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Optimisation of *Orthosiphon Stamineus* Loaded onto Nanostructured Lipid Carrier using D Optimal Mixture Design

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In this study, the formulation of *Orthosiphon stamineus* (OS) loaded onto nanostuctured lipid carrier was prepared using melt emulsification homogenisation technique. Glyceryl monostearate and triglyceride were used as solid and liquid lipids respectively. D-optimal mixture design was employed to optimise the formulation of OS loaded into nanostructured lipid carrier. The independent variables were OS extract, solid lipid and liquid lipid, while the dependent variables were particle size, polydispersity index (PDI) and encapsulation efficiency. Multiple correlation coefficient (R^2) obtained for particle size, PDI and encapsulation efficiency was 0.9404, 0.9138 and 0.8754. All three models are significant and the lack of fit for all dependent variables was not significant. Solid lipid was the most influential factor for particle size. For PDI, OS was the dominant factor. Meanwhile liquid lipid is the most influential factor toward the encapsulation efficiency. The optimum formulation of OS loaded nanostructured lipid carrier is 4 % of OS, 1 % of solid lipid and 5 % of liquid lipid.

1. Introduction

Orthosiphon stamineus (OS) or also known as misai kucing has been used widely to treat hypertension (Azizan et al., 2012), renal calculus (Adam et al., 2009) and diabetes (Rao et al., 2014). Tezuka et al., (2002) found that active compounds such as terpenoids and polyphenols are exist in OS. The presence of polyphenolic contents in OS act as health benefits of OS. Akouwah et al. (2004) reported that sinensetin, rosmarinic acid and eupatorin are presence in the leaves of OS. OS was found to have chemical compounds which exhibit anti-obesity effect such as terpenoids, polyphenols, sterols, orthosiphols, caffeic acid, ursolic acid and siphonols (Son et al., 2011).

Human skin consists of epidermis, dermis and hypodermis. Epidermis or also known as stratum corneum is located at the outermost layer of the skin. This stratum corneum acts as a barrier; therefore, most topical products cannot penetrate it.

Nanostructured lipid carrier (NLC) is a carrier-based topical and transdermal drug delivery which overcomes the limitation of solid lipid nanoparticle (SLN). NLC may increase the maximum amount of active ingredient being loaded compared to SLN. The production of NLC involves both solid and liquid lipids that exhibit low toxicity (Muller et al., 1997). Ultrasonication method was used in this study. Ultrasonic dispersion provides good alternative for laboratory scale because it involves low cost of apparatus and rapid nature of production (Schwarz, 2012).

Oral administration is more toxic than topical administration. Oral aid transports the medicine through the blood stream impacting the entire body along with the area in pain, while topical aid transports the medicine directly to the affected area. Numerous slimming cream and pills are available in the market and easily obtained. However, their efficacies and safety are uncertain. In this research, OS is chosen as a replacement to synthetic drug.

Experimental design is a method devised to develop and optimise the formulation of drugs and give the desired formulation. Main objective to design an experiment is to have a valid result at minimum effort, time and resources. In this research, D-optimal mixture design was employed in order to optimise the formulation of OS loaded into NLC. The D-optimal mixture design was used widely in product formulation of cosmetic industry (Borhan et al., 2014). In this study, the formulation of OS loaded into nanostructured lipid carrier was designed specifically for topical application.

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2. Materials and methods

2.1 Materials

White variety of OS was purchased from Ethno Resources Sdn Bhd, Sungai Buloh., glyceryl monostearate, triglyceride from Making Cosmetic (USA), Tween 80 and soy lecithin were purchased from Sigma Aldrich, distilled and deionised water.

2.2 Preparation of OS extract

OS extract was prepared following a method from Aisha et al. (2014). The OS ethanolic extract was prepared by maceration method. OS was added to 96 % ethanol and mixed continuously using magnetic stirrer for 48 h. Next, it was filtered and rotavapored at 60 ºC. Finally, the sample was freeze-dried.

2.3 Preparation of OS loaded into nanostructured lipid carrier

Method for preparation of OS loaded into NLC was employed based on Rosli et al. (2014) with slight modification. Lipid and aqueous phases were prepared separately. Lipid phase was made from glyceryl monostearate and triglyceride while aqueous phase was produced through a combination of water, tween 80 and soy lecithin. Both phases were heated individually. Next aqueous phase was added to lipid phase and mixed well. After that, OS was incorporated into the mixture. The mixture was homogenised using IKA Ultra Turrax® Homogenizer at 11,000 rpm for one minute. The acquired pre-emulsion was ultasonicated using probe sonicator (Fisher Scientific, FB705) for 20 minutes. The NLC was cooled in iced water bath.

