions to form a desired complex maybe due to the less amount of hydrogen bonding. The variation effect of solvent level showed that ciprofloxacin (not ciprofloxacin-HCl) tends to dissolve more in ethyl alcohol than in water which is in the agreement with previous works [17]. Ionic strength had a moderate effect on response in the way that increasing its level (higher KNO_3 concentrations) would result in more complexation reaction which has been also reported before [18]. This effect can be explained by the increase in reagents solubility and hence more effective interactions as a result of higher ionic strength. Similarly, an increase in contact time would lead to more rapid equilibrium attachments and hence, more complexation efficiency.Increasing temperature to ambient conditions would allow more thorough reaction owing to more collisions but as expected the effect was not as significant as others since higher values were not tested due the possible reagent degredation. Stirring rate had a positive effect on response since as expected provided better surface area for reactants to collide each other.

To conduct a Box-Behnken method four variables under study were chosen as pH, contact time, temperature and ionic strength which were designated as A, B, C and D. The design levels in terms of coded and actual forms and related response values are shown in Table 7. A Box-Behnken design matrix containing 29 experiments was planned to investigate all possible combinations of factors (Table 8).

With the Box-Behnken design methodology, major and interaction effects can be easily evaluated. The major effect refers to the effect caused by the varied factor, while the interaction effect is related to the case which the effect of one factor is dependent on the value of another factor [19].

The significant factors in the regression model can be estimated by performing analysis of variance [19]. A standard analysis of variance (ANOVA) showed the best fit with a quadratic model (values of Prob>F less than 0.0001). The Model F-value of 23.02 implied that the model was significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicated that the model terms were significant.
(See Table 8)

In this case A (pH), B (contact time), C (temperature) and C^2 are significant model terms. Values greater than 0.1 indicate the model terms are not significant.Therefore regarding the F-values the efficiency order of factors can be ordered as contact time > pH > Temperature > Ionic strength. It was revealed that the complexation process highly depended on contact time which also showed the robustness of the analysis method. The effect of pH was also significant as it is directly related to the ciprofloxacin structure (neutral, anion or cation) taking part in the chelation reaction with ferric ions.

The comparative study of different factor interactions revealed the order of importance as: $C^2 > A^2 > CD > D^2 > B^2 > AC, AD > AB \approx BC \approx BD$. Because the model contained significant and non-significant terms, it was reduced by elimination of insignificant terms to achieve the desired model (Table 10). Therefore the new model terms would be A, B, C, A^2 and C^2.

The modification of the model did not affect the adequacy of the model since the R^2 and the adjusted R^2 for reduced model were satisfactory and the predicted R^2 value enhanced (Table 11).

Where R^2 is a measure of the amount of deviation around the mean explaned by the model and adjusted R^2 is the R-squared adjusted for the number of terms in the model relative to the number of points in the design. Predicted R-squared is a measurment of the amount of variation in new data explained by the model.

The final regression equation expressing the dependence of Fe (III) concentration change (Δc) on significant variables in terms of coded factors was reduced to:

$$\Delta c = +48.41 - 4.92A + 12.92B + 2.25C + 2.13A^2 - 3.37C^2 \quad \text{.....(3)}$$

Where A is pH, B is the contact time and C is the temperature. Note that no two-factor interaction terms are included in this equation as they were insignificant model terms. This indicates that various experimental variables make their own effect on response without relatively no interaction with other variables. The relative effect of each factor in this equation is described by its coefficient and algebric sign [20]. It was revealed from regression equation that complexation reaction increased with decrease in pH (negative sign). In contrast increasing the reaction period and temperature will provide an apportunity for metal ions to bind to ciprofloxacin molecules.

Model graphs are represented in terms of coded factors and actual factor levels. In the model graphs (Figure 5) the most effective factor (B) can be demonstrated by its steep slope. While the variation of iron binding strength with pH was less and temperature (C) had no significant effect.

