

Process optimization for synthesis of Anti-Tuberculosis drug catalyzed by fluor apatite supported potassium fluoride

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Abstract: The optimization of the synthesis of anti-tuberculosis drug by thia-Michael addition between thiophenol and chalcone catalyzed using activated Fluorapatite supported potassium fluoride (KF/FAP) was studied using a 2 block central composite design including 4 factors (reaction time, solvent volume, catalyst weight and impregnation ratio). The high reactivity and regioelectivity of our catalyst coupled with their ease of use and reduced environmental problems makes them attractive alternatives to homogeneous basic reagents.

Keywords: Anti-Tuberculosis Drug, Fluorapatite Supported Potassium Fluoride, Heterogeneous Catalyst, Response Optimization; Central Composite Design

1. Introduction

In the last few years, tuberculosis (TB), caused by *Mycobacterium tuberculosis*, has again become one of the most dangerous and lethal infectious diseases [1]. According to the new WHO report in 2010, there were an estimated 8.8 million new cases of TB, 1.45 million TB deaths and more than two billion people infected with *M. tuberculosis*, of which approximately 10% will most likely become ill during their life [2]. This situation is due to HIV infection (1.1 million of all TB causes and 0.35 million of TB deaths are in people who are HIV positive), poor compliance of patients, population migration and increased drug resistance [3].

Recently, the Czech Authors [4] are showed that the 1, 3-Diphenyl-3-phenylsulfanyl-propan-1-one compound presented an anti-*Mycobacterium tuberculosis*. The conjugate addition of thiophenol to chalcone is a convenient route for synthesis of this sulfanyl organic compound [5].

In classic methods, this reaction catalyzed by strong bases such as alkali metal alkoxides [6], hydroxides [7] and amines [8]. The employment of these strong bases and acids in these reactions [9], however, leads to two main problems

affecting the environment; the necessity to dispose of huge amounts of organic waste due to formation of undesirable side products resulting from polymerization, bis-addition and self condensation, and total dissolved salts formed following the neutralization of soluble bases with acids. The replacement of liquid basic catalysts by solid bases in the synthesis of fine and intermediate organic chemicals allows one to avoid corrosion and environmental problems [10].

In view of this, the aim of the present study is investigate the application of experiments design for modeling and optimization of the synthesis of anti- tuberculosis drug 1,3-Diphenyl-3-phenylsulfanyl-propan-1-one catalyzed using activated Fluorapatite supported by potassium fluoride.

2. Materials and Methods

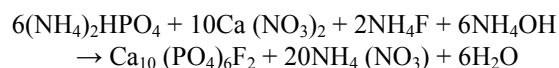
2.1. Chemicals and Instrumentations

All commercial reagents and solvents were used without further purification. X-ray diffraction (XRD) patterns of the

catalysts were obtained on a Philips 1710 diffractometer using Cu-K α radiation. Surface areas were determined at 77 K using a Coulter SA 31000 instrument with an automated gas volumetric method employing nitrogen as the adsorbate. NMR spectra were recorded on a Bruker ARX 300 spectrometer. Mass spectra were recorded on a VG Autospec spectrometer. FTIR spectra were recorded on an ATI Mattson-Genesis Series spectrophotometer using the KBr disc method.

2.2. Preparation and Characterization of Catalysts

The Fluorapatite catalyst (FAP) was prepared by the co-precipitation method [11], using the following reaction:



The activated Fluorapatite supported by potassium fluoride catalyst (KF/FAP) has been prepared by impregnation method [12]. The ratio of impregnation (*RI*) for KF/FAP catalyst was calculated according to equation (1):

$$\text{RI} = (\text{Weight of KF})/(\text{Weight of FAP}) \quad (1)$$

The analysis of the catalysts FAP and KF/FAP with X-ray diffraction gives the diffraction patterns almost identical. The comparison of images by scanning electron micrograph for FAP with KF/FAP shows a modification in the morphology of the doped material.

These results indicate that the structure of the apatite remains unchanged and that the potassium fluoride interacted with the surface of the support of catalyst.

2.3. General Procedure

The general procedure is reported in Figure 1, as follows: To a flask containing an equimolar mixture (1 mmol) of chalcone 1 and thiophenol 2 in methanol, the KF/FAP or FAP was added and the mixture was stirred at room temperature. The catalyst was filtered, washed with dichloromethane and the filtrate was concentrated under reduced pressure.

