Department of Pharmaceutical Sciences[1], Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand; Department of Pharmaceutics[2], Biopharmaceutics & NutriCosmetics, Freie Universität Berlin, Germany; Department of Applied Logistics and Polymer Sciences[3], University of Applied Sciences, Kaiserslautern, Pirmasens, Germany

Development of lycopene-loaded nanostructured lipid carriers: effect of rice oil and cholesterol

P. Riangjanapatee[1], R. H. Müller[2], C. M. Keck[3], S. Okonogi[1]

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Siriporn Okonogi, Ph.D., Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University Chiang Mai 50200, Thailand
siripon.okonogi@cmu.ac.th

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Nanostructured lipid carriers (NLC) were developed using a skin-compatible surfactant and natural lipid materials (rice oil, cholesterol) to incorporate lycopene. Characteristics of the NLC were explored in comparison with nanoemulsions and solid-lipid nanoparticles (SLN). Photon correlation spectroscopy, laser diffractometry (LD) and differential scanning calorimetry were used to determine particle size and thermal stability. Particle size expressed as LD (0.99) was 405 nm for the SLN, 350 nm for the NLC without cholesterol and 287 nm for the NLC with cholesterol. Rice oil and cholesterol enabled the formation of smaller particles, but cholesterol also reduced drug stability in the NLC. To preserve chemical stability of lycopene in the NLC, cholesterol should be avoided and storage should be at 4 °C or at room temperature.

1. Introduction

For people with sensitive skin, dermal application of skin products may result in problems such as acne, rosacea, burning and stinging, and high susceptibility to contact and irritant dermatitis. To avoid such problems, skin-friendly skin care ingredients or excipients are increasingly considered for topical formulations. In the present study, we demonstrate the development of a promising, skin friendly nanoparticle product manufactured by an emulsion technique using high pressure homogenization with skin friendly lipid materials, having lycopene as an active ingredient.

The cosmetic industry nowadays moves away from ethoxylated and polymeric surfactants. PEG-free is becoming a quality criterion (Cosmetic Ingredient Reviews 1999). Eumulgin SG, an anionic and skin friendly emulsifier based on sodium stearoyl glutamate enables high efficiency emulsification tolerating electrolytes (Cognis Corporation 2009). Emulsification by this surfactant could be achieved at low concentrations. Skin pH of about 5 or lower usually limits the utilization of anionic surfactants; however, Emulgin SG is superior to other anionic surfactants because it is effective over a wide pH-range, including pH values <5. Therefore, it was selected as the major emulsifier in this study.

The lipid phase of the nanoparticles developed in the present study is mainly composed of orange wax, a solid biodegradable lipid from the fruit peel of *Citrus*. It is acceptable for cosmetic use in the United States, Europe, and Japan. It was reported to have many activities such as sunscreen-enhancing, antioxidant, antimicrobial and anti-inflammatory properties (Reynhardt and Riederer 1991; Puleo and Rit 1992). The main constituents of orange wax are unsaturated monoesters, hydroxy-monoesters, free fatty acids (C12-C26), hydrocarbons (C21-C33), sterol esters, free sterols, free alcohols, carotenoids, glycolipids, phospholipids, and flavonoids (Puleo and Peters 1994). Orange wax can be classified as a skin friendly lipid material since its lipid composition is similar to that of skin.

Rice oil is the edible natural fixed oil obtained from the bran of the rice kernel during the process of rice milling. It is composed of unsaturated fatty acids, triterpene alcohols, phytosterols, tocotrienols, alpha-tocopherol, gamma-oryzanol, squalene, and other nutrients (Sugano and Tsuji 1997; Orthoefer 2005). These components are useful in protecting cells against the effects of free radicals. They aid in slowing down the effects of aging, e.g. by slowing the formation of skin wrinkles (Santa-María et al. 2010). Gamma-oryzanol impedes the progress of melanin pigmentation and is effective in keeping skin smooth while squalene supports the collagen within the skin (Lerma-García et al. 2009). Moreover, the high content of fatty acids in rice oil is beneficial for mature, delicate and sensitive skin.

Cholesterol is a lipid naturally produced by the body and is essential for the maintenance of healthy cell walls. It is one of three major lipid classes found in the skin (Bouwstra et al. 2001) that serve essential functions not only in terms of good skin health but also the health of the entire body. Skin cholesterol levels may decrease because of factors such as aging, various disorders, or use of certain drugs. Skin cells may deteriorate and perish due to lack of cholesterol. The *stratum corneum* becomes flaky, resulting in a severe dried skin condition called xerosis (Harding et al. 2000). Cholesterol imparts water-absorbing power with an emollient activity. Incorporation of cholesterol into topical products is necessary to soothe and plump up dry skin.

Using only one lipid component like orange wax might be insufficient to meet the requirements of a desirable formulation. Therefore, rice oil and cholesterol were incorporated as minor

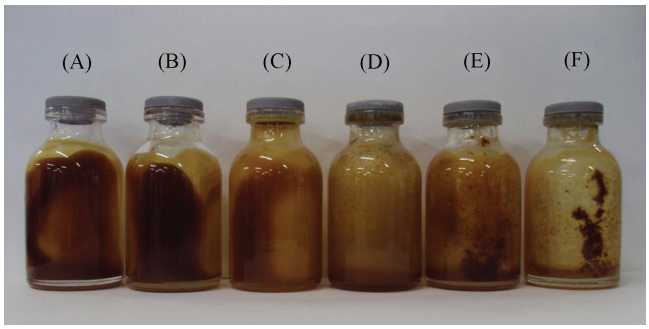

Fig. 1: The physical appearance of the melted orange wax (A) and orange wax - rice oil mixtures containing oil of 10% (B), 20% (C), 30% (D), 40% (E) and 50% (F).

constituents of the lipid phase of orange wax. The effect of these two components was investigated on the size, size distribution and zeta potential as well as the stability of the obtained nanoparticles. Lycopene, an acyclic carotene with 11 conjugated double bonds found in tomato, watermelon, and pink grapefruit, was reported to possess pronounced antioxidant, anti-inflammatory, anti-cancer, and anti-mutagenic properties (Stahl and Sies 1996; Giovannucci 1999; Heber and Lu 2002). However, its therapeutic usefulness is limited by certain disadvantages. Lycopene is water insoluble and hardly diffusible via the transdermal pathway when applied topically. Moreover, it is unstable and highly susceptible to oxidation when exposed to air or sunlight (Shi et al. 2003).

It was reported that the stability of many active ingredients such as vitamin A, coenzyme Q10, ascorbyl palmitate, and vitamin E is increased after incorporation into lipid nanoparticles (Dingler 1999; Jee et al. 2006; Teeranachaideekul et al. 2007a,b). The utilization of lipid nanoparticles to increase the stability of lycopene by using skin friendly materials as lipid carriers is considered a worthwhile challenge and has not yet been reported elsewhere. The aim of the present study was to develop suitable skin friendly NLC for entrapment of lycopene. The effects of the lipid materials on the characteristics and stability of the lycopene loaded nanoparticles were explored.

