DEVELOPMENT AND CHARACTERIZATION OF PRECIROL ATO 88 BASE IN NANOSTRUCTURED LIPID CARRIERS (NLC) FORMULATION WITH THE PROBE SONICATION METHOD

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Received: 16 Sep 2020, Revised and Accepted: 10 Oct 2020

ABSTRACT

Objective: Adapalene is a medicinal ingredient that can be used to treat acne. Adapalene has a Log P value of 8.6 and has high lipophilic and low solubility in water and is potentially degraded. Adapalene NLC can increase biphasic drug penetration at low doses but has good reactivity and provides occlusive properties that can increase skin hydration, thereby accelerating acne treatment.

Methods: Forming Adapalene NLC using the method of heat homogenization followed by ultrasonication probe. The NLC formulas used were Precirol ATO5 ® 4.0%, Myritol ® 2%, Cremophor RH 40 1%, Plantacare 1%, Tegocare 1%, and Adapalene 0.3%. Following that is the characterization of particle size, polydispersity index, zeta potential, efficiency entrapment.

Results: The results showed the measurement of particle size with a range (150-318 nm), index polydispersion showed (0.12-0.36) and zeta potential (-26)-(-60 mV) and efficient entrapment testing showed results (84-98%). In the TEM morphological evaluation images of spheres and evenly distributed forms, this is in line with the results of the adapalene NLC characterization.

Conclusion: These results suggest that NLC containing adapalene showed excellent result.

Keywords: Adapalene, Precirol® ATO5 ®, Nanostructured Lipid Carriers (NLC), Probe Sonication

INTRODUCTION

Nanostructured lipid carrier (NLC) is one of the drug delivery systems with modifications that is able to improve the physicochemical properties of an active ingredient [1]. Carrier lipid nanostructured is a second-generation nanocarrier as the first generation improvement, namely solid lipid carrier (SLN) [2]. SLN only consists of solid lipids without liquid lipids; this is a weakness of SLN because the matrix structure owned by SLN is very solid, resulting in low loading capacity and the possibility of the expulsion of the drug during storage and this system also has a high potential for explosive eruptions drug [3]. The liquid lipid component in NLC functions to overcome SLN weaknesses, namely to disrupt the conformation of the solid lipid matrix so that it does not become a perfect matrix of solid lipid crystals during storage and also the absorption of active substances increases [4, 5]. Adapalene (ADA) is a new synthetic retinoid (3rd generation) as an anti-inflammatory, redness on the skin when in direct contact with the epidermal layer.

MATERIALS AND METHODS

Study materials

The ingredients used consist of active ingredients namely Adapalene (PT. Oton spa), Glyceryl Palmitostarate (Precirol® ATO5) solid lipids from PT. Gatotse, liquid lipid Cetyltrimethylammonium (Myristol® 318), surfactant PEG-40-Hydrogenated Castor Oil (Cremophore RH 40®), surfactant Lauryl Triglyceride (Plantacare®), and Poliglycerol-3 Methil Disteatrate Glucose (Tegocare®) from Evonic Industries AG, Methanol Pro Analysis (Merck).

FTIR testing

FT-IR test is intended to determine the compatibility between the materials used [11, 12]. This test is carried out by analyzing the active substance Adapalene, Precirol® ATO5 solid lipid, and a mixture of Precirol® ATO5-Adapalene solid lipid. The sample is crushed and placed in the sample container until it meets the crystal part of the Agilent Cary 630 FT-IR spectrophotometer instrument then the sample spectrum results are compared [13].

X-Ray diffractometry (X-RD) testing

Analysis of the active substance Adapalene, Precirol® ATO5 solid lipids, and a mixture of Tretinoin-solid lipids using the X-RD Bruker D8 instrument to determine the crystallinity of the ingredients used [14, 15]. Samples were analyzed with a scanning speed of 1°/min.

