Jurnal Teknologi

Full paper

The Effect of Virgin Coconut Oil Loaded Solid Lipid Particles (VCO-SLPs) on Skin Hydration and Skin Elasticity

Norhayati Mohamed Noor[a]*, Azila Abd. Aziz[a], Mohamad Roji Sarmidi[a], Ramlan Aziz[a]

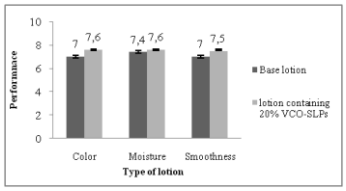
[a]*Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

*Corresponding author: norhayati@ibd.utm.my

Article history

Received :1 November 2012
Received in revised form :
10 April 2013
Accepted :15 April 2013

Graphical abstract



Abstract

Virgin Coconut Oil is usually extracted from well matured and fresh coconut through specialized processes without damaging its natural nutrition. In this work, formulation, characterization and efficacy of VCO-SLPs have been studied. VCO-SLPs were prepared using ultrasonification of molten stearic acid and virgin coconut oil in an aqueous solution and particles with the size of 0.608 µm have been obtained. 20% concentration of VCO-SLPs of 0.608 µm particle size was added into the base lotion. Sensory study and skin evaluation study was conducted to compare the difference between lotion containing VCO-SLPs and lotion without VCO-SLPs. Moisturizing lotion incorporated with VCO-SLPs was found to increase skin hydration and skin elasticity by 24.8% and 2.60% respectively from day 0 to day 28. This shows that solid lipid particles has the potential to be utilized as a carrier for improved dermal delivery of VCO.

Keywords: Solid lipid particles; virgin coconut oil; ultrasonification; moisturizer

Abstrak

Minyak Kelapa Dara kebiasaannya diekstrak daripada kelapa yang matang dan segar melalui proses khas tanpa merosakkan khasiat semulajadi. Dalam kajian ini, formulasi, pencirian dan keberkesanan VCO-SLPs telah dikaji. VCO-SLPs telah disediakan menggunakan ultrasonifikasi asid stearik lebur dan minyak kelapa dara dalam larutan akueus dan partikel dengan saiz partikel 0.608 µm telah diperolehi. 20% kepekatan VCO-SLPs yang bersaiz 0.608 um telah ditambah ke dalam losyen asas. Kajian sensori dan kajian penilaian ke atas kulit telah dijalankan untuk melihat perbezaan di antara losyen yang mempunyai VCO-SLPs dan juga losyen yang tiada VCO-SLPs. Losyen pelembap yang mengandungi VCO-SLPs didapati telah meningkatkan hidrat dan keanjalan kulit masing-masing sebanyak 24.8% dan 2.60% dari hari 0 sehingga hari ke 28. Ini menunjukkan bahawa, partikel lipid pepejal berpotensi sebagai agen pembawa VCO yang lebih baik ke bahagian kulit.

Kata kunci: Partikel lipid pepejal; minyak kelapa dara; ultrasonifikasi; pelembap

1.0 INTRODUCTION

Human skin needs care. From the moment of birth, the skin begins to age, as does the whole body, in a natural physiological process. Skin is the largest, heaviest and most versatile organ of the human body. The skin is also the outermost part of our sensitive system and it acts as a force transmitter and a sensor [1]. The major functions are the protection of body, regulation of body temperature and sensory perception. Biological skin aging is supposed to begin from age 25; however, physiological skin aging starts much earlier and is accelerated by many external factors such as sunlight, cold, UV radiation and air pollution [2].

Skin is permeable and has highly specialized structures such as the stratum corneum (SC), which is the outermost layer of the epidermis [3]. The barrier property of the skin lies mainly in the stratum corneum. This highly hydrophobic layer is composed of differentiated non-nucleated cells, corneocytes, which are filled with keratins and embedded in the lipid domain [4].

Skin cells will continue to divide throughout over entire lifetime. The skin renewal process takes about 28 days. By forming new cells continuously, the cells in the above layers are pushed increasingly upwards to the surface, slowly dry out and form the uppermost horny layer of stratum corneum [2]. The accumulation of corneoctyes can cause flaky, rough, dullness of skin, followed by rapid deterioration with cracking, inflammation, exudation, and bleeding. Hydration of stratum corneum would be the solution in the skin natural generation cycle.