2. Investigations, results and discussion

2.1. Effect of lipid combination

Materials of brown, homogeneous texture were obtained from the mixtures of rice oil and orange wax containing 10% and 20% oil as seen in Fig. 1 . Small fragments of separated solid lipid could be seen in the mixtures containing 30–50% oil, indicating incomplete miscibility of the wax and oil. The thermograms

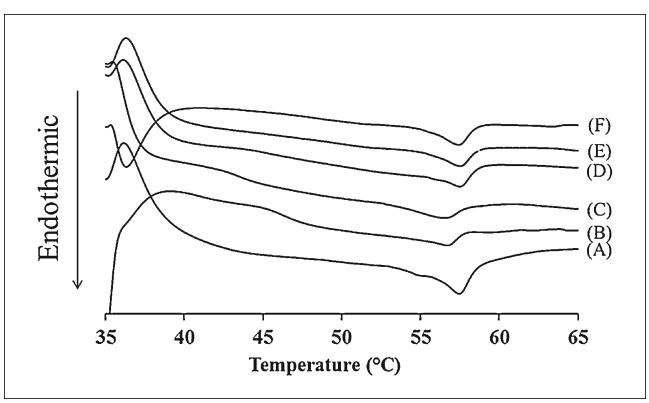

Fig. 2: DSC thermograms of the melted orange wax (A) and orange wax - rice oil mixtures containing 10% (B), 20% (C), 30% (D), 40% (E) and 50% oil (F).

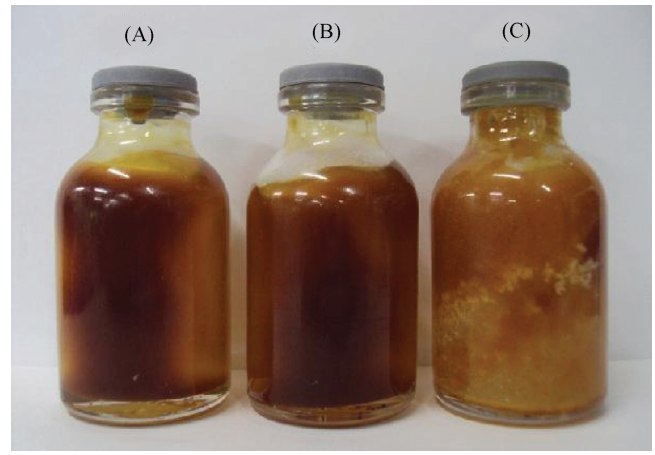

Fig. 3: Physical appearance of the melted orange wax/cholesterol mixtures of 4:1 (A), 3:1 (B), and 7:3 (C).

obtained by differential scanning calorimetry (DSC) of these mixtures running from 35 to 65 °C are shown in Fig. 2. The melting point of orange wax was observed at 57.8 °C. Adding 10% or 20% of rice oil results in decreased melting temperatures of the wax of 56.6 °C and 56.3 °C, respectively. Orange wax is composed of C21-C33 hydrocarbons, sterol esters, free sterols, glycolipids, phospholipids, carotenoids, and flavonoids. These compounds were well arranged with limited imperfection holes (Puleo and Peters 1994). Adding of certain oils might distort the formation of perfect lipid crystals of the wax by incorporation into these holes as a monolayer film and interaction with the molecules of wax substances resulting in imperfections of the crystal lattice of the mixtures and slightly decreased melting point of the wax. The endothermic peaks of the mixtures containing 30, 40, and 50% oil were found to be 57.6, 57.5, and 57.4 °C, respectively. These peaks were considered to be the melting peak of the pure orange wax. These results could be explained that the oil at first might spread through the surface of the wax and modified the interfacial tension to, at equilibrium, a lower value than that of the pure wax. The residual oil hence would gather together in a completely separate phase from the wax and cover the wax surface in a monolayer film. On the basis of these results, the completely miscible lipid mixtures containing rice oil and orange wax of 1:9 and 2:8 were selected for further study.

Considering the skin friendly effects of cholesterol, we attempted to incorporate as much cholesterol as possible in the formulations. The miscibility between orange wax and cholesterol was investigated prior to formulation. A heterogeneous

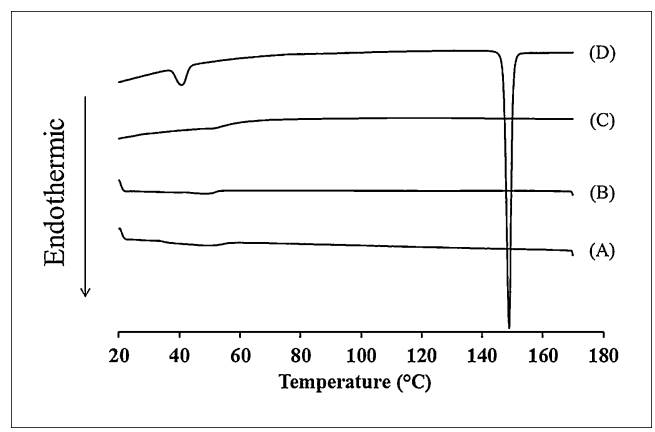

Fig. 4: DSC fusion curves for the mixtures of orange wax and cholesterol at ratios of 4:1 (A) and 3:1 (B), orange wax (C) and cholesterol (D).

Table 1: Composition of ingredients used in the lipid nanoparticle bases

Material	Amount of ingredients (% w/w)		
	Formula 1	Formula 2	Formula 3
Orange wax	5.0	4.5	4.0
Rice oil	0.0	0.5	1.0
Eumulgin SG	1.0	1.0	1.0
Milli Q water qs.	100	100	100

texture with separated lipid matter distributed through the system was found in the mixture of wax/cholesterol, 7:3 as seen in Fig. 3. A pale brown homogeneous texture was obtained from the mixtures of 4:1 and 3:1. Hence, mixing quantities of orange wax and cholesterol in these ratios was considered to result in homogeneous miscibility. The mixtures of 4:1 and 3:1 were selected for further investigation by DSC. The thermograms of these mixtures shown in Fig. 4 revealed that the melting peak of cholesterol at 147.9 °C was absent while melting endothermic peaks were observed at 48.1 °C and 48.3 °C for mixture ratios of 4:1and 3:1, respectively. These endothermic peaks are similar in shape to the melting peak of orange wax at 57.8 °C. These data suggested that cholesterol could completely dissolve in the melted orange wax. Considering the most suitable ratio, the mixture at a ratio of 4:1 was selected and used for further experiments.

