Original Article

การกักเก็บน้ำมันรำข้าวในโซลิดลิปิดนาโนพาร์ทิเคิลสำหรับเพิ่มความชุ่มชื้นและความยืดหยุ่นผิว

Encapsulation of Rice Bran Oil in Solid Lipid Nanoparticles (SLN) for Skin Hydration and Viscoelasticity

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Abstract

The purposes of this study were to encapsulate rice bran oil (RBO) in solid lipid nanoparticles (SLN) by high pressure homogenization technique and to investigate the influence of formulation compositions (lipid, surfactant, and RBO concentrations) and process parameters (homogenization pressures and size reduction times) on physical properties of the SLN. The system was also tested in 10 healthy human volunteers for potential skin hydration and viscoelasticity benefits. Results showed that the optimized SLN formulation with particle size below 200 nm could be achieved by using Compritol® 888 ATO, RBO, and Pluronic® F68 at the concentration below 2.5, 2.5 and above 2.5%w/w, respectively. Homogenization pressure at 500 to 700 bars significantly reduced particle size from 500 to 160 nm. The SLN mean particle size (160±20 nm, polydispersity index<0.2) remained unchanged for at least 28 days at 25 °C. Skin hydration of cream containing RBO encapsulated in SLN (1.28 %w/w RBO, SLN/cream base ratio was 51.2:48.8 %weight) was significantly higher than the cream base after applying at 7, 14 and 28 days and the skin viscoelasticity was significantly increased in the ranges of 4-5% at 7, 14 and 28 days, respectively ($p<0.05$). In conclusion, SLN represents a promising delivery system of RBO for skin hydration and viscoelasticity.

Keywords: Rice bran oil, Compritol® 888 ATO, Pluronic® F68, Solid lipid nanoparticles, Skin hydration, Skin viscoelasticity

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อกักเก็บน้ำมันรำข้าวในโซลิดลิปิดนาโนพาร์ทิเคิล (SLN) ด้วยเทคนิคโฮโมจีไนเซชันความดันสูง และได้ศึกษาปัจจัยของส่วนประกอบตำรับ (ความเข้มข้นของไขมัน สารลดแรงตึงผิวและน้ำมันรำข้าว) และกระบวนการผลิต (ความดันและเวลาที่ลดขนาดด้วยเครื่องไฮเพรชเชอร์โฮโมจีไนเซอร์) ต่อคุณสมบัติทางกายภาพของ SLN ตำรับที่พัฒนาได้นำไปศึกษาประสิทธิภาพในการเพิ่มความชุ่มชื้นและความยืดหยุ่นของผิวในอาสาสมัคร 10 คน พบว่าตำรับที่ดีที่สุดคือ SLN ที่มีขนาดอนุภาคเล็กกว่า 200 nm ที่เตรียมจาก Compritol® 888 ATO น้ำมันรำข้าว และ Pluronic® F68 ในความเข้มข้นต่ำกว่า 2.5, 2.5 และสูงกว่า 2.5%w/w ตามลำดับ การใช้ความดันในการปั่นผสมที่ 500 ถึง 700 บาร์ให้ขนาดอนุภาคลดลงอย่างมีนัยสำคัญจาก 500 เป็น 160 nm ค่าเฉลี่ยอนุภาคของ SLN (160±20 nm และดัชนีการกระจายอนุภาค<0.2) ที่ได้ไม่เปลี่ยนแปลงอย่างน้อย 28 วัน เมื่อเก็บที่อุณหภูมิ 25 ˚C ครีมที่มีส่วนผสมของน้ำมันรำข้าวที่กักเก็บใน SLN (น้ำมันรำข้าว 1.28%w/w, อัตราส่วน SLN ต่อครีมพื้นเท่ากับ 51.2:48.8 %น้ำหนัก) ทำให้ผิวมีความชุ่มชื้นสูงกว่าผิวที่ทาครีมพื้นหลังใช้ในวันที่ 7, 14 และ 28 ส่วนความยืดหยุ่นผิวเพิ่มขึ้นในราว 4-5 % อย่างมีนัยสำคัญหลังใช้ในวันที่ 7, 14 และ 28 ตามลำดับ (p<0.05) ดังนั้น SLN จึงสามารถใช้กักเก็บน้ำมันรำข้าวเพื่อเป็นระบบนำส่งในการเพิ่มความชุ่มชื้นและความยืดหยุ่นของผิวได้

คำสำคัญ: น้ำมันรำข้าว Compritol® 888 ATO Pluronic® F68 โซลิดลิปิดนาโนพาร์ทิเคิล ความชุ่มชื้นของผิว ความยืดหยุ่นของผิว

