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DESIGN AND PHYSICOCHEMICAL EVALUATION OF NANOSTRUCTURED LIPID CARRIER ENCAPSULATED *ZINGIBER ZERUMBET* OIL BY D-OPTIMAL MIXTURE DESIGN

Nur Ayshah Rosli, Rosnani Hasham*, Azila Abdul Aziz

Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310, Johor Bahru, Johor, Malaysia

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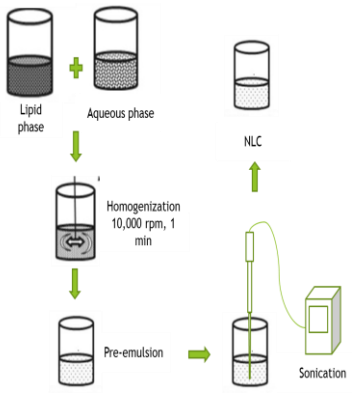
*Corresponding author
rosnani@ibd.utm.my

Graphical abstract



Abstract

The D-optimal mixture design was employed to evaluate the effect of different composition variables on particle size, polydispersity index, zeta potential and encapsulation efficiency for optimization of Zingiber zerumbet oil loaded nanostructured lipid carrier (NLC). The glyceryl monostearate were used as solid lipid, virgin coconut oil as liquid lipid, and Tween 80 as well as soy lecithin were used as surfactant to achieve stable NLC formulation. The hot homogenization and ultrasonication techniques were employed in preparation of NLC. The statistical evaluations by ANOVA revealed that optimum NLC formulation generated as 3.7% Zingiber zerumbet oil, 5% liquid lipid and 1.3% solid lipid. The optimal NLC formulation had an average diameter of 91.002 nm, PDI of 0.172, zeta-potential of -40.88 mV, and encapsulation efficiency of 94.45%. The transmission electron microscopy (TEM) observations exhibited spherical morphology of Zingiber zerumbet oil loaded NLC. Penetration through Strat-M® membrane shown an excellent diffusion coefficient of NLC-Zingiber zerumbet oil. Therefore, D-optimal mixture design has succeeded in generating optimum NLC formulation for encapsulation of Zingiber zerumbet oil. The stable formulation of NLC for encapsulating essential oil give promising future in various applications such as drug delivery, food, textile and cosmetics.

Keywords: D-optimal mixture design, *Zingiber zerumbet* oil, optimization, nanostructured lipid carrier, drug delivery

Abstrak

Rekabentuk campuran D-optimal telah digunakan untuk menilai kesan pembolehubah komposisi berlainan kepada saiz zarah, indeks kepoliserakan (PDI), kecekapan keupayaan zeta dan peratusan pengkapsulan untuk pengoptimuman pembawa lipid nanostruktur (NLC) mengkapsulkan minyak Zingiber zerumbet. Gliseril monostearate telah digunakan sebagai lipid pepejal, minyak kelapa dara sebagai lipid cecair, dan Tween 80 serta soya lesitin sebagai surfaktan untuk mendapatkan NLC yang stabil. Teknik penghomogen panas dan ultrasonik telah digunakan dalam penyediaan NLC. Penilaian statistik oleh ANOVA mendapati bahawa NLC yang optimum terdiri daripada komposisi 3.7 % minyak Zingiber zerumbet, 1.3 % lipid pepejal dan 5 % lipid cecair. NLC optimum mempunyai saiz purata berdiameter 91.002 nm, PDI 0.172, kecekapan keupayaan zeta -40.88 mV, dan peratusan pengkapsulan sebanyak 94.45 %. Pemerhatian menerusi mikroskop elektron transmisi (TEM) menunjukkan NLC mempunyai morfologi bulat. Manakala penembusan NLC menerusi membran Strat-M® membuktikan pekali resapan yang baik. Kesimpulannya, rekabentuk campuran D-optimal telah berjaya merangka rumusan NLC yang optimum untuk mengkapsulkan minyak Zingiber zerumbet. Rumusan NLC yang stabil dalam mengkapsul minyak pati menjanjikan masa depan yang baik

untuk pelbagai bidang seperti bidang penghantaran dadah, makanan, tekstil dan kosmetik.

