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Original Research Article

Optimization of Nanostructured Lipid Carriers (NLC) Extract *Crude Fucoidan Algae Brown* (*Sargassum chrysanthemum*): Design and Characterization

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ABSTRACT

Brown algae contain bioactive components *meroterpenoids, fucoidan, and phlorotannins*. Although research has explored the effects of fucoidan on cell differentiation and proliferation, studies on its topical application for hair growth, particularly in the treatment of androgenetic alopecia, remain limited. *The crude fucoidan extract from brown algae* has low solubility in water and acidic environments and possesses a high molecular weight, factors that reduce its skin penetration efficacy due to increased viscosity. This study aimed to develop a Nanostructured Lipid Carrier (NLC) formulation of *brown algae fucoidan extract* to enhance skin penetration, targeting hair follicles and dermal papilla cells, while maintaining high physical quality and stability. The NLC formulation of *brown algae fucoidan* using the emulsification sonication method with monostearin, oleic acid, and tween 80. The optimization method used is Simplex Lattice Design (SLD). Optimization parameters included particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, and morphology analyzed by transmission electron microscopy (TEM). Formula optimization and analysis were conducted using Design Expert software (version 10.0.1) and validated by one sample T-test. The results showed that NLC has good characterization with optimum formula components of monostearin, oleic acid and Tween 80 of 2.01%, 2.20% and 7.79%. Predicted responses yielded a mean pH of 5.10, particle size of 452.52 nm, PDI of 0.43, and entrapment efficiency of 94.83%. Verification of the optimized formula showed no significant differences in pH, particle size, or PDI, though enntrapment efficiency varied significantly. The zeta potential was measured at -23.08 mV, and TEM analysis confirmed a spherical morphology.

Keywords: *crude fucoidan extract brown algae*, Nanostructured Lipid Carriers (NLC), Simple Lattice Design (SLD), particle size, transmission electron microscopy (TEM)

Introduction

Sargassum species contain a variety of bioactive components with diverse functional properties, including *meroterpenoids, fucoidan (sulfated polysaccharides), and phlorotannins (polyphenols)*[1]. *Fucoidan*, a fucose-rich *sulfated polysaccharide*, can be extracted from *brown algae*, such as *Sargassum sp*.[2]. Previous studies, such as those by C.-Y. Huang, have analyzed *Sargassum glaucescens* extracts, which contain fucoidan compounds with a molecular weight exceeding 1 kDa. This study has shown that *Sargassum* extract can enhance hair growth and nourish hair follicles, with findings indicating that a concentration of 1 mg/mL of *Sargassum glaucescens* extract in mice can influence the signal transduction pathway for dermal papilla cell proliferation and hair growth factor-related gene expression [3].

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The high molecular weight of *fucoidan* affects cellular activation timing and influences skin penetration, as increased molecular size and viscosity hinder its permeability [3]. Effective drug delivery targeting hair follicles (HF) requires overcoming barriers in the dermis and subcutaneous fat beneath the stratum corneum (SC). These follicles are associated with lipid-rich sebum secreted by sebaceous glands, providing a hydrophobic barrier that protects both hair and skin. Thus, this research aims to enhance fucoidan penetration, reduce viscosity, and minimize pH sensitivity by incorporating it into a lipid nanoparticle drug delivery system, specifically Nanostructured Lipid Carriers (NLC) [4].

Lipid-based drug delivery systems, including solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), offer distinct advantages. Compared to SLN, NLC enhances drug absorption efficacy, promotes controlled release, and reduces drug leakage [5,6]. NLCs are designed to improve the dispersion of hydrophobic bioactive compounds in hydrophilic systems, thereby increasing stability and bioavailability [7]. As a second-generation lipid carrier, NLC combines solid and liquid lipids, creating additional space within the solid lipid matrix, which enhances drug loading capacity. The nanometer-scale particle size of NLCs allows for close interaction with the stratum corneum, promoting penetration, occlusion, and targeted drug accumulation in the dermal layer, making it ideal for topical delivery [8]. Critical to the efficacy of NLCs is the careful selection of lipid-phase components, which must be optimized for melting point, crystal structure, viscosity, and polarity [9].

Lipids serve as the fundamental framework in forming Nanostructured Lipid Carriers (NLC), significantly influencing their final characteristics, particularly in terms of stability. Solid lipids play a crucial role in enhancing system stability; in this study, monostearin (glyceryl monostearate) was selected as the solid lipid. Monostearin offers distinct advantages over other solid lipids, such as glyceryl behenate and cetyl palmitate, as it has a stable polymorphic form with low potential for polymorphic transformation [10]. Solid lipids will be combined with liquid lipids, one of the liquid lipids that is often used in the combination of NLC lipid matrices is oleic acid. The use of oleic acid as a liquid lipid plays an important role in reducing the crystallization process and is the main factor that affects the speed of release of active ingredients and the efficiency of drug entrapment in the NLC system [11]. Hence, this research aims to investigate the formulation of an NLC system containing a crude fucoidan extract, targeting androgenetic alopecia treatment. The formulation is optimized and characterized for physical properties and morphology using the Design Expert software (version 10.0.1) with a Simple Lattice Design (SLD).

