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Effects of Preparation Temperature and Liquid-Solid Lipid Composition to Curcumin-Nanostructured Lipid Carrier Characteristics Fabricated by Microfluidic Technique

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ABSTRACT

Nanostructured Lipid Carriers (NLC) are lipid-based carrier that uses a combination of liquid and solid lipids which is believed to deliver a higher amount of active substance to the human body. This study aimed to obtain the best formulation and evaluate the stability of curcumin-loaded NLC (C-NLC) using microfluidic technique at temperature of 40ºC and 60ºC with the ratios of liquid:solid lipids were 2 : 1 ; 3.5 : 1 ; 4 : 1 ; 6 : 1% w/w. Our results showed that the increase of process temperature and liquid lipid concentration reduced particle size. There was a non-linear relationship between lipid ratio and temperature to encapsulation percentage. At ratio of soybean oil:stearic acid 6 : 1 and, at 40°C, particles size (PS) obtained was 143.87 ± 3.36 nm, polydispersity index (PDI) obtained was 0.44 ± 0.01, zeta potential (ZP) obtained was -33.3 ± 6.53 mV with encapsulation percentage of 20.62%. At the same ratio at 60°C, the PS obtained was 60.21 ± 2.55 nm, PDI obtained was 0.72 ± 0.03, ZP obtained was -26.10 ± 1.83 mV and encapsulation percentage of 31.45%. Stability test showed that C-NLC produced at 60°C was more stable since the change of particle size and pH were lower than C-NLC produced at 40°C.

Key word: Nanostructured Lipid Carriers, curcumin, microfluidic, temperature, soybean oil, stearic acid

INTRODUCTION

Curcumin is categorized as a symmetrical molecule that has IUPAC name (1E,6E)-1,7-bis(-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dion with the molecular formula $C_{21}H_{20}O_6$, relative molecular mass of 368.38 gram/mol and has three reactive functional groups, one ditone group, and two phenolic groups [1]. Curcumin has potency as an anti-oxidant, anti-inflammatory, anti-mutagenic, anti-microbial, and anti-cancer [2]. Therefore, various countries utilize curcumin in turmeric as curry ingredients in India, herbal tea in Japan, cosmetics in Thailand and antiseptic in Malaysia [3]. However, the efficacy of curcumin is low due to limited bioavailability in the human body [4]. In order to enhance its bioavailability, curcumin has been formulated into nanoemulsion, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC) systems using liquid and solid lipids. These systems have particle sizes ranging from 50 to 1000 nm, and they have the potential to deliver a higher quantity of the drug within the human body [5–7]. The characteristics of nanoemulsion, SLN, and NLC systems are summarized in Table 1.

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According to Aditya et al. [8], both quercetin-loaded NLC and nanoemulsion exhibited the highest bioaccessibility (60%) compared to SLN (35%) and free quercetin solution (7%). López et al. [9], conducted a comparison of the penetration ability between glyceryl dibehenate-loaded SLN and glyceryl dibehenate and caprylic capric triglycerides-loaded NLC, and the NLC demonstrated significantly greater penetration (4.7 ± 1.3 µg) compared to SLN (1.7 ± 0.4 µg). In the study by Mura et al. [10], the effectiveness of hydrochlorothiazide in the form of SLN and NLC was evaluated, and it was concluded that NLC entrapped a higher amount of the drug (80%) compared to SLN (65%). Similarly, Borges et al. [11], investigated the encapsulation of sclareol (SC) in NLC and SLN systems, and NLC exhibited better encapsulation properties compared to SNL. Erwati et al. [12] encapsulated p-methoxy cinnamic acid (PMCA), an anti-inflammatory drug, in NLC, SLN, and nanoemulsion systems, and NLC demonstrated superior release and penetration compared to SLN. These findings indicate that NLC enhances the properties of SNL and nanoemulsion systems.

Table 1. Comparison of Nanoemulsion, SLN, and NLC

Properties	Nanoemulsion	SLN	NLC	Ref
Particle size	1 - 100 nm	50 - 1000 nm	50 - 1000 nm	[1,2]
Physical appearance	Transparent	Milky	Milky	[3]
Formulation in System	Liquid Lipid	Solid Lipid	Liquid Lipid and Solid Lipid	[4]
Structure in system	No Matrix	Brick Wall Structure	Unstructured Matrix	[5]
Drug percentage encapsulation	Relatively low when compared to NLC systems, but comparatively high when compared to SLN	Lower encapsulation due to highly ordered crystalline in system	Higher encapsulation with combining solid lipids and liquid lipids to increase drug loading in system	[6,7]
Storage	Maintains stability throughout the storage process	During the storage process, the drug may undergo release	The drug remains stable enough in the system	[5,13]

