Design of Experiment of Redispersible Spray Dried Powder of Fatty Acid-Nanostructured Lipid Carriers using a 2^3 Full Factorial Design

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Abstract. Fatty acid such as lauric and oleic acid have been used extensively either in cosmeceutical or pharmaceutical products as an emollient or moisturiser. In this study, fatty acids which combine lauric and oleic acids have been formulated into nanostructured lipid carriers (FA-NLCs) system in order to improve its physical stability hence, enhancing the topical effectiveness. In order to prolong the stability of the FA-NLCs, spray drying technique has been applied to produce FA-NLCs in a powder form. The current work describes the design of experiment of spray dried lipid-based nanosystems with the ability to acquire nanosystems after redispersion. FA-NLCs were prepared using melt-dispersion ultrasonication method. Three parameters were optimised with respect to spray drying; inlet temperature, flow rate and feed concentration using 2^3 Full Factorial Design. The responses evaluated were the powder yield, moisture content and size of the redispersed nanoaggregates. Results showed that in achieving small redispersed nanoaggregates size, high powder yield and low moisture content was come from formulation Standard Order No 6 (Run Order No 8). In conclusion, a spray dried FA-NLCs was successfully prepared with the smallest redispersed nanoaggregate size, highest powder yield and low moisture content, potentially suitable to be used as cosmetic ingredients.

INTRODUCTION

Fatty acids (FAs) are essential for maintaining the structure and function of the outer layer or epidermis (stratum corneum, SC) that contains glycolipids, intercellular lipids (cement), and a skin lipid coat called the natural moisturising factor (NMF) [1]. One of the famous major fatty acids that can be found in coconut oil (*Cocos nucifera*) is lauric acid. Lauric acid (C12) is an edible oil which comprise approximately 50% of the fatty acid composition, indicating its function as a primary fatty acid in the coconut oil [2]. Lauric acid, a minor component in the sebum, is the most potent antimicrobial saturated fatty acid [3]. A study by Nakatsuji et al. [4] showed that lauric acid becomes an alternative option for antibacterial therapy in acne treatment and showed a stronger antimicrobial activity against skin bacteria,

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including *P. acnes, S. aureus, and S. epidermidis* both *in vitro* and *in vivo*. A study conducted by Taylor et al. [5] produced a solid lipid nanoparticle (SLN) formulation which consisted of lauric acid, stearic acid, and oleic acid in the inner core and surrounded by phosphatidylcholine lipid and sodium taurocholate. The SLN was shown to be able to kill bacteria *Pseudomonas aeruginosa*.

Apart of the most commonly found lauric acid, oleic acid or also known as *cis-9-octadecenoic*, appears to be present to some extent in all oils and fats. Olive oil (*Olea europaea*) contains 55 – 83% of oleic acid which is the major monounsaturated fatty acid (MUFA) from the glyceride fraction [6]. Previous study found that unsaturated fatty acid such as linoleic and oleic acid can also act as antibacterial agent [7]. This shows that combination of both lauric and oleic acid can potentially be useful as antibacterial agent in cosmetic or pharmaceutical products.

Nanostructured lipid carriers (NLCs) and solid lipid nanoparticles (SLNs) have been used in several studies as drug delivery due to their robust constituent characteristics and drug encapsulation enhancement [8]. NLCs were developed by Muller et al. [9] as an enhancement in functionality that overcomes the limitations in SLNs. NLCs release drugs faster at low drug loading and are more stable at high drug loading compared to SLNs [10]. NLCs offer a closer contact with the superficial junctions of the corneocyte due to its small particle size [11]. In addition, SLNs and NLCs tend to be promising lipid carriers due to the fact that the skin barrier has a high lipophilic content [12,13]. This may therefore enable the lipid carrier to be exchanged and penetrates between the outermost layers of the stratum corneum and thus allowing the drug to be transmitted to the targeting sites.

