Optimization of Conditions for the synthesis of new Triphenylphosphonium-Substituted phthalocyanine from Tetrabrominated Phthalocyanine using Experimental Design Method

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Abstract: An experimental design has been drawn up to optimize the experimental conditions of the synthesis of new Triphenylphosphonium-substituted phthalocyanine from tetrabrominated phthalocyanine. Response surface methodology (RSM) was applied to study the influence of molar ratio of tetrabrominated phthalocyanine to triphenylphosphine, reaction time and the reaction temperature. Quadratic model was developed for yield of Triphenylphosphonium-substituted phthalocyanine using Design-Expert software NEMRODW. The model was used to calculate the optimum operating conditions for synthesis of Triphenylphosphonium-substituted phthalocyanine. The optimum conditions were obtained as follows: n (tetrabrominated phthalocyanine TTBPc) = 1:20, reaction time 12 h and reaction temperature 120°C. Under these conditions, the yield of Triphenylphosphonium-substituted phthalocyanine reached 70%.

Keywords: Triphenylphosphonium-substituted phthalocyanine; tetrabrominated phthalocyanine; Response surface methodology

1. Introduction

Phosphonium salts play important role in chemistry, especially as precursors of phosphorus ylides in synthesis of olefins via the Wittig-reaction [1, 2]. In most cases the required phosphonium salts are obtained from triphenylphosphane by heating it with the appropriate alkyl or benzyl halide in dry solvents.

For many years Arylpolonium salts are compounds that have both lipophilic and cationic character, allowing a facile transport through plasma membranes or cell walls. The nature of the mitochondrial membrane is prone to interact with positively-charge and lipophilic molecules. Recently, much attention has been directed towards targeting bioactive compounds to cell mitochondria, in order to remediate mitochondrial dysfunctions that are the cause of a number of diseases, including cancer [4, 5].

One approach typically employed to target drugs to mitochondrias to conjugate the bioactive molecule of choice directly to mitochondrialotropic ligands such as the triphenylphosphonium group (TPP) [6, 7]. So labelling and imaging of mitochondria is an important aspect for understanding the cellular processes and in vitro diagnostic assay. [8-15] Mitochondria targeting is also considered as therapeutic strategy for cancer [12] that involves their destruction via delivering drugs/nanomaterials followed by therapy. [16] for specific targeting of mitochondria.

Among them triphenylphosphonium (TPP) is widely used for mitochondria targeting, [8, 9] TPP is a polar cationic molecule with high lipophilicity which makes it suitable for penetrating the mitochondrial intermembrane potential barrier and have been used in different mitochondria targeting materials. [8, 9] TPP has been conjugated with nanoparticle, [17-19] dendrimer, [13] liposome [12] and reported for targeted drug delivery and imaging of mitochondria.

Mitochondrial targeting, in particular, now attracts increasing attention for subcellular targeting [20-21] is starting to be applied to PDT. The synthesis of a new family of Triphenylphosphonium-substituted with positively Phthalocyanines (Pcs) are gathering importance as effective photosensitizers in targeted PDT and imaging of tumors.

This work is a complement and a very large extension of the work we have previously reported concerning Biological activities on human cancer cells of Triphenylphosphonium-substituted phthalocyanine [22] by employing a rational approach to maximize the reaction yield of the desired product. Furthermore, a surface response methodology (RSM) approach was used to statistically identify critical reaction parameters with high yield.

The preparation of Triphenylphosphonium-substituted Phthalocyanines (Pcs) is influenced by many factors. For this reason a preliminary study on the effect of these factors on the preparation was carried out in order to determine the most important ones and their regions of interest. The most influential factors were found to be reaction time (X1), PPh3 to TTBPc molar ration (X2) and reaction temperature (X3).

Among them triphenylphosphonium (TPP) is widely used for mitochondria targeting, [8, 9] TPP is a polar cationic molecule with high lipophilicity which makes it suitable for
1) [22] with values of (X1), (X2) and (X3) included in the suitable range. Desirable preparation outputs based on reaction yield (Y) was considered as responses. Thus, an experimental design methodology is applied to relate the experimental conditions of the Synthetic route of Zn (II) phthalocyanine core with triphenylphosphonium units with high percent yield. Furthermore, response surface methodology (RSM), an efficiently statistical technique, was applied to determine the optimum operating conditions with minimum number of experiments.