Materials and Methods

The equipment used in this study includes Particle Size Analyzer (HORIBA SZ-100), Zeta Nanosizer (Zetasizer), Hot Plate Magnetic Stirrer (Thermo Scientific, China), pH Meter (Trans Instrument HP 9000), Sonicator (Elma Transsonic 570), IKA Ultra-Turrax T18, centrifuge (Gemmyco), UV-Vis Spectrophotometer (Shimadzu 1240), FT-IR (Agilent Technologies Cary 630 FTIR), and Transmission Electron Microscope (TEM) (JEOL/EO JEM-1400 version 1.0).
Materials include the *crude fucoidan extract from brown algae* collected in August 2023, glyceryl monostearate (Rikevita (Malaysia) SDN. BHD), oleic acid and Tween 80 as solid lipid, liquid lipid, and surfactant components, PEG 400 (Bratachem, Indonesia), and distilled water.

***Brown Algae Crude Fucoidan** Extraction*
Brown algae were collected in August 2023 at Empu Rancak Beach, Karang Gondang Village, Mlonggo District, Jepara Regency, Central Java 59452, Indonesia. *Brown algae* samples were identified by the Department of Pharmaceutical Biology, Faculty of Pharmacy, UGM, as the type *Sargassum cristaefolium C. Agardh*. The extraction process for obtaining *crude fucoidan* from *brown algae* followed a modified digestion method based on established protocols [12,13] and recent studies [14]. Ground, dried *brown algae* were soaked in distilled water at a ratio of 1:20 (w/v) and stirred continuously at 85°C for 4 hours. The resulting mixture was filtered through flannel cloth, collecting the filtrate. Solution $CaCl_2$ 2% was added to the filtrate at a 1:20 ratio while stirring at room temperature for 30 minutes, followed by centrifugation at 8,000 rpm for 15 minutes. The supernatant was retained, and the sediment was discarded. Ethanol was added to the filtrate in a 1:2 ratio, and the sediment obtained was dissolved in water until fully dissolved, then centrifuged again at 8,000 rpm for 15 minutes. Finally, the resulting solution was freeze-dried to yield the *crude fucoidan extract algae brown.*

Optimization of Experimental Design and Run Determination Using Simplex Lattice Design (SLD)
The optimal formulation for the NLC system containing *crude fucoidan extract from brown algae* was determined using the Simplex Lattice Design (SLD) approach via Design Expert software (version 10.0.1). This process involved experimental design, model analysis, prediction, and validation. Independent variables, specifically monostearin, oleic acid, and tween 80, were used as factors to optimize the formulation. Lower and upper concentration limits were set at 2% - 4% for monostearin, 1% - 3% for oleic acid, and 7% - 9% for tween 80. Response parameters for determining the NLC's physical properties included pH, particle size, polydispersity index (PDI), and adsorption efficiency. The formula for the results of determining the lower and upper limit values is shown in Table 1.
Table 1. SLD optimization design parameters used for formulating the NLC with *crude fucoidan extract brown algae* in Design Expert version 10.0.1.

Table 1: Simplex Lattice Design (SLD) formula NLC Optimization of *Brown Algae Fucoidan Crude Extract*

Formula	Run (%)												
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**
Fucoidan crude extract	1	1	1	1	1	1	1	1	1	1	1	1	1
Monostearin	2	2	2	3.33	2.67	2.33	2	2.33	3	4	2	4	2
Oleic Acid	3	1	3	1.33	1.67	2.33	2	1.33	1	1	1	1	2
Tweens 80	7	9	7	7.33	7.67	7.33	8	8.33	8	7	9	7	7
PEG 400	2	2	2	2	2	2	2	2	2	2	2	2	2
Aquadest ad	100	100	100	100	100	100	100	100	100	100	100	100	100

Preparation Method
The crude fucoidan extract of brown algae NLC was formulated using the melt emulsification method, the formula can be seen in table 1. Solid lipids (monostearin) and liquid lipids (oleic acid) were melted on a hot plate at a temperature of 65±5°C. After liquefaction, the *crude fucoidan extract brown algae* was incorporated into the lipid matrix. A pre-heated solution of tween-80, PEG 400, and distilled water was prepared at 65°C and subsequently emulsified with the lipid phase using an Ultra-Turrax homogenizer at 3400 rpm for 30 minutes. This mixture was then sonicated for 30 minutes to achieve further emulsification and nanoscale dispersion. The final NLC product was weighed to determine yield, followed by characterization of its physical properties, including pH, particle size, polydispersity index (PDI), and entrapment efficiency. For pH measurement, a calibrated pH meter was utilized. Nanoparticle distribution and size were determined using a Particle Size Analyzer (PSA) by diluting a 1 mL sample with 10 mL of mineral water at ambient temperature. Entrapment efficiency was assessed by adding 1 mL of NLC sample to 10 mL of ethanol in a test tube, centrifuging at 6000 rpm for 30 minutes, filtering the supernatant, and diluting it 25-fold. The resulting solution was analyzed using a UV-Vis spectrophotometer at a wavelength of 490 nm [15,10,16].

