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Box-Behnken Design for Optimization of Nanostructured Lipid Carrier Sonication Conditions from Mixtures of Palm Stearin and Palm Olein

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ABSTRACT

In this study we aimed to determine the optimum conditions for sonication in the manufacture of nanostructured lipid carriers (NLC). NLC were developed using a mixture of palm stearin and palm olein, water, and tween as surfactants. The optimum conditions of NLC is defined as having a size below 200 nm, a zeta potential of $\pm$30 mV, and a polydispersity index below 0.5. We used response surface methodology with a Box-Behnken experimental design to obtain the optimum conditions for the production of NLC. The independent variables used in this study were amplitude (X_1, kHz), pulse on pulse off (X_2, minute), and time of sonication (X_3, minute), each having three levels. The zeta potential (Y_1, mV), polydispersity index (Y_2), and particle size (Y_3, nm) were used as the dependent variables. The dependent variables were determined by dynamic light scattering using a Malvern Zetasizer Nano ZS. The closest to optimum formulation was obtained at a combination of 35 kHz amplitude, pulses on 9 pulses off 3, and 3 min 25 seconds sonication. This regime resulted in the production of NLCs with a particle size 127.9 nm, a polydispersity index of 0.191, and a zeta potential of −27.3 mV.

Keywords: nanostructured lipid carrier, sonicator probe, response surface methodology

INTRODUCTION

Crude palm oil is one of main commodities exported by Indonesia to many countries. The main fractions of palm oil are palm stearin (solid fraction) and palm olein (liquid fraction). To increase the benefits of palm oil in the pharmaceutical sector, further development of palm stearin and palm olein must be carried out, including the manufacture of Nanostructured Lipid Carriers (NLCs). NLCs are composed of a lipid core consisting of a mixture of solid and liquid lipids, in an aqueous phase with a surfactant or surfactant mixture (Shah *et al.*, 2017). NLCs have an average size of 10–500 nm (Sharma & Baldi, 2018). NLCs as drug delivery systems have many advantages, including excellent biocompatibility (Sharma & Baldi, 2018), controlled drug release, and high bioavailability. They can be produced on an industrial scale, and can be administered via several routes, such as oral, intravenous, pulmonary, and transdermal administration (Surya Tej *et al.*, 2016). An NLC composed of palm stearin solid lipid and palm olein liquid lipid has been produced by Rohmah *et al* 2020 (Rohmah, *et al.*, 2020; Rohmah, *et al.*, 2020).

To obtain NLCs with the best characteristics, optimization of the production methods must be performed (Subramaniam *et al.*, 2020). The NLC in this study was produced using the High Shear Homogenization and ultrasonication methods. High shear homogenization was performed using an Ultra-Turrax homogenizer to form NLCs of nano size, while ultrasonication is used to reduce the size of the NLC to less than 200 nm. The independent variables involved in the use of Ultra-Turrax are amplitude, pulse on pulse off, and duration of sonication. This experiment was performed to investigate the effects of amplitude, pulse on pulse off, and duration of sonication on the resulting NLC. The independent variable in the process of making NLC using a sonicator must be optimized to obtain an optimum response. The research design used a statistical approach, a Box-Behnken design, which can reduce the number of experiments to be performed to less than is necessary for the traditional method. Process optimization can be done using response surface methodology (RSM) (Stamenković *et al.*, 2018).

Table I. Variable and respective coded levels of BBD

Factors Independent variables	Low Level (−1)	Coded Levels Medium Level (0)	High Level (+1)
X_1= amplitude	30	45	60
X_2= pulse on pulse off	1 (3:3)	2 (6:3)	3 (9:3)
X_3= time	2	3	4
Dependent variables			
Y_1 = zeta potential	−30 kHz		
Y_2= PDI	Minimum		
Y_3= mean particle size (Zavg)	Minimum		
Y_4= particle size distribution D−90	Minimum		

To conduct the experiment, this study used the Box-Behnken design (BBD), which has been proven to minimize the number of experiments needed in order to find an optimum condition for manufacturing (Ferreira *et al.*, 2007). The Box-Behnken design is also suitable for analyzing quadratic response surfaces while allowing optimization of the process with a minimum of experimentation (Agrawal *et al.*, 2021; Kudarha *et al.*, 2015).

