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Application of vegetable oils as pharmaceutical ingredient: the impact of liquid lipid type on the characteristics of nanostructured lipid carrier

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ABSTRACT

Recently, drug encapsulation using a Nanostructured Lipid Carrier (NLC) has gained attention in formulation studies due to its high loading capacity and prevent drug expulsion during storage. Drug loading capacity is mainly affected by lipid type and composition, especially liquid lipids. Therefore, this research aimed to evaluate the potential of avocado oil as a liquid lipid of NLC replacing pure oleic acid. All components including oil, glyceryl monostearate, Tween 20®, and Span 60® were processed to NLC by solvent injection method. The colloidal characteristics of NLC dispersion in water and 20 mM PBS pH 7 were determined, including transmittance, particle size, size distribution, zeta potential, loading capacity (LC), and loading efficiency (LE) of capsanthin in NLC. The results showed that NLC containing oleic acid (F_{ola}) and avocado oil (F_{avo}) dispersion in PBS exhibited a similar transmittance and zeta potential of 69-74% and -51 to -58 mV, respectively, whereas the particle size and size distribution of F_{avo} were significantly higher than F_{ola}. Moreover, the 1.3-fold higher LC and LE of F_{avo} compared to F_{ola} was insignificant ($p>0.05$). Additionally, the Tween 20® and Span 60® ratio of F_{avo} should be improved to obtain an ideal particle size and size distribution as in F_{ola}. In conclusion, avocado oil indicated the potential to be utilized as a liquid lipid of NLC formulation regarding zeta potential and drug loading. However, the surfactant composition should be adjusted to reduce the particle size of the NLC, leading to permeability enhancement in delivery, particularly oral administration.

Keywords: nanostructured lipid carrier, nanocarrier, avocado oil, vegetable oil application

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INTRODUCTION

Recently, drug discovery has been moving toward two categories, small-molecule drugs and macromolecule biological products. The first category is dominated by lipophilic substances exhibiting low water solubility properties thereby categorized in Class II or IV of the Biopharmaceutics Classification System (BCS) leading to low oral bioavailability (Leeson, 2016; Lobo, 2020; Walters et al., 2011). Meanwhile, the second category meets a stability problem against the harsh environment of the gastrointestinal tract due to enzymatic activities and extreme pH. Moreover, biological product macromolecules also show low permeability across high-viscosity intestinal mucus and lipophilic-nature gastrointestinal membranes (Fuhrmann & Fuhrmann, 2017). One of the efforts to address such problems is the development of a nanocarrier system, including nanostructured lipid carrier (NLC), which has been proven to increase drug- dissolution and stability in gastrointestinal medium, and drug permeability across gastrointestinal barrier (Elmowafy et al., 2017; Mahor et al., 2023; Shahzadi et al., 2021).

NLC was developed to ameliorate the properties of solid lipid nanoparticles (SLN) by introducing liquid lipid, a key component that is not available in SLN formulation. This differentiating component modifies the characteristic of the lipid core leading to the enhancement of the capability of the nanocarrier to load the intended drug (Khan et al., 2022). The solubility of drugs in the core of SLN is affected by the crystal structure of the composing lipid. Therefore, the similar lipid composition of SLN might produce a different drug loading capacity due to lipid crystal polymorphism phenomena. A dense triglyceride polymorph, namely β-form, serves fewer crystal voids and spaces leading to decreased drug solubility, thereby loading capacity (Zhong & Zhang, 2019). Additionally, the drug can occupy the crystal defect (imperfection) in which much space is available due to crystal disorder (Mukherjee et al., 2009). Therefore, increasing the part of crystal defect of lipid or selecting an unstable solid form, including an amorphous state is proven to augment the solubility of the drug in the lipid core, leading to drug loading enhancement. However, during storage, the lipid molecules in the part of the crystal defect or amorphous self-arranges bringing the solid into perfect crystal leading to drug expulsion (Mukherjee et al., 2009). In NLC, alongside utilizing surfactants as in SLN, the liquid lipid is added producing a core consisting of a mixture of liquid and solid lipid. The introduction of liquid lipids in the lipid nanocarrier holds the imperfect condition of the lipid mixture, thereby enhancing the drug loading at a lower risk of drug expulsion (Chauhan et al., 2020; Ghasemiyeh & Mohammadi-Samani, 2018). Several types of liquid lipids could be used to formulate NLC, such as triglyceride, fatty acid, fatty alcohol, and vegetable or animal oil (Qushawy, 2021; Saedi et al., 2018; Soeratri et al., 2019; Veider et al., 2022).

