



## **Physicochemical Characteristics, Stability, and Irritability of Nanostructured Lipid Carrier System Stabilized with Different Surfactant Ratios**

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### **Abstract**

**Background:** One of the vital variables affecting the stability and the characteristics of the Nanostructured Lipid Carrier (NLC) is the surfactant concentration. Using the two combinations of surfactants can cause higher stability and a better characteristic of NLC. Tween 80 and Span 20 are anionic surfactants whose combination has not been studied for use in NLC systems. **Objective:** Determine the effect of different surfactant ratios of Tween 80 and Span 20 on the physicochemical characteristics, stability, and irritability of NLC using the High Shear Homogenization (HSH) method. **Methods:** Four different surfactant ratios were used in the NLC formulation, in which the ratio of Tween 80:Span 20 were 5:5, 6:6, 7:7, and 8:8, respectively. In this NLC system, cetyl palmitate served as solid lipid, medium-chain triglyceride (Crodamol™) as liquid lipid, Tween 80, and Span 20 as surfactant components. NLC was characterized for organoleptic, viscosity, pH, zeta potential, particle morphology, particle size, and polydispersity index (PDI), then evaluated for stability using the real-time and freeze-thaw method, and irritability effect. **Results:** The different ratios of Tween 80 and Span 20 had no significant effect on the particle size, PI, and irritation score of the NLC system. On the other hand, it influenced all formulas' pH value, viscosity, zeta potential, and stability. **Conclusions:** The different ratios of surfactant combination affect the characteristics and stability of the NLC system.

**Keywords:** irritability, NLC, physicochemical characterization, stability, surfactant ratios

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## INTRODUCTION

Over the past decade, many formulation studies about lipid carriers have increased. The rise in lipid carriers exploration as a nanotechnology delivery system is essentially due to the drawbacks of conventional drug delivery systems, like first-pass metabolism, which leads to a decrease of bioavailability, interaction due to food and drug, poor solubility drug, and high fluctuation of the drug concentration level in plasma (Brito *et al.*, 2019). Lipid nanoparticles are utilized as an alternative drug delivery system for the existing conventional particulate systems, like polymeric nanoparticles, or known as a liposome. This system enhances drug stability, increases the safety and the efficiency of drugs, provides targeted drug delivery, improves bioavailability for instance, and extends the drug's effect in the target tissue (Zahin *et al.*, 2020).

Nanostructured Lipid Carriers (NLC) is one of the nanoparticle lipid-based systems developed from the Solid Lipid Nanoparticles (SLN). The NLC structure is the most significant advantage of this system compared to SLN. NLC is composed of a blend of spatially incompatible liquid lipids along with solid lipids providing spaces to host the active compound. Those lipids can be utilized in a high concentration (up to 95%) when compared to SLN. Due to the lipid protection, NLC can avoid the degradation of drugs and promotes drug-controlled release (Natarajan *et al.*, 2017; Durán *et al.*, 2019). The utilization of liquid lipid also gives a better drug loading and it can avoid drug expulsion for a long period. NLC presents many other advantages such as the improved penetration of drugs due to the increased permeation on the skin and the occlusive effect while decreasing the transepidermal water loss and increasing skin hydration (Pivetta *et al.*, 2018). Thus, NLC formulations have been proposed to be suitable for cosmeceuticals, especially for poorly water-soluble (Ortiz *et al.*, 2021) and weak-acid drugs (Rahmasari, 2018).

The surfactant concentration is one of the important factors that affect the stability, particle size, particle size distribution, degree of crystallization, and polymorphism of the Nanostructured Lipid Carrier (NLC). The surfactant acts as a stabilizer (Ortiz *et al.*, 2021), and plays an important role in lipid nanoparticles formation. Surfactant reduces the surface tension and facilitates the particle partition during the homogenization process (de Souza *et al.*, 2021). To produce a great NLC with good characteristics and stability, it is necessary to select and use the proper surfactant concentration (Witayaudom & Klinkesorn,

