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Optimization of Reaction Conditions in the Production of Gadolinium Diethylenetriamine Pentaacetate-Folate

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Abstract. A previous study has performed the laboratory-scale synthesis and characterization of Gadolinium Diethylenetriaminepentaacetate-Folate. Some parameters associated with the synthesis have been defined. In current study was focused on establishment the parameters for scaling up the production of Gadolinium Diethylenetriaminepentaacetate-Folate as a targeted MRI contrast agent. For the purpose of subsequent scaling up the synthesis, the parameters particularly those determining the yield of the reaction product should be established. This report presents the use of The Placket Burman Design and the Response Surface Methodology in establishing the parameters. Thus, following the Placket Burman Design, a number of synthesis reactions were carried out, each with different reaction conditions, with respect to parameters to include: mole ratio of reactants (i.e mole of Gd^{3+} to DTPA-Folate), time of reaction, temperature, stirring rate, pH and solvent volume. Using this method, a conclusion could be drawn that the three factors were found to be significant. To get final conclusions on the optimal synthesis reaction conditions, the Response Surface Methodology was then applied. For this purpose, again, some synthesis reactions experiments were performed. These were done, in accordance to the Response Surface Methodology, verified by analysis of countour plots, helped to locate the optimal value of the factors. The resulted data showed that for optimal yield of the synthesis reaction there were three dominant parameters. They were mole ratio of reactants, stirring rate process, and the volume of water.

Keywords: Magnetic Resonance Imaging, contrast agent, Gd-DTPA-Folate, synthesis, scale-up

Introduction

MRI diagnosis technique has taken the advantage of using what so called contrast agents, which function to improve the quality of image produced. A contrast agent that has been recommended by the US FDA since 1988 is gadolinium diethylene triaminepentaacetate (Gd-DTPA), with its brand name Magnevist [1]. This contrast agent cannot, however, be used to specifically detect cancers [2]. Thus, for this purpose a new type of contrast agents, i.e. targeted contrast agents, have been developed. This new type of contrast agents contains, in their molecular structures, a ligand having a high affinity to receptors on cancer cells. The ligand functions to direct the whole molecule of the contrast agent to go to the targets, i.e. the cancer cells, and thus do imaging specifically [3]. In the case of folic acid is the ligand, this has taken placed through the folate-mediated endocytosis mechanism [4].

So far, three radiopharmaceutical preparations have been synthesized by conjugating folic acid with radioactive-labelled ligands; all of them have been evaluated for their use as tumor markers. They are ^{67}Ga-deferoxamine-folate [5], ^{111}In-DTPA-Folate [3] and ^{99}Tc-DTPA-Folate [6].

In our preliminary study, ^{153}Gd-DTPA-Folate has been successfully prepared and tested for its bio-distribustion [7]. An addition, Gd-DTPA-Folate has been synthesized and characterized through EDA-Folate

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conjugated synthesis [8], and characaterization using Ultraviolet Spectroscopy, Infrared Spectroscopy, and Mass Spectroscopy [9]. However, the yield of rendement has not resulted a good amount of product.

The experimental design must be done, to optimize the reaction condition of synthesis Gd-DTPA Folate, so the rendement of the product will have a good rendement. The Placket Burman design may be optimized in screened the parameters by using statistical and mathematical optimization tools such as Response Surface Methodology (RSM) [10]. This empirical technique enables to evaluate the relationship between independent variables and to predict the response in an effective experimental design.

The aim of this study was to screen and optimize the most important factors affecting the production of synthesis Gd-DTPA-Folat as novel targeted contrast agent for MRI using Plackett-Burman design and Response Surface Methodology-Box Behnken. so the yield will have a good rendement.

Methods

Syntesis of DTPA Folate.

DTPA Folate was synthesized from EDA Folate [8] and DTPA Dianhidrid based on the previous study [8,9]. EDA-Folate and DTPA Dianhidrid were dissolved in DMSO and neutralized by NaoH, then purified to get yellow solid roduct of DTPA Folate.

