Scientific paper

Complexation of Iron with Piroxicam – Evaluation via Response Surface Methodology

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Abstract

A response surface methodology (RSM) based on a Box-Behnken design was applied for study on ferrous ions binding ability to piroxicam in aqueous solution as a function of three numerical factors (extraction time, pH, piroxicam concentration) and extractant type as a categorical variable each in three levels. Analysis of variance (ANOVA) provided a supporting evidence for quadratic model to fit the experimental data with a correlation value squared (r^2) of 0.9433. All the experimental data resulted by a selective extraction-spectrophotometric method. The relative standard deviation (RSD) was found to be 0.63%.

Keywords: Piroxicam, iron, spectrophotometry, box-behnken design.

1. Introduction

Piroxicam with the IUPAC name of 4-Hydroxy-2-Methyl-N-2-Pyridyl-2H-1,2-Benzothiazine-3-Carboxamide-1,1 Dioxide belongs to the acidic, nonsteroidal and anti-inflammatory drugs without any side-effects.[1–3] Piroxicam is a colorless and odorless powder with a bitter taste possessing low acidity.[1,4] The structure of piroxicam which is shown in Figure 1, includes four different heteroatom sites that is promising for complex formation with metal ions.

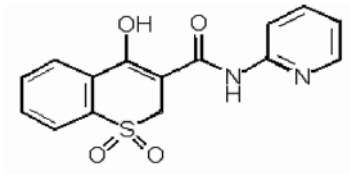

Figure 1. The structural formula of Piroxicam ($C_{15}H_{13}N_3O_4S$)

The existence of different piroxicam species is highly based on the pH of medium. The species percents in different pH values is depicted in Figure 2.[1]

Piroxicam behaves as a neutral bidentate ligand coordinated to the metal ions such as Cu^{II}, Cd^{II}, Fe^{II}, Co^{II}, Ni^{II} and Zn^{II} via the pyridyl nitrogen and the amide oxygen.[5–8] It has been revealed that metal complexes of anti-inflammatory drugs have lower toxicity and higher pharmaceutical effect.[9–13] Iron has significant biological importance. The presence of Iron in biosystems is necessary. The biochemical activity of Iron is attributed to its chelation by electron donors and participation in redox reactions.[14,15] However the large production and application of Iron in industry and the subsequent contamination by this

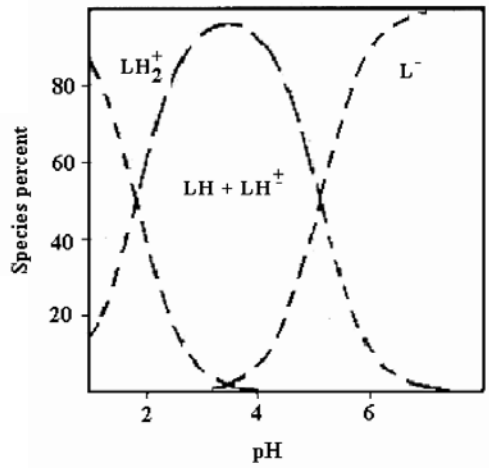

Figure 2. Diverse species of piroxicam in different pHs

element from industrial effluents has been the cause of concern for environmentalists. Therefore it is revealed that control of Iron contamination in the environment, water and food is important. For determination of Iron in foods, drugs, and biological tissues several photometric reagents have been introduced and mostly used.[16]

Chelation of metal ions by piroxicam have been reported in versatile literatures which indicates the effectiveness of this reagent for chelation with metal ions.[5,17–22] In the previous works the preparation of produced metal complexes were reported.[18] In the present work the focus was on the optimization and modeling of a spectrophotometric method in Fe-pir complexing as a function of effective variables. The selectivity of applied method was evaluated in the presence of versatile anionic and cationic species based on their tolerance limits.

Nowadays experimental designs have been regarded as one of the most favorable techniques in covering a large area of practical statistics and obtain unambiguous results with the least expense. Response surface method (RSM) designs help you quantify the relationships between one or more measured responses and the vital input factors. They include a category of statistical methods for model building and exploitation.

