Journal of Oil Palm Research Vol. 26 (3) September 2014 p. 232-239

NANOSTRUCTURED LIPID CARRIERS (NLC) FOR EFFICIENT DELIVERY OF PALM PHYTONUTRIENTS

LOO, C H*; R ISMAIL; M BASRI*; LAU, H L N*; B A TEJO‡; H ABU HASSAN**; CHOO, Y M** and M S KANTHIMATHI‡**

ABSTRACT

Palm phytonutrients found in crude palm oil consist of carotenes and tocols as well as other minor components including sterols, squalene, ubiquinones, coenzyme Q_{10} and phospholipids. Palm phytonutrients contains all the naturally occurring phytonutrients present in crude palm oil, whereas commercially available individual phytonutrients, such as Gold-tri E and Tocomin 50% mainly consist of palm tocotrienols. The encapsulation of palm phytonutrients by nanostructured lipid carriers (NLC) was investigated using Transmission Electron Microscopy. NLC was proven to effectively encapsulate palm phytonutrients in oil droplets. Based on the particle size analysis and rheological study, NLC was found to be the most physically stable delivery system when compared to the macro-emulsion and the nano-emulsion carriers. The long-term chemical stability of the palm phytonutrient using β-carotene as the prototype active in NLC was also determined. The degradation of β-carotene in NLC was lower when compared to the macro-emulsion and the nano-emulsion carriers. The efficacy of NLC as a delivery system and the effect of the addition of lecithin and propylene glycol to the NLC formulation were also studied. The parameters investigated were skin hydration and trans-epidermal water loss (TEWL). NLC with the presence of 1% lecithin and 2% propylene glycol were found to enhance skin hydration and prevent water loss.

Keywords: palm phytonutrients, nanostructured lipid carriers, physical stability, chemical stability, efficacy.

Date received: 10 July 2012; **Sent for revision:** 13 June 2013; **Received in final form:** 6 June 2014; **Accepted:** 23 June 2014.

INTRODUCTION

Palm oil is a rich source of naturally occurring phytonutrients. The major phytonutrients found in crude palm oil are carotenes, tocols, sterols and squalene (Goh *et al.*, 1985). Palm phytonutrients are a by-product produced during the conversion of palm oil to biodiesel. The first step in the production of palm phytonutrients is the trans-esterification of crude palm oil into alkyl ester. The following step, the distillation of alkyl esters, produces the phytonutrients concentrate (Choo *et al.*, 2002).

The use of phytonutrients as an antioxidant to combat skin ageing has been well documented. Researchers have found that vitamin A is an antioxidant that can decrease and reverse the sign of cutaneous aging and reduce fine and coarse facial wrinkles. It also increases the production

* Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
E-mail: mahiran@science.upm.edu.my

** Malaysian Palm Oil Board, 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.

‡ Department of Molecular Medicine, UMCPR (UM Centre for Proteomics Research), Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.

of collagen, elastin and fibronectin (Schlossman, 2002). Vitamin E (tocols) is also an antioxidant that can protect the skin against photoaging (Thiele *et al.*, 2005). It can also improve moisturisation, softness and smoothness of the skin (Schlossman, 2002). Coenzyme Q_{10} is a powerful antioxidant which is 10 times more powerful than vitamin E (Choo *et al.*, 2002). Squalene acts as a truly natural vehicle because its moisturising activity is related to its ability to act as an emollient, humectant and lubricant, whereas sterols formed liquid crystals in emulsified systems which greatly improved emulsion stability (Schlossman, 2002). Lastly, phospholipids, especially lecithin are well-known for their emulsifying properties and they also have synergistic antioxidant effect in the presence of tocols (Choo *et al.*, 2002).

The incorporation of phytonutrients into cosmetic formulations is a big challenge because of their photochemical instability. This is due to the presence of conjugated double bond systems that make them susceptible to oxidation cleavage and isomerisation by oxygen, light and heat. Therefore, any novel delivery systems that increases the chemical stability of chemically labile bioactives (carotenes and tocols) and enhances their penetration through the stratum corneum is crucial to improve their efficacy as topical agents (Kaur *et al.*, 2007).

