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Optimization of a Reconstitutable Suspension of Rifampicin Using 2^4 Factorial Design

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ABSTRACT

In this study 2^4 factorial design associated with surface response methodology was used to develop and optimize a reconstitutable suspension of rifampicin. The study illustrated the effect of the percentage of each of sucrose, avicel RC-591, hydrophilic aerosil, and aerosol-OT on the flowability and the bulk density of the dry mixture as well as the viscosity, the sedimentation volume, and the redispersibility of the suspension. An empirical equation developed for each of the above responses was used with the aid of computer software to plot a contour map of the most significant effects and interactions. Five replicates at the center of the design were used to independently calculate the experimental error and to detect any curvature in the response surface. Three formulas which are not included in the design were prepared to check the validity of the model equation.

INTRODUCTION

The development of a new pharmaceutical formulation is usually an optimization problem. The frequently applied trial-and-error technique, including a careful control of the variables one at a time in a series of logical steps, usually leads to a satisfactory formulation rather than an optimal one.

The application of an optimization technique consisting of statistically experimental design to pharmaceutical formulation development would provide an efficient and economical method to acquire the necessary information to understand relationship between controllable (independent) variables and performance or quality (dependent) variables (1). In addition, the optimization process provides a method to develop an empirical

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model equation to characterize the response as a function of the different independent variables.

Available response surface designs for fitting first-order models include simplex, super modified simplex, factorial and fractional factorial. Plackett–Burman, and koshal experimental designs. The second-order response surface designs include factorial, computer optimized. Box–Behnken, central composite design based on a factorial, fractional factorial, and simplex (2).

The technique of optimization is well reported in the literature for the development of tablet formulations (2–4), microcapsules (5,6) fluid bed spray coating (7), a dry powder blend to be filled into hard gelatin capsules (8), a hydrocolloid dressing (9), and suspension (10). The present study investigates the utility of a 2^4 factorial design and optimization process to develop and improve formulation for rifampicin reconstitutable suspension. The optimization process was used to generate a model equation that provides a means of evaluating changes in response due to changes in the independent variable levels.

MATERIALS AND METHODS

Materials

Materials used were: rifampicin (El-Nasr Pharmaceutical Chemicals Co., Cairo, ARE), Avicel (microcrystalline cellulose RC 591, FMC Co., Pennsylvania, USA), hydrophilic Aerosil-200 (Degussa Co., USA), Aerosol-OT (Atlas Chemicals, Wilmington, DE, USA), sodium citrate, citric acid and sodium benzoate (Prolabo), straw flavor (Roure Co., France), and pure grade of sucrose.

Preparation of the Dry Mixture

All the powder components were reduced to more or less the same particle size. They were passed through a sieve of 125 μm pore size and retained on 100 μm. Ingredients present in small quantities (buffer components, sodium benzoate, flavor, and Aerosol-OT) were mixed in two-stage mixing operations. The same procedure was followed for rifampicin as a colored material. Such ingredients were mixed with a portion of sucrose at the first stage. The whole powder was then blended using powder blender (Erweka-Apparatebau, Frankfurt, Germany). Formulas were prepared for 100 ml suspension containing 0.2% sodium benzoate, 0.1% flavor, 0.12% sodium citrate, and 0.24% citric acid. Buffer components were used to adjust the pH of the constituted suspension to pH 4.5, where rifampicin is considered to be almost insoluble.

The Experimental Design

A 2^4 full factorial design was utilized in this study. The four independent variables investigated are: the concentration of sucrose (X_1), Avicel RC 591 (X_2), Aerosil 200 (X_3), and Aerosol-OT (X_4) per the final suspension. Five replicates at the center of the design were investigated to allow for an independent estimation of the experimental error and to check the linearity of the factor effects (11). The levels of the four independent variables are shown in Table 1 and the design is presented in Table 2.