Spray drying technique had been used in both laboratory and industrial applications including manufacturing, research and development [14] due to its ability in generating dry pharmaceutical powder rapidly and continuously. The technique is reproducible, involving a single-step and the process can be measured. In the production of a nanostructured lipid-carrier (NLC) powder, application of spray drying method might cause aggregation due to the high temperatures and shear forces on the particles [15]. Due to that, in this study, the processing parameters of the spray dryer was optimised to obtain a redispersible spray dried powder of FA-NLCs for a long term storage stability.

One way of optimising the processing parameters is by using the design of experiment (DOE). DOE is a systematic method to determine the relationship between factors affecting a process and the output of that process [16]. To understand the variables and their interactions, many statistical experimental designs can be used. One of the designs is factorial design in which it evaluates effects of several factors by conducting a factorial experiment. Factorial design is defined as the most efficient technique to study the effects of two or more parameters [17] and has been successfully used in various fields including pharmaceutical and cosmetic ingredients production.

The aim of this study is to produce a redispersible spray dried powder of FA-NLCs using a 2^3 Full Factorial Design in order to get the smallest nanoaggregate size after redispersion, highest powder yield and lowest moisture content for cosmeceutical application.

METHODOLOGY

Materials: Lauric acid, oleic acid, Kolliphor P188 and L-alpha phosphatidycholine (granular, from soybean oil) were supplied by Sigma-Aldrich. Glyceryl monostearate (Capmul GMS-50K) were obtained from ABITEC Corporation (Jenesville, WI). Food grade maltodextrin was purchased from San Soon Seng Food Industries Sdn Bhd. Deionised water (Sortorius Arium 611) was prepared in-house.

Formulation of Fatty Acid-Nanostructured Lipid Carriers (FA-NLCs)

The FA-NLCs were comprised of oil phase (i.e. liquid and solid lipid) and aqueous phase. The formulation was prepared as described in the previous research [18]. Oil phase was made by weighing glyceryl monostearate (1250 mg), lauric acid (100 mg) and oleic acid (100 mg) into a glass beaker. Aqueous phase was made by weighing a combination of emulsifiers [i.e. soy lecithin (500 mg) and Kolliphor P188 (1250 mg)] with 100 ml water added into a glass beaker. Both solutions were heated separately at 80 – 90°C in a water bath until completely melted. Solutions were then mixed together and homogenised at 19,000 rpm using a high speed stirrer (IKA Ultra Turrax T25, Germany) for 5 minutes. The mixture in the beaker was further sonicated using a probe sonicator (Fisherbrand™ Model 705 Sonic Dismembrator. USA) with a ½ inch diameter probe at 50% amplitude for 5 minutes to obtain nanoparticles dispersion.

Measurement of Particle Size Distribution, Zeta Potential and Determination of Morphology

Hydrodynamic diameter and polydispersity index (PDI) of the prepared FA-NLCs formulation were analysed on the same day by dynamic light scattering technique using a particle size analyzer (Litesizer 500, Anton Paar instruments, GmbH, Austria). Prior to the measurement, 0.1 mL of FA-NLCs formulation were diluted with 100 ml of deionised water. Each sample was measured in triplicate. The size of redispersed nanoaggregates were evaluated by redispersing 100 mg of dried powder in 10 mL deionised water under gentle agitation prior to determination of size and PDI. The hydrodynamic diameter was measured as previously described. The spray dried FA-NLCs visualisation were performed at 15 kV using tabletop microscope scanning electron microscopy (SEM) (Hitachi TM3000, Japan). Samples were placed on carbon sticky tabs and spinned with gold-plated before imaging.