Response surface methods have been designed for factors with more than three levels in which quadratic models can be established. The main objective is to find a desirable location in the design space. This could be a maximum, a minimum or an area where the response is stable over a range of the factors. After clarifying the goal, next step is to figure out which responses will be measured and how to measure them. Quantifiable response is one of the most important steps in a prosperous design of experiments. The most popular response surface methodologies are Central Composite, Box-Behnken and Doehlert designs.[23–25]

Box-Behnken design is an efficient and creative three-level composite design for fitting second-order response surfaces. It is an independent quadratic design in that it does not contain an embedded factorial or fractional factorial design. The methodology is based on the construction of balance designs which are rotatable and enable each factor level to be tested several times. Each factor or independent variable can be placed at one of three equally spaced values (coded as –1, 0, and +1). In this design the treatment combinations are at the midpoints of edges of the cubical design region and at the center. The design is spherical with the design points for high and low levels located at a distance equal to the square root of 2 from the center of the design and the related shape is given in Figure 3. By avoiding the corners of the design space, they allow experimenters to work around extreme factor combinations. It should be noted that analyst should not view the lack of coverage of the cube as a reason not to use the Box-Behnken design. It is not meant to be a cuboidal design. However, the application of this design should be confined to conditions in which one is not interested in predicting response at the extremes, That is, at the corners of the cube. The spherical nature of the Box-Behnken design, combined with the fact that the designs are rotatable or near-rotatable, suggests that ample center runs have to be performed. In an experimental design, additional replicates of the center point as necessary in each design to estimate experimental error is considered. Box-Behnken designs provide excellent predictability within the spherical design space and require fewer experiments compared to the full factorial designs or central composite designs. The number of required experiments for Box-Behnken design can be calculated according to $N = k^2 + k + c_p$, where k is the factor number and c_p is the replicate number of the central point.

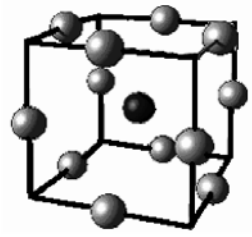

Figure 3. Cube derived Box-Behnken design

The Box-Behnken design matrix generated by Design Expert software displays factor levels in the experimental design in two ways: (i) the actual factor levels, which are the values from the experiment, and (ii) the coded factor levels, –1, 0, and +1 for low levels, center point, and high levels, respectively.

In the present work a Box-Behnken matrix design including 51 experiments was applied for evaluation of three numerical factors (extraction time, pH, piroxicam conc.), and one categorical factor (extractant type) each in three levels. An appropriate model which could fit the data was found.

2. Experimental

Piroxicam was purchased from RAZAC pharmaceutical Co. (Iran) with mp (melting point) of 198 °C and purity of 99.8%. All chemicals including Sodium acetate, hydroxylamine solution and all solvents were of analytical grade and purchased from Merck Company.

Three variables under study were pH, extraction time, and temperature each in three levels which are shown in Table 1.

A matrix of experiments based on Box-Behnken design including 51 experiments was planned by Design-Expert software-v.6 (state-ease, corp., minnesota) (Table 3). The concentration of Fe-pir complex was considered as response. The average of three replicates was used for each datum.

Table 1. Experimental factors with their actual and coded levels

Numerical factors	**Low level(–1)**	**Medium level(0)**	**High level(+1)**
Extraction time (sec), A	60	90	120
pH, B	3	4	5
Piroxicam conc. (mol L^{-1}), C	10^{-3}	5×10^{-3}	10^{-2}
Categorical factors	**Low level(1)**	**Medium level(2)**	**High level(3)**
Extractant type, D	Chloroform	Ethyl acetate	Dichloromethane

To an aqueous solution containing 2×10^{-2} g L^{-1} of Fe^{III}, 2 ml of hydroxylamine solution was added. Sodium acetate solution (10^{-2} mol L^{-1}) was used to adjust the pH. The solution was transferred to a 25 ml standard flask. 5 ml of piroxicam in methanolic hydrochloric acid (10%) solution with a definite concentration (initial piroxicam conc.) was added and diluted to the total volume with distilled water. The containing was held for 5 min. Deep red colored complex was transferred to a separation funnel containing 25 ml of organic solvent (extractant type) which was shaken vigorously for definite intervals in each case(extraction time). After separation of the phases, the organic phase was drained off into a 25-ml flask. The absorbance was measured using chloroform as reference at 500nm by a PDA-Multispect Shimadzu spectrophotometer; Uv-vis ($CHCl_3$) λ_{max}: 500 nm.