The effect of the previously mentioned variables was investigated on the following responses:

1. The bulk density calculated as the weight per unit volume of the dry mixture of powder.
2. The flowability of the powder calculated as πr^2, where r is the radius of the base of a 2 cm high cone.
3. The viscosity of the suspension 24 hr after constitution, measured in cps, using Brookfield viscometer (model DV-I$^+$, Brookfield Engineering Laboratories Inc., Stouchton, USA) connected to a thermostated circulating bath (model TC 200). The viscosity of the suspension was measured at 25° using spindle #21 at 100 rpm.
4. The sedimentation volume as a percentage of the original volume of the suspension (V_u/V_o %) measured 1 week after constitution.
5. The percentage ease of redispersibility was measured 1 week after constitution. The stored suspension in a measuring cylinder was inverted through 180° and the number of inversions nec-

Table 1

Level of the Investigated Variables

Independent Variables	Levels as % of Constituted Suspension		
	−1	0	+1
Sucrose (X_1)	30.0%	45.0%	60.0%
Avicel (X_2)	1.0%	1.5%	2.0%
Aerosil (X_3)	0.0%	0.5%	1.0%
Aerosol (X_4)	0.0%	0.05%	0.1%

Table 2

Design Points for the Investigated Variables

Random #	Level of Variables as % of Suspension			
	X_1	X_2	X_3	X_4
1	30	1.0	1.0	0.1
2	30	1.0	0.0	0.1
3	45	1.5	0.5	0.05
4	30	2.0	1.0	0.0
5	60	1.0	1.0	0.0
6	60	1.0	1.0	0.1
7	30	2.0	1.0	0.1
8	45	1.5	0.5	0.05
9	30	2.0	0.0	0.1
10	45	1.5	0.5	0.05
11	60	2.0	0.0	0.0
12	60	2.0	1.0	0.1
13	60	1.0	0.0	0.0
14	30	2.0	0.0	0.0
15	60	1.0	0.0	0.1
16	45	1.5	0.5	0.05
17	30	1.0	0.0	0.0
18	30	1.0	1.0	0.0
19	45	1.5	0.5	0.05
20	60	2.0	0.0	0.1
21	60	2.0	1.0	0.0

essary to restore a homogeneous suspension was determined. One inversion was considered 100% easy to be redispersed. Every additional inversion decreased the % ease of redispersibility by 5%.

Response Surface Analysis

A linear regression model equation was employed for fitting the response surface in the form:

$$y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + \ldots + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + \ldots + B_{123}X_1X_2X_3 + \ldots \quad (1)$$

where Y is the level of the response, B_i, B_{ij}, B_{ijk} . . . are the regression coefficients, and X_i is the coded level of the ith independent variables.

Data were analyzed using analysis of variance (ANOVA) and regression coefficients were calculated for factors having significant effect at $p < 0.01$ and/or 0.05.

The error mean square was calculated according to the following equation:

$$MS_E = \frac{SS_E}{n_{c-1}} \quad (2)$$

$$MS_E = \frac{\Sigma\{Y_i - \bar{Y}\}^2}{n_{c-1}} \quad (3)$$

where y_i is the response of the ith replicates at the center of the design, Y is the mean, and n is the number of the replicates.

The curvature sum of squares was calculated according to the following equation:

$$SS_{curvature} = \frac{n_f n_c\{\bar{Y}_f - \bar{Y}_c\}^2}{n_f + n_c} \quad (4)$$

Where n_f and n_c are the number of the factorial experiments and the experiments at the center, respec-

tively; $\bar{Y}_f$ and $\bar{Y}_c$ are the means of the corresponding observations.

$SS_{curvature}$ with 1 degree of freedom was compared with the error mean square to check any curvature in the response surface as a function of different factors effect.

RESULTS AND DISCUSSION

The effects of the different combinations of sucrose (X_1), Avicel (X_2), Aerosil (X_3), and Aerosol-OT (X_4), illustrated in Table 2, on the different measured characteristics of both the powder mixture (flowability and bulk density) and of the constituted suspension (viscosity, sedimentation volume, and redispersibility) are presented in Table 3.

Analysis of data was carried out using analysis of variance and the significance of the individual parameters was evaluated with F test. Results of the response surface analysis are summarized in Table 4.

It is obvious that only sucrose and Aerosil had a significant effect on the bulk density of the powder mixture. Figures 1(a) and 1(b) show both the increasing and decreasing effect of sucrose and Aerosil, respectively. A significant synergistic interaction between sucrose and Aerosil at $p < 0.01$ was observed. This interaction is reflected by the pattern of the lines of Fig. 1(c). Although, the $F_{curvature}$ for the response surface was significant at $p < 0.05$, the developed model equation having the parameters significant at $p < 0.01$ and 0.05 was a good predictor of the bulk density for the three formulas presented in Table 5.