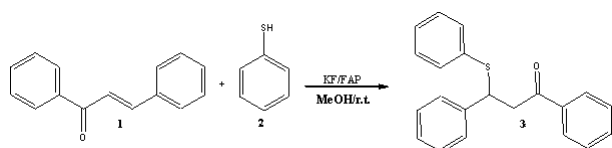


Figure 1. Synthesis of anti-tuberculosis drug

The crude product 3 was purified by recrystallization. The product was analyzed by ^1H , ^{13}C NMR and IR spectrometry.

2.4. Statistical Analysis

The synthesis of anti-tuberculosis drug 1,3-Diphenyl-3-phenylsulfanyl-propan-1-one from the

thia-Michael addition between chalcone and thiophenol catalyzed using KF/FAP catalyst was carried out and optimized using the Central Composite Design (CCD) and Response Surface Methodology (RSM) [13]. CCD helps in investigating linear, quadratic, cubic and cross-product effects of the four reaction condition variables on the reaction yield. The four independent variables studied were reaction time (X_1), solvent volume (X_2), catalyst weight (X_3) and impregnation ratio (X_4). Table 1 lists the range and levels of the four independent variables studied.

Table 1. Study field and coded factors

Un-coded variables x_1, x_2, x_3 and x_4	Unit	Coded variables X_1, X_2, X_3 and X_4		
		-1	0	+1
x_1 =Reaction time	min	3	4	5
x_2 =Solvent volume	mL	2	4	6
x_3 =Catalyst weight	mg	80	120	160
x_4 =Impregnation ratio		1/24	1/12	1/8

The experimental data obtained by following the above procedure were analyzed by the response surface methodology using the following second-order polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 \\ + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 \\ + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 \\ + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 \quad (2)$$

Where Y is the response (reaction yield); X_i and X_j are the coded independent variables and β_0 , β_i , β_{ii} and β_{ij} are intercept, linear, quadratic and interaction constant coefficients, respectively. The software stat graphics-Plus was used for regression analysis and analysis of variance (ANOVA). Response surfaces and contour plots were developed using the fitted quadratic polynomial equation obtained from regression analysis, holding two of the independent variables at a constant value corresponding to the stationary point and changing the other two variables. Confirmatory experiment was carried out to validate the equation, using combinations of independent variables which were not part of the original experimental design but within the experimental region.

3. Results and Discussion

3.1. Response Surface Methodology

By using a central composite design (CCD), a correlation between the reaction condition variables to the reaction yield was developed. Table 2 shows the complete design matrix and reaction yield at various reaction condition variables.

Selection of the levels was based on our preliminary

study. The value of α for this CCD was fixed at two. The complete design matrix of the experiments conducted and results are given in Table 2.

All variables at zero level constitute to the center points and the combination of each of the variables at either its lowest (-2.0) level or highest (+2.0) level with the order variables at zero level constitute the axial points. The

experiment sequence was randomized in order to minimize the effects of the uncontrolled factors.

In table 2 we can show, the reaction yield obtained was in the range from 32 to 95%. Experimental error was determined from run 25 to 26 at the centre point of the design.

Table 2. Experimental design and results

Standard order	Run order	Coded variables				Un-coded variables				Reaction yield
		X ₁	X ₂	X ₃	X ₄	x ₁	x ₂	x ₃	x ₄	
1	4	+1	+1	+1	+1	5	6	160	1/8	83
2	19	+1	+1	+1	-1	5	6	160	1/24	54
3	1	+1	+1	-1	+1	5	6	80	1/8	76
4	20	+1	+1	-1	-1	5	6	80	1/24	47
5	12	+1	-1	+1	+1	5	2	160	1/8	94
6	2	+1	-1	+1	-1	5	2	160	1/24	64
7	24	+1	-1	-1	+1	5	2	80	1/8	95
8	26	+1	-1	-1	-1	5	2	80	1/24	64
9	3	-1	+1	+1	+1	3	6	160	1/8	62
10	25	-1	+1	+1	-1	3	6	160	1/24	38
11	18	-1	+1	-1	+1	3	6	80	1/8	58
12	6	-1	+1	-1	-1	3	6	80	1/24	33
13	15	-1	-1	+1	+1	3	2	160	1/8	71
14	9	-1	-1	+1	-1	3	2	160	1/24	46
15	16	-1	-1	-1	+1	3	2	80	1/8	73
16	5	-1	-1	-1	-1	3	2	80	1/24	48
17	10	+2	0	0	0	6	4	120	1/12	81
18	8	-2	0	0	0	2	4	120	1/12	53