Optimization of process parameters

Identifying the significant variables using Plackett-Burman design. The aim of this study was to screening the important synthesis parameter with respect to the main efect using Placket Burman experiment design. Using Placket-Burman design was to identify the parameter required for Gd-DTPA Folate production by screening *n* variables in *n+1* experiments. The variables observed in this study, were ratio mol of reactans Gd^{3+} and DTPA Folate (which was synthesis adapted from Fuchs et al., 1999 and modified by Fauzia et al., 2014). All the variables were selected for this study, with each being at two levels, high (+1) and low (-1) as shown in Table 1.

Table 1. Variabels which affected in Gd-DTPA-Folate production.

No	Variabels	Symbol	Unit	Level	
				Low (-)	High (-)
1	Mol ratio (Gd: DTPA Folat)	X_1	Mol	1:1	10:1
2	Reaction temperature	X_2	°C	30	80
3	Stirring rate	X_3	Rpm	250	500
4	Reaction time	X_4	Hours	2	6
5	pH	X_5	-	5	7
6	The volume of water	X_6	mL	10	20
7	Dummy	X_7	-	-	+

Dummy factors was added in the design. Placket-Burman proposed a number of two level factorials, where the number of experiments is a multiple four. Hence the design exist for 8 experiments (n+1).

As per the design, various combinations of the six parameters used, along with the results obtained, are summarized in Table 2.

Table 2. Placket Burman design of Gd-DTPA-Folate production

Exp	Variabel								Yield
	X_0	X_1	X_2	X_3	X_4	X_5	X_6	X_7	
1	+	-	-	-	-	-	-	-	0,21
2	+	+	+	+	-	+	-	-	0,7161
3	+	-	+	+	+	-	+	-	0,269
4	+	-	-	+	+	+	-	+	0,2577
5	+	+	-	-	+	+	+	-	0,8357
6	+	-	+	-	-	+	+	+	0,2586
7	+	+	-	+	-	-	+	+	0,9474
8	+	+	+	-	+	-	-	+	0,3767
Coef	0,4839	0,2351	-0,0787	0,0635	-0,049	0,033	0,0937	-0,0237	

To obtain a proper model for the optimization of Gd DTPA-Folate production, the Box-Benhnken design, for three variables which has a positive coeficients (b) was then applied. They were X_1, X_3 and X_6. This design was preferred because relatively a few experimental combinations of the variables are adequate to estimate the response function.

Result and discussions

Experimental design and statistical analysis for optimization. Optimization of synthesis Gd-DTPA-Folate was doing in two stages. At the first stage, the components that have significant effect on production Gd-DTPA-Folate were identified. At the second stage, the optimum values of these components for Gd-DTPA-Folate production were determined. In the first stage, ratio mole of reactans Gd3+ and DTPA-Folate, stirring rate and the volume of water were identified affected in Gd DTPA-Folate production . Moreover the model coefficients allow to assess the influence of factors on the response (Table 2). It was found that coefficients with high values are the most important factors. In this design, it is assumed that the main factors have no interactions and a first-order multiple regression model is appropriate:

$$Y = \beta + iix \sum \beta \ (i = 1, \dots \dots, k) \quad \text{Equation (1)}$$

Where Y is the response function (Gd-DTPA-Folate production) and βi is the regression coefficient. In Table 2, the design matrix built for the evaluation of seven variables in eight experiments is presented. Variables X1 through X8 represent the variabel which affected for synthesis Gd-DTPA-Folate and D represents a dummy variable.

