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Research Article

FORMULATION OPTIMIZATION OF CITRONELLA GRASS OIL SOLID LIPID PARTICLES USING MIXTURE DESIGN

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ABSTRACT

Citronella grass oil is one essential oil which may have potential for acne control and help to relieve acne blemishes. However, formulations of aromatic oil in topical dosage forms are limited by volatility and oxidation problems. Objective: To optimize citronella grass oil solid lipid particle formulation. Methods: Factors, namely cetyl alcohol, citronella grass oil and mixed emulsifiers, affecting the citronella grass oil entrapment efficiency and the particle size of solid lipid particles (SLPs) were studied using an augmented simplex-centroid mixture design. Each batch of SLPs was prepared by a melt dispersion technique. Results: The response surface model of the percentage oil entrapment and volume distribution of particle size less than 10 µm fitted significantly to the linear mixture model with determination coefficients (R^2) of 0.8539 and 0.7088, respectively. The levels of mix emulsifiers and cetyl alcohol caused a positive effect, but the level of citronella grass oil caused a negative effect on percent oil encapsulation. Verification of predicted and experimental values of the oil encapsulation efficiency indicated the adequacy of the response surface model with less than 10% predicted error. Conclusion: The predicted optimal ratio of cetyl alcohol : citronella grass oil: mixed emulsifiers to reach the maximum oil entrapment of 28.69% and volume of particle size diameter less than 10 µm of 16.42% with desirability of 0.957 was 6.77 : 4.17 : 7.60.

Keywords: Solid lipid particles, Mixture design, Citronella grass oil, Melt dispersion technique

INTRODUCTION

Propionibacterium acnes is a major cause of acne vulgaris. Citronella oil is anti-candidiasis and has antifungal properties [1]. *In vitro* study demonstrated that citronella oil possesses a very good free radical scavenging activity and has anti-inflammatory action. In addition, at a concentration of 50% w/v, citronella grass oil was classified as a non-sensitizer [2-3]. In a previous study, citronella grass oil was identified as one essential oil which may have a potential to use for acne control and help to relieve acne blemishes [2]. However, formulations of aromatic oil in topical dosage forms are limited by volatility and oxidation problems. Therefore, encapsulation might need to be carried out to diminish these problems [4]. Lertsatitthanakorn et al. [5] demonstrated that a of citronella grass oil microemulsion was unable to mask its odor. Furthermore, the amount of citronella grass oil released from a microemulsion was practically low, leading to less anti-*P. acnes* efficacy. Therefore, a citronella grass oil microemulsion is a poor delivery system for acne control. However, when citronella grass oil was loaded into solid lipid particles, their volatility was prolonged and anti-acne activity was more stable than citronella grass oil alone [6].

Solid Lipid Particles (SLPs) are one encapsulation technique which is simple and easy to scale up. Moreover, incorporation of both hydrophilic and lipophilic drugs as well as increased drug stability can be obtained by this technique [7]. Unsystematic search for the best formulation by changing the level of each factor at a time involves a large number of experiments and is time-consuming. Therefore, the design of an experiment plays an important role in drawing the maximum information from the collected data in the smallest number of experimental runs [8-9]. In this study, mixture design was used to optimize pharmaceutical formulation with maximum oil encapsulation efficiency and appropriate mean particle size. Response surface methodology was used to determine the factors affecting the response as well as to predict the optimal mixture components corresponding to the fitting model.

The aim of this study was to explore the mixture component proportions of citronella grass oil SLPs corresponding to the maximum entrapment efficiency and to determine the volume of particle size distribution in the range of 1 to 100µm. Design-Expert® software was used to optimize the SLP formulation. In the present work, the procedure to find the optimal formulation having solid lipid particles size less than 10 µm with the maximum citronella grass oil entrapment has been performed. This finding would help to improve anti-acne efficacy by trans-follicular delivery of citronella grass oil into the sebaceous glands.

MATERIALS AND METHODS

Materials

Citronella grass oil was purchased from Thai China Flavors and Fragrances Industry Co. (Thailand). Cetyl alcohol and sodium dodecyl sulfate were provided by Sigma-Aldrich (Germany). Poloxamer 188 was a gift from BASF (Germany). All other chemicals were of reagent grade.