MATERIALS AND METHODS

The materials used were palm stearin (Chemical Point, Deisenhofen, Bayern, Germany), palm olein (Green Gaia Solutions, Kuala Lumpur, Malaysia), and Tween 80 (Kao, Jakarta, Indonesia). The equipment used were a homogenizer IKA-Ultra-Turrax T25 (Staufen, Germany), a sonicator probe (QSonica, Newtown, CT, USA), a vortex mixer (VM–300 Germany Industrial Corporation, USA), a hot plate, a thermometer, and a Malvern Zetasizer Nano ZS (Malvern, UK; Beckman Coulter, Brea, CA, USA).

Preparation of NLCs

NLC formulations were prepared using hot high shear homogenization and ultrasonication. A mixture of palm stearin and palm olein (7:3) was melted at 60°C. Tween 80 and water were also heated to 60°C. The aqueous phase was gradually added to the oil phase under continuous stirring at 700 rpm, and maintained at a temperature of 60°C. The mixture was homogenized at 12,000 rpm for 30 min using a high shear homogenizer (IKA T25 Digital Ultra-Turrax, Staufen, Germany) (Rohmah, Raharjo, Hidayat, & Martien, 2019). The NLC was sonicated using a sonicator probe for different amplitudes, pulse on pulse off times, and times of sonication.

Experimental Design

A Box-Behnken Design was used in the study for the optimization of NLC. A three-level design was employed for exploring responses, using Minitab software Version 19.

A non-linear, polynomial quadratic equation model explaining the three-factor three level design is given below:

$y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{11}X^2{}_1+\beta_{22}X^2{}_2+\beta_{33}X^2{}_3$ (Agrawal *et al.*, 2021; NIST/SEMATECH, 2012; Pinto *et al.*, 2019),

where *Y* represents the dependent variables, including the zeta potential (Y_1), PDI (Y_2), mean particle size (*Z* avg) (Y_3), and particle size distribution D90 ($Y4$). β_0 is the intercept, and β_1 to β_{33} are the regression coefficients. *X* represents the independent variables, where X_1 represents amplitude (kHz), X_2 is pulse on pulse off (min), and X_3 is the time of sonication (*C*, min) (Table I). The determination of the low level and the high level for the independent variables was based on preliminary studies. The determination of the low and high values of the independent variables was obtained based on observations of liquid moving at several amplitudes (QSonica, 2021). Based on these observations, samples could flow well at an amplitude of 30 kHz, while at an amplitude of more than 60 kHz the liquid movements and cavitation were too large.

Particle size analysis and Zeta potential

One drop of NLC was dissolved in 10 mL of water. The particle size and zeta potential were measured using a Malvern Zetasizer Nano ZS (Malvern, 2013; PT.DKSH Indonesia, 2019).

RESULTS AND DISCUSSION

NLC was prepared by heating the lipid phase (palm olein and palm stearin), and aqueous phase (Tween 80 and water) to a temperature of 60°C.

Table II. Box-Behnken Design Matrix and Response

STD order	Independent Variables						Dependent Variables			
	Coded variables			Real variables			Y_1 (mV)	Y_2	Y_3 (nm)	Y_4 (nm)
	X_1	X_2	X_3	X_1	X_2	X_3				
1	−1	−1	0	30	(3:3)	3	−21.6	0.243	120.8	216
2	1	−1	0	60	(3:3)	3	−15.1	0.201	129.7	256
3	−1	1	0	30	(9:3)	3	−22.6	0.271	128.9	267
4	1	1	0	60	(9:3)	3	−23.7	0.183	130.8	261
5	−1	0	−1	30	(6:3)	2	−28.7	0.183	134.2	247
6	1	0	−1	60	(6:3)	2	−30.7	0.397	184	597
7	−1	0	1	30	(6:3)	4	−24.6	0.226	131.8	282.7
8	1	0	1	60	(6:3)	4	−21	0.267	152.9	476
9	0	−1	−1	45	(3:3)	2	−26.5	0.207	122.4	228
10	0	1	−1	45	(9:3)	2	−28.1	0.221	123.1	217
11	0	−1	1	45	(3:3)	4	−23.6	0.337	179.4	635
12	0	1	1	45	(9:3)	4	−39.8	0.204	128.4	265
13	0	0	0	45	(6:3)	3	−38.7	0.182	132.9	268
14	0	0	0	45	(6:3)	3	−39.2	0.172	134.3	248
15	0	0	0	45	(6:3)	3	−38.9	0.357	181.8	604