Introduction

Rice bran oil (RBO) is a by product of rice milling process that is best recognized for its potential skin benefits. It contains high concentration of important bioactive compounds especially those with anti-aging properties such as vitamin E (α-tocopherol, α-tocotrienol, γ-tocopherol, γ-tocotrienol), γ-oryzanol and feruric acid (Bramley et al., 2000). Recent data also indicated that γ-oryzanol inhibited oxidation by hydrogen donating from the ferulic acid hydroxyl group (Renuka and Arumughan, 2007). Other interesting properties were anti-tumor (Iwatsuki, 2003) and anti-inflammatory (Akihisa et al., 2000). Bucci et al. (2003) reported that ferulic acid was an excellent ultraviolet-absorbing with no phototoxic and had antioxidant property. Besides, RBO at the concentration of 2-5 %w/w had also been used as antioxidant to stabilize oil phase (Taylor et al., 1997) and at 1-2 %w/w used as skin conditioner. Undiluted RBO had been reported as a safe cosmetic ingredient (Amended final report, 2006). Although there is quite a lot of information concerning the usefulness of RBO available, its application as active ingredient for cosmetics has been limited.

Solid lipid nanoparticles (SLN) have been introduced as an alternative drug and/or cosmetic delivery system since 1991 (Müller et al., 1996). The systems compose of solid lipids which melted and emulsified into aqueous external phase containing surfactants to form oil in water coarse emulsion droplets. After that the

emulsion droplets were passed through high pressure homogenizer and submicron size emulsion droplets were obtained. Upon cooling to room temperature, the lipids crystallize to form lipid shell matrixes, thus making the system more stable to conventional liquid emulsions and control drug release over time (Charcosset et al., 2005). These systems have been used to encapsulate several cosmetic actives such as α-lipoic acid (Souto et al., 2005), vitamin E (Dingler et al., 1999). Besides, Pople and Singh (2006) reported that topical gel containing vitamin A palmitate incorporated in SLN increased the thickness of stratum corneum and improved skin hydration. SLN particle sizes have been reported to be in the range of 50 to 1000 nm (Müller et al., 2000) which enhanced occlusive effect and increased penetration of active compounds into deeper skin. SLN offered several advantages for topical-route application over the conventional available formulations includes enhanced protection of labile active ingredients from chemical degradation and produced occlusive effects (Pople and Singh, 2006) with decreased total epidermal water loss (TEWL) from skin (Kuntche et al., 2007; Wissing and Müller, 2002). Furthermore, SLN has been reported to reduce systemic absorption as well as reduce dermal irritations (Jenning and Gohla, 2001).

Although RBO is an interesting active ingredient for skin benefit, some of its vital components such as vitamin E are oxidized upon exposed with oxygen from the air and in the formulation, light, alkali, and trace minerals (Bramley et al., 2000). Therefore, encapsulation of RBO in SLN can protect vitamin E and other of its components against degradation. In addition, SLN acts as physical sunscreen carrier for ultraviolet ray protection and also increases skin hydration. In this study, the system with RBO encapsulated in SLN was developed. Formulation as well as process parameters that affect SLN particle size and stability were investigated and skin hydration and viscoelasticity effect were tests in 10 healthy human volunteers during 28 days application time.

Materials and Methods

1. Materials

Rice bran oil was purchased from Namsiang, Ltd., Bangkok, Thailand. Compritol® 888 ATO (Glyceryl behenate, a mixture of approximately 15% mono-, 50% di-, and 35% triglycerides of behenic acid) was ordered from Gattefoss" , France. Pluronic® F68 was purchased from BASF, Germany. Propylene glycol, glyceryl monosterate, stearic acid, jojoba oil, silicone oil, and butylated hydroxytoluene were purchased from Pharma, Ltd., Bangkok, Thailand. Glycerin, sorbitol and mineral oil were purchased from S.P.Y Science Tech. Ltd., Ubonratchathani, Thailand. Germaben® II was purchased from International Specialty Products, Ltd., U.S.A. Isopropyl myristate was from Daily fat oil, Ltd., Malaysia. Cetyl alcohol was from Vidhayasom, Ltd., Bangkok, Thailand. Triethanolamine was from Italmar, Ltd., Bangkok, Thailand. Carbopol® 940 was from Pharmanex, Ltd., Khon Kaen, Thailand. Sodium chloride was from Thai Nakorn Pattana, Ltd., Nonthaburi, Thailand. All chemicals and reagents used were of analytical and cosmetic grade.

2. Formulations

2.1 Blank SLN and RBO encapsulated in SLN

Blank SLN and RBO encapsulated in SLN were prepared by high pressure homogenization technique (Müller et al., 1996) in 100 ml batches. Briefly, RBO (0, 2.5, 5, and 10%w/w) was mixed with lipid (Compritol® 888 ATO: 1.25, 2.5, 5, and 10%w/w) at 80 °C and emulsified into aqueous external phase containing surfactant (Pluronic® F68: 0.5, 1, 2.5, and 5%w/w) using the high speed mixer (ultra-turrax mixer model T25, IKA-Baortechnik, USA) at 11,000 rpm for 1 minute. The coarse emulsion was then passed through the high pressure homogenizer (APV 1000, Invensys APV Products, Denmark) at 500 to 800 bars for 5 minutes.