2.2. Preparation and characterization of the formulations

Lipid nanoparticle bases composed of ingredients as shown in Table 1 were prepared. Formula 1 yielded solid lipid nanoparticles (SLN) whereas in Formulae 2 and 3, lipid phases containing 1:9 and 1:4 weight ratios of rice oil to orange wax, respectively, were used to form nanostructured lipid carrier (NLC) formulations. These formulations were obtained in the form of homogenous, viscous pale yellowish liquids as shown in Fig. 5. Particle size analysis indicated that Formula 2 yielded the lipid nanoparticles of smallest size as shown in Fig. 6. Moreover, after 1 day at room temperature (25 °C), the SLN (Formula 1) and NLC (Formula 2) were unchanged whereas solid lipid fragments had obviously been formed from Formula 3, indicating the separation of the ingredients in the formula (Fig. 7). Formulae 2 and 3 correspond to NLC products and differ from each other by the quantity of rice oil. These results suggested that, in order to obtain homogenous formulations, the rice oil loading in

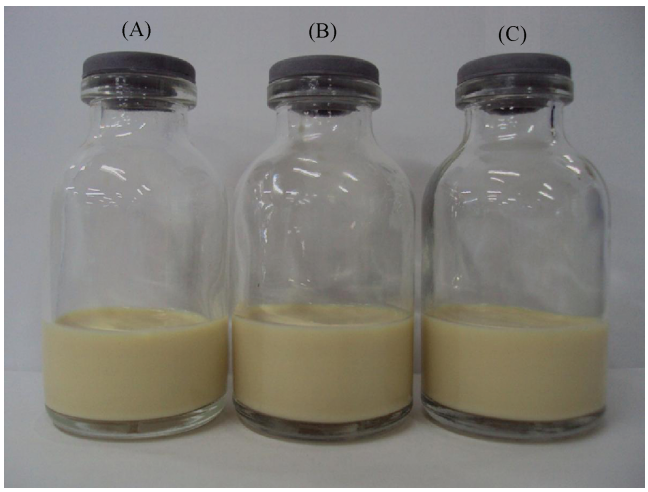

Fig. 5: Outer appearance of Formula 1 (A), Formula 2 (B) and Formula 3 (C).

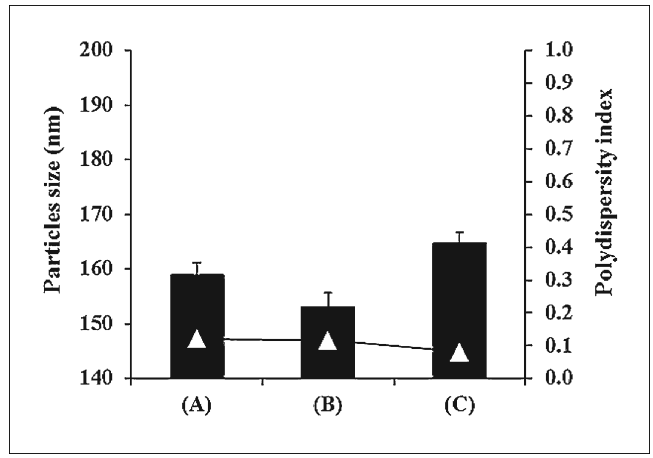

Fig. 6: Particle size and size distribution of Formula 1 (A), Formula 2 (B) and Formula 3 (C).

the NLC should be not more than 10%. It was known that incorporation of oil into the solid lipid could reduce the degree of organization of the lipid matrix (Muller et al. 2002). Lipid mixtures incorporating an optimum quantity of rice oil facilitated particle size reduction. The smaller particle size obtained was therefore considered to be due to the effect of rice oil incorporated into the system. However, increasing oil content to more than 20% resulted in bigger particles. The larger particle size could be mainly due to particle aggregation.

In order to obtain the maximum therapeutic efficacy *via* the transdermal route, the size of the carrier plays the most important role. With the extremely small size of the NLC, the amount of encapsulated drug reaching the site of action will be increased because the particle ensures close contact to the *stratum corneum* and thus, the bioavailability of drugs penetrating into viable skin can be enhanced (Souto et al. 2007). In the present study, we therefore focused our attention on the particle size of the products. According to our results, four lycopene loaded lipid nanoparticle formulations composed of the selected lipid combinations and ratios as shown in Table 2 were prepared. It was found that the outward appearance of the four emulsions obtained was that of opaque liquids with yellowish orange color corresponding to the color of lycopene. The emulsion type was tested by the dye-soluble method and conductivity measurements. All emulsions were miscible with the water soluble dye and showed conductivity values higher than 100 μS/cm as shown in Table 3. This result indicates that all emulsions obtained were of the o/w type.

2.3. Effect of lipid on the state of the internal phase

DSC provides information on the thermal behavior of a compound by measuring the heat loss or gain resulting from physical

Table 2: Composition of ingredients used in lycopene loaded lipid nanoparticle formulations

Material	Amount of ingredients (% w/w)			
	Formula 4	Formula 5	Formula 6	Formula 7
Lycopene	0.005	0.005	0.005	0.005
Orange wax	0.0	5.0	4.5	3.6
Cholesterol	0.0	0.0	0.0	0.9
Rice oil	5.0	0.0	0.5	0.5
Eumulgin SG	1.0	1.0	1.0	1.0
Milli Q water qs.	100	100	100	100

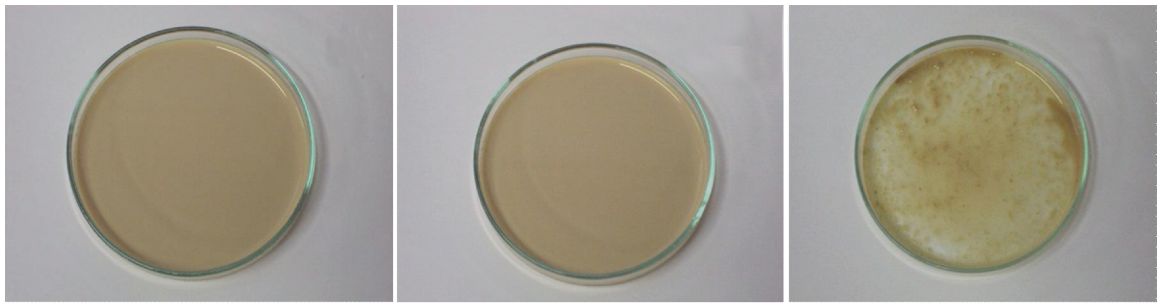

Fig. 7: Outer appearance of Formula 1 (A), Formula 2 (B), and Formula 3 (C) after 1 day's storage at room temperature.

Table 3: Conductivity values of lycopene loaded lipid nanoparticle formulations

Formula	Conductivity (μS/cm)
4	1208.33 ± 17.21
5	2060.05 ± 20.01
6	1851.00 ± 13.45
7	1293.67 ± 11.93

or chemical changes within a sample as a function of temperature. In order to investigate the state of the internal phase, the products were subjected to DSC and the results are shown in Fig. 8. Formulae 4 and 5 were compared as they differ from each other by one lipid component, the liquid state rice oil and the solid state orange wax, respectively. The DSC thermogram of Formula 4 shows no endothermic peak or melting point as seen in Fig. 8(A) whereas an endothermic peak was observed at 54.4 °C for Formula 5 as seen in Fig. 8(B). This result confirms that the state of the internal droplets of Formula 4 is liquid whereas that of Formula 5 is solid. As expected, the state of the lipid raw material used in the formula determines the state of the internal phase of the product.