2017). It has been reported that the nanoparticle surface can be covered efficiently using Poloxamer with an optimum concentration of 3%. The nanoparticles' surface will be well-covered, and the aggregation among particles reduces with an adequate concentration of surfactant (Zirak & Pezeshki, 2015). Another study reported that the surfactant type influenced the quality of lycopene-loaded NLC. Surfactant type moreover appeared to have a vital role in the zeta potential of the NLC (Riangjanapatee & Okonogi, 2012). Karn-Orachai *et al.* (2014) reported that smaller NLC particles, lower crystallinity, and also a more homogenous mixture of solid lipid and oil are obtained by the two surfactants system. It indicates that the stability of NLC of mixed two surfactant systems showed to be held over more extended periods than the one or the three-surfactant systems.

This study aimed to explore the physicochemical characterization, stability, and irritability of NLC prepared at different ratios of Tween 80 and Span 20. In expansion, we assessed which surfactant ratio could deliver great NLC with good characterization, and stability and had no irritability effect.

## MATERIALS AND METHODS

### Materials

Cetyl palmitate was bought from BASF (Germany). Medium-chain triglyceride (Crodamol<sup>TM</sup>) was a gifted sample from Croda (Singapore). Tween 80 was obtained from Kao Corporation (Japan). Span 20 was purchased from Brataco (Indonesia). All of these chemicals were in pharmaceutical grade.

### Tools

High Shear Homogenizer (T25 Ultra-Turrax IKA<sup>®</sup>), Zeta Potential and Submicron Particle Size Analyzer (Delsa <sup>TM</sup>Nano), Zetasizer Nano (Malvern Panalytical), Transmission Electron Microscope (Jeol JEM-1400), pH Meter (Schott Glass Mainz, GC 824 type), Viscometer (Brookfield), and Light Microscope (Nikon H600L).

### Methods

#### Preparation of NLC

This NLC preparation was made by the high shear homogenization method. Firstly, the cetyl palmitate, Crodamol<sup>TM</sup>, and Span 20 were put in a glass and dissolved using a hot plate at 70°C. This blend was then stirred by using a high-speed homogenizer at a speed of 3400rpm for 5min. On the other hand, a beaker glass containing acetate buffer solution and Tween 80 was blended by heating at 70°C. The lipid phase was then added with this hot aqueous phase gradually and

homogenized at a speed of 20,000 rpm for 3 mins in five cycles. This preparation then cooled while stirring at 500 rpm for 30 mins until the best NLC system was obtained (Table 1).

**Organoleptic test**

Organoleptic tests were carried out by visually determining the odor, color, and consistency of the NLC systems.

**pH evaluation**

About 1 g of NLC system was dissolved in 20 mL distilled water, then immersed the electrode into the sample. A digital pH meter was used to measure the pH value of the NLC preparations, which was already calibrated. The pH values were observed until the screen showed a stable result.

**Viscosity test**

A viscometer was used to determine the viscosity of the samples. The 150 g samples were poured into the container, and the spindle had to be sunk into it. At that moment, the viscometer was turned on and maintained in place until the steady measurement result was achieved.

**Zeta potential evaluation**

Zeta potential value was measured by using a zetasizer. About 0.5 g of NLC system was dissolved in 20 mL of distilled water. Approximately 3 mL sample was diluted in 10 mL distilled water, then shaken with a vortex to prevent aggregation. After that, the sample was placed in the sample holder until the measurement result was stable.

**Particle size and polydispersity index (PDI) evaluation**

The particle size and PDI analysis were performed using a dynamic light scattering instrument (Particle Size Analyzer). The variance of the average intensity of light scattering from this instrument will be calculated as the particle size. The PDI shows the particle size homogeneity within the sample population (Hendradi *et al.*, 2017).

**Particle morphology evaluation**

Transmission Electron Microscopy (TEM) was utilized to observe the morphology of the NLC systems. A sample drop was colored with 2% (w/v) of

phosphotungstic acid solution and placed on a copper grid for observation with TEM (Gokce *et al.*, 2012).