Experimental design

Our preliminary experiments indicated that cetyl alcohol and Poloxamer 188 were the main factors that affected the oil entrapment efficiency. An anionic surfactant, namely sodium dodecyl sulfate, was added to overcome the aggregation problem at the ratio of Poloxamer 188 : sodium dodecyl sulfate equal to 35:1 by weight. Therefore, a simplex-centroid mixture design was used to systematically investigate the influence of these three factors, including cetyl alcohol as a lipid wall, Poloxamer 188 and sodium dodecyl sulfate at a fixed ratio as the mixed emulsifiers, on percent volume of particle size distribution and oil encapsulation efficiency of citronella grass oil SLPs. Table 1 shows the factors and levels of the components used in the simplex-centroid mixture design. Each experiment was run with replication. All batches of SLPs were prepared according to the mixture design layout (Table 2). The sequence of experiments was perfomed randomly by run number.

Table 1: Variables and intervals selected to perform the simplex-centroid mixture design

Factors	Level	
	Low	High
A = percent by weight of cetyl alcohol (X_1)	6.5	9.6
B = percent by weight of citronella grass oil (X_2)	4.2	7.2
C = percent by weight of mixed surfactants * (X_3)	4.8	7.8

* Poloxamer 188 : sodium dodecyl sulfate = 35:1; Total (A+B+C) = 18.5 % by weight; The amount of water in all runs was fixed at 81.5% by weight

Table 2: Mixture design layout

Std No.	Run No.	Point Type	A:Cetyl alcohol	B:Citronella grass oil	C:Mixed emulsifiers
1	14	Vertex	9.6	4.2	4.8
2	6	CentEdge	8.1	5.7	4.8
3	5	CentEdge	8.1	4.2	6.3
4	10	Vertex	6.5	7.2	4.8
5	9	CentEdge	6.5	5.7	6.3
6	13	Vertex	6.5	4.2	7.8
7	11	Axial	8.6	4.7	5.3
8	12	Axial	7.0	6.2	5.3
9	8	Axial	7.0	4.7	6.8
10	3	Center	7.6	5.2	5.8
11	7	Vertex	9.6	4.2	4.8
12	2	Vertex	6.5	7.2	4.8
13	1	Vertex	6.5	4.2	7.8
14	4	CentEdge	8.1	5.7	4.8

Preparation of citronella grass oil SLPs using a melt dispersion technique

The citronella grass oil SLPs were prepared according to the method of Lertsatitthanakorn et al. [6]. Briefly, amounts of cetyl alcohol, citronella grass oil and the mixed surfactants were accurately weighed according to the mixture design layout (Table 2). Citronella grass oil was dissolved in molten cetyl alcohol at 65°C and vigorously vortex-mixed (Vortex Genie 2, Scientific Industries, USA) for 1 minute. The mixed surfactants consisting of Poloxamer 188 (Pluronic® F 68 NF Prill) and sodium dodecyl sulfate at a 35:1 weight ratio were dissolved in water, heated to 65°C, added into the essential oil-lipid mixture and mixed for 1 minute. The dispersion was cooled down in an ice bath (4°C) for 30 minutes giving citronella grass oil SLPs in aqueous medium.

Analytical methods

Determination of Citronella grass oil components by gas chromatography (GC)

An ethanolic solution of standard citronella grass oil (0.3-2.5 µL/mL) was prepared and analyzed using a Gas Chromatograph connected to a flame ionization detector (FID) (GC 1850, Agilent ®). The capillary column used was HP®-5 (30 m x 0.32 mm id. x 0.25 µm film thickness). The injector temperature was 250°C; oven temperature was started at 100°C and held for 1 minute. The temperature was increased from 100°C to 220°C at 10°C/minute and held for 1 minute. The carrier gas used was nitrogen at a flow rate of 2 mL/minute while the split ratio was 1:10. The effluent was detected by FID at 280°C. A calibration curve was constructed by plotting between concentration of standard citronella grass oil and total peak area. Citronella grass oil concentration in samples was determined by dissolving samples in absolute ethanol and analyzing using GC. Total peak area of the sample was backward calculated to concentration of citronella grass oil using a linear regression equation generated from a calibration curve.