2.4 Determinations of Particles Size and Polydispersity Index (PDI)

The determinations of particle size and PDI were performed using Malvern Zetasizer Nano S (Malvern instrument, UK). All samples were diluted using deionised water at 1 : 9 ratios respectively. The purpose of dilution was to prevent back-scattering phenomena (Ng et al., 2014). Each measurement was done in triplicate.

2.5 Encapsulation Efficiency (EE)

The encapsulation efficiency of OS loaded into NLC was measured based on the method reported by Barras et al. (2013) with slight modifications. Sephadex gel-50 was used to separate the NLC suspension. The concentration of suspension before and after filtration was analysed using HPLC.
The encapsulation efficiency was calculated according to the Eq(1):

$$\text{EE (\%)} = \frac{n_1}{n_2} \times 100\ \% \quad (1)$$

Where; n_1 = amount of encapsulated OS, n_2 = total amount of OS in the total suspension

2.6 Experimental Design

D-optimal mixture design from design expert software version 7.1.5 (STAT-EASE Inc., Minneapolis, USA) was used to find out the optimum formulation of OS loaded into NLC as well as to investigate the effect of independent variables to dependent variables. The independent variables are OS, solid lipid and liquid lipid as listed in Table 1. While the dependent variables are particle size, PDI and EE. The level of independent variables were based on the preliminarily study conducted.

Table 1: Summary for independent variable

Factor	Variable	Units	Level of Variable	
			Lower limit	Upper limit
A	OS	%	1	4
B	Solid lipid	%	1	3
C	Liquid lipid	%	3	8

3. Results and discussion

3.1 Model fitting

The effect of three independent variables namely active, solid lipid and liquid lipid on the particle size, PDI and encapsulation efficiency was investigated using D-optimal mixture design as shown in Table 2.

Table 2: Results of dependent variables

Run	Particle size (d, nm)	PDI	EE (%)
1	90.21	0.135	97.00
2	159.0	0.153	94.70
3	134.0	0.120	97.22
4	140.0	0.143	98.45
5	119.4	0.133	94.60
6	89.48	0.132	97.50
7	128.1	0.115	98.10
8	139.0	0.119	99.10
9	147.5	0.162	90.40
10	138.3	0.114	98.72
11	159.0	0.114	98.70
12	106.0	0.149	95.10
13	116.0	0.132	97.41
14	131.2	0.123	98.80
15	141.0	0.157	92.80
16	147.8	0.157	92.80

Analysis of variance (ANOVA) was used to determine the best fitted model for all the independent variables. The parameters include the adjusted multiple correlation coefficient (Adjusted R^2), multiple correlation coefficient (R^2), lack of fit, regression (P value), and regression (F value). ANOVA for each dependent variables are shown in Table 3, 4 and 5. Eq(2), (3) and (4) were analysed based on their magnitude and sign of regression coefficient. Higher value of coefficient of independent variable resulted in greater influence on the response. Positive sign of regression coefficient implied that the independent variable was directly proportional to response, while negative sign of regression coefficient indicated that the independent variable was inversely proportional to the response (Yunus et al., 2011).

$$\text{Particle size} = 3.36A + 370.22B + 149.28C - 20.69AB + 118.63AC - 387.26BC \quad (2)$$

$$\text{PDI} = 0.81A + 0.30B + 0.11C - 0.26AB - 0.070AC - 0.16BC \quad (3)$$

$$\text{EE} = 98.36A + 88.07B + 98.83C - 10.51AB - 5.03AC + 15.46BC \quad (4)$$

The distribution of nanoparticles was influenced by particle size since small particle resulted in narrow PDI. Eq(2) shows that all independent variables were directly proportional to the particle size and the amount of solid lipid had the biggest influence towards the particle size. Rosli et al. (2014) reported that an increase in lipid concentration led to higher viscosity of sample and lesser dispersion energy available per unit lipid in high concentration of solid lipid which results in higher particle size. In this study, it was found that as the concentration of active, solid lipid and liquid lipid was increased, the particle size increased as well. Uner (2006) found that higher active concentration contributed to higher particle size of nanoparticles. In addition, Emami et al. (2012) also discovered that changing the active content from 5 to 10 may increase the size of nanoparticles. Rosli et al. (2014) found that particle size of nanoparticle was increased as the concentration of solid lipid being increased in the *Zingiber zerumbet* loaded into nanostructured lipid carrier formulation, possibly due to less energy dispersion per unit lipid available. Table 3 shows ANOVA for particle size. The model is significant ($p < 0.05$) and the lack of fit is not significant. The adjusted R^2 is 0.8409 and R^2 is 0.9404.

Polydispersity index (PDI) implies particle size homogeneity. The range of PDI is usually between 0 and 1. It was reported that the closer the value of PDI to zero, the higher the homology of particle (Joshi and Patravale, 2008). From Eq(3), it was found that the amount of active being added presented the biggest effect to PDI. The interaction between A and B, A and C, and B and C gave negative value of PDI. The ANOVA for PDI is shown in Table 4. The model is significant ($p < 0.05$) and the lack of fit is not significant. The adjusted R^2 is 0.8706 and R^2 is 0.9138.

