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Formulation and Characterization of Tretinoin Nanostructured Lipid Carriers Using Apifil and Cremophore

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ABSTRACT

The most widely used vitamin A derivative for mild to severe acne is tretinoin. However, it is lipophilic (LogP 6.3). Tretinoin must be transformed into liquid lipids and NLC (nanostructured lipid carriers) based on Apifil® and stabilized by surfactants to address permeability and stability concerns. Heat homogenization and sonication with a sonicator probe were used to formulate tretinoin into NLC. Apifil®, Myritol®, and Chremophore® RH 40 were the materials utilized. Particle size, polydispersity index, zeta potential, adsorption effectiveness, and morphological measurements were then used to describe NLC. The characterization results showed that NLC tretinoin has a particle size of <200 nm for 2 measurements over 30 days, a polydispersity index value of 0.5, a zeta potential range of -7 mV to -19.3 mV, and an efficiency entrapment value of >80% for all formulas. Spherical shape emerged from the morphology data. **Conclusion:** The findings demonstrate that tretinoin's nanostructured lipid carriers provide favorable characterization outcomes.

Keywords: Diabetes, Metformin HCl, Extended release, Direct compressed, Hypromellose, Carboxymethylcellulose natrium.

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1. Introduction

At this time acne can usually be treated with topical skin cosmetic treatments or orally depending on the severity of the acne. Topical application is currently an effective method for acne, but it can cause redness, peeling, initial skin irritation, dryness, and photosensitivity resulting from poorly targeted drug delivery at the epicenter of acne, the polysebaceous state (1). One of the drugs used is the retinoid drug class and its derivatives such as tretinoin (2). The mechanism of action of tretinoin is activating a receptor called the RAR *(retinoic acid receptor),* forming and increasing the amount of NGAL protein *(Neutrophil Gelatinase-Associated Lipocalin)* which will result in the death of sabesa gland cells or sebum-producing cells by increasing and forming NGAL protein which can then reduce production sebum so that it can reduce the appearance of acne (3).

Tretinoin in lipids and surfactants in various literatures has a molecular weight of 300.4 g/mol, melting point of 180-182 °C, has a Log P value of 6.3, Pka value of 4.76 (Pubchem). Tritenoin is not soluble in water, it is difficult to dissolve in ethanol and in chloroform with the side effect of being very irritating and causing redness and peeling of the skin and also tretinoin has low solubility and also low permeability, therefore tretinoin requires a new drug delivery system (4). New drug delivery systems such NLC (nanostructured lipid carriers) are designed to enhance the therapeutic efficacy of tretinoin. The NLC system has a high encapsulation capacity, controlled release, thermodynamic stability, and can increase the bioavailability of bioactive compounds (5).

The use of nanostructured lipid carriers (NLC) for tretinoin is aimed at addressing the solubility and stability problems treatinoins face due to their lipophilic properties. By incorporating tretinoins into the NLC, the study has successfully improved the soluble and stableness of tretinoin, which can ultimately improve its clinical effectiveness in the treatment of acne. New drug delivery systems such as NLC are designed to enhance the therapeutic efficacy of tretinoin. The NLC system has high encapsulation capacity, controlled release, thermodynamic stability, and can increase the bioavailability of bioactive compounds.

2. Method

2.1. Instrumentation

The tools used in the research are digital scales (Mettlertoledo®), spray bottles, ultraturax homoginizer (IKA T25 digital), analytical balance (Mettler Toledo), magnetic stirrer (IKA® C-mag HS 10), probe sonicator (Ivymen System CY - 500), hot plate (Oxone®), particle size analyzer (PSA) instrument (Malvern instruments Ltd), pH meter (Mettlertoledo®), Vivaspin (Eppendrof, Germany), UV spectrophotometer (Shimadzu UV), Fourier Transform Infra-Red (FTIR) instrument (Aligent Technologies Cary 630 FTIR), Transmission Electron Microscopy (TEM, JEOL JEM 1400, Japan), Differential Scanning Calorimetry (DSC) instrument, X-Ray Diffraction (XRD) instrument, general purpose glassware (Pyrex®) and SPSS data analysis software (SPSS Inc. , Chicago, IL).

2.1.1. Materials

The materials used were the active substance Tretinoin (Beatue Lab), Methanol PA (PT. Merapi Utama Pharma), solid lipid PEG-8 Beeswax

(Apifil®) (PT. Gattefosse SAS France), liquid lipid Capric Triglyceride (Myritol®) (BASF Indonesia) and surfactant Polyoxyl 40 hydrogenated castor oil (Cremophore®) (Evonic Industries AG).