Validation of analytical method

a) Limit of detection (LOD) and limit of quantitation (LOQ)

Three concentrations of standard citronella grass oil consisting of 0.65, 0.90 and 1.80 µL/mL were assayed to determine total peak area using GC**.** Each concentration was assayed 7 times and the means and standard deviations (SD) were calculated**.** The SD were plotted with concentration and the Y-intercept was extrapolated to determine S_0. LOD and LOQ were described as $3S_0$ and 10 S_0, respectively [10] [1]. The results revealed that LOD ($3S_0$) and LOQ ($10S_0$) were 0.0075and 0.025 µL/mL, respectively.

b) Linearity

An ethanolic solution of standard citronella grass oil was prepared and a calibration curve was then constructed**.** The calibration curve between concentration of standard citronella grass oil and total peak area was linear in the range of 0.3 - 2.5 µL/mL with correlation coefficient (r) of 0.9999.

c) Accuracy and precision

Three target concentrations of standard citronella grass oil consisting of 0.65, 0.90 and 1.80 µL/mL were assayed to determine measured concentrations using GC. Precision of the analytical method was investigated by assaying these target concentrations 5 times for both intraday and inter-day aspects. The precision was expressed as the percent coefficient of variation (%CV) that was calculated from the following equation[1]:

$$\%CV = \frac{SD \times 100}{\bar{x}}$$

Where,

SD = standard deviation

$\bar{x}$ = mean value

The accuracy was expressed as the percent of inaccuracy (% inaccuracy) that was calculated from this equation:

$$\% \text{ inaccuracy} = \frac{(\text{measured concentration} - \text{target concentration}) \times 100}{\text{target concentration}}$$

As shown in Table 3, the intra- and inter-day precisions were less than 5% except for the inter-day analysis of the sample at a concentration of 0.65 µL/mL. Therefore, the GC condition used in this study has both precision and accuracy when the samples were prepared and analysed within the same day.

Determination of entrapment efficiency

The lyophilized SLPs (0.5 gm) were accurately weighed and absolute ethanol added to adjust to 25 mL. The sample was sonicated in an ultrasonic bath (Crest, Malaysia) for 30 minutes before centrifugation at 5,300 × g for 10 minutes. The supernatant was assayed to determine entrapped citronella grass oil concentration using GC. Percent entrapment was calculated from the amount of entrapped oil divided by the amount of theoretical oil.

Table 3 : Precision and accuracy of citronella grass oil analytical method

Concentration	Intraday analysis			Interday analysis		
(µL/mL)	Calculated concentration	%CV	% inaccuracy	Calculated concentration	%CV	% inaccuracy
0.65	0.6329	0.71	2.63	0.6170	7.63	5.07
0.90	0.8690	0.96	0.99	0.9229	4.05	2.54
1.80	1.7845	0.97	0.99	1.7711	2.38	1.61

Verification of models

To verify the accuracy of the selected model, 3 new batches of citronella grass oil SLPs were chosen at checked points. The selected check points, consisting of different ingredients generated by the Design- Expert® software, were prepared in triplicate and assayed to determine actual percent entrapment using GC.

Particle size analysis of citronella grass oil SLPs

All batches of citronella grass oil SLPs had particle size distributions verified by laser diffraction particle size analyzer (Beckman Coulter LS 230, Illinois, USA).

Data analysis

Design-Expert® software Version 7.1 (Sata-Esae, Inc., Minneapolis, USA) was used for analysis of a response surface model. Regression analysis and analysis of variance (ANOVA) was used to determine regression coefficients and statistical significance of model terms, as well as to fit the mathmetical models.

The model proposed for predicting the response variables of three component mixtures used to estimate the coefficients of the model is as follows;

$$Y = b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{123} x_1 x_2 x_3$$

Where, Y is the predicted response, b_1, b_2 and b_3 are the regression coefficients for linear effect terms, b_{12}, b_{13}, b_{23} and b_{123} are the interaction coefficients. X_1, X_2 and X_3 are the fractions of the first, second, and third mixture components, respectively. The significance of the equation parameters was determined by F-ratio at P value of 0.05. The adequacy of the models was determined by lack-of-fit test and coefficient of determination (R^2) and adjusted- R^2.