Research that has been conducted with the Box-Behnken model is then evaluated using RSM.

Table III. Summary of regression model.

Response	Model	ANOVA Testing		
		P value		R^2 (%)
		Linear	Square	
Zeta potential	$Y=86.7-4.251X_1-16.7X_2-8.1X_3+0.04759X_1^2+7.47X_2^2+1.97X_3^2-0.127X_1{*}X_2+0.093X_1{*}X_3-3.64\,X_2{*}X_3$	0.192	0.008	91.21
PDI	$Y=-0.264+0.0084\,X_1+0.208\,X_2+0.059\,X_3+0.000031\,X_1^2-0.0191X_2^2+0.0246X_3^2-0.00077X_1{*}X_2-0.00288X_1{*}X_3-0.0368X_2{*}X_3$	0.942	0.930	30.78
Mean particle size	$Y=-93+4.28\,X_1+107.9\,X_2+15.4\,X_3-0.0215\,X_1^2-17.2\,X_2^2+5.9\,X_3^2-0.117\,X_1{*}X_2-0.478\,X_1{*}X_3-12.9\,X_2{*}X_3$	0.679	0.627	48.57
D90	$Y=-990+26\,X_1+639\,X_2+2\,X_3-0.131\,X_1^2-93.9\,X_2^2+56.8\,X_3^2-0.77\,X_1{*}X_2-2.61\,X_1{*}X_3-89.8\,X_2{*}X_3$	0.594	0.706	48.28

The aqueous phase was gradually added to the oil phase under continuous stirring at 700 rpm, and maintained at a temperature of 60°C. This process resulted in mixture homogenization at 12,000 rpm for 30 min using an Ultra-Turrax high shear homogenizer. The mixture was then ultrasonicated with the sonicator probe under the conditions determined by the Box-Behnken design experiments. The particle size and zeta potential of the NLC were measured using a Malvern Zetasizer Nano ZS. Processing was performed using the software supplied with the equipment, and the particle size data were evaluated using the volume distribution.

The selected independent variables were found to influence the four responses measured. All batches showed particle sizes in the range 120.8–184 nm and zeta potential (−15.1 mV)–(−39.2 mV) (Table II). The Box-Behnken design is one of the tools used to reduce the amount of research required for optimizing research conditions in the manufacture of NLC. Based on the Box-Behnken design, the following results were obtained (Table II): Research that has been conducted with the Box-Behnken model is then evaluated using RSM. Based on the analysis using RSM, it was found that the regression equation that applied was the quadratic regression equation, as apparent by the P value of < 0.5 (0.008) (Table III).