Kata kunci: Rekabentuk campuran D-optimal, minyak Zingiber zerumbet, pengoptimuman, pembawa lipid nanostruktur, penghantaran dadah

1.0 INTRODUCTION

Nanostructured lipid carrier (NLC) is composed of solid lipid, liquid lipid and surfactant mixture which used to encapsulate bioactive ingredients or compounds [1]. As the name implies, NLC are having nanometer size and commonly range between 10-1000 nm. NLC is a successor to solid lipid nanoparticles (SLN) which improvised in term of bioactives loading capacity, solubility, and its bioavailability [2]. The lipid matrix of NLC has given space for bioactives loading as it is important to ensure effective bioactives incorporation into efficient carrier. Hence, carrier supposed to have high stability in term of storage and systemic behaviour. The degradation or agglomeration of nanocarrier could impair the purpose of nanocarrier and reduce efficacy of bioactives [3].

There were numerous research reporting the encapsulation of herbal-based bioactives for various application for example; compound [4, 5, 6], extracts [7, 8], and essential oil [9, 10, 11, 12]. Bioactives compounds in formulation commonly has high volatility, low availability and has physicochemical instabilities, for example, sensitive to exposure of high temperature and light. Therefore, the nano-encapsulation has overcome these limitations to improve bioavailability, stability and solubility which could be applied in various pharmaceutical and nutraceutical applications.

Zingiber zerumbet is traditionally used as spice and as medicine for so long in so many places throughout Southeast Asia, the Pacific, and the Oceania. It is well known as pinecone ginger, 'lempoyang' (Malaysia) and shampoo ginger, commonly found in south East Asia [13]. *Zingiber zerumbet* has anti-oxidant [14], anti-cancer [15, 16], anti-inflammatory [17, 18, 19], antimicrobial [20], antiobesity [21, 22, 23], and antipyretic [24] properties as reported in previous research. It is also reported that *Zingiber zerumbet* essential oil contains many active compounds which contributed to the properties reported before, for example, oleoresin, and phenolic compound [25]. However, *Zingiber zerumbet* essential oil were also very sensitive, volatile, and susceptible to physical and chemical degradation. Therefore, it is important to develop appropriate carrier to encapsulate *Zingiber zerumbet* oil, thus the physical and chemical properties could be preserved. Furthermore, *Zingiber zerumbet* oil has lower solubility and slow absorption [26]. There were few types of carrier to encapsulate oil which included nanoemulsion, lipid based formulations, liposome and complexation to enhanced bioavailability of this low soluble bioactives in topical application, respectively [27].

The optimization of NLC formulation has made easy using Design Expert 6.0.6 software. The software has mixture design option which generate combination of excipients in numbers of experimental run. In this experiment, the ratios of lipids and *Zingiber zerumbet* oil is varied according to range provided to the mixture design. The mixture design requires total proportion of each factor equals to one. The response of each experimental run were recorded and thus analyzed to get optimum combination of excipients [28]. The advantage of using this software is the ability to choose the range of response to get different optimized formulation. In this experiment, the responses were characterization of NLC particle size, polydispersity index, zeta potential and encapsulation efficiency. There was abundant of data available for encapsulation of actives for NLC using optimization method [29, 30, 31, 32]. In this study, we generated a model of mixture design using 11 different formulations and compared the effect of each composition on the nanolipid properties such as average particle size, encapsulation efficiencies, zeta potential, and polydispersity index in order to find the optimal NLC formulation encapsulated *Zingiber zerumbet* oil.

2.0 METHODOLOGY

Materials

Zingiber zerumber essential oil (ZZ) and virgin coconut oil (VCO) were obtained from Insitute of Bioproduct Development (Universiti Teknologi Malaysia, Malaysia). Glyceryl monostearate (GMS), soy lecithin, Tween 80, gallic acid, Sephadex G-50 were purchased from Sigma-Aldrich (Selangor, Malaysia). 2-propanol, Folin-Ciocalteu reagent, and ethanol were purchased from Merck chemicals (Selangor, Malaysia) and all were analytical reagent grade. The water in the formulation were distilled water.