Recently, a new delivery system known as nanostructured lipid carriers (NLC) has been introduced as an alternative to the conventional delivery systems especially to deliver lipophilic bioactives. NLC, consisting of a solid lipid matrix with a certain amount of liquid lipid, are considered as a new generation of solid lipid nanoparticles (SLN) (Hu *et al.*, 2006). It has a great attraction in the cosmetic fields as it is able to enhance the chemical stability of bioactives, provide an occlusive effect to the skin and therefore increase skin hydration, enhance the skin bioavailability of actives and increase the physical stability of topical formulations (Müller *et al.*, 2007).

The important criteria for the development of any new cosmetic products are their physical stability and the efficacy of the formula. For the development of new cosmetics, positive effects on skin hydration and lack of skin occlusivity are crucial. The moisturisation effect of a product leads to the increase in skin hydration, which contributes to wrinkle smoothing and enhancing the penetration of compounds into specific skin layers. In the present work, we developed and characterised an NLC loaded with palm phytonutrients and the efficacy of this NLC as a delivery system was also studied. The potential use of NLC as a delivery system for palm phytonutrients was studied and compared with macro-emulsion and nano-emulsion carriers. The hydrating and occlusivity effect of the NLC were investigated *in vivo* after repetitive application for seven days.

MATERIALS AND METHODS

Materials

Span 40 (sorbitan monopalmitate) and Tween 80 (polysorbate 80) were obtained from Croda (Goole, England). Isopropyl palmitate was purchased from Intermed Sdn Bhd (Kuala Lumpur, Malaysia). Lipocire DM (hydrogenated palm kernel and palm glycerides) were purchased from Gattefossé Inc. (Toronto, Canada). Palm phytonutrients (major components being tocols and carotenoids) were obtained from the Malaysian Palm Oil Board (Bangi, Malaysia).

Preparation of the Macro-emulsion

Melted lipid phase (20% lipid); solid:liquid phase (90:10) with the solid lipid consisting of hydrogenated palm kernel and palm glycerides, and liquid lipid consisting of isopropyl palmitate; 3% lipophilic surfactant (Span 40); 1% lecithin; and 0.1% bioactive compounds (palm phytonutrients) were added to a hot aqueous solution containing 3% hydrophilic surfactant (Tween 80) and 2% propylene glycol. Both phases (lipid and water phases) were heated to 70ºC. The mixture was homogenised using a Polytron PT 3100 homogeniser (Kinematica Inc, Switzerland) at 10 000 rpm for 2 min. The macro-emulsion formed was cooled in an ice bath to room temperature (25ºC).

Preparation of the NLC and the Nanoemulsion

A pre-emulsion for NLC and nano-emulsion were prepared in a similar manner to the preparation of the macro-emulsion. For the nano-emulsion, only liquid lipid was used in the preparation whereas for NLC, a mixture of solid lipid and liquid lipid (90:10) was used. The hot pre-emulsion was further homogenised at 85ºC using a high pressure homogeniser for three cycles at 750 bars. The lipid dispersion was then cooled in an ice bath to room temperature (25ºC).

Particle Size Analysis

Particle size analysis of the macro-emulsion was carried out by a laser diffraction particle analyser, *i.e.*, the Malvern Hydro 2000S (Worcestershire, England). The instrument was able to measure the particle size of the sample in the range of 0.02 to 2000

μm. Wet samples were prepared for the analysis by diluting the pre-emulsions with de-ionised water. The centrifugal pump was set at 1645 rpm to mix the sample. The wet samples were then added to the Hydro 2000S and segregated by ultrasound for a few seconds. Sample quantity was adjusted to obtain laser beam obscuration in the range of 10 to 20%. The particle size of the macro-emulsion was described by the cumulants mean diameter (Stanley-Wood and Lines, 1992).

Particle size analyses for the NLC and nano-emulsion samples were performed using photon correlation spectroscopy (PCS) by employing a Malvern hpp5001 High Performance Particle Sizer (Malvern Instruments Ltd, United Kingdom). In PCS, the intensity fluctuations of scattered light arising from Brownian motion were measured (Malvern, 2003). The size distribution of the particles was measured by the Stokes-Einstein equation (Malvern, 2003). The mean particle size was obtained from the average of five measurements (number of runs = 10 and run duration = 30 s) at an angle of 90°. All samples were diluted in distilled water to an optical level of weak opalescence. The dispersant (water) had a refractive index of 1.333 and a viscosity of 0.9 cP at 25°C.

