Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 12 No. 1 April 2025, 1-14
DOI: 10.20473/jfiki.v12i12025.1-14
Available online at https://e-journal.unair.ac.id/JFIKI/

Optimization Using D-Optimal Design of Nanostructured Lipid Carrier (NLC) with Variation of Surfactants and Co-surfactant

Wafa'ul Athiyyah[1], Widji Soeratri[2,3], Noorma Rosita[2,3]*, Siti Hartini Hamdan[4]
[1]Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[3]Skin and Cosmetic Technology Centre of Excellent, Universitas Airlangga, Surabaya, Indonesia
[4]Technical Foundation Section, Universiti Kuala Lumpur Institute of Chemical & Bioengineering Technology (MICET), Melaka, Malaysia

*Corresponding author: noorma-r@ff.unair.ac.id
Orcid ID: 0009-0006-5802-7742

Submitted: 29 August 2024
Revised: 13 January 2025
Accepted : 22 January 2025

Abstract

Background*: Nanostructured lipid carriers (NLC) are topical delivery systems designed to address the challenges associated with active ingredients, such as poor solubility and limited skin penetration. NLCs incorporate surfactants, such as sorbitan monooleate and lauryl glucoside, to stabilize the system, while the addition of soy lecithin as a co-surfactant further enhances NLC stability. A D-optimal design was employed to optimize the NLC components, ensuring that the formulation achieved the desired characteristics.* ***Objective:*** *To determine the optimal NLC formulation.* ***Method:*** *Optimization was conducted using the D-optimal design method. The NLCs were prepared using the high-shear homogenization method with an Ultra-Turrax device. Characterization included measuring the particle size, polydispersity index (PDI), pH, and creaming index.* ***Results:*** *All formulations resulted in homogeneous emulsions with a white color, slight aroma of castor oil, smooth texture, and thick consistency. The particle sizes ranged from 200 to 500 nm, although the polydispersity index was not significantly influenced by surfactants or co-surfactants. All the formulations maintained an appropriate pH range for skin compatibility and product stability. The %creaming index demonstrated that the co-surfactant effectively reduced creaming in the NLCs.* ***Conclusion:*** *The optimal formulation consisted of 0.284% sorbitan monooleate, 3.429% lauryl glucoside, and 0.287% soy lecithin.*

Keywords: *d-optimal design, lauryl glucoside, nanostructured lipid carrier, sorbitan monooleate, soy lecithin*

How to cite this article:
Athiyyah, W., Soeratri, W., Rosita, N. & Hamdan, S. H. (2025). Optimization Using D-Optimal Design of Nanostructured Lipid Carrier (NLC) with Variation of Surfactants and Co-surfactant. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 12(1), 1-14. http://doi.org/10.20473/jfiki.v12i12025.1-14

INTRODUCTION

Nanostructured Lipid Carriers (NLC) are lipid-based delivery systems consisting of a mixture of solid and liquid lipids in an oil-in-water (O/W) system with particle sizes ranging from to 50-1000 nm (Mohammadi et al., 2019). NLCs are typically used to improve physicochemical stability, such as difficulty in dissolving and penetrating the skin (Apostolou et al., 2021). The NLC system contains a combination of solid and liquid lipids of various types and concentrations that affect the HLB. It must be stabilized with surfactants and co-surfactants so that it can be stable in aqueous solvents. Emulsion stability is achieved if the required-HLB (rHLB) and HLB of surfactants/co-surfactants are close to or almost the same (Wang et al., 2023).

In a previous study by De Barros et al. (2022), NLC Quercetin was prepared using only one type of surfactant, sorbitan monooleate (HLB=4.3), and liquid lipids of five types of oils and solid lipids of myristic acid. In the characteristic test, various particle sizes were obtained (160–505 nm), while the results of the stability test showed that there was a fluctuation in PDI from 0.5 0.6. This is certainly an interesting finding because the HLB of the surfactant used had a range of values far from the rHLB of the lipid used.

The lipids used in this study were castor oil (HLB=14), glyceryl monostearate (HLB=5.8) and cetearyl alcohol (HLB=15.5). In the present study, the required HLB was 14.21. The combination of sorbitan monooleate (HLB=4.3) and lauryl glucoside (HLB=16) as surfactants and soy lecithin as a co-surfactant (HLB=7) can obtain a HLB that is close to the HLB required for lipids to stabilize the NLC.

Surfactants are used to reduce the surface tension between lipids and water, prevent particle aggregation, and maintain small particle sizes (Sar et al., 2019). Non-ionic surfactants such as sorbitan monooleate and lauryl glucoside are often used because they are considered safer and have lower skin irritation effects (Lechuga et al., 2023). Surfactants can affect NLC characteristics because they reduce the surface tension. When surfactants reach the critical micelle concentration (CMC), the cohesive forces between particles decrease, encapsulating the particles in micelles and forming smaller particles (Moulik et al., 2024). To obtain the appropriate HLB, sorbitan monooleate can be combined with a lauryl glucoside surfactant to produce a more stable emulsion.

Lauryl glucoside is a bio-based surfactant derived from natural sources with good solubility in water (HLB=16). It belongs to the Alkyl Polyglucoside (APG) group and can be used to replace ethoxylated nonionic surfactants such as polysorbate (Tween) because it has a similar HLB (HLB=13-16) (Pavoni et al., 2020). Alkyl polyglucoside surfactants are better than polysorbate surfactants owing to their natural biodegradability, resistance to oxidation at room temperature, and low dermatological irritation (Wieczorek and Kwasniewska, 2020). APG surfactant also provides skin softness and hydration due to its glucose content, is gentle on the skin (Das et al., 2024), and may enhance skin penetration due to the interaction of the alkyl tail of the surfactant with lipids in the stratum corneum. The use of lauryl glucoside in leave-on cosmetic products is generally at concentrations of 0.03-8%, but it is often used below 5% to prevent excess foam.

Jafar et al.(2022) reported that the use of 2% (w/v) lauryl glucoside produces NLC with vitamin E with small particle size (280 nm), low polydispersity index (0.44), zeta potential of -28 mV, and entrapment efficiency of 95%. However, based on patch tests conducted on 24,097 people in North America, 470 people showed allergies to APG surfactants, only 35 of whom showed allergies to lauryl glucoside (Warshaw et al., 2022). In the guinea pig sensitization test, APG solution resulted in an irritation (Bhoyrul et al., 2019). Moreover, several cosmetic products containing a combination of sorbitan monooleate and lauryl glucoside are available in the market, indicating that these two surfactants are compatible when combined in a product (INCI decoder, 2024).

