Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 11, No. 1, January 2026

Research Paper

Surfactant Variations in Capsanthin-loaded Nanostructured Lipid Carrier: Formulation, Characterization, and Stability Study

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Abstract
The stability of drugs and nanocarriers is a crucial factor in guaranteeing their efficacy and quality. Nanostructured lipid carriers (NLCs) as lipid-based nanocarriers provide benefits in overcoming the stability and other issues associated with the drug. Surfactants play a crucial role in imparting the desired characteristics of lipid-based systems, including stability, particle size, size distribution, and zeta potential. This study aimed to develop a capsanthin-loaded NLC formulation with an optimal surfactant combination that exhibits the required properties and stability. Three formulations were prepared applying Tween 20 and Span 80 in ratios of 5:1, 2:1, and 1:1 for F1, F2, and F3, respectively. Capsanthin, as an active ingredient, originates from paprika powder. NLC properties regarding transmittance, particle size, polydispersity index (PI), zeta potential, loading capacity (LC), and loading efficiency (LE) were characterized. Furthermore, stability studies were also conducted in this research. The results showed that F1 exhibited desirable characteristics, including transmittance percentage, particle size, PI, zeta potential, LC, and LE of 87.22±2.64%, 255.4±46.88 nm, 0.54±0.13, -39.51±3.02 mV, 12.59±0.35%, and 35.40±3.07%, respectively. Moreover, the physical stability studies exhibited that F1 provided a more stable system compared to other formulations. Additionally, the chemical stability indicated that degradation occurred in the paprika powder, not in the NLC. In conclusion, NLC can protect capsanthin, as evidenced by the stable capsanthin levels in NLC compared to those in paprika powder.

Keywords
Stability, Surfactant, Capsanthin, Nanostructured Lipid Carrier

Received: 30 July 2025, Accepted: 20 December 2025
https://doi.org/10.26554/sti.2026.11.1.335-344

1. INTRODUCTION

The stability of the drug and carrier is a crucial factor that must be considered to maintain product quality, including the active pharmaceutical ingredients and finished pharmaceutical products. Stability refers to the absence of any changes in the product that affect its therapeutic effect. Capsanthin, an active ingredient derived from paprika, exhibits several potential activities; however, it has low water solubility (Kennedy et al., 2021; Kulkarni et al., 2020) and is unstable to light, oxygen, and heat, resulting in low oral bioavailability (de Oliveira et al., 2021). Therefore, it is necessary to develop a lipid-based nanocarrier for capsanthin. Development of nanocarriers for drugs offers several benefits, such as the ability to improve the bioavailability and stability of active ingredients (Bhalani et al., 2022; Chenxi et al., 2025; Gupta et al., 2020; Madaniyah et al., 2025; Nahrowi et al., 2024; Yeo et al., 2022; Zhu et al., 2020).

Surfactant is an important component in the lipid-based nanocarrier formulation (Pink et al., 2021; Shah et al., 2025). The selection of surfactant type, concentration, and ratio of surfactant mixture is a critical step in the nanocarrier formulation. The type of surfactant determines the characteristics of lipid-based systems, including stability, particle size, size distribution, and zeta potential (Bnyan et al., 2018). A general rule can be adopted in selecting a surfactant for lipid-based nanocarriers. The safety of surfactant, namely non-ionic surfactant, is safer than ionic surfactant (Gloxhuber, 1974). Usually, the cationic surfactant produces a positively charged carrier, and vice versa for the anionic surfactant (Baig et al., 2020; Baig and Siddiqui, 2020; Botto et al., 2017; Knoll et al., 2022). Negatively charged lipid nanocarriers are also formed by several subtypes of non-ionic surfactants, which are classified based on functional groups of hydrophilic sub-structure, including sorbitan,

polyoxyethylene, polyoxyethylene sorbitan, sugar, and glycerol (Eh Suk et al., 2020; Liu et al., 2020; Varela-Fernández et al., 2022; Yousaf et al., 2023; Zhang et al., 2021). Regarding surfactant concentration, increasing the surfactant-to-lipid ratio serves to increase the water and oil interphase area, thereby decreasing the droplet size. However, the regulatory body limits the permissible concentration of a surfactant due to toxicity issues. Therefore, to achieve the targeted properties of the lipid nanocarriers' internal phase, i.e., small size and small size distribution, it is necessary to optimize the surfactant variation and concentration in the formulation. Among these various ratios, a specific ratio produces the ultimate nanocarrier properties (Mahor et al., 2023). This specific ratio of surfactant mixture yields a Hydrophilic-Lipophilic Balance (HLB) value close to the required Hydrophilic-Lipophilic Balance (r-HLB) value of the lipid/oil that makes up the lipid nanocarrier (Smejkal et al., 2024).