Transmission Electron Microscopy (TEM)

A drop of the sample was dispersed in deionised water in a 10 ml screw-capped test tube. The test tube was shaken for several minutes using a vortex mixer (Heildoph reax top, Germany). Several drops of dispersed sample were dropped onto parafilm. A formvar-coated copper grid was placed within a droplet of sample and left in the open air for 10 min. The grid was then stained using 2% phosphotungstic acid (PTA) at pH value 7.2 for 10 min. The grid was then dried by evaporation at room temperature. Characterisation of the sample was conducted by TEM (Hitachi H-7100, Japan).

Rheology Measurement

Oscillatory stress sweep analysis was carried out between 0.1-100.0 Pa using Paar-Physica MCR 300 rheometer, using a cone and plate geometry CP 50-1 (Lim *et al.*, 2009).

In the stress sweep test, the oscillation frequency was fixed at 1 Hz in order to stay in the linear viscoelastic region. The region where the G' (storage modulus) is independent of applied shear stress up to critical strain value, γ_c is known as the linear viscoelastic region. The purpose of this test is to determine the limits of the linearity (critical strain value) of the delivery systems (Bais *et al.*, 2005). Critical strain is the minimum strain where the structures of the delivery systems start to break down which also indicates minimum energy required to disrupt the structure of the delivery system. The magnitude of critical strain is dependent on the delivery system's resistance to flow.

Chemical Stability of β-carotene in Delivery Systems

The chemical stability of β-carotene in all the tested delivery systems was investigated over a period of two months. All the investigated delivery systems (0.5 g) were weighed; β-carotene was extracted with a mixture of 2 ml ethanol and 3 ml of n-hexane. The extraction was repeated twice and the removed hexane phases were combined in a 25 ml volumetric flask. The mixture was then diluted with hexane until the 25 ml mark. For the determination of the β-carotene content in macro-emulsion, NLC and nano-emulsion systems, the absorbance of the extracted sample at 446 nm in a 1 cm cuvette was measured against the blank (solvent). The β-carotene content was calculated using equation 1 below (Tang, 1988).

$$\beta\text{-carotene content (ppm)} = \frac{A_{446} \text{x} 383 \text{x} 25}{100W} \quad (1)$$

A_{446} = absorbance of sample at 446 nm
W = weight of sample in gramme

The absorbance was converted to concentration (ppm) by multiplying by the universally accepted factor of 383 based on the molar extinction coefficient of pure β-carotene in organic solvents (Chong, 1987).

Efficacy Testing (skin hydration and transepidermal water loss)

The effect of the samples on the skin hydration was evaluated using a Corneometer® CM 825 (Courage und Khazaka, Germany). The measuring principle of the instrument is based on the capacitance measurement of a dielectric medium. The dielectric constant of the skin changes with the water content. The changes in water content of the stratum corneum are converted to arbitrary units of hydration. Transepidermal water loss (TEWL) from the skin was investigated using a Tewameter® TM 300. The principle of this test is based on the diffusion principle in an open chamber (Wissing and Müller, 2002). Three measurements for skin hydration and skin occlusion were performed in each testing area on a volunteer forearm. The results are given in 'arbitrary units' (arb. units). All the data were statistically analysed by one-way analysis of variance (ANOVA). Differences between sample and the untreated control group were evaluated by Tukey t-test. The $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Particle Size Analysis

The particle size of the NLC sample remained constant over two month storage, whereas the particle size of the macro-emulsion sample increased gradually with time (*Figure 1*). Similar results were reported by Fernandez *et al.* (2004). When the droplet size of macro-emulsion is larger than 1 μm, the emulsion is susceptible to gravitational forces.

The particle size of the nano-emulsion increased from the first month to the second month, whereas the particle size of the NLC remained unchanged throughout the course of study (*Figure 1*). The NLC system was found to be more stable compared to the nano-emulsion due to the presence of solid core in the particles making it difficult to coalesce, and therefore showed better physical stability than liquid droplets (Benita, 1996).