On the flowability of the powder mixture, only sucrose and Aerosil had a significant effect. The decreasing effect of sucrose was significant at $p < 0.05$, this being observed in the slope of the flowability lines of Fig. 2(a) toward lower sucrose contents. As all formulas were kept in desiccator after preparation and up to the time of evaluation, the decreasing effect of sucrose on the flowability of the powder may be due to its sticking characteristics. The increasing effect of Aerosil, which was highly significant ($F_{1,4} = 535.829$), is obvious from both Fig. 2(a) and Fig. 2(b).

Table 3

Effect of Different Combinations on the Characteristics of the Dry Mixture and Suspension

Exp. Number	Bulk Density	Flowability, πr^2	Viscosity, cps	% Sedimentation Volume	% Ease of Redispersibility
1	0.665	17.524	6.0	52	90
2	0.813	14.318	3.5	28	70
3	0.833	17.520	9.0	44	70
4	0.719	17.898	6.5	64	80
5	0.858	17.898	22.5	92	95
6	0.788	17.524	22.5	58	95
7	0.725	18.181	8.0	60	90
8	0.841	17.898	9.5	44	75
9	0.792	14.683	4.5	32	55
10	0.835	18.460	9.5	48	70
11	0.790	12.560	18.0	24	40
12	0.809	17.898	27.0	72	95
13	0.818	12.560	13.0	18	50
14	0.815	13.357	4.0	24	40
15	0.781	12.560	16.5	26	70
16	0.797	17.330	9.0	48	70
17	0.829	12.874	3.5	18	35
18	0.725	19.040	6.0	66	95
19	0.841	17.710	8.5	48	75
20	0.837	12.249	21.0	34	40
21	0.856	19.236	25.5	100	95

Table 4

Response Surface Data

Parameters	Bulk Density	Flowability, πr^2	Viscosity, cps	% Sedimentation Volume	% Ease of Redispersibility
B_0	0.796**	15.648**	13.000**	44.06**	70.875**
B_1	0.028**	-0.337*	7.750**	5.00**	—
B_2	—	—	1.312**	3.25**	-4.060**
B_3	-0.021*	2.502**	2.500**	22.50**	20.937**
B_4	—	—	0.625**	-2.75**	4.687**
B_{12}	—	—	0.812**	—	—
B_{13}	0.031**	0.326**	1.125**	5.00**	—
B_{14}	—	—	0.373*	-2.75**	-2.187*
B_{23}	—	—	—	—	2.187*
B_{34}	—	-0.338*	—	-7.25**	-4.062**
B_{123}	—	—	—	—	2.81*
B_{134}	—	—	0.375*	-2.75**	—
B_{234}	—	—	—	—	3.437*
Error MS	0.34	0.749	0.175	4.73	7.5
Curv. MS	4.9	17.332	57.94	0.002	4.3
$F_{\text{curvature}}$	14.41*	23.140**	331.08**	0.004	0.573

*$p < 0.05$.
** $p < 0.01$.

While sucrose decreased the flowability of the powder, it improved the effect of Aerosil, which was viewed as a synergistic interaction. This may be due to increasing the bulk density of the powder by sucrose in addition to smoothing the surface of the particles induced by Aerosil. A significant ($p < 0.05$) antagonistic interaction was observed between Aerosil and Aerosol-OT. Figure 2(b) shows that Aerosil-OT had a mild increasing effect on the flowability of the powder up to 0.5% aerosil, whence an antagonistic interaction effect started as observed from the change in the slope of the lines to the opposite direction.

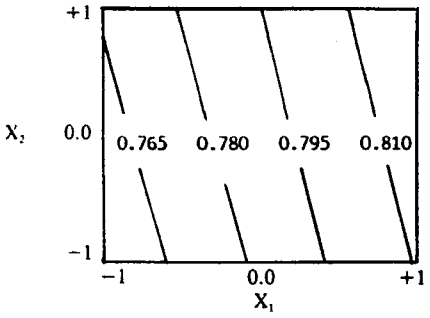
The $F_{\text{curvature}}$ (23.14) was very close to the tabulated value at $P < 0.01$ (21.2); however, the developed model equation with the linear parameters significant at $P < 0.01$ and 0.05 was still giving a reasonable prediction of the flowability of the powder, as presented in Table 5.