*Optimization of the Optimum NLC Formula of Brown **Algae Fucoidan Crude Extract***
To identify the optimal NLC formulation for *crude fucoidan extract brown algae*, the Simplex Lattice Design method was applied using Design Expert software version 10.0.1. This involved processing physical properties data from 13 experimental formulations. The optimization focused on monostearin, oleic acid, and tween-80 as independent variables, with pH, particle size, PDI, and entrapment efficiency as response parameters. The optimal formula was determined based on the desirability function, ranging from 0 to 1, with values closer to 1 indicating a more favorable outcome in meeting the targeted parameters. Optimization aimed to identify conditions that satisfy all response objectives [17].

Verification of the Optimum NLC Formula of ***Brown Algae Fucoidan Crude Extract***

Verification of the optimal formula was conducted by comparing the predictive software results with empirical testing across five replicates. The physical properties assessed included pH, particle size, PDI, entrapment efficiency, zeta potential, and morphological analysis via Transmission Electron Microscopy (TEM). The predicted physical properties of the optimal formula were compared with the experimentally derived properties using a one sample T-test with a 95% confidence interval. Data analysis was performed using IBM SPSS Statistics Version 23 software.

Statistical Analysis

The physical characteristics of the NLC prepared with *crude fucoidan extract from brown algae* were evaluated across 13 different formulations (Run 1 to Run 13) and analyzed using Design Expert software version 10.0.1, employing the Simplex Lattice Design method to determine the optimal formula. The selected optimal formula was then reproduced, and the same set of physical characterization tests was applied to validate consistency. Results of the optimum formula were compared with theoretical predictions generated by Design Expert, and validation of the optimization was performed using a one-sample T-test in IBM SPSS Statistics version 23 to confirm the accuracy and reliability of the optimized results.

Results and Discussion

Formulation of NLC from Crude Fucoidan Extract of Brown Algae

Nanostructured Lipid Carriers (NLC) are lipid-based delivery systems composed of a blend of solid and liquid lipids, resulting in a partially crystallized matrix. These carriers offer several advantages over solid lipid nanoparticles, including enhanced drug-loading capacity, flexible modulation of drug release, and improved stability. Lipid nanoparticles generally consist of a lipid matrix that remains solid at body temperature [18,19].

The NLC formulation containing the *brown algae crude fucoidan extract* was prepared using the emulsification method to achieve nanoscale particle sizes (10–1000 nm). Compared to other methods, emulsification offers simplicity and efficient drug entrapment [20,16]. During the manufacturing process, an ultra-turrax high-shear homogenizer was employed, which functions by grinding and dispersing particles within a mixture of lipids, surfactants, and water, maintained at a temperature 5–10°C above the lipid melting point, to form an emulsion. This emulsion is subsequently sonicated to further reduce particle size [21]. High-pressure homogenization [16] is a widely utilized technique in the pharmaceutical industry for producing nutrient emulsions [21], making it suitable for large-scale production. Sonication promotes the formation of nanoparticles by applying sound energy, which generates a cavitation effect that disaggregates particle agglomeration [23].

The incorporation of solid lipids in NLC formulations helps minimize molecular diffusion, thereby enhancing the retention and chemical stability of bioactive components. In contrast, liquid lipids improve the encapsulation efficiency of bioactive compounds [24]. This research employed optimized materials: monostearin (solid lipid), oleic acid (liquid lipid), and tween 80 (surfactant). Monostearin, with its relatively irregular crystal lattice, supports high encapsulation efficiency. The crystal structure of monostearin includes hexagonal, orthorhombic, and triclinic arrangements, indicating the presence of polymorphism [25]. Prior studies by [11] and [26] suggests that oleic acid plays a critical role in limiting crystallization, thus altering the structural regularity of stearic acid crystals, which significantly impacts the release rate of active ingredients in the NLC system. The distinct melting points of solid and liquid lipids facilitate an earlier crystallization of solid lipids, positioning liquid lipids at the matrix periphery along with the drug substance, creating a drug-enriched shell that promotes an immediate release profile. Structurally, oleic acid ($C_{18}H_{34}O_2$) is a straight-chain fatty acid with 18 carbon atoms and a carboxylate group at one end. It is a monounsaturated fatty acid, which enhances the stability of emulsions in the system, thus supporting greater NLC stability [27].

The formulation and testing of NLC preparations containing *crude brown algae fucoidan extract* were conducted using Design Expert Software, applying the Simplex Lattice Design (SLD) method. The results from 13 experimental runs indicated that all NLC formulations exhibited a thick consistency, a brownish hue, and maintained stability without solidification during storage (see Figure 1 and Table 2).