The selectivity of method was evaluated in terms of tolerance limits for diverse cations and anions.[24] For doing this, versatile ions were added to the solution containing 20 μg ml^{-1} of Fe^{II} ion in the presence of ligand. Iron concentration was measured then in λ_{max} of 500 nm (Table 2).

3. Results and Discussion

Piroxicam showed two maximum spectrophotometric bands; Uv-vis (MeOH–HCl) λ_{max}: 242, 339 nm. A broad band at 500 nm is attributed to Fe–Pir complex and no spectral interferences were observed as can be understood from Figure 4.

The significant increase in Fe-pir complex absorbance as a function of pH showed that complex formation was highly depended on the acidity which will be demonstrated later through ANOVA estimations.

Optimum pH for complex formation was found to be 4.0 which was in good consistent with previous reported works. The related plot is shown in Figure 5. Piroxicam molecules are mostly (70%) in the form of neutral species ($LH^{\pm}$) at pH = 4.0 which revealed that the binding of ferrous ions by piroxicam mostly happened by neutral species. Variation of pH caused the cationic species to be more dominant and hence lowered the binding ability with Iron ions.

The selectivity of a definite method is based on the degrees of freedom from interferences. Tolerance limit values for versatile cations and anions showed that acetate, bromide and chloride anions did not have any interferen-

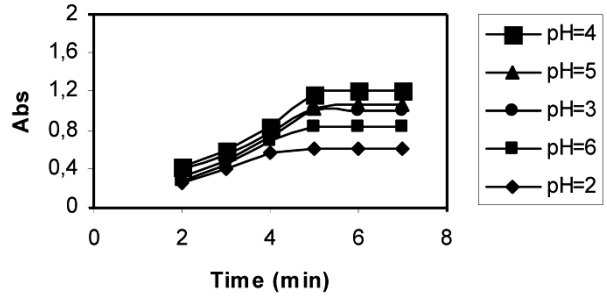

Figure 5. Effect of pH on the complexation of Fe^{2+} ions with piroxicam within different intervals at 25 °C

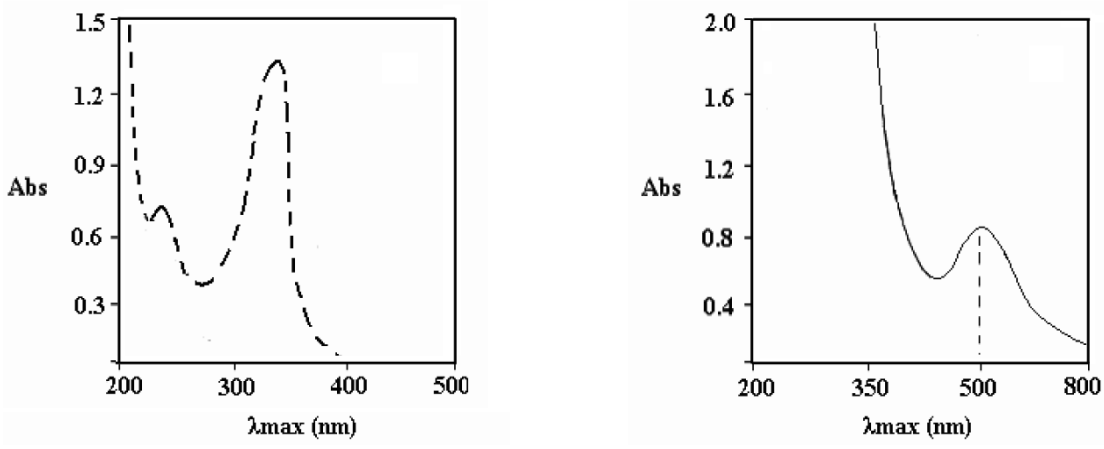

Figure 4. Ultraviolet spectrum of (a) 2×10^{-2} g L^{-1} methanolic hydrochloric acid solution of piroxicam, (b) 2×10^{-2} g L^{-1} solution of Fe–Piroxicam complex in 0.1 mol L^{-1} methanolic hydrochloric acid solution

ces even up to high concentrations. Other ions also had no significant interferences with Iron. Ethylene diamine tetra acetic acid (EDTA) is a serious interference which can be tolerated up to 0.75 mg. The boiling of a solution by nitric acid could be an efficient resolve in this case.