In general, NLC, SLN and nanoemulsion preparation techniques are divided into two, namely high and low energy methods. High-energy techniques comprise pressure homogenization, ultrasonication, wet ball milling, and microfluidization, while low-energy methods include phase inversion temperature (PIT) and self-nano emulsification. High-pressure homogenization, a high-energy method, generates small particle sizes by applying pressure in the range of 500-5000 psi using a homogenizer. This approach is capable of producing nanoemulsion particles smaller than 100 nm. However, it is costly, limited in availability, and generates excessive heat, which can affect the stability of the nanoemulsion. Moreover, high-pressure homogenization is not suitable for viscous nanoemulsion formulations. Ultrasonication employs ultrasonic waves to induce cavitation forces that break down microemulsion samples into nanoemulsions using an ultrasonicator. The emission of

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waves can be adjusted to achieve the desired particle size and enhance nanoemulsion stability. This method can produce particle sizes below 200 nm. However, the stability of the resulting nanoemulsion is limited due to shear-induced coalescence. Regarding scalability, ultrasonication is not highly effective for large-volume production of nanoemulsion. The wet ball milling method involves continuously milling particles using milling beads to reduce their size compared to the original material. Increasing the milling time leads to a decrease in particle size. However, after a certain point, the particle size may increase due to friction between particles, indicating the stability of the nanoemulsion [14–16].

Previous studies have been done to produce curcumin-NLC. Hamano et al. [17], conducted a study of curcumin loaded-liposomes production using microfluidic techniques to produce a particle size of 120 nm, a polydispersity index of less than 0.2 and drug loading capacity of up to 17%. Puglia et al. [18], developed curcumin NLC, in order to reduce the process of histone acetylation in the central nervous system, using ultrasonication techniques. Similarly, Rapalli et al [19] attempted to produce curcumin NLC using sonication technique in treating psoriasis vulgaris. Using a different technique, emulsion-evaporation and low-temperature-solidification, Wang et al [20], developed a curcumin NLC as an anti-cancer therapy for hepatocellular carcinoma (HCC).

From the methods that have been described above, methods in producing NLC offer advantages as well as challenges in its application. Our study tries to propose the production of NLC using microfluidic technique which is capable of producing very small-sized monodispersed particles. It is one of the high-energy methods which employs the formation of NLC in microchannels. The emulsification step using this process occurs via collision between two immiscible streams moving in a single microchannel under high pressure. The stability of the emulsion formed with this system depends on the wetting of the canal walls by the emulsion components [21]. Factors that affect the size of the particles include the properties of the emulsified liquid, flow rate, channel geometry and channel surface properties [22]. By raising the temperature during the pre-emulsion and microfluidization procedures, it is possible to decrease the particle size in the NLC system. Previous research suggests that manipulating the temperature during emulsion formation can effectively reduce the emulsion size to the nano-scale and enhance drug penetration [23]. The increase in temperature influences the NLC system in the form of a decrease in oil viscosity, an increase in collision between droplet particles, an increase of phase changes rate between emulsion components [24].

Our experiment produces curcumin loaded NLC (C-NLC) using soybean oil as the liquid lipid and stearic acid as the solid lipid. Soybean oil has a high abundance of omega acids which plays an active role in increasing anti-inflammatory effect while stearic acid has a hydrophile-lipophile balance (HLB) value of 17 which has the potential as an emulsifier material in oil in water (O/W) based NLC. Variation of liquid-solid lipid ratio and temperature are applied to determine the optimized condition. Variables observed were encapsulation percentage, morphology, particle size, polydispersity index, zeta potential, and the physical stability of the NLC system.

EXPERIMENT

Chemicals and instrumentation

Materials used in this research were curcumin powder (Health Ingredients, Pharmaceutical Grade, China), stearic acid (Merck, CAS No. 57-11-4, USA), food grade soybean oil (Jinyuone Co., Ltd, BPOM RI ML 209509061028, Hwaseong, South Korea),

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Tween-80 (Pharmaceutical Grade, CAS No. 9005-65-6), ethanol 96% (Technical Grade, Malang City, Indonesia), and methanol (Smartlab, CAS No. 67-56-1, Indonesia).