Spray Drying Process, Percentage of Powder Yield and Measurement of Moisture Content

In order to obtain a spray dried powder of FA-NLCs, maltodextrin as a carrier was added in different concentrations (total solid content: 10% - 30% w/v). The formulation was spray dried using a Labultima LU-228 spray dryer (Labultima Process Technologies Pvt, India) according to the experimental design. The acquired powders were collected from the collection chambers and stored in a humidity controlled cabinet before further characterisation [19].
The percentage of powder yield was calculated based on the following equation,

$$\text{Percentage of powder yield (\%)} = \frac{W_{obtained}}{W_{initial}} \times 100 \qquad (1)$$

Where:

$W_{obtained}$ = weight of powder attained from the drying process

$W_{initial}$ = initial weight of all solids in the sample

The moisture content of FA-NLCs was determined by Mettler Toledo Deluxe Halogen Moisture Analyzer HR83 (Mettler-Toledo, Belgium). 1 g of FA-NLCs spray dried powder was weighed on the pan and analysed at 105ºC. The result of moisture content was obtained, and the experiment was repeated 3 times.

Experimental Design

For experimental design, 2^3 Full Factorial Design was used to analysed the data. The effect of three independent processing variables which were inlet temperature (*A*), flow rate (*B*) and feed concentration (*C*) were analysed based on the selection of three factors with two levels design with center point per block as discussed previously [19] with modification (Table 1). Powder yield (%), moisture content (%) and size of redispersed nanoaggregates (nm) were indicated as the responses of the factors. A design that consist of 9 runs were generated and analysed using a statistical software known as Design Expert 6.0.8 (StatEase Inc., USA). Interactions and effects among variables were determined using factorial model. A model with a *p*-value smaller than 0.05 was considered as a statistically significant model.

TABLE 1. Selected variable parameters and levels.

Factors	Coded Units	Level (coded value)	
		Minimum	Maximum
Inlet temperature (°C)	*A*	115 (-1)	160 (1)
Flow rate (mL/min)	*B*	3 (-1)	5 (1)
Feed concentration (%)	*C*	10 (-1)	30 (1)

RESULTS AND DISCUSSIONS

Based on the result from Litesizer 500, the mean hydrodynamic diameter of freshly prepared FA-NLCs was found to be 230.1 ± 27.0 nm with a narrow PDI (0.270 ± 0.042). The formulation was spray dried by varying the process parameters. Values of all variable parameters and responses obtained from the experiment were presented in Table 2. The results was analysed using a 2^3 Full Factorial Design. Table 2 shows the responses of the FA-NLCs spray dried powder and the morphology of spray dried FA-NLCs for Standard Order No 6-Run Order No 8. Inlet temperature of 160°C, flow rate of 3 mL/min and feed concentration of 30% had produced a powder with the highest powder yield (80.7%), a low moisture content (4.98%) and the smallest size of redispersed FA-NLCs (472 nm). Although the size of redispersed FA-NLCs was increased approximately 2-times (i.e. from 230 nm to 472 nm), it was still within the target size for topical application, which should be small, as supported by previous research [11]. The increment in the particle size after the spray drying process may be caused by particle aggregation due to the high temperatures and shear forces on the particles during the drying process [15]. SEM micrograph of spray dried of FA-NLCs particles (Standard Order No 6) show nearly spherical-like shape with wrinkled surface.

TABLE 2. Analysis results for each run from 2^3 Full Factorial Design and scanning electron micrograph for Standard Order No 6.

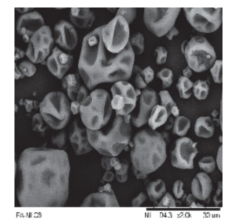
Standard Order (Run order)	Variable parameters			Responses			Scanning Electron Micrograph of Standard Order No 6 at 2000x magnification
	Inlet temperature (°C)	Flow rate (mL/min)	Feed concentration (%)	Powde r yield (%)	Moisture content (%)	Size of redispersed nanoaggregates (nm)	
1 (9)	115	3	10	57.0	6.08	5689	
2 (7)	160	3	10	77.0	4.78	2092	
3 (5)	115	5	10	59.0	6.33	4572	
4 (4)	160	5	10	65.0	5.16	4703	
5 (1)	115	3	30	80.7	6.50	626	
6 (8)	160	3	30	80.7	4.98	472	
7 (3)	115	5	30	75.7	7.52	3028	
8 (6)	160	5	30	78.3	5.36	598	
9 (2)	137.5	4	20	77.5	5.38	5134	

