International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 2, 2016

Original Article

DEVELOPMENT OF NANOSTRUCTURED LIPID CARRIER FORMULATION CONTAINING OF RETINYL PALMITATE

JESSIE SOFIA PAMUDJI, RACHMAT MAULUDIN, NASYA INDRIANI

School of Pharmacy, Institut Teknologi Bandung
Email: jessie@fa.itb.ac.id

Received: 06 Nov 2015 Revised and Accepted: 30 Dec 2015

ABSTRACT

Objective: The purpose of this research is to develop a Nanostructure Lipid Carrier (NLC) formulation containing of Retinyl Palmitate.

Methods: Preparation of NLC was carried out by ultrasonication method. The formulas of NLC were developed by using virgin coconut oil and oleic acid as a liquid lipids, cetyl palmitate, and stearic acid as solid lipids, Tween 80 and Poloxamer as a surfactant and glycerine as co-surfactant. Characterization of NLC consisted of visual appearance, morphology, particle size, polydispersity index (PI), and physical stability test using freeze-thaw, centrifugation, and accelerate stability test method.

Results: Obtained NLC revealed a good characterization with the formulation of 7.2% of cetyl palmitate, 4.8% of oleic acid, 10% of Tween 80, 10% of glycerin, and 2% of retinyl palmitate. This NLC has a pale yellow appearance, globule size of 258±15.85 nm; and a polydispersity index of 0.31±0.09. It was also physically stable after centrifuged at 13,000 rpm for 30 min, during 4 cycles of freeze-thaw and storage at room temperature for 28 d. During storage for 28 d, retinyl palmitate in NLC had been degraded only about 15% in comparison with macroemulsion destroyed almost 50% of retinyl palmitate in the same time.

Conclusion: NLC formulations with 7.2% cetyl palmitate, 4.8% of oleic acid, 10% of Tween 80, 10% of glycerol, and 2% of retinyl palmitate is the most optimal formula that showed a good characteristic. Stability study revealed that NLC provided better stability than macroemulsion.

Keywords: Retinyl palmitate, NLC, Ultrasonication Methode

INTRODUCTION

Nanostructure Lipid Carrier (NLC) is the second generation of lipid nanoparticle having a structure like nanoemulsion. The first generation was solid lipid nanoparticle (SLN). The difference between both of them is in its core. Both of them are a colloidal carrier in submicron size in the range of 40-1000 nm. SLN has a core which consists of one of solid lipid or a mixture of solid lipids while the core of NLC consists of a mixture of solid lipid and liquid lipid. Usually, SLN formulation can stable up to 3 y, has a good reproducibility, and can be delivered for various routes of administration such as intravenous, dermal, peroral or topical [1]. SLN also has the capability to protect degradation of active ingredient, to modulate of drug release, enhance the stability of sensitive active ingredients such as Co-enzyme Q10, Vitamin E, and Vitamin A, and has an ability to control the release of active ingredients [2]. Besides all of the advantages above, SLN still has any disadvantages such as a limitation of drug loading that depend on the solubility of active ingredient in the solid lipid and expulsion of drugs during storage caused by lipid crystallization [3].

NLC is developed to improve the disadvantages of SLN. The unique advantages of NLC such as enhanced of drug loading capacity and prevention of drug expulsion during storages make NLC is more favorable than SLN [2]. Recrystallization in NLC was less then SLN due to crstalline order in NLC was disturbed by oil particle to maintain the system in the form of liquid phase [1]. There were several method to construct NLC such as: High-Pressure Homogenization [4], O/W Microemulsion [5], Emulsification-solvent evaporation [6], multiple emulsion water/oil/water [6]. The high shear homogenization and/or ultrasonication were a dispersion technique which was not use an organic solvent, a large amount of surfactant or additive compound. The melting lipid was added and dispersed in a solution of surfactant using ultrasonication method [7]. The kind of ultrasonic equipment which usually use was probe sonicator [8].