The tools/instruments used included hot plate magnetic stirrer Heidolph (Schwabach, Germany), analytical balance, centrifuge, ultrasonic cleaner (Skymen China), UV-Vis Spectrophotometer Shimadzu Type 1601 (Waltham, USA), Zetasizer Nano (ZS Malvern, UK), and a set of microfluidic devices described in Figure 1. Microfluidic plate made of acrylic plate with modified-Y design channel was printed using Computer Numerical Control (CNC) technique. The Y-design channel was modified into five branches with the same path length separated by 30° angle. These five branches became the input of the pre-emulsion. The channel had a diameter of 2 mm and a length of 150 mm. At the end of the channel, there was an exit point or system output. Figure 1. shows the illustration of the microfluidic device setting. We applied a simple microfluidic plate made of acrylic (4 mm thick) with a modified Y-design channel connected to 5 pumps. The channel width was 2 mm and the channel length was 15 cm.

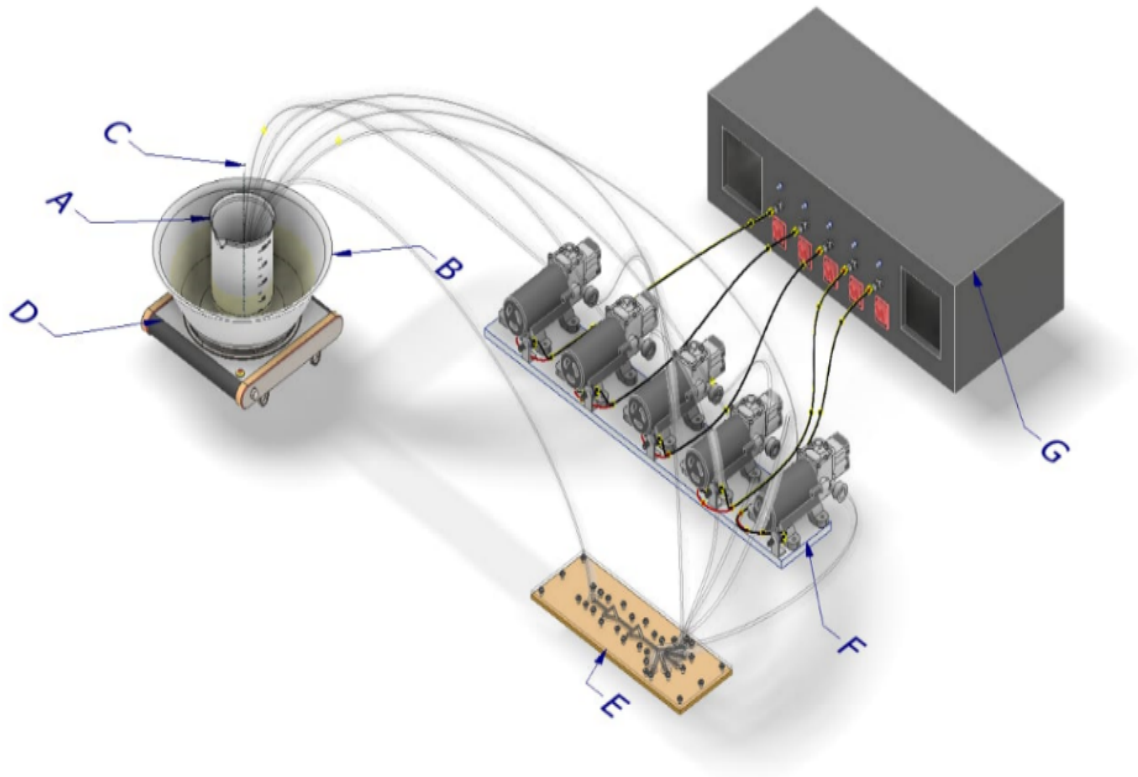

Figure 1. Microfluidic device setting consisting of A. beaker glass as sample holder, B. oil bath, C. thermometer, D. hot plate, E. microfluidic plate, F. pump DC 12 Volt Type 2201, G: power supply. Sample was fed through polytetrafluoroethylene (PTFE) tubing with a length of 100 cm in the input of a modified Y-design channel to the output.

Curcumin loaded - NLC Synthesis

C-NLC was made based on the formulation summarized in Table 2. Each formula is processed at temperature variations of 40°C and 60°C. These temperatures are chosen in accordance with the melting point of stearic acid which ranges at 69-71°C. [25,26]. The temperature applied does not exceed the melting point to maintain the encapsulation capacity of stearic acid as the solid lipid. Meanwhile, 40°C is chosen as an effort to examine process

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efficiency using lower preparation temperature. Two phases, aqueous and oil phase, were prepared with continuous stirring. The two phases were then homogenized for 120 minutes while being heated as the temperature variation to form a pre-emulsion. Pre-emulsion was then immersed in the oil bath at temperature variation and being circulated in the microfluidic channel using pressure of 80 psi for 1 hour. The NLC formed was then stored at room temperature (27 ± 2°C).