Sorbitan monooleate and lauryl glucoside, as non-ionic surfactants, offer formulation advantages for cosmetic and pharmaceutical products. Both are stable under various conditions, such as acidic and mildly basic electrolytes, and do not react with ionic substances, especially lauryl glucoside, which is stable across pH 4-5.5 (Seweryn et al., 2019). The combination of sorbitan monooleate and lauryl glucoside allows the preparation of various emulsion systems, both oil-in-water (O/W) and water-in-oil (W/O). Sorbitan monooleate (sorbitan ester) is produced by dehydration of sorbitol. The HLB value of this surfactant decreases with increasing esterification, providing a higher solubility in lipophilic substances (Hong et al., 2018). However, sorbitan monooleate, which has a low HLB, is mostly used to prepare water-in-oil (W/O) emulsions.

Another component of NLC is the co-surfactant, which stabilizes the surfactant performance at low concentrations, making the resulting emulsion more thermodynamically stable. An example of a co-surfactant is soy lecithin. Soy lecithin has an HLB of 7-

P-ISSN: 2406-9388
E-ISSN: 2580-8303

9, which has HLB value between the HLB of sorbitan monooleate (HLB=4.3) and lauryl glucoside (HLB=16). In leave-on products, soy lecithin is usually used at low concentration (1-2%), which can stabilize 10% of the surfactant. It is commonly used at ratios of 1:1, 1:3, and 1:5. In this study, only up to 0.8% surfactant was used, assuming that the surfactant was used in the range of 3.5-4% (ratio 1:5). Thus, a combination of sorbitan monooleate and lauryl glucoside surfactants, along with the co-surfactant soy lecithin, is expected to produce NLC with optimal characteristics for nanolipid-based topical applications.

This study aims to optimize NLC formulations using castor oil, glyceryl monostearate, and cetearyl alcohol as lipid matrix with a combination of sorbitan monooleate, lauryl glucoside, and soy lecithin to achieve a stable and effective topical delivery system. A D-Optimal Design was applied to determine the optimal formula of mixture ingredients consisting of three factors (sorbitan monooleate, lauryl glucoside, and soy lecithin) and four responses (particle size, polydispersity index, pH, and creaming index). Particle size is an important characteristic parameter because it can affect the stability of NLC. Smaller particles reduce the effects of gravity and prevent sedimentation and creaming. While PDI also affects stability, a low PDI (<0.5) has a narrow particle size distribution, indicating good size uniformity, which can ensure homogeneity in the NLC systems. pH is also considered as NLC preparations are used for topical delivery; a pH closer to the skin's physiological range (4.5–5.5) ensures reduced irritation and improves skin compatibility.

MATERIALS AND METHODS

Materials and instruments

Materials used in this research are Castor Oil (Thai Castor Oil Industries Co.Ltd., Thailand), Cetearyl Alcohol (Lexemul®, Inolex, USA), Glyceryl Monostearate (CIMS, Surabaya, Indonesia), Lauryl Glucoside (Plantacare®1200UP, BASF, China), Sorbitan Monooleate (Elotant™, LG Healthcare, South Korea), Soy Lecithin Granule (Hongwan Biotechnology, China), Citric Acid Monohydrate pro analysis (Merck, Germany), Natrium Citric Dihydrate pro analysis (Merck, Germany), NaOH pellets pro analysis (Merck, Germany), Aquadest (CIMS, Surabaya, Indonesia). Instruments used in this research are Digital Ultra-Turrax IKA®T25 (Germany), Thermo Scientific Cimarec+ (USA), Analytical Balance OHAUS® (USA), Delsa™Nano C Particle Analyzer Beckman Coulter® (USA), pH meter SI Analytics Lab 865 (Germany), Ruler.

Methods

Application of D-optimal design for NLC optimization

In this study, blank NLC containing various surfactants and cosurfactants were optimized. Different NLC formulations were prepared using a high-shear homogenization method. Owing to the mixed nature of the components under investigation, a mixed statistical design was deemed appropriate. D-Optimal was chosen as the mixture design because it does not require 1 in the total mixture (Annisa et al., 2022). In fact, if the total components are set to 1, then one of the components will be set to 1, so that in a 100% mixture containing only that ingredient, it will affect the HLB value of the system. Therefore, we prepared a system of 4% surfactant (w/w) and co-surfactant.

The three components of the surfactant and co-surfactant mixture investigated were sorbitan monooleate, lauryl glucoside, and soy lecithin. Upper and lower limits were applied to the components of the mixture to ensure that each mixture contained all three components. The same range was specified for each component of the mixture, as listed in Table 1. The responses resulting from the optimization process are listed in Table 2.

The D-optimal mixed design minimizes the variance associated with the approximate regression coefficient for the model described and is used to select the design points (Montgomery, 2013). This model was built using Design Expert software (Version 13.0.5.0, Windows 64-bit, Stat-Ease Inc., Minneapolis, 2021). A total of 15 experiments were performed (12 different design points and three replications). Design Expert is used to analyze the generated data by describing the appropriate regression model for each response, explaining how it is affected by mixed components. The significance level was set at 0.0005. The results of 15 trials (F1–F15) are presented in Table 2. The preparation and characterization of the different trials were performed accordingly.

An ANOVA test was conducted to determine the suitability of the model and the strength of the correlation between factors and responses. The model was considered suitable if it had a ‘Not Significant’ lack of fit (P-value >0.0005). The correlation between the factors and responses was considered strong if the correlation coefficient (R-square) was close to 1. A positive correlation coefficient indicated a positive correlation between the factors and the resulting

P-ISSN: 2406-9388
E-ISSN: 2580-8303

response. Table 5, 6, 7, and 8 present the ANOVA results.

Contour plots and 3D surfaces are essential in the Design of Experiments (DoE) for visualizing and analyzing the interactions between different factors and their effects on responses. Color variations depicted response values, with lighter shades (yellow or red) indicating higher response values and darker shades (blue or green) indicating lower response values. The contour lines represent areas with equal response values. Tightly packed contour lines indicate rapid changes in response to changes in factors, while widely spaced contour lines indicate slower changes in response.

The steps for working using D-Optimal Design are as follows:

1. The upper and lower limits of the independent variables and factors were determined for sorbitan monooleate, lauryl glucoside, and soy lecithin.
2. The responses to the test were recorded. In this study, the parameters were particle size, PDI, pH, and creaming index.
3. Fifteen optimization formulas were prepared according to their applications.
4. The obtained data were analyzed.
5. Four solutions were generated using the optimization formula. Observations were then performed again (wet-lab observations) to compare the predicted and observed data.