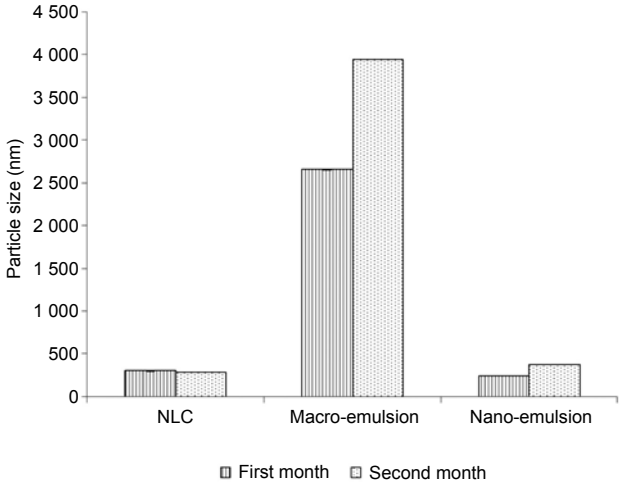

Figure 1. Particle size of nanostructured lipid carriers (NLC), macro-emulsion and nano-emulsion within two months.

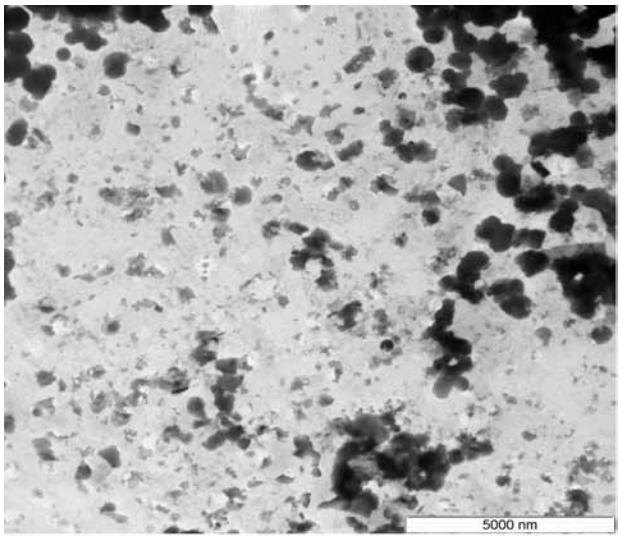

Figure 2. Transmission electron microscopy (TEM) image of nanostructured lipid carriers (NLC) loaded with palm phytonutrients.

Transmission Electron Microscopy (TEM)

TEM analysis was performed to obtain information about the encapsulation and the shape of the particles in the NLC. *Figure 2* shows the image of phytonutrients-loaded NLC. The particles shown in the image have an anisometric shape which is in agreement with the finding of Teeranachaideekul *et al.* (2007). The shape deviated from sphericity might be due to the lipid modification during the drying process of sample treatment.

Rheological Study

By extrapolating the linear region in the graph, the critical strains of the delivery systems can be obtained as shown in *Figure 3*. The critical strain of NLC was higher compared to the macro-emulsion and nano-emulsion samples which indicate that