Optimization design. After selecting the most important components influencing Gd-DTPA-Folate production, Box-Behnken response surface methodology was used to determine the optimum levels of these variables. Selected variables (ratio mole of reactans Gd3+ and DTPA-Folate, stirring rate and the volume of water) were studied at three different concentrations coded as -1, 0, and 1. The coded and actual values of the variables are given in Table 3. According to the Box-Behnken design matrix generated by MINITAB 17 software. Three levels, such as low, medium and high, denoted as -1, 0, +1, were employed to fit a full quadratic response surface model and later approximated to obtain the optimal response (Table 3).

Table 3. Parameter Response Surface Methodology Box-Behnken

Symbols	Variabels	Units	Level		
			Low	Med	High
			-1	0	1
X_1	Mol ratio (Gd^{3+}: DTPA Folate)	mol	1:01	1:05	1:10
X_3	Stirring rate	Rpm	375	427,5	500
X_6	The volume of water	mL	10	15	20

The design variables selected in this study with actual and coded levels along with response variables with 3 replicates are shown in Table 4. The aim of the study is to find the best reaction conditions to obtain the highest yield. All of the 15 designed experiments were performed and the results were multi-regression analyzed. Coefficients were evaluated by regression analysis and tested for their significance (Table 5). Finally, the best-fitting model was determined by regression.

Table 4. Response Surface Methodology Box-Behnken three variables experimental design

Run	Variabels			Yield
	X_1	X_3	X_6	
1	-	-	0	0,2449
2	+	-	0	0,7997
3	-	+	0	0,2556
4	+	+	0	0,9376
5	-	0	-	0,2596
6	+	0	-	0,2893
7	-	0	+	0,7733
8	+	0	+	0,6451
9	0	-	-	0,4368
10	0	+	-	0,3946
11	0	-	+	0,6101
12	0	+	+	0,5442
13	0	0	0	0,4198
14	0	0	0	0,4033
15	0	0	0	0,2755

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The obtained results of Response Surface Methodology Box Behnken were analyzed by ANOVA using MINITAB 17 Software (Table 5).

Table 5. Analysis of variance of Resoponse Surface Methodology Box-Behnken three variables

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	0,415446	0,046161	0,82	0,625
Linear	3	0,339812	0,113271	2,02	0,230
A	1	0,161966	0,161966	2,88	0,150
B	1	0,000208	0,000208	0,00	0,954
C	1	0,177638	0,177638	3,16	0,136
Square	3	0,065212	0,021737	0,39	0,768
A*A	1	0,032825	0,032825	0,58	0,479
B*B	1	0,036161	0,036161	0,64	0,459
C*C	1	0,003626	0,003626	0,06	0,810
2-Way Interaction	3	0,010422	0,003474	0,06	0,978
A*B	1	0,004045	0,004045	0,07	0,799
A*C	1	0,006233	0,006233	0,11	0,753
B*C	1	0,000144	0,000144	0,00	0,962
Error	5	0,280959	0,056192		
Lack-of-Fit	3	0,268483	0,089494	14,35	0,066
Pure Error	2	0,012476	0,006238		
Total	14	0,696405			

The resulting fitted second-order polynomial equation with the coded factors values is given below (Table 6):

Table 6. Coded Coeficients of Resoponse Surface Methodology Box-Behnken three variables.

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		0,366	0,137	2,68	0,044	
A	0,2846	0,1423	0,0838	1,70	0,150	1,00
B	0,0102	0,0051	0,0838	0,06	0,954	1,00
C	0,2980	0,1490	0,0838	1,78	0,136	1,00
A*A	0,189	0,094	0,123	0,76	0,479	1,01
B*B	0,198	0,099	0,123	0,80	0,459	1,01
C*C	0,063	0,031	0,123	0,25	0,810	1,01
A*B	0,064	0,032	0,119	0,27	0,799	1,00
A*C	-0,079	-0,039	0,119	-0,33	0,753	1,00
B*C	-0,012	-0,006	0,119	-0,05	0,962	1,00

Regression Equation in Uncoded Units

Response = 0,366 + 0,1423 A + 0,0051 B + 0,1490 C + 0,094 A*A + 0,099 B*B + 0,031 C*C + 0,032 A*B - 0,039 A*C - 0,006 B*C

The response was 0,366 + 0,1423 A + 0,0051 B + 0,1490 C + 0,094 A*A + 0,099 B*B + 0,031 C*C + 0,032 A*B – 0,039 A*C – 0,006 B*C (where A, B and C mean X_1 , X_5 and X_6).