Table 1. Factors (mixture components) and responses to their targets

Factors and responses			**HLB Values**	**Range of HLB values from mixed surfactants/co-surfactant**	**Actual values (% w/w)**		**Real values (Proportions)**	
Variable/Mixture components					Lower bond	Upper bond	Lower bond	Upper bond
Sorbitan monooleate			4.3		0	0.5	0	0.125
Lauryl glucoside			16	13.41 – 14.74	3	3.5	0.75	0.875
Soy lecithin			7-9		0	0.8	0	0.2
Responses	**Model**	**Goals**	**Constraints**					
Particle Size	Special Quartic	Minimize	Below 300 nm					
Polydispersity Index (PDI)	Special Quartic	In range	0.2 up to 0.4					
pH	Linear	In range	4.5 up to 4.8					
Creaming Index	Cubic, with transform Base ^{10}Log, k=0.0093	Minimize	Below 10%					

Table 2. Observed response of NLC prepared according to D-optimal design

Trial formula code	**Run**	**Mixture Components**			**HLB Values**	**Y_1 = Particle Size (nm)**	**Y_2 = Polydispersity Index**	**Y_3 = pH**	**Y_4 = Creaming Index (%)**
		X_1 = Sorbitan monooleate (SMO)	**X_2 = Lauryl glucoside (LG)**	**X_3 = Soy lecithin (SL)**					
F1	1	0.5	3.5	0	14.54	538.8	0.447	4.86	9.3
F2	2	0.1	3.23539	0.664607	14.2	295.5	0.374	4.67	0
F3	3	0.5	3.24975	0.250253	13.97	322.8	0.363	4.7	0
F4	4	0	3.41163	0.588371	14.67	362.4	0.374	4.64	0
F5	6	0	3.41163	0.588371	14.67	290.7	0.395	4.65	0
F6	7	0.164554	3.36776	0.467686	14.47	352.3	0.42	4.72	0
F7	8	0.37944	3.44052	0.180043	14.48	234.3	0.388	4.76	0
F8	9	0.332123	3.30842	0.359453	14.22	271.1	0.421	4.51	0
F9	10	0.2	3.5	0.3	14.74	205.9	0.441	4.6	2.32
F10	11	0.130975	3.06902	0.8	13.82	355.6	0.476	4.5	0
F11	14	0.317798	3	0.682202	13.53	477.4	0.375	4.55	9.3
F12	15	0.5	3.5	0	14.54	512.4	0.455	4.82	9.3
F13	13	0.130975	3.06902	0.8	13.82	356.8	0.429	4.6	0
F14	12	0.5	3	0.5	13.41	488.2	0.435	4.52	2.32
F15	5	0.270637	3.19759	0.531775	14.01	364.7	0.46	4.53	2.32

The total amount of surfactant and co-surfactant components were 4% (w/w)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 3. NLC optimization formula

Formula	Materials						
	Oil Phase (% w/w)				Water Phase (% w/w)		
	Castor Oil	Glyceryl Monostearate	Cetearyl Alcohol	Sorbitan Monooleate	Lauryl Glucoside	Soy Lecithin	Citrate Buffer pH 4.5±0.3
F1	6	1	3	0.5	3.5	0	86
F2	6	1	3	0.1	3.23	0.67	86
F3	6	1	3	0.3	3.25	0.25	86
F4	6	1	3	0	3.41	0.59	86
F5	6	1	3	0	3.41	0.59	86
F6	6	1	3	0.16	3.37	0.47	86
F7	6	1	3	0.38	3.44	0.18	86
F8	6	1	3	0.33	3.31	0.36	86
F9	6	1	3	0.2	3.5	0.3	86
F10	6	1	3	0.13	3.07	0.8	86
F11	6	1	3	0.32	3	0.68	86
F12	6	1	3	0.5	3.5	0	86
F13	6	1	3	0.13	3.07	0.8	86
F14	6	1	3	0.5	3	0.5	86
F15	6	1	3	0.27	3.2	0.53	86

Preparation of NLC

The NLC were prepared using a high-shear homogenization method with Ultra-Turrax with modifications (Azzahrah et al. 2022). Solid lipids (cetearyl alcohol and glyceryl monostearate) were melted at 60°C. Once the solid lipid melted, castor oil was added. The lipid phase was stirred at a low speed (200-300 rpm) using a magnetic stirrer.

Lauryl glucoside was heated until it melted (at 40°C) while the lipid phase was homogenized. Soy lecithin was dissolved in citrate buffer and homogenized using an Ultra-Turrax at 15,000 rpm for 2 min. At a temperature of 55-60°C, the melted lauryl glucoside was dissolved in the lipid phase until it was well-homogenized. Subsequently, the water and lipid phases were homogenized at 5,000 rpm using an Ultra-Turrax for two cycles of 5 min each. After homogenization, the emulsion was stirred at 16,000 rpm for 2 min. The NLC were then cooled to room temperature (25°C) while continuously stirring with a magnetic stirrer at 500 rpm.

All prepared NLCs were divided into two bottles. One set was stored at room temperature (22-25°C) for 24 h for further characterization tests (particle size, PDI, and pH), whereas the other set was stored at room temperature (22-25°C) for 14 days to assess the stability of the NLCs by observing the creaming index.

Characterization of NLC

Particle size (PS) and polydispersity index (PDI) testing

The PS and PDI of the NLC were determined as reported by Mayangsari et al. (2021) by weighing 50 mg of NLC and diluting it with 50 ml of water (1:1). The mixture was stirred at a moderate speed (400-500 rpm) for 10 min. Then, 2 ml of the dilution was mixed with 8 ml of water and stirred again at a low speed (100-200 rpm). The mixture was then placed into a cuvette and filled to ¾ of its volume with distilled water. The analysis was performed using dynamic light scattering (DLS) with a DelsaNano instrument.

pH testing

The pH was measured by weighing 1 g of the NLC preparation and diluting it with 10 mL of CO_2-free water in a glass beaker, followed by stirring until it was evenly mixed. The pH of the solution was measured at 25 °C using a pH meter (Suzliana et al., 2020).

Creaming index testing

The creaming index was measured based on the methods described by Tan et al. (2020) and Wangpradit et al. (2022), with modifications. The NLC formulation was stored at room temperature (22-25℃) for 14 d. Changes in the organoleptic properties of the formulation were visually observed, including phase separation (Rohmah et al., 2019). Phase separation was naturally observed between the serum (transparent appearance) and the total emulsion height using a ruler. The height of the emulsions prepared in this study was 4.3 cm. The %creaming index was calculated using the following formula (Haji et al., 2023; Wangpradit et al., 2022):

$$\%\text{Creaming Index (\%CI)} = \frac{Hc}{He}\ x\ 100\%$$

Hc = Height of cream layer (cm)

He = Height of emulsion prepared (cm)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

RESULTS AND DISCUSSION

All the prepared samples appeared as white, homogeneous emulsions. All the formulas had an opaque white color, a slight characteristic odor of castor oil, a smooth texture, and a thick consistency.

Particle size testing

Measuring the particle size is essential for determining the dimensions of the particles and ensuring that they meet the desired size range. Typically, the diameter of Nanostructured Lipid Carriers (NLC) ranges from 10 to 1000 nm, with a preferred size of 50–300 nm for targeted drug delivery. For topical applications, such as hair and skin, NLCs are generally the most effective when their maximum particle size is approximately 300-500 nm (Amasya et al., 2019; Ghasemiyeh et al., 2019; Shinde et al., 2019). Although the human pore diameter ranges from 40–80 μm to 0.05–0.2 mm, achieving an NLC particle size of approximately 300 nm is crucial for optimal product stability and effectiveness.