Table 2: Tolerance limits for different ions in the presence of Fe^{II} ion (20 μg ml^{-1})

Tolerance limit (TL)	Ions
High	CH_3COO^-, Cl^-, Br^-
0.5 mg	EDTA
160 mg	NO_3^-
80 mg	Pb^{2+}
140 mg	Cr^{3+}
40 mg	Cu^{2+}
160 mg	Mg^{2+}
20 mg	Ni^{2+}
10 mg	Al^{3+}
80 mg	Cd^{2+}

A Box-Behnken design containing 51 experiments was applied. These designs have fewer runs than 3-level factorial designs which reveals to be more economic, convenient and time fluent. The relative standard deviation (RSD) for seven replicate measurements at the absorbance of 0.434 (minimum instrumental error) in solutions containing 2 ppm of Fe^{2+} was found to be % 0.63. Four variables under study 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 3. As described before Fe-pir complex concentration (mg L^{-1}) was taken as response. If categorical factors are added, the Box-Behnken design will be duplicated for every combination of the categorical factor levels.

Results showed that optimum pH for the color reaction was 4.00. The extraction yield would be maximum within 90 sec and the most efficient extractant was chloroform. Desirable ligand concentration was found to be within the range of 5×10^{-3}–10^{-2} mol L^{-1} while the most efficient value was found to be 10^{-2} mol L^{-1}. The optimum condition for complex formation is highlighted in Table 3.

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 in which the effect of one factor is dependent on the value of another factor.[26] The significant factors in the regression model can be estimated by performing analysis of variance.[26] It seems necessary to have an understanding about different statistical terms applied here. So the definition for each term is shown in appendix.

Table 3. Box–Behnken design with actual and coded levels

Run	Extraction time (sec)	pH	Piroxicam conc. (mg L^{-1})	Extractant type	Conc. of Fe–pir complex (mg L^{-1})
1	60(–1.00)	3(–1.00)	5×10^{-3}(0.00)	Ethyl acetate(2)	36.1
2	60(–1.00)	4(0.00)	10^{-3}(–1.00)	Dichloromethane(3)	38
3	90(0.00)	5(1.00)	10^{-2}(1.00)	Ethyl acetate(2)	64
4	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Chloroform(1)	85
5	**90(0.00)**	**4(0.00)**	**10^{-2}(1.00)**	**Chloroform(1)**	**90.3**
6	120(1.00)	4(0.00)	10^{-3}(–1.00)	Chloroform(1)	83.5
7	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Dichloromethane(3)	64
8	60(–1.00)	4(0.00)	10^{-3}(–1.00)	Ethyl acetate(2)	45.7
9	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Dichloromethane(3)	66.7
10	60(–1.00)	4(0.00)	10^{-2}(1.00)	Chloroform(1)	63.3
11	90(0.00)	3(–1.00)	10^{-2}(1.00)	Ethyl acetate(2)	62.5
12	120(1.00)	3(–1.00)	5×10^{-3}(0.00)	Chloroform(1)	76.8
13	60(–1.00)	4(0.00)	10^{-3}(–1.00)	Chloroform(1)	56
14	60(–1.00)	5(1.00)	5×10^{-3}(0.00)	Dichloromethane(3)	36.7
15	120(1.00)	4(0.00)	10^{-2}(1.00)	Dichloromethane(3)	70.8
16	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Chloroform(1)	84.9
17	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Chloroform(1)	82.5
18	90(0.00)	3(–1.00)	10^{-2}(1.00)	Dichloromethane(3)	66
19	90(0.00)	5(1.00)	10^{-3}(–1.00)	Ethyl acetate(2)	71
20	120(1.00)	5(1.00)	5×10^{-3}(0.00)	Ethyl acetate(2)	73
21	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Ethyl acetate(2)	73.7
22	120(1.00)	4(0.00)	10^{-3}(–1.00)	Ethyl acetate(2)	75
23	120(1.00)	4(0.00)	10^{-2}(1.00)	Ethyl acetate(2)	79.1
24	60(–1.00)	3(–1.00)	5×10^{-3}(0.00)	Dichloromethane(3)	26.8
25	60(–1.00)	3(–1.00)	5×10^{-3}(0.00)	Chloroform(1)	52
26	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Ethyl acetate(2)	75.2
27	120(1.00)	5(1.00)	5×10^{-3}(0.00)	Dichloromethane(3)	73