Lipid-based nanocarriers cover a wide range of systems from liquid-phase to solid. Nano-emulsions and microemulsion droplets are liquid-phase nanocarriers containing oil covered by surfactant, which is dispersed during storage. This condition is detrimental due to its voluminous and physical instability risk. Moreover, the loaded drugs have the potential to be released to the medium, the unprotected phase of the nano/microemulsion (Khan et al., 2023). These issues are the reasons for developing solid lipid-based nanocarriers, which can be easily dispersed once introduced into water. Nanostructured lipid carrier (NLC) is a lipid-based system combining various advantages of other lipid-based carrier systems, such as liposomes, nano-emulsions, polymeric nanoparticles, as well as solid lipid nanoparticles (SLN), namely, higher stability and drug loading (Dhiman et al., 2021). The NLC preparation procedures include a step in which a lipid dispersion in water as a continuous phase is presented. The internal phase is constructed by a mix of solid and liquid lipids covered by a single layer of surfactant or surfactant mixture. These internal phases are the candidates of NLC. Thus, the characteristics of the internal phase in this preparation step, including zeta potential, particle size, and size distribution, reflect the resulting NLC properties, such as stability once dispersed during administration. Currently, there is no study performed on the development of NLC containing capsanthin with various surfactants. Therefore, this study aims to develop a carrier for capsanthin, specifically a lipid-based nanocarrier, namely a nanostructured lipid carrier (NLC), with varying surfactant concentrations to achieve a formulation that provides the required properties and stability.

In this study, NLC formulations were prepared using various surfactant ratios of Tween 20 and Span 80, followed by characterization including transmittance, particle size, polydispersity index, and zeta potential. The ability of NLC to incorporate capsanthin was determined by calculating the capsanthin loading capacity (LC) and loading efficiency (LE). Furthermore, the physical and chemical stability studies of capsanthin-loaded NLC were performed.

2. EXPERIMENTAL SECTION

2.1 Materials

Capsanthin standard (purity >95%) was purchased from BOC Sciences. Paprika powder (Loss on Drying/LOD: 2.6%; 100% pass 80 mesh) originated from Qin Health Industry (Shaanxi) Co., Ltd. (the concentration of capsanthin in the paprika powder was 37% as determined previously using the spectroscopic method). Glyceryl monostearate (GMS) was obtained from Multi Jaya Kimia, Indonesia. Oleic acid, Tween 20, Span 80, ABTS, and potassium persulfate were supplied by Nitrakimia, Indonesia.

2.2 Methods

2.2.1 Preparation of Capsanthin-Loaded NLC

The preparation of capsanthin-loaded NLC was carried out using a modification of the previously published method by Duong et al., namely, the solvent injection method (Duong et al., 2019). The preparation of capsanthin-loaded NLC was conducted according to Table 1 by melting the GMS as a solid lipid in ethanol at 60°C, followed by adding the liquid lipid and Span 80. The mixture was stirred in a magnetic stirrer for about one hour at the same temperature. Afterward, the paprika powder solution in ethanol was added to the above lipid mixture (after the temperature was decreased to room temperature), and shaking was continued for one hour. Subsequently, the aqueous phase containing Tween 20 was added to this lipid phase and mixed at room temperature for one hour. Afterward, to obtain the NLC product, the mixture was centrifuged at 4000 rpm, at 4°C for 15 minutes. The supernatant was collected and stored in a freezer for lyophilization.

2.2.2 Characterization of Capsanthin-Loaded NLC

2.2.2.1 Measurement of Transmittance

Transmittance measurement was performed by dispersing the capsanthin-loaded NLC at approximately 10 mg in 5 mL of 20 mM Phosphate-Buffered Saline (PBS) at pH 7 using a magnetic stirrer at 37°C at a speed of 300 rpm for 30 minutes. Afterwards, the transmittance of the sample dispersion was determined using a UV-VIS spectrophotometer (Shimadzu) at a wavelength of 650 nm (Fitria et al., 2021).

2.2.2.2 Measurement of Particle Size, Polydispersity Index, and Zeta Zotential

The particle size, polydispersity index, and zeta potential of capsanthin-loaded NLC were measured using a modified method of Duong et al. (2019). Briefly, 10 mg of capsanthin-loaded NLC was dispersed in 5 mL of 20 mM Phosphate Buffer Saline (PBS) pH 7. The mixing was carried out using a magnetic stirrer at 37°C with a speed of 300 rpm for 60 minutes, followed by measuring the sample using Zeta sizer (Malvern).

2.2.2.3 Calculation of Loading Capacity (LC) and Loading Efficiency (LE)

Table 1. Formulation Composition of Capsanthin-Loaded NLC with Various Ratios of Tween 20 and Span 80 as Zurfactants. All Materials are in mg; Each Formulation is in 10 mL of Water in a Production Batch. GMS and Oleic Acid are Solid and Liquid Lipids, Respectively

Formulations	GMS	Oleic Acid	Tween 20	Span 80	Propylene Glycol	Paprika Powder
F1	120	60	150	30	75	100
F2	120	60	120	60	75	100
F3	120	60	90	90	75	100

The measurement of LC and LE was conducted using a method developed by Ocampo Osorio et al. (2023) with slight modifications. An adequate amount of capsanthin-loaded NLC was completely dissolved in ethanol using a magnetic stirrer at 500 rpm. Subsequently, capsanthin concentration was analysed using a UV-VIS spectrophotometer at a wavelength of 476 nm. Loading capacity (LC) and loading efficiency (LE) were calculated using Equations 1 and 2, respectively.

$$\text{LC } (\%) = \frac{C_c \times V_e}{M_p} \times 100\% \quad (1)$$

$$\text{LE } (\%) = \frac{\left(\frac{M_t}{M_p}\right) C_c \times V_e}{M_c} \times 100\% \quad (2)$$

Notes: Mp = Amount of dissolved sample (capsanthin-loaded NLC); V_e = Ethanol volume to dissolve the sample; C_c = Dissolved capsanthin concentration in ethanol; M_c = The amount of capsanthin in a batch formulation; M_t =The total amount of capsanthin-loaded NLC produced in a batch formulation.