The essential cosmetic care for normal skin is moisturization. Moisturizers are expected to increase skin hydration and to modify the chemical nature of the skin surface to one that is smooth, soft and pliable [5]. Moisturizers are externally applied compounds comprising multiple components, including occlusive ingredient, emollients and humectants. Occlusive moisturizing

ingredients are the oily substances that impair the evaporation of skin moisture by forming an epicutaneous greasy film that impedes water loss. Emollients act by filling the spaces between corneocytes to increase hydration and can be occlusive if applied heavily. Humectants are compounds that attract water from the dermis into stratum corneum, as opposed to trapping water found in the environment. Moisturization of the stratum corneum occurs from below, with the dermis contributing moisture to the skin [6].

In this work, virgin coconut oil is used as the active ingredient for a topical skin moisturizer. Virgin coconut oil is a popular ingredient in traditional skin care application especially in the South East Asia region. Virgin coconut oil (VCO) has a high content of fatty acids, particularly lauric acid and has higher phenolic content and antioxidant activity compared to regular coconut oil [7]. In a moisturizer, VCO may act as an emollient and even as an occlusive agent if applied at the right concentrations.

Solid lipid particles were developed at the beginning of the 1990s as an alternative carrier system for emulsions, liposomes and polymeric nanoparticles. Solid lipid particles are widely used as delivery vehicles for pharmaceutical and cosmetic active agents. Solid lipid particles have a high affinity to the stratum corneum, thus enhancing the bioavailability of the encapsulated material to the skin. Solid lipid particles can enhance the penetration and transport of active substances, particularly lipophilic agents, and thus intensify the concentration of these agents in skin [8]. In this study, VCO is incorporated in solid lipid particles to enhance its effectiveness. This study will focus on the efficacy of the VCO-SLPs incorporated into the base lotion moisturizer.

■2.0 EXPERIMENTAL

2.1 Materials for preparation of VCO-Solid Lipid Particles (SLPs)

Stearic acid (n-octadecanoic acid) 95%, Soy lecithin, Tween 80 and Sephadex G-50 were obtained from Sigma-Aldrich (Selangor, Malaysia). Virgin coconut oil was obtained from Institute of Bioproduct Development (Universiti Teknologi Malaysia, Malaysia). Other chemicals and solvents used such as Carbomer, Tetrasodium EDTA, Sodium citrate, Methylisothiazolinone & caprylyl glycol, Glyceryl monostearate, refined coconut oil, glycerin, propylene glycol and essential oil were of cosmeceutical, pharmaceutical grade or analytical reagent grade. Dionized water was obtained on site from a Sartorius Arium 611 water system.

2.2 Preparation of VCO-SLPs

VCO-SLPs were prepared by ultrasonication method based on HLB value. The lipid phase consisting of stearic acid (10%) and 5% (w/w) virgin coconut oil was melted in a double boiled beaker and was dispersed in a warm aqueous solution with the addition of emulsifier. The weight percentage of the surfactants used was 2.5% out of the total formulation of VCO-SLPs. The amount of surfactants blend used was 29% and 71% for Soy lecithin and Tween 80, respectively. A pre-emulsion was obtained using a high speed stirrer (IKA Ultra Turrax® T25) for 2 minutes at 12000 rpm. The particle size was narrowed down using a sonicator probe (Fisher Scientific Sonic Dismembrator Model 500) at 60% power intensity while ultrasonication time was constant at 180 s. The emulsion was cooled down at room temperature to obtain lipid particle dispersions. Samples were kept at 4ºC.

2.3 Measurement of Particle Size Distribution

The VCO-SLPs sample were analyzed for particle size distribution (volume weighted mean) measurement using Mastersizers 2000S from Malvern Instruments (UK). The VCO-SLPs samples was added to the sample dispersion unit containing a stirrer and then stirred to minimize the interparticle interactions, and the laser obscuration range was maintained between 10% and 20% [9]. The analysis was performed 3 times, and the average values were taken.