RESULTS

Determination of the response surface model fitting

Citronella grass oil entrapment efficacy

In this study, the response surface models fitting of citronella oil entrapment efficacy and % by volume of particle size diameter less than 10 µm was performed using multiple regression analysis and the goodness of fit was assessed by ANOVA. As shown in Table 4, the % oil entrapment possesses a non-significant lack of fit (P >0.05) and a significant linear mixture model (P<0.05) with determination coefficient (R^2) of 0.8539, thus ensuring the fitness of the regression model to the experimental data (Table 5). The linear model obtained for predicting the percent oil entrapment in SLPs is shown in Equation 1.

$$\text{Percent oil entrapment} = 1.874x_1 - 3.433x_2 + 3.960x_3 \quad (1)$$

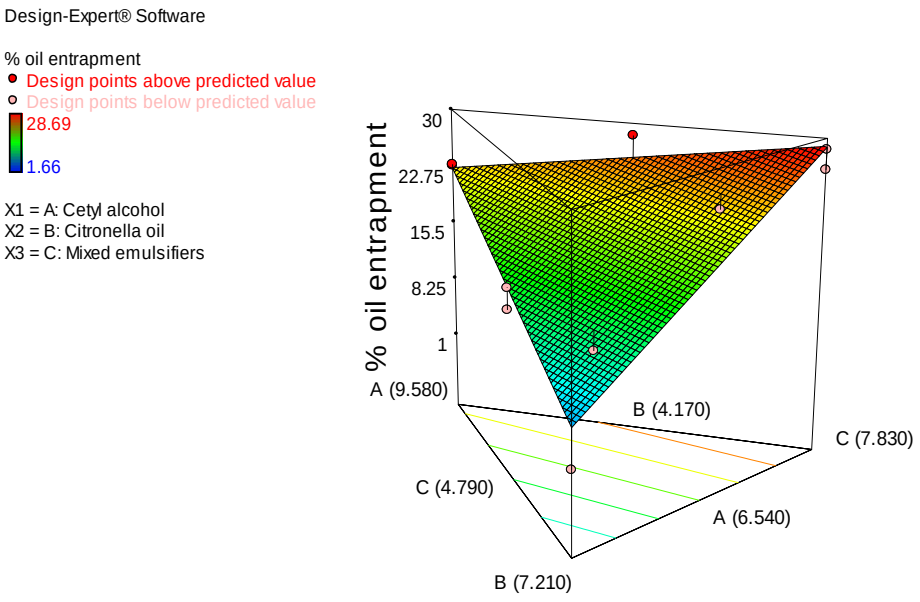
The response surface model of oil entrapment efficacy is shown in Fig 1a and the correlation between actual and predicted values is shown in Fig 1b. The area in the triangle of Fig 1a represents percent entrapment (response) which varies from 1.66 to 28.69 %.

Table 4: Regression coefficients, R^2, adjusted-R^2 probability values and lack of fit for mixture components

Regression coefficient	Estimated Effect of Actual Components	
	% oil encapsulation	% by volume of particles < 10µm
1. Cetyl alcohol, *A* (*X1*)	22.61	7.01
2. Citronella grass oil, *B* (*X2*)	6.48	30.09
3. Mixed emulsifiers, *C* (*X3*)	29.95	17.18
Model linear mixture (P value)	<0.0001*	0.0011*
Lack of Fit (F value)	32.23	13.39
Lack of Fit (P value)	0.6947	0.6050
Coefficient of determination (R^2)	0.8539	0.7088
Adjusted-R^2	0.8273	0.6559

*$p < 0.05$

a)



b)