D-optimal Mixture Design Experiments

In order to determine an optimum mixture of excipients that provides a desired response, the D-

optimal mixture design from Design Expert 6.0.6 was utilized in which it produces a minimum number of experimental runs. In the NLC-ZZ (NLC-*Zingiber zerumbet*) optimization formulation, three variables used were *Zingiber zerumbet* oil (X_1), glyceryl monostearate(X_2), and virgin coconut oil (X_3) which varied from range 0.10 to 0.50 in ratio. The responses were listed as NLC-ZZ particle size, polydispersity index, zeta potential and NLC-ZZ encapsulation efficiency, respectively.

NLC-ZZ Preparation

The preparation of NLC-ZZ was done following the formulation matrix tabulated in Table 1 [33]. Firstly, lipid phase were form by mixing glyceryl monostearate (solid lipid) with virgin coconut oil (liquid lipid). At 50°C, the lipid phase mixture were melted to form a uniform and clear mixture of lipid. As lipid mixture were completely melted, Zingiber zerumbet oil were added to the mixture and the heating temperature was maintained above melting temperature of solid lipid. On the other hand, distilled water, Tween 80 and soy lecithin were thoroughly mixed according to pre-determined ratio to form an aqueous phase. The aqueous mixture was then heated to 50°C, and subsequently added to lipid mixture to form a pre-emulsion mixture. IKA Ultra Turrax® homogenizer was used to homogenized the pre-emulsion mixture at 11 000 rpm for one minute. The pre-emulsion mixture was ultrasonicated using probe sonicator at 50 amplitudes for 20 minutes. The NLC dispersion produced were then cooled in ice water bath to room temperature and were stored at 4°C.

Particle Size, Polydispersity Index Analysis, and Zeta Potential Analysis

The particle size, polydispersity index (PDI) and zeta potential analysis of NLC-ZZ were performed using dynamic light scattering (DLS) method, also known as photon correlation spectroscopy (PCS) method using a Malvern Zetasizer nano ZSP (Malvern instrument, UK). The NLC-ZZ sample was put in a standard capillary electrophoresis cell equipped with gold electrodes. The NLC-ZZ suspension was diluted and vortexed to avoid multiple scattering effects and directly placed in the module. Each measurement was performed in triplicate at 25°C. Refractive indices of nanoparticles and water were set at 1.54 and 1.33, respectively.

Encapsulation Efficiency Analysis

The encapsulation efficiency percentage was determined using Folin-Ciocalteu colorimetric method. The NLC-ZZ suspension was separated by Sephadex gel-50 using mini spin column. The sephadex gel-50 was employed to separate encapsulated *Zingiber zerumbet* oil to unencapsulated *Zingiber zerumbet* oil based on size. The collected encapsulated ZZ sample and NLCs suspension were each diluted with a solvent mixture (ethanol and 2-propanol) with a ratio of 1:3 and sonicated in a sonicator bath for 20 min to break the NLC wall. 500µL distilled water was added to 100 µL sample (separated encapsulated ZZ and NLC-ZZ suspension) and 2 mL 10% Folin-Ciocalteu reagent. The mixture was left to stand for six minutes. Afterwards, 2 mL of 7% Na_2CO_3 was added to the mixture. The solution was incubated for 90 minutes in the dark before the measurement was taken. The measurement was done at 760 nm wavelength (using gallic acid as reference) using a *uv-vis* spectrophotometer. A standard curve for gallic acid was prepared by dissolving 25 mg gallic acid in 25 mL distilled water. Concentrations of 0 to 450 µg/mL gallic acid were used to construct a calibration curve. Therefore, the percentage of encapsulation efficiency was calculated using the following equation:

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

where:
n_1 = total concentration of phenolic content in nanostructured lipid carrier encapsulated *Zingiber zerumbet* oil
n_2 = total concentration of phenolic content in nanostructured lipid carrier encapsulated *Zingiber zerumbet* suspension

Transmission Electron Microscopy

Transmission electron microscopy (TEM) was used to observe NLC-ZZ dispersion by using negative staining method. NLC dispersion was spread on a 200-mesh copper grid coated with carbon membranes for about 3 min. Subsequently, any excess droplets were removed using filter paper. The grid was then placed above a drop of phototungstic acid solution (2%, w/w) for about 2 min and excess droplets were removed using filter paper. The grid was observed using Hitachi H-7110 electron microscope (Japan).