2.4 Measurement of Zeta Potential

In order to measure the stability of the particle, zeta potential measuring equipment was used. In this work, the zeta potential of the samples were measured using Zetasizer Nano Z (Malvern Instrument, UK). The samples were diluted with distilled water before being analyzed and the measurements were recorded at 25ºC. This equipment uses micro-electrophoresis/electrophoretic light scattering technology to measure zeta potential and electrophoretic mobility.

2.5 Entrapment Efficiency of VCO-SLPs

Entrapment efficiency of VCO-SLPs samples were calculated based on the analysis done by Zhang *et. al.,* [10] with modifications. The VCO-SLPs suspension was separated by Sephadex gel-50 column (20 mm × 130 mm) chromatography by washing with distilled water at a flow rate of 2.0 ml/min. The VCO-SLPs suspension was diluted with distilled water at a ratio of 1:5. Then 1 ml of solution was pipetted in the column. In order to get the concentrated part (cloudy), the first 3 ml of liquid was removed then 25 ml of liquid was collected. The collected sample was diluted with ethanol (1:1) and sonicated for 20 minutes to break the particle before being filtered using 2 µm pore size syringe filter. The particle was evaluated by determining the amount of entrapped ferulic acid in SLPs using HPLC (Waters, USA). The column used was Synergy hydro-RP 80A (particle size: 250 mm x 4.60 mm x 4µm) and it was thermostated to 25ºC using a column temperature control module. For analysis, elution was carried out with solvent (water/acetonitrile/acetic acid (80:20:0.25 v/v)) as the mobile phase. During HPLC analysis, the solvent was programmed at flow rate of 1.5 mL/min. Phenolic compounds (ferulic acids) will be analyzed as a marker compound The concentrations of ferulic acid in the virgin coconut oil in the suspension (n_1) and SLPs (n_2) were assayed by the HPLC detector at 321 nm (Equation 1).

EE% could be obtained from the following equation:

$$\text{Entrapment efficiency (\%,w/w)} = \frac{n_2}{n_1} \times 100 \qquad (1)$$

where;

n_1 = total concentrations of ferulic acid in the virgin coconut oil (total amount of VCO in the starting solution)

n_2 = concentrations of ferulic acid in encapsulated virgin coconut oil

2.6 Preparation of Base Lotion

Distilled water was heated up to 70ºC in a glass beaker (beaker 1). Other water base materials (Carbomer, Tetrasodium EDTA, Glycerin, Methylisothiazolinone & caprylyl glycol) were added in to beaker 1 in order. While adding, the mixture was stirred using glass rod until homogenous. In a separate beaker (beaker 2), all oil phase (Glyceryl monostearate, refined coconut oil) were added and heated up to 60-70ºC until a homogenous mixture observed. Beaker 1 was homogenized using a high speed homogenizer to mix well. Then, the mixture in beaker 2 was added into beaker 1 and homogenized until a homogenous lotion was obtained. Once the temperature of the base lotion is dropped to 40 ºC, essential oil is added. The VCO-SLPs were incorporated into the base lotion at 20% for sample and blank as placebo. The efficacy of the VCO-SLPs moisturizing lotion was evaluated in vivo using human volunteers. The evaluated parameters on skin are the moisture content and elasticity throughout 28 days of application.

2.7 Accelerated Stability Testing of VCO-SLPs Moisturizing Lotion

2.7.1 Centrifugation Test

Centrifugation tests were performed to observe phase separation in extreme condition. 30g of the VCO-SLPs moisturizing lotion sample was loaded to a centrifuge tube. The sample was centrifuged directly at room temperature at 3000 rpm for 30 minutes.

2.7.2 Freeze-Thaw Test

150g of VCO-SLPs moisturizing lotion sample was filled into a container. The sample was first put into the freezer at -5°C for 24 hours. The frozen sample was then left at room temperature for 24 hours to thaw. One cycle of freeze-thaw was completed in 48 hours. Colour change and phase separation change were observed. The properties of the sample were evaluated before the freeze-thawing process and after every cycle until the fourth cycle.