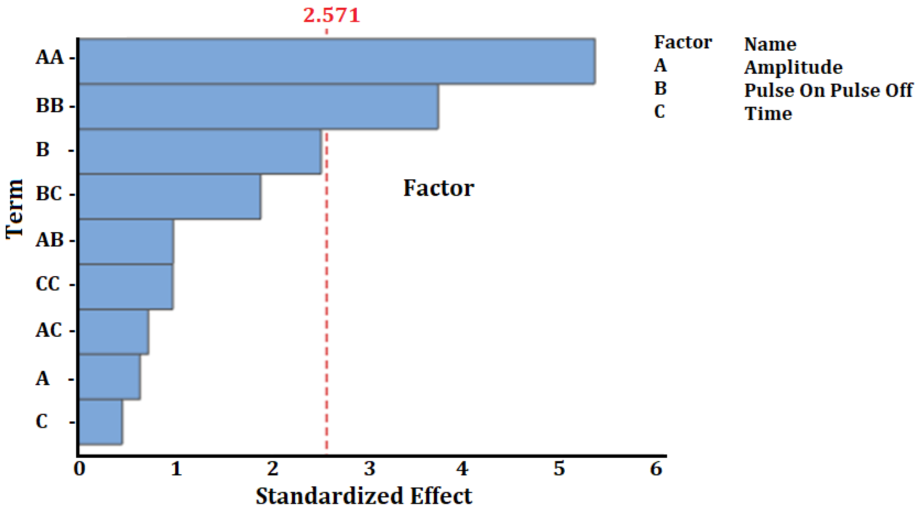

Figure.1. Pareto chart showing the standardized effect of the independent variables and their interactions on the zeta potential (Y_1), α = 0,05

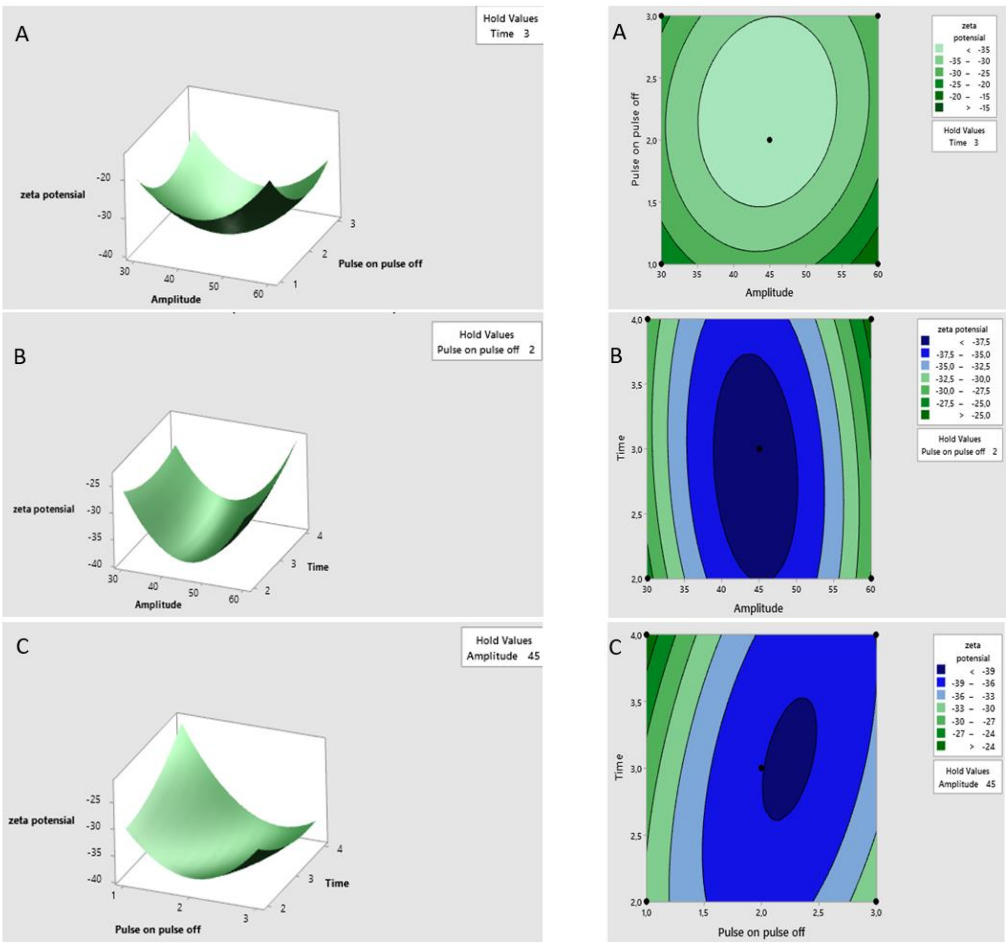

Figure 2. Response surface and contour plots showing the effects of independent variables with major influence on the zeta potential (Y_1). A. amplitude (X_1) and pulse on pulse off (X_2), B. amplitude (X_1) and time (X_3), C. pulse on pulse off (X_2) and time of sonication (X_3)

Based on the zeta potential factor, the factors that had the most significant influence were amplitude and pulse on pulse off. Time did not have a significant effect on zeta potential. Based on this model, amplitude, pulse on and pulse off did not significantly affect PDI and particle size (Table III).