2.2.3 Morphology Analyses of Capsanthin-Loaded NLC Using Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

The morphology of the NLC containing capsanthin of selected formulation (F1) was analysed using TEM (JEOL JEM-1400). Firstly, the sample was dispersed in the water, followed by dropping onto the grid. After drying, 10 μL of 2% uranyl acetate was added, kept standing, and observation was conducted. Further analysis was the observation of particles using an SEM instrument (JEOL JCM-7000). Briefly, the sample was put on the carbon tape in the holder. The bubble air was removed previously before the measurement.

2.2.4 Analyses of Capsanthin-Loaded NLC Using FTIR

The FTIR analysis was carried out to observe the presence of functional groups in the capsanthin-loaded NLC of the selected formulation (F1). Samples, the NLC containing capsanthin and each lipid component, were measured at 4000-500 cm-1 using FTIR (BRUKER) to find the spectra that were presented on the computer. The resulting spectra were observed regarding the similarity of each functional group .

2.2.5 Measurement of Antioxidant Activity Using ABTS

The Antioxidant activity of capsanthin was measured using the ABTS assay. Briefly, the ABTS reagent was prepared by mixing an equal volume of ABTS and potassium persulfate at concentrations of 0.48 mM and 0.168 mM, respectively, followed by storage at room temperature in the dark for 16 hours. This reagent was used to measure the antioxidant activity of samples, including capsanthin loaded in NLC, capsanthin in the paprika powder, and vitamin C as a standard. Each sample was mixed with ABTS reagent at an equal volume ratio, followed by standing in the dark at room temperature for 10 minutes. Thereafter, the absorbance of the samples was measured at a wavelength of 734 nm. As a blank, a mixture of ABTS reagent and ethanol at an equal volume ratio was used (Geng et al., 2020).

2.2.6 Stability Studies of Capsanthin-Loaded NLC

Stability testing was conducted by storing samples at room temperature (25°C ± 2°C / 60% RH ± 5% RH) for about 6 months based on the ICH Guideline Stability Testing of New Drug Substances and Products (European Medicines Agency, 2003). The physical stability study was evaluated by measuring the characteristics, including particle size, PI, and zeta potential, whereas the chemical stability study was determined by quantifying the amount of capsanthin both in the capsanthin-loaded NLC (selected formulation) and paprika powder using a UV-VIS spectrophotometer before and after storage.

2.3 Data Analysis

The three compositions in this research were distinguished based on the Tween 20 and Span 80 ratios. The effect of the ratio on particle size, polydispersity index, zeta potential, and drug loading was statistically analysed using One-way Analysis of Variance (ANOVA) followed by Tukey HSD post hoc test at a 95% confidence level. The stability study was analysed using an independent sample t-test, including before and after storage.

3. RESULTS AND DISCUSSION

3.1 Preparation of Capsanthin-Loaded NLC

The preparation of capsanthin-loaded NLC was carried out using a modified solvent injection method. This method was chosen due to several advantages, including a simple process and flexibility in temperature adjustment according to drug stability. An organic solvent, namely ethanol, was used in this

preparation to dissolve paprika powder and assist the melting process of GMS. At the end of the process, the solvent and organic solvent were removed through evaporation, followed by lyophilization to obtain a solid product of capsanthin-loaded NLC, as shown in Figure 1. In the formulation optimization, the ratio of surfactants, including Tween 20 and Span 80, was varied into three combinations, namely 5:1, 2:1, and 1:1 for F1, F2, and F3, respectively. As shown in the molecular structure depicted in Figure 2, the hydrophilic mass fraction of Tween 20 is greater than that of Span 80. According to such mass fractions, Tween 20 and Span 80 are denoted as having the HLB values of 16.7 and 4.3, respectively.

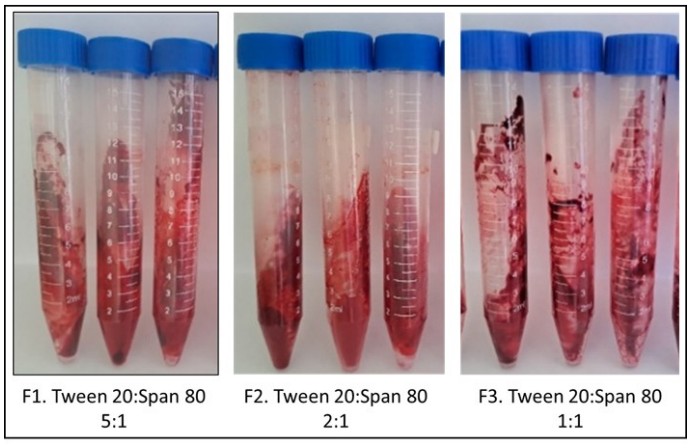

Figure 1. Photograph Of Capsanthin-Loaded NLC Product After Lyophilization, Including F1, F2, And F3