2.8 Sensory Evaluation Test

Sensory evaluation test was conducted in Institute of Bioproduct Development, Universiti Teknologi Malaysia by distributing a questionnaire to 30 volunteers. The test was conducted based on the hedonic scale scoring by Jones *et al.* [11].

2.9 Efficacy Study of VCO-SLPs Moisturizing Lotion

30 female volunteer testers in the age range 20-25 years and with normal skin conditions were signed up for the efficacy study. The volunteers signed informed consent and were asked to undergo patch testing before commencing with the study. The patch testing was based on the method described by Barbaud [12] with slight modification. A small sample of lotion was dabbed behind the ear of a volunteer with a clean cotton swab and left for 24 hours. Volunteers with no inflammation or allergy at the site of testing were considered to have passed patch testing.

Double-blinded test was used to study the efficacy of the VCO-SLPs lotions (based on the Helsinki Declaration of 1964). Volunteers were instructed not to apply any topical products such as moisturizers or sunscreens to the test sites for one week before and during the study. Two group of respondent in total of 15 persons for each group were supplied with one types of lotion containing VCO-SLPs moisturizing lotion (A) or placebo moisturizing lotion (B). They were required to apply the lotion on their volar forearm at specific area respectively, twice a day, in the morning and at night for 28 days. Two skin parameters, corneometry and elasticity, were studied using Corneometer CM825 and Cutometer MPA580. The testing was done by scanning the skin using the specific probes. The tester was asked to rest for at least 15 minutes at the testing room before any measurement was taken. All measurements were done in triplicates. The sampling times were day 0 (before application), day 7, day 14 and day 28. The efficacy testing was modified from the method described by Gaspar *et al*., [11] on evaluation of dermatological effects of cosmetic formulations.

■3.0 RESULTS AND DISCUSSION

3.1 Particle Size, Zeta Potential and Entrapment Efficiency

Table 3.1 showed the result for the analysis based on formulation and processing parameters described earlier. Based on the particle size, the size was approximately 0.608 µm. Based on Verma *et. al*. [13], larger vesicles may not penetrate well into the deeper layers of the skin and stay in/on the SC. These results showed that larger particle size would be good for an occlusive agent.

Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle [14]. The magnitude of the zeta potential gives an indication of the potential stability of the system, where high zeta potentials (either negative or positive) indicates electrically stabilized particles, while colloids with low zeta potentials tend to coagulate or flocculate [15]. From the result, it showed that the particle size were stable based on the zeta potential values. The zeta potential for the sample was -47.5 mV. Zeta potential values of ±30 mV and above characterize a stable formulation [16].

The entrapment efficiency, expressed as percent of the starting active ingredients, was determined after separation of the free active ingredients by the mini column Sephadex G50. Entrapment Efficiency for this sample was 99.97% meaning that overall of the virgin coconut oil was encapsulated inside the solid lipid particles.

Table 3.1 Characterization of VCO-SLPs

Analysis	Value	Unit
Particle Size	0.608 ±0.002	µm
Zeta Potential	-47.5±0.14	mV
Entrapment Efficiency	99.97±0.014	%

3.3 Performance of VCO-SLPs based Moisturizing Lotion: *in vivo* Study

Moisturizing lotion based on VCO-SLPs was prepared for the *in vivo* performance study. VCO-SLPs at 0.608 µm sizes was incorporated into the lotion at 20% concentration of VCO-SLPs [17]. Accelerated stability testing was conducted to test the stability of the formulation. Then, sensory evaluation tests were performed to measure the acceptability of the lotion to end users. Finally, efficacy of the VCO-SLPs lotion was evaluated by measuring skin hydration and skin elasticity. Efficacy of the VCO-SLPs based moisturizers was determined based on the effect of the lotion on skin hydration and skin. Volunteers were asked to apply the lotions on their volar forearms at a specific area twice a day, in the morning and at night for 28 days.