Strat-M® Membrane Diffusion Analysis

The diffusion experiment was conducted in static transdermal diffusion system (PermeGear, Germany), composed of three horizontal Franz-style diffusion cells, a thermally controlled circulation water bath and magnetic stir console. Strat-M® (Sigma Aldrich) were used for the penetration study. The Strat-M® was mounted with the shiny part facing the donor cell. The receptor compartments were filled with 2.5 mL fresh phosphate buffer solution (PBS) (pH 7.4) and donor compartments were filled with 1.25 mL of the same buffer. The diffusion cell was calibrated at 37°C using recirculating water bath and the fluid in the donor and receptor compartments were stirred at 300 rpm for 30 minutes before the test sample was added to the donor compartments. The samples

(1.25 mL) were then gently pipetted in the donor compartments. At pre-determined time intervals, aliquots were withdrawn (1 mL) from the receptor compartment and replaced by the same amount of fresh buffer to maintain a constant volume. The collected withdrawn sample were extracted with ethanol (1 mL) for 1 hour in an ultrasonic bath. The sample were filtered and evaluated using uv-vis spectrophotometer. Fick's law equation is used to calculate the amount of NLC-*Zingiber zerumbet* penetration through the Strat-M®.

3.0 RESULTS AND DISCUSSION

Designing the Models

In the present work, nanostructured lipid carrier encapsulated *Zingiber zerumbet* were successfully prepared using hot homogenization and ultrasonication technique. To evaluate the optimum excipients in preparation of NLC-*Zingiber zerumbet*, a total of 11 experiments were performed, producing the same NLC formulation for each independent experiment (Table 1). The selected parameters for preparation of all formulations were: (i) 60 s of pre-homogenization with Ultra Turrax, (ii) 20 min ultrasonication, and (iii) 50 Hz ultrasonication power. To date, the use of experimental design is a common method for simultaneously analysing the influence of different variables on the properties of drug delivery system being studied [34]. Using a D-optimal mixture design, the effect of various ratio of solid lipid (X_1), liquid lipid (X_2) and *Zingiber zerumbet* oil (X_3) to mean size, polydispersity index, zeta potential and encapsulation efficiency of NLC-ZZ has been investigated (Table 1). In mixture design optimization, the mixture proportion must add up to one and the setting of various factors must satisfy $x_i \geq 0$, for all i.

The statistical parameters computed by design-expert software indicated that quadratic polynomial is best fitted for the experimental data for all response. ANOVA (Analysis of Variance) statistical test were performed for each parameter to identify the significant effects and interactions. It would be time-consuming in order to perform all the experiments to figure out the relationships between the parameters. Therefore, the understanding of statistical design of experiments techniques were capable to comprehend the relationship between parameters and responses.

Table 1 NLC encapsulates Zingiber zerumbet oil: preparation matrix

NLC formulation	NLC-ZZ preparation variables		
	Zingiber zerumbet oil, X_1	Solid lipid, X_2	Liquid lipid, X_3
1	0.10	0.40	0.50
2	0.33	0.33	0.33
3	0.25	0.50	0.25
4	0.42	0.37	0.22
5	0.40	0.10	0.50
6	0.10	0.50	0.40
7	0.50	0.25	0.25
8	0.40	0.50	0.10
9	0.40	0.10	0.50
10	0.25	0.25	0.50
11	0.22	0.42	0.37