Effect of independent variables on zeta potential

The value of the zeta potential (*Y1*) ranged between −15.1 mV and (−)39.8 mV (Table II). The interaction amplitude in the quadratic response, interaction pulse on pulse off in the quadratic response, and interaction time in the quadratic response had positive effects (β_{11} = 0.04759, p = 0.008; β_{22} = 7.47, p = 0.014; β_{33} = 1.97, p = 0.371) (Figure 1). The increase in the amplitude, pulse on pulse off, and sonication time resulted in the increase in the zeta potential value.

In this research, the zeta potential response target was ±30 mV. In the graph, the relationship between amplitude and pulse on pulse off, amplitude with time, pulse on pulse off with time, and zeta potential ± 30 mV, is shown in the green area of the contour plot (Figure 2).

Effect of independent variables on PDI

The value PDI (*Y2*) ranged between 0.172 and 0.397. In this model, the amplitude, pulse on and pulse off did not significantly affect the PDI (Figure 3).

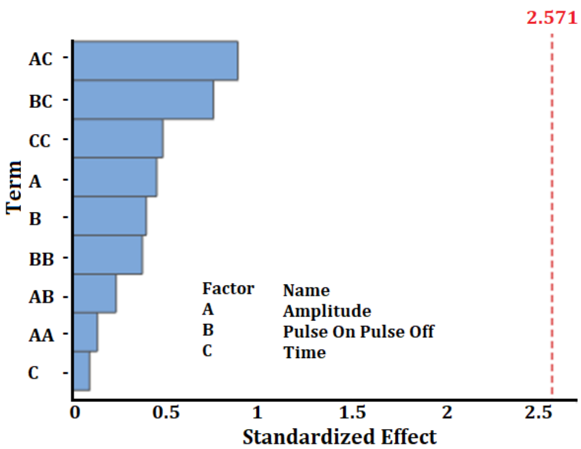

Figure 3. Pareto chart showing the standardized effect of independent variables and their interactions on PDI (Y_2), α = 0.05

Effect of independent variables on mean particle size

The mean particle size (*Y3*) ranged between 120.8 and 184 nm. In this model, amplitude and pulse on and pulse off did not significantly affect the mean particle size (Figure 4).

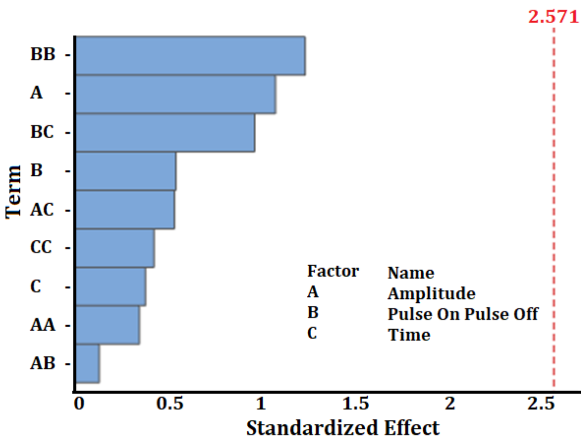

Figure 4. Pareto chart showing the standardized effect of independent variables and their interactions on mean particle size (Y_3), α = 0.05

Effect of independent variables on particle size distribution D–90

The value particle size D–90 (*Y4*) ranged between 216 and 635 nm. In this model, amplitude and pulse on and pulse off did not significantly affect particle size distribution D–90 (Figure 5).