3.3.1 Stability of the Lotions

Stability is usually studied by a visual inspection that an emulsion would become creaming or flocculate. This analysis is necessary to show whether the active ingredient is degrading or uniformity distribution. Stability tests require a long period of time whereas few companies can afford the luxury of three years testing before marketing new cosmetic products. Demand of the industry require that a reduction in time from product concept to its appearance on the retail shelf is critical, and anything that reduces this time is an advantage. Reducing this period is called accelerated ageing and the most commonly used methods are testing at elevated temperature and humidity or light cabinets and by using freeze/thaw cycles [18]. Two different procedures of accelerated stability testing, centrifugation and freeze-thawing were performed on the lotions. The results of the stability testing are shown in Table 3.2 where no phase separation was observed for all samples. This indicated that stable formulations had been obtained and no colour changes in the both lotions were observed (Figure 3.1).

Table 3.2 : Accelerated stability testing of VCO-SLPs based moisturizing lotion

Sample	Observation	
	Centrifugation Test	Freeze Thaw Test
Base lotion without VCO-SLPs	No phase separation	No phase separation
Base lotion with 20% of Sample A (0.608µm)	No phase separation	No phase separation

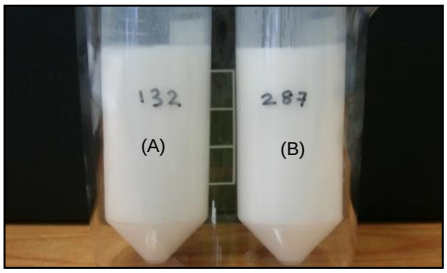


Figure 3.1 Lotion with VCO-SLPs (A) and without VCO-SLPs (B) after centrifugation

3.3.2 Sensory Evaluation Result

The sensory evaluation based on the after feel effect of the lotion (moisture), easiness of application (smoothness), odor and color was conducted to assess the performance of VCO-SLPs. Two types of lotions which were either blank or added with VCO-SLPs were prepared and given to the respondents. Lavender oil was added in the formulation to enhance the smell of the lotions. The respondents preferred moisturizers containing VCO-SLPs compared to the blank moisturizer. Besides that, the respondents also preferred the after feel effect and the spreadability of the moisturizing lotion with VCO-SLPs compared to blank lotion (Figure 3.2).

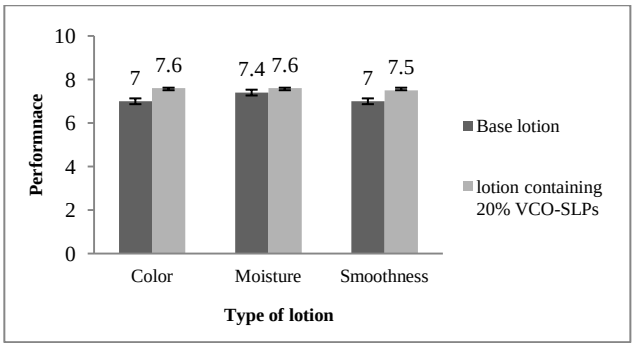


Figure 3.2 Sensory evaluation of the VCO-SLPs moisturizing lotions

3.3.3 Comparison Study on Skin Hydration

The skin moisture in the volunteers were recorded and the results are shown in Figure 3.3. The application of lotion with VCO-SLPs resulted in a significant increase of skin moisture compared to the use of the blank lotion. There is an increase of 24.8% of skin moisture for lotion with VCO-SLPs as compared to an increase of 12.7% in the use of the blank lotion for the duration of a 28 day application. The use of lotion with VCO-SLPs has a higher moisture retention and this could be due to the high occlusion factors of the smaller particle [19, 20]. The significant increase in skin hydration was also found by Müller *et al.*, [21] for an NLC-containing cream compared to conventional cream.

The moisturizing lotion containing VCO-SLPs with 0.608 µm showed a better performance by prolonging the effect of the moisture on the skin. This effect is probably due to the smaller particle size that allows the particle to be retained in the SC layer. The lotions with SLP had better results in increasing the moisture content according to Pardeike *et. al.*, [22]. The most important criteria for a moisturizer are to prevent water loss from the skin and to keep the skin moisture of the SC at about 20% to 35% or more [23]. Maintaining moisture at the optimum condition can prevent the effects of wrinkles [24].