Based on the experimental results, four formulations had particle sizes above 300 nm, six had particle sizes of approximately 300 nm, and five had particle sizes below 300 nm. It was also found that increasing the concentration of soy lecithin resulted in larger particle sizes. However, the stability of the emulsion improved, as evidenced by the decrease in the creaming index (no creaming was observed). The amphiphilic nature of lecithin, which possesses both hydrophobic and hydrophilic regions, makes it an ideal surfactant for stabilizing oil/water emulsions. By modifying the interfacial properties, lecithin helps create a stable oil/water interface, which is crucial for maintaining emulsion stability (Tabaniag et al., 2023).

Higher concentrations of lecithin also resulted in larger particle size. This can be attributed to the formation of complex systems that grow larger owing to the dynamic exchange of lecithin molecules between particles. As this exchange occurred, larger aggregates were formed, which increased the overall particle size. The increased size occurs because lecithin molecules move from one aggregate to another, causing structural modifications in the aggregates, leading to their growth. When the lecithin concentration was high, more of these dynamic exchanges occurred, leading to the growth of larger vesicles. This exchange can lead to phase separation and structural modifications, contributing to an increase in particle size (Tabaniag et al., 2023).

Moderate amounts of lecithin helped stabilize the emulsion because excessive concentrations decreased stability. This is because excess lecithin molecules form a denser layer at the droplet interface, intensifying the attractive forces between droplets and leading to coalescence. Furthermore, aggregated molecules can act as nuclei for new droplet growth, destabilizing the emulsion (Tabaniag et al., 2023).

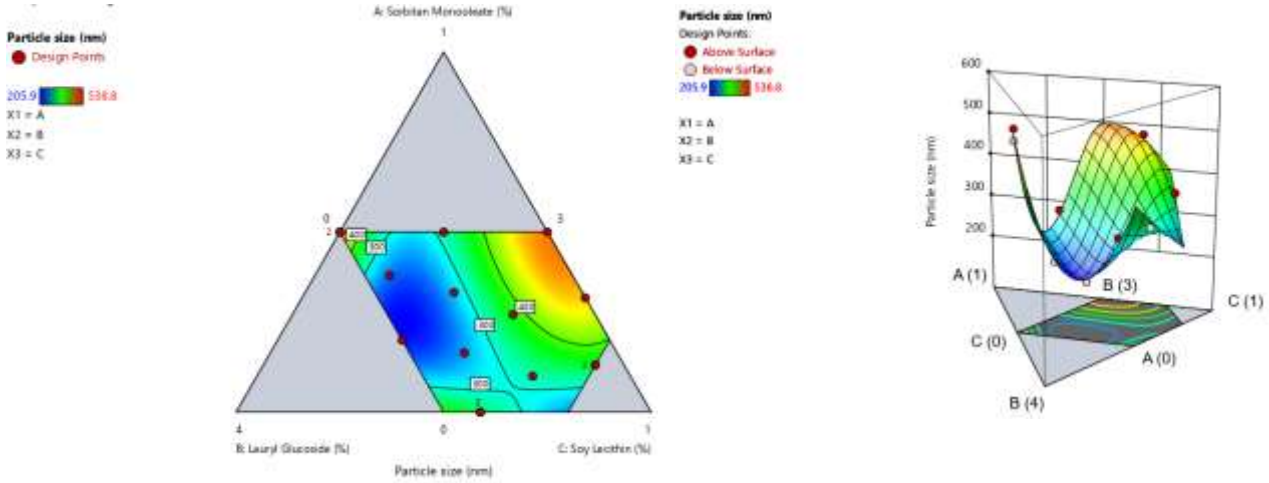
The analysis of variance (ANOVA) in Table 5 shows that the model is a linear mixture with a P-value of 0.0011; thus, there are significant differences in the particle size of each formula, and the Lack of Fit is "Not Significant" meaning that the model can be used for further research. An R^2 value of 0.9819 indicated a very strong relationship between the surfactant/co-surfactant and particle size.

Figure 1. The 15 NLC optimization formulas

Table 5. Analysis of variance (ANOVA) for particle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.337E+05	8	16712.34	18.44	0.0011	significant
[(1)]Linear Mixture	29615.96	2	14807.98	16.34	0.0037	
Residual	5436.51	6	906.09			
Lack of Fit	4552.67	3	1517.56	5.15	0.1057	not significant
R^2	0.9819					

P-ISSN: 2406-9388
E-ISSN: 2580-8303



(a) **(b)**

Figure 2. Contour plot (a), 3D surface diagram (b) of NLCs particles size

As shown in Figures 2 (a) and (b), the color gradient represents different particle sizes. The blue areas (205.9 nm) indicate smaller particles, whereas the red areas (538.8 nm) represent larger particles. The 3D plot shows how the particle size changed across the different factor levels. Widely spaced contour lines suggest a gradual change in particle size, indicating that small changes in factor levels do not significantly affect particle size.

Polydispersity index (PDI) testing

The polydispersity index (PDI) describes the homogeneity of a dispersion and ranges from 0 to 1 (Garcia et al., 2019; Hoseini et al., 2023). A PDI value close to 0 indicates a homogeneously dispersed formulation with monodisperse particles. The particle size and PDI were influenced by the surfactants. Theoretically, a polydisperse system is formed when the surfactant concentration is 100–1000 times greater than the minimum concentration required to form micelles. This is because excess surfactant leads to the elongation and formation of molecular links, thereby increasing the mobility of the tail groups. This destabilizes the aggregates, resulting in larger micelles with varying sizes. Consequently, this results in a high PDI, indicating a broad particle size distribution (Nazarova et al., 2022).

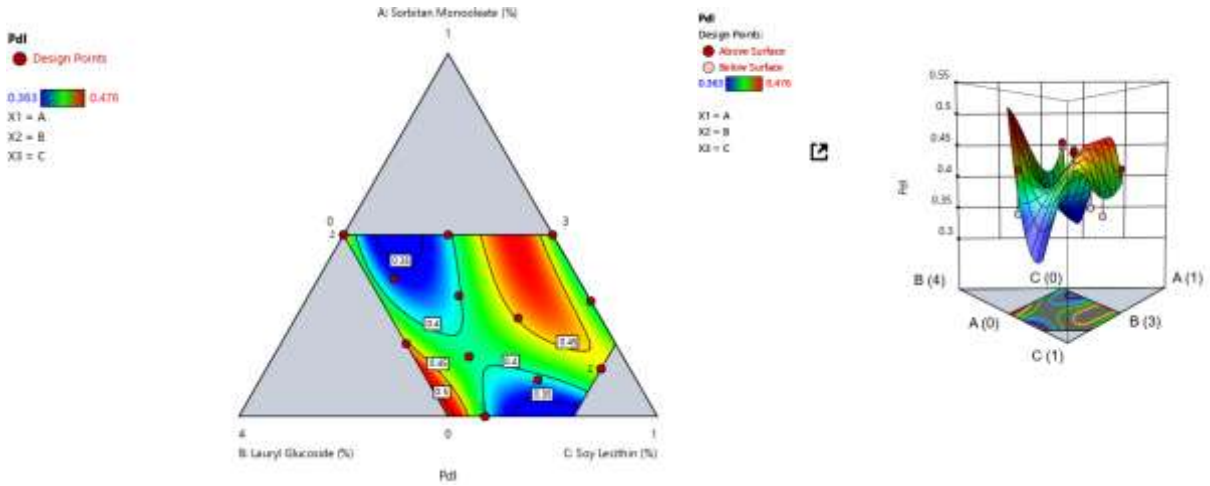
Various acceptable limits have been reported, allowing the particle size distributions to be classified as narrow, relatively broad, or broad. A PDI of 0.2 is often considered indicative of a narrow distribution, and a value of 0.5 is generally regarded as the upper limit for a relatively broad distribution (Garcia et al., 2019). Based on the experimental results, all formulations had PDI values of < 0.5. However, in this study, the upper limit of the PDI was set at 0.4. Six formulations had a PDI below 0.4, and nine formulations had a PDI above 0.4. However, variations in PDI were not significantly correlated with changes in surfactant and co-surfactant concentrations.