Table 2. Composition of C-NLC Formula

Liquid Lipid	**Soybean Oil**	
	mass (g)	Concentration (M)
2 : 1	0.813	0.117
3.5 : 1	0.871	0.136
4 : 1	0.975	0.140
6 : 1	1.045	0.150
Solid Lipid	**Stearic Acid**	
	mass (g)	Concentration (M)
2 : 1	0.406	0.057
3.5 : 1	0.348	0.049
4 : 1	0.244	0.034
6 : 1	0.174	0.024
Bioactive Compound	**Curcumin (g)**	
	mass (g)	Concentration (M)
2 : 1	0.125	0.014
3.5 : 1	0.125	0.014
4 : 1	0.125	0.014
6 : 1	0.125	0.014
Emulsifier	**Tween-80 (g)**	
	mass (g)	Concentration (M)
2 : 1	3.656	0.112
3.5 : 1	3.656	0.112
4 : 1	3.656	0.112
6 : 1	3.656	0.112

Percent Encapsulation Measurement (% E)

Percent encapsulation (%E) can be calculated by determining the amount of free curcumin outside the NLC system. As much as. 1 mL of C-NLC was taken and then diluted 1000x. A total of 10 mL of the diluted solution was centrifuged for 45 minutes at a speed of 3500 rpm. Supernatant, 5 mL, was taken and measured using UV-Vis spectrophotometer to determine free curcumin (C_f). The remaining solution was dissolved in 5 mL of methanol and sonicated for 5 minutes to completely dissolve curcumin which was then measured using UV-Vis spectrophotometer as total curcumin (C_t) [29]. The equation to calculate %E is presented in equation 1.

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$$\% \, E = \frac{C_t - C_f}{C_t} \times 100 \, \% \qquad (1)$$

Morphological Analysis

C-NLC was visually observed using a Confocal Laser Scanning Microscopy (CLSM) based on the intensity of curcumin. The wavelengths used for the excitation and emission processes in the sample were 420 nm and 539 nm with 400x magnification [16].

Particle Size Analysis (PS), Polydispersity Index (PDI), Zeta Potential (ZP)

Measurement PS, PDI, and ZP were conducted using Dynamic Light Scattering (DLS) technique which refers to photon correlation spectroscopy at room temperature (± 25ºC) through a sample dilution process of 10 times using distilled water. The measurements were conducted using distilled water at a temperature of 25ºC, with a refractive index of 1.3328, viscosity of 0.8878 N.s/m^2, dielectric constant of 78.3, and using disposable cells. For each measurement, a solution of C-NLC was prepared by dissolving 50 µL of the solution in 2450 µL of distilled water. Prior to this, an optimization method was carried out to compare the composition of the curcumin nanoemulsion with the solvent used.

C-NLC Stability Test

The criteria for a stable NLC were based on the resistance of the system to a phase separation or coalescence. Unstable NLC tends to separate into two distinct phases. C-NLC stability tests were done through three procedures. First, C-NLC sample was put into a 10 mL tightly closed screw tube and centrifuged at 3500 rpm for 15 minutes. Changes in sample appearance indicated sample instability which was predicted up to 1 year. Second, sample was situated at room temperature (27 ± 2°C) for 14 days. Particle size changes were monitored based on the images taken with digital microscope periodically, followed with image analysis using ImageJ software (National Institutes of Health, Maryland, U.S). The third procedure was the measurement of C-NLC pH at day 1 and day 30. Samples were stirred in a beaker glass with a stirring speed of 100 rpm at 25ºC during pH measurement [18]. Any changes in pH value indicated system instability.

RESULT AND DISCUSSION

C-NLC Morphology

C-NLC produced in our research are displayed in Figure 3. From the physical appearance, C-NLC produced became more transparent as the temperature of the process increased. At 40ºC and, at all lipid ratio, the samples showed a milky appearance. At 60ºC, in a certain lipid ratio, the mixture started to be more transparent. It indicated that the particle size decreased as the temperature of the process increased. In general, oil and water are very difficult to mix due to differences in viscosity, density and polarity, however, if the temperature increases gradually, it causes a decrease in viscosity which ease the mixing of lipids and curcumin to form an encapsulated curcumin in NLC structure [27]. Higher temperature facilitates the dispersion of NLC components to form smaller particle sizes. Figure 4 shows the morphology of C-NLC observed using CLSM. Curcumin, as a natural fluorophore, has excitation and emission wavelengths at 420 and 539 nm respectively. Figure 4(a) displays the fluorescence image of curcumin in lipid ratio of 2 : 1 processed at 40ºC while figure 4(b) shows curcumin distribution in the same lipid ratio processed at 60ºC. At lower temperatures,

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curcumin formed aggregate and was seen to be less dispersed in the system. Stearic acid, which tended to recrystallize, formed a dark needle-like structure. In contrast, at 60 °C, curcumin was more dispersed in the system. These results signified that temperature played an important role in increasing curcumin dispersion in the system.