2.2 Cream base, cream containing RBO, and cream containing RBO encapsulated in SLN

Lipid phase (isopropyl myristate, glyceryl monostearate, stearic acid, cetyl alcohol, jojoba oil, silicone oil, and butylated hydroxytoluene) was heated separately to 80 °C and mixed with an aqueous phase (deionized water, propylene glycol, and triethanolamine). The mixture was cooled down to 40 °C while stirring and then Carbopol® 940 and Germaben® II were added and stirred continuously until the cream base was homogeneously mixed. In order to prepare cream containing RBO, 1.28 %w/w of RBO was added to the cream base at 40 °C. The SLN containing 2.5 %w/w RBO were dispersed into cream base at 51.2:48.8 %weight ratio to make final RBO concentration of 1.28 %w/w which was in the recommended concentration for a skin conditioner (Amended final report, 2006).

3. Particle size and zeta potential measurements

Physical properties of SLN such as particle size and zeta potential (ζ) were performed after 30 minutes of preparation and 1, 3, 5, 7, 14, 21, and 28 days after storage at 25 °C. Particle size distributions were determined using dynamic light scattering via a Zetasizer® nano Series (Model 3000 HS, Malvern Instruments, United Kingdom), covering the size range of 0.6 nm to 6000 nm with a wavelength of 670 nm and detection under a fixed angle of 90 ° Prior to measurement, the samples were diluted with ultra-pure water with conductivity adjusted to 50 μS/cm to obtain optimum scattering intensity. Each sample was measured in triplicate at 25 °C. The data were expressed as mean particle size and polydispersity index (PDI) was the width of particle size distribution, and by mean of ζ from three measurements.

4. Accelerated stability study

The physical stability testing of cream was tested by heating-cooling cycle at 4 °C for 48 h and 45 °C for 48 h, for 6 cycles. Changes in the physical stability such as texture, phase separation, color, pH and odor were visually observed.

5. Skin hydration and viscoelasticity evaluations

The effectiveness of RBO encapsulated in SLN was investigated in 10 healthy human volunteers. All volunteers were informed about the study and their written informed consents were obtained after being acquainted with the tested procedure. The measurements were carried out one-side blind test using

Corneometer® CM 825 and Cutometer® S EM 575 (pressure at 450 mbar), which were connected on a Multi Probe Adapter® MPA 580 (Courage and Khazaka, Electronic GmbH, Germany) for measurements of skin hydration and viscoelasticity, respectively (Courage and Khazaka Electronic GmbH, 2001). The viscoelasticity parameter was determined using the relative parameter UA/UF, the relation between maximum deformation during the first cycle and back formation. All evaluations were performed under the control environmental conditions at 20±2 °C and 45±2% relative humidity. All measurements were recorded after volunteers rested at least 10 - 15 minutes. The evaluations were classified into long and short term as followed;

5.1 Long term study of skin hydration and viscoelasticity

All healthy human volunteers were informed to avoid applying any topical formulations on the volar forearm at least 1 day before and during investigation period. Three formulations: cream containing RBO encapsulated in SLN, cream containing RBO, and cream base were applied twice daily on the marked volar forearm at 0.1 ml tested formulation per 1 cm^2 for 28 days and using untreated area of each volunteer served as control. The measurement started after 10-16 hours of the previous formulation application. The skin of volunteers was measured before and after the formulation application at 0, 3, 7, 14 and 28 days.

5.2 Short term study of skin hydration

Six formulations includes (1) cream containing RBO encapsulated in SLN, (2) cream containing SLN, (3) cream containing RBO, (4) cream base, (5) blank SLN, and (6) RBO encapsulated in SLN were investigated and glycerin was served as a positive control. The volar left forearm was divided into seven blocks in which an area of 1 cm^2. Each tested area was measured before formulation application as baseline. After 30 minutes of formulation application, all tested areas were wiped with cotton bud and skin hydration was measured at 1, 2, 3, and 6 hours.

5.3 Data calculations

Three measurements were performed in each area. The results of % changes of skin hydration and skin viscoelasticity were calculated with the following equation;

$$\%Changes = \left(\frac{\overline{X}_{tv}}{\overline{X}_{0v}} - 1 \right) \times 100$$

Where $\overline{X}_{tv}$ is the mean of the measured values of treated or untreated skin area after application time of healthy human volunteers, and $\overline{X}_{0v}$ is the mean of the measured values of treated or untreated skin area before application time of healthy human volunteers.

6. Data and statistical assessments

All data were expressed as means ± standard deviation (S.D.) or standard error of mean (S.E.M.) and n value indicated the number of experiments. The statistical significances between the treated groups and the respective control groups were analyzed by student's-paired t test. Differences were considered statistically significant when $p<0.05$.