Based on Eq(4), all independent variables were directly proportional to the encapsulation efficiency. Liquid lipid contributed the greatest effect on encapsulation efficiency. This finding was in agreement with Yuan et al. (2007) who found that by increasing liquid lipid such as oleic acid, an increase in encapsulation efficiency of the nanostructured lipid carrier was obtained. The interaction between solid and liquid lipids administered positive sign hence implying that it was directly proportional to the encapsulation efficiency. In this research, it was discovered that increasing the amount of liquid lipid gave higher value of encapsulation efficiency. Liquid

lipid played an important role in the encapsulation efficiency since any addition of liquid lipid deformed the perfect crystal formed by solid lipid nanoparticles hence providing more space to fit the active. The encapsulation efficiency was increased (Muller et al., 2007). As for ANOVA analysis, the model is significant ($p < 0.05$) and the lack of fit is not significant. The adjusted R^2 is 0.8131 and R^2 is 0.8754.

Table 3: Analysis of variance (ANOVA) for particle size

Source	Sum of square	dF	Mean Square	F-value	P-value	Significance
Model	6,399.04	5	1,279.81	31.58	< 0.0001	Significant
Linear Mixture	3,706.35	2	1,853.18	45.73	< 0.0001	
AB	1.89	1	1.89	0.047	0.8332	
AC	342.91	1	342.91	8.46	0.0156	
BC	774.62	1	774.62	19.12	0.0014	
Residual	405.22	10	40.52			
Lack of fit	136.01	5	27.20	0.51	0.7642	Not significant
Pure error	269.22	5	53.84			
Cor total	6,804.26	15				
R^2	0.9404					
R^2 (Predicted)	0.9107					
R^2 (Adjusted)	0.8409	15				

Table 4: Analysis of variance (ANOVA) for PDI

Source	Sum of square	dF	Mean Square	F-value	P-value	Significance
Model	3.769×10^{-3}	5	7.539×10^{-4}	21.19	< 0.0001	Significant
Linear Mixture	3.406×10^{-3}	2	1.703×10^{-3}	47.87	< 0.0001	
AB	3.026×10^{-4}	1	3.026×10^{-4}	8.51	0.0154	
AC	1.200×10^{-4}	1	1.200×10^{-4}	3.37	0.0961	
BC	1.396×10^{-4}	1	1.396×10^{-4}	3.92	0.0757	
Residual	3.557×10^{-4}	10	3.557×10^{-5}			
Lack of fit	2.417×10^{-4}	5	4.835×10^{-5}	2.12	0.2145	Not significant
Pure error	1.140×10^{-4}	5	2.280×10^{-5}			
Cor total	4.125×10^{-3}	15				
R^2	0.9138					
R^2 (Predicted)	0.7886					
R^2 (Adjusted)	0.8706					

Table 5: Analysis of variance (ANOVA) for encapsulation efficiency

Source	Sum of square	dF	Mean Square	F-value	P-value	Significance
Model	81.06	5	16.21	14.05	0.0003	Significant
Linear Mixture	66.92	2	33.46	29.01	< 0.0001	
AB	0.49	1	0.49	0.42	0.5301	
AC	0.62	1	0.62	0.53	0.4818	
BC	1.23	1	1.23	1.07	0.3254	
Residual	11.53	10	1.15			
Lack of fit	8	5	1.60	2.26	0.1953	Not significant
Pure error	3.53	5	0.71			
Cor total	92.59	15				
R^2	0.8754					
R^2 (Predicted)	0.7224					
R^2 (Adjusted)	0.8131					

3.2 Verification of the models

The comparison between experimental and predicted values of the response was conducted in order to check the adequacy of the equation. Results obtained (Figure 6) show good agreement with the predicted values.

Table 6: Experimental and predicted values of the response

Independent variable			Dependent variables					
A (%)	B (%)	C (%)	Y_1	Y'_1	Y_2	Y'_2	Y_3	Y'_3
4	1	5	90.204	88.570±1.187	0.1348	0.135±0.007	97.340	98.10±1.101

Y'_1: Experimental value for particle size
Y'_2: Experimental value for PDI
Y'_3: Experimental value for encapsulation efficiency

4. Conclusion

The optimum formulation of OS loaded onto NLC can be obtained using D-optimal mixture design. Multiple correlation coefficient (R^2) obtained for particle size, PDI and encapsulation efficiency was 0.9404, 0.9138 and 0.8754. All three models are significant and the lack of fit for all three responses was not significant.