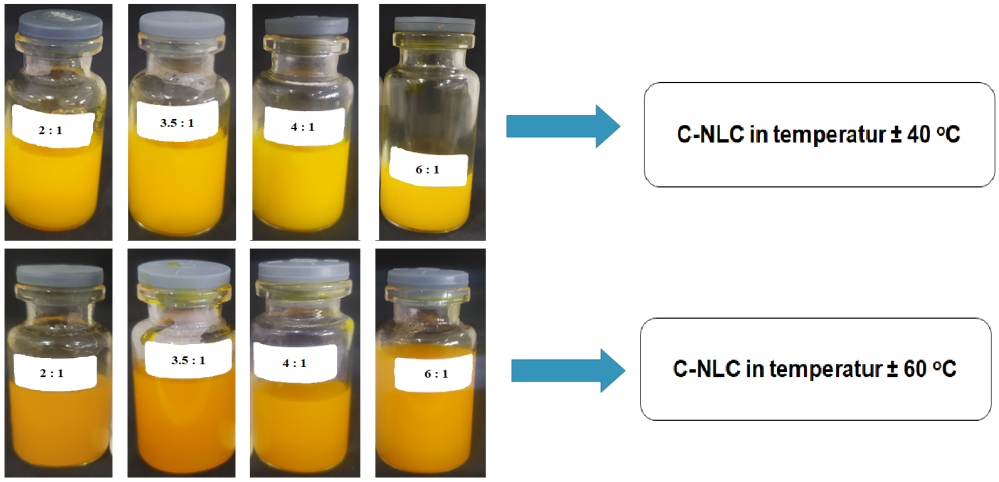
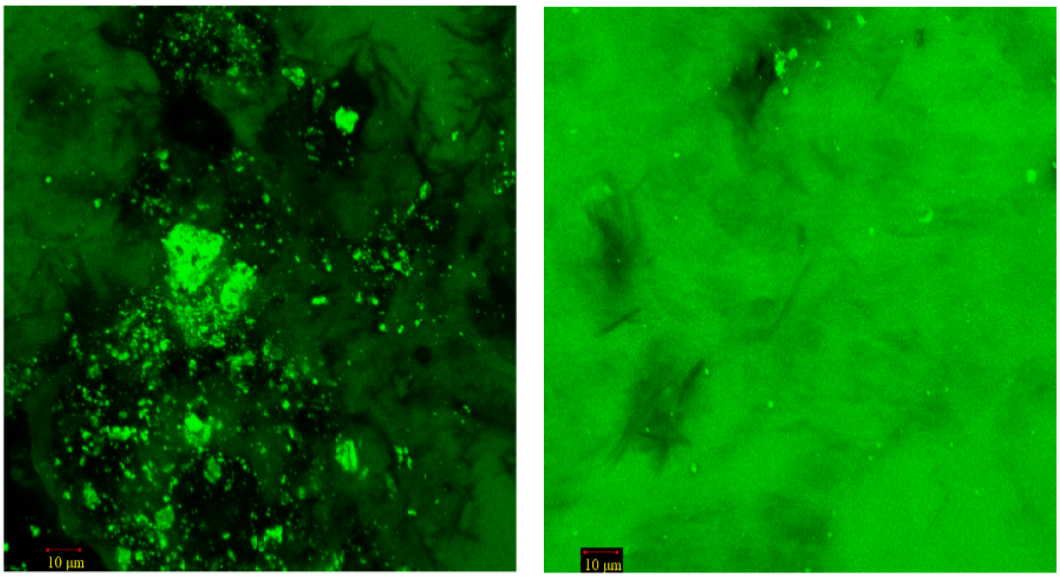


Figure 3. C-NLC produced using microfluidic technique at temperature 40°C and 60°C



(a) (b)

Figure 4. Curcumin dispersion in NLC system at lipid ratio of 2 : 1 and at 40°C (a) and at 60°C (b)

C-NLC Physicochemical Properties and Encapsulation Percentage

To further examine the properties of C-NLC produced, we measured PS, PDI, ZP and curcumin encapsulation percentage (Table 3). At 60°C, the particle size produced was relatively smaller than at 40°C. Interestingly, at 40°C, PDI values were smaller, while ZP values were higher compared to those obtained at 60°C. Our further measurement showed an irregular

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pattern of encapsulation percentage. We observed a non-linear relationship between lipid ratio and encapsulation percentage.