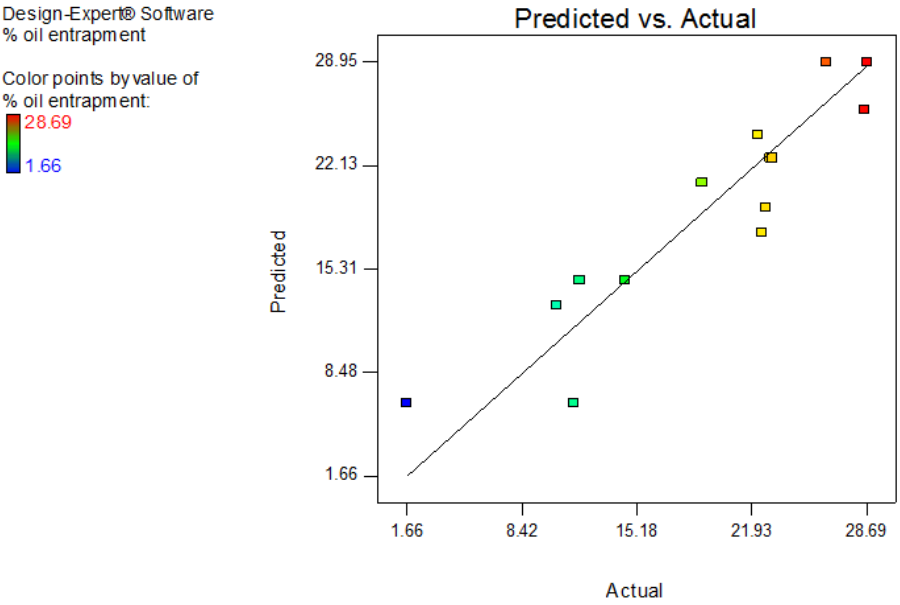
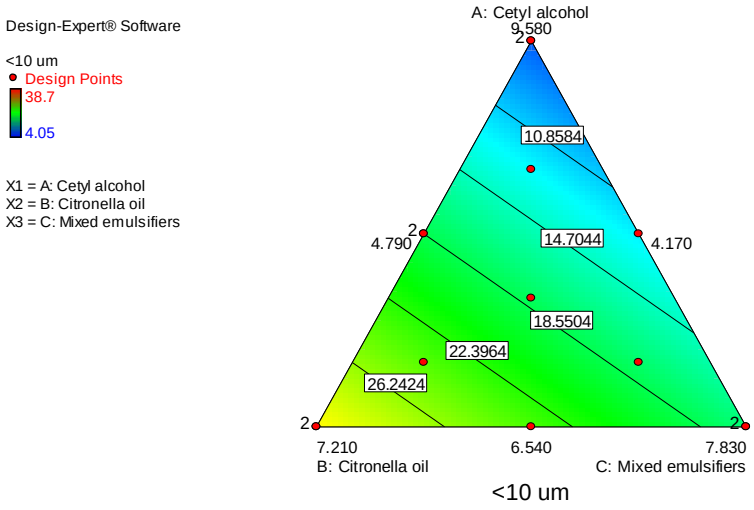


Fig. 1: Response surface analysis of the 3 mixture components (a) and the correlation between predicted and actual values of % oil entrapment (b).

Table 5: Percentages of citronella grass oil encapsulation and volumetric size distribution of the SLPs

Run no.	Percent entrapment	% by volume of particle diameter range			
		<1µm	<3µm	<10µm	<100µm
1	13.01	0.23%	1.08%	4.05%	89.60%
2	11.82	8.19%	31.70%	68.60%	100%
3	28.55	1.01%	4.30%	21.60%	98.50%
4	1.66	2.10%	24.90%	24.90%	65.70%
5	22.50	1.70%	8.11%	21.90%	84.10%
6	26.30	0.80%	4.90%	15.10%	94.30%
7	19.00	1.16%	4.71%	14.30%	87.70%
8	10.46	3.15%	18.80%	27.70%	57.10%
9	22.27	1.14%	5.13%	15.50%	91.50%
10	22.76	1.19%	5.09%	14.20%	82.00%
11	23.14	0.61%	2.00%	8.71%	96.80%
12	11.45	4.21%	20.20%	38.70%	88.60%
13	28.69	1.07%	5.48%	17.20%	95.50%
14	14.47	1.35%	5.84%	13.80%	84.40%

a)



b)