2.4. Effect of rice oil and cholesterol on the state of the internal phase

To investigate the effect of rice oil, Formula 5 was compared with Formula 6, and Formula 6 was compared with Formula 7 when the effect of cholesterol was investigated. The DSC thermograms of Formulae 6 and 7 exhibited melting peaks at 48.4 °C and 46.1 °C, respectively, as shown in Figs. 8(C) and 8(D). This result clearly indicates that the internal phases of these emulsions are solid state. Replacement of some solid orange wax

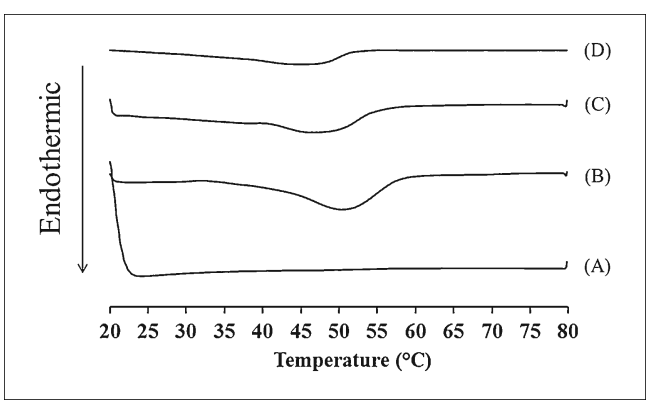

Fig. 8: DSC thermograms of Formula 4 (A), Formula 5 (B), Formula 6 (C) and Formula 7 (D).

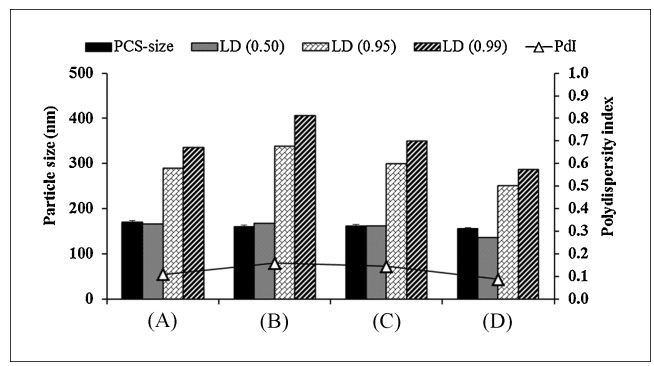

Fig. 9: Partcle size and size distribution of Formula 4 (A), Formula 5 (B), Formula 6 (C) and Formula 7 (D).

with liquid rice oil reduced the melting point of the internal phase. Further replacement of some orange wax with cholesterol led to further reduction of the melting point. According to the solid lipid nanoparticle classification (Müller et al. 2006), the lipid particle internal phase of Formula 5 is classified as a simple SLN whereas that of Formulae 6 and 7 is categorized as NLC.

2.5. Effect of rice oil and cholesterol on particle size of the internal phase

In order to obtain maximum physical stability and skin penetration, the particle size of the internal phase should be extremely small and homogeneous without any aggregation (Mäder and Mehnert 2005). The small size (<1 μm) of the internal phases of all formulae was determined by photon correlation spectroscopy (PCS) whereas the larger size (>1 μm) was measured by laser diffractometry (LD). The PCS yielded the mean particle size (z-ave) and the polydispersity index (PdI) which indicates the width of the size distribution. The LD data of 50%, 90%, and 99% cumulative undersize of median volume weighted diameters expressed as LD (0.50), LD (0.90), LD (0.99), respectively, indicate the percentages of particles possessing a diameter equal to or lower than the given value. Mie theory was used for LD data evaluation. The real refractive index and the imaginary refractive index were set as 1.456 and 0.01, respectively. These values were assessed to be valid for our lipid nanoparticle formulations (data not shown) by using the methods and equipment cited in previous reports with similar oil variation (Muller and Schuhmann 1997; Kovacevic et al. 2011). There are slight variations in the indices depending on the nature of matrix lipid and stabilizer used, but for the envisaged development of a dermal formulation with lipid variation as in our study range, these effects can be neglected (Pardeike et al. 2010; Muchow et al. 2011). The PCS diameter of Formula 4 was 170 nm as shown in Fig. 9. The LD diameters of all formulae were found to be less than 1 μm. According to the state of the internal phase and droplet size,

