Effect of Lipid and Oil Compositions on Physicochemical Properties and Photoprotection of Octyl Methoxycinnamate-loaded Nanostructured Lipid Carriers (NLC)

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Abstract: This study aimed to evaluate the effect of solid lipid and oil structures on the physicochemical properties, kinetic release, photostability, and photoprotection of nanostructured lipid carriers (NLC) containing octyl methoxycinnamate (OMC). OMC was used as a model compound since it is an effective sunscreen agent and is widely used in sunscreen products; however, it is unstable after ultraviolet radiation (UVR) exposure. OMC-loaded NLC were prepared from different solid lipids (cetyl palmitate (CP) or tristearin) and oils (caprylic/capric triglyceride, isopropyl myristate or isononyl isononanoate) at a 4:1 ratio. After production, the particle size (z-ave) and polydispersity index (PDI) of OMC-loaded NLC ranged from 190 to 260 nm and were lower than 0.25, respectively, and the zeta potential (ZP) values were higher than [50 mV]. The Fourier transform infrared (FTIR) spectroscopy results indicated no interaction among the components. Data obtained from differential scanning calorimetry (DSC) and X-ray diffraction showed that the incorporation of oil into solid lipids disturbed the crystallinity of the lipid matrix, depending on the structure of the oil molecule. OMC loaded in tristearin-based NLC (OMC-tristearin-NLC) showed higher release of OMC than OMC loaded in CP-based NLC (OMC-CP-NLC). For photostability properties, OMC-CP-NLC prepared from isononyl isononanoate showed the highest stability owing to the less-ordered structure, providing space for accommodation of OMC, whereas the percentage of OMC remaining in tristearin-based NLC was comparable. Therefore, the degree of protection was dependent on the type of solid lipid and oil. As a result, branched-chain fatty acids provided a higher degree of disturbance than linear-chain fatty acid.

Key words: nanostructured lipid carriers, NLC, *in vitro* release study, branched-chain hydrocarbons, photostability, photoprotection

1 Introduction

Ultraviolet radiation (UVR) can be divided into three categories based on wavelength: UVA (320-400 nm), UVB (290-320 nm) and UVC (100-290 nm). The UV radiation reaching the Earth's surface is mainly composed of UVA (approximately 95%) and UVB (approximately 5%), while UVC is absorbed by the ozone layer¹⁻³. Excessive exposure to UVR is harmful to the skin, resulting in cancer,

sunburn and skin aging⁴⁻⁶. Therefore, the application of sunscreen before exposure to UVR is necessary to prevent sunburn and skin aging and reduce the risk of skin cancer^{1,7}.

Octyl methoxycinnamate (OMC) or ethylhexyl methoxycinnamate is one of the most widely used organic UVB filters due to its good protection potential⁸. OMC is used in a large number of sunscreen products; however, it is not stable after exposure to UVR, especially when used in

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combination with butyl methoxydibenzoylmethane (BMDBM) which may cause skin irritation and sensitization^{8–10}. This is due to the degradation of OMC resulting in toxic degradation products. Ideally, UV filters should remain on the skin surface or in the stratum corneum after application to maximize product efficacy and safety without deep penetration into the skin and subsequently into the blood circulation.

Many studies have reported the successful incorporation of UV filters into lipid nanoparticles, including solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and nanoemulsions (NE)^{8, 11–18}. In addition, an increase in photostability and a decrease in skin permeability of UV filters could be achieved after incorporation into lipid nanoparticles^{12, 15–17}. Puglia *et al.* indicated that OMC loaded in NLC and microemulsion (ME) showed the highest photostability, followed by OMC loaded in SLN and gel. The higher photostability of OMC in NLC and ME than in SLN resulted from higher OMC encapsulation, suggesting that NLC were an effective and promising drug delivery system for UV filters⁸. Liu *et al.* reported that encapsulation of OMC in SLN resulted in higher photostability compared to conventional emulsions¹⁶. Therefore, lipid nanoparticles are a promising carrier for sunscreen agents. The performance of lipid nanoparticles reported in many studies depends on many factors such as the compositions of lipids, oils and emulsifiers, as well as the preparation techniques¹⁹. The aims of this study were to evaluate the effect of lipid and oil structures on the physicochemical properties and on photoprotection of OMC loaded in NLC. As a result, using different structures of oil molecules (e.g., linear-chain or branched-chain fatty acids) to prepare NLC may affect the properties of the NLC. Therefore, NLC prepared from different solid lipids (i.e., cetyl palmitate (CP) or tristearin) and oils (i.e., caprylic/capric triglyceride, isopropyl myristate or isononyl isononanoate) were developed and OMC was used as a model compound due to its instability. The physicochemical properties including particle size (z-ave), polydispersity index (PDI), zeta potential (ZP),