Particle size of NLC-ZZ

In the development of a drug delivery system for topical administration, the particle size and distribution are important factor to affect the penetration pathway and mechanism. Generally, smaller particle exhibits higher bioactives uptake in comparison to those larger size particle, depicting the ability to penetrate across complex skin structures. By using dynamic light scattering method, the particle size of nanoparticles was measured. In this context, the particle size is presented as z-average diameter and also indicated as hydrodynamic diameter of particles. All formulation in Table 1 has produced mean particle size of less than 200 nm with low PDI. Nanometer size range of NLC can lead to occlusive effect and able to increase skin hydration due to close contact to stratum corneum. The skin hydration will subsequently increase skin penetration to deeper skin layer [1]. The analysis was taken within 48 h after preparation. Hence, the possibility of particle aggregation occurs may cause slight increase in particle size of NLC-ZZ. ANOVA has suggested linear mixture model for analysis particle size of NLC with Prob>F 0.0005 and consider significant with not significant lack of fit. The R^2 obtained for this model is 0.8504 and adjusted R^2 of 0.8130. From the analysis, concentration of solid lipid gave the most significant influence to NLC's particle size, follows by *Zingiber zerumbet* oil concentration and least influence were given by liquid lipid. Figure 1 shows contour plot of NLC particle size as a function of the three mixture components. The concentration of *Zingiber zerumbet* oil however did not influence the particle size as much as GMS and virgin coconut oil. It is probably due to aggregation of solid lipid while solidification of NLC occurred [1]. The selection of the lipid was based on lipid screening solubility of *Zingiber zerumbet* oil (data not shown). Through the trace plot (data not shown), the liquid lipid has inverse effect on nanoparticle size as it increases, the particle size decrease. The liquid lipid could be located near or at

the surface of the lipid nanoparticles, therefore influencing the physicochemical characteristic of NLC [35].

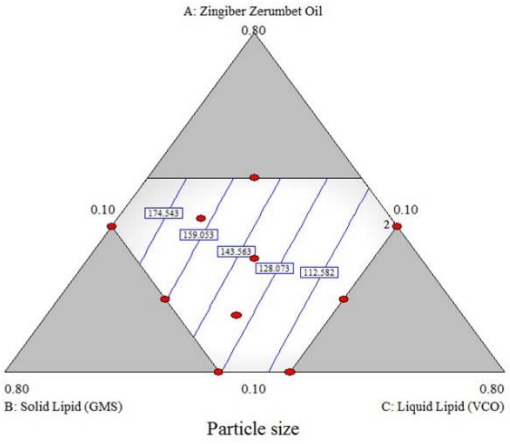


Figure 1 Contour plots illustrating the effect of (a) Zingiber zerumbet oil concentration, (b) liquid lipid concentration and (c) solid lipid concentration on particle size of NLC-Zingiber zerumbet

In this study, ultrasonication method is employed to prepare nanostructured lipid carrier and hot homogenization technique were used prior to ultrasonication [3, 36]. The energy produced by ultrasonic probe has permits dispersion of two immiscible phases such as lipid and water. The probe also collapsing cavitational bubble of inner phase and leads to NLC size reduction. It is practical to use sonicator probe as it is self-cleaning and has almost negligible sample losses.

Polydispersity Index of NLC-ZZ

Polydispersity index (PDI) less than 0.20 indicated narrow size distribution of nanoparticles. PDI also serve as stability indicator in which large PDI may lead to nanoparticle aggregation. The formulation in Table 1 has polydispersity index which range of 0.159 ± 0.02 to 0.360 ± 0.06 in which indicate good homogeneity of nanoparticles. ANOVA analysis suggested model of polydispersity index analysis to be significant with Prof>F less than 0.0500 in which linear mixture components are significant model terms. However, R^2 was only 0.6782 with adjusted R^2 was 0.5977. This is probably due to small range of results and it is assumed not much variation or pattern observed. Additionally, solid lipid has given more effect compared to *Zingiber zerumbet* oil and liquid lipid in term of coefficient values for each components. These give insight on how solid lipid influence the stability and homogeneity of nanostructured lipid carrier. The interaction between independent variables can be observed in Figure 2.

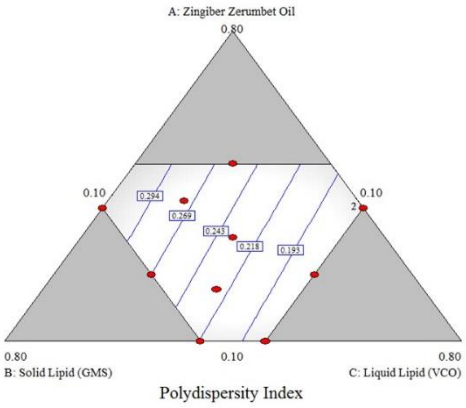


Figure 2 Contour plots illustrating the effect of (a) Zingiber zerumbet oil concentration, (b) liquid lipid concentration and (c) solid lipid concentration on polydispersity index of NLC-Zingiber zerumbet

The response observed for polydispersity index were reported similar with particle size whereas solid lipid ratio gives higher effect compared to *Zingiber zerumbet* oil and liquid lipid ratio. This could be due to particle aggregation cause by excess solid lipid which influence homogeneity and increases PDI value [29]. Therefore, it could be suggested that there's been relation between polydispersity index and NLC particle size since smaller size of NLC will subsequently have low PDI.