Results

1. Effect of compositions

Effect of SLN compositions on the physical properties of SLN such as particle size and zeta potential were evaluated. The measurements were performed after storage at 25 °C at 0, 1, 3, 5, 7, 14, 21, and 28 days. First, SLN with possible highest concentration of Compritol® 888 ATO as lipid shell was attempted because of its higher tendency of encapsulation capacity. Results showed that the formation of SLN with Compritol® 888 ATO at concentration higher than 10 %w/w could not be achieved due to highly viscous lipid phase. As shown in Figure 1, an increase in concentration of Compritol® 888 ATO increased in the particle size of SLN. The particle size of SLN with 10 %w/w Compritol® 888 ATO was significantly altered after 1 day storage while that of SLN prepared from 5 %w/w Compritol® 888 ATO was stable up to 3 weeks. Concentration of Compritol® 888 ATO between 1.25-2.5 %w/w gave good SLN stability up to 4 weeks (investigated time).

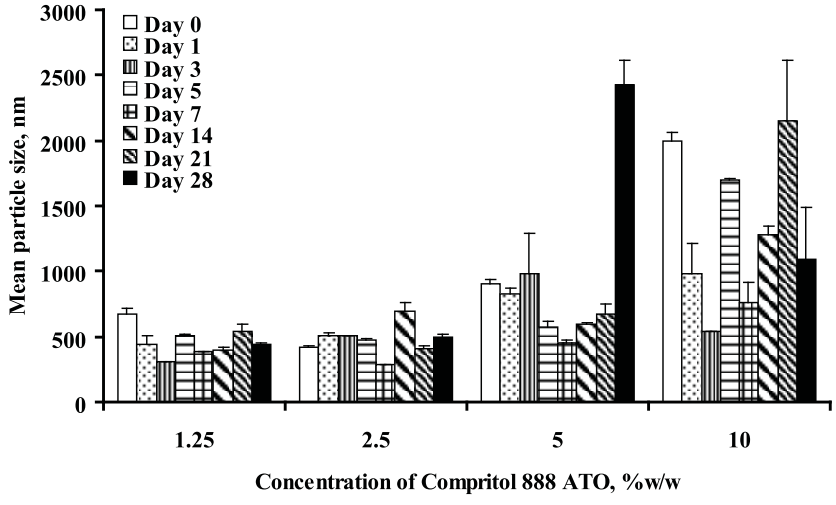

Figure 1 Effect of Compritol® 888 ATO concentrations on the mean particle size of SLN at 25 °C (5%w/w Pluronic® F68, 0%w/w RBO, homogenization pressure at 500 bars for 5 minutes). Data were expressed as mean±S.D. (n=3).

The effect of surfactant concentrations on particle size of the SLN was investigated using 0.5, 1, 2.5, and 5%w/w Pluronic® F68 and results are shown in Figure 2. As expected, increasing the surfactant concentration led to the smaller SLN mean particle size. The SLN prepared from Pluronic® F68 concentration at 2.5 and 5% w/w showed interesting particle size range below 500 nm. This result suggested that SLN which composed of 2.5 %w/w Compritol® 888 ATO and 2.5 %w/w Pluronic® F68 were the suitable formulations concerning the particle size

and physical stability. The unstable SLN were the formulations which prepared from Compritol® 888 ATO and Pluronic® F68 at concentration higher than 2.5 and less than 2.5 %w/w, respectively. They were change in particle sizes during storage at 25 °C. Effect of other compositions such as mineral oil (0, 5, and 10 %w/w), glycerin (0, 5, and 10 %w/w), 70 %v/v sorbitol (0, 5, 10 %w/w) and mixtures of glycerin and 70 %v/v sorbitol (5:5) on the size and stability of SLN were also investigated. Use of these highly viscous excipients led to increase of particle sizes after kept at 25 °C. Therefore, SLN should not contain these excipients higher than 5 %w/w in order to obtain the particle size smaller than 500 nm. This observation was in good agreement with results previously reported (Asautiarit et al., 2007).

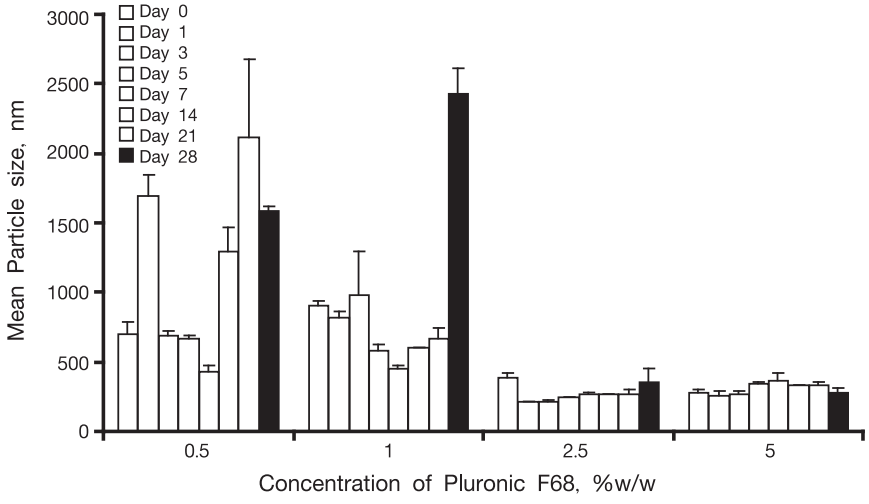

Figure 2 Effect of Pluronic® F68 concentrations on the mean particle size of SLN at 25 °C (2.5 % w/w Compritol® 888 ATO, 0 %w/w RBO, homogenization pressure at 500 bars for 5 minutes). Data was expressed as mean±S.D. (*n*=3).