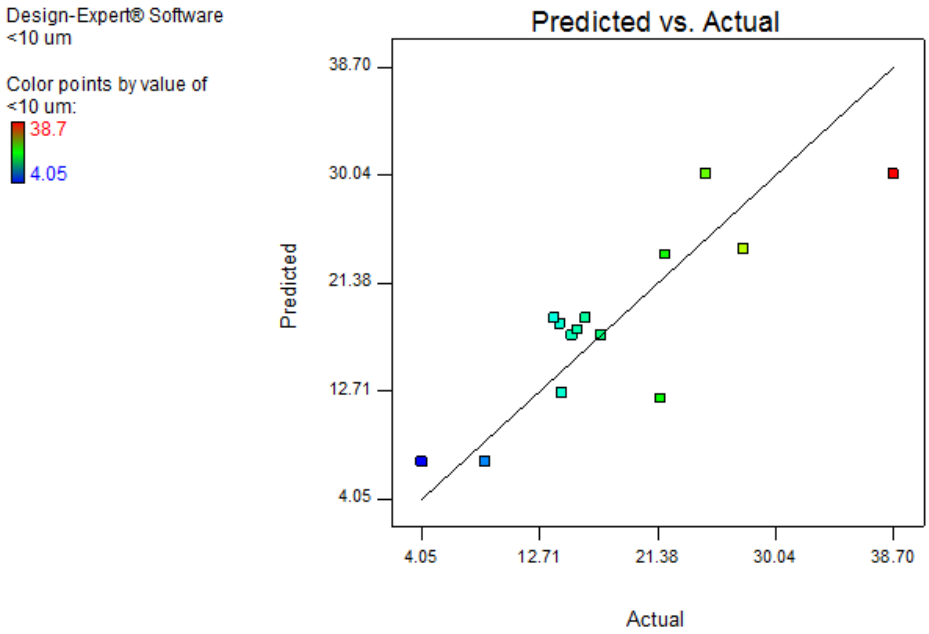


Fig. 2: Contour plot (a) and the correlation between predicted and actual values of cumulative percent SLPs having size less than 10 µm (b).

Volume of particle size distribution

As shown in Table 5, the volume of particle size ranges were examined at size less than 1, 3, 10 and 100 µm. In this study, the target size of SLPs required to penetrate into the pilosebaceous glands should be less than 10 µm. Therefore, the response surface model of volume of particle size in this target site was investigated. Results in Table 4 show that coefficients of determination (r^2) of the linear model fitting were 0.7088 with adjusted-R^2 of 0.6559 indicating a moderate fitness for the model. The linear model obtained for predicting the volume of particle size less than 10 µm is shown in Equation 2.

$$\%\ \text{Volume of SLPs (particle size} < 10\ \mu m\ ) = -2.193x_1 + 5.398x_2 + 1.151x_3 \quad (2)$$

The response surface model of cumulative percent SLPs having size less than 10 µm is shown in Fig 2a and the correlation between actual and predicted values is shown in Fig 2b. The percent volumes of SLPs diameter less than 10µm were from 4.05 to 68.60 % (Table 5).

Verification of linear mixture model of oil entrapment efficacy

The predictive model equation was verified by selecting predicted points at low, medium and high oil entrapment efficacy according to the software recommendation. Table 6 shows that the predicted values and actual values were not significantly different (P>0.05) with the correlation coefficient of 0.9880 suggesting the adequacy of the model.

Table 6 : Verification of the linear mixture model for oil entrapment efficacy

% by weight	K[a]	L[a]	M[a]
A: Cetyl alcohol	7.6	7.5	6.7
B : Citronella grass oil	5.9	4.9	4.3
C : Mixed surfactants	5.1	6.1	7.6
Predicted percent oil entrapment	13.54	20.66	27.97
Actual percent oil entrapment (Mean ±S.D., n=3)	14.47±0.69	22.17±1.30	29.74±1.87
% Predicted Error [b]	6.87	7.31	6.32

[a] P >0.05 by Mann-Whitney U test

[b] $\%\ \text{Predicted Error} = \frac{(\text{Actual value} - \text{Predicted value}) * 100}{\text{Predicted value}}$

DISCUSSION

The values of the coefficients A (cetyl alcohol), B (citronella grass oil) and C (mixed emulsifiers) are related to the effect of these variables on the responses. A positive sign for the coefficient indicates a synergistic effect while a negative term demonstrates an antagonist effect on the response. A higher coefficient means the factor possesses more potent influence on the response. As seen in equation (1), the higher the levels of cetyl alcohol and mixed surfactants, the greater the entrapment efficiency. In contrary, the higher the amount of citronella grass oil, the lower the entrapment efficiency. As a result, SLPs should be prepared using relatively higher concentrations of the mixed surfactants and cetyl alcohol and lower concentrations of citronella grass oil. On the contrary, as shown in equation (2), in order to increase the number of particle sizes less than 10 µm, the levels of citronella grass oil and mix emulsifiers possess a positive effect, while the level of cetyl alcohol shows a negative effect. In addition, there was no significant interaction effect between the independent variables on both responses.