Retinoid is a widely used ingredient in dermatology therapies of wrinkles. Retinol and retinyl palmitate are two compounds from the retinoid group which have an ability to induce the thickness of epidermis and effective as anti wrinkle agent. These substances is widely used as active ingredient in cosmetic products. Retinyl palmitate in the skin wills hydrolyze into retinol, which plays an important role on the regulation of epidermis cell growth. It is iniatate the final step of keratization, involve in synthesizer of collagen, prevent the atropi of connective tissue, increase the synthesizer of glicoaminoglican and essential substance in the reproduction of membrane basale cell [9]. Retinoid is an unstable drug which is easily to oxidized by light, oxygen and metal. Therefore it's needed a special formula to prevent the retinoid degradation [10]. The aims of this study were to develop a good characteristic of NLC containing of retinyl palmitate.

MATERIALS AND METHODS

Materials

Retinyl palmitate was provided from PT Kimia Farma, Cethyl palmitate and Poloxamer 188 were purchased from BASF Indonesia, Oleic acid, Tween 80, Glycerin, and stearic acid were purchased from local market, Virgin Coconut Oil (VCO) was purchase from LIPI.

Instruments

Analytical balance (Mettler Toledo, USA), Heater stirrer, Ultraturax, Sonicator probe, High Pressure Liquid Chromatography (Hewlett Packard Agilent Series 1100), Climatic Chamber (Hotpack), pH Meter (Mettler Toledo S20), particle size analizer (Delsa™ Nano C Particle Analyzer, Beckman Coulter, USA), Transmission Electron Microscopy (Dept Physic of Gajah Mada University), microcentrifuge (Eppendorf), micropipette (Eppendorf), and other general laboratory glasswares.

NLC formula optimization

NLC formula optimization was done using various combinations of lipid, oil and surfactant concentration. The used lipid compounds in this study were stearic acis and cethyl palmitate. Meanwhile the oil compounds were VCO and oleic acid, surfactant were Tween 80 and

Poloxamer 188 and as co-surfactant was glycerine. For oil phase: First the lipid component was melting then mixed with the oil. Water phase: Surfactant and co-surfactant were dissolved in deionized water. Then both phases were heated up until 60-70 °C, mixed and stirred using ultra turax at 9800 rpm for 5 min. The next step the emulsion was sonicated using probe sonicator at an amplitude of 60 kHz for 1 minute and 45 seconds which on-off period of 30 seconds and 5 seconds respectively. The emulsion were kept at room temperature for 24 h and the nanoparticles size were measured. The NLC formula with particle sizes in the range of 200-300 nm is choosen for further investigation. Retinyl palmitate was added to the choosen formulas and optimization was carried out again until the target particle size were obtained.

Particle sizes analysis

The nanoparticles size of NLC were measured using Delsa Nano-C Particle Analyzer. The obtained data were polydispersity index and means of nanoparticles size. The polydispersity index (PI) measured nanoparticles size distribution [2]. The good value of PI was ≤ 0.5.

Morphology of nanoparticles

The nanoparticles morphology were observed by using Transmission Electron Microscope (TEM) Jeol JEM 1400. Samples were mixed with aqua bidest and keep in the carbon film with 1% of Phosphotungstate acid and dried. Then the samples were ready to observe by TEM.

NLC physical stability test [11]

NLC was kept at room temperature. Different parameters, namely nanoparticles sizes, PI, and pH were determined during stability studies. All parameters were measured at the day of 0, 7, 14, 21, 28 after storage.

The effect of centrifugation to the physical stability of NLC was also observed at 13,000 rpm for 30 min. The NLC is said to be stable if there was no separation of oil phase occurred after centrifugation is processed.

The physical stability of NLC against extreme fluctuation of temperature was also observed by doing Freeze-Thaw test for six cycles. Each cycle consist of 48 h storage at a temperature of 4 °C followed by 48 h storage at a temperature of 40 °C. At the end of each cycle the nanoparticles size and PI of NLC were measured. The value of zeta potential of the system was also determined after 0, 3, and 6 cycles. The NLC is said to be stable if there was no oil phase separation and significant change of its nanoparticles size.