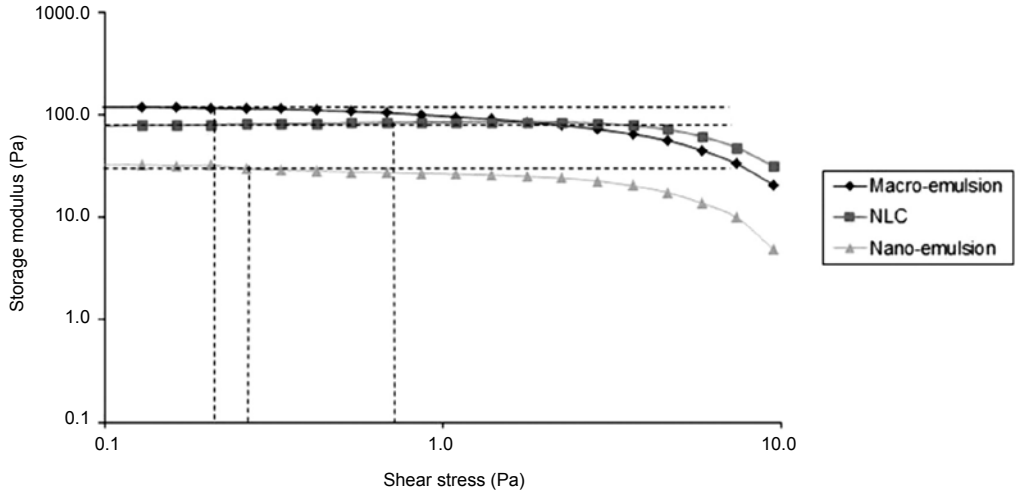

Figure 3. Plot of storage modulus (Ps) as a function of shear stress (Pa) of macro-emulsion, nanostructured lipid carriers (NLC) and nano-emulsion.

macro-emulsion and nano-emulsion broke down more easily with small movements, and therefore NLC was predicted to have longer shelf-life compared to macro-emulsion and nano-emulsion (*Figure 3*). This was due to the smaller size of the NLC compared to the macro-emulsion, which provided greater attractive forces between the droplets and resulted in higher critical strain. The cohesive force, E_c, can be used to explain interdroplet interactions in the delivery systems; it is directly proportional to the critical strain, γ_c (Equation 1):

$$E_c = \frac{1}{2} G' \gamma_c^2 \quad \text{Equation 1}$$

Small droplets have shorter distances between them, resulting in more interdroplet interactions which hold the droplets firmly and withstand deformation forces applied during the test (Tadros, 2005). The NLC had higher critical strain compared to the nano-emulsion. This might have been due to the presence of solid lipids in the NLC which provided stronger interdroplet forces between droplets compared to the nanoemulsion, which was mainly composed of liquid lipids.

Chemical Stability of β-carotene in the Delivery Systems

The residual β-carotene content in the tested delivery systems after two months of exposure to fluorescence light is as shown in *Table 1*. The NLC system significantly improved the chemical stability of β-carotenes compared to the macro-emulsion and nano-emulsion systems. The percentage degradation of β-carotene in the NLC sample from the first month to the second month was only 8.29%, whereas the percentages degradation of β-carotene in the macro-emulsion and nano-emulsion samples were 33.09% and 28.47% respectively (*Table 1*).

Efficacy Testing

Skin hydration. The application of the NLC for seven days produced a significant increase in average skin hydration from 29.67±0.41 (arb. units) to 42.20±0.27 (arb. units) (P<0.001), whereas the untreated control remained almost unchanged, *i.e*., 30.65 (arb. units) on Day 1 and 31.80 (arb. units) on Day 7 (*Figure 4*) (P>0.05). The application of the NLC sample led to an increase in skin hydration by 42.23%. The presence of partially occlusive agents found in the NLC, *i.e*. solid lipid (hydrogenated palm kernel and palm glycerides) and liquid lipid (isopropyl palmitate) produced a further increase skin hydration. After the seventh day application of the NLC containing 1% lecithin on the skin, the increase in skin hydration was comparable to the NLC without lecithin. Percentage increase in skin hydration was 49.09% and 42.62% for NLC with and without lecithin, respectively. Treatment with NLC containing 1% lecithin increased skin hydration slightly higher than NLC without lecithin, which could be due to the addition of occlusive agent (lecithin) to the formulation.

TABLE 1. β-CAROTENE CONTENT IN MACRO-EMULSION, NANO STRUCTURED LIPID CARRIERS (NLC) AND NANO-EMULSION WITHIN TWO MONTHS EXPOSURE TO FLUORESCENCE LIGHT

Sample	Carotenes content (ppm)	
	First month	Second month
Macro-emulsion	94.33 ± 0.34	63.12 ± 0.36
NLC	92.20 ± 0.56	84.56 ± 0
Nano-emulsion	92.61 ± 0.17	66.24 ± 0.47

Application of the NLC with 2% propylene glycol increased the skin hydration from 30.33 (arb. units) on Day 1 to 43.97 (arb. units) on Day 7 (*Figure 5*) (P<0.001), whereas the untreated control remained almost unchanged, *i.e*. 30.65±0.68 (arb. units) on Day 1 and 31.80±0.98 (arb. units) on Day 7 (*Figure 5*) (P>0.05).