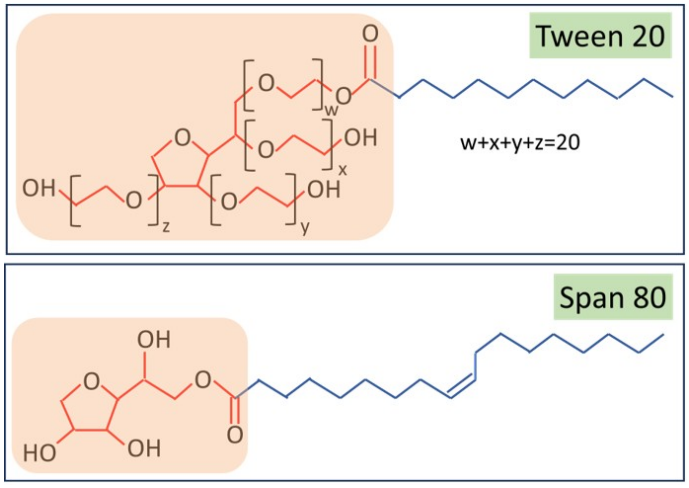

Figure 2. Molecular Structure of Surfactants Used in Various Ratios for Capsanthin-NLC Formulation, Including Polysorbate 20 (Polyoxyethylene (20) Sorbitan Monolaurate (Tween 20®, HLB 16.7) And Sorbitan Monooleate (Span 80®, HLB 4.3). The Substructure Highlighted in Orange Constructs the Hydrophilic Head, Whereas the Remaining Part is the Lipophilic Tail

In the formation of lipids structured in an aqueous dispersion system, a high HLB of surfactant is required (Smejkal et al., 2024). Tween 20 and Span 80 combinations provide several mixed HLB values depending on the ratio and concentration of surfactants in each formulation, resulting in different properties of NLC obtained from development. Along with determining the dispersion stability with the HLB - (r-HLB) matching theory (Wang et al., 2023). The addition of Span 80 containing oleic tails, showing cis isomers of unsaturated bonds, is expected to facilitate drug release after the nanocarrier reaches the target. The incorporation of oleic acid in the lipid layer increases the fluidity of the membrane, leading to permeability enhancement (Binarjo and Nugroho, 2019; Subramaniam et al., 2020). The produced capsanthin-loaded NLC was further characterized, including the transmittance, particle size, PI, zeta potential, and loading capacity.

3.2 Characterization of Capsanthin-Loaded NLC

3.2.1 Measurement of Transmittance

Transmittance (T%) measurement is one of the characterizations performed in this study, describing the transparency and clarity of a colloidal system. The greater the transmittance percentage value, the clearer the system, indicating that the particle size is getting smaller. As exhibited in Table 2, F1 showed the highest T%. A transmittance value approaching 100% indicates that the formed nanoparticles produce a clear dispersion with a size in the nanometer range. Based on Table 2, the results show that the ratio of Tween 20 and Span 80 affects the T%, where the greater the use of Tween 20, the greater the T% and the clearer the NLC dispersion obtained.

Table 2. Transmittance (T%) of Capsanthin-Loaded NLC Formulations with Various Ratios of Tween 20 and Span 80 as Surfactants after Dispersing in 20 mM PBS pH 7. Data Are Presented as Mean ± SD (n = 3)

Formulations	Transmittance (%)
F1 (5:1)	87.22 ± 2.64
F2 (2:1)	84.10 ± 2.49
F3 (1:1)	73.04 ± 1.94*

*The results showed a significant difference from F1

3.2.2 Measurement of Particle Size, Polydispersity Index (PI), and Zeta Potential

Further characterization of capsanthin-loaded NLCs included particle size measurement, as shown in Figure 3A (F3A). F1 provides the smallest particle size, i.e., about 250 nm, is is likely due to the HLB value of the surfactant mixture in F1, namely 14.6, which is closer to the required HLB (r-HLB) of the lipid combination of GMS and oleic acid compared to the HLB of the surfactant mixture in F2 and F3, namely 12.6 and 10.5, respectively. At the same lipid-water-surfactant ratio, the lipid colloidal dispersion shows smaller particles if the HLB value of the surfactant is as close as possible to the r-HLB of the lipid.

The particle size of NLC inversely reflects the produced colloidal dispersion stability. According to Stokes' law, a bulk of small particles is sedimented more slowly than the large ones. Another factor influencing the stability of the nanocarrier system is the polydispersity index (PI), describing the homogeneity and distribution of particle sizes. A dispersion system showing a smaller PI is more stable than a higher one due to the inhibition of particle flocculation. Based on Figure 3A (F3A), the PI of F1 exhibited the smallest value. The PI values closer to 0 indicate a narrower particle size distribution and a more homogeneous system. A PDI value greater than 0.7 indicates a wider particle size distribution and an inhomogeneous system (Danaei et al., 2018). A small PI value (close to zero) is an important factor that impacts the system stability by regulating the particle size stability during storage and application. The nature of particle size and PI is a crucial factor affecting the quality of the nanocarrier system, including its stability and ability to pass through the membrane. Particle sizes less than 300 nm are more suitable for oral drug delivery systems due to their ease of internalization by enterocytes, thereby improving intestinal transport (Peczek et al., 2023). The higher concentration of the hydrophilic surfactant, in the present research, namely, Tween 20, resulted in the formation of smaller particle sizes. This is in line with the previous research, which used a higher concentration of Poloxamer 188 (HLB of more than 15) in Solid lipid nanoparticle (SLN) formulation, indicating the smaller particle size (Hariyadi et al., 2024). Another research performed by Witayaudom and Klinkesorn (2017) also exhibited that the use of more concentration of hydrophilic surfactant, such as Tween 80, could decrease the particle size.