The perturbation plot of reduction in Fe (III) concentration against all four investigated factors (Figure 6) showed supporting evidence of the importance of contact time (factor B) and pH (factor A) on the complexation process. Perturbation plot shows variations in the Fe (III) concentration as each variable moves from the chosen reference, with all other factors held constant at the middle of the design space (The coded zero level) [21]. Ionic strength produced an insignificant effect whereas the temperature effect was developed through moving from middle level.

Considering different statistical methods revealed that all of them produced nearly similar trends. In Ruggedness testing, Taguchi design and factorial method, acidity of the midium was considered as the most determinant variable. Ruggedness testing, Taguchi design and factorial methods considered nearly no significant effect for ciprofloxacin concentration. Contact time for reactants had a positive effect on response in all methods while the

estimated effect was more tangible in factorial design and less pronounced in Taguch design (Figure 4). For stirring rate, again all the methods considered a moderate positive effect on complex formation except for Taguchi design that represented low effect. For temperature, the trend was almost the same which could indicate the increase in effective collisions of metal ions and drug via much higher kinetic energies. The effect of ionic strength was best pronounced via factorial method with the net positive effect for all methods. Type of applied solvent had the greatest influence in Ruggedness method, little effect in Taguchi design and no observable effect in factorial design. Consequently, one may deduce that various statistical methods for evaluation of effective factors on response values represent the more compatible and trusty results for most and least significant variables. Of course this can not be attended as a general rule and more experimental and statistical investigations need to be done.

The relationship among the variables (pH, contact time, temperature and ionic strength) and response was described by quadratic response model. High resulted coefficient of regression values proved the fitness of the selected model in analyzing the experimental data. The complexation of ferric ions with ciprofloxacin was strongly influenced by the contact period and pH while no significant effective two-factor interaction terms could be observed except A^2 and C^2 which demonstrated that each factor under study affected the response relatively independently. Except for contact time and medium acidity, the predicted order for temperature and ionic strength were the same as Ruggedness testing, Taguchi design and factorial methods. The effect of temperature could be interpreted as before while the complex formation could be negatively affected in much higher temperature maybe due to the instability of drug molecule.

Conclusion

The effect of various categorical and numerical variables on metal–drug complex formation could be well evaluated via developing simple mathematical methods such as Ruggedness testing, factorial design and Taguchi method and more advanced response surface techniques. Based on such calculations, it was found that complex formation between ciprofloxacin molecule and Fe (III) ions was mostly responsive to the contact time of reactants and also pH of the chemical medium, hence, needed to be well adjusted. However, the outputs of different statistical methods were in reliable consistency with each other. The best results could be expected for the most and least determinant variables which maybe due to their sensitivity level. According to the obtained data, ciprofloxacin can be sequestered fairly well by ferric ions provided that the levels for experimental factors held at optimum levels. These and similar findings are important in interaction of metal ions with typical drug molecules posing significant effects on drug interactions and the pharmacokinetic profile. Drug interactions with versatile exogenous substances are under further investigation.

Acknowledgement

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Table 1: Actual levels of experimental factors for factorial, Taguchi and Ruggedness methods applied for interaction of ciprofloxacin with ferric ions

Factors	Low level	High level
pH	2	4
Temperature (°C)	15	25
Ciprofloxacin concentration (M)	0.01	0.02
Contact time (min)	3	5
Ionic strength (M)	0.001	0.005
Solvent type	water	ethanol
Stirring rate (rpm)	90	120

Table 2: Experimental design of 7 factors each in two levels according to Ruggedness testing

Run	Factors combination							Response
1	A	B	C	D	E	F	G	R_1
2	A	B	c	D	e	f	g	R_2
3	A	b	C	d	E	f	g	R_3
4	A	b	c	d	e	F	G	R_4
5	a	B	C	d	e	F	g	R_5
6	a	B	c	d	E	f	G	R_6
7	a	b	C	D	e	f	G	R_7
8	a	b	c	D	E	F	g	R_8

Table 3: Ordered factor effects on ciprofloxacin- ferric ions interaction

Factor	Effect
pH	$E_A = 0.320$
Contact time	$E_D = 0.275$
Solvent type	$E_F = 0.195$
Stirring rate	$E_G = 0.180$
Temperature	$E_B = 0.030$
Ionic strength	$E_E = 0.025$
Ligand concentration	$E_C = 0.020$