The application of NLC which showed an increase in skin hydration, might be due to the fact that NLC is able to dry to form a film on the skin and thus preventing the water from evaporating from the skin. The smaller particle size of NLC is the main contributing factor to this effect due to the capillary forces of nanometer pores between the NLC particles are contractive promoting fusion and dense film formation (Wissing and Müller, 2003). Humectant such as propylene glycol increases the hydration of the stratum corneum by coordinating water and reducing its evaporation (Prendergast and Shiffman, 2011).

Transepidermal water loss. The average water loss from the untreated test area remained the same, 8.11±0.08 (arb. units) on Day 1 and 8.48±0.12 (arb. units) on Day 7 (*Figure 6*) (P>0.05). Application of NLC on the skin helped to reduce water loss from the skin compared to the untreated control. Total average water loss from the treated area with NLC was 8.02±0.08 (arb. units) on Day 1 and 6.12±0.11 (arb. units) on Day 7 (*Figure 6*) (P<0.001). The addition of 1% lecithin into NLC formulation was found to be more effective to reduce water loss from the skin compared to NLC without the presence of 1% lecithin.

The percentage reduction of water loss from the skin for NLC without 1% lecithin was 23.69%, whereas for NLC with 1% lecithin was 68.68%. Besides, the addition of 2% propylene glycol helped to reduce water loss from the skin dramatically compared to NLC without the addition of 2%

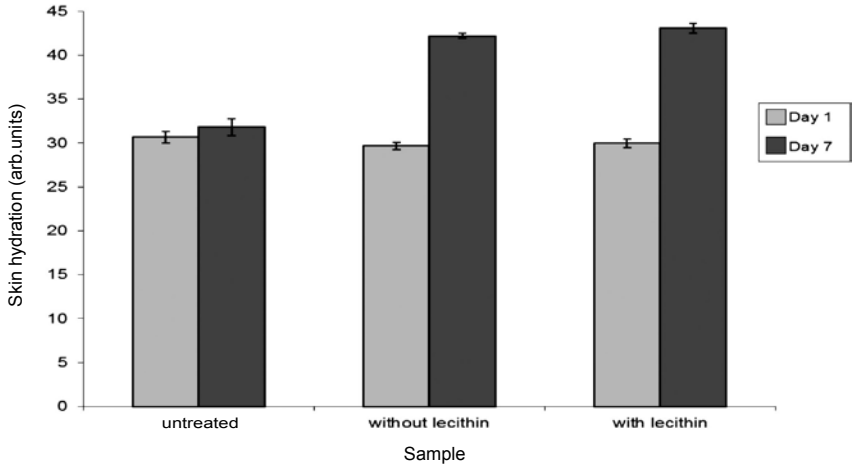

Figure 4. Skin hydration of untreated control, nanostructured lipid carriers (NLC) without 1% lecithin, and NLC with 1% lecithin within seven days.

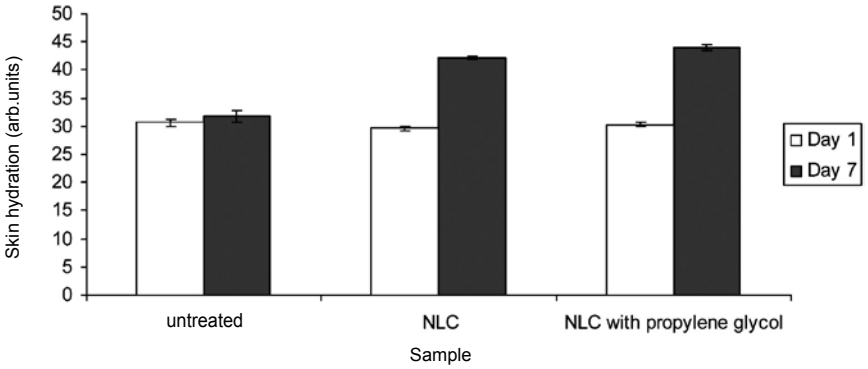

Figure 5. Skin hydration of untreated control, nanostructured lipid carriers (NLC) without 2% propylene glycol, and NLC with 2% propylene glycol within seven days.