All the factors significantly ($p < 0.01$) affect the viscosity of the constituted suspension. While sucrose increased the viscosity of the solution, Avicel, Aerosil, and Aerosol-OT had their effect by hydration and swelling, increasing solid content, and inducing flocculation, respectively. The most pronounced increase in viscosity was induced by sucrose and Aerosil ($F_{1,4}$ = 5491.4 and 571.43, respectively). The increasing effect of sucrose is observed in the high coefficient (Table 4) as well as from Fig. 3(a), 3(b), and 3(c). The increasing effect of Avicel and Aerosil is presented in Fig. 3(a) and 3(b), respectively.

Figures 3(a) and 3(b) reveal the significance of the interaction between sucrose and each of Avicel and Aerosil, respectively. This interaction was reflected by the lack of parallelism between lines of viscosity which is even more prominent in sucrose-aerosil interaction.

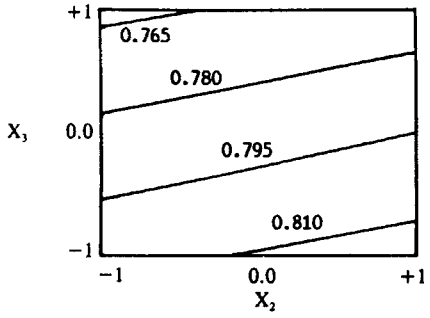
A linear equation was constructed so that it only has those parameters significant at $p < 0.01$. The equation was suitable for predicting the viscosity at the two extremes. However, it was insufficient to predict viscosity at the middle of the surface, indicating a high curvature in the response plane confirmed by the high $F_{\text{curvature}}$ (331.08) compared to the tabulated one at $P < 0.01$.

It was observed that all factors had a significant effect on the sedimentation volume of the suspension at $p < 0.01$ (Table 4). the most influential effect was due to Aerosil, as observed from Fi. 4(b) ($F_{1,4}$ = 1712.473). This effect was mainly due to its antipacking



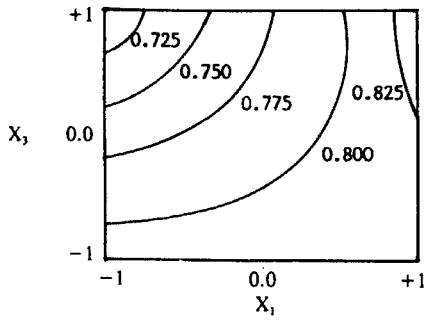
X_3=0.50%
X_4=0.05%

Figure 1a



X_1=45.00%
X_4=00.05%

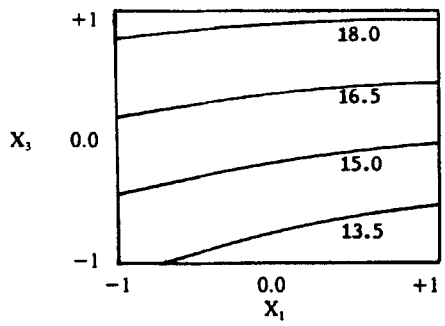
Figure 1b



X_2= 1.50%
X_4= 0.05%

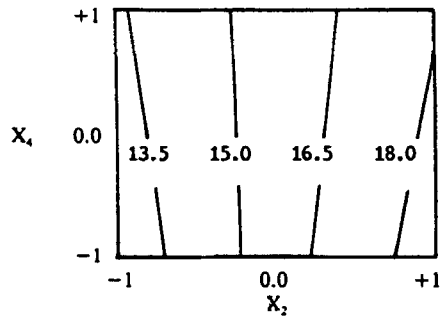
Figure 1c

Figure 1. Effect of different variables on bulk density of powder mixture.