2. Materials and Methods

All solvents and reagents were of reagent grade quality and obtained commercially from Aldrich, Fluka or Merck. FT-IR spectra were recorded between 4000 and 650 cm-1 using a PerkinElmer Spectrum 100 FT-IR spectrometer. NMR spectra were recorded in deuterated solvents on a Varian 500 MHz spectrometer at 298 K. 31P NMR spectrum was recorded with a 202.26 MHz magnetic field, in proton-decoupling conditions. Mass spectra were recorded on a MALDI (matrix assisted laser desorption ionization) BRUKER Microflex LT.

Phthalocyanine bearing Triphenylphosphonium-functions was prepared as reported [22].

Synthesis of Triphenylphosphonium-substituted Zn (II) phthalocyanine and characterization

The Zn (II) phthalocyanine core with triphenylphosphonium units was synthesized using a procedure previously described by Tarhouni and coworkers [22]. All classical analyses (FT-IR, MS-MALDI-TOF, 1H and 31P NMR) have been performed and confirmed the proposed structure.

The formation of Triphenylphosphonium-substituted Zn (II) phthalocyanine reaction undergoes based on the following reaction:

![Scheme 1: Synthetic route of Zn (II) phthalocyanine core with triphenylphosphonium units](image)

A $2^3$ full-factorial Box-Behnken experimental design with coded levels augmented by $2^3$ axial points ($\pm X_1$, 0, 0), (0, $\pm X_2$, 0), (0, 0, $\pm X_3$), and 2c center points (0, 0, 0) [13-14]. Hence, the total number of tests (N) required for the three independent variables is:

$$N = 2^3 + (2 \times 3) + 2 = 16.$$  

The center points are made up of all variables at zero level which are crucial in determination of experimental error and the reproducibility of the data. On the other hand, combination of variables consisting of one at its lowest (-a) level or highest (a) level with other variables at zero level constitutes the axial points, a is the distance of the axial point from center and makes the design rotatable and is calculated by $a = 2n/4$, where $n$ is the factor numbers (in this study, three factors are being evaluated, so a is equal to 1.68).

Replicates of the test at the center are very important as they provide an independent estimate. Variables were designed at levels by associated plus signs (+1) with high levels, zero (0) indicating center value and minus signs (-1) with low levels. The coded values of these factors were obtained according to equation 1 as follows:

3. Experimental Design and Mathematical Model

Central composite design

RSM was a collection of mathematical and statistical techniques that was utilized to design experiments, build models and analyze the effects of the several independent variables. RSM was an effective tool to study the individual and interactive effects of these factors in order to find the target value [23-27].

The Box-Behnken experimental design was chosen to study the combined effects of reaction time, PPh3 to TTBPc molar ration and reaction temperature (on the yield of phthalocyanine by RSM. The experimental design was carried out by three chosen independent process variables at three levels including reaction time ($X_1$), PPh3/ TTBPc mole ratio ($X_2$) and temperature ($X_3$) shown in Table 1.

![Table 1: Parameter levels and coded values in the experimental design](image)
The predicted quadratic model related the yield of Zn (II) phthalocyanine core with triphenylphosphonium units with three independent factors (reaction time, PPh3/TTBPc molar ratio, reaction Temperature) expressed in equation 3 as follows:

\[ Y = 63.242 + 0.420X_1 + 0.807X_2 - 1.445X_3 - 0.776X_1X_1 - 11.188X_2X_2 - 2.476X_3X_3 - 5.663X_1X_2 - 0.787X_1X_3 + 0.937X_2X_3 \]

where \( x_i, x_2 \) and \( x_3 \) were the coded values of the best variables reaction time, PPh3/TTBPc molar ratio and reaction Temperature, respectively; \( Y \) was the response of yield of Zn (II) phthalocyanine core with triphenylphosphonium units.

Positive sign in front of the terms indicates synergistic effect, whereas negative sign indicates antagonistic effect. The coefficient of the model for the response was estimated using multiple regression analysis technique included in the RSM. Fit quality of the models was judged from their coefficients of correlation and determination.