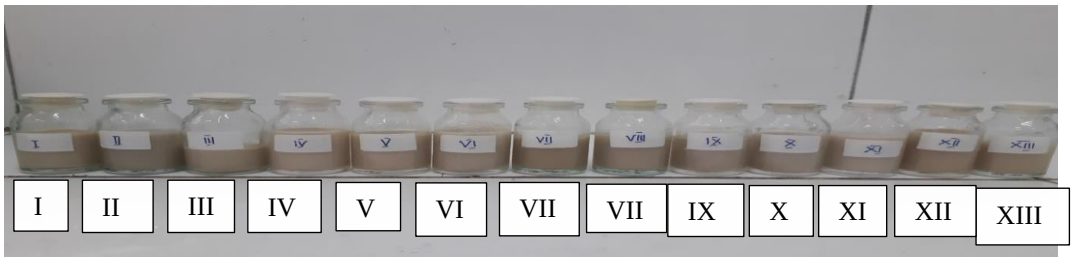

Figure 1: Run formula for NLC *extract crude fucoidan brown algae*

The crude extract of fucoidan exhibits low solubility in water, as the medium for the adsorption of the active ingredient is the lipid phase. As a result, a more intense coloration is observed in lipids at lower concentrations, leading to an increased dissolution of the compound in the aqueous phase. Conversely, an elevated lipid phase concentration results in a greater absorption of compounds, which diminishes the intensity of the coloration. Visual observations indicate that the consistency of the nanostructured lipid carrier (NLC) formulation is significantly influenced by lipid concentration, with the preparation becoming more solid as the lipid concentration increases. For the assessment of homogeneity, a visual inspection was conducted to determine the uniformity of the NLC formulation. The findings revealed the absence of lumps or phase separation, suggesting that the preparation is homogeneous, likely due to adequate mixing of the components and sufficient emulsifier content to stabilize the emulsion [28].

The ratio of liquid lipid concentration that was highest yielded the smallest particle size. This phenomenon may be attributed to the potential effects of increased solid lipid concentrations, which could lead to liquefaction and the subsequent formation of agglomerates during NLC preparation. Moreover, a higher concentration of solid lipids may predispose the formulation to coalescence or aggregation during the solidification phase. Such aggregates may resist disaggregation, resulting in the formation of larger particles characterized by a broad size distribution, as indicated by an increasing polydispersity index (PDI) with increased solid lipid ratios [6].

The pH measurements obtained across all experimental runs conform to the scalp product specifications outlined in SNI 16-4955-1998, which stipulate a pH range of 3-7 [29]. The pH regression equation presented in Table 3 indicates that all components and their combinations influence the pH value, with the exception of the interaction between monostearin and tween 80, which yields a negative response. The regression coefficient for tween 80 is notably high, indicating that it exerts the most substantial influence on the increase in pH compared to other components. This may be attributable to the stability of monostearin at elevated temperatures, which can lead to an increase in acidity and consequently a notable reduction in pH. The pH contour plot depicted in Figure 2A illustrates a gradient from low pH (represented by blue) to

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high pH (represented by red), highlighting the dominance of monostearin in elevating pH levels, while higher concentrations of tween 80 correlate with decreased pH levels. It is important to note that monostearin has a pH range of 8-10, oleic acid has a pH of 4.4, and tween 80 surfactant exhibits a pH range of 5-7 [30].

Table 2: Results of the NLC Run test response of *crude fucoidan brown algae extract* based on *Simplex Lattice Design using Design Expert software version 10.0.1*

Run	Monostearin (%)	Oleic Acid (%)	Tween 80 (%)	pH	Particle size (nm)	PDI	Entrapment Efficiency (%)
1	2	3	7	4.28	718.1	0.43	96,02
2	2	1	9	4.54	705.2	0.75	94,99
3	2	3	7	4.5	726.2	0.47	97,19
4	3.33	1.33	7.33	5.12	728.1	0.68	93,55
5	2.67	1.67	7.67	4.77	265.5	0.52	95,93
6	2.33	2.33	7.33	5.05	720	0.43	96,91
7	2	2	8	5.12	417	0.53	94,43
8	2.33	1.33	8.33	4.55	541.4	0.45	95,63
9	3	1	8	5.04	718	0.43	97,17
10	4	1	7	4.55	559.6	0.54	94,55
11	2	1	9	4.6	770.7	0.74	95,17
12	4	1	7	4.38	437.8	0.46	93,69
13	3	2	7	4.11	1169	0.80	92.99

The results of the particle size test for runs 1-12 of the nanostructured lipid carrier (NLC) formulation utilizing the *crude extract of brown algae fucoidan* conformed to the established parameter standards. In contrast, run 13 exhibited a particle size response that exceeded the specified limit of 1000 nm, failing to meet the nanoscale requirements, which are defined as 10-1000 nm [31]. Particle size is a critical characteristic of NLCs, as it influences the homogeneity of the formed system, drug entrapment efficacy, drug release kinetics, membrane penetration, and the overall stability of the NLC formulation. According to the particle size equation presented in Table 3, the coefficients associated with each component monostearin, oleic acid, tween 80, and their combinations demonstrated a negative response, indicating that these components contribute to a reduction in particle size. Conversely, the coefficients of certain mixtures exhibited a positive response, suggesting that these combinations can increase particle size. Notably, oleic acid displayed a higher coefficient value than the other components, underscoring its predominant role in reducing particle size.

