FULL FACTORIAL DESIGN APPROACH FOR FORMULATION OF FLEXIBLE LIPOSOMES LOADED *LABISIA PUMILA*: A STORAGE STABILITY STUDY OF THE OPTIMIZED FORMULATION

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ABSTRACT

Labisia pumila (*Lp*), is a medicinal herb contained high antioxidant and phenol compound. Due to its high antioxidant properties, *Lp* extract is beneficial for anti-aging and could be applied topically. In this study, *Lp* was encapsulated into Flexible liposomes (FlexLipo) to protect its stability which comprises lecithin, β-sitosterol and surfactants as an edge activator. The present work focus on the design of FlexLipo-*Lp* nanocarrier and its storage stability. A 2^3 full factorial design was used to investigate the interaction of FlexLipo formulation and five parameters on the particle size, zeta potential, polydispersity index, elasticity and encapsulation efficiency. Results indicated that the particle size, zeta potential and polydispersity index affect the interaction among the factors. The final optimized nanocarrier formulation was obtained and used to study its stability during storage. The important outcomes of this research revealed that the formulation prepared from the design demonstrated a stable structure, high encapsulation efficiency and desired particle size. It could be concluded that Flexible liposomes loaded *Labisia pumila* are a new strategy in herbal transdermal delivery.

Keywords: Full-factorial design, *Labisia pumila*, flexible liposomes, nanoparticles, stability.

1. INTRODUCTION

Flexible liposomes are known as deformable liposomes, transfersomes or elastic liposomes consist of phospholipid along with surfactant as an edge activator. Edge activators can increase the fluidity of bilayers in the stratum corneum by lowering the interfacial tension of the bilayer, thus enhancing the permeability of encapsulated active ingredients into the skin [1]. Mostly, a single chain surfactant was used such as SPAN 80, TWEEN 80 and sodium deoxycholate.

Labisia pumila (*Lp*) is also known as Kacip Fatimah (KF) was traditionally used in post –partum treatment to enhance energy and get stronger after giving birth [2, 3]. The extract contained estrogen – liked substituent which is believed will assist collagen synthesis and has strong antioxidant activity comparing ascorbic acid [4].They also indicated that *Lp* extract may have great potential to be developed as novel anti – inflammatory drug. Materials having anti-inflammatory efficacy could be used not only for inflammatory skin conditions but also as soothing ingredients for sensitive skins. In consequence, *Lp* extract shows a great potential as an active ingredient for an anti-aging cosmetic that is suitable for any types of skin.

In order to enhance the stability of the nanoparticles and its permeability in transdermal delivery, a stable formulation is crucial. A 2^3 full factorial design was applied to develop a stable formulation for FlexLipo loaded *Labisia pumila*. The formulation was optimized by the application of the design to evaluate the major interaction among the factors on the characteristics of FlexLipo-*Lp* such as encapsulation efficiency, zeta potential, particle size, deformability and polydispersity index. Furthermore, the optimized formulation

was evaluated in terms of its storage stability. This approach is to be known much more reliable as it is based on the mathematical model of the combined effects of the processing factors.

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2. MATERIALS AND METHODS

2.1 Formulation of Flexible Liposomes using 2^3 Full Factorial Design

Formulation of flexible liposomes was developed by employing two level full factorial design using Design Expert (version 6.1). A total of 32 experiments were generated by Design Expert software to evaluate the interaction effect of L-α-phosphatidylcholine (PC), β-sitosterol, surfactants and time of sonication in the formulation. Table 1 defines the five adjustable factors and responses for the formulation of flexible liposomes.

Table 1: Experimental parameters for 2^3 Full Factorial Design for Flexible liposomes formulations.

Independent variables	Coded unit	Low (-1)	High (+1)
Phosphatidylcholine	X_1	250mg	500mg
B-sitosterol	X_2	50mg	100mg
Tween 80	X_3	12.97µl	39.95µl
Span 80	X_4	17.52µl	49.07µl
Time of sonication	X_5	3 minutes	7 minutes
Dependent variables		Unit	
Encapsulation efficiency	Y_1	%	
Particle size	Y_2	d.nm	
Zeta potential	Y_3	mV	
Polydispersity index	Y_4		
Deformable index	Y_5		

The assumption of linearity in the factor effect was expressed using regression equation with five parameters and the interaction can be given by the following equation;

$$Y_i = b_0 + b_1X_{1i} + b_2X_{2i} + b_3X_{3i} + b_4X_{4i} + b_5X_{5i}\ldots + b_{12345}X_{1i}X_{2i}X_{3i}X_{4i}X_{5i} \quad \text{(Eq. 1)}$$

Where Y_i is the response, X_{ji} values j = 1, 2, 3, 4, 5 and i = 1, 2, 3, 4, 5 indicated the corresponding parameters; b_0 is the average value of the result, b_1, b_2, b_3, b_4 and b_5 are the linear coefficient; and b_{12}, b_{123}, b_{1234}, b_{12345} represent the interaction coefficient [5]. Optimised formulation was validated and analysis of variance (ANOVA) was used to analyse the results.