The inhibition of lipid crystallization as well as directing the crystallization to a specific polymorphic form by liquid lipid producing a more accommodating crystal are determined by the type of liquid lipid (Bertoni et al., 2021; Yang et al., 2024). In the same liquid lipid type, for instance, free fatty acid, the carbon-chain length also determines the crystal packing of the lipid mixture (Beddoes et al., 2021). The similar aforementioned situations are likely to appear in NLC preparation, explaining the effect of liquid lipids on drug loading in NLC. Moreover, since emulsification is one of the steps in NLC preparation, the conformity between surfactant HLB and lipid mixture RHLB determines the diameter of NLC. Changing the liquid lipid type of the emulsion means shifting the RHLB resulting in an alteration of the internal phase (the pre-NLC) characteristics (Griffin, 1949). In previous study implemented various types of lipid fatty acid ester, namely triglyceride, ester propylene glycol, ester long chain alcohol, and polyethylene glycol in NLC formulation, concluded that the type of fatty acid ester affected the particle size and drug loading (Houacine et al., 2020).

The role of liquid lipid type in determining the characteristics of NLC underlies the needs of this research. Avocado oil was employed as a liquid lipid in NLC formulation to increase vegetable oil utilization. Avocado oil contained in NLC not only acts as an oil but also as an active ingredient since it shows various biological activities (Lin & Li, 2024). Therefore, as a first stage, this study aimed to identify the effect of lipid types, namely avocado oil and oleic acid on the characteristics of NLC. Avocado oil is a crude vegetable oil containing mostly triglyceride showing an esterified oleic acid substructure (Liu et al., 2023). Therefore, an NLC formulation containing oleic acid as a free fatty acid form was developed as a comparator formulation. Several characteristics of NLC formulations were

determined including transmittance of NLC dispersion, particle size, polydispersity index, and zeta potential. Moreover, capsanthin derived from paprika extract was used as a model drug to calculate loading efficiency (LE) and loading capacity (LC). The pharmacological activities of capsanthin and avocado oil are expected to support each other in delaying degenerative diseases, for instance, cardiovascular disease and diabetes (Jo et al., 2017; Kim et al., 2022).

MATERIALS AND METHOD

Materials

Avocado oil used as the liquid lipid of the first formulation of NLC was a cold-press product of PT Tamba Sanji Wani, Bali, Indonesia, whereas oleic acid was purchased from Alfa Kimia, Yogyakarta. Other materials used in both NLC formulations were glyceryl monostearate (GMS, Multi Jaya Kimia, Indonesia), Tween 20®, and Span 60® were purchased from (Nitrakimia, Yogyakarta). Standard capsanthin is of analytical grade purchased from BOC Sciences (USA). Capsanthin was purchased from the Qin Health Industry (Xi'an, China) as a paprika extract. The capsanthin concentration in the extract determined by spectrophotometric method is 37%. All components of the phosphate buffer saline (PBS), namely potassium phosphate mono- and dibasic and sodium chloride, were analytical grade of Sigma Aldrich (Singapore). Purified water and 95% ethanol were purchased from General Labora, Yogyakarta.

Methods

Preparation of NLC

NLC was prepared by solvent injection method (Duong et al., 2020) with slight modification according to the formulation as listed in Table 1. Firstly, a paprika extract containing about 185 mg of capsanthin was dissolved in 1000 µL of ethanol 70% at room temperature assisted by a magnetic bar stirring at about 300 rpm for 30 minutes. A similar stirring method was also applied to dissolve GMS and Span 60® in 70% ethanol, each of 700 µL, and the temperature was set at 60 °C. These three solutions were mixed at room temperature by stirring at 1000 rpm for 30 minutes before transferring into a vial containing liquid lipids. Subsequently, about 2000 µL of ethanol 70% was added to the rinse and the solution containing capsanthin, GMS, and Span 60®. The solution was added to the main mixture under continuous stirring at room temperature to get a homogenous oil phase. Afterward, Tween 20® solution in 50 mL of water was added dropwise into the continuously stirred oil phase to produce NLC dispersion in water. The unstructured and coarse structured lipids were separated by centrifugation at 4000 rpm for 15 minutes. The supernatant, colloidal dispersion of NLC, was collected and freeze-dried to remove the solvent. The NLC powder was stored in the fridge until further experiment.