**Stability test**

Stability testing was held using the real-time and freeze-thaw method. In the real-time method, the NLC samples were stored at  $30 \pm 2^\circ\text{C}$  for one month (30 days) (Dantas *et al.*, 2016). In the freeze-thaw method, the NLC samples were stored at  $4 \pm 2^\circ\text{C}$  for 24 hours and moved at  $40 \pm 2^\circ\text{C}$  for 24 hours (counted as one cycle), then repeated up to six cycles (12 days) (Kumar & Dua, 2018). The organoleptic, pH values, particle size, and PDI were evaluated on the last day of storage.

**Irritation test**

The irritation test was held using in vivo histopathological scoring method. Male mice were sedated with ketamine (50 mg/Kg) intraperitoneally one hour before use. Then the back hair was shaved, and the samples applied. After 48 hours, the mice were sacrificed with the dislocation method. The back skin was cut using a microtome, then stained with hematoxylin-eosin (HE) and observed with a light microscope. Observation of skin irritation was carried out with histopathological scoring on several indicators of irritation, which are liquefaction, edema, collagen fibre swelling, inflammatory cell infiltration, and skin appendages degeneration (Shoviantari *et al.*, 2020).

**Ethics consideration**

The Research Ethics Committee approved this research of Veterinary Medicine Faculty of Airlangga University (Animal Care and Use Committee, Approval Code: No. 738-KE).

**Statistical analysis**

The characterization, which includes pH, viscosity, zeta potential, particle size and PDI, stability, and irritability of NLC system, was analyzed using a one-way Analysis of Variance (ANOVA) method for parametric data and Kruskal-Wallis method for non-parametric data, at a 95% confidence interval, statistically. The data results were further analyzed using the Honestly Significant Difference (HSD) test (parametric data) and the Mann-Whitney test (non-parametric data).

**Table 1.** Composition of the NLC preparations (% w/w)

Composition	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)	Formula 4 (F4)
Cetyl palmitate	1	1	1	1
Crodamol™	4	4	4	4
Tween 80	5	6	7	8
Span 20	5	6	7	8
Acetate buffer (4.5 ± 0.5)	until 100	until 100	until 100	until 100

**RESULTS AND DISCUSSION**

**Organoleptic test**

The organoleptic observation showed that the NLC was white, odorless, had a liquid consistency and soft texture, as shown in Figure 1.



**Figure 1.** NLC systems (A) formula 1; (B) formula 2; (C) formula 3; (D) formula 4

**pH evaluation**

The pH of all formulas was found about 4.36 to 4.57 (Table 2). It can be demonstrated that NLC systems can be utilized for topical preparation, in line with normal skin pH, which is 4 - 6 (Prakash *et al.*, 2017). Based on the measurable examination of ANOVA followed by the post-hoc Tukey HSD test, the results revealed a significant difference (sig. value 0.010 < 0.05) in pH values, especially between Formula 4 with the other formulas. These results may be due to the acetate buffer solution (pH = 4.5) used in NLC, and in Formula 4 there was a substantial change in micellar molecular weight, which caused a change in pH (Bloor *et al.*, 1970).

**Viscosity test**

The proper viscosity is required to enable NLC to adhere to the skin surface, thus increasing the drug penetration across the skin and the residence time (Hendradi *et al.*, 2017). As can be seen in Table 2, the results showed a significant difference (sig. value 0.000 < 0.05) in viscosities among all formulas. It represents that different ratio of surfactant (Tween 80 and Span 20) affect the NLC systems' viscosity. This result was in line with the theory that viscosity increases with the addition of more surfactants because surfactants can change the

morphology of the micelle from spherical form to cylindrical form. It causes electroviscous surfactant effects, leading to a bigger molecular weight (El Aferni *et al.*, 2020).

