Original Article

Thai Journal of Pharmaceutical Sciences

A comparison of model fitting using different runs in Box–Behnken design for alginate film formulation

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Received: Mar 05, 2020
Accepted: Jun 23, 2020
Published: Jul 23, 2020

ABSTRACT

Introduction: Box–Behnken design (BBD) is a response surface method used for experimental design in several applications such as pharmaceutical and food. BBD experiment can be run using different central points (CPs) for analyzing optimal model fitting for responses. **Objective:** This study aimed to compare the model fitting between 3-CPs (15 runs) and 5-CPs (17 runs) BBD in preparation of alginate film. **Materials and Methods:** The concentrations of trehalose, sodium alginate, and calcium chloride were dependent variables, and film properties, thickness, opacity, and water vapor permeability were responses. **Results:** The results showed that the model fitting was quadratic. Although values of adjusting R^2 of each response in 17-run BBD were higher than 15-run BBD, analysis of variance of model fitting indicated the "lack of fit" in thickness, and WVP of 17-run BBD was significant (P = 0.0084, P = 0.0148, in orderly). It seemed that the 17-run BBD might have a higher number of erroneously observed model predictions. **Conclusion:** Therefore, the 3-CP BBD was more suitable to be used as a model for evaluation of response and prediction of further optimized film formulation.

Keywords: Alginate, Box–Behnken design, film, response surface method

INTRODUCTION

Response surface methodology (RSM) is a combination of statistical and mathematical methods created on the fit of the polynomial model to the data with the purpose of setting statistical predictions. The method is useful for designing, optimizing, developing, and improving processes in various applications where one response or more than one response is affected by several factors.[1] The RSM such as Central composite design (CCD) and Box–Behnken design (BBD) provides less time, effort, and resources in experimental design and facilitates the gathering of a large amount of data while minimizing the number of experiments.[1] In the number of factors, more than two, CCD and BBD have the appropriate efficiency in the quadratic model. However, BBD provides a smaller number of experimental runs.[2] BBD is the one of RSM for quadratic response surfaces as three-level factorial (level −1, 0, 1) for each factor. In BBD, the experimental points are situated on a hypersphere equally distant from the central point (CP).[1] This design could decrease the number of experiments needed to conduct research, thus leading to reduced cost and time.[2] A number of CPs is numbers of replications at a CP in BBD (level 0). For model fitting using BBD, the 3-CP BBD (15 runs) are more commonly used than 5-CP BBD (17 runs) based on modeling validity criteria.[2] However, some studies suggested that using different runs might affect the model fitting due to interaction between each factor (independent variable) and responses (dependent variable).[3,4] The replication of the experiment should provide the precision of the experiment and reduce the error of the experiment; however, none of the study has been compared to the different model fit between 3-CP BBD (15 runs) and 5-CP BBD (17 runs). This study aimed to compare the validity of model fitting using different runs in 3-factor BBD for alginate film preparation. The model analysis was done for prediction the film properties (responses), namely, opacity, thickness, and water vapor permeability (WVP) from the film composition (factors) which were concentrations of alginate, calcium chloride ($CaCl_2$) and trehalose.

MATERIALS AND METHODS

Chemicals and Reagents

Sodium alginate was supplied from Sigma-Aldrich, USA. $CaCl_2$ and glycerol were purchased from Daejung, South Korea. Trehalose as a dihydrate form was derived from Hayashibara, Japan. Deionized water obtained from distillation using a water purification system (Pacific TII 12 UV, Thermo Scientific, Hungary).