X_2=1.50%
X_4=0.05%

Figure 2a



X_1=45.00%
X_2= 1.50%

Figure 2b

Figure 2. Effect of different variables on the flowability of powder mixture.

characteristics, while sucrose and Avicel increased the sedimentation volume by increasing the viscosity of the medium. Contrary to what was expected the flocculating agent Aerosol-OT had a negative coefficient. This decreasing effect can be explained on the basis of the highly significant antagonistic interaction observed between Aerosil and Aerosol-OT as reflected by the high negative coefficient and Fig. 4(c).

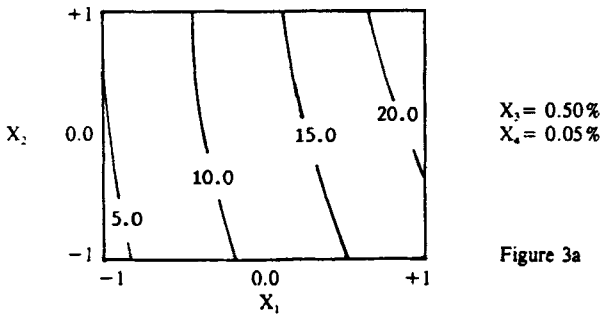
A synergistic interaction was observed between sucrose and aerosil [Fig. 4(b)]. This synergistic effect can be explained on the basis that while aerosil surrounds

Table 5

Comparison of the Experimental and Predicted Powder and Suspension Properties

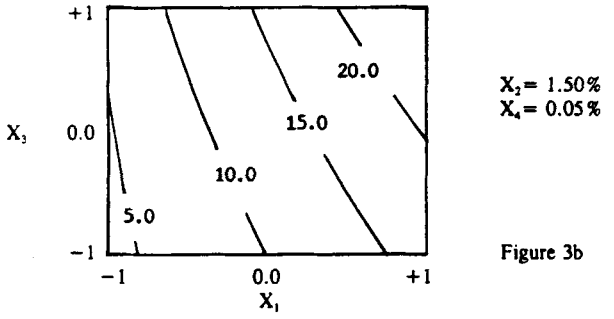
	Composition %				Bulk Density		Flowability		Viscosity		% Sed. Vol.		Ease of Red.	
Formula	X_1	X_2	X_3	X_4	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
(1)	30.0	2.0	0.5	0.0	0.785	0.768	16.23	15.925	4.5	5.49	48.0	42.31	65.0	62.13
(2)	45.0	2.0	0.5	0.0	0.798	0.796	15.54	15.648	9.5	13.69	54.0	57.31	70.0	62.13
(3)	60.0	1.5	0.0	0.1	0.815	0.814	11.33	12.821	20.0	18.50	30.0	26.06	60.0	58.69

Note. Exp., experimentally determined; Pred., predicted through the model equation.



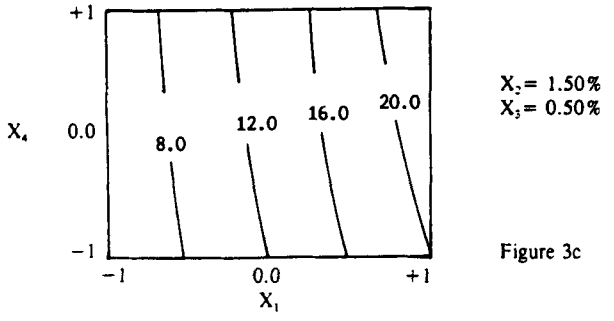
X_3= 0.50%
X_4= 0.05%

Figure 3a



X_2= 1.50%
X_4= 0.05%

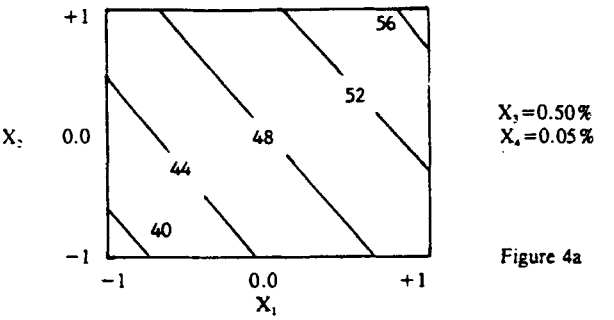
Figure 3b



X_2= 1.50%
X_3= 0.50%

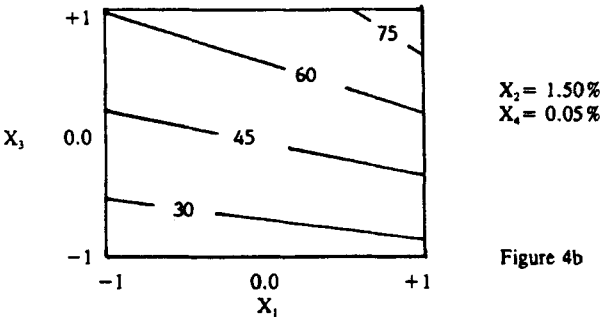
Figure 3c

Figure 3. Effect of different variables on the viscosity of the constituted suspension.