crystallinity and polymorphism were evaluated. The interaction among the compounds was also analyzed by Fourier transform infrared (FTIR) spectroscopy. The release of OMC from the formulations was achieved using Franz diffusion cells. The photostability of OMC was analyzed by high-performance liquid chromatography (HPLC) and photoprotection was evaluated by sun protection factor analyzer before and after exposure to UVR.

2 Materials and Methods

2.1 Materials

Octyl methoxycinnamate (OMC) (>98%), isopropyl myristate, isononyl isononanoate and DMDM hydantoin were purchased from Namsieng (Bangkok, Thailand). Tristearin (Dynasan® 118) (Triglycerides >95%) was a gift from IOI Oleo GmbH (Hamburg, Germany). Polyceryl-3 methylglucose distearate (Tego® Care 450) was obtained from Evonik (Darmstadt, Germany). Cetyl palmitate (>98%) was acquired from SABO S.p.A. (Levate, Italy). Caprylic/capric triglyceride was purchased from Inolex (Philadelphia, USA). Acetonitrile was purchased from Burdick & Jackson (Seoul, Korea). Acetic acid was obtained from Sigma-Aldrich (St. Louis, MO, USA). Sterile water for injection (pH ~5.5–5.7) was acquired from Thai Nakorn Patana (Bangkok, Thailand). All chemicals were used as received without modification.

2.2 Preparation of NLC

One hundred and fifty grams of each NLC formulation was produced by high pressure homogenization (HPH) according to the method described by Müller *et al.*¹⁹ based on the proportions listed in Table 1. Briefly, lipid phases containing solid lipid, oil and OMC were first melted at 80°C, then added to hot aqueous solutions (85°C) comprising Tego® Care 450 and mixed at 8,000 rpm for 1 min using a high speed homogenizer (IKA T25 Ultra-Turrax®, Germany). The obtained pre-emulsions were then passed

Table 1 Compositions of OMC-loaded NLC.

Composition	NLC1	NLC2	NLC3	NLC4	NLC5	NLC6
Cetyl palmitate	7.2	7.2	7.2	—	—	—
Tristearin	—	—	—	7.2	7.2	7.2
OMC	1.0	1.0	1.0	1.0	1.0	1.0
Caprylic/capric triglyceride	1.8	—	—	1.8	—	—
Isopropyl myristate	—	1.8	—	—	1.8	—
Isononyl isononanoate	—	—	1.8	—	—	1.8
Tego® Care 450	1.8	1.8	1.8	1.8	1.8	1.8
DMDM hydantoin	0.3	0.3	0.3	0.3	0.3	0.3
Water q.s. to	100.0	100.0	100.0	100.0	100.0	100.0

through a high pressure homogenizer (APV 2000, UK) at pressures of 500 bar and 80°C three times. Finally, the hot nanoemulsions were cooled to room temperature under ambient conditions to obtain NLC particles.

2.3 Particle size and zeta potential (ZP) analysis

The particle size in terms of the z-average diameter (z-ave) and polydispersity index (PDI) of OMC-loaded NLC were assessed by photon correlation spectroscopy (PCS) using a Malvern Zetasizer Nano Series ZS (Malvern Instruments, UK). PCS yields the z-ave and the PDI which is defined as a measure of the width of the particle size distribution. Prior to the measurement, the samples were diluted with sterile water to obtain a suitable scattering intensity. Measurements were performed at a scattering angle of 173°, and the z-ave and PDI values were calculated as the mean of three evaluations.