Preparation of Composite Films

The film formulation was RSM designed and conducted according to a 3-factor, 3-level BBD [Table 1] in either 15 runs (3-CP) or 17 runs (5-CP), as shown in Table 2. The effect of trehalose (X_1), sodium alginate (X_2), and $CaCl_2$ (X_3) on thickness (Y_1), opacity (Y_2), and WVP (Y_3) of the film were studied. It was noted that the concentrations of each factor (X_i) were preliminary tested and then the results to the narrower level of each factor were selected.[5-8] The film solution was prepared by dissolving sodium alginate (1.2, 1.6, and 2.0% w/v) in distilled water and heating (70°C) (VELP Scientifica, Italy) to obtain a clear solution before adding glycerol (1% w/v) as a plasticizer. Trehalose (3, 6, 9% w/v) was then added to the mixture. Then, $CaCl_2$ (0.2, 0.4, 0.6% w/v) as a cross-linking agent was added and the mixture was stirred continuously until completely solubilized. The film solution was poured onto a petri dish with a Teflon sheet (INDY Supply & Service Ltd., Thailand) and kept for drying in an oven at 60°C for 24 h. All films were stored in a desiccator for 6 h before analysis.

Table 1: Factors used in BBD for film preparation

Symbol	Factors	Levels		
		−1	0	1
X_1	Trehalose (% w/v)	3	6	9
X_2	Sodium alginate (% w/v)	1.2	1.6	2.0
X_3	$CaCl_2$ (% w/v)	0.2	0.4	0.6

BBD: Box–Behnken design, $CaCl_2$: Calcium chloride

Determination of Film Physical Properties

Thickness

The thickness of films was measured using a digital Vernier Caliper (Intro TSC Co., Ltd., Thailand) at three different areas on each film and a mean value was calculated.[9]

Opacity

The opacity of films based on the CIE L* a* b* was determined using UltraScan XE colorimeter (Hunterlab, Inc., Reston, USA) which was calibrated with standard white and black backgrounds. The EasyMatch QC software version 4.62 (Hunterlab, Inc., USA) was used for calculation of opacity values. The experiment was done in three measurements.[9]

WVP

WVP was done by cutting the film into a 2-cm diameter and sealing on top of the glass bottle containing 5 ml of distilled water. The bottle was kept in a desiccator containing a saturated solution of $MgCl_2.6H_2O$ at 25°C/33% RH. The bottles were weighed and recorded every 1 h for 8 h, then water vapor transmission rate (WVTR) was calculated using a slope of the linear regression of weight loss versus time (g/h) divided by the exposed area of film (m^2). WVP (g mm/m^2 h kPa) was determined as follows;

$$WVP = L \times WVTR/(P_i - P_a) \quad (1)$$

Where P_i was the partial pressure (kPa) of water vapor in the air and P_a was the partial pressure of water vapor in the air saturated to 25°C/33% RH. L was the average film thickness (mm).[9]

Table 2: Coding and decoding factors used in BBD for film formulation

Film code	Three-CP						Five-CP					
	Coding			Decoding			Coding			Decoding		
	X_1	X_2	X_3	X_1	X_2	X_3	X_1	X_2	X_3	X_1	X_2	X_3
1	−1	−1	0	3	1.2	0.4	−1	−1	0	3	1.2	0.4
2	1	−1	0	9	1.2	0.4	1	−1	0	9	1.2	0.4
3	−1	1	0	3	2.0	0.4	−1	1	0	3	2.0	0.4
4	1	1	0	9	2.0	0.4	1	1	0	9	2.0	0.4
5	−1	0	−1	3	1.6	0.2	−1	0	−1	3	1.6	0.2
6	1	0	−1	9	1.6	0.2	1	0	−1	9	1.6	0.2
7	−1	0	1	3	1.6	0.6	−1	0	1	3	1.6	0.6
8	1	0	1	9	1.6	0.6	1	0	1	9	1.6	0.6
9	0	−1	−1	6	1.2	0.2	0	−1	−1	6	1.2	0.2
10	0	1	−1	6	2.0	0.2	0	1	−1	6	2.0	0.2
11	0	−1	1	6	1.2	0.6	0	−1	1	6	1.2	0.6
12	0	1	1	6	2.0	0.6	0	1	1	6	2.0	0.6
13	0	0	0	6	1.6	0.4	0	0	0	6	1.6	0.4
14	0	0	0	6	1.6	0.4	0	0	0	6	1.6	0.4
15	0	0	0	6	1.6	0.4	0	0	0	6	1.6	0.4
16							0	0	0	6	1.6	0.4
17							0	0	0	6	1.6	0.4