Accelerated stability test

The dosage forms were kept in Climatic Chamber at a temperature of 40±2 °C and humidity of 75±5% for 25 d. The remaining of retinyl palmitate is determined using HPLC method using mobile phase of acetonitrile and UV detector at wavelenght of 325 nm. First retinyl palmitate NLC is dissolved in acetone and centrifuged. The clear acetone phase is withdraw and evaporated. Then the residue is dissolved in acetonitrile and injected to HPLC instrument [12].

Statistical analysis

The difference of mean particle sizes between NLC and microemulsion was analyzed statistically using one-way ANOVA during 28 d of storage. Furthermore, stability profile of retinyl palmitate in NLC is compared with retinyl palmitate in macroemulsion, and the result is analyzed statistically using one-way ANOVA at α=0.05 (significance level of 0.05).

RESULTS AND DISCUSSION

Preparation of *Nanostructure Lipid Carrier* (NLC) using ultrasonication method

The materials for constructed NLC are consist of solid lipids such as stearic acid and cetyl palmitate. Virgin Coconut Oil (VCO) and oleic acid were used as liquid lipids and Tween 80 and Poloxamer 188 were used as surfactants. Glycerine was also used in the NLC formula as co-surfactant. Optimation of the formulation to obtained the stable NLC were completed using various combination of all the components and the results obtained from each formula is shown at table 1.

Table 1: Optimization of NLC formulation

S. No.	Stearic acid (%)	Cetyl Palmitate (%)	VCO (%)	Oleic acid (%)	Tween 80 (%)	Poloxamer 188 (%)	Glycerine (%)	Results
1	3		2		1			Breaking
2	2.40		1.60		1			Breaking
3	1.80		1.20		1			Breaking
4	2.40		1.60		2			Breaking
5	1.80		1.20		2			Breaking
6	1.20		0.80		2			Breaking
7	1.80		1.20			1		Semisolid
8	1.80		1.20			2		Semisolid
9	1.20		0.80		1.50	1.50		Breaking
10	1.20		0.80		2.50	2.50		Breaking
11	1.20		0.80			5	4	Semisolid
12	1.20		0.80		5		4	Breaking
13		1.20	0.80		1			Breaking
14		1.20	0.80		2			Breaking
15		1.20	0.80			2		Semisolid
16		1.20		0.80	5		5	Breaking
17		1.20		0.80		5	5	Semisolid
18		1.20		0.80	10		10	Stable
19		1.20		0.80		10	10	Semisolid

n= 3 samples observation

The NLC formulation was prepared using ultrasonication method. First the mixture of melting lipids components were blended with a surfactant, co-surfactant, glycerin and deionized water at 60-70 °C. This mixture is stirred with Ultraturax at 9800 rpm for 5 min. Immediately this pre-emulsion is sonicated using Probe Ultrasonicator at an amplitude of 50 kHz for 2 min. Then the obtained NLC was kept at room temperature for 24 h.

Base on the results of optimation formulation at table 1, it was found that Poloxamer 188 was not suitable for formation of NLC. The results obtained from the formula that using Poloxamer 188 was semisolid dosage forms, so its nanoparticles size can not be measured. The formation of semisolid dosage forms by Poloxamer 188 was due to its thermogelling properties. Poloxamer consist of block copolymer of ethylen oxyde and proylen oxyde. This

temperature-dependent self-assembling and thermogelling characters has already known

In water, Poloxamer has an ability to change its form, namely from an individual block of co-polymer (unimer) into a group of micelles. At the concentration below the Critical Micelle Concentration (CMC), the unimer will disperse in water while at the concentration over the CMC, they unimer will agregate to form micelles. The formation of micelles is depends on temperature or Critical Micellization Temperature (CMT). Below the CMT, the molecule of Poloxamer is in the form of unimer, while over the CMT the aggregation will occur following by the formation of gel [13].