Table 3: *Simplex Lattice Design* Formula NLC *extract crude fucoidan algae brown*

Response Optimization	Optimization Equation	P-Value	Model	Information
pH	Y = 4.47 (A)+ 4.40 (B) + 4.58 (C)- 1.17 (AB) + 2.19 (AC) + 2.66 (BC) + 0.79 (ABC)	0.0474	Cubic	Significant
Particle size	Y = -10146.16 (A) – 18272.25 (B) – 1049.81 (C) + 11101.53 (AB) + 1620.57 (AC) + 2714.13 (BC) -1502.48 (ABC)	0.0070	Special cubic	Significant
PDI	Y = 0.89 (A) + 0.03 (B) + 0.38 (C) + 0.30 (AB) – 0.21 (AC) – 0.14 (BC)	0.0223	Quadratic	Significant
EE	Y = 94.12 (A) + 96.60 (B) + 95.08 (C) – 9.53 (AB) + 10.23 (AC) – 5.69 (BC) – 1.06 (ABC)	0.0206	Special cubic	Significant

Oleic acid, a liquid lipid, significantly influences the reduction of particle size due to its partition coefficient value exceeding 6.5, which facilitates its binding to the lipophilic groups of other compounds [32]. In addition, the cosurfactant properties [30] of monostearin contribute to this effect. The presence of monostearin in the formulation lowers the interfacial tension between the lipid matrix and the aqueous phase, thereby further decreasing the particle size of the NLC. Increasing the surfactant concentration usually causes a decrease in the particle size of lipid nanoparticles, that is, if a higher surfactant/lipid ratio is chosen, the particle size obtained will be smaller. The smaller particle size enhances the interaction between the drug substance and the biological membrane, resulting in accelerated penetration [10]. The contribution of tween 80 to particle size reduction is also noteworthy, as it comprises 18 hydrophobic chains that influence the solubility of the drug in aqueous environments. According to the Gibbs equation, a substantial reduction in surface tension correlates with a significant decrease in surface free energy, resulting in smaller droplet sizes [33]. The concentration of surfactants can affect the size of the droplets; higher surfactant concentrations effectively decrease globule size by lowering surface tension, stabilizing smaller particles through steric hindrance, and preventing aggregation into larger particles.

Figure 2B illustrates that the blue coloration indicates smaller particle sizes, while the progression from green to yellow and red denotes

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increasing particle sizes. The variation in liquid lipid concentration directly impacts the particle size of the NLC formulation. Specifically, as the ratio of solid lipid to liquid lipid diminishes, particle size increases. The inclusion of liquid lipids in the formulation contributes to size reduction, whereby an increased concentration of liquid lipids correlates with smaller NLC particle sizes [34]. The addition of liquid lipids to the formula plays a role in reducing the size, by increasing the concentration of liquid lipids, the size of NLC particles decreases. It has also been reported that the addition of liquid lipids to solid lipids tends to encourage the formation of small particles, which may be caused by increased mobility of the lipid phase matrix after the addition of liquid lipids.