19	21	0	+2	0	0	4	8	120	1/12	44
20	11	0	-2	0	0	4	0	120	1/12	61
21	23	0	0	+2	0	4	4	200	1/12	64
22	7	0	0	-2	0	4	4	40	1/12	36
23	17	0	0	0	+2	4	4	120	1/6	95
24	14	0	0	0	-2	4	4	120	0	32
25	22	0	0	0	0	4	4	120	1/12	73
26	13	0	0	0	0	4	4	120	1/12	71

3.2. Regression Analysis

A regression analysis was performed to fit the response function and predict the outcome of reaction yield with a simple equation. The model is expressed by Eq. (3) which takes their coded value.

$$Y = 72 + 8.5X_1 - 5.75X_2 + 3.08X_3 + 14.33X_4 - 0.48X_1^2 - 4.12X_2^2 - 4.73X_3^2 - 1.36X_4^2 - 0.63X_1X_2 + 0.5X_1X_3 + 1.25X_1X_4 + 1.75X_2X_3 - 0.25X_2X_4 - 0.13X_3X_4 \quad (3)$$

The summary of the analysis of variance (ANOVA) result is shown in Table 3.

The regressors or term incorporated in the model are those statistically tested to be significant. The 'Prob > F' value indicates the probability equals the proportion of the area under the curve of the *F*-distribution that lies beyond

the observed *F* value. The small probability values called for the rejection of the null hypothesis, in other words, the particular term significantly affected the measured response of the system. In this case, the linear terms (X_1 , X_2 , X_3 and X_4) and the squared terms (X_2^2 and X_3^2) were significant model terms whereas the squared terms (X_1^2 and X_4^2) and the interaction terms (X_1X_2 , X_1X_3 , X_1X_4 , X_2X_3 , X_2X_4 and X_3X_4) were insignificant to the response. On the other hand, from this ANOVA the model *F*-value of 599.46 implied that the model was significant. Values of Prob. > *F* less than 0.01 indicated that the model terms was significant.

The final empirical model in term of coded factors after excluding the insignificant terms for reaction yield is shown in Eq. (4):

$$Y = 72 + 8.5X_1 - 5.75X_2 + 3.08X_3 + 14.33X_4 - 4.12X_2^2 - 4.73X_3^2 \quad (4)$$

Table 3. Regression variance analysis for the model

Source of variation	Coefficient	Sum of Squares	v	Mean square	F _{exp}	Significance test
Regression	-	8392.38	14	0599.4600	017.40	***
β ₀	72.00	-	1	-	-	
β ₁	08.50	1734.0000	1	1734.0000	050.33	***
β ₂	-05.75	0793.5000	1	0793.5000	023.03	***
β ₃	03.08	0228.1670	1	0228.1670	006.62	*
β ₄	14.33	4930.6700	1	4930.6700	143.11	***
β ₁₁	-00.48	0004.0076	1	0004.0076	000.12	NS
β ₁₂	-00.63	0006.2500	1	0006.2500	000.18	NS
β ₁₃	00.50	0004.0000	1	0004.0000	000.12	NS
β ₁₄	01.25	0025.0000	1	0025.0000	000.73	NS
β ₂₂	-04.12	0294.0080	1	0294.0080	008.53	**

β_{23}	01.75	0049.0000	1	0049.0000	001.42	NS
β_{24}	-00.25	0001.0000	1	0001.0000	000.03	NS
β_{33}	-04.73	0390.3710	1	0390.3710	011.33	***
β_{34}	-00.13	0000.2500	1	0000.2500	000.01	NS
β_{44}	-01.36	0032.0076	1	0032.0076	000.93	NS
Residue	-	0379.0000	11	0034.4600		
Total	-	8771.3800	25	-		

*** : $p \leq 0,01$; ** : $p \leq 0,025$; * : $p \leq 0,05$; NS : No significant.

The coefficient of determination, R^2 for the model was 95.68%. This indicates that only 4.32% of the total variability was not explained by the regressors in the model. The high R^2 value specifies that the model obtained will be able to give a convincingly good estimate of response of the system in the range studied.

3.3. Model Analysis

The influence of each independent variable on the response is shown in Figure 2.

This figure shows that the reaction time and the impregnation ratio have a positive effect on the reaction yield, while the solvent volume has a negative effect on the response. The catalyst weight also has a positive effect on the reaction yield, but by looking at this figure, we see that it has a negative effect in the positive area, which can be explained by the negative effect of the interaction of the squared term.