**Zeta potential evaluation**

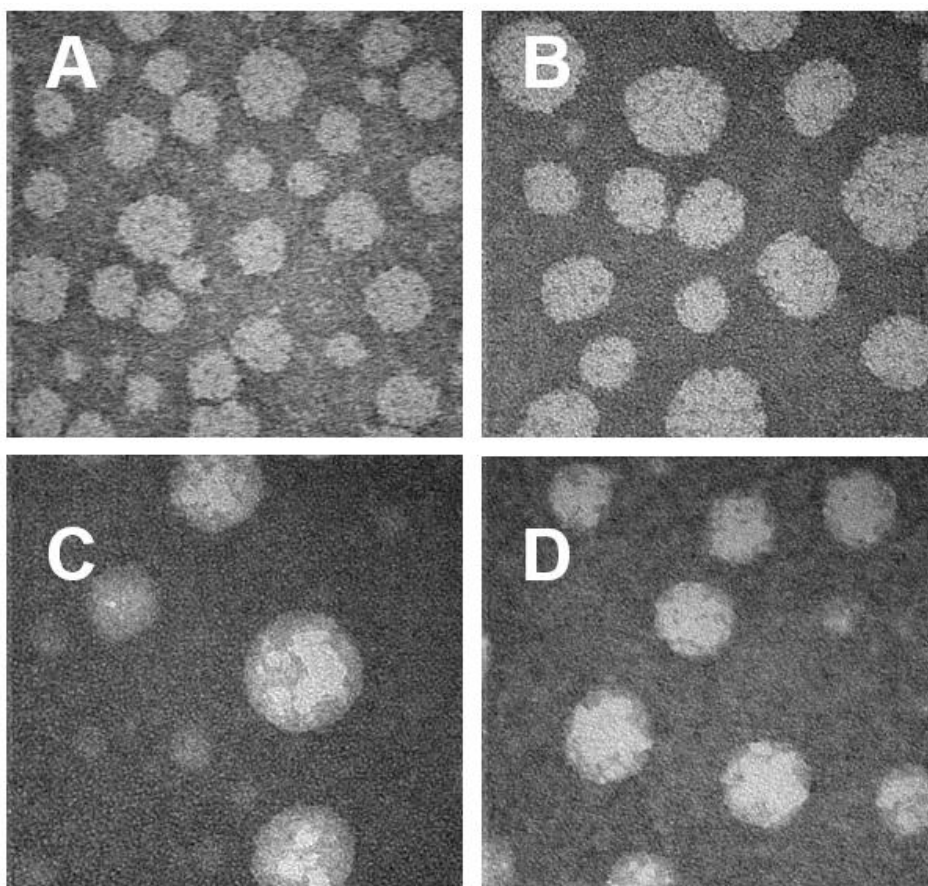
The zeta potential of all formula was found < (-)25mV (Table 2). Based on the statistical analysis of Kruskal-Wallis followed by the Mann-Whitney test, these results represented that there was a significant difference (sig. value 0.021 < 0.05) in zeta potential values among all formulas. It could indicate that NLC systems had been thought to be stable colloid dispersion. The NLC systems were considered to have sufficient repulsive force to attain a high degree of physical colloidal stability (Shnoudeh *et al.*, 2019).

**Particle size and polydispersity index (PDI) evaluation**

The size of particles is a critical factor in producing nano-sized particles. It depicts the stability of the formulation. One of the factors which affects the particle size is the added surfactant (Suhaimi *et al.*, 2015). This study found that the increment in the surfactant ratio contributed to bigger particle size (Table 2). Statistically, it showed no significant difference (sig. value 0.168 > 0.05) among all formulas. Polydispersity Index (PDI) exhibited the width of particle size distribution. The PDI range extended from 0 to 1. As the PDI value got to be closer to zero, the particles got to be more homogenous. From Table 2, it was indicated that all formulae has a homogenous particle and were considered to be acceptable (PDI < 0.3) (Danaei *et al.*, 2018). It statistically showed no significant difference (sig. value 0.243 > 0.05) among all formulae. As can be seen, the high amount of surfactant produced smaller PDI. This may occur due to increasing surfactant, which strengthens the steric resistance effect by forming an adsorption layer on the particle surface (Wang *et al.*, 2019), thereby preventing drug particles from aggregating (Pan *et al.*, 2015) and making the size among particles homogenous.