Table 4: Experimental design of 7 factors according to two-level factorial design

Run	pH	Temperature	Ligand conc.	Contact time	Ionic strength	Solvent type	Stirring rate
1	1	-1	-1	-1	-1	-1	1
2	-1	1	1	-1	-1	-1	-1
3	1	1	-1	1	-1	1	-1
4	-1	1	-1	-1	1	1	1
5	-1	-1	-1	1	1	-1	-1
6	1	1	1	1	1	-1	1
7	1	-1	1	-1	1	1	-1
8	-1	-1	1	1	-1	1	1

Table 5: Experimental design according to L8 Taguch design

Run	pH	Temperature	Ligand conc.	Contact time	Ionic strength	Solvent type	Stirring rate
1	-1	-1	-1	-1	-1	-1	-1
2	-1	-1	-1	1	1	1	1
3	-1	1	1	-1	-1	1	1
4	-1	1	1	1	1	-1	-1
5	1	-1	1	-1	1	-1	1
6	1	-1	1	1	-1	1	-1
7	1	1	-1	-1	1	1	-1
8	1	1	-1	1	-1	-1	1

Table 6: Levels of experimental factors in response surface study of ciprofloxacin-Fe (III) interaction

Factors	Low level	Medium level	High level
pH, A	2.5	4	5
Contact time (min), B	3	5	7
Temperature (ºC), C	15	25	40
Ionic Strength (M), D	0.005	0.01	0.02

Table 7: Box-Behnken design with actual and coded factor levels for ciprofloxacin-Fe (III) interaction

Run	pH	contact time (min)	Temperature (°C)	Ionic strength (M KNO_3)	Reduction in Fe (III) concentration (%)
1	2.5(-1)	5(+1)	25(0)	0.05(0)	38
2	5(+1)	5(+1)	25(0)	0.05(0)	34
3	2.5(-1)	25(0)	25(0)	0.05(0)	65
4	5(+1)	25(0)	25(0)	0.05(0)	60
5	4(0)	15(0)	15(0)	0.01(-1)	44
6	5(+1)	15(0)	40(+1)	0.01(-1)	48
7	4(0)	15(0)	15(0)	0.1(+1)	41
8	4(0)	15(0)	40(+1)	0.1(+1)	51
9	2.5(-1)	15(0)	25(0)	0.01(-1)	57
10	5(+1)	15(0)	25(0)	0.01(-1)	46
11	2.5(-1)	15(0)	25(0)	0.01(-1)	59
12	5(+1)	15(0)	25(0)	0.1(+1)	50
13	4(0)	5(-1)	15(0)	0.05(0)	32
14	4(0)	25(+1)	15(0)	0.05(0)	56
15	4(0)	5(-1)	40(+1)	0.05(0)	35
16	4(0)	25(+1)	40(+1)	0.05(0)	58
17	2.5(-1)	15(0)	15(0)	0.05(0)	52
18	5(+1)	15(0)	15(0)	0.05(0)	36
19	2.5(-1)	15(0)	40(+1)	0.05(0)	55
20	5(+1)	15(0)	40(+1)	0.05(0)	41
21	4(0)	5(-1)	25(0)	0.01(-1)	33
22	4(0)	25(+1)	25(+1)	0.01(-1)	60
23	4(0)	5(-1)	25(+1)	0.1(+1)	34
24	4(0)	25(+1)	25(+1)	0.1(+1)	62
25	4(0)	15(0)	25(0)	0.05(0)	48
26	4(0)	15(0)	25(0)	0.05(0)	47
27	4(0)	15(0)	25(0)	0.05(0)	48
28	4(0)	15(0)	25(0)	0.05(0)	49
29	4(0)	15(0)	25(0)	0.05(0)	49

Table 8: ANOVA for response surface quadratic model representing effective factors on ciprofloxacin-Fe (III) interaction