Differential scanning calorimetry (DSC)

The DSC test was carried out to determine the melting point and properties of the material, adapalene, Precirol®, ATO5 solid lipid, and a solid-Adapalene lipid mixture placed in an aluminum plate on the DSC instrument Thermal Analysis License (USA), then heated from 20-400 °C at speed heating 10°C/min. The data obtained in the form of thermograms with thermal parameters such as onset, offset, and maximum peak.

Preparation of NLCs

NLC was made using the hot homogenization method followed by probe sonication [16]. As much as 0.1% of adapalene is put into Precirol® ATO5 and liquid lipids (Cetyltrimethylammonium (Myristol® 318) then melted (Phase A). Campervan surfactant with a water phase using magnetic-stirrer (IKA® C-Mag HS4) (Phase B). Phase B is added to phase A, then stirred using a high-shear homogenizer (Ultra Thurrax IKA® T25) to form pre-emulsions [17-19]. The next
step is to reduce particle size using a sonicator probe (Ivymen Ultrasonic Homogenizers CY-500) [20].

Physicochemical characterization of particles

Adapalene NLC formulations were characterized, consisting of particle size, index polydispersion, and zeta potential using Malvern ZSP (England) zeta sizer particle size gauges at room temperature (25 °C) [21]. Adapter NLC samples were taken 5 drops and then added with water up to 10 ml and put into a disposable cuvette[22]. To confirmed the formation of NLC adapalene, do to characterization Test X-Ray diffractometry (X-RD Bruker D8) and Differential Scanning Calorimetry testing is perform to recognize changes that occur in NLC formulated with adapalene after probe sonication methods [23].

The morphological formula of NLC adapalene was formed using transmission electron microscopy (TEM) Jeol Japan JEM 1400. A total of 1 g of the adapalene NLC oil phase is dispersed in 5 ml of deionized water before analysis. The mixture is then stirred and dropped 10 µl on the specimen. A lat grid of 400 mesh then dropped 10 ml of uranyl acetate over the grid and the rest of the droplets is cleaned again using filter paper, allowed to stand for 30 min to dry, and put into a TEM tool to capture [24].

Entrapment efficiency (EE)
The adsorption efficiency calculation is performed by 1 ml Adapalene NLC inserted in Vivaspin filter tubes (Vivaspin, Goettingen, Germany) with a membrane filter that has a 5 kDa molecular weight cut-off and centrifuged at 14,500 rpm for 3 h. Then the supernatant is taken and diluted, then measured by the UV Spectrophotometry method (Shimadzu 1800) at the maximum wavelength to obtain levels of Adapalene that is not absorbed (unentrapped). Then the entrapment efficiency (EE) can be calculated using the following equation [25]:

\[
\% \text{EE} = \frac{\text{Total adapalene} - \text{free adapalene}}{\text{Total adapalene}} \times 100
\]

RESULTS AND DISCUSSION

Evaluation and characterization of adapalene NLC

FT-IR test is a means of qualitative identification by involving the infrared ray spectrum, which provides information on the presence or absence of interactions on the mixture of materials used in the NLC formula [28]. This test consisted of a single adapalene analysis, a single Precirol® ATO5 solid lipid and a mixture of both. The fig. above shows that a single Adapalene has sharp peaks at frequencies of 2914.73 cm-1 and 2849.74 cm-1, while Precirol® ATO5 sharp peaks are at frequencies 2912.54 cm-1 and 2848.30 cm-1. This proves that both have similar characteristics in terms of the type of bond even though they have different frequencies; this difference is caused by the difference in vibration between the two. Similar to the Precirol® ATO5 spectrum, the mixture spectrum of Adapalene and Precirol® ATO5 has sharp peaks at 2912.54 cm-1 and 2848.30 cm-1 without the formation of new peaks, this revealed that Adapalene was not detected as a whole because it had absorbed in Precirol® ATO5. This becomes a reference that the two sample materials tested are mutually compatible and besides, the absence of new peaks in the FT-IR spectrum pattern also provides information that there is no interaction between Adapalene and Precirol® ATO5 so that it does not form new molecular groups (fig. 1).