The formulation that using a combination of Tween 80 and Poloxamer 188 as surfactants is also yielded a semisolid or unstable NLC. The addition of co-surfactant glycerine into the formulation can not improve the performance of NLC. Therefore, the formulations that using Tween 80 as the surfactant is selected for further development. The best formulation was showed by a combination of cetyl palmitate as solid lipid and oleic acid as liquid lipid or oil (table 1). The other formulas were in the form of unstable emulsions. It is suggested that the surfactant and cosurfactant used in these formulas were not able to construct the densed layer surrounding the nanoparticles. Therefore its have a tendency to become coalescence.

The preparation of NLC was performed using ultrasonication method. This instrument produced a strong vibration that can break lipid nanoparticles into a smaller diameter of nanoparticles, hence increases the surface area and surface free energy. It is needed a large amount of surfactant to cover all the surface of the nanoparticles and stabilized the system. If there was an uncovered surface of nanoparticles, it can be a trigger to cause a coalescence of the nanoparticles [14].

The next optimation was to find the concentration of each material in the formulation that can produce the NLC with a diameter of nanoparticles in the range of 200-300 nm, because the target that has to be reached was retinoic acid responsive elements (RAREs) in the epidermis [15]. The ratio between solid and liquid lipid was constant at 60:40. The NLC formulas were developed with various concentration of oil phase and surfactants as shown in table 2. The next day after preparation the diameter of nanoparticles and polydispersity index form each formulation were analyzed. The measurement of diameter nanoparticles was very important. It can be used to observe the physical instability of the products. The increasing of nanoparticles size during the storage is indicated the unstable of NLC. The diameter of nanoparticles can be affected by various factors suh as the composition of the formulation, production method, and concentration of surfactant and co-surfactant. In general, the smaller of the nanoparticles size, the higher of the ratio between surfactants and lipids needed to stabilize the formula [1].

Table 2: Optimation of concentration of oil phase, surfactants, and cosurfactant used in the NLC formulation

Formula	Oil phase (%)	Surfactant (%)	Co-surfactant (%)	Particles size (nm)	PI
F20	3	10	10	24.60±0.82	0.41±0.05
F21	4	10	10	26.37±2.36	0.34±0.08
F22	4	8	8	247.33±24.90	0.45±0.05
F23	5	8	8	312.87±16.16	0.31±0.04
F24	10	10	10	73.33±6.00	0.34±0.03
F26	12	10	10	230.60±38.41	0.36±0.09
F27	15	10	10	400.53±48.31	0.28±0.02

n= 3 time measurement

Base on the result at table 2, it was found that the formulas of F20 and F21 have the smallest of nanoparticles size. The concentration of surfactant and co-surfactant in these formulas were relatively high so it can improve the surface area that can cover the small nanoparticles. Therefore, in the formulation of F 22 the concentration of surfactant-co-surfactant were reduced. The result obtained from this formulation was fulfill the size target of nanoparticles. However, the concentration of oil phase in the formula was too low to load the active ingredient. Therefore, the next step of optimation was to improve the concentration of oil phase. The F26 (consist of 12 % of oil phase) fulfilled the nanoparticles size target (table 2). The PI value from all of the formulation was ≤ 0.5. These value indicated that the distribution of particle size of the NLC was narrow distribution. The lower of the value of PI the higher of homogeneity in the dosage forms [15].

Optimation of retinyl palmitate loading into NLC

It has to be considered that loading of retinyl palmitate into the NLC can increase the particle size. So next optimation was to find the formulation that can load high concentration of retinyl palmitate but still fulfill the particle size target. The formula of F25 and F26 were chosen for this purpose. The result obtained from this optimization is shown in table 3 and fig. 2.