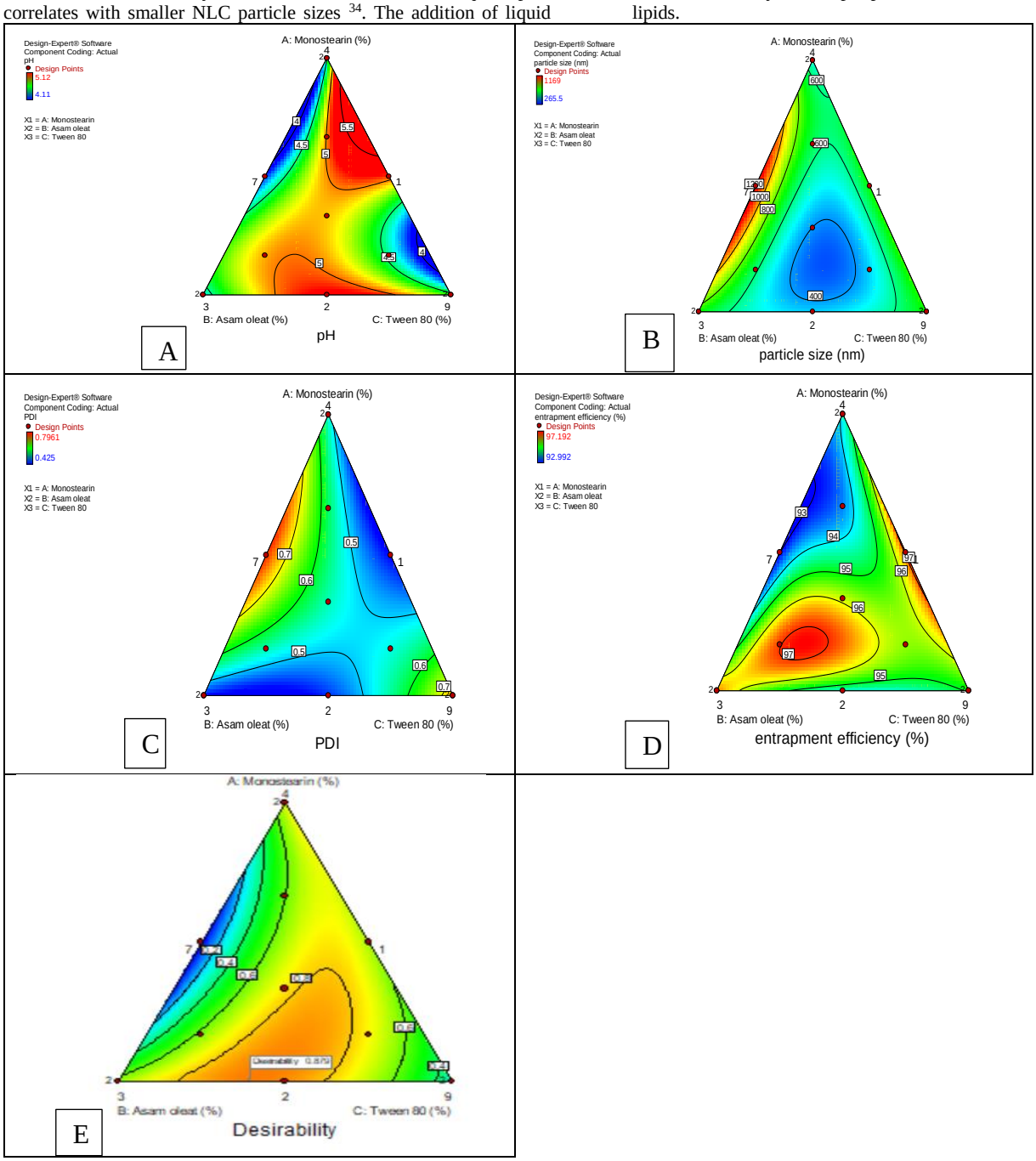

Figure 2: Contour plot graph of the test results of the response (A) pH response, (B) particle size response, (C) PDI response, (D) Entrapment efficiency, (E) Desirability of the Optimum NLC Formula of *Brown Algae Fucoidan Crude Extract*

The polydispersity index (PDI) value of the nanostructured lipid carrier (NLC) formulated with *crude extracts of brown algae fucoidan* ranged from 0.4 to 0.5, remaining below the threshold of 0.7, which indicates a monodisperse and homogeneous system [35]. PDI serves as a metric for assessing the uniformity within a system; thus, lower PDI values signify a more uniform particle distribution within a monodisperse system [36]. The coefficient equation presented in Table 4 reveals that the components monostearin, oleic acid, tween 80, and their mixture positively influence the PDI, resulting in an increase in the polydispersity value. Conversely, the mixtures of monostearin with tween 80 and oleic acid with tween 80 exhibit a negative effect, leading to a decrease in polydispersity. The coefficient value of monostearin indicates a greater impact on increasing the polydispersity value, while the interaction between monostearin and tween 80 is more pronounced in its ability to decrease the NLC polydispersity value. This relationship is illustrated in Figure 2C, where a closer proximity to the red color gradation corresponds to higher PDI values for the monostearin and oleic acid components. In contrast, the blue color signifies a lower PDI

value resulting from the interaction of the monostearin-tween 80 mixture and the oleic acid-tween 80 mixture.

The NLC entrapment efficiency for the *crude extract of brown algae fucoidan* was found to be between 92% and 97%, indicating that it meets the characteristic criteria for NLC [37]. The incorporation of monostearin and oleic acid results in an imperfect crystal lattice due to the differing lipid chain lengths, which provides substantial accommodation space for drug entrapment and enhances entrapment efficiency [38]. The coefficient equations in Table 3 demonstrate that monostearin, oleic acid, tween 80, and the mixture of monostearin with tween 80 yield a positive response, suggesting that these components enhance adsorption efficiency. Conversely, the interactions among the mixtures of monostearin-oleic acid, oleic acid-tween 80, and the tri component mixture generate a negative response, indicating a reduction in entrapment efficiency. Based on the coefficient values, oleic acid is more influential in improving entrapment efficiency due to its larger coefficient value. The presence of oleic acid as a liquid lipid is crucial in mitigating the crystallization process and serves as a key factor in enhancing the release kinetics of active ingredients and the efficiency of drug entrapment within the NLC system [11].

The accompanying 2D representation illustrates that a shift towards red corresponds to increased entrapment efficiency, while the gradation from yellow to green and blue signifies decreasing entrapment efficiencies. The red color is prominently associated with the interaction between the oleic acid component and tween 80. The observed reduction in entrapment efficiency can be elucidated through the partition phenomenon. Elevated surfactant concentrations in the external phase can enhance the partitioning of the drug from the internal to the external medium. This augmented partitioning occurs due to the increased solubility of the drug in the external aqueous phase, facilitating greater drug dispersion and dissolution within it [39].