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Reference

Adam Y., Somchit M.N., Sulaiman M.R., Nasaruddin A.A., Zuraini A., Bustamam A.A., Zakaria Z.A., 2009, Diuretic properties of Orthosiphon stamineus Benth, Journal of Enthnopharmacology 124 (1), 154-158.

Aisha A.F.A, Abdul Majid A.M.S., Ismail Z., 2014, Preparation and characterization of nano liposomes of Orthosiphon stamineus ethanolic extract in soybean phospholipids, BMC Biotechnology 14 (23), 1-11.

Akouwah G.A., Zahari I., Norhayati I., Sadikun A., Hamsha S.M.K., 2004, Sinensetin, eupatorin, 3-hydroxy-5,6,74-tetra methoxy flavone and rosmarinic acid content and anti oxidative effect of Orthosiphon stamineus from Malaysia, Food Chemis 82, 556-559.

Azizan N.A., Ahmad R., Mohamed K., Ahmad M.Z., Asmawi Z., 2012, The in vivo antihypertensive effects of standerdized methanol extracts of orthosiphon stamineus on spotaneous hypertensive rats: A preliminary study, African Journal of Pharmacology 6 (6), 376-379.

Barras A., Boussekey L., Courtade E., Boukherroub R., 2013, Hypericin-loaded lipid nanocapsules for photodynamic cancer theraphy in vitro, The Royal Society of Chemistry 5 (21), 10562-10572.

Borhan F.B., Abdul. Gani S.S., Shamsuddin R., 2014, The use of D-optimal mixture design in optimising okara soap formulation for stratum corneum application, The Scientific World Journal, DOI: 10.1155/2014/173979.

Emami J., Rezazadeh M., Varshosaz J., Tabbakhian M., Aslani A., 2012, Formulation of LDL targeted nanostructured lipid carriers loaded with paclitaxel: a detailed study of preparation, freeze drying condition, and in vivo cyotoxicity, Journal of Nanomaterials 12, 1-10.

Joshi M., Patravale V., 2008, Nanostructured lipid carrier (NLC) based gel celecoxib, International Journal of Pharmaceutics 346 (1-2), 124-132.

Müller R.H., Petersen R.D., Hommoss A., Pardeike J., 2007, Nanostructured lipid carriers (NLC) in cosmetic dermal products, Advanced Drug Delivery Reviews 59 (6), 522–530.

Ng W.K., Saiful Yazan L., Yap L.H., Wan Nor Hafiza W.A.G., How C.W., Abdullah R., 2014, Thymoquinone-loaded nanostructured lipid carrier exhibited cytotoxicity towards breast cancer cell lines (MDA-MB-231 and MCF-7) and cervical cancer cell lines (HeLa and SiHa), BioMed Research International, DOI: 10.1155/2015/263131.

Rosli N.A., Mohamed Noor N., Abdul Aziz R., Abdul Aziz A., Hasham R., 2014, Formulation and characterization of [6]-gingerol loaded nanostructured lipid carrier (NLC), International Science Postgraduate Conference, Faculty of Science, Universiti Teknologi Malaysia, Johor Bahru, Malaysia, L40.

Rao N.K., Bethala K., Sisinthy S.P., Rajeswari K.S., 2014, Antidiabetic activity of Orthosiphon Stamineus Benth roots in streptozotocin induced type 2 diabetic rats, Asian Journal of Pharmaceutical and Clinical Research 7 (1), 149-153.

Schwarz J.C., Weixelbau A., Pagitsch E., Low M., Resch G.P., Valenta C., 2012, Nanocarriers for dermal drug delivery: Influence of preparation method, carrier type and rheological properties, International Journal of Pharmacy 437 (1-2), 83-88.

Son J.Y., Park S.Y., Kim J.Y., Won K.C., Kim Y.D., Choi Y.J., Zheng M.S., Son J.K., Kim Y.W., 2011, Orthosiphon stamineus Reduces Appetite and Visceral Fat in Rats, Journal of the Korean Society for Applied Biological Chemistry 54 (2), 200–205.

Tezuka Y., Stampoulis P., Banskota A., Awale S., Tran K., Saiki I., Kartoda S., 2002, Constituent of the vietnamese medicinal plants of Orthosiphon stamineus, Chemis Pharmaceutical Bul 48, 1711-1719.

Uner M., 2006, Preparation, characterization and physico-chemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carrier (NLC): Their benefit as colloidal drug carrier system, Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Istanbul, Turkey, 61, 375-386.

Yuan H., Wang L.L., Du Y., You J., Hu F.Q., Zeng S., 2007, Preparation and characteristic of nanostructured lipid carriers for control releasing progesterone by melt emulsification, Colloids and Surfaces B. Biointerfaces, 60 (2), 174-179.

Yunus M.A.C, Lee C.Y., Idham Z., 2011, Effects of variables on the production of red-fleshed pitaya powder using response surface methodology, Jurnal Teknologi 56, 15-29.