2.2 Preparation of Flexible liposomes

Flexible liposomes were prepared by a standard thin –film hydration method. Chloroform and ethanol (1:1) were used as a solvent to produce lipid thin film. β -sitosterol was dissolved in ethanol [6] prior mixed with chloroform. L – α – phosphatidylcholine was dissolved in chloroform and followed by TWEEN 80 and SPAN 80 as an edge activator to enhance the elasticity of the vesicles. *Labisia pumila* extract was dissolved in a hydration medium using phosphate buffer solution (PBS), 0.1mM at 6.8 pH before added to the dried thin film. After complete hydration, the suspension then sonicated using probe sonicator at 30% amplitude to obtain smaller vesicles size. The sample was respectively stored at 4°C for analysis.

2.3 Characterization of the nanoparticles and Stability Study

Average particle size, zeta potential (ζ) and polydispersity index (PDI) were measured using Zetasizer Nano ZS (Malvern, United Kingdom). A comparative measurement of elasticity for flexible liposomes and liposomes were carried out using extrusion method [7]. To analyse the stability of the nanoparticles, the samples were stored at different temperature (4°C, 25°C) and the physical stability was examined.

2.4 Encapsulation Efficiency and Leakage Rate

Freshly prepared flexible liposomes encapsulated *Labisia pumila* was separated from unentrapped extracts using mini-column centrifuge method [8,9]. The released *Labisia pumila* extracts were assayed using Total Phenolic Content (TPC). The TPC was expressed as Gallic acid equivalent (GAE) in mg of dry weight *Labisia pumila* extract. All assays were done in triplicate [10]. The amount of entrapment extract and leakage ratio were expressed in % and calculated from the following equation:

$$\text{Encapsulation Efficiency (EE) \%} = (\text{Encapsulated extract} / \text{Total extract}) \times 100 \quad \text{(Eq.2)}$$

$$\text{Leakage ratio} = (1 - LE_{\text{during storage}} / LE_{\text{before storage}}) \times 100\% \quad \text{(Eq.3)}$$

**LE = Loading efficiency*

3. RESULTS AND DISCUSSION

The accuracy and the convenient of the model was expressed by the determination of coefficient value (R^2). The R^2 value of the model was calculated, indicating that the developed mathematical equation defined the model as shown in Table 2. The formulation of FlexLipo-*Lp* affected Y_1, Y_2 and Y_3 statistically.

Table 2: Results of regression analysis of variance for all dependent variables.

	Y_1	Y_2	Y_3	Y_4	Y_5
F	4.03	26.95	379.35	2.15	3.92
P value	0.0446	0.0028	0.0406	0.0728*	0.1429*
R^2	0.9438	0.9945	0.9999	0.6966	0.9734
Adjusted R^2	0.7097	0.9576	0.9973	0.373	0.7252
Predicted R^2	0.5978	0.6501	0.91	0.3808	2.0262
Adeq. Precision	7.2940	23.2780	77.108	6.078	8.7592

*Y_4 and Y_5 models were considered statistically not significant where $p>0.05$.

The formulation was assessed for 120 days of storage stability and the formulation was observed not stable at 25°C after 120 days. Table 3 shows the storage stability outcomes of the storage stability of FlexLipo-*Lp*.

Table 3: Stability of Flexible liposomes loaded *Labisia pumila*

Parameters	At first day	After 120 days	
		4°C	25°C
Particles size	182.4 d.nm	111.2 d.nm	
Polydispersity index	0.216	0.217	
Zeta potential	-31.3	-31	Not Available
Encapsulation efficiency	87.12%	-	
Loading efficiency	-	68.36%	
Leakage ratio		21.5%	

It was observed that a leakage ratio during storage was 21.5% from the first day prepared, due to saturating of lipid compartment and aqueous compartment with the active ingredients. The low polydispersity index (0.216) confirmed the homogeneity of developed nanocarrier and it was stable throughout the storage.

4. CONCLUSION

According to ANOVA results, the formulation of FlexLipo-*Lp* was designated as the optimum formulation due to its physiochemical properties. This research demonstrated that the optimum FlexLipo-*Lp* nanoparticles designed in this study may be considered for transdermal delivery application. To evaluate the effectiveness of this nanocarrier, further studies are strongly required to identify its pharmacological properties.

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