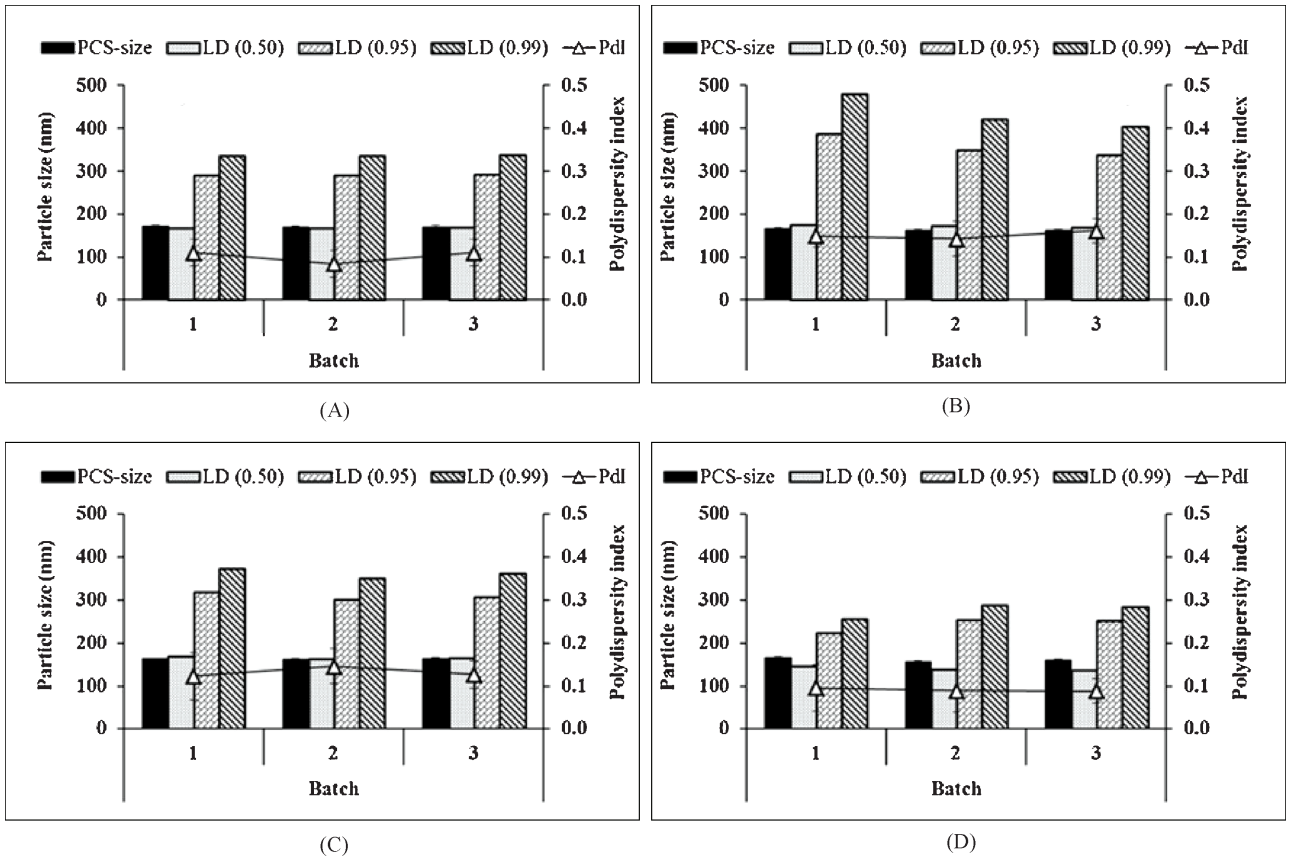

Fig. 10: Particle size and size distribution of the three batches of Formula 4 (A), Formula 5 (B), Formula 6 (C) and Formula 7 (D).

Formula 4 is classified as nanoemulsion. The PCS diameters of the SLN (Formula 5) and NLC (Formula 6) were insignificantly different, amounting to ca.160–162 nm. The NLC of Formula 7 is slightly smaller with a diameter of 156 nm. Considering the LD diameters at LD (0.95) and LD (0.99) of solid particles in each system, it was found that the lipid particles of the NLC (Formulae 6 and 7) are significantly smaller than those of the SLN (Formula 5). The incorporation of oil into the solid lipid could reduce the degree of organization of the lipid matrix as described above. The resulting smaller particle size obtained in both NLC formulae was therefore considered to be due to the incorporation of rice oil. The effect of rice oil could be seen clearly when Formula 5 was compared with Formula 6. The rice oil disrupts the structure of the orange wax and facilitates size reduction by high pressure homogenization. This result is in accordance with Mitri et al. (2011) who have reported that the incorporation of oil into solid lipid results in smaller particle size. In Formula 7, some more orange wax was replaced with cholesterol but the particles obtained were still classified as NLC. The effect of cholesterol can be seen when comparing the LD (0.95) and LD (0.99) sizes of Formula 6 (without cholesterol) and Formula 7 (with cholesterol). Addition of cholesterol significantly reduces the LD diameters of the NLC. Cholesterol is composed of hydrophilic and lipophilic parts, enabling it to act as a surface active agent. Its use was reported as co-emulsifier in a bioactive delivery system (Mu and Feng 2001). Therefore, the small particle size obtained with Formula 7 was considered to be due to the surface active function of cholesterol. The results of the present study confirm the ability of cholesterol to facilitate emulsification. The PdI for all formulations were below 0.16, indicating a good narrow size distribution. Fig. 10 reveals that the mean PCS diameters of the three batches of each formula were insignificantly different ($p < 0.05$). This result illustrates the high batch-to-batch reproducibility of the formation of small size particles. The LD diameters of each formula shown in this figure suggest that good reproducibility of the production process can be achieved with Formulae 4, 6, and 7.

2.6. Effect of rice oil and cholesterol on zeta potential of the internal phase

Zeta potential plays an important role in the prevention of particle agglomeration. Fig. 11 shows that the zeta potentials of all formulae were not significantly different. Therefore, rice oil or cholesterol has no influence on the zeta potential of the internal phase. Zeta potential values below −30 mV indicate good physical stability of lipid nanoparticles. The zeta potential values measured ranged from −66 mV to −74 mV. These high zeta potential values are considered to be due to the anionic groups of the surfactant used.

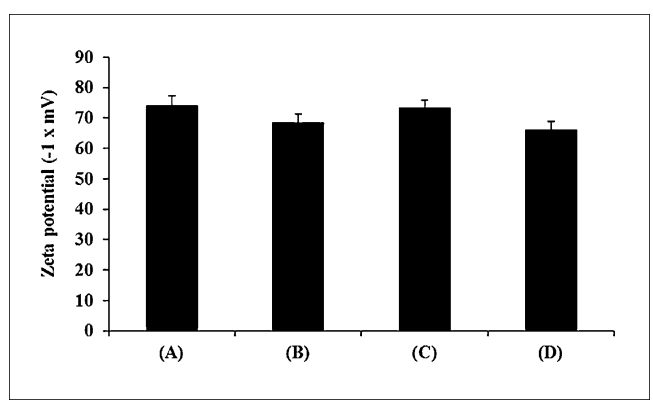

Fig. 11: Zeta potential of Formula 4 (A), Formula 5 (B), Formula 6 (C) and Formula 7 (D).