2. Effect of RBO concentrations

Effect of RBO concentrations at 0, 2.5, 5, and 10% w/w on the particle size of SLN was investigated. Figure 3 showed that the mean particle size of SLN with 2.5%w/w RBO remained unchanged up to 4 weeks after kept at 25 °C. SLN with RBO concentrations higher than 5%w/w gave particle size larger than 500 nm after preparing and the mean particle size was increased after 5 days kept at 25 °C. This result suggested that SLN with RBO concentration at 2.5% w/w gave particle size below 500 nm which may lead to skin occlusive effect as had been reported previously (Wissing and Müller, 2003). The zeta potential values of all formulations were in the range of -5 to -10 mM. The means values were not significantly changed after kept for 28 days.

3. Effect of homogenization

The particle size, polydispersity index has been optimized by varying the homogenization pressure and time. As observed in Figure 4, the higher homogenization pressure gave the lower particle size and narrower polydispersity index. Increasing in homogenization pressure from 500 to 700 bars reduced the particle size from 500 to 160 nm while increasing homogenization pressure from 700 to 800 bars could not further reduce the particle size. The particle size of SLN prepared at 700 bars for 5 minutes was 160±20 nm and polydispersity index was 0.213±0.021. In addition, the particle size was unchanged when kept at 25 °C for 5 days. Thus, the homogenization pressure at 700 bars was selected as optimum for SLN preparations. To investigate SLN reproducibility, 6 batches were prepared by the selected SLN formulations using high speed mixer at 11,000 rpm for 1 minute and high pressure homogenizer at 700 bars for 5 minutes. The SLN exhibited good batch to batch reproducibility as the mean particle sizes of all the 6 batches significantly not different from one another.

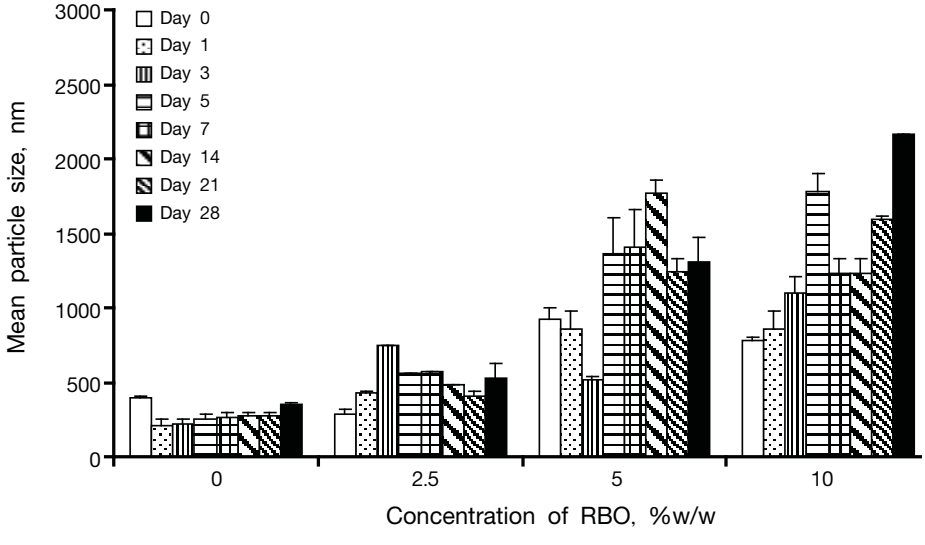

Figure 3 Effect of RBO concentrations on the mean particle size of SLN at 25 °C (2.5 %w/w Compritol® 888 ATO, 2.5 %w/w Pluronic® F68, homogenization pressure at 500 bars for 5 minutes). Data were expressed as mean±S.D. (n=3).