The analysis of variance (ANOVA) in Table 6 shows that the model is a linear mixture with a P-value of 0.4216; thus, there are no differences in the polydispersity index of each formula, and the Lack of Fit is "Not Significant" meaning that the model can be used for further research. An R^2 value of 0.6165 indicated a strong relationship between the surfactant/co-surfactant and the polydispersity index.

Table 6. Analysis of variance (ANOVA) for polydispersity index

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	0.0113	8	0.0014	1.21	0.4216	not significant
[(1)]Linear Mixture	0.0002	2	0.0001	0.0812	0.9230	
Residual	0.0070	6	0.0012			
Lack of Fit	0.0038	3	0.0013	1.16	0.4519	not significant
R^2	0.6165					

P-ISSN: 2406-9388
E-ISSN: 2580-8303



(a) **(b)**

Figure 3. Contour plot (a), 3D surface diagram (b) of NLCs polydispersity index

According to Figure 3 (a) and (b), the blue areas (0.363) indicate a lower PDI, which indicates a more uniform particle size distribution. The red areas (0.476) indicate a higher PDI, indicating a broader particle-size distribution. Widely spaced contour lines suggest a gradual change in the polydispersity index, indicating that small changes in factor levels do not drastically affect the polydispersity index.

pH testing

The pH of the Nanostructured Lipid Carrier (NLC) system is crucial for ensuring its safety and compatibility with the body, particularly in the skin and mucous membrane areas. Based on the experiment, all formulations had a pH range of 4.5–4.8, which is within the safe limits for skin and formula stability. An increase in pH was observed with an increase in lauryl glucoside concentration. This was evident in F1 and F12, which exhibited higher pH levels, although they remained within the physiological pH range of the skin.

Ethoxylated surfactants, such as sorbitan monooleate, contain carboxyl groups (–COOH) with acidic properties; thus, increasing their concentration results in a lower pH. In contrast, alkyl glycoside surfactants, such as lauryl glucoside, contain sugars and fatty alcohols and tend to have a higher pH than ethoxylated surfactants because of their hydroxyl groups (–OH). The pH of dilute lauryl glucoside is typically neutral to slightly acidic or basic; however, it does not significantly alter the overall pH of the formulation. Therefore, the use of a buffer solution is recommended to maintain a stable pH level in topical products without compromising their stability.

The ideal pH range for NLC systems intended for skin use is 4.5–5.5. Recent studies have shown that the normal pH of the skin surface is moderately acidic, typically ranging from 4.1–5.8, with an average value of 4.9. This acidic environment is vital for maintaining the physical, chemical, and microbiological barrier functions of the skin. Therefore, topical formulations designed to preserve and support the physiological pH of the skin at more acidic levels may provide significant benefits.

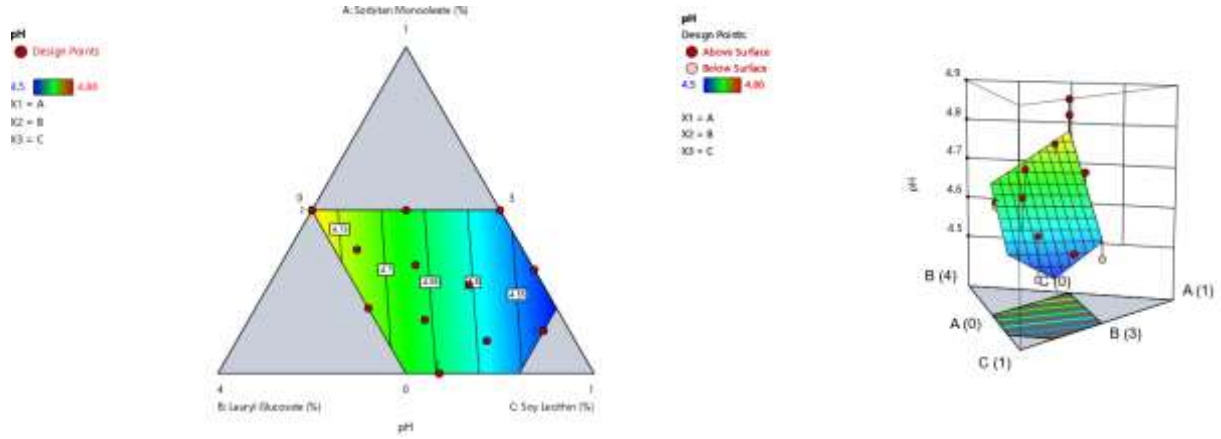
Topical formulations with low pH and sufficient buffer capacity are specifically designed to align with the skin's natural acidic environment, typically ranging from pH to 4.5-5.5. A low pH helps maintain the optimal acidic state of the skin, which is essential for preserving its barrier function and preventing microbial growth. An adequate buffer capacity ensures that the pH remains stable, even when exposed to external factors that can alter it. By combining a low pH with a robust buffer capacity, these products effectively support and stabilize the natural pH of the skin, enhance barrier integrity, promote hydration, and reduce the risk of irritation (Mehlich et al., 2021).

The analysis of variance (ANOVA) in Table 7 shows that the model is a linear mixture with a P-value of 0.0046; therefore, there are significant differences in the pH of each formula, and the Lack of Fit is “Not Significant” meaning that the model can be used for further research. The R^2 value of 0.5924 indicates a strong relationship between the surfactant/co-surfactant and the pH.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 7. Analysis of variance (ANOVA) for pH

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.1069	2	0.0534	8.72	0.0046	significant
[(1)]Linear Mixture	0.1069	2	0.0534	8.72	0.0046	
Residual	0.0736	12	0.0061			
Lack of Fit	0.0677	9	0.0075	3.86	0.1470	not significant
R^2	0.5924					



(a) **(b)**

Figure 4. Contour plot (a), 3D surface diagram (b) of NLCs pH

As shown in Figures 4 (a) and (b), the blue areas (pH 4.5) indicate a lower pH, whereas the red areas (pH 4.86) represent a higher pH. The lack of curvature and tightly

packed lines suggest that pH is relatively stable across a range of factor combinations and shows minimal sensitivity to changes in these factors.