In the physical stability study after storage, as shown in Figure 3B (F3B), the increase in particle size of F1 was not significantly different, whereas F2 and F3 indicated a significantly higher. An increase in particle size is likely due to mechanisms such as aggregation or flocculation within the system, resulting in larger particles. The PI value was also increased for F1 and F2. However, the values are still not more than 0.7.

Regarding zeta potential, as shown in Figure 4A (F4A), F1 and F2 provide similar zeta potential values of -30 to -40 mV, whereas F3 indicates a value less negative than F1 and F2. Particles with a zeta potential more negative than -30 mV or more positive than +30 mV will repel each other (Chauhan et al., 2020), leading to minimal aggregation and thus increasing stability. NLC with non-ionic surfactants exhibits negative charges due to the molecular polarization, resulting in the formation of charges in water. The charges are formed by ion adsorption at the particle/water interface on the surfactant surface, and finally, an electric double layer is formed, similar to ionic (Lasoń et al., 2013). Previous studies also showed that the use of Tween in NLC-L-ascorbic acid-Gold Tri. E30 produced a negative zeta potential (Eh Suk et al., 2020).

Figure 4B (F4B) exhibited the zeta potential of the capsanthin-loaded NLC formulation after storage. The zeta potential of F1 is almost the same before and after storage; however, the zeta potential of F2 and F3 showed a change to be more negative. It is likely due to the decrease in Tween 20 in those formulations (F2 and F3). This is in line with previous research, indicating that the enhancement of Tween 80 concentration in the NLC formulation results in a change of the zeta potential to be less negative (Witayaudom and Klinkesorn, 2017).

3.2.3 Calculation of Loading Capacity (LC) and Loading Efficiency (LE)

Further important data, as shown in Figure 5, include the drug loading, specifically LC and LE. LC is a characteristic of the nanocarrier informing the concentration of drugs in the nanocarrier. As shown in Figure 5, F1 produced the NLC exhibiting the significantly ($p<0.05$) highest loading capacity. Typically, the loading capacity is affected by the lipid composition, which determines drug solubility. Since the lipid components of all formulations were the same, the surfactant composition influenced the difference in LC. It is assumed that Span 80 untightened the monolayer of the surfactant mixture due to the availability of the cis-isomer oleate tail (Subramaniam et al., 2020) as described previously in Figure 2, which leads to the enhancement of drug movement out from NLC during processing. Therefore, F1, containing the least fraction of Span 80 in the surfactant mixture, contained more capsanthin in the NLC than other formulations. A high loading capacity is desirable, particularly for drugs that require high doses for administration. Low LC means the NLC-containing drug should be formulated in voluminous units, which is inconvenient for administration.

Loading efficiency determines the effectiveness of the composition and preparation procedure for loading the drug. The sedimentation of unstructured lipids containing lipophilic drugs during the centrifugation step of the preparation usually causes low loading efficiency in NLC formulation, simultaneously with low yield. In this research, all formulations produced a similar yield, namely 60-65%, indicating that the separated lipids were almost equivalent. This is in line with the loading efficiency values, which were not different for all formulations (Figure 5). For expensive drugs, high loading efficiency is a key parameter to reduce drug wastage.

Three NLC compositions were successfully prepared in the optimization step, concluding that F3 did not show appropriate characteristics regarding particle size, PI, and zeta potential, significantly different from other formulations ($p<0.05$). Therefore, F3 was not selected for further experiments. Moreover, F1 and F2 showed a similar particle size and zeta potential as indicated by the post-hoc test, which was not significantly different. However, F1 indicated the more stable characteristics regarding the particle size and zeta potential after stability study by storing for 6 months. In addition, F1 showed a significantly ($p<0.05$) higher loading capacity than F2, suggesting that this composition should be selected for further experimentation, including transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analyses, FTIR analysis, antioxidant activity measurement, and chemical stability study.