Table 3 shows the analysis of variance for powder yield (%) on the redispersible spray dried powder of fatty acid-nanostructured lipid carrier and model equation in coded factors. Based on the equation in Table 3, positive values of inlet temperature (*A*) and feed concentration (*C*) indicate positive impact of both parameters towards the powder yield. The positive impact means an increase in the value of *A* and *C* will cause an increase in the powder yield. This result is in agreement with the previous research [19]. There was also an interaction between the inlet temperature (*A*) and feed concentration (*C*), indicated by *AC* in the equation, in order to produce the powder yield and moisture content.

TABLE 3. Analysis of variance for powder yield (%)

Source	Sum of Squares	DF	Mean Square	F value	Prob > F	
Model (R2FI)	582.54	3	194.18	8.69	0.0317	significant
A	102.25	1	102.25	4.58	0.0992	
C	411.85	1	411.85	18.43	0.0127	
AC	68.45	1	68.45	3.06	0.1550	
Powder yield (%)			$= 71.67 + 3.58*A + 7.18*C - 2.93*A*C$			

(A: Inlet temperature (°C), C: Feed concentration (%))

Based on the equation in Table 4 shows moisture content (%, on the redispersible spray dried powder of fatty acid-nanostructured lipid carrier and model equation in coded factors. A negative value of inlet temperature (*A*) indicates a negative impact of the parameter towards the moisture content. A negative impact means an increase in the value of A will cause a decrease in the moisture content, as supported by previous research [20].

TABLE 4. Analysis of variance table for moisture content (%)

Source	Sum of Squares	DF	Mean Square	F value	Prob > F	
Model (R2FI)	5.42	3	1.81	10.38	0.0234	significant
A	4.73	1	4.73	27.18	0.0065	
C	0.51	1	0.51	2.90	0.1636	
AC	0.18	1	0.18	1.05	0.3630	
Moisture content (%)			$= 5.84 - 0.77*A + 0.25*C - 0.15*A*C$			

(A: Inlet temperature (°C), C: Feed concentration (%))

Further, feed concentration (*C*) contributed a significant effect ($p<0.05$) on the size of redispersed nanoaggregates shown in Table 5, redispersed nanoaggregates size (nm) with their respective model equations. It can be concluded that high inlet temperature (*A*) and high feed concentration (*C*) were able to produce a powder with a high powder yield and low moisture content as can be seen for Standard Order No 6. The 3D surface plots in Fig. 1 can also be used to see the influence of all parameters towards the responses (i.e. powder yield, moisture content and size of redispersed nanoaggregates).

TABLE 5. Analysis of variance table for redispersed nanoaggregates size (nm) on the redispersible spray dried powder of fatty acid-nanostructured lipid carrier and model equation in coded factors. (A: Inlet temperature (°C), C: Feed concentration (%))

Source	Sum of Squares	DF	Mean Square	F value	Prob > F	
Model (Main effect)	2.561E+007	3	8.536E+006	6.83	0.0472	significant
A	4.574E+006	1	4.574E+006	3.66	0.1284	
B	2.023E+006	1	2.023E+006	1.62	0.2723	
C	1.901E+007	1	1.901E+007	15.20	0.0176	
Size of redispersed nanoagregates (nm)			= 2722.47 - 756.15**A* + 502.90**B* - 1541.50**C*			