Creaming index testing

The creaming index was assessed by monitoring the extent of creaming in emulsions. A more stable emulsion exhibits less visible phase separation, whereas an unstable emulsion exhibits more pronounced separation (Nollet et al., 2019). Creaming occurs when oil droplets rise to the surface of an emulsion because they are less dense than the water. Creaming stability refers to an emulsion's ability to resist this separation; however, the cream layer can be easily redispersed into the emulsion by shaking it (Loi et al., 2019). The creaming indices of the emulsions were determined by visual observations. An ideal emulsion has a creaming index of 0%. However, the maximum creaming index tolerated by an emulsion is 20% (Wangpradit et al. 2022).

For some formulations, an increase in the creaming index was observed as the HLB decreased. The higher the HLB, the greater the emulsion stability, as a highly hydrophilic surfactant content forms a thick layer at the interface. An increase in the interfacial film thickness indicates the presence of van der Waals forces, which form steric hindrance and prevent particle coalescence. However, the ability to reduce interfacial tension is considered more important than the HLB value alone (Yan et al., 2023).

Emulsion stability is evaluated by observing the extent of creaming, a phenomenon in which droplets of the dispersed phase rise to the top or settle at the bottom owing to gravitational forces, depending on their density relative to the continuous phase. A stable emulsion maintains a uniform distribution of droplets with minimal macroscopic phase separation, meaning that the droplets remain well dispersed without forming distinct layers over time. Conversely, the unstable emulsion exhibited significant creaming, where the droplets coalesced and separated from the continuous phase, resulting in visible layers. Thus, less creaming indicates a more stable emulsion, whereas more creaming indicates greater instability and a higher propensity for phase separation.

Based on the experimental results, the addition of a co-surfactant in optimal amounts prevented creaming, indicating better stability of the Nanostructured Lipid Carriers (NLC) compared to formulations without a co-surfactant. A co-surfactant is used to lower the interfacial tension and improve drug solubility in NLCs more effectively than surfactants alone (Javed et al., 2022). The use of co-surfactants with shorter chain lengths reduced the rigidity of the system and enhanced its flexibility. This decrease in particle size results from

P-ISSN: 2406-9388
E-ISSN: 2580-8303

the increased fluidity of the interfacial film, which promotes better intermicellar exchange, leading to smaller particle sizes (Waghule et al., 2019).

Soy lecithin has carbon chain lengths ranging from medium to long. To enhance the flexibility of the interfacial film, soy lecithin can be combined with other co-surfactants of short to medium chain lengths, such as poloxamer 188, which can further reduce particle size (Azzahrah et al., 2022).

The analysis of variance (ANOVA) in Table 8 showed that the model was a linear mixture with a P-value <0.0001; thus, there were significant differences in the creaming index of each formula, and the Lack of Fit was not calculated because the degree of freedom (df) was too small. The creaming index was identified as a number that was too small; therefore, it was not included as a response in subsequent research.

As shown in Figures 5 (a) and (b), the blue areas (0%) indicate no creaming, whereas the red areas (9.3%) indicate a higher degree of creaming or phase separation. Tightly packed contour lines suggest a steep change, indicating that the creaming index is highly sensitive to changes in the formulation factors. Thus, a minor modification in the concentration of surfactants, co-surfactants, or other components can significantly affect the stability of the NLC system.

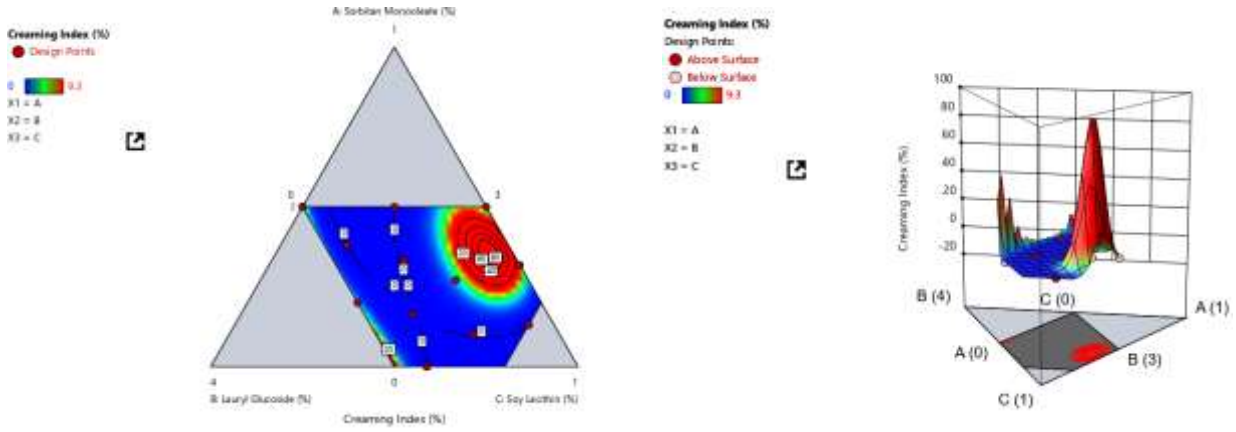
Verification of the models

A design of experiments (DoE) model was used to ensure that the solutions or formulas derived from the experiments were effective and accurate under real conditions compared to the predicted values (Vinayaka et al., 2021). The verification process involved several steps: the formulas obtained from the experiments were applied in the actual process or system to observe whether the expected results were achieved; the experiments were repeated under the same conditions used in the model to confirm that the obtained results were consistent and reproducible; and the results were compared with the predicted values from the model to verify that the difference between the predicted and actual values was minimal. This verification was crucial to ensure that the DoE model was reliable, provided real observed benefits, and enabled a more effective process optimization.

Based on the application used, the design recommended formula 1 as the optimum formula, with a desirability value of 0.976. For Equations 3 and 4, the particle sizes were not in accordance with their creaming index. Stokes' law states that the smaller the particle size, the lower the flocculation rate, which makes coalescence difficult. However, the creaming index from the observation results was still within the acceptance limit of 10%, so that the resulting emulsion was still physically stable.