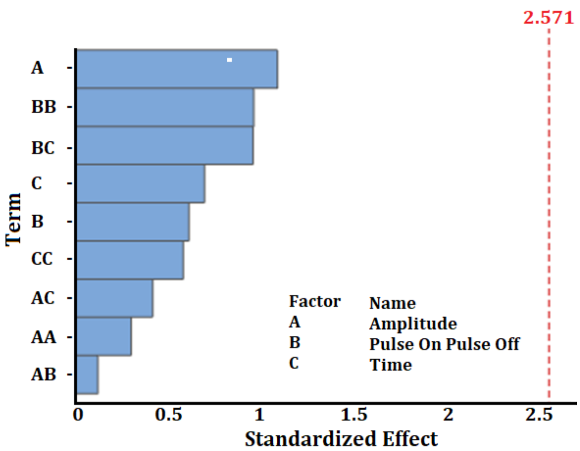

Figure 5. Pareto chart showing the standardized effect of independent variables and their interactions on D–90 (Y_4), α = 0.05

Optimization and response prediction

Optimization of the response was performed using Minitab software Version 19 (Table IV). Based on the RSM, the optimum conditions were obtained at amplitude 35 kHz, pulse on 9 pulse off 3, and time 3 min 25 seconds. The composite desirability was 0.5023, while the desirability values for zeta potential, PDI, mean particle size, and D–90 were 0.96206; 0.4893; 0.82030 and 0.16480, respectively (Figure 6).

Table IV. Components and optimized response

Response component	Target/ goal	Lower limit	Upper limit
Zeta potential (mV)	Range	−30	−29
PDI	Minimum	0.01	0.397
Mean particle size (nm)	Minimum	100	200
D90 (nm)	Minimum	100	200

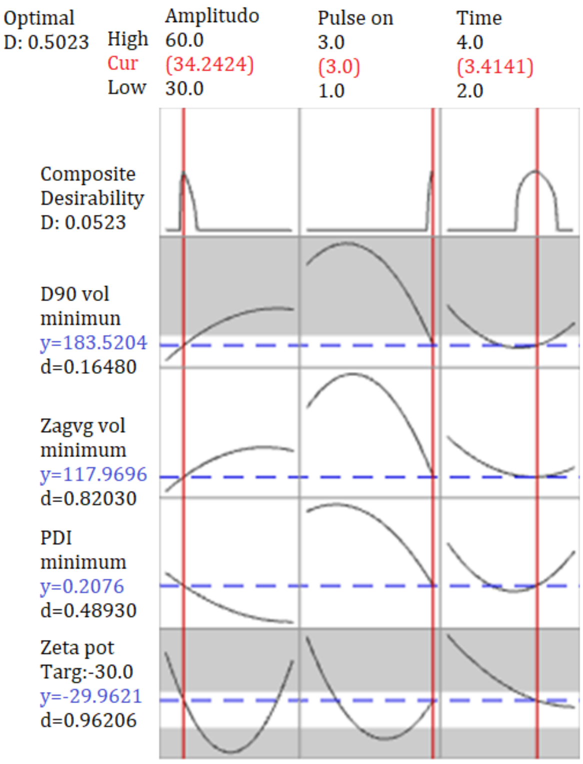

Figure 6. Prediction of optimum conditions.

Verification of optimum conditions

To confirm the validity of the optimum conditions, the experiment was carried out under the optimum conditions. The results of the zeta potential, mean particle size, and polydispersity index of the predictions and experiments were very similar. However, the result of the D90 value was above 200 nm (Table 5). There have been several reports of average particle size, and some of these are based on the particle distribution of D–90 (Patel *et al*., 2012). In this study, the mean particle size was used as an indicator of particle size (Lakhani *et al*., 2019)

TableV. Results verification optimum condition

	Prediction	Results
Zeta potential (mV)	−29.96	−27.3±1
Polydispersity Index	0.2076	0.191±0.027
Mean particle size (nm)	118	127.9±0.9
D–90 vol (nm)	183.52	241

CONCLUSION

In this study, the major factor that affected the particle size, polydispersity index, and zeta potential in the sonication process was the amplitude. The closest condition for optimum formulation was obtained at a combination of an amplitude of 35 kHz, pulse on 9 pulses off 3, and time of sonication of 3 min 25 seconds. This set of conditions produced NLC with a particle size of 127.9 nm, a polydispersity index of 0.191, and a zeta potential of −27.3 mV. The zeta potential was significantly affected by the amplitude, pulse on pulse off, and time of sonication. Further research is needed to optimize the zeta potential parameters.

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