BBD: Box−Behnken design

Model Fitting Analysis

The effects of factors (X_1, X_2, and X_3) on the responses (Y_1 = thickness, Y_2 = opacity, and Y_3 = WVP) were analyzed to optimize composite film formulation by BBD using Design-Expert® Software version 12.0 (Stat-Ease, USA). Parameters which were significant at least the 95% confidence level were considered in the prediction model.[10] For evaluation of the relationship between the response and independent variables, the generalized polynomial equation of quadratic model can be written as follows:

$$Y_i = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 \quad (2)$$

Y_i is a calculated response. X_1, X_2, and X_3 are factors influencing the response of Y_i; β_0 is the constant coefficient; β_1, β_2, and β_3 indicate linear coefficients; β_{12}, β_{13}, and β_{23} represent interaction coefficients; and β_{11}, β_{22}, and β_{33} present coefficients of the quadratic term.[2]

For statistical analysis, lack of fit test and coefficient of determination (adjusted R^2) were performed to determine the adequacy of the model.[10] The statistical significance of the factors in 3-CP or 5-CP BBD was compared at $P < 0.05$.

Table 3: Physical properties of film

Film code*	Thickness (mm)	Opacity	WVP (g mm/ m² h kPa)
1	0.06±0.01	17.3±0.1	42.03±0.04
2	0.12±0.02	17.5±0.1	72.86±0.21
3	0.13±0.01	17.8±0.4	62.74±0.39
4	0.17±0.02	18.5±0.3	44.99±0.29
5	0.07±0.01	16.7±0.1	44.68±0.18
6	0.09±0.01	17.2±0.1	39.23±0.23
7	0.16±0.01	20.5±0.1	94.65±0.36
8	0.20±0.02	19.9±0.2	133.89±0.19
9	0.04±0.01	16.2±0.2	20.55±0.37
10	0.08±0.01	17.2±0.2	46.08±0.31
11	0.18±0.02	20.7±0.2	78.46±0.44
12	0.20±0.03	19.8±0.2	87.18±0.39
13	0.08±0.01	17.6±0.1	37.36±0.30
14	0.09±0.01	17.9±0.1	36.24±0.25
15	0.09±0.02	18.1±0.1	37.83±0.36
16	0.09±0.01	17.8±0.1	39.76±0.41
17	0.09±0.02	17.6±0.1	38.69±0.23

*(1–15 for 3-CP; 1–17 for 5-CP). CP: Central point

The equation indicating the significant relationship by analysis of variance between each factor and responses was obtained.

RESULTS AND DISCUSSION

The physical properties of film such as thickness, opacity, and WVP were measured and presented in Table 3, film code 1–15 for 3-CP BBD and film code 1–17 for 5-CP BBD. The thickness of all films was in varied between 0.04 ± 0.01 mm and 0.20 ± 0.03 mm. The opacity of all films was found to be in the range of 16.2 ± 0.2–20.7 ± 0.2. The WVP of all films ranged from 20.55 ± 0.37 g mm/m² h kPa to 133.89 ± 0.19 g mm/m² h kPa. Experimental data (film code 1–15 for 3-CP BBD and 1–17 for 5-CP BBD) were fitted to the quadratic model, as shown in Table 4. The suitability of the model was evaluated by (1) the sequential *P* value, $P < 0.05$; (2) the insignificance ($P > 0.05$) of "lack of fit," erroneously observed model prediction;[11] and (3) coefficient of determination for non-linear regression or adjusted R^2.