Table 4: Determination of Optimum Formula and Targets for Determining Optimal Formula

Trial Response	Limitation		
	Minimum parameters	**Maximum parameters**	**Goal**
pH	4.11	5.11	None
Particle size (nm)	265.5	1169	Minimize
PDI	0.43	0.79	Minimize
Entrapment Efficiency (%)	92.99	97,19	None

Optimization of NLC Formulation from ***Crude Fucoidan Extract of Brown Algae***

The determination of the optimum nanostructured lipid carrier (NLC) formula was derived from the analysis of the contour plots concerning pH response, particle size, polydispersity index (PDI), and entrapment efficiency, as illustrated in Figure 2E.

Based on these predictive analyses, an optimal formulation was achieved with a desirability value of 0.879, comprising the following component concentrations: monostearin at 2.01%, oleic acid at 2.20%, and tween 80 at 7.79%, as depicted in Figures 2E and 3. The Design Expert 10 software facilitated this assessment, as shown in Figure 3 and Table 4, wherein various responses to the optimal formula parameters were obtained, aligning closely with the defined targets. The contour plot in Figure 2E illustrates a desirability value characterized by a reddish hue, indicative of its proximity to 1, specifically 0.879. This suggests that the predictive capability of the program to produce the desired formulation is increasingly refined. The less intense red coloration on the contour plot arises from the dual objectives set for pH response and entrapment efficiency; these goals were established based on the significant results from ANOVA. However, the pH results yielded an adjusted R-squared value that, in conjunction with the predicted R-squared, indicated that the overall mean may serve as a more reliable predictor of the response than the employed model. In contrast, the predicted R-squared for entrapment efficiency demonstrated good alignment with the adjusted R-squared, exhibiting a difference of less than 0.2, although the model selection for this response was not feasible due to its non-hierarchical nature. To validate the predicted optimum formula derived from the Design Expert software, a repreparation of the NLC formulation was conducted utilizing the same manufacturing methodology, followed by testing for pH, particle size, and entrapment efficiency. The optimal formula was also subjected to zeta potential and transmission electron microscopy (TEM) assessments. The verification outcomes were subsequently analyzed statistically to compare the software predictions with the experimental results to ascertain the presence of any significant differences.