2.1.2. Detailed Procedure

2.1.2.1. FT-IR (Fourier transform infra-red)

Samples to be tested (tretinoin, apifil; a mixture of apifil®® and tretinoin). The sample is melted first and then waited for it to freeze again, then the snippet is taken until smooth and inserted into the FTIR tool. Then the sample is read using the Agilent Cary 630 FTIR Spectrometer instrument (6).

2.1.2.2. XRD (X-Ray Difraction)

Samples to be tested (tretinoin, Apifil; a mixture of Apifil® and tretinoin). The sample is melted first and then waited for it to freeze again, then taken snippets are crushed until smooth and analyzed (7).

Manufacture of NLC Tretinoin

The manufacture of NLC Tritenoin was carried out using the heat homogenization method using a probe sonicator at a temperature of approximately 70 °C (8). Preparation by mixing Tretinoin, solid lipids, and liquid lipids in a vessel and then melted at 70 °C (lipid phase). Surfactants are partially dissolved with aqua deion with a magnetic stirrer (aqueous phase) (8,9).

1. Characterization of NLC Tretinoin

This evaluation was conducted to determine the stability of tretinoin NLC during storage at room temperature (25°C) using Malvern ZSP Zetasizer (UK). NLC characterizations include particle size, polydispersity index (PDI) and zeta potential. Efficiency entrapment (%EE) testing using ultracentrifuges and vivaspin. Morphological testing using the Transmission Electron Microscope (TEM) instrument (6).

2. Measurement of particle size and polydispersity index

The test takes 10 drops from an NLC sample of tretinoin to add water up to 10 ml and put into a disposable cuvette and inserted into a particle size measuring device (8).

3. Zeta potential measurement

The test takes 10 drops from an NLC sample of tretinoin to add water up to 10 ml then put into a disposable cuvette and closed using a dip cell electrode then measured into a zeta potential measuring device (8).

4. Determination of Efficiency entrapment (%EE)

The calculation of efficiency entrapment was carried out by means of 1ml of NLC Tretinoin inserted into vivaspin and centrifuged at a speed of 12000 rpm for 3 cycles, each cycle of 60 minutes. Next, the supernatant was taken and diluted, measured by UV spectrophotometry at the maximum wavelength to obtain unentrapped Tretinoin levels (6,8).

Efficiency entrapment (EE) is identified by the equation:

%EE = (Total active substance-free active substance) / (Total active substance) x 100%

5. NLC tretinoin morphological testing

Testing using transmission electron microscope (TEM-1400 Flash Electron Microscope JEOL Ltd.). A total of 1 g of the oil phase of NLC tretinoin was dispersed into 5 ml of deionized water

before analysis. The mixture is then stirred and dripped onto the specimen. The grid on 400 mesh is dripped into 10 ml of uranyl acetate on the grid; then the remaining droplets are cleaned again using filter paper and left for 30 minutes to dry before being inserted into the TEM tool for shooting (8).

3. Result

The following is a table of characteristics of NLC Tretinoin for one month.

Table 1. Formulation and characterization of tretinoin NLC D-1.

D-1 Formulation					Z-Ave		ZP	Efficiency Entrapment
					nm	PdI	mV	EE%
Code	TRN (%)	APF (%)	Myt (%)	CRE (%)				
F1	0.05	4	1	2	97.27±9.47	0.12±0.10	-18.5±0.36	83.87642
F2	0.05	2	1	3	96.73±1.73	0.34±0.04	-15.4±0.23	84.8419
F3	0.05	5	1	1.5	124.60±3.47	0.14±0.09	-18.6±0.46	84.8419
F4	0.05	3	1	2.5	111.73±2.05	0.25±0.02	-14.7±0.06	83.87642
F5	0.05	6	1	1	111.53±0.97	0.16±0.02	-16.3±0.32	85.80739