Source	Sum of squares	DF	Mean square	F- value	Probe>F
Quadratic model	2517.32	14	179.81	23.02	<0.0001
A	290.08	1	290.08	37.13	<0.0001
B	2002.08	1	2002.08	256.29	<0.0001
C	60.75	1	60.75	7.78	0.0145
D	6.75	1	6.75	0.86	0.3684
A^2	29.52	1	29.52	3.78	0.0723
B^2	8.09	1	8.09	1.04	0.3262
C^2	73.52	1	73.52	9.41	0.0083
D^2	8.33	1	8.33	1.07	0.3192
AB	0.25	1	0.25	0.032	0.8606
AC	1.00	1	1.00	0.13	0.7258
AD	1.00	1	1.00	0.13	0.7258
BC	0.25	1	0.25	0.032	0.8606
BD	0.25	1	0.25	0.032	0.8606
CD	9.00	1	9.00	1.15	0.3013
Residual	109.37	14	7.81		
Lack of fit	104.17	1	10.42	8.01	0.0298
Pure error	5.20	4	1.30		
Cor total	2626.69	28			

Table 9: ANOVA for response surface modified quadratic model for effective factors on ciprofloxacin-Fe (III) interaction

Source	Sum of squares	DF	Mean square	F- value	Prob>F
Quadratic model	2478.57	5	495.71	76.98	<0.0001
A	290.08	1	290.08	45.04	<0.0001
B	2002.08	1	2002.08	310.89	<0.0001
C	60.75	1	60.75	9.43	0.0054
A^2	31.33	1	31.33	4.87	0.0377
C^2	78.35	1	78.35	12.17	0.0020
Residual	148.12	23	6.44		
Lack of	142.92	19	7.52	5.79	0.0504
Pure error	5.20	4	1.30		
Cor total	2626.69	28			

Table 10: Regression coefficients (R^2) for full and reduced quadratic models from ANOVA analysis in ciprofloxacin-Fe (III) interaction

Types of Coefficient of regression	Full quadratic model	Reduced quadratic model
R^2	0.9584	0.9436
Adjusted R^2	0.9167	0.9314
Predicted R^2	0.7685	0.9020

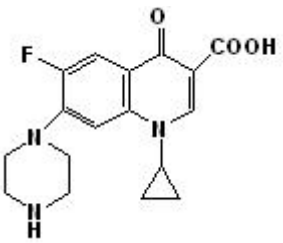

Figure 1: Chemical Structure of Ciprofloxacin

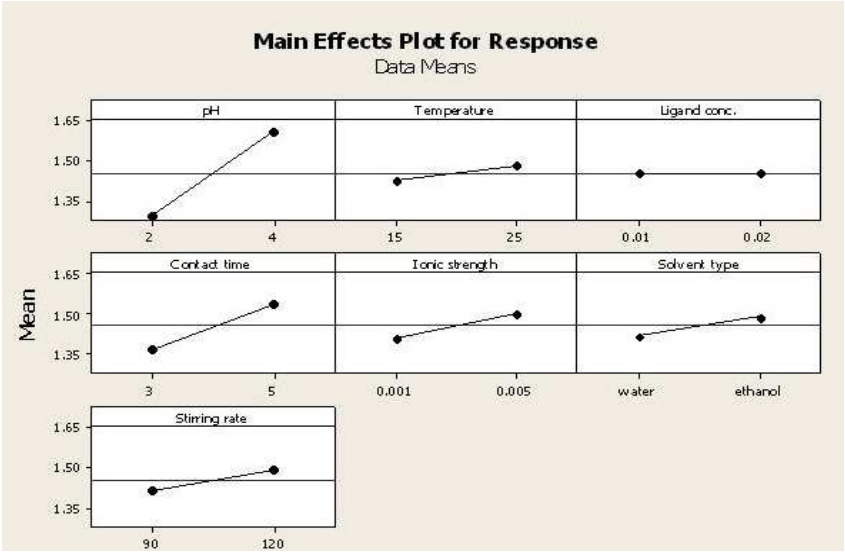

Figure 2: Main effect plots for ciprofloxacin–Fe (III) Complex formation in two-level factorial design