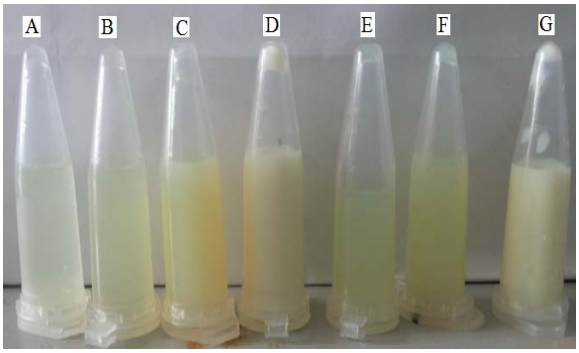

Fig. 1: Visual appearence of l NLC retinyl palmitate: (A) F25-1, (B) F25-2, (C) F25-3, (D) F25-4, (E) F26-1, (F) F26-2, (G) F26-3

Table 3: Optimation of retinyl palmitate loading into NLC

Oil phase (%)	Surfactant (%)	Formula	Co-surfactant (%)	RP (%)	Particle size (nm)	PI
10	10	F25-1	10	0.5	55.43±7.65	0.31±0.15
10	10	F25-2	10	2	135.33±11.34	0.38±0.05
10	10	F25-3	10	3	343.00±9.53	0.28±0.02
10	10	F25-4	10	4	628.90±32.29	0.31±0.07
12	10	F26-1	10	1	159.03±20.66	0.36±0.02
12	10	F26-2	10	2	258.00±15.85	0.31±0.09
12	10	F26-3	10	4	737.60±29.20	0.27±0.02

n= 3 time measurement.

Base on the result in table 2 and fig. 2, the higher concentration of retinyl palmitate loading NLC, the most cloudy appearance of NLC. The translucent of the NLC performance is indicated that its particle size was smaller than the wave length of visible light [16]. The best formula obtained from this optimization was showed by the formula of F26-2 that consists of oil phase of 12%, the surfactant of 10%, Co-surfactant of 10%, and retinyl palmitate of 2%. This formula will be further characterized.

Morphology of NLC core

The morphology of NLC lipid core sample was determined using Transmission electron micrograph (TEM*)*. The result obtained from this study showed that the lipid core of NLC was spherical with a diameter in the range of 200-300 nm.

Physical stability study of NLC

The study was performed by keeping the NLC samples at room temperature. During storage, particle sizes, polydispersity index, and pH of the samples were analyzed at days of 0, 7, 21, 28. The result obtained from this study is shown at table 4.

The particles size of retinyl palmitate NLC were increased during 28 d of storage, but this alteration was still in the range of targeted diameter of NLC. The difference of mean particle sizes was analyzed statistically using one-way ANOVA and it was found that there were no significant changes between the particle size of NLC particles during 28 d of storage. The value of PI was still less than 0.5. It means that the distribution of particle size was still good.

The physical instability of emulsion can be detected if there are phenomena of creaming, separation of oil phase or sedimentation of a component that has a high density occurred in the emulsion. The formation of these phenomena can accelerate by using centrifugation [17]. There were no such phenomena observed for F26-2 after centrifugation at 3000 RPM for 30 min.

The freeze-thaw test was carried out to observe the physical stability of emulsion during the fluctuation of temperature. One cycle of the freeze-thaw test was consist of 48 h of storage at 4 °C and 48 h storage at 40 °C. The parameters of particles sizes, polydispersity index, and zeta potential of the samples were measured during 4 cycles of freeze-thaw test, and the result is shown at table 5.