Table 1. Formulation of NLC containing oleic acid (F_{ola}) and avocado oil (and F_{avo})

Components	Functions	Amount (mg)	
		F_{ola}	F_{avo}
Paprika extract (containing 37% of capsanthin)	Active substance	500	500
Glyceryl monostearate	*Solid lipid*	600	600
Oleic acid	*Liquid lipid*	300	-
Avocado oil	*Liquid lipid*	-	300
Tween 20®	Hydrophilic Surfactant	600	600
Span 60®	Lipophilic Surfactant	300	300

Note: The composition is for a processing batch of 50 mL in an emulsification (dispersion) step using purified water

Measurement of transmittance, particle size, polydispersity index, and zeta potential of NLC dispersion

About 10 mg of NLC was dispersed in 5 ml water as well as in 20 mM PBS pH 7 by stirring at 300 rpm for 30 minutes at 37°C. The transmittance of dispersed NLC was measured at a wavelength of 650 nm using a Shimadzu UV1900 spectrophotometer. Moreover, the characteristics of the NLC, namely

particle size, polydispersity index, and zeta potential were determined using Zetasizer® (Malvern Panalytical).

Loading Capacity (LC) and Loading Efficiency (LE) Determination

Loading capacity reflects the concentration in percent mass of drug loaded in the carrier. In this research, LC was determined by direct method (Garms et al., 2021) by measuring the concentration of capsanthin dissolved from the NLC in ethanol. Briefly, a precise weight of about 10 mg of NLC sample containing capsanthin (Mp) was dissolved in ethanol up to 5 mL (Ve). Thereafter, the absorbance of the solution was measured at its maximum wavelength (λ_{max}) of 476 nm to calculate the capsanthin concentration (Cc) using a calibration curve. Loading capacity (LC) was calculated using Equation 1 (Garms et al., 2021).

$$LC(\%) = \frac{(Cc\ x\ Ve)}{Mp}\ x\ 100\% \quad \text{.....(1)}$$

The amount of capsanthin loaded (CcxVe) in a precise weight of NLC (Mp) in LC determination was also used to calculate loading efficiency (LE). Some additional data was needed in the LE calculation (Equation 2) (Garms et al., 2021), including the mass of total capsanthin processed in a batch as stated in Table 1 (Mc) and the mass of total NLC recovered from a process (Mt). To define Mt, we pre-weighed the mass of the plastic tube for the freeze-drying procedure and determined the mass of the plastic tube containing NLC once the freeze-drying step was finished. Mt was calculated as mass differences of such values.

$$LE(\%) = \frac{(Mt/Mp\ x\ Cc\ x\ Ve)}{Mc}\ x\ 100\% \quad \text{.....(2)}$$

Data Analysis

Independent sample t-test at a 95% confidence interval was performed using SPSS Statistics 17 (IBM, 2008) to analyze the differences between two groups regarding the effect of medium disperse (water and PBS) on NLC colloid characteristics, including transmittance, particle size, polydispersity index, and zeta potential of each formulation, as well as the effect of liquid lipid type constructing NLC on above characteristics either in water medium or PBS. Moreover, the same statistical approach was also used to determine the effect of liquid lipid type on loading capacity and loading efficiency.

RESULTS AND DISCUSSION

The development of vegetable oil extraction technology increases the variety of oils to be used as nutrients as well as pharmacological materials (Saputra et al., 2024). Avocado oil extracted by cold-pressing preserves its composition as original (Çakaloğlu et al., 2018; Wandhekar et al., 2023) showing potential as anticancer, anti-inflammatory, antidiabetic, and anti-cholesterol (Alkhalaf et al., 2019; de Oliveira Marques et al., 2022; Del Toro-Equihua et al., 2016; Ericsson et al., 2023; Ilesanmi et al., 2022). Utilization of vegetable oil, as a component of lipid nanocarrier, namely avocado oil, is expected to obtain two advantages including physical and biological characteristics. Before this application, several pre-studies should be conducted as in the present research. The NLC formulation products were subjected to characterization (Figure 1). Both NLC formulations showed a red color due to capsanthin and could not be distinguished visually.