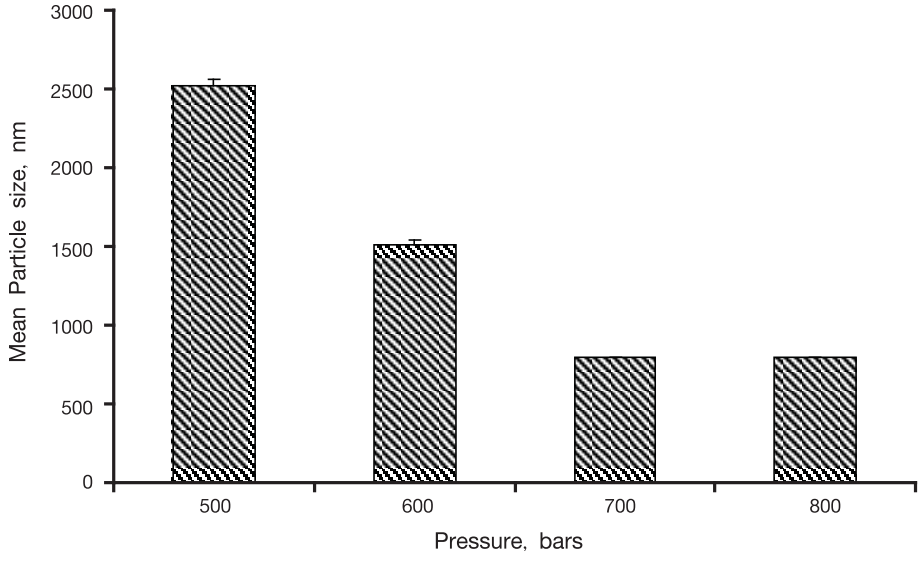

Figure 4 Effect of homogenization pressures on the mean particle size of SLN at 25 °C (2.5%w/w Compritol® 888 ATO, 2.5 %w/w Pluronic® F68, and 2.5 %w/w RBO) after 1 day of formulation preparation. Data were expressed as mean±S.D. (n=3).

4 *In vivo* evaluation of skin hydration and viscoelasticity

4.1 Long term study of skin hydration and viscoelasticity

Long term study of skin hydration and viscoelasticity of the tested formulations were investigated using Corneometer® CM 825 and Cutometer® SEM 575 by comparison between cream containing RBO encapsulated in SLN versus cream containing RBO, cream base, and the untreated area. Figure 5 showed no significant difference between cream containing RBO and cream base. Interestingly, cream containing RBO encapsulated in SLN was significantly more effective in increasing skin hydration after application for 7, 14, and 28 days (+17.06, +28.21, and +40.38 %) when compared to cream base. Figure 6 showed that cream containing RBO encapsulated in SLN gave higher skin viscoelasticity than that of cream base (+3.87, +4.85, and +5.66 %) (p<0.05). Moreover, skin viscoelasticity from cream containing RBO was higher than from cream base after application for 14 and 28 days.

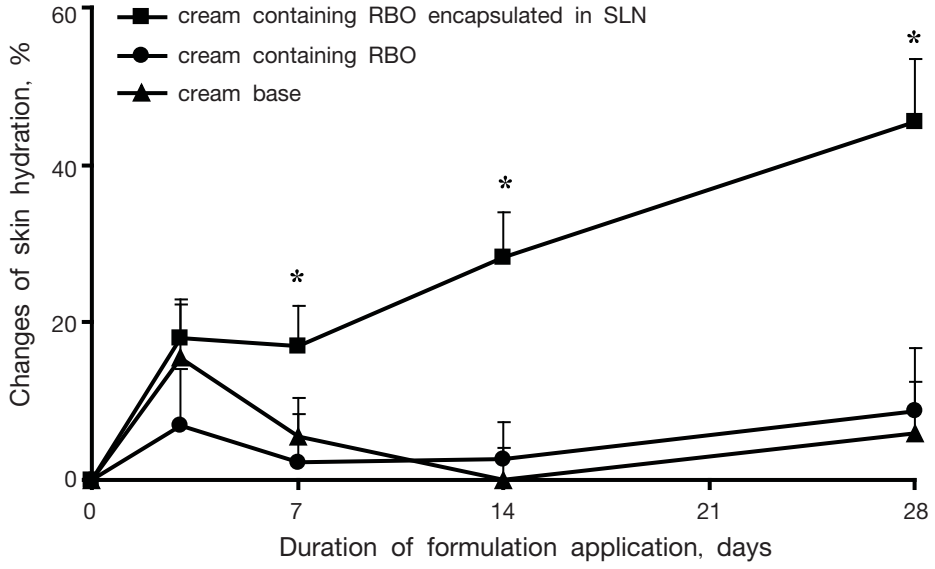

Figure 5 Percent changes of skin hydration of cream containing RBO encapsulated in SLN, cream containing RBO, and cream base. Data points with (*) are significantly different from cream base at $p<0.05$. Data were expressed as mean±S.E.M. (n=10).