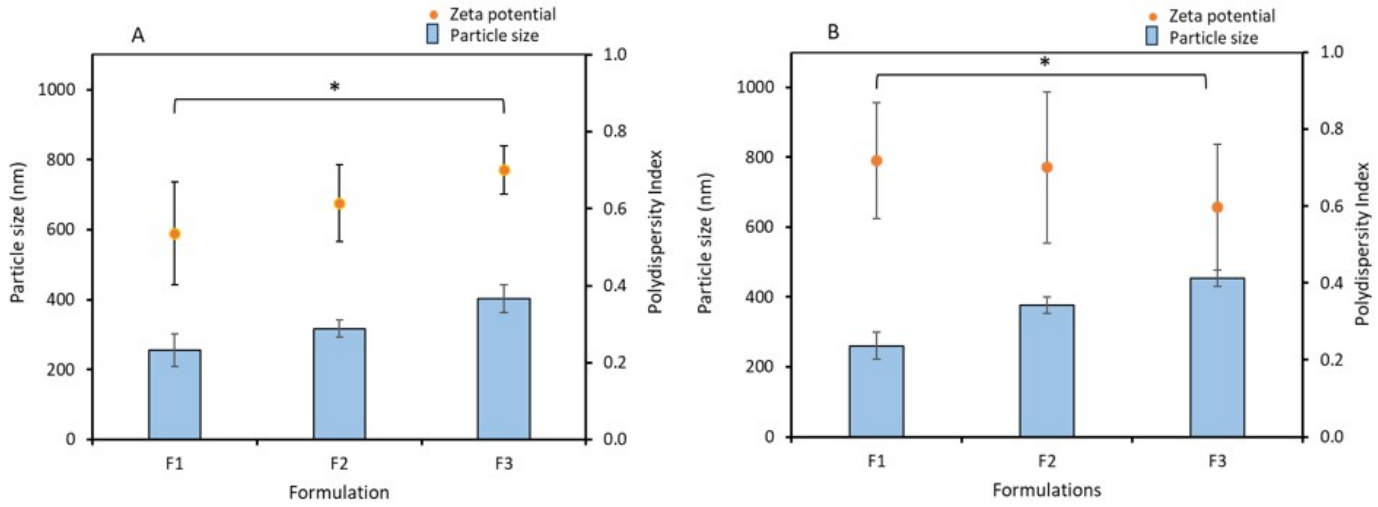

Figure 3. Particle Size and Polydispersity Index of Capsanthin-Loaded NLC Formulations After Dispersing in 20 mM PBS pH 7, before Storage (F3A) and After Storage (F3B). Data are Presented as Mean ± SD (n = 3)

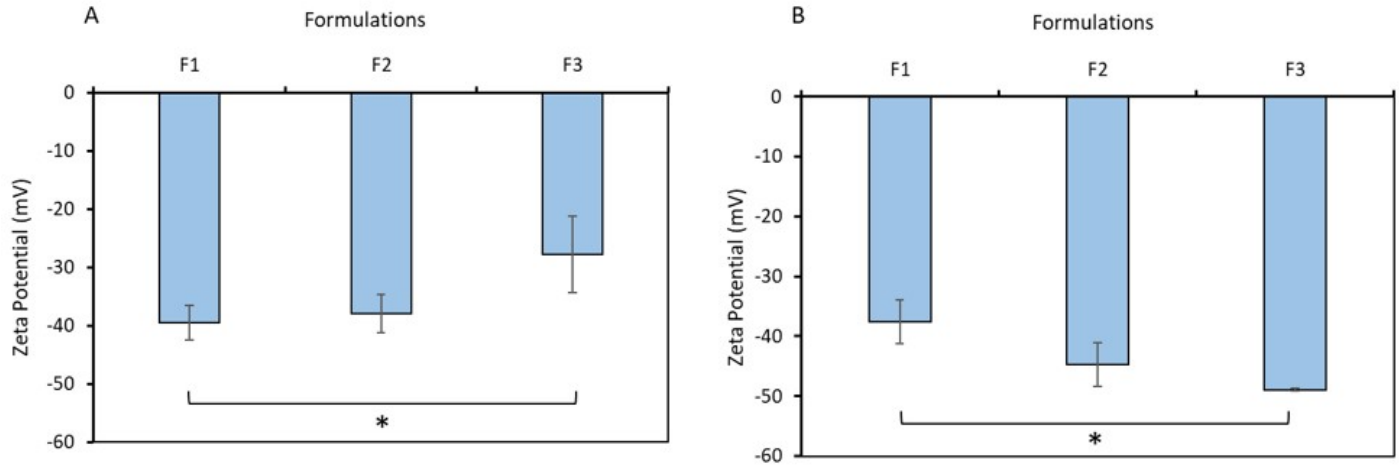

Figure 4. Zeta Potential of Capsanthin-Loaded NLC Formulations After Dispersing in 20 mM PBS pH 7, Before Storage (F4A) and After Storage (F4B). Data are Presented as Mean ± SD (n = 3)

3.3 Morphology Analyses of capsanthin-loaded NLC using Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

The selected formulation, F1, was further characterized regarding the morphology of the nanoparticles using TEM. Based on Figure 6, the particles appear to have a spherical shape with a diameter of approximately 200 nm. This follows the results of the particle size measurement of F1 using Zetasizer.

Based on the SEM analyses shown in Figure 7, the capsanth in- loaded NLC of the selected formulation (F1) appears as spherical particles with a uniform particle size distribution, indicating homogeneous particle size. This result aligns with the measurement of the polydispersity index of F1, as shown in Figure 3.

3.4 FTIR Analyses of Capsanthin-Loaded NLC

Based on the FTIR analyses as shown in Figure 8, it is evident that there is a change in the capsanthin-loaded NLC spectrum (d) compared to the main components of NLC-capsanthin (F1), including paprika powder containing capsanthin (a), GMS (b), and oleic acid (c). The paprika powder contains hydroxyl groups, shown as the broad spectrum at 3200-3550 cm^{-1} (O−H stretching) and conjugated ketone (–C=O) at a wave number of 1750-1680 cm^{-1}, which is also found in the capsanthin-loaded NLC spectrum (d). This is evidence of the presence of the active ingredient capsanthin. GMS, as a solid lipid, and oleic acid, as a liquid lipid, contain many methylene groups, $-CH_2^-$ (C−H stretching) as exhibited in the spectra at a wave number of 2950-2850 cm^{-1}, originating