Table 3. Physicochemical properties of C-NLC

Temperature °C	Parameter	Liquid-Solid Lipid Ratio			
		2 : 1	**3.5 : 1**	**4 : 1**	**6 : 1**
40	Percent Encapsulation (%)	38.32	19.15	26.25	20.62
	Particle Size (nm)	481.00 ± 150.06	162.27 ± 12.57	199.24 ± 30.66	143.87 ± 3.36
	Polydispersity Index (PDI)	0.75 ± 0.06	0.47 ± 0.03	0.46 ± 0.04	0.44 ± 0.01
	Zeta Potential (mV)	-26.13 ± 5.35	-30.60 ± 2.20	-30.83 ± 3.74	-33.33 ± 6.53
Temperature °C	**Parameter**	**Liquid-Solid Lipid Ratio**			
		2 : 1	**3.5 : 1**	**4 : 1**	**6 : 1**
60	Percent Encapsulation (%)	22.62	2.93	7.07	31.45
	Particle Size (nm)	330.87 ± 67.92	438.60 ± 165.43	87.57 ± 21.19	60.21 ± 2.55
	Polydispersity Index (PDI)	0.93 ± 0.06	0.78 ± 0.02	0.64 ± 0.02	0.72 ± 0.03
	Zeta Potential (mV)	-26.47 ± 1.66	-24.10 ± 1.15	-24.53 ± 0.84	-26.10 ± 1.83

Our results show that the increasing amount of liquid lipid affected the particle size. In general, particle size decreased both at 40°C and 60°C and at high liquid lipid concentration. This could be due to the fact that liquid lipid reduces the viscosity as well as the surface tension of the system. Ebtavanny et.al [28], reported that the more liquid lipid was added in the Q10 NLC system, the smaller particle size produced. They reported that the optimum solid (cetyl palmitate)-liquid (caprylic) lipid ratio to produce the smallest particle size was 7 : 3. Similarly, Savic et.al [29], stated that higher solid lipid composition in tacrolimus-loaded lecithin-based NLC increased the particle size. Furthermore, temperature increase can reduce the viscosity and surface tension of the system [30]. These properties help the dispersion of NLC components, thus forming a smaller particle. Lower deviation standard at high liquid lipid concentration signifies the formation of a more uniform system. Our results of curcumin distribution observation using CLSM confirm the formation of a more dispersed and uniform system in higher temperature. Meanwhile, temperature increments in each liquid-solid lipid ratio affected differently toward curcumin encapsulation percentage. At ratio 2 : 1, 3.5 : 1, 4 : 1, encapsulation percentage decreased at higher temperatures. Unlike other ratios, at 6 : 1, where liquid lipid is in excess compared to liquid lipid, encapsulation percentage reaches the highest value at 60°C. These results signified that the liquid-solid lipid ratio played an important effect on the encapsulation percentage. Moreover, the encapsulation percentage was susceptible to temperature changes.

The encapsulation percentage was influenced by various factors, including the ratio of solid-liquid lipids, total lipid concentration, drug solubility, volume of the aqueous phase,

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temperature, volume of surfactant and co-surfactant, and nanoparticle stability [31,32]. The more liquid lipids were present in the NLC system, the less likely solid lipid matrices will recrystallize, resulting in an expected increase in the percentage of encapsulation [21]. Referring to the previous studies, soybean oil acts as the main matrix for trapping curcumin resulting in a higher encapsulation percentage [33]. Thus, it is expected that the encapsulation percentage is high by applying soybean oil as the liquid lipid in C-NLC [34]. However, the increasing amount of soybean oil did not linearly ascend the encapsulation percentage. We observed a fluctuating pattern in encapsulation percentage as we increased the amount of soybean oil. In contrast to our results, Pan et al. stated that the encapsulation of beta-carotene in the glyceryl trioctanoate-eicosane NLC showed a linear correlation with liquid lipid concentration [35]. Nevertheless, Ruktanonchai et al. reported that the highest encapsulation of alpha-lipoic acid in the NLC system of apifil and Miglyol 8 was achieved at a solid:liquid lipid ratio of 8 : 1 [7]. Ruktanonchai et al. showed that lipid ratio affects differently in different formulations. In their experiment, the highest encapsulation efficiency was reached at a composition in which solid lipid is higher than liquid lipid. These results indicate that the lipid ratio significantly determines the encapsulation efficiency in each system.