The sequential *P* value of film properties analyzed by 3-CP and 5-CP BBD was significant ($P < 0.05$) except for film thickness of 3-CP BBD; however, it may be not necessary if the model can accurately describe the response of the data.[10] For the quadratic model of 3-CP BBD for thickness, the lack of fit *P* value was insignificant ($P = 0.08$) and adjusted R^2 was nearly 1 (0.916). By comparison, "lack of fit" *P* value of all responses derived from 3-CP BBD was insignificant ($P > 0.05$), while only *P* value of opacity analyzed using 5-CP BBD was found to be insignificant. As a principle, the precision of experiment and experimental error was reduced by the replication of the experiment, so the 5-CP BBD should have better model fitting than 3-CP BBD. In contrast, in previous studies reported that the 3-CP BBD was generally an appropriate efficiency model.[3,12,13] In this study showed that 3-CP BBD had better model fitting than 5-CP BBD based on modeling validity criteria as a lack of fit. The insignificant *P* value of "lack of fit" in 5-CP BBD that it might be implied that the residual error was happened in the experiment, however, in a previous study[14] showed that the 5-CP BBD was an appropriate efficiency model with a significant *P* value of lack of fit. In all responses for 3-CP and 5-CP BBD, the adjusted R^2 was greater than 0.86, indicating the suitability of the non-linear quadratic model.[15] The adjusted R^2 of 5-CP model fit was higher than those of 3-CP model fit. Although the use of 5-CP BBD for model fitting was suggested to be better than 3-CP BBD,[2,16] under criteria such as "lack of fit" and sequential *P* value it was, however, not definitely true in this study.

From the analysis, the quadratic equations of the model fitting are presented in Table 5, as detailed in equation (2).

Table 4: Model fitting analysis of the quadratic model of film properties (response)

Response	BBD model	Sequential *P*-value	Lack of fit *P*-value	Adjusted R²
Thickness	3-CP	0.0515	0.0845	0.9160
	5-CP	0.0064*	0.0084*	0.9329
Opacity	3-CP	0.0425*	0.3294	0.9443
	5-CP	0.0082*	0.1532	0.9509
WVP	3-CP	0.0281*	0.1267	0.8658
	5-CP	0.0030*	0.0148*	0.8961

*Significant; $P<0.05$. WVP: Water vapor permeability. CP: Central point

Table 5: Quadratic equation of model fitting for film properties using 3-CP and 5-CP BBD (significant terms in bold letter)

BBD	Model fit equation
	Thickness (Y_1)
3-CP	$= 0.087 + 0.02X_1 + 0.023X_2 + 0.058X_3 – 0.005X_1X_2 + 0.005X_1X_3 – 0.005X_2X_3 + 0.019X_1^2 + 0.014X_2^2 + 0.024X_3^2$
5-CP	$= 0.088 + 0.02X_1 + 0.023X_2 + 0.058X_3 – 0.005X_1X_2 + 0.005X_1X_3 – 0.005X_2X_3 + 0.019X_1^2 + 0.014X_2^2 + 0.024X_3^2$
	Opacity (Y_2)
3-CP	$= 17.87 + 0.10X_1 + 0.20X_2 + 1.70X_3 + 0.13X_1X_2 – 0.28 X_1X_3 – 0.48 X_2X_3 + 0.0042X_1^2 – 0.096X_2^2 + 0.70X_3^2$
5-CP	$= 17.80 + 0.10X_1 + 0.20X_2 + 1.70X_3 + 0.13X_1X_2 – 0.28 X_1X_3 – 0.48 X_2X_3 + 0.038X_1^2 – 0.063X_2^2 + 0.74X_3^2$
	WVP (Y_3)
3-CP	$= 40.48 + 5.86X_1 + 3.39X_2 + 30.46X_3 – 12.14X_1X_2 + 11.17X_1X_3 – 4.20X_2X_3 + 17.61X_1^2 – 2.43X_2^2 + 20.02X_3^2$
5-CP	$= 39.98 + 5.86X_1 + 3.39X_2 + 30.46X_3 – 12.14X_1X_2 + 11.17X_1X_3 – 4.20X_2X_3 + 17.86X_1^2 – 2.18X_2^2 + 20.27X_3^2$