Run	Extraction time (sec)	pH	Piroxicam conc. (mol L^{-1})	Extractant type	Conc. of Fe–pir complex (mol L^{-1})
28	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Ethyl acetate(2)	72.6
29	90(0.00)	3(–1.00)	10^{-2}(1.00)	Chloroform(1)	74.6
30	60(–1.00)	4(0.00)	10^{-2}(1.00)	Dichloromethane(3)	38.7
31	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Dichloromethane(3)	76.1
32	60(–1.00)	5(1.00)	5×10^{-3}(0.00)	Chloroform(1)	56.6
33	60(–1.00)	4(0.00)	10^{-2}(1.00)	Ethyl acetate(2)	52.3
34	90(0.00)	5(1.00)	10^{-2}(1.00)	Chloroform(1)	71.1
35	120(1.00)	4(0.00)	10^{-2}(1.00)	Chloroform(1)	88
36	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Dichloromethane(3)	66.6
37	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Dichloromethane(3)	65
38	120(1.00)	3(–1.00)	5×10^{-3}(0.00)	Dichloromethane(3)	64.4
39	60(–1.00)	5(1.00)	5×10^{-3}(0.00)	Ethyl acetate(2)	51
40	90(0.00)	3(–1.00)	10^{-3}(–1.00)	Chloroform(1)	67
41	90(0.00)	5(1.00)	10^{-3}(–1.00)	Chloroform(1)	73.5
42	90(0.00)	3(–1.00)	10^{-3}(–1.00)	Ethyl acetate(2)	60
43	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Chloroform(1)	88.5
44	120(1.00)	4(0.00)	10^{-3}(–1.00)	Dichloromethane(3)	73.3
45	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Ethyl acetate(2)	77
46	90(0.00)	3(–1.00)	10^{-3}(–1.00)	Dichloromethane(3)	51.1
47	120(1.00)	3(–1.00)	5×10^{-3}(0.00)	Ethyl acetate(2)	67.5
48	90(0.00)	4(0.00)	5×10^{-3}(0.00)	Ethyl acetate(2)	71.3
49	120(1.00)	5(1.00)	5×10^{-3}(0.00)	Chloroform(1)	79.5
50	90(0.00)	5(1.00)	10^{-2}(1.00)	Dichloromethane(3)	66.5
51	90(0.00)	5(1.00)	10^{-3}(–1.00)	Dichloromethane(3)	62.3

Table 4. ANOVA for response surface Quadratic model

Source	Sum of squares	DF	Mean square	F value	Prob>F
Model	10131.48	17	598.91	53.65	<0.0001
A	5124.60	1	5124.60	459.04	<0.0001
B	224.48	1	224.48	20.11	<0.0001
C	89.64	1	89.64	8.03	0.0078
D	2127.34	2	1063.67	95.28	<0.0001
A^2	1303.15	1	1303.15	116.73	<0.0001
B^2	808.03	1	808.03	72.38	<0.0001
C^2	37.22	1	37.22	3.33	0.0769
AB	13.23	1	13.23	1.19	0.2842
AC	6.02	1	6.02	0.54	0.4679
AD	117.39	2	58.69	5.26	0.0104
BC	76.00	1	76.00	6.81	0.0135
BD	38.09	2	19.04	1.71	0.1973
CD	17.23	2	8.61	0.77	0.4704
Residual	368.41	33	11.16		
Lack of fit	236.58	22	10.75	0.90	0.6041*
Pure error	131.83	11	11.98		
Cor total	10549.89	50			

* not significant

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 53.65 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. The lack of fit F-value of 0.90 implies that the lack of fit is not significant relative to the pure error.