The ZP values of all samples were analyzed by determining the particle electrophoretic mobility by the Malvern Zetasizer Nano ZS. The sample was diluted with sterile water for injection adjusted the conductivity to 50 µS/cm using a 0.9% w/v sodium chloride solution to avoid the variations in the ZP values due to variations in the water conductivity²⁰⁾. All samples were measured in triplicate.

2.4 Differential scanning calorimetry (DSC) measurement

Thermal analysis was performed using a Mettler DSC 1 (Mettler Toledo, Gießen, Switzerland). The NLC dispersions (approximately 10–20 mg) were accurately weighed in 40 µL aluminum pans. The instrument was calibrated with an indium standard. For the mixture of lipids and oils, the sample was heated at 80°C for 1 h and cooled down to room temperature under ambient condition before DSC measurement for 24 h. For the DSC measurement, the samples were heated from 20°C to 85°C and then cooled to 20°C at a rate of 5°C/min. An empty aluminum pan was used as a reference. The DSC parameters including onset temperature, melting point and enthalpy were evaluated using STAR[®] Windows 9.13 Software (Mettler Toledo, Switzerland). The crystallization index (CI%) was calculated as follows²¹⁾.

$$CI(\%) = \left(\frac{\Delta H_{\text{NLC aqueous dispersion}}}{\Delta H_{\text{bulk material}} \times \text{Concentration}_{\text{lipid phase}}} \right) \times 100$$

where ΔH_{NLC} and $\Delta H_{\text{bulk material}}$ are the melting enthalpy (J/g) of the NLC dispersions and bulk lipid, respectively.

2.5 X-ray diffraction analysis

X-ray diffraction measurements were performed by wide-angle X-ray scattering (WAXS, 2 Theta 3–40°, Miniflex 600, Rigaku, USA) with a copper anode (Cu-K_α radiation, 40 kV, 15 mA, $\lambda = 1.54056 \text{ \AA}$), using a goniometer as a detector. The sample was mounted on a quartz sample holder before measurement by WAXS. The data used were typically collected with a step width of 0.02° and a count

time of 60 s. Short spacing diffractograms were calculated using the Bragg equation²²⁾.

2.6 Fourier transform infrared (FTIR) spectroscopic analysis

An FTIR spectrophotometer (Nicolet iS5 + iD5 ATR, USA) was employed for analysis of the possibility of interaction in the formulations. FTIR spectra of the bulk lipids (tristearin and CP), oils (isopropyl myristate, isononyl isononanoate and caprylic/capric triglyceride), OMC, a physical mixture of oil, solid lipid and OMC, and OMC-loaded NLC dispersions were analyzed using the attenuated total reflection (ATR) technique. The samples were placed directly on the diamond crystal and FTIR spectra were measured over the range of 4000–400 cm^{−1}.

2.7 Encapsulation efficiency (EE) measurement

The EE of OMC in NLC was determined by the ultrafiltration method. Briefly, 1 g of OMC-loaded NLC dispersion was placed in a centrifuge tube (Amicon Ultra-4, molecular weight cut-off (MWCO) of 30 kDa) and centrifuged at 5,000 rpm for 60 min at room temperature. Afterwards, the filtrate was collected for measurement of free OMC in the NLC dispersion using HPLC. The EE was calculated using the following equations:

$$EE = \frac{\text{Total amount of OMC} - \text{Amount of OMC in filtrate}}{\text{Total amount of OMC}} \times 100$$

2.8 *In vitro* release and kinetic release studies

In vitro release studies were performed using static Franz diffusion cells. The volume of an acceptor chamber was approximately 12 mL and the temperature of the diffusion cells was controlled at 32 ± 0.5°C to mimic that of the skin surface²³⁾. A dialysis membrane with a MWCO of 12,000–14,000 Daltons was mounted between the receptor and donor chambers. The receptor medium was composed of a solution of methanol and 0.02 M phosphate buffer pH 5.5 at a ratio of 20 and 80, respectively, and maintained under stirring using magnetic bars at 300 rpm. The samples, 300 µL of OMC-loaded NLC, were spread homogeneously onto the dialysis membrane surface on the donor compartment. Five hundred microliters of the receptor medium was sampled at 2, 4, 6, 8, and 10 h and an equal volume of freshly prepared receptor phase was replaced after each sampling. OMC was analyzed by an HPLC-UV detector (Shimadzu LC-10AD, Japan) connected with a Hypersil[®] ODS C18 column (Thermo Scientific, USA, 4.6 mm × 250 mm, 5 µm particle size). The injection volume was 20 µL, and the mobile phase was acetonitrile and 2% acetic acid (80:20 v/v), with a 1.2 mL/min constant flow rate using an LC-10AD isocratic pump (Shimadzu, Japan). The retention time of OMC was 7.0 min, as observed by the UV detector at 310 nm. All experiments were carried out in triplicate at ambient temperature.