Zeta Potential of NLC-ZZ

The prediction of physical stability of nanoparticles can also be determined using zeta potential measurement. The electrostatic attraction between the oppositely charged particles has assist the mechanism of cellular uptakes of nanoparticles. Cell surface commonly has negative charge where cationic nanoparticles can be attracted which leads to increase in cellular uptake of either nanoparticles or released drugs. Apart from that, NLC with high surface charge (> ±30mV) is desirable as nanoparticles appears stable against flocculation and aggregation [37]. The zeta potential for studied formulation as in Table 1 were in range of -38.9±5.23 to -43.2±1.62 mV which indicated good stability of nanoparticles. The result was in good agreement with [38]; Norhayati *et al*., [2013] which found good zeta potential range for encapsulation of virgin coconut oil. Small range of variations were observed for zeta potential causes no pattern of result observed due to small range of ratio studied. This also suggested that the optimization could be done without including zeta potential factor, however it is still undeniably important for NLC surface characterization. The stabilized NLC suspension were also due to repulsion of charged particle, hence prevent tendency to aggregate. The interactions between the components influencing zeta potential were best shown by contour plot as depicted in Figure 3. As

Zingiber zerumbet oil and solid lipid increases, the zeta potential slightly increases.

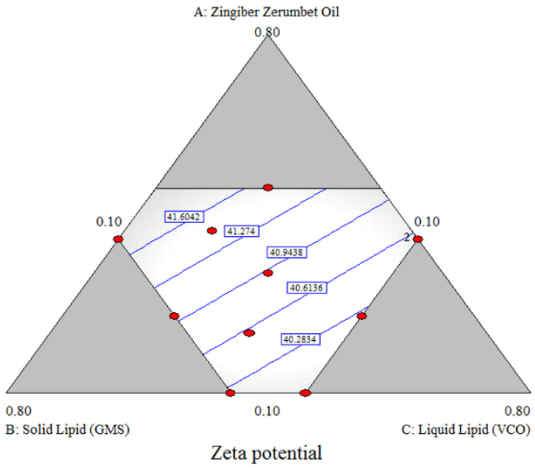


Figure 3 Contour plots illustrating the effect of (a) Zingiber zerumbet oil concentration, (b) liquid lipid concentration and (c) solid lipid concentration on zeta potential of NLC-Zingiber zerumbet

Encapsulation Efficiency of NLC-ZZ

Encapsulation efficiency and drug loading capacity is important in characterization of NLC as it implies how much active ingredients can occupy inside the NLC and how long the NLC can hold the active ingredient in given time. The encapsulation efficiency is expressed in percentage (Eq. 1) which calculates the free-actives concentration (non-encapsulated inside the lipid core). The quantification of actives ingredient was done using UV-VIS spectrophotometry. In this study, all formulation showed encapsulation efficiency values greater than 69.16 ± 7.56% and up to 97.03 ± 3.62%. Good encapsulation efficiency values denote that the lipid and surfactant compositions employed were adequate for encapsulation of *Zingiber zerumbet* oil in nanostructured lipid carrier. Referring to ANOVA analysis, the model for encapsulation efficiency were significant with Prob>F was 0.0181 with insignificant lack of fit. Based on the trace plot analysis and coefficient (data not shown), liquid lipid has greatest influence to encapsulation characteristics of NLC, followed by solid lipid and virgin coconut oil give has least influence. The interaction between liquid lipid and solid lipid has also posed high influence to encapsulation efficiency of NLC.

According to Figure 4, high percentage of *Zingiber zerumbet* oil shown less efficiency in term of encapsulation, might be due to excessive active ingredient could not be accommodated into lipid matrix. The increase in lipid content however creates more disturbance in lipid particle matrix, The disturbance in lipid particle matrix which due to increase in lipid content has caused imperfection of lipid matrix, subsequently improvised drug loading capacity and encapsulation efficiency. It is important to carefully select the surfactant used in the formulation as it affects bioactives solubility in melted lipid. In this study, Tween 80 and soy lecithin has been used as surfactant, in which selected based on hydrophilic-lipophilic-balance value. The addition of surfactant has helped in formation of stable lipid formulation and influencing the crystallization of lipid and leaving spaces in lipid lattice [39].