Although citronella grass oil appears as an oily liquid, it is able to partially dissolve in water [11]. In other words, citronella grass oil shows "amphiphilic" properties due to its possessing various semipolar constituents such as monoterpene alcohols (i.e. geraniol and β-citronellol). Thus, the factors affecting entrapment efficiency

of citronella grass oil SLPs might be similar to those of both lipophilic and hydrophilic drugs entrapped in lipid particles. In general, lipophilic drugs are likely to be entrapped in SLPs while hydrophilic compounds such as curcuminoids are less soluble in melted lipid causing lower entrapment efficiency [12].

In this experiment, citronella grass oil could be solubilized in an aqueous mixed surfactants solution that is a mixture of Poloxamer 188 and sodium dodecyl sulfate. When the concentration of the mixed surfactants increased, its ability to solubilize citronella grass oil was also increased, which might lead to a higher entrapment efficiency. A similar effect of surfactant upon entrapment efficiency was found when an amphiphilic drug namely 3'-azido-3'-deoxythimidine (AZT) palmitate was incorporated in SLPs by a high pressure homogenization technique. Entrapment efficiency of AZT palmitate loaded SLPs was clearly increased when the concentration of phospholipids, either dipalmitoyl-phosphatidyl choline (DPPC) or a mixture of DPPC and dimyristoylphosphatidyl glycerol used as surfactants increased [13].

Verification of the linear mixture model of oil entrapment efficacy demonstrated that formulation optimization using this linear model could possess both satisfactory reliability and accuracy to predict percent oil entrapment efficacy. However, the maximum percent entrapment of citronella grass oil SLPs was only 28.69 %. The viscosity of the lipid phase might account for the percentage of oil entrapment and the particle size distribution. Although little is known about essential oils encapsulated in SLPs, it might be assumed that the higher viscosity of the lipid phase, which acts as a useful barrier between the inner (incorporated drug) and the outer aqueous phase, could protect the diffusion of the drug into the outer phase during SLPs production [14]. In addition, methods of preparation would be responsible for particle size distribution. For example, SLPs prepared by a high pressure homogenization technique would lead to consistent-submicron range particles. In contrast, the vibrating force offered by a vortex mixer might not provide a sufficiently constant pressure, resulting in a broad particle size range. Furthermore, the concentration of surfactant also caused the reduction of SLPs [15].

The ingredients of citronella grass oil SLPs providing maximum percent entrapment (28.69%) were found at the right-end corner of the triangular area (Fig. 1a), consisting of 6.54 % cetyl alcohol, 4.17 % citronella grass oil and 7.83 % mixed surfactants. These citronella grass oil SLPs possessed a unimodal size distribution. Furthermore, Rolland et al. [16] demonstrated that drug delivery to pilosebaceous glands should have particle size in a range of 3 to 10 µm. Thus, the optimal formulation of citronella grass oil SLPs should have maximum oil entrapment and the percentage volume of particle having the size less than 10 µm should be enough to penetrate into the target site. Formulation optimization of citronella grass oil SLPs can be done by setting the criteria of desired reponses and selecting the mixture components around the highlighted area. Figure 3 shows the overlay graph of the percent oil entrapment (20-28.6%) and the volume of SLPs (20-38%).

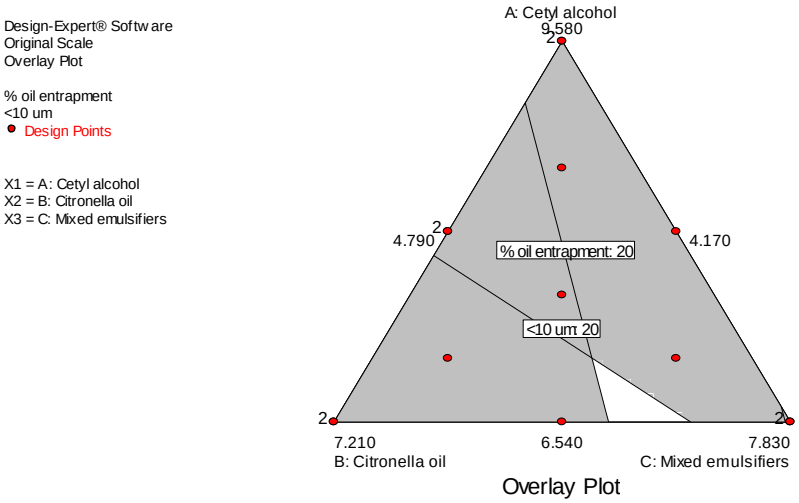


Fig. 3: Overlay graph of formulation optimization highlighting an area of operability.