Table 8. Analysis of variance (ANOVA) for creaming index

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	26.76	9	2.97	716.47	< 0.0001	significant
[(1)]Linear Mixture	8.16	2	4.08	982.61	< 0.0001	
Residual	0.0207	5	0.0041			
Lack of Fit	0.0207	2	0.0104			
R^2	0.9992					



(a) **(b)**

Figure 5. Contour plot (a), 3D surface diagram (b) of NLCs creaming index

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Figure 6. NLCs have been prepared

Table 9. Solutions of optimal formula

No.	SMO (%)	LG (%)	SL (%)	HLB	Particle size (nm)		PDI		pH		Creaming Index (%)		Desirability
					Prediction	Observed	Prediction	Observed	Prediction	Observed	Prediction	Observed	
1	0.284	3.429	0.287	14.52	210.433	220.4±11.28	0.388	0.382±0.004	4.708	4.69±0.017	0.000	0	0.976
2	0.000	3.200	0.800	14.20	253.060	258.3±9.2	0.320	0.365±0.0012	4.554	4.51±0.025	0.000	0	0.706
3	0.125	3.352	0.523	14.46	279.817	288.1±10.33	0.400	0.374±0.002	4.644	4.67±0.031	0.000	2.3	0.463
4	0.500	3.327	0.173	14.15	282.561	277.8±10.7	0.342	0.454±0.0025	4.705	4.68±0.022	0.000	2.3	0.430

P-ISSN: 2406-9388
E-ISSN: 2580-8303

CONCLUSION

The optimal formula contained 0.284% sorbitan monooleate, 3.429% lauryl glucoside, and 0.287% soy lecithin, which showed close alignment between the observed and predicted data. This formulation achieved the desired characteristics for Nanostructured Lipid Carriers (NLC), as evidenced by their homogeneous consistency, suitable particle size, pH range, and polydispersity index. The physical stability of NLC, as indicated by the creaming index, showed that this combination of surfactant and co-surfactant effectively improved the stability of NLC and produced an optimal formula for further development.

ACKNOWLEDGMENT

We thank our supervisors for their guidance and support throughout this study. We also thank the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga for providing the necessary resources and facilities.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Amasya, G., Aksu, B., Badilli, U., Onay-Besikci, A. & Tarimci, N., (2019). Qbd Guided Early Pharmaceutical Development Study: Production of Lipid Nanoparticles by High Pressure Homogenization for Skin Cancer Treatment. *International Journal of Pharmaceutics;* 563*;* 110-121. doi: 10.1016/j.ijpharm.2019.03.056.

Annisa, R., Mutiah, R., Yuwono, M. & Hendradi, E. (2022). The Development Formulation of *Eleutherine palmifolia* Extract-Loaded Self-Nanoemulsifying Drug Delivery System (SNEDDS) using D-Optimal Mixture Design Approach. *International Journal of Applied Pharmaceutics*; *15*; 1-8. doi: 10.22159/ijap.2023v15i5.47645.

Apostolou, M., Assi, S., Fatokun, A.A. & Khan, I., (2021). The Effects of Solid and Liquid Lipids on the Physicochemical Properties of Nanostructured Lipid Carriers. *Journal of Pharmaceutical Sciences;* 110*;* 2859-2872. doi: 10.1016/j.xphs.2021.04.012.

Azzahrah, R., Rosita, N., Purwanto, D.A. & Soeratri, W. (2022). The Effect of Decyl Glucoside on Stability and Irritability of Nanostructured Lipid Carriers-Green Tea Extract as Topical Preparations. *Pharmacy & Pharmaceutical Sciences Journal/Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia; 9;* 220-228. doi: 10.20473/jfiki.v9i32022.220-228.

Bhoyrul, B., Solman, L., Kirk, S., Orton, D. & Wilkinson, M. (2019). Patch Testing with Alkyl Glucosides: Concomitant Reactions are Common but not Ubiquitous. *Contact Dermatitis; 80(5);* 286-290. doi: 10.1111/cod.13186.

Das, R., Mallik, N., Adhikari, A. & Bhattarai, A. (2024). A Comprehensive Review on the Creation, Description, and Utilization of Surfactants Containing Multiple Hydroxyl Groups. *International Journal of Polymer Science; 2024;* 1-31. doi: 10.1155/2024/6120535.

Garcia, N.O., Fernandes, C.P. & da Conceição, E.C. (2019). Is it Possible to Obtain Nanodispersions with Jaboticaba Peel's Extract Using Low Energy Methods and Absence of Any High Cost Equipment? *Food Chemistry; 276;* 475-484. doi: 10.1016/j.foodchem.2018.10.037.

Ghasemiyeh, P., Azadi, A., Daneshamouz, S., Heidari, R., Azarpira, N. & Mohammadi-Samani, S. (2019). Cyproterone Acetate-Loaded Nanostructured Lipid Carriers: Effect of Particle Size on Skin Penetration and Follicular Targeting. *Pharmaceutical Development and Technology; 24;* 812-823. doi: 10.1080/10837450.2019.1596133.

Haji, M., Hosseinzadeh, M., Jahangiri, Y., Farajzadeh, Z., Sufiani, A.M., Olyaei, Y.A., Namarvar, B.B., Ghaffari, T. & Olyaei, N.A. (2023). Ghee Based Nanostructured Lipid Carriers (Nlcs) with Improved Wound-Healing Effects: Ghee Based Nanostructured Lipid Carriers (Nlcs). *Iranian Journal of Pharmaceutical*

Sciences; 19; 293-303. doi: 10.22037/ijps.v19i4.42194.

Hoseini, B., Jaafari, M.R., Golabpour, A., Momtazi-Borojeni, A.A., Karimi, M. & Eslami, S. (2023). Application of Ensemble Machine Learning Approach to Assess the Factors Affecting Size and Polydispersity Index of Liposomal Nanoparticles. *Scientific Reports*; *13;* 1-11. doi: 10.1038/s41598-023-43689-4.

Jafar, G., Salsabilla, S. & Santoso, R. (2022). Development and Characterization of Compritol Ato® Base in Nanostructured Lipid Carriers Formulation with the Probe Sonication Method. *International Journal of Applied Pharmaceutics; 14;* 64-66. doi: 10.22159/ijap.2022.v14s4.PP04.

Javed, S., Mangla, B., Almoshari, Y., Sultan, M.H. & Ahsan, W. (2022). Nanostructured Lipid Carrier System: A Compendium of Their Formulation Development Approaches, Optimization Strategies by Quality by Design, and Recent Applications in Drug Delivery. *Nanotechnology Reviews; 11;* 1744-1777. doi: 10.1515/ntrev-2022-0109.

Lechuga, M., Avila-Sierra, A., Lobato-Guarnido, I., García-López, A.I., Ríos, F. & Fernández-Serrano, M. (2023). Mitigating the Skin Irritation Potential of Mixtures of Anionic and Non-Ionic Surfactants by Incorporating Low-Toxicity Silica Nanoparticles. *Journal of Molecular Liquids; 383;* 1-10. doi: 10.1016/j.molliq.2023.122021.

Loi, C.C., Eyres, G.T. & Birch, E.J. (2019). Effect of Mono-and Diglycerides on Physical Properties and Stability of a Protein-Stabilised Oil-In-Water Emulsion. *Journal of Food Engineering; 240;* 56-64. doi: 10.1016/j.jfoodeng.2018.07.016.