To evaluate the kinetic release of OMC from NLC, different kinetic release models were applied including the zero-order model, Higuchi model, Hixon-Crowell and Korsmeyer-Peppas model^{24–26}. The equations for each kinetic release model are as follows:

Zero-order kinetics: $Q_0 - Q_t = kt$

Higuchi kinetics: $Q_0 - Q_t = kt^{1/2}$

Hixon-Crowell kinetics: $Q_0^{1/3} - Q_t^{1/3} = kt$

Korsmeyer-Peppas kinetics: $\log(Q_0 - Q_t) = \log k + n \log t$ where Q_0 and Q_t are the amount of active compound at the initial time and remaining at a particular time, respectively; k is the rate constant; and t is the time.

The cumulative amount of OMC release per area ($\mu\text{g}/\text{cm}^2$) through the membrane was plotted as a function of time. The kinetic model that best expressed the release profile of OMC was that with the highest determination coefficient (r^2).

2.9 Photostability studies of OMC-loaded NLC

The photostability of OMC-loaded NLC was ascertained by placing the test samples in a solar simulator (Atlas Suntest CPS+, USA). The samples were filled into glass bottles and exposed to solar radiation at 100 and 200 J/cm². The percentage of OMC remaining was analyzed before and after irradiation by an HPLC-UV detector as described above.

2.10 Photoprotection measurement

2.10.1 Sample preparation

Each sample was applied onto 5 × 5 cm polymethylmethacrylate (PMMA) plates on a standardized rough side at an application rate of 1.3 mg/cm² (27, 28). Briefly, the sample was applied to a large number of small droplets on the PMMA plate with a syringe. The specification of the PMMA plate complied with the guidelines of ISO 24443: Determination of sunscreen UVA photoprotection *in vitro*²⁹. After

spreading, the test sample was kept in the dark and allowed to dry for 15 min before measurement.

2.10.2 Photoprotection evaluation before and after UV radiation

The absorbance of the test samples was measured between 290 and 400 nm and recorded using an SPF-290S Analyzer (Optometrics 290S, USA). Before the measurement, the wavelength accuracy, linearity and absorbance limits were checked following the ISO 24443 guidelines. The plates were then positioned in the solar simulator and exposed to solar radiation at 100 J/cm². Afterward, a change in absorbance was detected by the SPF-290S Analyzer. Absorption curves were plotted before and after exposure to UV light, and the area under the curve (AUC) was calculated. The percentage of AUC remaining was computed by the following equation:

$$\% \text{ AUC} = \left(\frac{\text{AUC after irradiation}}{\text{AUC before irradiation}} \right) \times 100$$

2.11 Data analysis

The data were expressed as the mean ± standard deviation (SD) and were analyzed for statistical significance by Student's *t*-test or one-way ANOVA. The statistical significance of the difference was set at the probability level of 0.05.

3 Results and Discussion

3.1 Particle size, size distribution and zeta potential

The OMC-loaded NLC were prepared using CP or tristearin as a solid lipid, and isopropyl myristate, isononyl isononanoate or caprylic/capric triglycerides as a liquid lipid. After production, the z-ave of all formulations ranged from 190 to 260 nm and the PDI was lower than 0.25 (Fig. 1). This indicated that HPH was a suitable technique for

Fig. 1 Mean particle size (z-ave) and PDI values of all OMC-loaded NLC formulations after production.