CONCLUSION

The simplex-centroid mixture design was a good tool for investigating the optimized proportions of citronella grass oil SLPs ingredients providing the maximum oil entrapment efficiency. Among the models proposed by Design-Expert® software, the linear mixture model fitted the surface response with less than 10% predicted error. The result of the linear equation revealed that to obtain a high percent oil entrapment, citronella grass oil SLPs should be prepared using a relative higher concentration of the mixed surfactants and cetyl alcohol while using a relative lower concentration of citronella grass oil. The predicted optimal ratio of cetyl alcohol : citronella grass oil : mixed emulsifiers proposed by Design- Expert software (Fig. 3) to reach the maximum oil entrapment of 28.69% and volume of particle size diameter less than 10 µm of 16.42% with desirability of 0.957 was 6.77 : 4.17 : 7.60. However, stability studies as well as the anti-acne activity of citronella grass oil SLPs need to be investigated further.

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REFERENCES

1. Nand P, Drabu S, Gupta RK. In vitro antibacterial and antioxidant potential of medicinal plant used in the treatment of acne. Int J Pharm Pharm Sci 2012;4:185-190.
2. Lertsatitthanakorn P, Taweechaisupapong S, Aromdee C, Khunkitti W. In vitro bioactivities of essential oils used for acne control. Int J Aromather 2006;16:43-49.
3. Lertsatitthanakorn P, Taweechaisupapong S, Arunyanart C, Aromdee C, Khunkitti W. Effect of citronella oil on time kill profile, leakage and morphological changes of *Propionibacterium acnes.* J Essent Oil Res 2010;22:270-274.
4. Deasy PB, editor. Microencapsulation and related drug processes. New York: Marcel Dekker; 1984.
5. Lertsatitthanakorn P, Khunkitti W. Anti-acne activity of citronella oil microemulsion In: 9th Scientific Conference of the Asian Societies of Cosmetic Scientists (ASCS) 2009 March 2-4; Yokohama, Japan; 2009 p. 1-3.
6. Lertsatitthanakorn P, Taweechaisupapong S, Aromdee C, Khunkitti W. Antibacterial activity of citronella oil solid lipid

particles in oleogel against *Propionibacterium acnes* and its chemical stability. Int J Essent Oil Ther 2008;2:167-171.

7. Mehnert W, Mader K. Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Deliv Rev 2001;47:165-196.
8. Dhiman S, Verma S. Optimization on melt-in-mouth tablets of levocetrizine dihydrochloride using response surface methodology. Int J Pharm Pharm Sci 2012;4:176-84.
9. Kincl M, Turk S, Vrecer F. Application of experimental design methodology in development and optimization of drug release method. Int J Pharm 2005;291:39-49.
10. Taylor JK, editor. Quality assurance of chemical measurments. Michigan: Lewis Publishing; 1989.
11. Gennaro AR, editor. Remington: The science and practice of pharmacy. 21st ed. Philadelphia: Lippincott Williams and Wilkins; 2006.
12. Tiyaboonchai W, Tungpradit W. Formulation and characterization of curcuminoids loaded solid lipid nanoparticles. Int J Pharm 2007;337:299-306.
13. Heiati H, Tawashi R, Shivers RR, Phillips NC. Solid lipid nanoparticles as drug carriers. I. Incorporation and retention of the lipophilic prodrug 3'-azido-3'-deoxythymidine palmitate. Int J Pharm 1997;146:123-131.
14. Reithmeier H, Herrmann J, Gopferich A. Development and characterization of lipid microparticles as a drug carrier for somatostatin. Int J Pharm 2001;218:133-143.
15. Wang G, Mu Y, Yu H-Q. Response surface analysis to evaluate the influence of pH, temperature and substrate concentration on the acidogenesis of sucrose-rich wastewater. Biochem Eng J 2005;23:175-184.
16. Rolland A, Wagner N, Chatelus A, Shroot B, Schaefer H. Site-Specific drug delivery to pilosebaceous structures using polymeric microspheres.Pharm Res 1993;10:1738-1744.