Test X-ray diffractometry (X-RD) is carried out so that the diffraction data obtained as a result of the collision between the sample material with electromagnetic rays such as X-rays [27]. This test consists of a single adapalene analysis, a single Precirol® ATO5 solid lipid, a mixture of both, and an Apalene NLC using the X-RD Bruker D8 instrument to find out the crystallinity of the material used. Samples were analyzed with a scanning speed of 1°/min.

XRD test results show that a single Adapalene (plot A) it can be seen that Adapalene has 8 crystalline peaks with 1 high-intensity peak. The more peaks that are formed, the more lattices that can refract X rays. When more rays are transmitted than rays are refracted, the peaks formed will slope so that they have low crystalline properties, conversely the narrower the width of the peaks, the higher the crystalline properties a sample material because the higher the intensity of the light that is refracted.

Based on the diffractionogram, it shows that single and has sharp peaks that resemble the phenomenon of grass, whereas a single Precirol® ATO5 (plot B) has blunt peaks with low intensity, this shows that the Precirol® ATO5 dense lipids have the level of crystallinity is lower than that of Adapalene so that when Adapalene is encapsulated by Precirol® ATO5 the Plot D displays peaks which indicate that the mixture is amorphous (fig. 2).

Fig. 1: FTIR absorption spectra of adapalene, precirol, mixture adapalene and precirol

Fig. 2: X-Ray Diffraction pattern of adapalene, precirol, mixture adapalene and precirol
DSC testing is performed to determine the melting point and properties of materials used with endothermic and exothermic phenomena [28]. A total of 10 mg samples (solid lipids Precirol ATO5®, Adapalene, a mixture of solid lipids and Adapalene) were placed in aluminum plates on the DSC instrument. The sample is heated from 20-400 °C with a heating speed of 10 °C/minute. Adapalene has crystalline properties, a mixture of solid lipids and Adapalene formed a curve in the range of 20.5 °C-83.3 °C with an endothermic energy needed to be able to influence the system inside [29]. Precirol® ATO5 experienced a narrowing, ie in the range of 19.4 °C-77.7 °C and had a peak at 62.4 °C. This indicates a shift in the melting point that occurs in the mixed sample material after Precirol® ATO5 and Adapalene. Mixture thermogram and Adapalene NLC did not form a new peak which states that Adapalene can be completely absorbed on its solid lipid matrix, Precirol® ATO5, which is indicated by the loss of the Adapalene peak (59.6 °C) on the mixed thermogram (fig. 3).

The development of Adapalene NLC formula using Precirol Ato solid lipid with liquid Myritol lipid and using three types of surfactants, namely Plantacare, Cremophore RH 40 and Tegocare was successfully produced. The parameters used to characterize the adapalene NLC were particle size, index polydispersion, zeta potential, and efficient entrapment. The results showed the measurement of particle size with a range (150-318 nm), index polydispersion showed the homogeneity of the particle size distribution. The data shows (0.12-0.36) a number below 0.5, this indicates that NLC formula has a good particle size distribution. The zeta potential evaluation provides an overview of the repulsive force between particles that has the potential for particle aggregation, the zeta potential value is close to zero indicating aggregation, causing instability. The data shows that the potential zeta value is more than and result zeta potential showed (-26) to (-60 mV) so the NLC formula has good stability and efficient entrapment testing showed results (84-98%) (table 1).