Values of "Prob > F" less than 0.0500 indicated that the model terms were significant. In this case A, B, C, D, A^2, B^2, AD, and BC were significant model terms. Values greater than 0.1000 indicate the model terms are not significant. Regarding the F-values extraction time was the most determinant factor. Extractant type was the second effective variable while pH and initial piroxicam concentration were the following. The comparative study of different factor interactions revealed the following order of importance:

$$A^2 > B^2 > BC > AD > C^2 > BD > AB > CD > AC$$

Table 5. ANOVA for response surface modified Quadratic model

Source	Sum of squares	DF	Mean square	F value	Prob>F
Model	10071.02	7	1007.10	84.12	<0.0001
A	5124.60	1	5124.60	428.06	<0.0001
B	224.48	1	224.48	18.75	<0.0001
C	89.64	1	89.64	7.49	0.0092
D	2104.93	2	1052.46	87.91	<0.0001
A^2	1320.67	1	1320.67	110.32	<0.0001
B^2	821.50	1	821.50	68.62	<0.0001
AD	117.391	2	58.69	4.90	0.0125
BC	76.00	1	76.00	6.35	0.0158
Residual	478.87	40	11.97		
Lack of fit	14.50	29	11.97	1.00	0.5313*
Pure error	2.80	11		11.97	
Cor total	3254.94	50			

* not significant

Because the model contained significant and non-significant terms (Table 4), it was reduced by elimination of insignificant terms to achieve the desired model (Table 5). Therefore the new model terms would be A, B, C, A^2 and C^2.

The lack of fit F-value of 1.00 implies that the lack of fit is not significant relative to the pure error. Lack of fit test for different models was obtained and is shown in Table 6.

Table 6. Sequential Model Sum of Squares

Source	Sum of squares	DF	Mean square	F value	Prob>F
linear	2803.78	34	82.46	6.88	0.0008
2FI	2518.68	25	100.75	8.41	0.0004
Quadratic	**236.58**	**22**	**10.75**	**0.90**	**0.6041**
Cubic	98.45	6	16.41	1.37	0.3079
Pure error	131.83	11	11.98		

Obtained data have revealed that quadratic model having insignificant lack of fit F-value could be suggested and is highlighted in the Table 6. Whenever there are fewer independent points in the design than there are terms in a model, there would be parameters which can not be estimated independently therefore, the model is aliased which means inappropriate as in the case of Cubic model.

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 7).

Table 7. Values of coefficient of regression (r^2) for the full and reduced quadratic models from ANOVA analysis

Types of Coefficient of regression	Full quadratic model	Reduced quadratic model
r^2	0.9651	0.9546
Adjusted r^2	0.9471	0.9433
Predicted r^2	0.9163	0.9259

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.

Different model statistics could demonstrate the preference of suggested quadratic model in comparison with other models due to the maximum adjusted r^2 and predicted r^2 values (Table 8).

The final regression equation expressing the dependence of Fe-pir concentration (c) on significant variables in terms of coded factors was obtained as:

Table 8. Model summary statistics

Source	Standard deviation	r–squared	Adjusted r–squared	Predicted r–squared
Linear	8.08	0.7217	0.6908	0.6533
2FI	8.58	0.7488	0.6511	0.5379
Quadratic	3.34	0.9651	0.9471	0.9163
Cubic	3.68	0.9782	0.9358	

$$c = +74.95 + 14.61A + 3.06\,B + 1.90\,C + \\ + 8.42\,D\,[1] - 1.24\,D\,[2] \\ -10.21\,A^2 - 8.06\,B^2 - 2.12\,AD\,[1] - \\ - 0.93AD\,[2] - 2.52\,BC \quad (1)$$

Where A is extraction time in sec, B is pH, C is initial piroxicam concentration in mol L^{-1}, and D represents extractant type. Terms possessing D have been considered as two cases; [1] represents the case when chloroform is used as organic phase while [2] is a representative of ethyl acetate. Note that C^2, AB, AC, BD, and CD interactions were not included in this equation as they were insignificant model terms. The relative effect of each factor in this equation can be described by its coefficient and algebric sign.[27] It was revealed from regression equation that complexation efficiency increased with increasese in all four factor levels (positive sign).