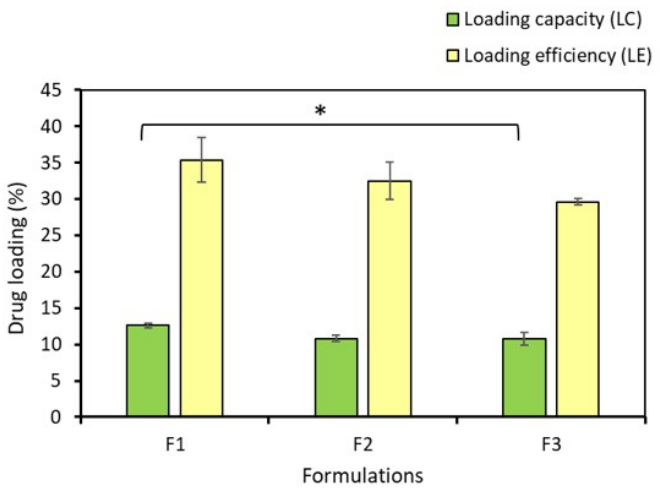

Figure 5. Loading of Capsanthin in NLC Formulation Showing Loading Capacity (LC) and Loading Efficiency (LE). Data are Presented as Mean ± SD (n = 3). The * Denotes That the Samples Have a Significant Difference (p<0.05)

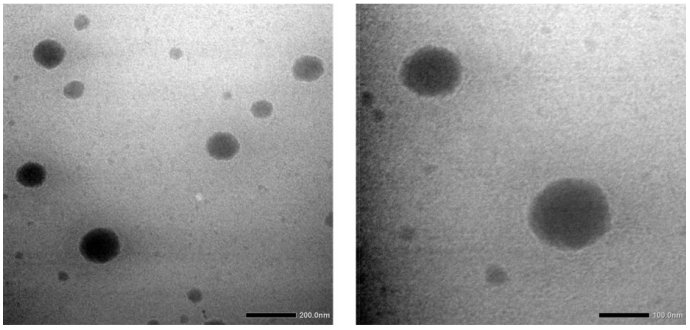

Figure 6. The Transmission Electron Microscopy (TEM) Analyses of Capsanthin-Loaded NLC (F1)

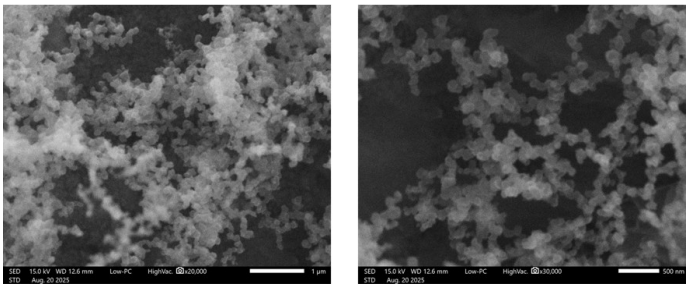

Figure 7. The Scanning Electron Microscopy (SEM) Analyses of Capsanthin-Loaded NLC (F1)

from fatty acid contained in the lipid. These methylene groups appear in the capsanthin-loaded NLC spectrum (d) after the interaction of those components, indicating the formation of NLC capsanthin (Khan et al., 2018).

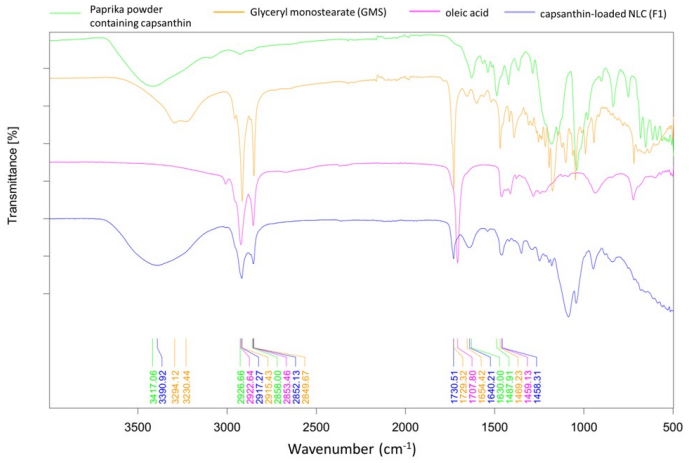

Figure 8. FTIR Spectra of Paprika Powder Containing Capsanthin, Glyceryl Monostearate GMS as a Solid Lipid, Oleic Acid as a Liquid Lipid, and Capsanthin-Loaded NLC (F1)

3.5 Antioxidant Activity of Capsanthin

The ABTS method Geng et al. (2020) was conducted to measure the free radical scavenging activity of capsanthin dissolved from an NLC formulation (F1) as well as capsanthin from paprika powder without any formulation. The same method of antioxidant measurement was also applied to ascorbic acid and blank NLC, serving as positive and negative controls, respectively. The negative control did not show any radical scavenging (data are not shown), while the activity of other groups is shown in Figure 9. Capsanthin and vitamin C quenched the ABTS radical in a concentration-dependent manner, indicating precision in the selection of drug concentration. The exponential positive correlation curve shown in Figure 9 indicates that there will be a maximum concentration at which the radical scavenging reaches its maximum. The slope of the curve correlating percent scavenging as a function of concentration in the logarithmic scale (log C vs % RSA) is the first indication of antioxidant activity (Rodriguez-Ruiz et al., 2018). The curve showed great correlation, as indicated by a high r^2. The average slopes of the curves were 43.44 (r^2=0.931), 36.31 (r^2=0.993), and 33.30 (r^2=0.960) for vitamin C, capsanthin in NLC, and capsanthin in paprika powder, respectively, which is also the order from the highest activity.