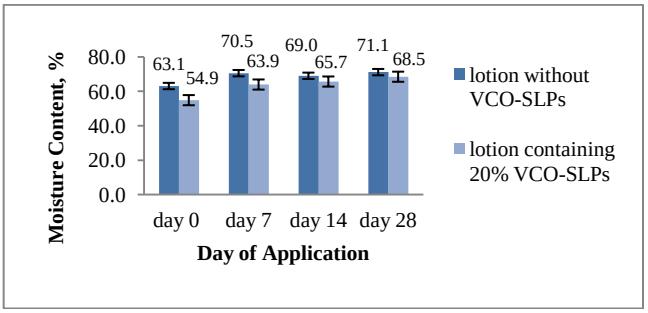


Figure 3.3 Skin moisture content

3.3.4 Comparison Study on Skin Elasticity

Figure 3.9 shows the gross elasticity (UA/UF) increases with continuous applications of the moisturizer. The increasing elasticity is more pronounced with the addition of VCO-SLPs to the moisturizer. The blank moisturizer exhibits the smallest increase in skin elasticity which is only 0.76% after 28 days. The moisturizer containing 20% VCO-SLPs shows the highest increase in skin elasticity of 2.60% from day 0 and day 28. This might due to higher skin hydration and reduced transepidermal water loss [25]. Moisturizing lotion that contains predominantly

water and small molecules of virgin coconut oil allow for easy absorption through skin. Therefore, the moisture from the particulate carrier will be absorbed effectively into the skin with the function of solid lipid particle [26].

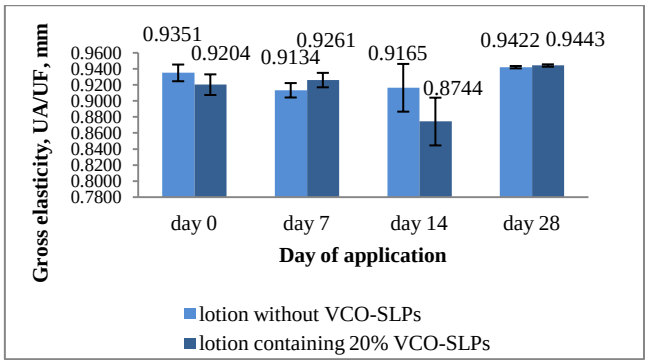


Figure 3.4 Skin elasticity result

4.0 CONCLUSION

Based on the result above, the objectives of this research have been achieved. Moisturizing lotion loaded with VCO-SLPs was found to be effective in increasing skin moisture and improving skin elasticity. VCO-SLPs have the potential to be used as topical delivery vehicles due to their biodegradability, biocompatibility and low toxicity. They also exhibit excellent hydration, occlusiveness and can prolong the release of VCO transcendently

Acknowledgement

The authors are thankful to the Universiti Teknologi Malaysia for giving the Short Term Grant (No Vote:77319).

References

[1] Ramalho, A., Silva, C.L., Pais, A. A. C. C., Sousa, J. J. S. 2007. In Vivo Friction Study Of Human Skin: Influence Of Moisturizers On Different Anatomical Sites. *Wear*. 263(7–12): 1044–1049.

[2] Brummer, R. 2006. *Rheology Essentials of Cosmetic and Food Emulsions*. 1st ed. New York Unnastr, Hamburg: Springer Berlin Heidelberg.

[3] Wickett, R. R. and Visscher, M. O. 2006. Structure and Function of the Epidermal Barrier. *Am J Infect Control*. 34(10, Suppl 1): S98–S110.

[4] Godin, B. and Touitou, E. 2007. Transdermal Skin Delivery: Predictions for Humans from in Vivo, Ex Vivo and Animal Models. *Adv. Drug Deliv. Rev*. 59(11): 1152–1161.

[5] Elsner, P. and Maibach, H. I. 2000. Eds. *Cosmeceuticals: Drugs vs. Cosmetic* (Cosmetic Science and Technology Series/Volume 23) New York: Marcel Dekker Inc.

[6] Flynn, T. C., Petros, J., Clark, R. E. Viehman, G. E. 2001. Dry Skin and Moisturizers. *Clin Dermatol*. 19: 387–392.