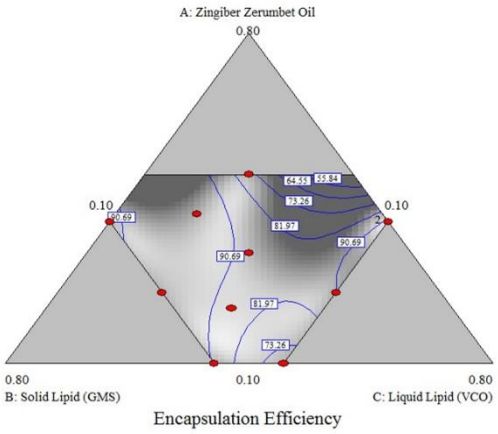


Figure 4 Contour plots illustrating the effect of (a) Zingiber zerumbet oil concentration, (b) liquid lipid concentration and (c) solid lipid concentration on encapsulation efficiency of NLC-Zingiber zerumbet

Optimization of NLC Formulation with Mixture Design

The optimization parameter suggested by D-optimal mixture design were further validated with experimental values. Numerical optimization has suggested optimized experimental condition with desirability of 0.974. Model desirability approaching unity denoted applicability of model. The suggested responses were having insignificant difference with experimental response as recorded in Table 2. The statistical evaluations by ANOVA revealed that optimum NLC formulation generated as 3.7% *Zingiber zerumbet* oil, 1.3% solid lipid and 5% liquid lipid. The optimal NLC formulation had an average diameter of 91.002 nm, PDI of 0.172, zeta-potential of -40.88 mV, and encapsulation efficiency of 94.45%. Therefore, the optimization can be used for further analysis and application.

Table 2 Comparison between mixture design response and experimental result achieved for optimized parameter

Factors	Size, nm	PDI	zeta-potential, mV	Encapsulation efficiency, %
Mixture design response	97.325	0.168	-40.48	97.03
Experimental result	91.002± 4.24	0.172± 0.01	-40.88± 1.50	94.45± 1.76
Error (%)	6.49	2.5	0.98	2.64

Morphological Analysis

The NLC- *Zingiber zerumbet* exhibited spherical shape and size distribution similar with measurement by DLS method. This observation confirms that the size of NLC-*Zingiber zerumbet* were in range of less than 200 nm.

Figure 5 shows distribution of NLC- *Zingiber zerumbet* oil depicted good distribution of size measured by DLS method. While Figure 6 shows TEM imaging of NLC-*Zingiber zerumbet*. There was no expulsion observed in TEM imaging of NLC which denotes stability of NLC encapsulating *Zingiber zerumbet* oil. Similar observation was reported by Gomes *et al*., 2014 and Manea *et al*., 2013 [40-41].

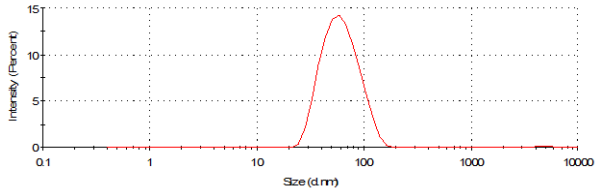


Figure 5 Size distribution of NLC encapsulates Zingiber zerumbet oil by intensity