Effect of liquid lipid on the transmittance of colloidal dispersion

Fast and cheap investigation of particle size can be performed by measuring the transmittance of colloidal dispersion of NLC using a Spectrophotometer set up at 650 nm. The type of dispersed medium did not give any significant effect (at $p<0.05$) on the transmittance of NLC dispersion. Generally, NLC dispersion showing transmittance at above 70% is accepted regarding the particle size, namely, less than 500 nm (McClements & Rao, 2011). This submicron-sized particle is still possible to be absorbed by

the gastrointestinal membrane via pinocytosis mechanism (Wang et al., 2023). Based on transmittance data available in Figure 2, both formulations were predicted to exhibit an acceptable size (McClements & Rao, 2011; Wang et al., 2023). However, since other factors alongside particle size also determine the transmittance of colloidal dispersion (Zhang & Reineccius, 2016), a confirmation from a standard instrument for particle size measurement was conducted.

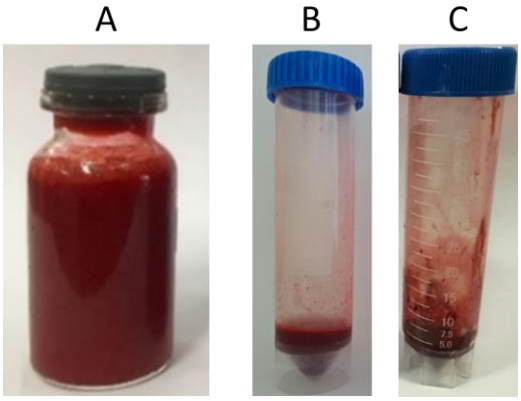

Figure 1. Photograph of NLC: emulsification step of NLC formulation (A), the supernatant of NLC formulation before freeze drying (B), The freeze-dried product of NLC (C)

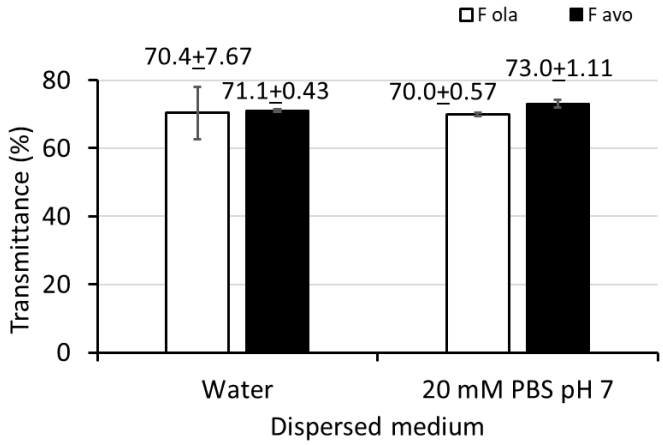

Figure 2. The transmittance of colloidal dispersion in water and PBS at a solid concentration of 2 mg/mL. The data is presented as mean ± SD of three replications

Effect of liquid lipid type on NLC characteristic

Liquid lipid type affects the particle size, size distribution, and zeta potential

In this research, two lipid types, namely, pure oleic acid and avocado oil, were utilized. In explaining the effect of liquid lipid type on NLC characteristics, the chemical information of avocado oil is provided in Figures 3 and 4. The lipid composition of avocado oil and the general molecular structure of the main lipid type are depicted in Figure 3 (Liu et al., 2023), whereas the variety of fatty acids present in avocado oil as either free or bonded to glycerol is shown in Figure 4 (Nasri et al., 2023).