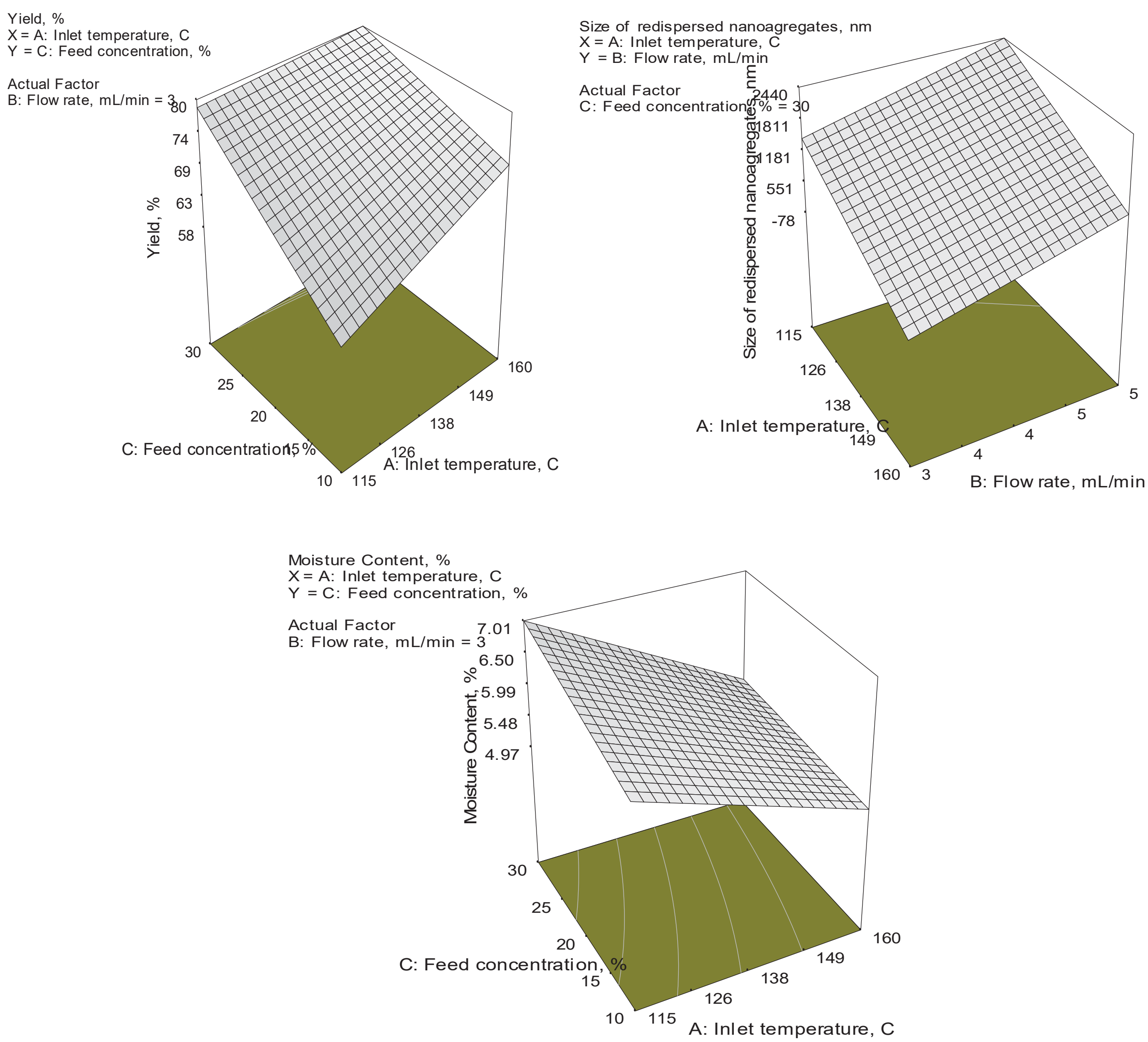


FIGURE 1. Graphical representation of powder yield, moisture content and size of redispersed nanoaggregates in 3D surface plots of the spray dried FA-NLCs.

CONCLUSIONS

In this study, redispersible spray dried powder of fatty acid-nanostructured lipid carrier (FA-NLCs) for cosmeceutical application has been successfully prepared and analysed using a 2^3 Full Factorial Design. Based on the result, significant mathematical models were obtained. It can be concluded that a spray dried FA-NLCs formulation with the highest yield, lowest moisture content and smallest redispersed nanoaggregate size could be obtained by using following spray drying parameters; inlet temperature of 160°C, flow rate of 3 mL/min and feed concentration of 30%.

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