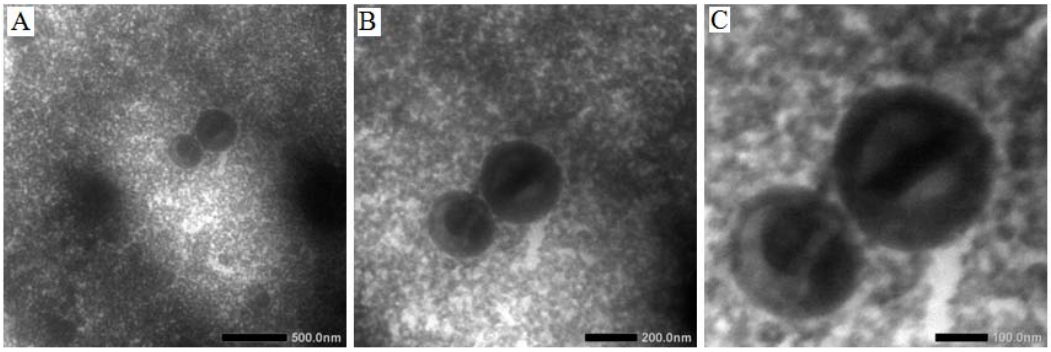

Fig. 2: The morphology of retinyl palmitate NLC: (A) magnification of 10000x, (B) magnification of 20000x, and (C) magnification of 40000x

Table 4: Physical stability of NLC at room temperature for 28 d

Days of	Particle sizes (nm)	PI	pH
0	259.27±20.14	0.29±0.01	4.76±0.04
7	261.47±23.15	0.34±0.02	4.6±0.03
21	268.2±0.34	0.34±0.02	4.5±0.11
28	283.33±24.71	0.33±0.05	4.32±0.11

n= 3 time measurement.

Table 5: Particles size, polydispersity index (PI) and zeta potential of retinyl palmitate NLC during freeze-thaw test

Cycles	Nanoparticles size (nm)	PI	Zeta Potential (mV)
0	259.3±20.14	0.29±0.01	-0.27
1	283.33±24.71	0.33±0.04	-
2	375.5±12.14	0.31±0.05	-
3	584.1±22.55	0.32±0.04	-1.19
4	728.93±52.25	0.31±0.05	-

n= 3 time measurement.

At the end of first cycles, the diameter of nanoparticles was still in the range of the target. But the size of the nanoparticles was continuously increase after the next cycles. After 4th cycles, the NLC was broken. These phenomena were presumed because of the redissolution rate of the emulsifying agent was slow after freezing, and induced the coalescence of the nanoparticles and finally the phase separation was occurred.

The value of zeta potential can be used to predict the stability of dispersing system potential during the storage. The decreasing of zeta potential value is indicate the decreasing of physical stability of the system. However, this rule was not valid for the disperse system which is stabilized by steric mechanism [1]. Although there was an alteration of zeta potential during the freeze-thaw test, it can not be use to predict the stability of NLC. The value of zeta potential of F26-2 NLC was very small (table 5) because the used emulsifying agent in this formula works as sterical mechanism.

Accelerated stability test

Chemical stability study was accomplished in Climatic *Chamber* at temperature of 40±2 °C and humidity of 75±5% for 28 d.

According to an accelerated stability study at fig. 3 reveals decreasing the concentration of retinyl palmitate in macroemulsion

was faster in comparison with NLC. During storage for 28 d retinyl palmitate in NLC was only about 15% destroyed in comparison with macroemulsion was destroyed almost 50% of retinyl palmitate in the same time.

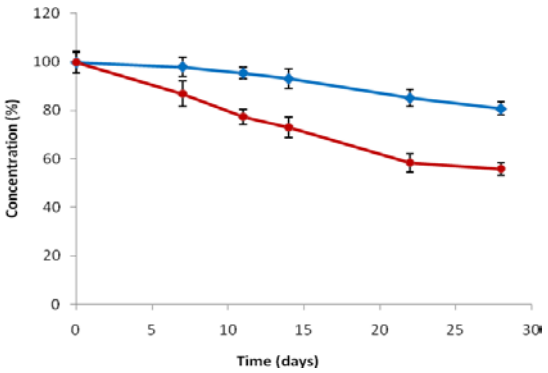

Fig. 1: Remaining retinyl palmitate concentration in NLC (◆) and macroemulsion (-●) after stability study in period of 28 d (n= 3 time measurement)

In this case indicate that NLC was better to protect retinyl palmitate against stressing from the environment such as light, temperature or hydrolysis degradation by water surrounding lipid nanoparticles. Stability profile of the both formulation was different according to one-way ANOVA with a significance level of 0.05 (α=0.05). The tested NLC formulation was a standard formulation that needed to improve the stability of retinyl palmitate against environment stressing. During maximum storage retinyl palmitate degradation is considered below 5%, therefore improving stability can be accomplished by adding antioxidant, chelating agent or another specific preparation.