the production of OMC-loaded NLC. The ZP values of all developed OMC-loaded NLC were higher than $|\ 50\text{ mV} |$, as shown in Fig. 2. From the literature, ZP values higher than $|\ 60\text{ mV} |$ are required for excellent physical stability, and higher than $|\ 30\text{ mV} |$ are considered physically stable(30). Although all formulations were stabilized by a nonionic emulsifier (i.e., polyglyceryl-3 methylglucose distearate), the ZP values were highly negative ($> |\ 50\text{ mV} |$). This could be explained due to the dissociation of free fatty acids at the surface and the strong interaction between the hydrophilic chain of the emulsifier (polyglycerol linked with methyl glucose) and water, resulting in negatively charged hydroxyl ions³⁰⁾. Subsequently, an electric double layer could form around the particles. In addition, the nonionic surfactant could enhance the stability of the colloidal

system by steric hindrance which could prevent particle aggregation, coalescence and phase separation.

3.2 DSC

DSC measurements of bulk CP, bulk tristearin and OMC-loaded NLC were performed to evaluate the effect of oil incorporation on the inner structure of the lipid matrix (e.g., melting point, crystallinity and polymorphism). Table 2 shows the DSC parameters of bulk CP, bulk tristearin, mixtures of solid lipid and oil at the same ratio of NLC formulations after tempering at 80°C for 1 h, and OMC-loaded NLC formulations prepared from different solid lipids and oils. The DSC thermogram of bulk CP consisted of two crystal forms corresponding to α -modification for the melting endotherm at 44°C and β' -modification for the melting endo-

Fig. 2 ZP values of all OMC-loaded NLC formulations.

Table 2 DSC parameters of CP, tristearin, mixtures of lipids and oils after tempering at 80°C for 1 h and OMC-loaded NLC.

Formulation	Onset (°C)	Melting point (°C)	Enthalpy (J/g)	CI (%)
CP	49.17	49.66	188.43	100.00
Tristearin	69.85	73.76	217.04	100.00
CP: Caprylic/capric triglyceride	39.33	47.33	124.79	66.23
CP: Isopropyl myristate	32.35	44.58	107.71	57.16
CP: Isononyl isononanoate	22.36	45.25	66.22	35.14
Tristearin: Caprylic/capric triglyceride	66.28	69.42	103.14	47.54
Tristearin: Isopropyl myristate	66.84	68.17	113.71	52.39
Tristearin: Isononyl isononanoate	63.84	67.75	86.12	39.68
NLC1	42.23	46.33	1.50	7.96
NLC2	37.04	45.84	1.10	5.84
NLC3	36.29	45.18	1.05	5.57
NLC4	63.14	67.06	9.40	43.31
NLC5	62.02	66.18	6.48	29.86
NLC6	60.72	65.15	4.50	20.73

therm at 50°C (Fig. 3)¹⁸⁾. For the DSC thermograms of CP-based NLC (i.e., NLC1, NLC2 and NLC3) only a melting endotherm in the range of 45–47°C was observed, corresponding to β' -modification. For tristearin-based NLC (i.e., NLC4, NLC5 and NLC6), the melting endotherm decreased to between 60°C and 64°C compared to that of bulk tristearin (74°C), as indicated in Fig. 3. This decrease in the melting endotherm of the nanoparticles compared to bulk lipids was a result of the small particle size in the nanometer range, the high specific surface area and the presence of surfactant rather than a result of polymorphic changes³¹⁾. The melting endotherm depression owing to the decrease in particle size to the nanometer range can be explained by the Kelvin effect described by the Thomson equation³²⁾. Furthermore, the melting point of all developed NLC was higher than the skin temperature (around 32°C), which is the general requirement for topical application of lipid nanoparticles. As a result, they were suitable for topical application.

The DSC results of the mixtures of solid lipid and oil after tempering at 80°C for 1 h are shown in Table 2. Incorporation of the oil into the solid lipid decreased the CI(%) and onset temperature, depending on the structure of the oil molecule. This effect was also observed in OMC-loaded NLC. Depression of the onset temperature of OMC-loaded NLC was more pronounced with the incorporation of isononyl isononanoate into CP and tristearin matrices than with isopropyl myristate and caprylic/capric triglyceride. In addition, the CI(%) of all developed OMC-loaded NLC dispersions, prepared from either CP or tristearin matrices, was lower than 100% and depended on the structure of oil loading. Concerning the chemical structure of solid lipids and oils, CP is an ester wax derived from palmitic acid and cetyl alcohol that both contain a 16-carbon backbone. Tristearin is a triglyceride derived from three units of stearic acid (C18). According to the CI(%), incorporating isononyl isononanoate, which is the ester of a branched-chain nonyl

