Application of Simplex Lattice Design on the Optimization of Andrographolide Self Nanoemulsifying Drug Delivery System (SNEDDS)

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ABSTRACT
Optimization of self-nanoemulsifying drug delivery system (SNEDDS) formulation is an important step to obtain optimal formulation with desired characteristics. This present study was aimed to utilize simplex lattice design in optimizing andrographolide SNEDDS. Simplex lattice design was employed to optimize andrographolide SNEDDS in which component of SNEDDS was selected as the independent factor while the characteristics of SNEDDS was used as the responses. Capryol-90, Kolliphor RH 40, and propylene glycol were selected as the oil, surfactant, and co-surfactant, respectively. Optimization of andrographolide SNEDDS formulation was based on their characteristics including emulsification time, droplet size, and drug content. The optimized SNEDDS formulation was evaluated for emulsification time, droplet size, drug content, and zeta potential. The emulsification time, droplet size, drug content, and zeta potential of the optimized andrographolide SNEDDS was found to be 1.21±0.03min, 44.02±0.67nm, 6.69±0.08mg/g, and -40.63±0.76mV, respectively. This result suggested that simplex lattice design is a suitable for efficiently optimizing the formulation of andrographolide SNEDDS.

Keywords: andrographolide; snedds; simplex lattice design

INTRODUCTION
Andrographis paniculata is a herbaceous plant which belongs to family of Achantaceae that is easily found across Asian countries. This plant was also known as king of bitter due to the bitter flavour (Lim et al., 2012). Despite the unpleasant flavor, this plant is traditionally used as the treatment for common cold, diarrhea, respiratory infection, and treat for the insect or bug bites (Richard et al., 2017). One of its chemical constituents is andrographolide, a diterpene lactone compound, which is reported to has various biological activities including anti-diabetic, anti-inflammatory, anti-viral, anti-microbial, and hepatoprotective activity (Lim et al., 2012; Subramanian et al., 2012). Andrographolide has poor water solubility. Futhermore, it was extensively metabolized that resulted in its low bioavailability and limit its potential use (Pawar, et al., 2016; Ye et al, 2011). These characteristics lead researchers to find the most suitable drug delivery system to enhance the bioavailability of this compound.

SNEDDS is a lipid based formulation that are considered promising to deliver poorly soluble active pharmaceutical ingredient. It is an isotropic system that comprise of oil, surfactant, and cosurfactant. When introduced into the water, SNEDDS will form fine emulsion with nanometer-sized droplets that approximately less than 200nm (Sanka et al., 2016; Senapati, et al., 2016; Villar et al., 2012). Due to this small size droplet, the
nanoemulsion become transparent after dilution. In recent years, SNEDDS is extensively developed and studied due to its various advantages including improving solubility, stability and also bioavailability of poorly soluble drug substances (Bandyopadhyay, et al., 2012; Shakeel, et al., 2013; Venkata et al., 2008). In addition, SNEDDS can be filled into gelatine capsule that is more convenient for oral administration. A trial and error approach on the formulation development is time consuming, cost ineffective, and also does not guarantee to obtain the real optimum formulation (Marasini et al., 2012). Thus, experimental design has remarkably used in the drug development and optimization process (Aslam et al., 2016; Das, et al., 2015; Duangjit and Kraisit, 2018; Patel, et al., 2017; Sanka et al., 2016; Shiyan, et al., 2018; Zhao et al., 2014). A simplex lattice design (SLD) is an optimization design that the total quantity of independent variable (ingredients of formulation) have to be constant (Bolton and Bon, 2010). This method has been successfully employed to optimize other drug delivery system including solid-SNEDDS of clonazepam (Sanka, et al., 2016), microspheres of ketoprofen (Das, et al., 2016), microemulsion (Duangjit, et al., 2014), and orodispersible tablet (Duangjit and Kraisit, 2018). As for SNEDDS, the percentage of oil, surfactant and co-surfactant in the formulation influence the characteristics of the SNEDDS itself. Hence, it is important to determine the optimal amount of oil, surfactant, and co-surfactant to obtain SNEDDS with desired characteristics. The present study was intended to design and optimize andrographolide-containing SNEDDS. The components of the SNEDDS were selected as the independent factor in a 3-factor 3-level simplex lattice design. A simplex lattice design on Design Expert software was employed to obtain experimental runs and optimize the formulation.

MATERIAL AND METHODS
Andrographolide was purchased from Sinobright Pharmaceutical (China) while standard andrographolide was obtained from Sigma-Aldrich. Capryol 90 was brought from Gattefosse (France), Kolliphor RH 40 was purchased from BASF, while propylene glycol and methanol for HPLC was obtained from Brataco (Indonesia).