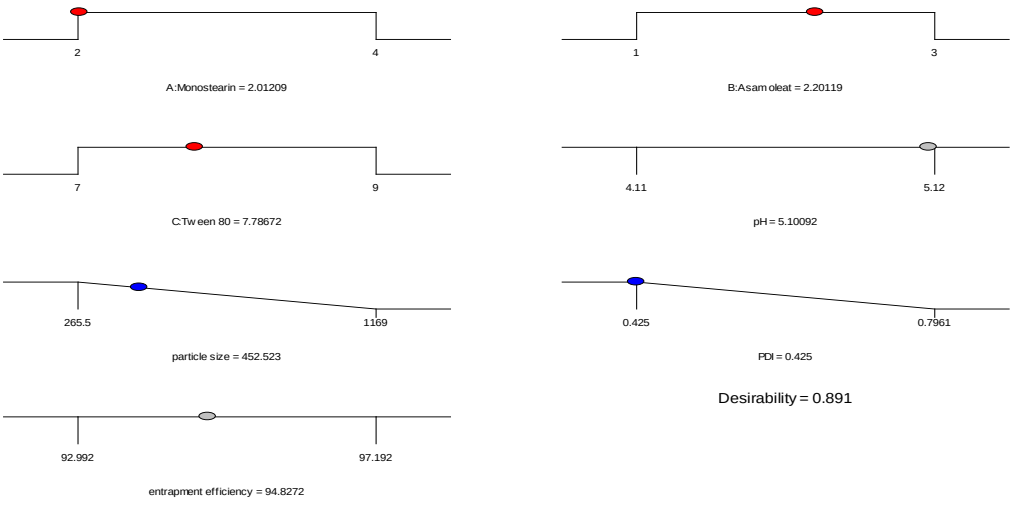

Figure 3: Diagram of Determining Optimum Formula and Predicted Response Values

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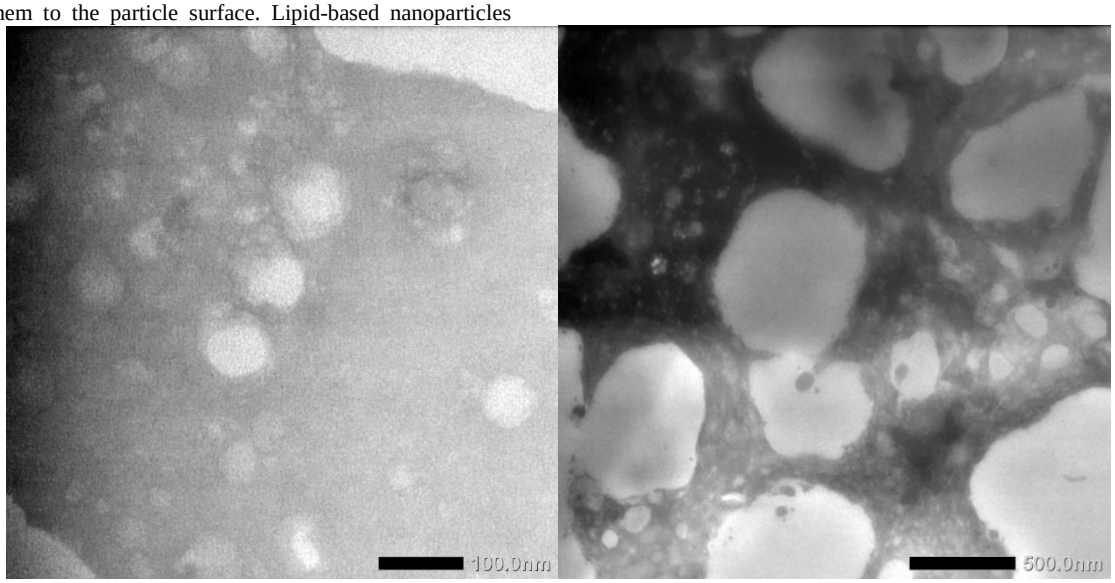
Statistical analysis revealed significance values greater than 0.05 for all responses, indicating no significant differences between the physical test values of the optimum gel formula and the predicted values from the software, thereby validating the reliability of the software predictions. However, a significant difference ($p < 0.05$) was observed between the predicted absorption efficiency and the observed value, with the latter exhibiting greater efficacy. The results of the One-sample T-test re presented in can be seen in table 5

The zeta potential of the NLC, as obtained from the study, was recorded at -23.08 mV, which suggests a tendency for particle aggregation due to attractive forces. The relatively low zeta potential value is attributable to tween 80 (an anionic surfactant), as its hydrophobic chain lacks a charge, resulting in an uncharged surface on the coated oil droplets [40]. Tween 80 serves as a non-ionic surfactant and stabilizer within the NLC system by adsorbing anionic ions (OH^-) from the aqueous phase and transporting them to the particle surface. Lipid-based nanoparticles typically exhibit negative zeta potential due to the lipid molecules capacity to absorb OH^- ions from water. The amount of charge on the particle surface is one of the important characteristics that provides information about the tendency of nanoparticles to agglomerate and their long-term stability. Despite the low zeta potential, the NLC system does not undergo flocculation during storage, attributable to the steric hindrance phenomenon, where the anionic surfactant forms a protective film on the droplet surface, preventing coalescence [33].

Zeta Potential (ZP) refers to the electrical potential of a particle located away from its surface within a diffusion layer, associated with particle mobility in a liquid, known as the shear plane. ZP is intimately linked to particle surface morphology and suspension stability, distinguishing it from metrics such as particle size or molecular weight, as ZP is influenced by the particle's environment, including pH, ionic strength, and the types of ions present [41].



a b

Figure 4: TEM results of NLC morphology of *crude brown algae fucoidan extract* observed using TEM. Scale bars = a. 100 nm b. 500 nm

Observational data obtained from TEM imaging, utilizing bar scales of 100 nm and 500 nm, reveal that the particle morphology within the NLC system, as depicted in Figure 4, is predominantly spherical (amorphous type). The high surface-to-volume ratio of lipid nanoparticles distorts crystalline structures. This distortion induces depression of the melting point and recrystallization point of lipid nanoparticles. A small particle size may cause the formation of liquid, amorphous, or only partially crystallized metastable systems. A less ordered arrangement of the lipid crystals increases the drug-loading capacity. NLCs, which have special structures, provide an increase in drug payload and prevent drug expulsion with better drug accommodation.

Table 5: Verification Test of Optimum Formula of NLC *Extract Crude Fucoidan Algae Brown* with *One Sample T-Test*

Response Parameters	Results Prediction	Results Observation	Significant (P.value)	Conclusion
pH	5.10	5.01	0.29	not significant
Particle size (nm)	452.52	389.94	0.10	not significant
PDI	0.43	0.49	0.05	not significant
EE (%)	94.83	98.37	0.00	significant

Conclusion

The development of a nanostructured lipid carrier (NLC) delivery system for the *crude extract of brown algae fucoidan* has been successfully achieved using the emulsification-sonication method. The optimized formulation consists of 2.01% monostearin, 2.20% oleic acid, and 7.79% tween 80. The resulting NLCs exhibit a negatively charged spherical morphology (amorphous type), with a pH of 5.10 and a particle size of approximately 389.94 nm. The particle size distribution is fairly homogeneous, characterized by a polydispersity index value of less than 0.5. Furthermore, the lipid-based delivery system demonstrated a high entrapment efficiency of 98.37%, indicating its capability to encapsulate significant quantities of *crude brown algae fucoidan extract*. In general, the NLC of *brown algae fucoidan crude extract* produced has high potential to be further developed for use as a therapeutic agent for topical use of androgenetic alopecia therapy.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby affirm that the work presented in this article is original and accept full responsibility for any claims related to its content.

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