alcohol (C9) with a branched-chain nonanoic acid (C9), would greatly disrupt the crystalline structure of the NLC lipid matrix compared to caprylic/capric triglyceride containing linear-chain hydrocarbons of 8 and 10 and isopropyl myristate which is the ester of a branched chain propyl (C3) with a linear chain myristic acid (C14). The incorporation of isononyl isononanoate (branched chain) into CP or tristearin could weaken the hydrophobic interaction due to the steric hindrance caused by branched hydrocarbon chains, leading to a less-ordered structure. The results showed that the structure of the oil molecule affected the crystallinity of the lipid matrix.

3.3 X-ray diffraction

Figure 4 shows X-ray diffractograms of bulk lipids (i.e., CP and tristearin) and OMC-loaded NLC dispersions. The X-ray diffractograms of all OMC-CP-NLC (Fig. 4A) revealed peak reflections at 0.38 and 0.42 nm, indicating β' -modification similar to that of bulk CP (data not shown). This suggested that incorporation of OMC and oils did not alter the polymorphic change in CP. For tristearin-based NLC, X-ray diffractograms of all OMC-tristearin-NLC showed patterns similar to those of bulk tristearin (Fig. 4B). Peak reflections were detected at 0.37, 0.38 and 0.46 nm, indicating β -modification. Generally, triacylglycerols exhibit several polymorphic forms with diverse orderly arrangements of unit cell structures and provide different thermodynamic behaviors. The literature review indicated that the main polymorphic forms of triacylglycerols are α , β' and β ³³⁾. The α -form is a hexagonal subcell with a short spacing of 0.42 nm, the β' -form is an orthorhombic perpendicular subcell with short spacings of 0.42–0.43 and 0.37–0.40 nm, and the β -form is a triclinic parallel subcell with a short spacing of 0.46 nm³⁴⁾.

The peak intensity was affected by the structure of oil loading as it relates to the crystallinity of lipid matrix, with higher peak intensity suggesting higher crystallinity. The

Fig. 3 DSC thermogram of CP and tristearin.

peak intensity of OMC-loaded NLC prepared from isononyl isononanoate was slightly lower than that prepared from isopropyl myristate and caprylic/capric triglyceride, indicating a less-ordered structure. Therefore, the data obtained from X-ray diffraction agreed with the DSC results which indicated that isononyl isononanoate disturbed the lipid matrix more than isopropyl myristate and caprylic/capric triglyceride.

3.4 Fourier transform infrared (FTIR) spectroscopy

FTIR studies were conducted to obtain information regarding the interaction among active, lipid and other compositions as well as the encapsulation efficiency of the active into the lipid matrix^{35–37}. FTIR spectra of OMC-CP-NLC and OMC-tristearin-NLC and their compositions are shown in Figs. 5A and 5B, respectively.

Compared to other excipients, OMC showed characteristic peaks between 1600 and 1650 cm^{-1} , indicating the

presence of a cyclic alkene and monosubstituted alkene. From Figs. 5A and 5B, all NLC dispersions showed the characteristic peaks of OMC. This suggested that no interaction between OMC and the excipients in the NLC formulation occurred.

3.5 Encapsulation efficiency (EE)

The EE of all formulations was determined by evaluating the amount of OMC in the filtrate. The NLC particles and filtrate were separated using a dialysis tube and the filtrate was analyzed by HPLC. The OMC peak was not observed in the chromatogram. Therefore, 100% EE of OMC was assumed in all NLC formulations.

3.6 *In vitro* release and kinetic release

In vitro release was evaluated using static Franz diffusion cells. The sink condition was maintained throughout the experiment. Figures 6A and 6B show the release pro-

A

B

Fig. 4 X-ray diffractograms of NLC1, NLC2 and NLC3 (A) and NLC4, NLC5 and NLC6 (B).