Optimization of SNEDDS
A three factor SLD was used to explore and optimize the ingredients of andrographolide-loaded SNEDDS. Design expert software (DX ver 10, State Ease Inc, Minnesota) was employed to generate and evaluate the formulation. There are 3 replications for the SLD hence this study requires 14 experimental runs to assess the impact of independent variables. The selected independent variables are Capryol-90 (A) as the oil phase (20-50%), Kolliphor RH 40 (B) as the surfactant (40-70%), and propylene glycol (C) as the cosurfactant (10-40%). Meanwhile, the responses used to assess the quality of andrographolide-loaded SNEDDS are emulsification time (Y1), droplet size (Y2), and andrographolide content (Y3).

Preparation of andrographolide-loaded SNEDDS
Andrographolide-loaded SNEDDS was prepared by mixing andrographolide with Capryol-90, Kolliphor RH 40, and propylene glycol using ultrasonic homogenizer. The amount of these three excipients was determined based on the formulations that were generated by software. The excess amount of andrographolide was removed by filtering using whatman membrane. The pre-concentrate SNEDDS was then placed in the glass container for further evaluation.

Emulsification time determination
One mililiter of andrographolide SNEDDS was added to 500mL of distilled water and wasmixed using stirrer at 50rpm. The emulsification process was evaluated visually and the time required to form emulsion was then noted as emulsification time.

Droplet size determination
Determination of droplet size and polydispersity index was carried out using particle size analyzer (Horiba SZ100, Japan). Pretconcentrate SNEDDS were diluted 100x using distilled water and then the droplet size were determined.

Drug content determination
Andrographolide content was determined using high performance liquid chromatography. A C-18 Sunfire column with length of 150mm and diameter of 46mm was used as the stationary phase. Meanwhile, the mobile phase comprise of methanol and water (67:33 %v/v) was pumped at flow rate of 1mL/min with isocratic elution mode. Detection was done at 224nm using ultraviolet detector. The studied HPLC method was validated for linearity, specificity, precision, accuracy, limit of detection, and limit of quantification.
Andrographolide was weighed and dissolved in methanol to produce stock solution (250μg/mL). Linearity was tested within an interval of standard solutions (6–64μg/mL of andrographolide) in order to calculate the calibration curve. Each level of concentration was prepared in triplicate. The experimental results were graphically plotted, obtaining a calibration curve. Accuracy was tested by mean percentage recoveries of three samples of andrographolide at three different concentrations precisely prepared and by determination of the relative standard deviation (RSD). Precision was assessed by testing the repeatability of the standard solutions in the same day and by intermediate precision analyzing the same three standard solutions on different days. The detection and quantitation limits were based on the standard deviation of the response and slope.

Accuracy was assessed by calculating the recovery percentage, obtained from the relation between the quantity found and the real quantity contained in the spiked placebo in nine determinations, corresponding to three different concentration levels (within the range used in the linearity), assessed three times and interpolated in a calibration curve.

**RESULT AND DISCUSSION**

**Emulsification time**

SNEEDS should be dispersed quickly in the aqueous medium. Rapid drug release was expected from the fast self-emulsified SNEEDS formulation (Xue, 2018). The self-emulsification time of andrographolide SNEEDS were in the range of 0.7-3min (Table I). The regression equation for the emulsification time is $Y_1 = -0.11908A - 0.05790B + 0.25054C + 0.005512AB - 0.003565AC - 0.003260BC$. The equation suggest that the factor PG has more significant effect on increasing the emulsification time. On the contrary, Capryol 90 and Kolliphor RH 40 decrease the emulsification time.

**Droplet size**

Droplet size is a critical parameter for assessing SNEEDS formulation since the rate and extent of drug release was mainly relies on it.
The droplet size of SNEDDS was found to range between 12 to 95 nm (Table I). The regression equation for the droplet size is $Y_2 = + 1.70228 A - 0.26505 B - 0.27471 C$. The equation implied that Capryol 90 increase the droplet size of the nanoemulsion. When the concentration of capryol 90 as the oil phase is higher, the surfactant and co-surfactant is not enough to cover the oil droplet hence coalescence tend to increase. On the other hand, Kolliphor RH 40 and PG decrease the droplet size.