Mayangsari, F.D., Erawati, T., Soeratri, W. & Rosita, N. (2021). Karakteristik dan Stabilitas Fisik NLC-Koenzim Q10 dalam Sleeping Mask dengan Minyak Nilam. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia; 8;* 178-186. doi: 10.20473/jfiki.v8i22021.178-186.

Mohammadi, M., Assadpour, E. & Jafari, S.M. (2019). Encapsulation of Food Ingredients by Nanostructured Lipid Carriers (Nlcs). in Jafari, S.M. (Ed.). *Lipid-based Nanostructures for Food Encapsulation Purposes*. *Academic Press; 2;* 217-270 doi: 10.1016/B978-0-12-815673-5.00007-6.

Moulik, S.P., Rakshit, A.K. & Naskar, B. (2024). Physical Chemical Properties of Surfactants in Solution and Their Applications: A Comprehensive Account. *Journal of Surfactants and Detergents; 27;* 895-925. doi: 10.1002/jsde.12757.

Nazarova, A., Yakimova, L., Filimonova, D. & Stoikov, I. (2022). Surfactant Effect on the Physicochemical Characteristics of Solid Lipid Nanoparticles Based on Pillar [5] Arenes. *International Journal of Molecular Sciences; 23;* 1-14. doi: 10.3390/ijms23020779.

Nollet, M., Boulghobra, H., Calligaro, E. & Rodier, J.D. (2019). An Efficient Method to Determine The Hydrophile‐Lipophile Balance of Surfactants Using the Phase Inversion Temperature Deviation of Ciej/N‐Octane/Water Emulsions. *International Journal of Cosmetic Science; 41;* 99-108. doi: 10.1111/ics.12516.

Pavoni, L., Perinelli, D.R., Ciacciarelli, A., Quassinti, L., Bramucci, M., Miano, A., Casettari, L., Cespi, M., Bonacucina, G. & Palmieri, G.F., (2020). Properties and Stability of Nanoemulsions: How Relevant is the Type of Surfactant?. *Journal of Drug Delivery Science and Technology; 58;* 1-9. doi: 10.1016/j.jddst.2020.101772.

Rohmah, M., Raharjo, S., Hidayat, C. & Martien, R. (2019). Formulasi dan Stabilitas Nanostructured Lipid Carrier dari Campuran Fraksi Stearin dan Olein Minyak Kelapa Sawit. *Jurnal Aplikasi Teknologi Pangan*; *8;* 23-30. doi: 10.17728/jatp.3722.

Seweryn, A., Klimaszewska, E. and Ogorzałek, M., 2019. Improvement in the Safety of Use of Hand Dishwashing Liquids Through the Addition of Sulfonic Derivatives of Alkyl Polyglucosides. *Journal of Surfactants and Detergents; 22;* 743-750. doi: 10.1002/jsde.12299.

Sar, P., Ghosh, A., Scarso, A. & Saha, B. (2019). Surfactant for Better Tomorrow: Applied Aspect of Surfactant Aggregates from Laboratory to Industry. *Research on Chemical Intermediates; 45;* 6021-6041. doi: 10.1007/s11164-019-04017-6.

Shinde, U.A., Parmar, S.J. & Easwaran, S. (2019). Metronidazole-Loaded Nanostructured Lipid Carriers to Improve Skin Deposition and

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Retention in the Treatment Of Rosacea. *Drug Development and Industrial Pharmacy; 45;* 1039-1051. doi: 10.1080/03639045.2019.1569026.

Suzliana, R.M., Erawati, T., Prakoeswa, C.R.S., Rantam, F.A. and Soeratri, W. (2020). Pengaruh Asam Hialuronat-Space Peptide terhadap Karakteristik, Stabilitas Fisik Gel Amniotic Membran-Stemcell Metabolite Product. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*; *7;* 66-73. doi: 10.20473/jfiki.v7i22020.66-73.

Tabaniag, J.S.U., Abad, M.Q.D., Morcelos, C.J.R., Geraldino, G.V.B., Alvarado, J.L.M. & Lopez, E.C.R. (2023). Stabilization of Oil/Water Emulsions Using Soybean Lecithin as a Biobased Surfactant for Enhanced Oil Recovery. *Journal of Engineering and Applied Science; 70;* 1-26. doi: 10.1186/s44147-023-00322-5.

Tan, C.C., Karim, A.A., Uthumporn, U. & Ghazali, F.C. (2020). Effect of Thermal Treatment on the Physicochemical Properties of Emulsion Stabilized by Gelatin From Black Tilapia (Oreochromis Mossambicus) Skin. *Food Biophysics; 15;* 423-432. doi: 10.1007/s11483-020-09638-8.

Vinayaka, A.S., Mahanty, B., Rene, E.R. & Behera, S.K. (2021). Biodiesel Production by Transesterification of a Mixture of Pongamia and Neem Oils. *Biofuels; 12;* 187-195. doi: 10.1080/17597269.2018.1464874.

Waghule, T., Rapalli, V.K., Singhvi, G., Manchanda, P., Hans, N., Dubey, S.K., Hasnain, M.S. & Nayak, A.K. (2019). Voriconazole Loaded Nanostructured Lipid Carriers Based Topical Delivery System: Qbd Based Designing, Characterization, in-Vitro and ex-Vivo Evaluation. *Journal of Drug Delivery Science and Technology; 52;* 303-315. doi: 10.1016/j.jddst.2019.04.026.

Wang, Q., Zhang, H., Han, Y., Cui, Y. & Han, X. (2023). Study on the Relationships Between the Oil HLB Value and Emulsion Stabilization. *RSC Advances; 13;* 24692-24698. doi: 10.1039/D3RA04592G.

Wangpradit, N., Macha, S., Phooteh, N., Yusohyo, N., Waedoloh, A. & Manee, S. (2022). Determination of Required Hydrophilic-Lipophilic Balance of Amesiodendron Chinense (Merr.) Hu Oil and Development of Stable Cream Formulation. *OCL; 29;* 1-8. doi: 10.1051/ocl/2022011

Warshaw, E.M., Xiong, M., Atwater, A.R., DeKoven, J.G., Pratt, M.D., Maibach, H.I., Taylor, J.S., Belsito, D.V., Silverberg, J.I., Reeder, M.J. & Zug, K.A. (2022). Patch Testing with Glucosides: The North American Contact Dermatitis Group Experience, 2009-2018. *Journal of the American Academy of Dermatology; 87;* 1033-1041. doi: 10.1016/j.jaad.2022.04.058.

Wieczorek, D. & Kwasniewska, D. (2020). Novel Trends in Technology of Surfactants. In: Chemical Technologies and Processes 223-250. Berlin: Walter de Gruyter GmbH.

Yan, G., Wang, S., Li, Y., He, L., Li, Y. & Zhang, L. (2023). Effect of Emulsifier HLB on Aerated Emulsions: Stability, Interfacial Behavior, and Aeration Properties. *Journal of Food Engineering; 351;* 1-8. doi: 10.1016/j.jfoodeng.2023.111505.

P-ISSN: 2406-9388
E-ISSN: 2580-8303