The regression equation in terms of actual levels was obtained in three different conditions; level 1 of factor D (chloroform), level 2 of factor D (ethyl acetate), and level 3 of factor D (dichloromethane). The related equations are respectively as follows:

$$c = + 83.37427 + 12.48750\,A + 3.05833\,B + \\ + 1.89703\,C \\ - 10.21414\,A^2 - 8.05581\,B^2 - 2.51667\,BC \quad (2)$$

$$c = + 73.71527 + 13.68750\,A + 3.05833\,B + \\ + 1.89703\,C \\ - 10.21414\,A^2 - 8.05581\,B^2 - 2.51667\,BC \quad (3)$$

$$c = + 67.77409 + 17.66250\,A + 3.05833\,B + \\ + 1.89703\,C \\ - 10.21414\,A^2 - 8.05581\,B^2 - 2.51667\,BC \quad (4)$$

The perturbation plot of complex concentration against numerical factors could be shown in three different levels of extractant type which is depicted in Figures 6 to 8. All the plots provided a supporting evidence of the importance of extraction time (factor A) effect on the response. Complex concentration can be followed as each variable moves from the chosen reference, with all other numerical factors held constant at the middle of the design space (coded zero level).[28] Initial ligand concen-

tration produced insignificant effect compared to other variables. The curve related to the effect of pH would be distinguished which could be attributed to the pH value of 4.00 in which the Fe-pir complex formation is highly efficient.

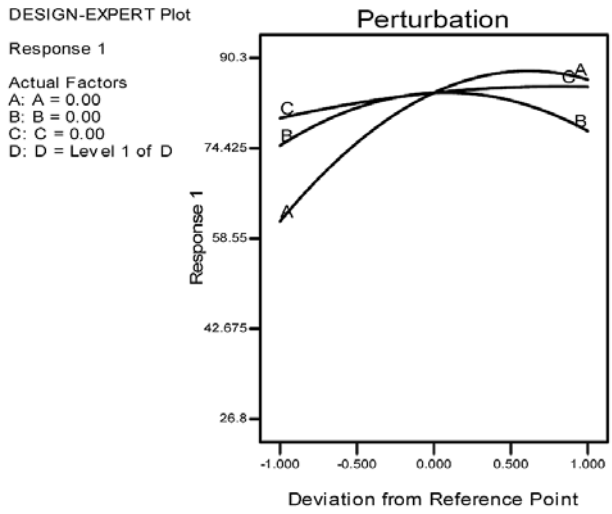

Figure 6. The perturbation plot of Fe-pir complex conc. against three numerical variables using chloroform as extractant. A represents extraction time, B is pH, and C is piroxicam conc.

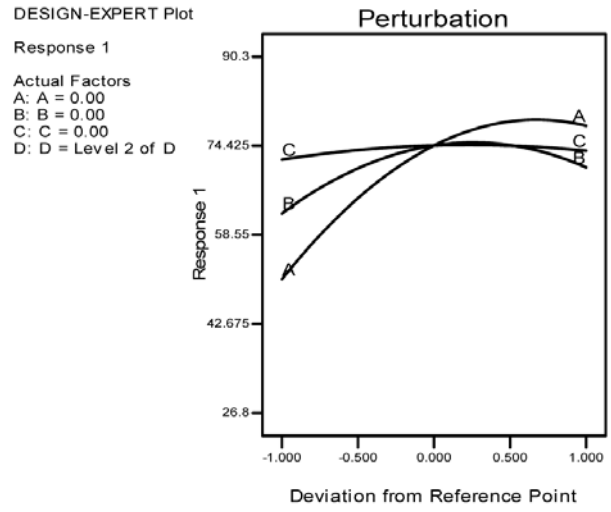

Figure 7. The perturbation plot of Fe-pir complex conc. against three numerical variables using ethyl acetate as extractant. A represents extraction time, B is pH, and C is piroxicam conc.

The thing which seems important is that the response values would decline as the factor D shifted from its lower level to higher level (chloroform > ethyl acetate > dichloromethane). So using chloroform as organic solvent would lead to the higher complex extractions.