Temperature is also expected to increase curcumin encapsulation percentage since it provides thermal energy to facilitate more molecules movement and interaction. At 40ºC, the encapsulation percentage showed a gradual decrease as more liquid lipid was added in the system. Similarly, the increment of liquid lipid concentration at 60ºC decreased the encapsulation percentage. These results indicated that the NLC system formed might undergo release of curcumin from the NLC system as the liquid lipid was added. Temperature increase might also create a possibility that curcumin was prone from escaping out of NLC entrapment as well as damaging the NLC system and forming smaller particles. Nonetheless, at 6 : 1 lipid ratio and at 60ºC, we observed a significant increase in the encapsulation percentage [36]. Thus, the encapsulation percentage of curcumin in the NLC system was not always directly proportional to temperature increase.

In their study, Sentosa et al. [37], observed changes in the encapsulation percentage of NLCs containing curcumin at different temperatures. For pure curcumin, the encapsulation percentage decreased from 10% to 6% when the temperature increased from 35°C to 45°C, however increased to 8% at 55°C. Similarly, NLCs formulated with curcumin from temulawak (*Curcuma zanthorrhiza*) showed an increase in encapsulation percentage from 0.05% to 0.15 % between 35°C and 45°C, but no further improvement at 55°C (0.11%). Another study by Ren et al. [38], focused on polymethoxyflavone loaded with orange oil and found a consistent decrease in encapsulation percentage with increasing temperature using the spray-drying method. The encapsulation percentage dropped from 75.8% at 110°C to 45.2% at 190°C. Overall, these results demonstrated a non-linear relationship between temperature and encapsulation percentage, emphasizing the need for optimization in the manufacturing process of NLC systems to achieve desired encapsulation levels.

The influence of temperature on nanoparticle synthesis has been studied by previous researchers. According to M. Nejadmansouri et al. [39], a nanoemulsion that contained free antioxidant samples, utilizing liquid lipid like fish oil, achieved a reduction in particle size from 102 ± 1.80 nm at 4°C to 60 ± 1.42 nm at 25°C. The viscosity also decreased from 1.68 ± 0.002 mPa•S at 4°C to 1.58 ± 0.001 mPa•S at 25 °C, and the surface tension decreased from 31.97 ± 0.10 mN/m at 4°C to 30.70 ± 0.35 at 25°C. Ansari et al. [25], also observed that nanoparticles loaded with liquid crystals experienced a decrease in particle size below 175 nm at

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temperatures of 45°C, 55°C, and 65°C, and a decrease in particle size distribution below 0.37 at temperatures of 45 °C and 55 °C [14].

C-NLC Stability

To observe C-NLC stability, we measured the PDI and ZP of the systems. PDI values indicate the distribution of particle shape and size in the range of 0-1 in which a small value of PDI represents a monodisperse system. Meanwhile, ZP values less than -30 mV or more than +30 mV signify a stable system since there is high repulsion force between particles to avoid coalescence, thus maintaining system stability. Based on data in Table 3, in general, the value of PDI at 40°C was smaller than at 60°C which indicated the distribution of particles at 40°C was more monodisperse. Likewise, the value of ZP at 40°C was higher than the value at 60°C.

At 40°C, the value of PDI tended to decrease as liquid lipid quantity increased which implied that the more liquid lipid added in the system, the more monodisperse the system produced. As more liquid lipids exist in the system, PDI value linearly descended from 0.75±0.06 to 0.44±0.01. Similarly, ZP value linearly descended from -26.13±5.35 to -33.3±6.53 mV. At 40°C, the addition of liquid lipid improved the stability of the systems.

Meanwhile, the PDI value of C-NLC produced at 60°C showed a high value more than 0.5 which signified that the system was a non-monodisperse system. Regardless of the smaller particle size produced in this temperature, PDI value implies that the distribution of the particle size in the system is broad. Higher temperature applied during NLC production, supplies more heat which facilitates abrupt movement and collision among C-NLC. This opens possibilities of broad particle size formation [40,41].