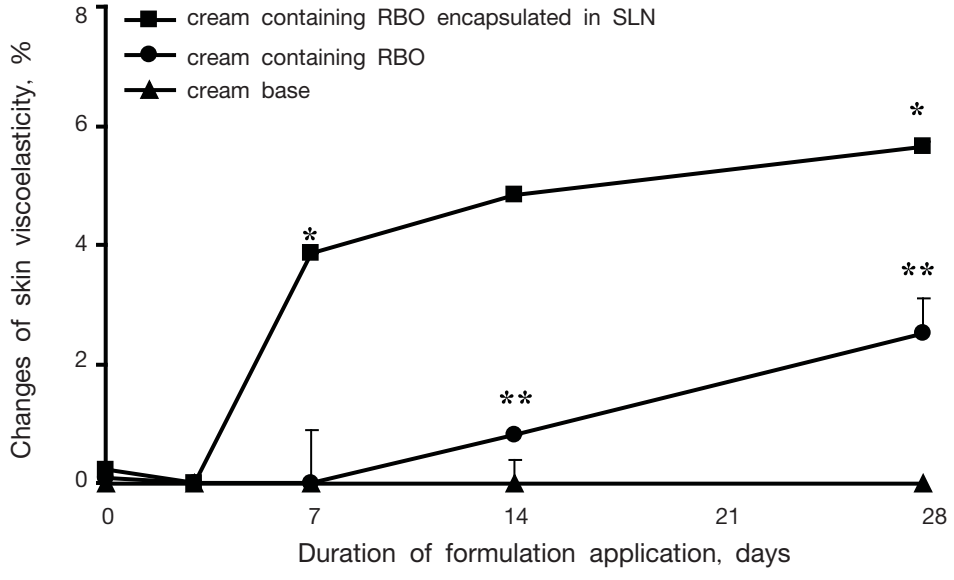

Figure 6 Percent changes of skin viscoelasticity of cream containing RBO encapsulated in SLN, cream containing RBO, and cream base. Data points with (*) are significantly different when compared between cream containing RBO encapsulated in SLN with cream base and (**) compared between cream containing RBO with cream base at $p<0.05$. Data were expressed as mean±S.E.M. (n=10)

4.2 Short term study of skin hydration

Effect could be achieved within 1 hour after formulation application and the resulting humidity remained constant up to 6 hours. Incorporation of RBO encapsulated in SLN into cream bases resulted in significantly higher skin humidity than that of the cream containing SLN (p <0.05) (Figure 8). This result implied that the system with RBO encapsulated in SLN gave more potentially skin hydration than from the blank SLN.

Discussions and Conclusion

1. Effect of compositions on physical property

The negative charge value (-5 to -10 mV) of SLN system was attributed from the free fatty acid of Compritol® 888 ATO or RBO. Despite relatively low zeta potential value, SLN system showed good stability. This was due to the Pluronic® F68, a block copolymer, stabilized SLN by steric stabilization mechanism (Lourenco et al., 1996).

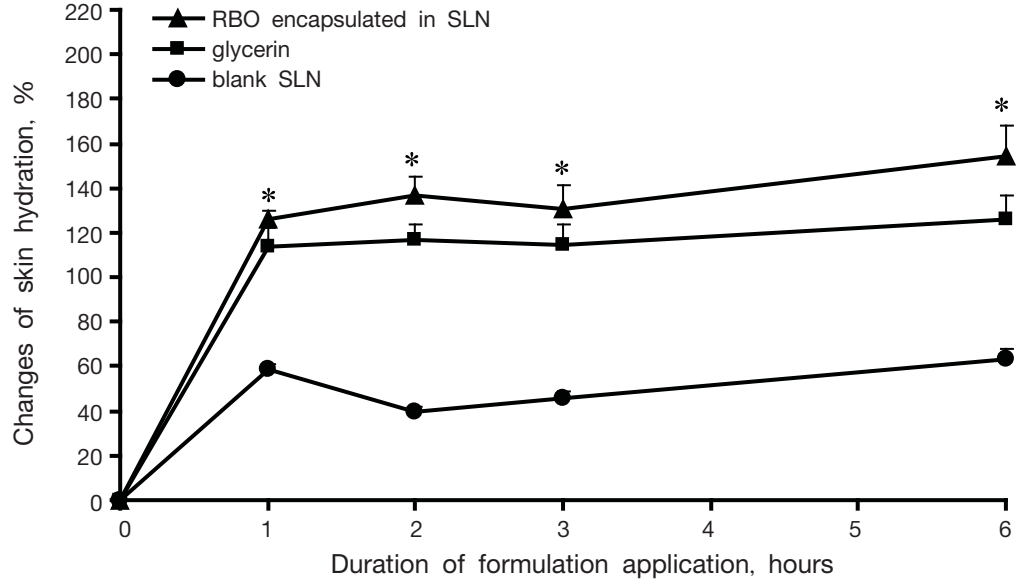

Figure 7 Percent changes of skin hydration of RBO encapsulated in SLN, blank SLN, and glycerin. Data points with (*) are significantly different when compared between RBO encapsulated in SLN and blank SLN at p<0.05. Data were expressed as mean±S.E.M. (n=10).