As shown in Figure 5, dispersing NLC containing avocado oil, either in water or in PBS produced a significantly ($p<0.05$) higher particle size compared to that of F_{ola}. The higher particle size of NLC containing crude vegetable oil compared to oleic acid was also determined previously (Soeratri et al.,

2019). Two possibilities likely arose to argue the situation as follows. Firstly, as shown in Figure 4, the mass fraction of the polar substructure of oleic acid liquid lipid, namely COOH, is higher than that in the corresponding triglyceride, the most abundant lipid type of avocado oil (Figure 3). Exhibiting about 16% of the polar group, alongside as liquid lipid, oleic acid exhibits surfactant properties with a low HLB of about 1 (Martin & Bustamante, 1993; Sinko, 2006). This liquid lipid can assist Tween 20® and Span 60® in emulsifying the other lipid, leading to the production of small particles. The second argument is by applying the HLB-RHLB conformity theory suggesting that stable emulsion and structured lipid suspension are achieved in the nearest value between the HLB of surfactant/surfactants mixture and RHLB of lipid/lipids mixture. As a liquid lipid, oleic acid was assumed to have an RHLB of about 14-16, the value of general fatty acids (Pasquali et al., 2009), whereas the RHLB of avocado oil could be approximated from other vegetable oils by about 7 (Pham et al., 2022; Rave et al., 2020). Since the HLB of Tween 20® and Span 60® mixture at a 2:1 ratio is calculated to be about 13, it is closer to oleic acid than avocado oil. This second argument can also be used as a basis to improve the future formulation of NLC containing avocado oil, particularly in particle size issues. Once the exact value of the RHLB of avocado oil is known, the composition of the surfactant could be adjusted to reach the HLB approaching the RHLB. Moreover, the surfactant-to-lipid ratio and surfactant concentration in the dispersion system of preparation could be increased to reduce particle size in a targeted value, for instance, less than 200 nm to ensure permeability and stability during administration or delivery.

Showing a particle size of about 600 nm, the NLC applying avocado oil as liquid lipid is in the border of colloidal and coarse dispersion. Since the 600 nm value is the mean from a bulk NLC, some part of the bulk particle is in the colloidal range namely below 500 nm, and the other part is in the nanoparticle range, i.e. 500-1000 nm. In oral application, the colloidal particles below 200 nm are absorbed by nonspecific pinocytosis transport in all regions of the intestine. The larger particles are absorbed via M-cell contained in Peyer's patch and delivered to the lymphatic system where the particles are destroyed. Then, the smaller particles are delivered to the systemic circulation by-passing the liver (Cai et al., 2011; Delon et al., 2022; Trevaskis et al., 2007). In F_{ola} formulation, along with pinocytosis transport as in F_{avo}, some parts of NLC particles are below 50 nm which can be absorbed via a paracellular route in between intestinal cell (Mok, 2024).

Figure 5 likely disproves Figure 2 since Figure 5 shows that the particle size of F_{avo} was significantly ($p<0.05$) higher than F_{ola} in both mediums, while Figure 2 informs that the transmittance of colloidal dispersion predicted the equal particle size. However, they are not contradictory as explained in the following. The curve of transmittances as a function of particle size slews in a certain particle size depends on the dispersion type. For lipid/oil nano-carrier, the turning point usually lies in the range of 500-1000 nm, in which the border of the size-based dispersion type rests. Previous studies about lipid nanocarriers determined that the transmittance vs particle/droplet size curve shows a negative slope meaning the larger the particle/droplet the lower the transmittance is (Jan et al., 2022; Khan et al., 2020; Negi et al., 2014; Ziani et al., 2012). Inversely, coarse dispersion containing greater particle/droplet size, for instant emulsion, shows a greater transmittance (Linke & Drusch, 2016; Orafidiya & Oladimeji, 2002) It means that around 500 nm, for instance, 300-600 nm as in the present research, the transmittance significantly did not change.

Regarding the higher zeta potential in the negative direction of F_{ola} than F_{avo} dispersion in water medium as shown in Figure 6, it is explained by Figure 4 that the ionization ability of the hydrophilic substructure of oleic acid which is on the surface of lipid particles is higher than that of avocado oil consisting mostly of triglyceride. Consequently, it was likely that the solid surface of NLC containing oleic acid was more negative than that of avocado oil. The presence of electrolyte in dispersed medium likely donated the cation adsorbed on the particle surface of F_{ola} explaining the equal zeta potential of both NLC formulations in PBS dispersion.

The more negative zeta potential of NLC formulation containing oleic acid in comparison to that of lipid type other than free fatty acid which also evaluated in previous research (Andalib et al., 2012; Chinsriwongkul et al., 2012; Soeratri et al., 2019) indicates that oleic acid lays on the particle interphase. This strengthens the evidence that oleic acid has surfactant properties as mentioned previously. The

change in zeta potential by introducing a specific surfactant is adopted in the formulation of zeta potential changing nanocarrier. For instance, introducing a cationic surfactant in a lipid-based nanocarrier system, trimethyl tetradecyl ammonium bromide, brought the zeta potential of the nanocarrier to be positive, and *vice versa* for the addition of anionic surfactant (Efiana et al., 2022).