[7] Marina, A. M., Man, Y. B. C., Nazimah, S. A. H. and Amin, I. 2009. Chemical Properties of Virgin Coconut Oil. *J Am Oil Chem Soc*. 86: 301–307.

[8] Müller, R.H., Radtke, M. and Wissing, S. A. 2002. Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) In Cosmetic And Dermatological Preparations. *Adv Drug Deliv Rev*. (54: Suppl 1): S131–S155.

[9] Mulla, J. S. and Khazi, I. M. 2009. Short Communication : Influence of Process Variables on Particle Size of Solid Lipid Nanoparticles. *Indian Journal of Novel Drug Delivery*. 1(1): 47–49

[10] Zhang, D., Tan, T. and Gao, L. 2006. Preparation of Oridonin-Loaded Solid Lipid Nanoparticles and Studies on Them *In vitro* and *in vivo*. *Nanotechnology*. 17: 5821–5828

[11] Jones, L.V., Peryman, D. R. and Thurstone, L. L. 1955. Development of a Scale for Measuring Soldiers Food Preferences. *Food Res*. 20: 512–520.

[12] Barbaud A. 2005. Drug Patch Testing in Systemic Cutaneous Drug Allergy. *Toxicol*. 209: 209–16

[13] Verma, D. D., Verma, S., Blume, G. and Fahr, A. 2003. Particle Size of Liposomes Influences Dermal Delivery of Substances into Skin. *International Journal of Pharmaceutics*. 258: 141–151.

[14] Aramwit, P., Kanokpanont, S., Nakpheng, T. and Srichana, T. 2010. The Effect of Sericin from Various Extraction Methods on Cell Viability and Collagen Production. *Int. J. Mol. Sci*. 11: 2200–2211

[15] American Society for Testing and Materials. 1985. *Zeta Potential of Colloids in Water and Waste Water ASTM Standard D 4187-82*. West Conshohocken, PA, USA.

[16] Freitas, C. and Müller, R. H. 1998. Effect of Light and Temperature on Zeta Potential and Physical Stability in Solid Lipid Nanoparticles (SLN) Dispersions. *Int J Phar*. 168: 221.

[17] Souto, E. B., Muller, R. H. and Alemieda, A. J. 2007. Topical Delivery of Oily Actives Using Solid Lipid Particles. *Pharmaceutical Technology Europe*. 19(12).

[18] COLIPA. 2004. *Guidelines on Stability Testing of Cosmetics Product*.

[19] Wissing, S. A, and Muller R. H. 2002. The Influence of the Crystallinity of Lipid Nanoparticles on their Occlusive Properties. *Int J Pharm*. 242: 377–379.

[20] Hommoss, A. 2008. Nanostructured Lipid Carriers (NLC) in Dermal and Personal Care Formulations. Free University Berlin. Ph. D Dissertation.

[21] Müller, R.H., Hommoss A., Pardeike, J., and Schmidt, C. 2007. Lipid Nanoparticles (NLC) as Novel Carrier for Cosmetics - Special Features & State of Commercialisation. *SÖFW*. 9: 40–46

[22] Pardeike, J., Hommoss, A. and Müller, R. H. 2009. Lipid Nanoparticles (SLN, NLC) in Cosmetic and Pharmaceutical Dermal Products. *International Journal of Pharmaceutics*. 366: 170–184.

[23] Flynn, T. C., Petros, J., Clark, R. E., Viehman, G. E. 2001. Dry Skin and Moisturizers. *Clinics in Dermatology*. 19: 387–392.

[24] Baran, R. and Maibach, H. 1998. *Textbook of Cosmetic Dermatology*. 2nd edition. Singapore: Kyodo Printing Co (S'pore) Pte Ltd.

[24] Wissing, S. A. and Müller, R. H. 2003a. Cosmetic Applications for Solid Lipid Nanoparticles (SLN). *International Journal of Pharmaceutics*. 254: 65–68.

[25] Wissing, S. A. and Müller, R. H. 2003b. The Influence of Solid Lipid Nanoparticles on Skin Hydration and Viscoelasticity-*In Vivo* Study. *Eur J Pharm Biopharm*. 56(1): 67–72.