CONCLUSION

The best formula of this study was the NLC formulation that consists of 7.2% of cetyl palmitate, 4.8% of oleic acid, 10% of Tween 80, 10% of glycerine, and of 2% retinyl palmitate. This formula has a pale yellow appearance with a particle size of 258±15.85 nm, and a polydispersity index of 0.31±0.09. It was also stable at room temperature for 28 d, after centrifugation at 13000 rpm for 30 min and 4 cycles of the freeze-thaw test. NLC was better to protect retinyl palmitate against stressing from the environment in comparison with macro emulsion.

CONFLICTS OF INTERESTS

All authors have none to declare.

REFERENCES

1. Üner M. Preparation, characterization and physicochemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC): Their benefits as colloidal drug carrier systems. Pharmazie 2006;61:375–86.
2. Beck R, Guterres S, Pohlmann A. Nanocosmetics and Nanomedicines: New Approaches for Skin Care, Springer-Verlag, Berlin Heidelberg; 2011.
3. Mŭller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm 2002;242:121–8.
4. Mŭller RH, Runge SH. Solid lipid nanoparticles (SLN1) for controlled drug delivery. In: Benita S, ed. The submicron emulsion in drug targeting and delivery. Harwood Academic Publishers, the Netherlands; 1998. p. 219–34.
5. Gasco MR. Solid lipid nanospheres from warm micro-emulsions. Pharm Technol Eur 1997;9:52–8.
6. Cortesi R, Esposito E, Luca G, Nastruzzi C. Production of lipospheres as carriers for bioactive compounds. Biomaterials 2002;23:2283–94.
7. Kržič M, Sentjurc M, Kristl J. Improved skin oxygenation after benzyl nicotinate application in different carriers as measured by EPR oximetry *in vivo*. J Controlled Release 2001;70:203–11.
8. Mei Z, Chen H, Weng T, Yang Y, Yang X. Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. Eur J Pharm Biopharm 2003;56:189–96.
9. Moyano MA, Segall A. Vitamin a palmitate and α-lipoic acid stability in o/w emulsions for cosmetic application. J Cosmet Sci 2011;62:405-15.
10. Froix M, Pukshansky M, Nacht S. Retinoid formulation in porous microspheres for reduced irritation and enhanced stability. United States Patent Application; 1998.
11. Mauludin R, Mohamad SFB, Suciati T. Formulation and characterization of ascorbyl palmitate are loaded o/w microemulsion. Int J Pharm Pharm Sci 2014;6:294-8.
12. Asean Guideline on Stability Study of Drug Product. 5th Draft. version 6.0; 2013. p. 1-40.
13. Bonacucina G, Marco C, Giovanna M, Gianfabio G, Giovanni FP. A thermosensitive self-assembling block copolymer as drug delivery systems. Polymers 2011;3:779-811.
14. Shekhawat PB. Preparation and evaluation of clotrimazole nanostructured lipid carrier for topical delivery**.** Int J Pharma Bio Sci 2013;4:407-16.
15. Baumann L, Saghari S, Weisberg E. Cosmetic dermatology principles, and practice. 2nd Ed. The McGraw-Hill Companies. Inc. United States; 2009.
16. Ahmed K, Li Y, McClements D, Xao H. Nanoemulsion and emulsion-based delivery system for curcumin: encapsulation and release properties. Food Chem 2012;132:799-807.
17. Andre V, Willenbcher N, Debus H, Börger L, Fernandez P, Frenchen T, *et al.* Prediction of emulsion stability: facts and myth. In: Cosmetics and Toiletries Manufacture Worldwide, Aston Publishing Group. Birkenhead. North Shore City; 1994. p. 1-7.