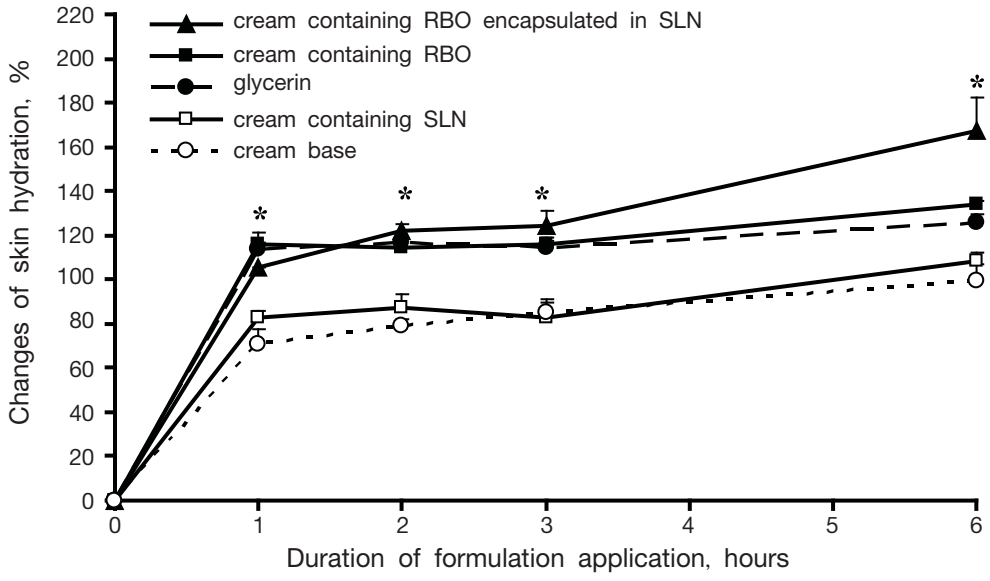

Figure 8 Percent changes of skin hydration of cream containing RBO encapsulated in SLN, cream containing RBO, cream containing SLN, and cream base, and glycerin. Data points with (*) are significantly different when compared between cream containing RBO encapsulated in SLN and cream containing SLN at $p<0.05$. Data were expressed as mean±S.E.M. (n=10).

2. Effect of RBO encapsulated in SLN on skin hydration and viscoelasticity

This study found that only RBO encapsulated in SLN increased skin hydration after application at least 7 days when compared with that from cream containing RBO and cream base. This could be attributed to occlusive effect of the SLN systems with smaller particles size. After cream containing RBO encapsulated in SLN was applied on skin, the SLN droplets were fused together and become dense film cover the skin by capillary forces of nanometer pores between particles (Wissing and Müller, 2003). This film reduced water evaporation from skin to environments (Kuntche et al., 2007; Wissing et al., 2001). On the other hand, this water retention has been reported to be promoted by the SLN properties such as size diameters, crystalline status, and lipid concentration (Wissing and Müller, 2002). The SLN with highly crystalline lipid contents and smaller sizes increased skin hydration higher than the lower crystalline lipid with larger sizes. Besides, polyunsaturated fatty acids including oleic acid, linoleic acid, palmitic acid, stearic acid, and linoleic acid acted as an emollient. Therefore, these factors may reduce

the trans-epidermal water loss (TEWL) from the stratum corneum to the environments and may increase skin hydration. Moreover, the increasing in skin humidity from the system may concurrently contribute to the skin viscoelasticity.

The skin viscoelasticity of cream containing RBO encapsulated in SLN and cream containing RBO were increased as comparing with the cream base. Several studies reported that RBO composed of several active ingredients including tocopherol, tocotrienol, γ-oryzanol, or ferulic acid which may lead to scavenging of free radicals (Bramley et al., 2000, Renuka and Arumugham, 2007) and may contributed to the skin viscoelasticity. Gamma-oryzanol at 1 to 2 %w/w was served as a natural antioxidant which enhanced skin protection from free radicals (Bramley et al., 2000), consequently can increase skin viscoelasticity better than the cream base. However this hypothesis should be further investigated. In addition, cream containing RBO encapsulated in SLN showed higher skin viscoelasticity than cream containing RBO. It may be due to the increase in skin penetration of the SLN system.

Although the entrapment efficiency of RBO was not investigated in this study, the lipophilic property of RBO made it prefers to stay in the lipophilic inner phase rather than in the aqueous external phase. In order to determine the entrapment efficiency of RBO, tocopherol, tocotrienol, γ-oryzanol, or ferulic acid can be analyzed as nature chemicals as marker chemicals.

In conclusion, this study showed that SLN formulations composed of Compritol® 888 ATO, Pluronic® F68, and RBO in the concentration of 2.5% w/w exhibited good physical stability and batch to batch reproducibility. The incorporation of SLN into cream base increased better skin hydration and viscoelasticity than cream containing RBO and cream base. Thus, the RBO encapsulated in SLN prepared by high pressure homogenization technique represents an alternative promising cosmetic delivery system for skin hydration and viscoelasticity.

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