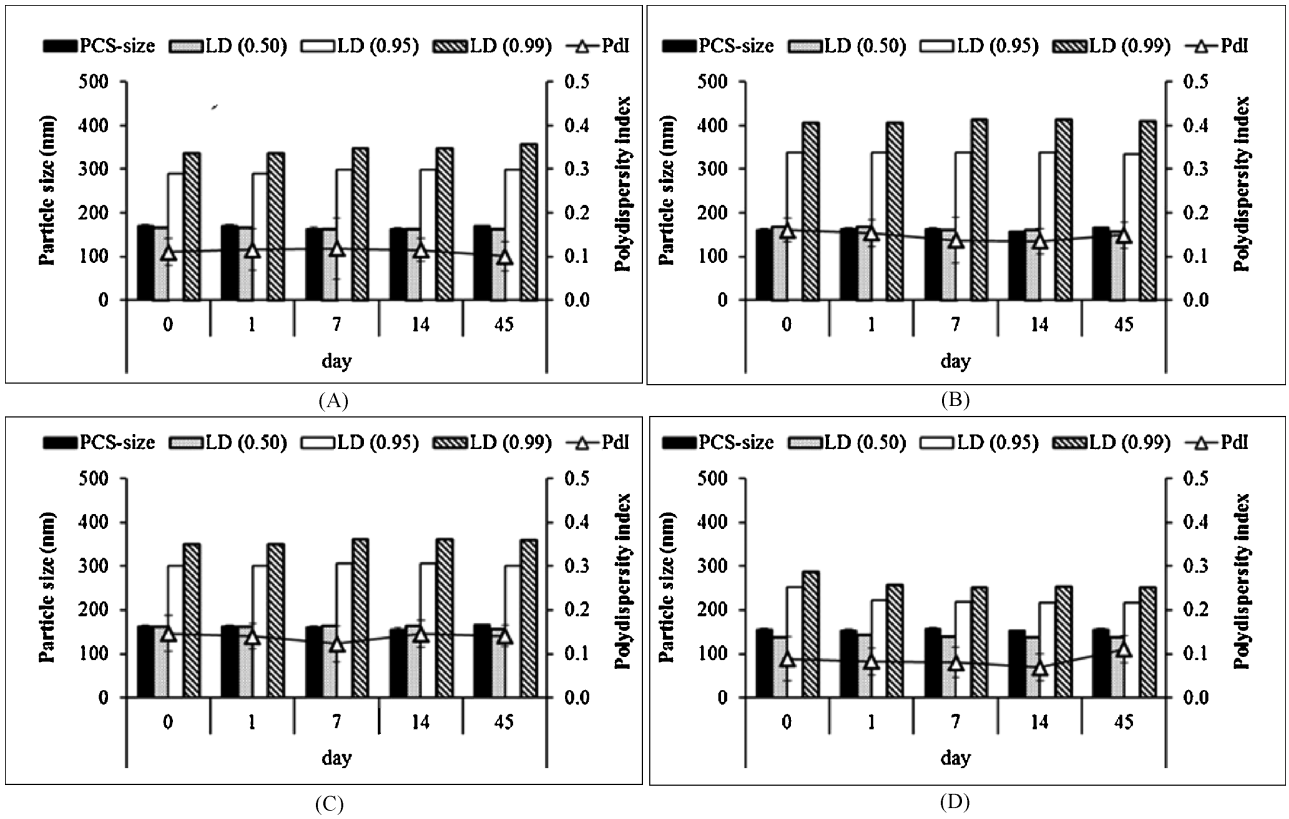

Fig. 12: Particle size and size distribution of Formula 4 (A), Formula 5 (B), Formula 6 (C) and Formula 7 (D) during stored at 25 °C for 45 days.

2.7. Stability

The developed formulations showed constant particle size during 45 days of storage at 25 °C as shown in Fig. 12. This is considered to be due to the high zeta potential causing the particles to repel each other, resulting in a stable colloidal system. According to the small particle size, Formulae 6 and 7 were selected for further stability study at different temperature settings.

The color of the formulations was found to change from pale yellow to brown after 14 days' storage at 40 °C and became dark brown at the end of the test period (45 days). The determinations of size and zeta potential for the formulations kept at 40 °C after 14 days were omitted in view of the obvious degradation of the products. The particle size of Formula 6 kept at 4 °C and 25 °C stayed almost constant at 158–163 and 156–162 nm, respectively, as seen in Figs. 13(A) and 13(B), whereas that of Formula 7 kept at both temperatures stayed at 156–163 and 152–156 nm, respectively as seen in Figs. 13(D) and 13(E). The PdI values of the two formulas kept at 4 and 25 °C are below 0.2. Formulae 6 and 7 exhibited stable zeta potential values at ca. −73 and −66 mV, respectively during the study period (Fig. 14).

Lycopene is an unstable molecule. It was reported that more than 95% of lycopene is degraded from a lycopene oil solution stored at room temperature for 24 h (Riangjanapatee and Okonogi 2012). In the present study, lycopene loaded nanoemulsion, SLN, and NLC formulations kept at 25 °C for 45 days were compared with that of a lycopene oil solution. No color change was observed in the SLN and NLC formulations while the color faded rapidly in the nanoemulsion and oil solution, indicating the loss of lycopene content in these systems. This result underscores the potential of lipid nanoparticle systems for protecting lycopene from degradation in skin formulations.

Chemical stability profiles of lycopene in the NLC formulations are shown in Fig. 15. Formula 6 kept at 4 °C and 25 °C showed high stability of lycopene compared with that at 40 °C as shown in Fig. 15(A). This result is in agreement with a previous study of the effect of thermal processing on lycopene degradation (Stahl and Sies 1992). Müller et al. (2011) reported that the addition of oil to a solid lipid can prevent or retard the recrystallization of the lipid to form a less stable modification. Hence, over time, no changes or fewer changes in modification occur in NLC and thus no or minimized drug expulsion is observed. This can explain the retention of lycopene inside the nanoparticles of Formula 6, resulting in high chemical stability.

Formula 7 kept at 4 °C could protect lycopene from degradation for a week. However, lycopene was almost totally degraded after 3 weeks in all tested conditions of 4 °C, 25 °C, and 40 °C as shown in Fig. 15(B). Considering the components in Formula 7 which include the highly crystalline cholesterol, it must be assumed that after preparation, at least a part of the particles crystallizes in a higher energy modification (α or β′). During storage, these modifications can revert to the low energy, more ordered β modification. Due to its high degree of order, the number of imperfections in the crystal lattice is reduced. The drug can therefore be expelled from the nanoparticles, leading to a large quantity of drug in the water phase where it is easily degraded. The results of this study suggest that the suitable storage temperature for the developed NLC systems should not be above 25 °C.

2.8. Conclusion

Lycopene-loaded NLC formulations were successfully developed using skin-friendly materials. The observed particle diameter by LD (0.99) of SLN was 405 nm whereas that of NLC without cholesterol was 350 nm. The NLC with cholesterol had a diameter by LD (0.99) of 287 nm. Rice oil and cholesterol facilitated the reduction of particle size. Both NLC systems showed high zeta potential and physical stability. Nevertheless, the chemical stability profile of lycopene was unfavorable when