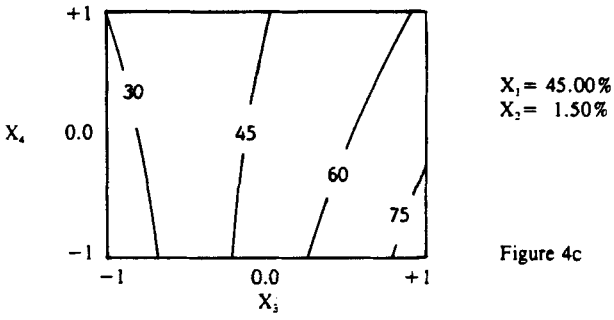
X_3=0.50%
X_4=0.05%

Figure 4a



X_2= 1.50%
X_4= 0.05%

Figure 4b



X_1= 45.00%
X_2= 1.50%

Figure 4c

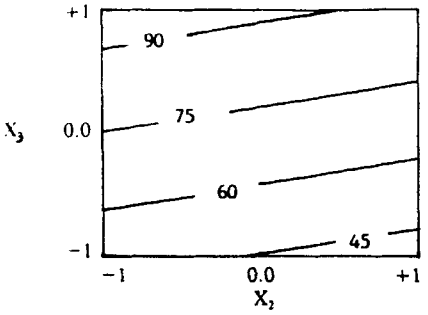
Figure 4. Effect of different variables on the sedimentation volume of the constituted suspension.

the particles of rifampicin preventing them from settling, this effect with the increased viscosity was improved, leading to higher sedimentation volume. A less influential interaction was observed between sucrose and Aerosol-OT and between sucrose, Aerosil, and Aerosol-OT.

A typical linear relationship was observed between the sedimentation volume of the suspension and the level of the different variables, as reflected by the negligible $F_{\text{curvature}}$ (Table 4). The polynomial equation constructed with all parameters significant at $p < 0.01$ was a good predictor of the sedimentation volume of the suspension, as presented in Table 5.

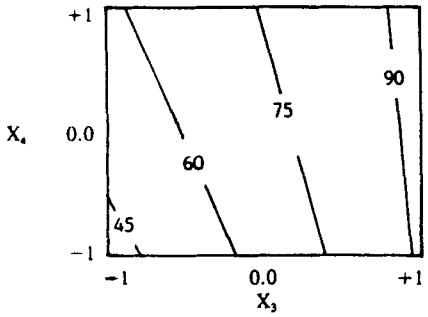
On the case of redispersibility of the suspension, only Avicel, Aerosil, and Aerosol-OT had a significant effect ($p < 0.01$). The highest improving effect shown with Aerosil ($F_{1,4} = 935.208$) was due to its antipacking characteristics. The decreasing effect of Avicel is observed in the slope of the line of Fig. 5(a) toward lower Avicel content as well as from the negative coefficient (Table 4). This effect may be due to the difficulty of redispersing a viscous suspension.

A significant antagonistic interaction was observed between Aerosil and Aerosol-OT [Fig. 5(b)]. The slope of the lines toward lower Aerosol-OT content indicates



X_1=45.00%
X_4= 0.05%

Figure 5a



X_1=45.00%
X_2= 1.50%

Figure 5b

Figure 5. Effect of different variables on the ease of redispersibility of the constituted suspension.

that Aerosil as anticompacting agent works better in the presence of lower concentration of Aerosol-OT.

No significant curvature was observed in the response surface of the redispersibility as a function of the different variables. This was reflected by the low $F_{curvature}$ and was confirmed by the good prediction of the response for the three checked formulas presented in Table 5.

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