**Drug content**

Andrographolide content on pre-concentrate SNEDDS was evaluated and selected as the response on this study. A liquid chromatography method was developed to determine the amount of andrographolide soluble in the oil-surfactant-cosurfactant system. The calibration curve of analytical method was validated in the range of 6 to 64 μg/mL of andrographolide with correlaton coefficient ($r$) of 0.99 (Table II). The precision of the method was demonstrated by repeatability study. The %RSD value of repeatability was less than 2% that indicated good precision of the developed method.

The accuracy of the analytical method was determined by recovery experiments using standard addition method. Percentage of recovery was 100.32±1.48% that indicates the method was accurate.

The regression equation for the drug content is $Y_3 = + 0.03232 A + 0.046712 B + 0.15336 C$. The equation indicated that the factor PG has more significant effect on increasing the drug content since andrographolide has greater solubility in PG compared to Capryol-90 and Kolliphor RH 40 (Syukri, et al., 2018). It can be concluded that PG has a more important role in increasing the amount of andrographolide in the SNEDDS system.

Three dimentional surface plots show the relationship between the variables and the response of experimental design. Contour lines and graduated color shading of the plot are beneficial to understand this relationship easier and more quickly. The 3D plots for the emulsification time, droplet size, and drug content (Figure 1).

The statistical analysis of variance (ANOVA) from the software showed that the model for the response of emulsification time was quadratic while the model for the droplet size and andrographolide content are both linear.
The model for each responses are all significant which was shown by the p-values less than 0.05 (Table III). It can be concluded that the responses fitted the models well. Moreover, lack of fit of all three responses were non-significant which was observed from the p value (more than 0.05). This data implied that the model fit the data within observed replicate variation.

The goodness of fit for the regression model can be seen from the $R^2$ value. All of the three responses has quite high $R^2$ value (Table IV) that indicated there were good correlation between independent variables and responses (Khanolkar, et al., 2017). Furthermore, there was a reasonable agreement between adjusted $R^2$ value and predicted $R^2$ value of all three models. All the responses were then used to optimize the SNEDDS formulation since they meet the requirement of good model for predicting the responses.

The optimization of the SNEDDS formulation was done by employing a numerical optimization technique based on desirability approach (Malakar, Sen, et al., 2012; Shi et al., 2015). The goal for independent variables including capryol-90 was setted as “maximize”, while Kolliphor RH 40 and PG was setted as “in range” to obtain desired quality of the SNEDDS formulation. Meanwhile the responses were also setted with the goal responses of “minimize” for emulsification time, “in range” for droplet size, and “maximize” for drug content. The highest desirability value (0.538) was obtained by the software from an optimal SNEDDS formulation containing combination of Capryol-90 (35.35%), Kolliphor RH 40 (40%), and PG (24.65%). The optimized formula was prepared in triplicate and was then evaluated in order to validate its predicted reponse (Table V).

Based on the observation of optimal SNEDDS formulation (Table V), it can be concluded that andrographolide can be formulate into SNEDDS with nanometer scale droplet size and rapid emulsification time. Furthermore, it has zeta potensial of -40.63±0.76mV (Figure 2) which indicated that the formulation has good stability. Nanoemulsions are considered stable when the zeta potential values exceed +30mV or below -30mV. Zeta potential value represents the surface charge of the emulsion droplets. The surface charge make the particle tend to repel each other hence they do not have the tendecy to come together and inhibit the aggregation and fusion of the droplets. Large positive of negative zeta potential implies good stability of the emulsion (Tran et al., 2014).

### CONCLUSION

The present study demonstrated that andrographolide-loaded SNEDDS were succesfully optimized by employing simplex lattice design. The measured values of optimized formula comprised of Capryol-90 (35.35%), Kolliphor RH 40 (40%), and polyethylene glycol (24.65%) were found to be in close agreement with the predicted value.

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**Table V. Comparison of Predicted and Observed Experimental Values of Andrographolide SNEDDS Prepared under Optimum Conditions (n=3)**

<table>
<thead>
<tr>
<th>Response</th>
<th>Predicted value</th>
<th>Observed value</th>
<th>p-value (one sample t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsification time</td>
<td>1.22</td>
<td>1.21±0.03</td>
<td>0.421</td>
</tr>
<tr>
<td>Droplet size</td>
<td>42.81</td>
<td>44.02±0.67</td>
<td>0.088</td>
</tr>
<tr>
<td>Andrographolide content</td>
<td>6.79</td>
<td>6.69±0.08</td>
<td>0.163</td>
</tr>
</tbody>
</table>

**Figure 2. Droplet size and zeta potential of andrographolide SNEDDS optimum formulation**
generated by the software. Our study concluded that simplex lattice design is suitable for efficiently optimizing the SNEDDS formulation.

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REFERENCES


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