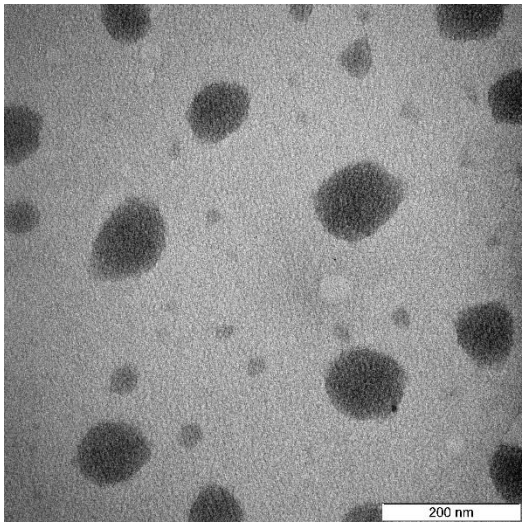


Figure 6 TEM image of NLC encapsulates Zingiber zerumbet oil

Strat-M® Diffusion Study

The Strat-M® diffusion study has been conducted using Franz diffusion cell as an alternative for human or animal skin. Strat-M® membrane significantly mimic human skin layer and useful as screening of chemical penetration properties through skin layer [42]. The receptor chamber also consist buffer solution which mimics condition of skin in terms of pH and temperature. The optimized NLC-ZZ were compared with unencapsulated *Zingiber zerumbet* oil in term of permeation through Strat-M® membrane and the result were shown in cumulative bioactives permeated over time in Figure 7. Fick's law of diffusion has been proposed to describe the diffusion parameters, which relates the amount of solute diffuses across the skin membrane area, its thickness and diffusion coefficient over a period of time, to a concentration gradient within the skin membrane [43]. The permeation parameters also shown in Table 3 including diffusion coefficient, lag time and partition coefficient. These diffusion parameters are important for tailoring the NLC for specific application. It assists us in evaluating the usefulness and safety of topically applied chemical compounds. The increase in flux corresponds to an increase in the product of the diffusion coefficient (D) by the partition coefficient and the concentration in the vehicle (C_o). Small size of carrier, in this case nano-size, causes good contact of nanoparticles on the membrane surface, therefore significantly increase permeation of actives. Upon application of NLC or pure *Zingiber zerumbet* oil, they immediately distributed or partitioned into the Strat-M® membrane surface. Thus the diffusion and partition coefficients is determined to identify the penetration profile of carrier NLC and pure oil. From Figure 7, the lag phase for NLC was observed shorter than non-encapsulated oil indicated shorter time needed for diffusion through the membrane. Whereas D measures the penetration of permeant (encapsulated and non-encapsulated *Zingiber zerumbet* oil) through the specific area of the membrane. NLC also acts as bioactive reservoir which only release the bioactives once reach the targeted site.

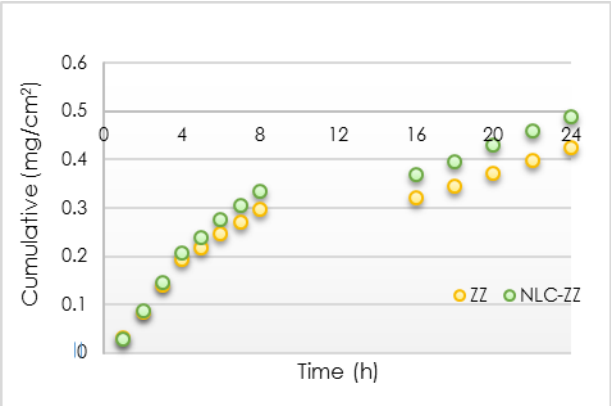


Figure 7 Cumulative amount (mg/cm²) of actives permeated. Comparison between NLC-Zingiber zerumbet and Zingiber zerumbet oil without carrier

Table 3 Strat-M® diffusion parameters for NLC-*Zingiber zerumbet* and *Zingiber zerumbet* oil

Sample	T_L (hr)	P (ml/hr)	D (cm^2h^{-1})
NLC-*Zingiber zerumbet*	0.11	1.5954	7.1×10^{-4}
Zingiber Zerumbet oil	0.21	1.4461	5.4×10^{-5}

4.0 CONCLUSION

The NLC-*Zingiber zerumbet* oil were successfully optimised by using D-optimal mixture design from Design Expert software. The D-optimal mixture design has successfully assisted the optimization process of NLC by varying the factors in the mixture. The optimised NLC-*Zingiber zerumbet* had a size of 91.002 nm, PDI of 0.172, zeta potential of -40.88 mV, and encapsulation efficiency exceeded 90%. Based on this characterization, NLC has potential to be applied as a carrier in topical and transdermal drug delivery. The potential of NLC-*Zingiber zerumbet* may need to be further discovered in term of efficacy for in vitro and in vivo study, respectively.

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