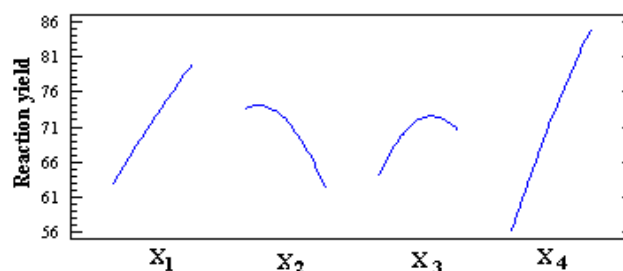


Figure 2. Main effects plot for reaction yield.

The geometric representation of the reaction yield according to the catalyst weight and the solvent volume is shown in Figure 3.

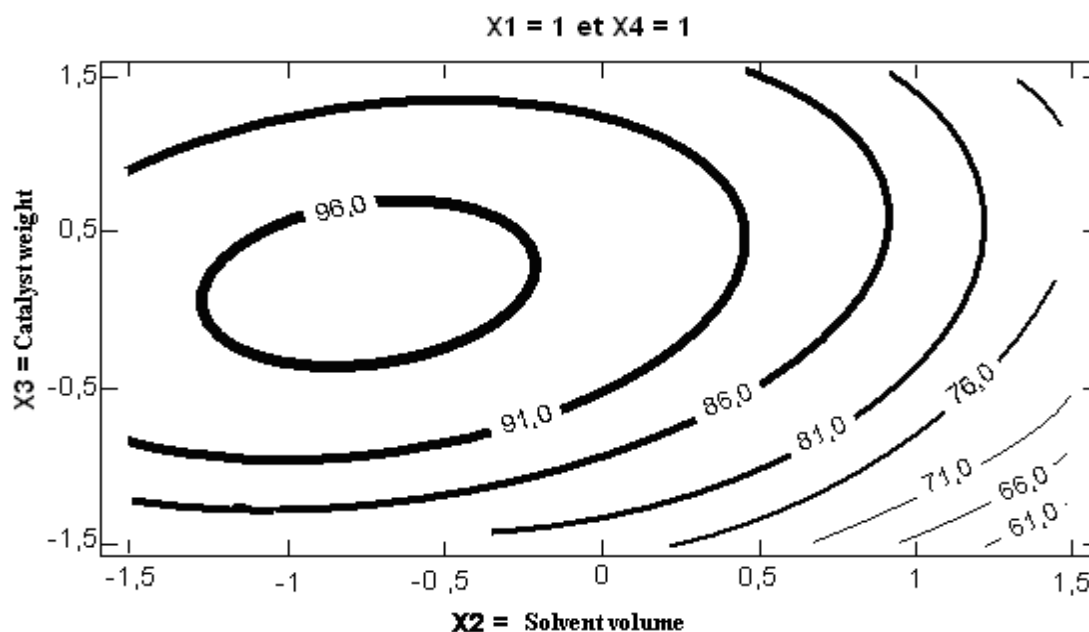


Figure 3. Contour plot of the combined effects of catalyst weight and solvent volume on the reaction yield at fixed reaction time and impregnation ratio

Analysis of the contour plot shows that for a fixed solvent volume, the increase in catalyst weight increases

the reaction yield. Moreover, for a fixed catalyst weight, increasing the solvent volume decreases the reaction yield

in the experimental field.

3.4. Process Optimization

Basically, the CCD was able to optimally design the desired response of the system based on the model obtained and the input criteria. The optimization of reaction yield was carried out based on the four variables which were in the range of experimental runs. The software predicted that optimized conditions for reaction yield were obtained when the reaction time, solvent volume, catalyst weight and impregnation ratio, were at 5min, 2mL, 110mg, ½, respectively with predicted reaction yield of 96%. The experimental reaction yield was 94%. This shows that the experimental value obtained was in good agreement with the value calculated from the model.

4. Conclusion

In summary, the optimization for synthesis of 1, 3-Diphenyl-3-phenylsulfanyl-propan-1-one catalyzed by activated Fluorapatite supported by potassium fluoride has been studied using central composite design. The model equation for the optimization of the reaction conditions for this synthesis was established. From this equation it was possible to forecast the optimal reaction conditions for synthesis of anti-tuberculosis drug in high yield.

This process bring advantages such as high catalytic activity and selectivity under mild reaction conditions, easy separation of the catalyst by simple filtration, use of non-toxic and inexpensive catalysts and especially, elimination of salts and by-product pollutants. This new solid base catalyst becomes then a practical alternative to soluble bases.

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