Zeta potential plays a crucial role regarding the stability during storage and the permeability in administration. The high zeta potential, either in a positive or negative direction hinders the flocculation of the dispersed phase of liquid formulation due to high electron repulsion, thereby hindering the increase in particle size and size distribution (Deryabin et al., 2015). Since both NLC formulations showed more than 30 mV (in a negative direction) then it is categorized as stable formulations (Malvern-Instrument, 2015). The negative value of zeta potential also provides a benefit in permeation across the mucus layer of the gastrointestinal membrane. The mucus of GI is constructed by oligosaccharides containing sialic acid sub-structure capable of ionizing. This negatively charged molecule attracts positively charged nanocarrier inhibiting nanocarrier permeation to the GI membrane (Griffin et al., 2016).

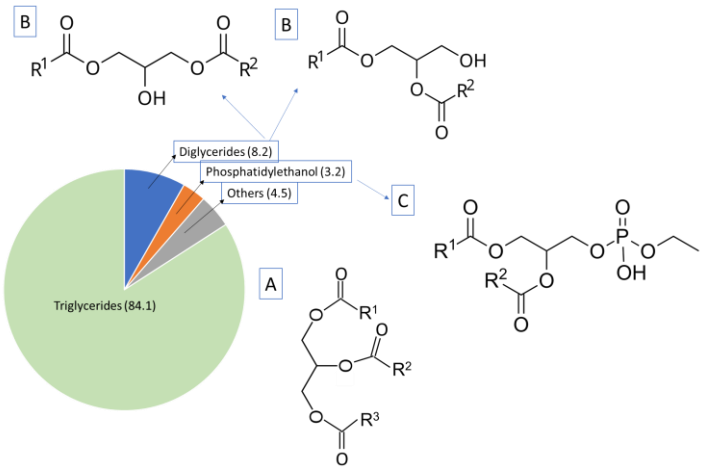

Figure 3. Lipid composition of avocado oil (Liu et al., 2023) and general molecular structure of triglyceride (A), diglyceride (B), and phosphatidylethanol (C). R^1-R^3 are carbon chains with or without double bonds which can be the same or different in carbon number

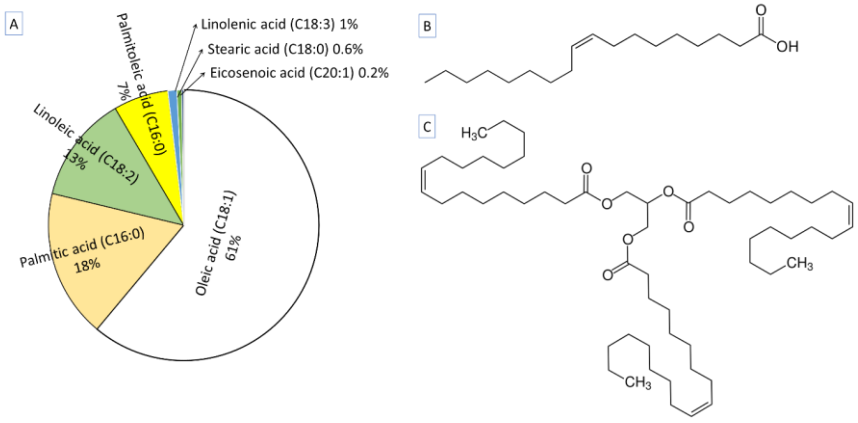

Figure 4. Average composition of fatty acid from 8 varieties of avocado oil (A) (Nasri et al., 2023), molecular structure of oleic acid (B) and glyceryl trioleate (C)