Table3. Analysis of variance (ANOVA) for response surface quadratic model for Zn (II) phthalocyanine core with triphenylphosphonium units

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>Fexp</th>
<th>Signif</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>926.3612</td>
<td>9</td>
<td>102.9290</td>
<td>53.3568</td>
<td>0.0176 **</td>
</tr>
<tr>
<td>Residual</td>
<td>11.5788</td>
<td>6</td>
<td>1.9298</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of fit</td>
<td>11.4775</td>
<td>5</td>
<td>2.2955</td>
<td>22.6716</td>
<td>16.5</td>
</tr>
<tr>
<td>Pure error</td>
<td>0.1013</td>
<td>1</td>
<td>0.1013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation total</td>
<td>927.9400</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

***Significant at 99.9% level confidence

The parameter levels and coded values were given in table 2. The graphical representations of the distribution of these experimental points are given in Fig. 1. The measured responses are defined as Reaction yield in %.

Figure 1 Response surface plots showing the predicted values of yield of Zn (II) phthalocyanine core with triphenylphosphonium units, (a) effect of reaction temperature and PPh3/TTBPc molar ratio (b) reaction time and reaction temperature, (c) reaction time and PPh3/TTBPc molar ratio.

Table2: Experimental design and response value

<table>
<thead>
<tr>
<th>Experimental No</th>
<th>Reaction time / h</th>
<th>PPh3/TTBPc</th>
<th>Reaction Temperature</th>
<th>Yield of Reaction /%</th>
<th>Expt</th>
<th>Calc.</th>
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<td>-1</td>
<td>-1</td>
<td>-1</td>
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<td>43.507</td>
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<td>1</td>
<td>-1</td>
<td>54.76</td>
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<tr>
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<td>1</td>
<td>-1</td>
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<tr>
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<td>40.49</td>
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</tr>
<tr>
<td>6</td>
<td>1</td>
<td>-1</td>
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<td>50.49</td>
<td>55.861</td>
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<td>1</td>
<td>1</td>
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<td>1</td>
<td>1</td>
<td>44.18</td>
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<tr>
<td>9</td>
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<tr>
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<tr>
<td>13</td>
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<td>0</td>
<td>-α</td>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>62.91</td>
<td>63.24</td>
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</tr>
</tbody>
</table>
Model fitting and statistical analysis
The statistical software package Design-Expert, NEMRODW, was used for regression analysis of experimental data to fit the equations developed and also to plot response surface. The quality of the developed model can be determined from the value of correlation (R²) while evaluation of the statistical significance of the equations developed can be determined by using an analysis of variance (ANOVA).

4. Results and Discussion

RSM experiments and studying
Analysis of variance (ANOVA) of the regression model was employed to seek out the significance of the effects of parameters on reaction process (Table 3). The significance of regression model was examined by F-test and p-value. As shown in (Table 3), the computed F-value (53.33) was much larger than the tabular F-value (3.70), which was desirable as it indicated that the model obtained from equation 3 gave a good prediction at 1% level of significance. In addition, the p-value for the model less than 0.0001 also meant that the model term was significant. In the study, $x_1$, $x_2$, $x_3$, $x_1x_2$, $x_1x_3$, and $x_2x_3$ were significant factors. Furthermore, the coefficient of determination ($R^2$) of the model was 0.9016, which implied that 90.16% of the variation could be explained by the fitted model. In general, the quadratic regression model with the $R^2$ value higher than 0.90 was considered as possessing a considerable high correlation [29, 30]. On investigating $R^2$ value, the predicted $R^2$ of 0.7399 was in reasonable agreement with the adjusted $R^2$ of 0.9016. The adequacy precision of 53.33 was much greater than 4, which indicated that the model could be used to navigate the design space. The desirable results represented that the chosen quadratic model was appropriate in predicting the response variables for the experimental data.

Statistical analysis
The quality of the model developed was evaluated based on the correlation coefficient value. The $R^2$ values for Eqs 1 was 0.939. Both the $R^2$ values obtained was relatively high (close to unity), indicating that this was a good agreement between the experimental and the calculated values of the determination coefficients of the multilinear regression from the models.