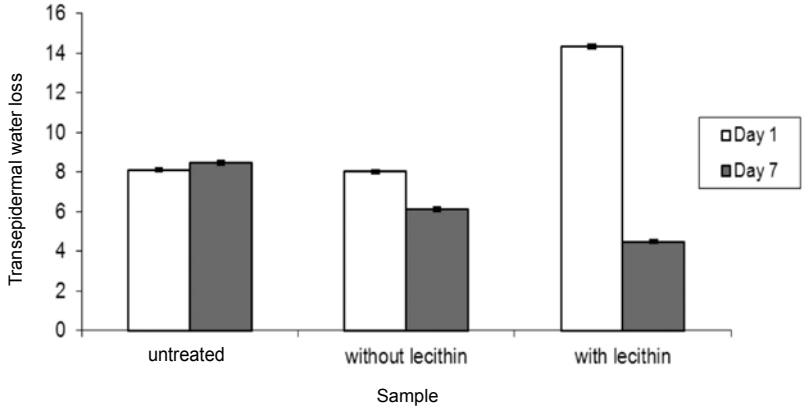

Figure 6. Transepidermal water loss of untreated control, nanostructured lipid carriers (NLC) without 1% lecithin, and NLC with 1% lecithin within seven days.

propylene glycol. The water loss from the skin at treated area with NLC containing 2% propylene glycol decreased from 13.08 (arb. units) on Day 0 to 4.19 (arb. units) on Day 7 (*Figure 7*). Percentage reduction of water loss from the skin for NLC with 2% propylene glycol was 67.97% compared to 23.69% for NLC without 2% propylene glycol. The presence of emollients in NLC formulation (lecithin, solid lipid and liquid lipid) helps to replace the absence of natural skin lipids in the space between corneocytes in the stratum corneum to prevent excessive transepidermal water loss (Draelos, 2010).

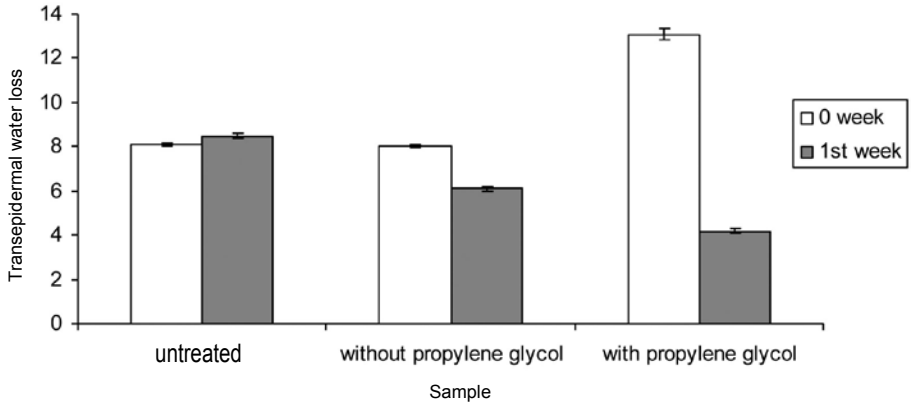

Figure 7. Transepidermal water loss of untreated control, nanostructured lipid carriers (NLC) without 2% propylene glycol, and NLC with 2% propylene glycol within seven days.

CONCLUSION

The NLC-loaded with palm phytonutrients showed a good physical stability compared to the nano-emulsion and macro-emulsion. This observation was proven by particle size analysis and stress sweep test analysis. NLC was seen by TEM image to effectively encapsulate palm phytonutrients in oil droplets. Also, the chemical stability of β-carotene was found higher compared to the nano-emulsion and macro-emulsion samples. The addition of 1% lecithin and 2% propylene glycol to the NLC sample also helped to increase the hydration. In conclusion, NLC represent a highly stable delivery system (*i.e.* physically and chemically) and a highly effective system to increase the skin hydration and prevent water loss from the skin.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Malaysian Palm Oil Board Graduate Students' Assistantship Scheme for the research grant. The authors would also like to thank MPOB and Universiti Putra Malaysia (Faculty of Science and Institute of Bioscience) for the facilities used in the project.