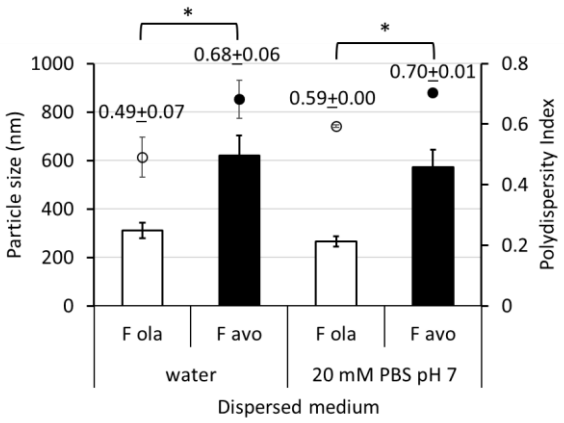

Figure 5. Particle size (bar) and polydispersity index (dot) of NLC particle dispersed in water and PBS of F_{ola} (white) and F_{avo} (black). The data is presented as mean ± SD of three replications. The star notation (*) indicates that the differences are significant at $p<0.05$, either for size or polydispersity index

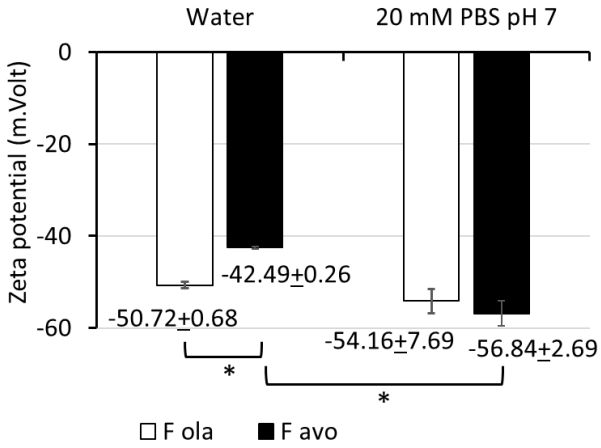

Figure 6. Zeta potential of NLC particle dispersed in water and PBS of F_{ola} (white) and F_{avo} (black). The data is presented as mean ± SD of three replications. The star notation (*) indicates that the difference is significant at $p<0.05$

Effect of liquid lipid type on NLC's drug loading

Drug loading in lipid nanoparticles (loading capacity, LC) reflects the solubility of drug molecules in the solid lipid constructing structured lipid carrier. The solubility of a drug in solid lipid is mostly determined by drug solubility in melted lipid at its melting point as an intrinsic property of drug and lipid (Alskär et al., 2016; Xu et al., 2022). Additionally, the crystallinity of solid lipids also plays a role in loading capacity in which low crystallinity solid, namely high defect lipid crystal, adopts more drug molecules leading to LC enhancement (Chauhan et al., 2020; Rosenblatt & Bunjes, 2017), thereby increasing the process efficiency. To induce and maintain lipid crystal defect, two liquid lipids were introduced in NLC formulation containing glyceryl monostearate solid lipid, namely oleic acid and vegetable oil. Capsanthin was used as a drug model to study the effect of resulted crystal defect on drug loading, and the data is shown in Figure 7.

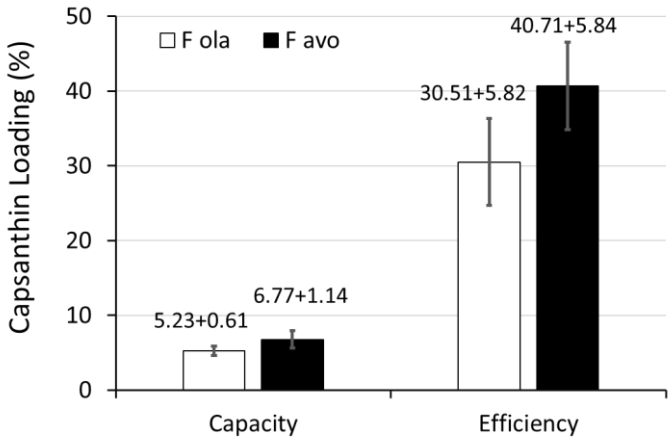

Figure 7. Loading capacity and loading efficiency of capsanthin in NLC formulation containing oleic acid (white bar) and avocado oil (black bar). The data is presented as mean ± SD of three replications

Figure 7 shows the slight differences in LC and LE of NLC due to the differences in liquid lipid components. It is predicted that a single-component liquid lipid, for instance, oleic acid, could be adopted more orderly during the crystallization of monoglyceride compared to a random molar ratio of multi-component liquid lipid as in vegetable oil. Therefore, the incorporation of avocado oil liquid lipids intensively decreased the crystallinity of solid lipids, higher than that of oleic acid. However, the high coefficient variation of the replication caused the differences are not significant (in $p<0.05$). Additionally, the physical characteristics might be used to indicate such lipid crystal imperfection (Aragão & Maximo, 2024; Flakemore et al., 2014; Folayan et al., 2019).