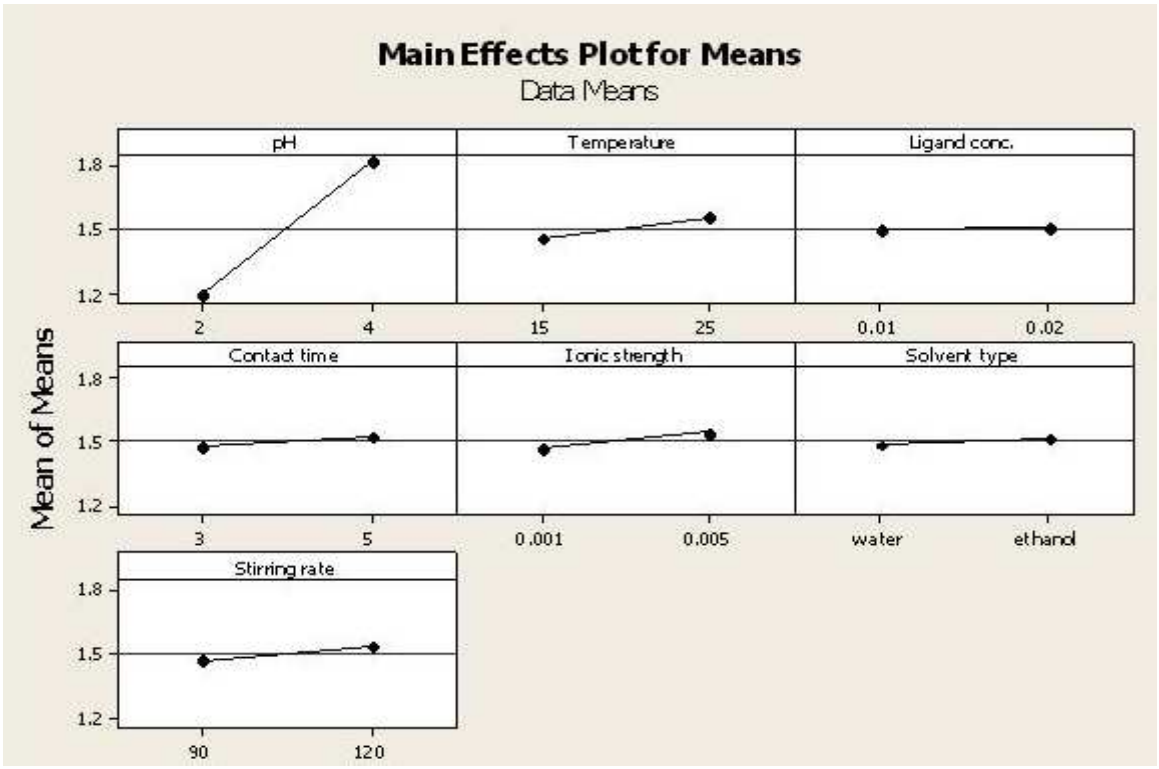

Figure 3: Main effect plots for ciprofloxacin–Fe (III) Complex formation in L8 Taguchi design