Contour plots

The final step of this experiments consists in finding the values of factors that give the optimal response . From the validated mathematical model, using the software, we performed graphically 2D contours. The boundary curves are generated using MINITAB software 17 by the combination of three factors induced. The three factors which influenced the Gd-DTPA Folate synthesis were ratiom mole of reactans, stirring rate and the volume of water. Countour plots were generated for the fitted model that displays the effect that combined variables. Figure 1 shows the response surface plots as function of ratio mole of reactants (Gd^{3+} and DTPA Folate), stirring rate (rpm) and the volume of water (mL).

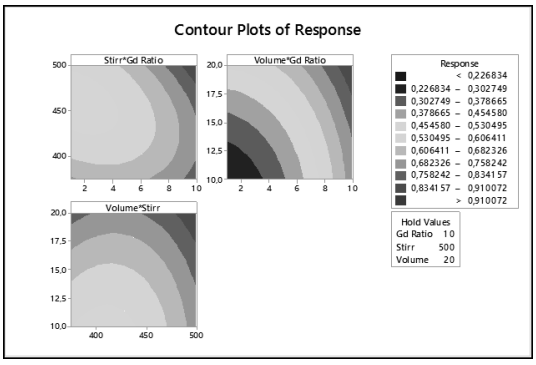

Figure 1. Analysis of countour plot the combined three variables

According to the interpretation of the contour diagrams, the highest values of the conversion (response > 91%) are obtained when the three factors are fixed at high levels. Consideration of the set of graphs allowed the selection of the optimal point: ratio mole of reactants (Gd^{3+} and DTPA Folate) = 1:10 (2,5 x 10^{-3} mol and 2,5 x 10^{-4}), stirring rate = 500 rpm and the volume of water = 20 mL and the conversion value at this point is equal to response = 91,0072 % . The 3D countour can be seen in Figure 2.

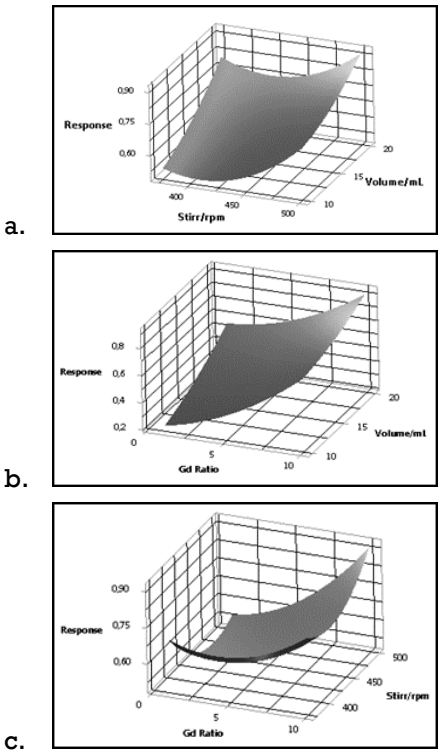

Figure 2. 3D countour plot of the combine three variables

Conclusions

Based on the discussion above, the applied statistical metodolgy, proved to be efficient in optimizing the synthesis of Gd-DTPA Folate for the aimed of scale-up production. The result showed that the combined of three variables in a high level: ratio mol of reactans (Gd^{3+} and DTPA Folate), stirring rate and the volume of water, have a response above 91 %. It means that the three variables were significant variables affected in Gd DTPA Folate production. Further work on optimization of reaction condition using other parameters and focused in the purity of product are still in progress.

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