A

B

Fig. 5 FTIR spectral of bulk CP and NLC1, NLC2 and NLC3 (A) and bulk tristearin and NLC4, NLC5 and NLC6 (B).

files of OMC-CP-NLC and OMC-tristearin-NLC, respectively. Regarding the release of OMC-CP-NLC, NLC1 showed the highest release, followed by NLC2 and NLC3. The lowest amount of OMC released from NLC3 was due to the less-organized lipidic structure, as indicated by the lower CI(%), providing space for OMC accommodation³⁸⁾. Similar findings were also found for OMC-tristearin-NLC (Fig. 6B). The amount of OMC released from tristearin-based NLC was higher than that released from CP-based NLC. During the experiment, it was observed that film formation was initially perceived within 4 h for tristearin-based NLC and after approximately 8 h for CP-based NLC. This was due to the difference in CI(%). OMC-tristearin-NLC showed higher CI(%) than OMC-CP-NLC (Table 2). The film formation might be due to the polymorphic transition of the lipid matrix. As a result, OMC was expelled from the NLC, resulting in a high percentage of OMC in the acceptor

medium³⁹⁾.

Concerning the effect of oils on the release of OMC, the amount of OMC released was related to the crystallinity. For CP-based NLC, NLC3 having the lowest crystallinity showed the lowest OMC release followed by NLC2 and NLC1. This result confirmed that the type of oil affected the amount of OMC released. However, the effect of oils on the release of OMC from tristearin-based NLC was less pronounced than that from CP-based NLC. This might be due to the homogeneous distribution of OMC in the lipid matrix of tristearin-based NLC. The obtained results, therefore, indicated that the release of OMC could be modified by varying either the types of oils or solid lipids in the NLC formulations.

To evaluate the release kinetics of OMC from NLC, four different release kinetic models including the zero order, Higuchi, Hixon-Crowell and Korsmeyer-Peppas models

A

B

Fig. 6 Release profiles of OMC-CP-NLC (A) and OMC-tristearin-NLC (B).

Table 3 Determination coefficient (r^2) of OMC-loaded NLC formulations fitted to zero order, Higuchi, Hixon-Crowell and Korsmeyer-Peppas models.

Kinetic model	NLC1	NLC2	NLC3	NLC4	NLC5	NLC6
Zero-order model	0.8038	0.9845	0.9780	0.9387	0.9401	0.8932
Higuchi model	0.9697	0.8891	0.8786	0.9721	0.9870	0.9938
Hixon-Crowell kinetics model	0.5492	0.5869	0.5418	0.6588	0.6123	0.5827
Korsmeyer-Peppas model	0.9015	0.9215	0.9394	0.9455	0.9821	0.9768

were applied and the determination coefficients (r^2) were compared. **Table 3** presents the determination coefficient of OMC released from NLC. The release pattern of OMC from NLC depended on the composition of the NLC. The release kinetics of tristearin-based NLC were best fitted to

Higuchi's model. For CP-based NLC, the release kinetics of NLC1 followed Higuchi's model, whereas those of NLC2 and NLC3 fit the zero order model. The difference in release kinetics may reflect the encapsulation model due to different disturbances of the lipid matrix by oil mole-

cules.

3.7 Photostability studies of OMC-loaded NLC evaluated by HPLC

The photostability of OMC-loaded NLC was evaluated before and after exposure to solar radiation at 100 and 200 J/cm². The percentage of OMC remaining depended on the composition of the formulation (Fig. 7). NLC3 showed the highest percentage of OMC remaining, followed by NLC2 and NLC1. This might be because OMC was encapsulated in the less-ordered structure of NLC3 compared to NLC2 and NLC1, as confirmed by DSC. As aforementioned above, the less-ordered structure provided space for OMC accommodation which could help protect the OMC from UVR, leading to the highest percentage of OMC in NLC3. This indicated that disturbance of the inner structure of the lipid matrix enhanced OMC stability. In contrast, the percentages of OMC remaining in OMC-tristearin-NLC prepared from different oils were comparable, referring to the homogeneous distribution of OMC in the lipid matrix. The obtained data were in line with the data obtained from the *in vitro* release study.