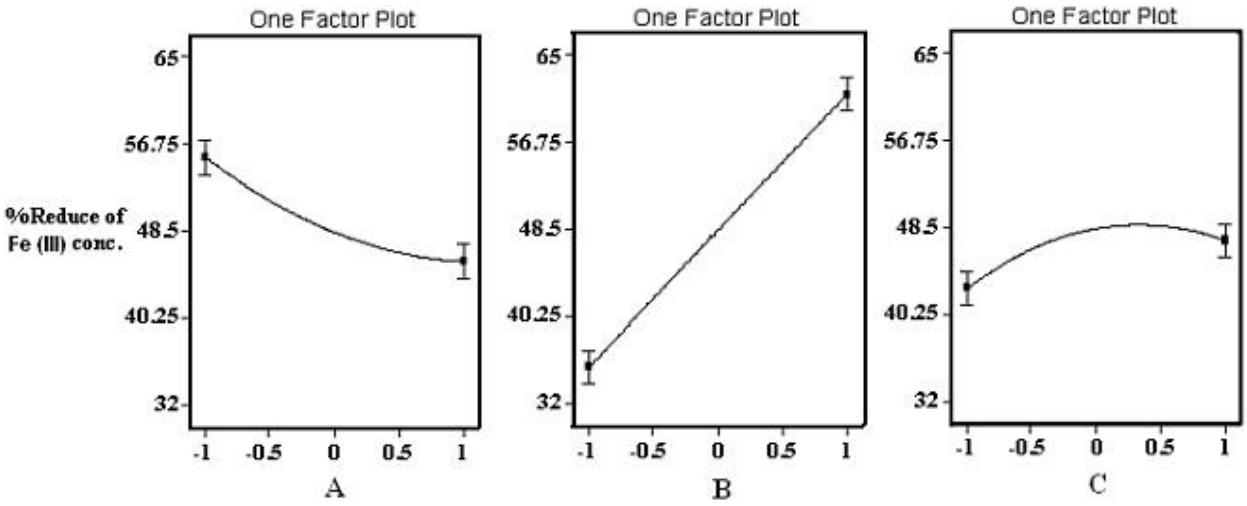

Figure 4: Model graphs expressing coded levels of significant factors vs. reduce in ferric ion concentration as the result of ciprofloxacin- Fe (III) Complex formation, (A): pH, (B): contact time, (C): Temperature. In each factor plot the other factors are held at their mean level.

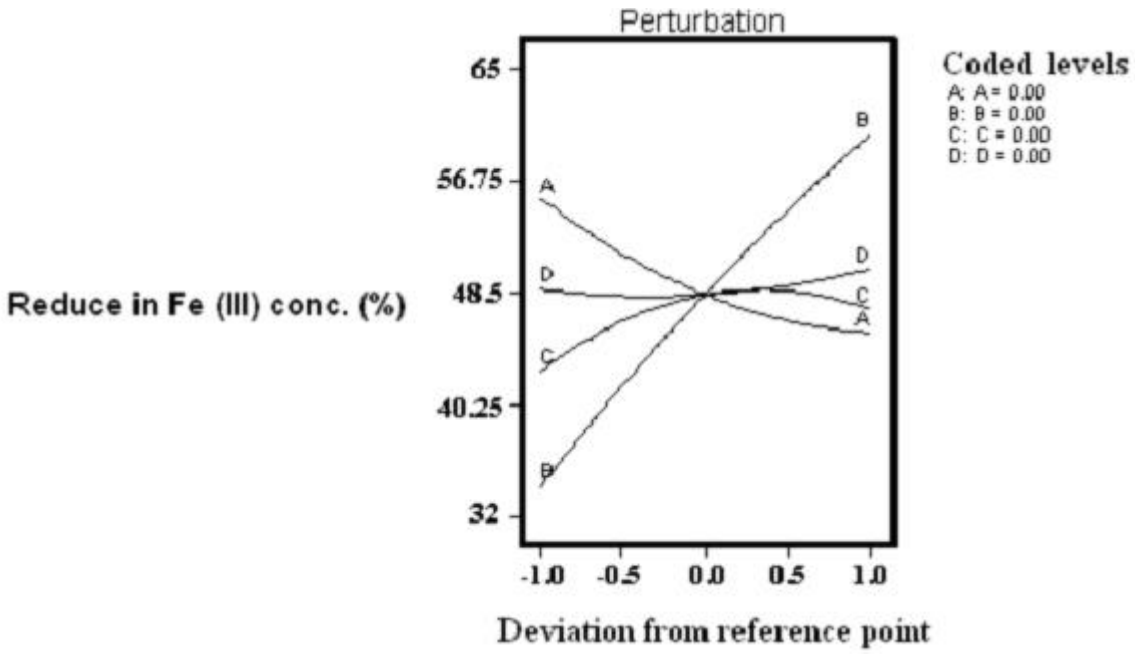

Figure 5: The perturbation plot of reduce in Fe (III) concentration as the result of ciprofloxacin- Fe (III) complex formation against four investigated variables. A represents pH, B is the contact time. C is temperature and D is the ionic strength.