WVP: Water vapor permeability

A smaller *P* value and larger F value of the coefficients (β) represent a more important impact on the response Y_i.[1] The factors influencing thickness (Y_1) of films in 3-CP and 5-CP BBD model were the linear term of trehalose (X_1) ($P = 0.0140$ and $P = 0.0033$, respectively), sodium alginate (X_2) ($P = 0.0088$ and $P = 0.0017$, respectively), and $CaCl_2$ (X_3) ($P = 0.0001$ and $P < 0.0001$, in orderly), followed by the quadratic term of $CaCl_2$ (X_3^2) ($P = 0.0287$ and $P = 0.0075$ in orderly). In 5-CP BBD, the quadratic term of trehalose (X_1^2) influenced the thickness ($P = 0.0222$). The results showed that increasing the concentrations of trehalose, sodium alginate, and $CaCl_2$ increased the thickness of film. The addition of sodium alginate and $CaCl_2$ led to thicker films due to their film-forming properties.[17] The addition of trehalose could increase film thickness due to hydrophilicity since the addition of sucrose was evident to increase the thickness of the starch-based film.[18] For the opacity response (Y_2), the linear term and the quadratic term of $CaCl_2$ (X_3 and X_3^2) in both 3-CP BBD ($P < 0.0001$ and $P = 0.0093$, respectively) and 5-CP BBD ($P < 0.0001$ and $P = 0.0013$, respectively) had significant effect. Furthermore, the interaction between sodium alginate and $CaCl_2$ (X_2X_3) in 3-CP BBD ($P = 0.0343$) and 5-CP BBD ($P = 0.0139$) was significant. The results represented that the opacity value was higher when $CaCl_2$ concentration increased. It was possibly due to increased film thickness in the presence of $CaCl_2$, resulting in less diffusion of light and the film appeared more opaque.[17,19] The last response, WVP (Y_3) was found to be significantly affected by linear term of $CaCl_2$ (X_3) of 3-CP BBD ($P = 0.0005$) and of 5-CP BBD ($P < 0.0001$), quadratic term of $CaCl_2$ (X_3^2) of 3-CP BBD ($P = 0.0159$), and of 5-CP BBD ($P = 0.0026$), quadratic term of trehalose (X_1^2) of 3-CP BBD ($P = 0.0255$) and of 5-CP BBD ($P = 0.005$). In 5-CP BBD, the trehalose-alginate interaction term (X_1X_2) and trehalose-$CaCl_2$ interaction term (X_1X_3) were significant ($P = 0.0321$ and $P = 0.0439$, respectively). From the results, the addition of $CaCl_2$ and trehalose increased WVP value. Hydrophilic compounds like trehalose might increase WVP when incorporated into films and coating by reducing the intermolecular bonds between the alginate polymer chain.[20,21] For increasing $CaCl_2$ concentration, the higher Ca^{2+} ions mostly reacted with alginate (G block) and thus forming the "egg-box" formation which led to stronger films, the higher WVP value and increased thickness.[22,23] In addition, some studies reported that the thickness of the film can influence the value of WVP. When the thickness increased, the film provided higher resistance to mass transfer through it; thus, the partial vapor pressure in equilibrium in the internal surface of the film increased.[19,23,24]

CONCLUSION

The findings showed that for alginate film formulation, the model fitting using different runs in BBD the 3-CP (15 runs) was comparative to 5-CP (17 runs). Even less experiment runs, the better values of "lack of fit" parameter were found in 3-CP design which could be used for further optimization of film formulation.

ACKNOWLEDGMENTS

The authors would like to gratefully acknowledge Chulalongkorn University Graduate School Thesis Grant, Dental Material Science Research Center, and Faculty of Pharmaceutical Sciences for the equipment support. We are grateful to Hayashibara, Nagase Group, Japan, for generous donation of trehalose.

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