The adequacy of the models was further justified through analysis of variance (ANOVA). The ANOVA for the quadratic model for of yield of Zn (II) phthalocyanine core with triphenylphosphonium units yield is listed in Table 3.

Reading the data in Table 3 reveals the validity of the model since the value of Fexp (22.67), which is the ratio between the lack of fit and the pure error, is much lower than the critical value of Fisher (F0.001 (5,1) =5764) at a 99.9% level of confidence with 5 and 1 degrees of freedom.

The results of the ANOVA (Table 3) also shows that the experimental value of Scnedecor (Fexp= 53, 33), which is the ratio between the square of the Response surface plots facilitated the interaction study of the process variables for tetra-triphenylphosphonium phthalocyanine yield. The three dimensional (3-D) response plots of reaction time, PPh3/TiBPC molar ratio and reaction temperature are shown in Figure 1.

The variation of yield of Zn (II) phthalocyanine core with triphenylphosphonium units with (a) effect of reaction temperature and PPh3/TiBPC molar ratio was presented in Figure 1 - (a) and. The interaction between reaction time and PPh3/TiBPC molar ratio reaction time is given in Figure 1 - (c). It could be seen that the yield of Zn (II) phthalocyanine core with triphenylphosphonium changed little compared to the effect of time. With increasing temperature, the yield increased, passing through a maximum and then decreased slightly. Certainly, the reaction with PPh3/TiBPC molar could not be conducted at a very high molar ratio. As it could be seen, the yield reached a maximum value around 120°C and then declined.
Figure 1- (c) and 1- (a) presented the three dimensional related to yield of Zn (II) phthalocyanine core with triphenylphosphonium units as a function of reaction time and amount of PPh3/TTBPC molar ratio under constant temperature. As it could be seen on the three-dimensional response surface, the yield of Zn (II) phthalocyanine with triphenylphosphonium changing slightly to the maximum meant that the reaction time on the response showed a more significant influence in comparison to temperature. With a further increase in temperature, a significant decrease in yield occurred what be explained by decomposition temperature for the phosphonium salt.

The reaction paths of tetraphenylphosphonium de phthalocyanine degradation can be explained by the following assumptions:

At temperatures >160 °C, reaction pathways involving reactive intermediates, namely free radicals, are more probable. Thus, in the case involves the homolysis of the P-phenyl bond to generate triphenylphosphonium radical and phenyl radical, which can subsequently either combine with another phenyl radical to form biphenyl or a bromine atom to form bromoalkyltriphenylphosphonium de phthalocyanine (Scheme 2)

**Scheme 2:** Degradation route of Zn (II) phthalocyanine core with triphenylphosphonium units at temperature higher than 160°C

The yield increased significantly before amount of PPh3/TTBPC molar ratio reached 20, but insignificant change was observed for molar ratio over 20. Meanwhile, the yield decreased gradually as temperature increased but changed slightly when temperature exceeded 120°C. The saddle shape of the plots showed that the relationships between PPh3/TTBPC molar ratio and temperature are likely to affect the reaction. The plot indicated that the maximum value (69%) was associated with molar ratio value 20 and temperature 120°C.

**Attaining optimum conditions and model verification**

Based on the 16 experimental data, the model predicted the optimum yield for triphenylphosphonium phthalocyanine of PPh3 over TTBPC could reach 72%, with the optimal process conditions of reaction time 12 h, alcohol/acid molar ratio 20 and reaction temperature 120°C. In order to verify the adequacy of the model, three parallel experiments were conducted, under the conditions above and the average yield reached 70.9%, which meant that the predicted and experimental data had a good agreement.

**5. Conclusions**

The response surface methodology based on Box-Behnken experimental design was employed for optimization the experimental conditions of the synthesis of new Triphenylphosphonium-substituted phthalocyanine from tetrabrominated phthalocyanine

The optimum conditions were obtained as follows: n (tetrabrominated phthalocyanine TTBPC): n (triphenylphosphinePPh3) = 1:20, reaction time 12 h and reaction temperature 120°C. Under these conditions, the yield of Triphenylphosphonium-substituted phthalocyanine
reached 70%, in close agreement with values predicted by the mathematical model (97.82%).

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