**Table 2.** Physicochemical characterization of NLC systems

Formula	pH Value	Viscosity (cps)	Zeta Potential (mV)	Particle Size (nm)	PDI
1	4.57 ± 0.03	0.38 ± 0.02	-38.1 ± 0.29	161.60 ± 51.35	0.205 ± 0.04
2	4.54 ± 0.02	0.43 ± 0.02	-35.6 ± 0.44	115.93 ± 50.32	0.265 ± 0.22
3	4.52 ± 0.07	0.48 ± 0.02	-35.0 ± 0.15	174.90 ± 3.16	0.125 ± 0.03
4	4.36 ± 0.08	0.51 ± 0.02	-37.9 ± 0.12	186.90 ± 11.12	0.160 ± 0.02



**Figure 2.** Particle morphology of NLC systems (A) formula 1; (B) formula 2; (C) formula 3; (D) formula 4 using Transmission Electrone Microscope (TEM) on 50.000x magnification

**Particle morphology evaluation**

Figure 2 shows TEM images of the NLC systems. As indicated in these figures, particles depicted a mono-dispersed spheroid-like appearance with a clear boundary among each particle. The particles showed no visible aggregation, a uniform and spherical shape. These spherical particles have an uneven surface. Probably, this matter is formed from a liquid lipid that coats the inner particle of the systems and includes a flip-flop structure.

**Stability test**

In the real-time method (Table 3), a stability test was conducted to physically determine the system

resilience of NLC when stored at room temperature. The NLC systems showed no significant changes in pH and PDI values, but a significant change in particle size, statistically with paired t-test method. There was an increment in the size of the particles, which indicates the incorporation of small particles or coalescence. After 30 days of storage with the real-time method, it can be concluded that the NLC system formed represents the stability of the NLC system in the absence of pH and PDI change. It can also be concluded that the surfactant concentration does not affect in ‘real-time’ method stability.

**Table 3.** Stability testing results of NLC systems in real-time method

For mula	Organoleptic	Parameters					
		pH		Particle Size (nm)		PDI	
		Before	After	Before	After	Before	After
1	No color	4.57 ± 0.03	4.62 ± 0.02	161.60 ± 51.35	384.73 ± 3.71	0.205 ± 0.04	0.227 ± 0.09
2	change, odor	4.54 ± 0.02	4.66 ± 0.06	115.93 ± 50.32	175.93 ± 39.12	0.265 ± 0.22	0.145 ± 0.06
3	change,	4.52 ± 0.07	4.66 ± 0.02	174.90 ± 3.16	380.37 ± 73.27	0.125 ± 0.03	0.235 ± 0.04
4	precipitation and phase separation	4.36 ± 0.08	4.70 ± 0.02	186.90 ± 11.12	276.47 ± 62.73	0.160 ± 0.02	0.256 ± 0.02

In the freeze-thaw method (Table 4), a stability test was conducted to physically determine the system resilience of NLC when stored in extreme conditions. The NLC systems statistically showed significant changes in pH values, particle size, and PDI values with the paired t-test method. There was a decrease in PDI values and an increment in the pH values and size of the particles. An increase in temperature s crystal growth, indicating the aggregation of nanoparticles when the temperature increases, which tends to increase particle size (Catauro *et al.*, 2018). Besides that, preparations containing Tween 80 and stored at 40°C undergo autoxidation to form more peroxides (Kishore *et al.*, 2011). This autoxidation leads to the destabilizing effect of Tween 80 and increases the aggregation of particles (Agarkhed *et al.*, 2013). After 12 days of storage with a freeze-thaw method, it can be concluded that the NLC system formed represents the instability of the NLC system in the presence of pH, particle size, dan PDI change. It can also be concluded that the surfactant

concentration does not affect in ‘freeze-thaw’ method stability.