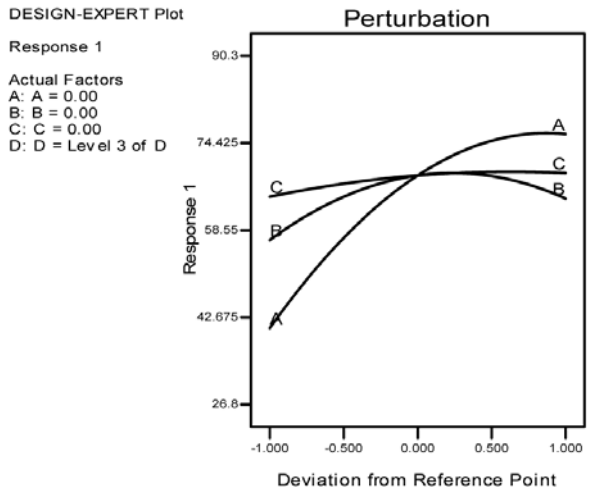

Figure 8. The perturbation plot of Fe-pir complex conc. against three numerical variables using dichloromethane as extractant. A represents extraction time, B is pH, and C is piroxicam conc.

4. Conclusion

Piroxicam molecules reacted in a moderately acidic medium with Ferrous ions to yield a deep red colored complex. The effect of numerical and categorical variables (extraction time, pH, piroxicam concentration, and extractant type) was described by quadratic response model. High resulted coefficient of regression values (r^2 = 0.9433) proved the fitness of the selected model in analyzing the experimental data. The binding of Ferrous ions with piroxicam reagent was strongly influenced by all the studied factors while piroxicam concentration had slightly lower effect. The ANOVA estimations revealed that the optimum process condition could be achieved at pH 4.0 during 120 sec extraction using chloroform as an extractant. Desirable ligand concentration was found to be within the range of 5×10^{-3}–10^{-2} mol L^{-1} while the most efficient value was found to be 10^{-2} mol L^{-1}. The results from perturbation curves also demonstrated the ANOVA data.

5. References

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Appendix

Statistical term	Definition
Sum of squares	The sum of the squared distances from the mean due to the variation in in average response when a factor shifts from low level to its high level
Model sum of squares	Total of the sum of squares for the model terms
Residual sum of squares	Total of the sum of squares of all the terms not included in the model
Lack of fit sum of squares	Residual sum of squares after subtracting the pure error sum of squares
Pure error sum of squares	Some of squares from replicated points
Degrees of freedom (DF)	The number of independent comparisons available to estimate a parameter. Usually the number of model parameters minus 1
Model DF	Number of model terms icluding minus 1
Residual DF	Adjusted total DF minus the model DF
Lack of fit DF	Amount of information available after accounting for blocking model terms and pure error
Pure error DF	Amount of information available from replicated points
Lack of fit	The result of experimentation should be a model which will adequately predict the response within the design space. The variation between the model prediction and the design points is defined as lack of fit.
Pure error	The normal variation in the response which appears when an experiment is repeated
Mean square	The sum of squares divided by the degrees of freedom analogous to variance
F value	The ratio of model mean square to the appropriate error mean square
Model F value	A test for comparing model variance with residual variance
Lack of fit F value	Test for comparing lack of fit variance with pure error variance
Prob > F (probability of a larger F value)	If the F value (the ratio of variances) lies near the tail of the <F> distribution then the probality of a large F is small and the variance ratio is supposed to be significant
Core. Total (corrected total)	The total sum of squares corrected for the mean . It is the sum of squared differences between the individual observations and overall average

Povzetek

Za študij zmožnosti vezave železovih ionov na piroksikam v vodnih raztopinah smo uporabili metodo površinskih odzivov, ki temelji na Box-Behnkenevem modelu. Le-ta upošteva tri numerične parametre (čas ekstrakcije, pH in koncentracijo piroksikama) ter vrsto ekstraktorja kot kategorično spremenljivko. Analiza variance (ANOVA) podpira kvadratni model za fit eksperimentalnih podatkov (R^2 = 0.9433), relativna standardna deviacija (RSD) pa je 0.63 %.