Table 1: Formulation and characterization of adapalene loaded NLC

| Formulation | Particle size (nm) | PdI | ZP (mV) | Entrapment efficiency (%) |
|-------------|--------------------|-----|---------|--------------------------|
| Code | ADA (%) | PRC (%) | MYR (%) | CRE (%) | PLA (%) | TGO (%) |
| 1 | 0.1% | 2 | 2 | 1 | - | - | 177.4±7.05 | 0.12±0.00 | -60.4±9.21 | 95.24±0.07 |
| 2 | 0.1% | 3 | 1.75 | 1 | - | - | 175.3±0.82 | 0.11±0.00 | -40.7±12.37 | 88.80±0.59 |
| 3 | 0.1% | 4 | 1.5 | 1 | - | - | 218.4±4.57 | 0.25±0.00 | -50.9±10.15 | 93.20±1.04 |
| 4 | 0.1% | 5 | 1.25 | 1 | - | - | 277.6±13.32 | 0.31±0.00 | -26.9±1.88 | 94.70±0.00 |
| 5 | 0.1% | 6 | 1 | 1 | - | - | 299.8±2.24 | 0.26±0.00 | -35.3±27.26 | 98.46±0.00 |
| 6 | 0.1% | 2 | 2 | - | 1 | - | 156.0±6.10 | 0.26±0.00 | -51.7±4.12 | 92.72±0.21 |
| 7 | 0.1% | 3 | 1.75 | - | 1 | - | 173.1±1.80 | 0.28±0.00 | -49.3±8.9 | 84.93±0.00 |
| 8 | 0.1% | 4 | 1.5 | - | 1 | - | 160.9±1.71 | 0.24±0.00 | -53.6±8.92 | 94.67±0.00 |
| 9 | 0.1% | 5 | 1.25 | - | 1 | - | 223.6±2.50 | 0.29±0.00 | -48.8±17.84 | 92.27±0.42 |
| 10 | 0.1% | 6 | 1 | - | 1 | - | 233.6±2.50 | 0.49±0.00 | -44.3±4.18 | 98.67±0.00 |
| 11 | 0.1% | 2 | 2 | - | - | 1 | 189.7±14.62 | 0.29±0.00 | -35.1±5.66 | 94.10±0.58 |
| 12 | 0.1% | 3 | 1.75 | - | - | 1 | 198.8±4.07 | 0.26±0.00 | -50.8±1.32 | 91.91±0.04 |
| 13 | 0.1% | 4 | 1.5 | - | - | 1 | 215.8±3.37 | 0.22±0.00 | -59.3±5.94 | 92.12±1.17 |
| 14 | 0.1% | 5 | 1.25 | - | - | 1 | 227.1±1.22 | 0.28±0.00 | -44.3±1.8 | 98.58±0.00 |
| 15 | 0.1% | 6 | 1 | - | - | 1 | 318.7±3.73 | 0.36±0.00 | -58.6±20.65 | 98.50±0.00 |

ADA: Adapalene, PRC: Precirol, MYR: Myritol, CRE: Cremophore, PLA: Plantacare, TGO: Tegocare, PdI: Polydispersity Index, ZP: Zeta Potential

Fig. 3: DSC thermograms of adapalene, precirol, mixture adapalene and precirol

Fig. 4: Tranmision electron microscopic photograph imaging of, A (Blank NLC), B (Adap-NLC)
The results of Microscopic Electronic Transmission showed that the form of NLC with Precirol Ato solid lipid has a spherical shape and has a size below 300 nm this shows that the NLC produced has a size below 500 nm and has a correlation in particle size testing and is well distributed. This is related to the results of the NLC characterization of particle size and index polydispersion (fig 4).

CONCLUSION
The development of Adapalene NLC formula using Precirol Ato solid lipid with liquid Myrtil lipids and using three types of surfactants, namely Plantacare, Ceremophore RH 40 and Tegocare were successfully produced. The parameters used to characterize the adapalene NLC were particle size (90-300 nm), index polydispersion (<0.5), zeta potential -20 mv up to -60 mv, and efficient entrapment (84-98) % and morphological evaluation using Transmission electron microscopy (TEM) showed spherical shape.

ACKNOWLEDGEMENT
The authors are thankful to the Faculty of Pharmacy, Padjadjaran University, for providing the support and their facilities, the Faculty of Pharmacy, Bhakti Kencana University, PT DKSH Indonesia, PT Sagara Purnama Indonesia.

FUNDING
Nil

AUTHORS CONTRIBUTIONS
All the authors have contributed equally.

CONFLICT OF INTERESTS
All the authors have contributed equally.

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