The main parameter of antioxidant activity, i.e., the IC_{50}, the drug concentration showing 50% free radical scavenging, was calculated using the same linear regression to determine the slope previously, and the data are presented in Table 3. In line with the slopes, the IC_{50} of the tested samples decreased from capsanthin in the paprika powder, followed by capsanthin in NLC and vitamin C. Statistical analyses using independent sample t-test indicated that capsanthin from NLC formulation showed antioxidant activity as strong as vitamin C, whereas the capsanthin in the paprika powder was significantly (p<0.05) less

potent compared to vitamin C. Therefore, it can be stated that in addition to the benefits of NLC, namely, drug dissolution and permeation enhancement (Shevalkar and Vavia, 2019; Yu et al., 2021), NLC also shows another benefit in formulating unstable drugs, such as antioxidants sensitive to oxygen and light. NLC formulation improved capsanthin stability by protecting the drug from factors inducing degradation, resulting in preserved antioxidant activity. This is in line with previous research applying NLC formulation containing α-tocopherol to protect camelina oil from oxidation (Yousaf et al., 2023).

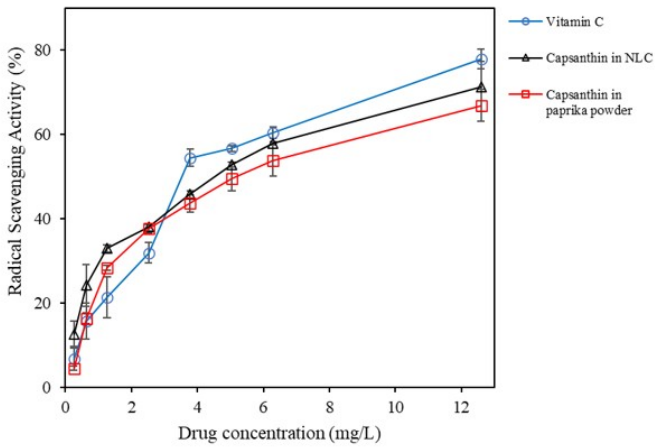

Figure 9. Radical Scavenging Activity of Samples in Various Drug Concentrations, Including Vitamin C, Capsanthin in Paprika Powder, and Capsanthin in NLC. Data are Presented as Mean ± SD (N = 3)

Table 3. IC_{50} of Capsanthin in NLC, Capsanthin in Paprika Powder, and Vitamin C. Data Are Presented as Mean ± SD (n = 3)

Samples	IC_{50} (mg/L
Capsanthin in NLC	4.01 ± 0.46
Capsanthin in Paprika Powder	5.11 ± 0.79
Vitamin C	3.77 ± 0.20

3.6 Chemical Stability Studies of Capsanthin-Loaded NLC

A stability study is a crucial step in ensuring the efficacy and quality of drugs and NLC products under various storage conditions, including temperature, light, and humidity. In the chemical stability study, the concentration of capsanthin was measured to determine whether capsanthin could be properly protected after being incorporated into NLC. The results, as shown in Figure 10, indicated a significant decrease in capsanthin levels in the paprika powder. In contrast, the capsanthin concentration in the NLC remained stable, indicating that the NLC can protect capsanthin during storage. The presence of surfactants in the NLC formulation can affect the nanocarrier stability in both conditions, namely, during storage and after application. Additionally, the use of cosolvents in NLC formulations can increase the solubility of drugs in the lipid matrix and enhance their stability as well.

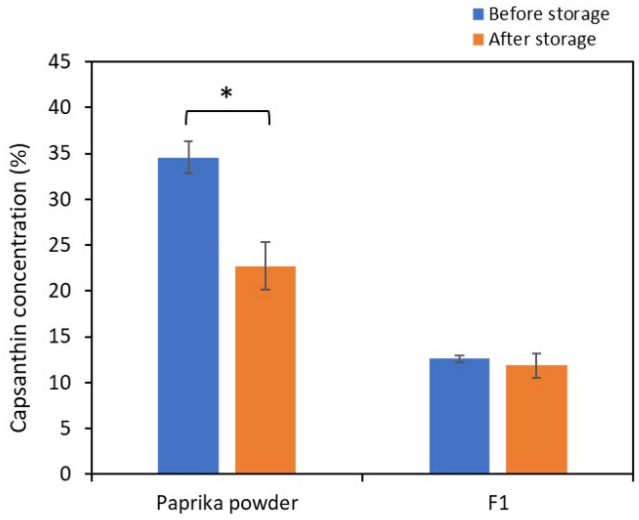

Figure 10. Capsanthin Concentration in the Paprika Powder and Capsanthin-Loaded NLC Formulation (F1) Before and After Storage. Data are Presented as Mean ± SD (n = 3). The * Denotes That the Samples Have a Significant Difference (p>0.05)

4. CONCLUSIONS

In this study, a desirable capsanthin-loaded NLC formulation was obtained. Variations in surfactant ratios, specifically Tween 20 and Span 80, impact the NLC properties, including particle size, polydispersity index, zeta potential, LC, and LE. Formulation 1 (F1), utilizing Tween 20 and Span 80 with a ratio of 5:1, exhibited the required properties. Based on physical stability studies, data showed that F1 exhibited better properties compared to other formulations. Furthermore, the chemical stability showed that degradation occurred in the paprika powder, whereas NLC can protect capsanthin, as evidenced by the stable capsanthin level in NLC compared to the paprika powder.

5. ACKNOWLEDGMENT

The authors would like to thank LPPM UAD for supporting this research.

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