**Irritation test**

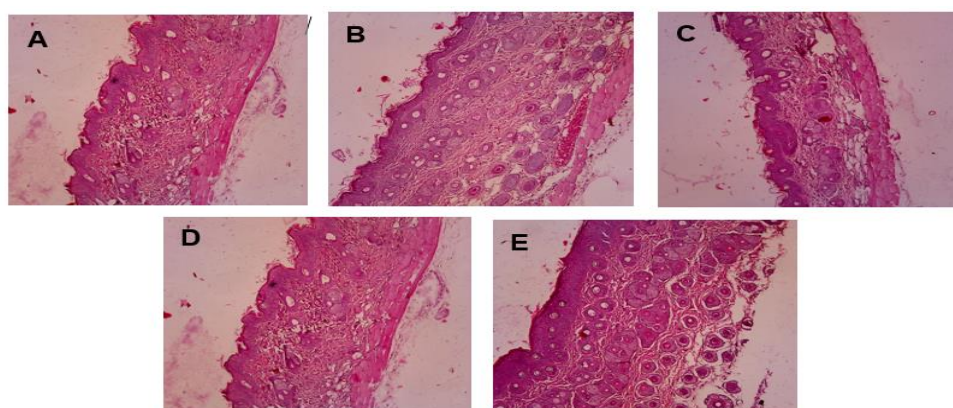
Safety of use is an important factor in developing such topical preparations. One of the safety parameters can be illustrated by the absence of skin irritation and can be done with histopathological observation (Figure 3). The histopathological scores of the back skin of mice after 48 h indicates that NLC systems had an average score that did not cause structural changes while Crodamol™ had a slight irritation (Table 5). Crodamol™ is a medium-chain triglyceride oil that acted as a skin sensitizer or caused dermal irritation in several studies (Traul *et al.*, 2000). This result represents that the NLC system has less irritation risk than the liquid lipid. This phenomenon was probably caused by the addition of cetyl palmitate, which has the ability to moisturize to minimize the occurrence of irritation (Shoviantari *et al.*, 2020).

**Table 4.** Stability testing results of NLC systems in freeze-thaw method

For mula	Organoleptic	Parameters					
		pH		Particle Size (nm)		PDI	
		Before	After	Before	After	Before	After
1	No color	4.57 ± 0.03	5.46 ± 0.03	16.60 ± 51.35	289.4 ± 24.93	0.205 ± 0.04	0.138 ± 0.00
2	change, odor	4.54 ± 0.02	5.43 ± 0.02	115.93 ± 50.32	275.47 ± 19.13	0.265 ± 0.22	0.123 ± 0.00
3	change,	4.52 ± 0.07	5.20 ± 0.04	174.90 ± 3.16	264.93 ± 17.14	0.125 ± 0.03	0.139 ± 0.00
4	precipitation and phase separation	4.36 ± 0.08	4.70 ± 0.05	186.90 ± 11.12	244.33 ± 44.92	0.160 ± 0.02	0.159 ± 0.00

**Table 5.** Histopathological score NLC systems irritation test on male mice’s back after 24 hours

Formula	Irritation Score	Classification
1	0.2 ± 0.28	Almost no change
2	0.4 ± 0.28	Almost no change
3	0.73 ± 0.19	Almost no change
4	0.87 ± 0.19	Almost no change
Crodamol™	1.6 ± 0	Slight irritation



**Figure 3.** Microscopic images of mice skin backs after 48 h of NLC systems (A) formula 1; (B) formula 2; (C) formula 3; (D) formula 4 and (E) crodamol™, using the nikon H600L light microscope at 100x magnification



## CONCLUSION

In this research, it can be concluded that the different surfactant ratios of Tween 80 and Span 20 affect the characteristics and stability of the NLC system and did not affect the irritability. There was no significant difference in particle size, PDI, and irritation score. There was a significant difference in the pH value, viscosity, zeta potential, and stability of samples for all formulas. The authors recommend Formula 3 with the surfactant ratio of Tween 80:Span 20 (7:7), as the best formula, due to the good physicochemical properties in the NLC system. These results also suggest the potential formula of NLC as a drug delivery system for weak-acid and poorly water-soluble drugs.

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## AUTHOR CONTRIBUTIONS

Conceptualization, N.R., W.S.; Methodology, N.R.; Software, D.R.; Validation, W.S.; Formal Analysis, D.R.; Investigation, W.S.; Resources, D.R.; Data Curation, N.R.; Writing - Original Draft, D.R.; Writing - Review & Editing, D.R.; Visualization, N.R.; Supervision, W.S.; Project Administration, N.R.; Funding Acquisition, D.R.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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