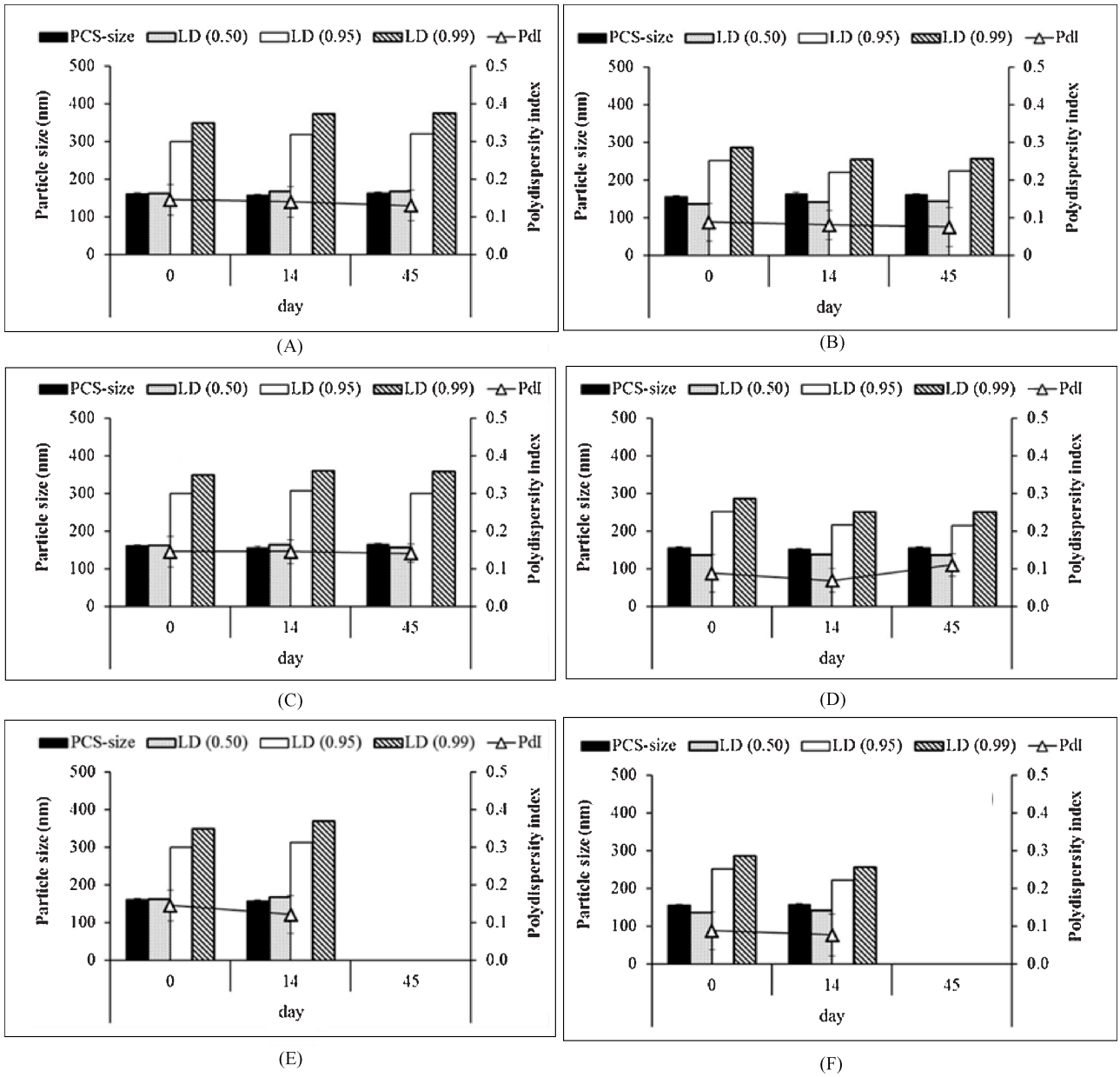

Fig. 13: Particle size and size distribution of Formula 6 stored at 4 °C (A), 25 °C (B), and 40 °C (C) and Formula 7 stored at 4 °C (D), 25 °C (E), and 40 °C (F).

cholesterol was incorporated in the NLC. To prolong the chemical stability profile of lycopene, the NLC should be stored below 25 °C and free of cholesterol.

3. Experimental

3.1. Materials

Orange wax and rice oil were kindly donated by Koster Keunen, LLC (Connecticut, USA) and Surin Bran Oil Co. Ltd. (Bangkok, Thailand), respectively. Eumulgin SG was obtained from Cognis (Düsseldorf, Germany). Cholesterol was from Merck (Darmstadt, Germany). Lycopene (90.25%) was purchased from Xian Guanyu Bio-technique Co.,Ltd. (China). Ultra-purified water was obtained from a MilliQ Plus system, Millipore (Schwalbach, Germany). Methanol and tetrahydrofuran were of HPLC grade and obtained from Mallinckrodt Baker, Deventer, Netherlands. Other ingredients were of the highest grade available.

3.2. Effect of lipid combination

Two lipid combinations were studied. One was composed of rice oil and orange wax, the other, of cholesterol and the wax. The exact weight of orange wax and rice oil were physically mixed to obtain lipid mixtures with oil contents of 10, 20, 30, 40, and 50%. For the orange wax and cholesterol combination, weight ratios of the wax and cholesterol of 4:1, 3:1, and 7:3 were investigated. The mixtures were gradually heated with gentle stirring until the temperature was up to approximately 80 °C or all ingredients were melted and completely mixed. The melted mixtures were slowly cooled to room temperature. The miscibility of wax and oil or wax and cholesterol in the mixtures as well their physical appearance were visually observed. The thermal behavior of the mixtures obtained was investigated by DSC.

3.3. Preparation of lycopene nanoparticles

The lipid nanoparticles were prepared by means of emulsification using high pressure homogenization. The oil phase and the water phase were firstly heated separately to 75 °C. The hot water phase was added to the oil phase at the same temperature and subjected to high speed stirring at 8,000 rpm for 1 min using an Ultra Turrax T25 (Janke and Kunkel GmbH, Staufen, Germany). This pre-emulsion obtained was then subjected to high pressure homogenization according to the method reported by Riangjanapatee and Okonogi (2012) for three cycles at 500 bars at 75 °C. The lipid dispersion was cooled at ambient condition to room temperature. A total of three batches of 40 ml of each formula were produced under the above described production parameters.

3.4. Particle size and zeta potential measurement

Analysis of the small particle size and zeta potential was carried out by means of PCS using a Malvern Zetasizer IV (Malvern Instruments, UK) and