Effect of medium disperse on NLC characteristic

In particle size analysis using the dynamic light scattering method, particle size is represented by hydrodynamic diameter. The size is not only determined by solid-state diameter but also includes the electric Stern layer, an ionic solution extending from the surface of the solid to the slip plane when the particle is moving (Gordillo-Galeano & Mora-Huertas, 2021; Maguire et al., 2018). Since the thickness of the Stern layer is affected by ionic strength determined by the salt concentration in the dispersed medium (Brown et al., 2016), measuring the particle size in different mediums might yield different results. Moreover, the electrolyte concentration in the medium also affects the electric repulsion determined by the zeta potential of particles (Hutin et al., 2023). In this research, the characteristics of colloidal dispersion of NLC in 20 mM PBS pH 7 representing the condition in the gastrointestinal medium were measured and compared to those in water.

Figure 5 indicates that the particle size of both NLC formulations was decreased by the availability of electrolytes in the dispersed medium. Since the dispersed particle was the same, the differences in particle size after dispersion are affected by at least two possibilities, namely the particle aggregation and Stern layer shrinking or extending. In this study, the second likelihood is preferred since the particle size in the electrolyte medium is lower than in water which is in line with a theory suggesting that the thickness of the Stern layer is reduced by enhancing the ionic strength of the medium (Brown et al., 2016). The decrease in particle size due to the enhancement of ionic strength up to 3% was also evaluated in emulsion (Narukulla et al., 2020). The mechanism of electric layer compression due to electrolyte addition into medium disperse was explained by Guerrero-Garcia and co-workers. Briefly, the electrolyte presented in medium disperse increases the colloidal surface charge density, thereby inducing the deswelling of the electric field (Guerrero-García et al., 2019). Nonetheless, several previous studies identified that the particle size of NLC dispersed in electrolyte solution was higher than that in water. It

is explained that electrolytes induce particle aggregation (Choi et al., 2014; Gordillo-Galeano & Mora-Huertas, 2021). It is also possible that aggregation in PBS medium increasing the particle size has occurred but in a less pronounced than Stern layer shrinking. The higher size distribution of both NLC formulations dispersed in PBS in comparison to that in water correlated to aggregation. The electrolyte induced some parts of the NLC particle to aggregate, while another part remained as separate particles. This situation produced a higher size distribution in the PBS medium (Figure 5).

Regarding the higher zeta potential of both NLC formulations when dispersed in PBS (Figure 6), it is justified as follows. Potential-determining ion, the cation in this case, adsorbed onto the solid surface, followed by anionic counterions (*gegenions*) adsorbed on the next-outer layer by the slip plane. In the water medium, the availability of potential-determining ions and *gegenions* is not enough to decrease the negatively charged surface resulting in a maintaining of the more negative of F_{ola} until the slip plane. The more negative of the solid surface of F_{ola} formulation could be countered by cation and anions available in PBS medium resulting in equal zeta potential to F_{avo}. It should be noted that the thickness of the Stern layer in the PBS medium is less than in water, explaining why the similar zeta potential is more negative in the PBS medium than in water. However, it should be considered that based on statistical analysis the effect of the dispersed medium was only significant ($p<0.05$) on the zeta potential of F_{avo} and the polydispersity index of F_{ola}.

CONCLUSION

Pre-formulation studies on the use of avocado oil in NLC formulations have been conducted to improve the utilization of this oil for pharmaceutical applications as an alternative to oleic acid. This oil has the potential to be used in NLC formulation since the zeta potential, loading capacity, and loading efficiency are in an acceptable range and close to the NLC standard applying oleic acid liquid lipid. However, the higher particle size and size distribution of NLC-containing avocado oil in comparison to NLC-containing oleic acid should be addressed. It is suggested to adjust the surfactant ratio in the formulation of NLC containing avocado oil to reduce particle size and size distribution. Alternatively, the nonionic surfactant type could be used in further research, including in the NLC formulation applying other vegetable oils.

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