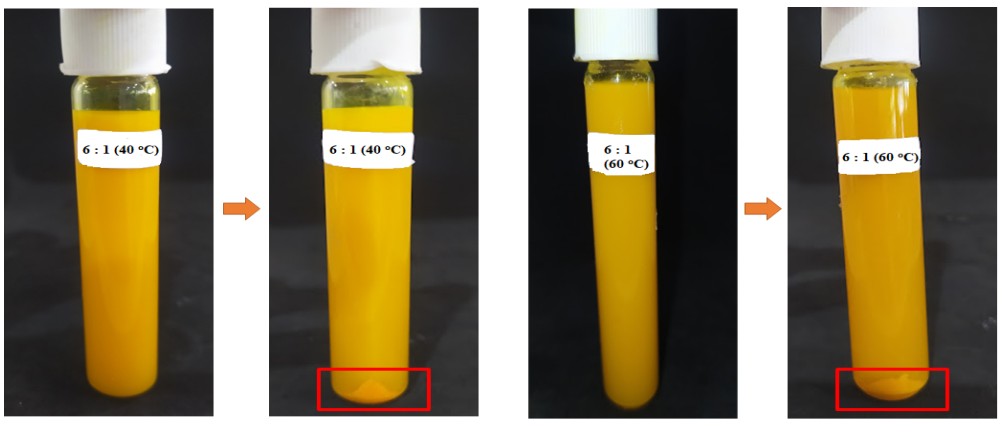

Figure. 5 Precipitate was formed after the centrifugation (a) C-NLC formed at 40°C (b) C-NLC formed at 60°C

Physical stability of C-NLC was monitored through physical appearances changes after 15 minutes centrifugation at 3500 rpm. As shown in Figure 5, before centrifugation no precipitate was observed, however, after the centrifugation precipitate was formed in both systems. This suggests that the system stability is expected to last in less than one year. To further determine the system's stability, we monitor particle size changes during 14 days of storage. Figure 6 exhibits that particle size in both C-NLC systems fluctuates during storage. Nonetheless, fluctuation in C-NLC system produced at 60°C is less prominent than in the system produced at 40°C. This is however, in contrast with the ZP observed in which ZP values are higher in

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the C-NLC produced at 40°C. ZP values were measured immediately after the samples were prepared and might be altered during storage. C-NLC produced at 60ºC displays a better stability in the long run.

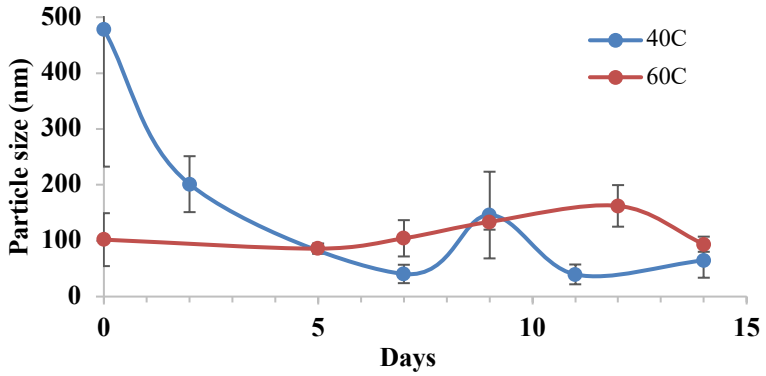

Figure 6. Particle size as a function of time of storage (days) at 40 and 60°C.

Table 4 shows that the pH value of the 6 : 1 formulation gradually increased after 30 days of storage. The pH value plays a crucial role in the physical stability of colloidal systems and significantly affects the ZP value [42–44]. The increase or decrease of pH correlates with the changes in ZP value. In our system, pH changes in C-NLC produced at 40°C (0.26) was higher than in C-NLC produced at 60°C (0.10). The changes in pH value indicated the alteration of chemical's structure involved in the system, thus, the small changes of pH value signify the stability of the system. It can be concluded that C-NLC system produced at 60ºC was stable up to 30 days of storage.

Table 4. pH C-NLC 6 : 1 at 40ºC and 60ºC

Temperature ºC	pH	
	6 : 1 (0 Days)	6 : 1 (± 30 Days)
40	5.980	6.240
60	5.930	6.030

CONCLUSION

In general, the increase of process temperature and liquid lipid concentration reduce particle size. We observed a non-linear relationship between lipid ratio and encapsulation percentage. Likewise, the encapsulation percentage of curcumin in the NLC system was not always directly proportional to temperature increase. These results signified that the liquid-solid lipid ratio played an important effect on the encapsulation percentage. Moreover, the encapsulation percentage was susceptible to temperature changes. At the best ratio of soybean oil:stearic acid of 6 : 1, at process temperature of 40°C, particles size obtained was 143.87 ± 3.36 nm, PDI 0.44 ± 0.01, ZP -33.3 ± 6.53 mV with encapsulation percentage of 20.62 %. At the same ratio with process temperature of 60°C, the particle size obtained was 60.21 ± 2.55 nm, PDI 0.72 ± 0.03, ZP -26.1 ± 1.83 mV and encapsulation percentage of 31.45%. Stability tests showed that C-NLC produced at 60°C is more stable since the change of particle size (during 14 days) and pH (during 30 days of storage) were lower than C-NLC produced at 40°C.

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