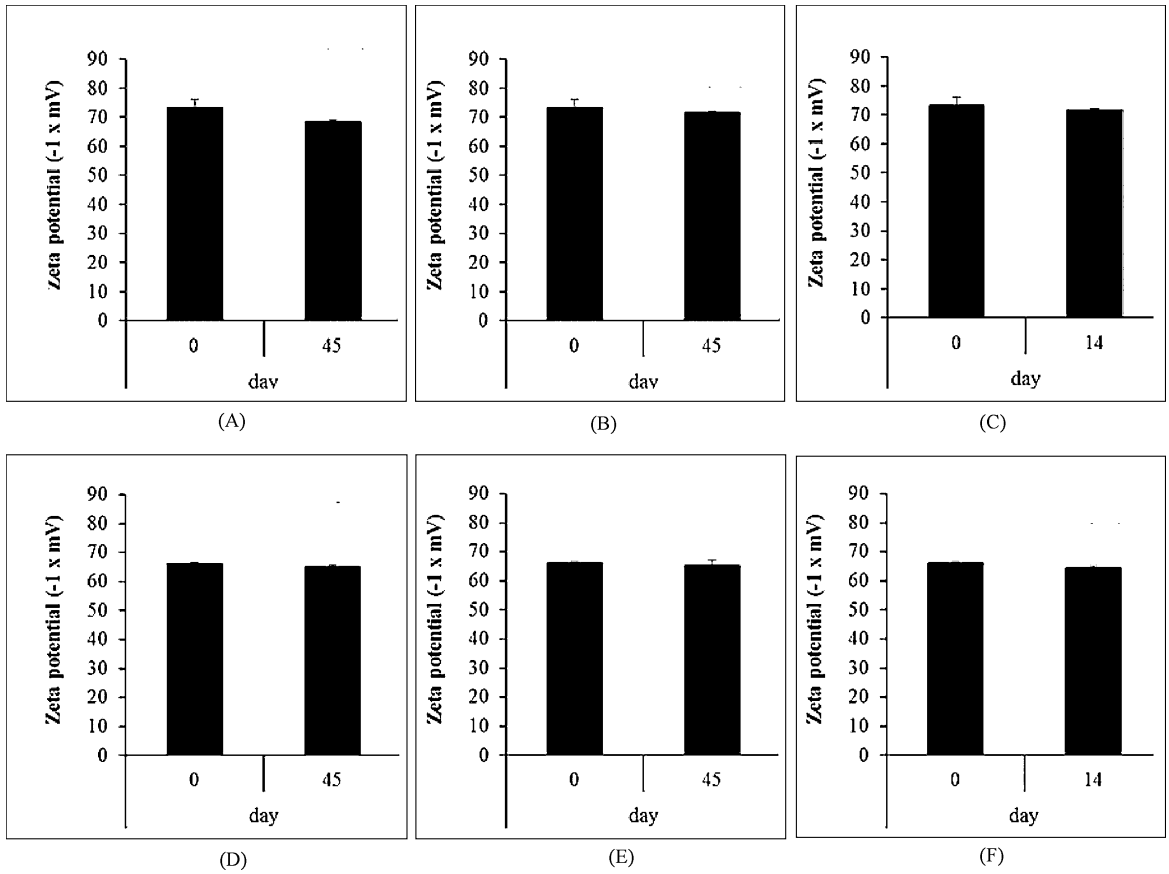

Fig. 14: Zeta potential of Formula 6 stored at 4 °C (A), 25 °C (B), and 40 °C (C) and Formula 7 stored at 4 °C (D), 25 °C (E), and 40 °C (F).

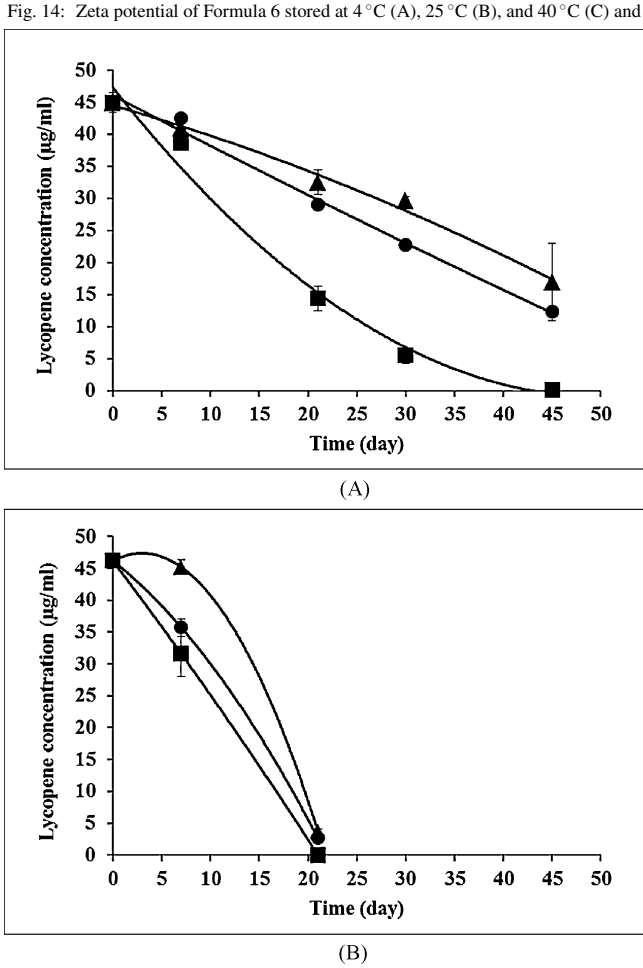

Fig. 15: Effect of temperature on lycopene stability of Formula 6 (A) and Formula 7 (B) stored at 4 °C (▲), 25 °C (●), and 40 °C (■).

the method of determination was according to Riangjanapatee and Okonogi (2012). The z-ave and PdI values were obtained by averaging of at least ten measurements at a fixed angle of 90° in 10-mm diameter cells at 25 °C. All samples were diluted with purified water to provide suitable scattering intensity before measurement. Zeta potential indicates the electric charge on the particle surface and the physical stability of colloidal systems. The sample was dispersed in purified water adjusted with 0.9% w/v NaCl to a conductivity of 50 µS/cm. The experiment was done in triplicate. The large particle size was measured by means of LD using a Coulter LS 230 (Beckman-Coulter, Germany).

3.5. Electric conductivity measurements

The electrical conductivity of the formulae was measured by using Cyberscan CON 11: hand-held conductivity meter (Eutech Instruments, Singapore) connected with a conductivity/TDS electrode cell. The experiment was performed at 25±10 °C by dipping the electrode into the test sample until equilibrium was reached and reading became stable. The measurements were done in triplicate.

3.6. DSC

DSC measurements were carried out using a DSC 821e (Mettler Toledo GmbH, Gießen, Germany). An accurately weighed amount of approximately of 2 mg tested sample was used. An empty aluminum pan was used as a reference. DSC scans were recorded from 20 to 80 °C at a heating rate of 10 K/min, using nitrogen gas to purge the system at the rate of 80 ml/min. The onset and melting temperature of each sample was recorded.

3.7. Stability test

The developed lycopene nano-products were kept at room temperature (25 °C) for 45 days. In this study, lycopene oil solution (0.05 mg/ml) was prepared and kept in the same condition. Color change of the lycopene nano-products in comparison with lycopene oil solution was observed by visualization. Particle size and size distribution of lycopene nano-products were determined by using PCS and LD. The formulas that showed high physical stability were selected for further stability study of the effect of temperature. The products were kept in different temperatures of 4 °C, 25 °C and 40 °C for 45 days and their physical stability, e.g. particle size, size distribution, and zeta potential vs. storage time and temperature were compared. To investigate the chemical stability, the selected formulations

stored in each condition were periodically determined for lycopene using an auto-sample HPLC system model 360 with a pump system model 420 and a UV visible detector model 430 (Kontron Instruments, Groß-Zimmern, Germany) linked to a KromaSystem 2000 v. 1.83 computerized data acquisition and process system. A portion of 20 µl of the tested sample was injected into a Eurosphere-100 C18 (5 µm) endcapped 250x 4.6 mm column with a matching pre-column (Knauer, Berlin, Germany). The column was kept at 25 °C during the measurement. The mobile phase, composed of methanol/tetrahydrofuran/water (60:33:7) was run with a flow rate of 1.5 ml/min. The peak at a retention time of 11.4 min detected at a wavelength of 475 nm was recorded and calculated for quantity of lycopene by using a standard curve.

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