REFERENCES

BAIS, D; TREVISAN, A; LAPASIN, R; PARTAL, P and GALLEGOS, C (2005). Rheological characterization of polysaccharide-surfactant matrices for cosmetic o/w emulsions. *J. Colloidal Interface Science*, *290*: 546-556.

BENITA, S (1996). *Microencapsulation: Methods and Industrial Applications*. Informa Health Care, New York.

CHONG, C L (1987). Ultraviolet-visible light spectroscopy: instrumental parameters scope of application and experimental precautions in the analysis of vegetable oils**.** *PORIM Bulletin No. 15*: 18-26**.**

CHOO, Y M; LAU, H L N; PUAH, C W; NG, M H; BONG, S C; MA, A N and YUSOF, B (2002). Production of phytonutrients (carotenes, vitamin E, sterols, squalene, co-enzyme Q_{10}, and phospholipids) from palm methyl esters. *MPOB Information Series No. 168*: 1-2.

DRAELOS, Z D (2010). *Cosmetic Dermatology: Products and Procedures*. Wiley-Blackwell, West Sussex.

FERNANDEZ, P; ANDRÉ, V; RIEGER, J and KÜHNLE, A (2004). Nano-emulsion formation by emulsion phase inversion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *251*: 53-58.

GOH, S H; CHOO, Y M and ONG, A S H (1985). Minor constituents of palm oil. *J. Amer. Oil Chem. Soc.*, *62*: 237-240.

HU, F; JIANG, S; DU, Y; HONG, Y; YE, Y and SU, Z (2006). Preparation and characteristics of monostearin nanostructured lipid carriers. *International Journal of Pharmaceutics*, *314*: 83-89.

KAUR, I P; KAPILA, M and AGRAWAL, R (2007). Role of novel delivery systems in developing topical antioxidants as therapeutics to combat photoageing. *Ageing Research Reviews*, *6*: 271-288.

LIM, H N; KASSIM, A; HUANG, N M; YARMO, M A and YEONG, S K (2009). Study of highly concentrated olive oil-in-water emulsions stabilized

by palm-based nonionic surfactant. *Sains Malaysiana, 38 (1)*: 95-102.

MALVERN (2003). *HPPS Operators Guide*. Worcestershire, United Kingdom.

MÜLLER, R H; PETERSON, R D; HOMMOSS, A and PARDEIKE, J (2007). Nanostructured lipid carriers (NLC) in cosmetic dermal products. *Advanced Drug Delivery Reviews*, *59*: 522-530.

PRENDERGAST, P M and SHIFFMAN, M A (2011). *Aesthetic Medicine: Art and Techniques*. Springer, New York.

STANLEY-WOOD, N and LINES, R W (1992). *Particle Size Analysis*. Royal Society of Chemistry, Cambridge.

SCHLOSSMAN, M L (2002). *Chemistry and Manufacture of Cosmetics*. 3rd Ed. Vol. 3. Book 2. Allured Publishing Corporation, Carol Stream.

TADROS, T F (2005). *Applied Surfactants: Principles and Applications*. Mörlenbach: Wiley-VCH Verlag GmbH & Co.

TANG, T S (1988). Quality parameters – carotene content. *Engineering News, 011*: 18.

TEERANACHAIDEEKUL, V; SOUTO, E B; JUNYAPRASERT, V B and MÜLLER, R H (2007). Cetyl palmitate-based NLC for topical delivery of coenzyme Q10 – development, physicochemical characterization and *in vitro* release studies. *European Journal of Pharmaceutical and Biopharmaceutics*, *67*: 141-148.

THIELE, J J; HSIEH, S N and EKANAYAKE-MUDIYANSELAGE, S (2005). Vitamin E: critical review of its current use in cosmetic and clinical dermatology. *Dermatologic Surgery*, *31*: 805-813.

WISSING, S A and MÜLLER, R H (2003). The influence of solid lipid nanoparticles on skin hydration and viscoelasticity – *in vivo* study. *European Journal of pharmaceutics and Biopharmaceutics*, *56*: 67-72.