3.8 Photoprotection measurement

UV absorption of OMC-loaded NLC was measured and compared to that of an OMC solution. After application of OMC-loaded NLC on the PMMA plates, absorbance was measured between 290 and 400 nm before and after exposure to solar radiation at 100 J/cm². The area under the curve (AUC) of the absorbance between 290 and 320 nm of OMC-loaded NLC was calculated before and after exposure to UV radiation, and the percentage of AUC remaining after exposure to UVR was computed and compared to the initial value. Figure 8A depicts the percentage of AUC remaining after exposure to UV radiation. The AUCs of all OMC-loaded NLC and OMC solutions after UV radiation

were significantly lower than the initial values ($p < 0.05$). This indicated that OMC was not stable after exposure to UV radiation. Compared to that of the OMC solution, the AUCs of all OMC-loaded NLC were significantly higher ($p < 0.05$). Thus, improvement of OMC photostability was achieved by means of encapsulation of OMC into lipid nanoparticles; however, the percentage of OMC remaining was lower than 90%. Generally, sunscreen is considered photostable when the percentage of UV sunscreen remaining is higher than 90% after exposure to light⁴⁰. Similar findings were also reported for OMC and avobenzone encapsulated in NLC¹¹.

For OMC-CP-NLC, the percentage of AUC remaining after exposure to 100 J/cm² UVR was reduced and depended on the type of oil loading. NLC3 (79.1% $\pm$ 9.6%) showed the highest percentage of AUC remaining, followed by NLC2 (70.4% $\pm$ 16.4%) and NLC1 (65.6% $\pm$ 8.5%) as shown in Fig. 8A. The data were in agreement with the results obtained from photostability studies as previously mentioned. For OMC-tristearin-NLC, the percentage of AUC remaining after exposure to 100 J/cm² UVR among NLC4 (68.8% $\pm$ 8.0%), NLC5 (70.3% $\pm$ 1.8%) and NLC6 (74.6% $\pm$ 5.9%) was comparable. The percentage of AUC remaining for the OMC solution (46.4% $\pm$ 7.6%) dramatically decreased compared to the initial value.

Concerning the absorbance spectra before exposure to UVR, the absorbance of OMC-CP-NLC was higher than that of OMC-tristearin-NLC (Fig. 8B). It has been reported that incorporation of a UV filter into lipid nanoparticles may offer a synergistic effect in terms of UV absorbance due to the solid property of lipid nanoparticles compared to conventional o/w nanoemulsions^{41, 42}. Nikolic *et al.* prepared NLC formulations containing UV filters from different solid lipids (i.e., Atowax, Compritol® 888 ATO, carnauba wax and beeswax). The NLC prepared from different solid lipids showed a difference in UV absorbance (carnauba wax >

Fig. 7 Percentage of OMC remaining analyzed by HPLC before and after solar irradiation at 100 and 200 J/cm².

A **B**

Fig. 8 Percentage of AUC remaining before and after exposure to UVR (A) and absorbance spectral of OMC-loaded NLC and OMC solution before exposure to UVR (B).

beeswax > Compritol® 888 ATO ~ Atowax). This might be due to the differences in the distribution of actives in the particles¹²⁾. Compared to the OMC solution, all NLC formulations showed lower UV absorbance. The OMC molecules in the OMC solution could freely interact with the UVR while those in the NLC particles were entrapped in the lipid, possibly preventing the interaction between OMC and UV radiation, resulting in low UV absorbance. Based on our observations, the difference in absorbance between CP-based NLC and tristearin-based NLC could be explained by the differences in crystallinity and OMC distribution in NLC, i.e., core or shell models. Thus, the types of oils and solid lipids had an impact on the photoprotection of OMC-loaded NLC.

4 Conclusions

The physicochemical properties, photostability and release kinetics of OMC-loaded NLC depended on the lipid and oil compositions. The types of both oils and solid lipids affected the inner structure of the obtained NLC, resulting in different properties such as particle size, size distribution, *in vitro* release, photostability and photoprotection. The addition of a branched-chain oil had a greater effect on the disturbance of the inner structure than the addition of a linear-chain liquid oil, resulting in a lower % CI. Encapsulation of OMC in NLC could may enhance the photostability.

Conflict of Interest

The authors confirm no conflict of interest for this article.

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