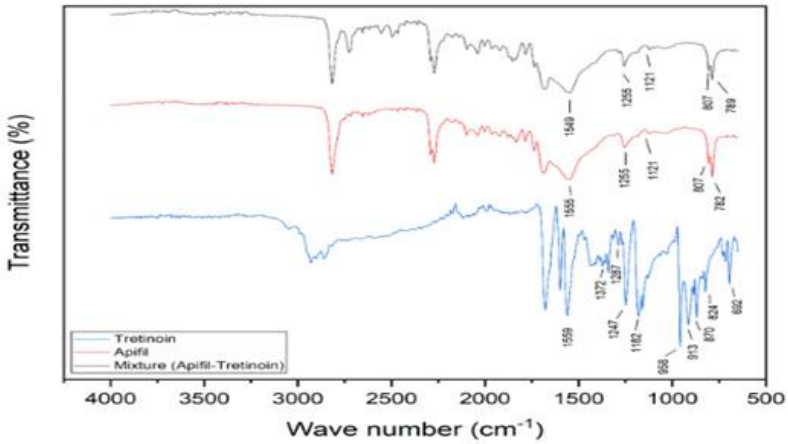
Table 2. Formulation and characterization of tretinoin NLC D-30.

D-30 Formulation					Z-Ave		ZP	Efficiency Entrapment
					nm	PdI	mV	EE%
Code	TRN (%)	APF (%)	Myt (%)	CRE (%)				
F1	0.05	4	1	2	129.33±1.10	0.40±0.01	-10.9±0.84	80.9799662
F2	0.05	2	1	3	101.41±1.63	0.36±0.07	-17.5±1.15	85.807386
F3	0.05	5	1	1.5	141.37±1.59	0.30±0.01	-19.3±1.52	82.9109341
F4	0.05	3	1	2.5	137.40±7.13	0.44±0.04	-14.5±0.21	82.9109341
F5	0.05	6	1	1	112.97±1.86	0.13±0.08	-7.1±0.37	82.9109341

4. Discussion

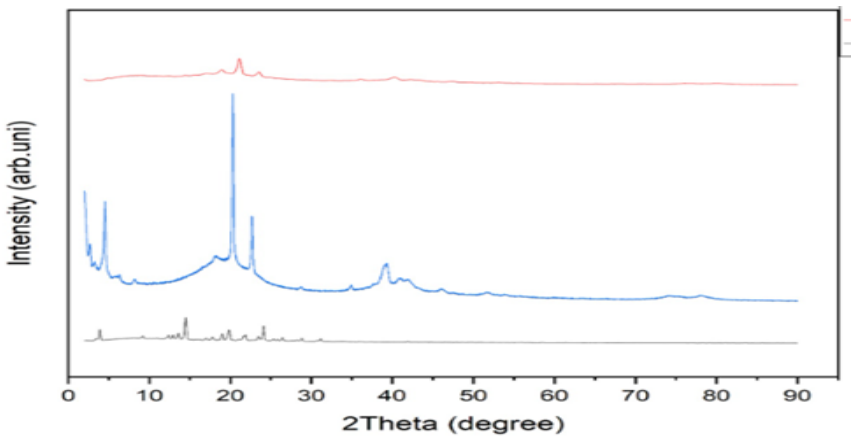
The FT-IR results can be seen in Figure 1 in the range 500-1500 cm^{-1}, the results show that it does not show significant new peaks in Figure 1.c. The purpose of this test is to determine whether or not there is an interaction between each of the ingredients used. The results showed that there were no significant new peaks in Figure 1(c) after lipid addition. This indicates that the ingredients used in the tretinoin NLC formula are suitable and have good compatibility and there is no interaction between the two samples (8).

Figure 1. FTIR Tretinoin (a), Apifil (b), Mixtures (c)



XRD test results figure 2 shows that a single tretinoin (b) shows Tretinoin has multiple crystal peaks with 3 high intensity peaks. Purpose used to characterize the crystal structure of a solid material, the results of this test are expected to occur interaction or the presence of graphical changes in X-ray diffraction patterns (10). Based on the diffractogram, it appears that single and sharp peaks resemble grass phenomena, whereas when Tretinoin is encapsulated with Apifil® (A) displays blunt peaks with low intensity indicating that the mixture is amorphous. This indicates a reduction in crystallinity after mixing tretinoin with apifil. Therefore, the structural changes in the mixture will potentially increase the solubility which is characterized by a sloping chromatogram peak which originally had a sharp peak or grass phenomenon (8).

Figure 2. X-ray Diffractogram of (a) Tretinoin, (b) Apifil, Mixtures (c)



Based on the results of measurements twice in 30 days, the F2 and F5 formulas tend to be stable, the increase in particle size in all formulas is due to the solid lipids used having a low melting point, although the increase in particle size in the five formulas still shows good characterization results where the particle size range (<200 nm), determining the average size of NLC particles aims to ensure effective absorption by the body and better penetration through biological membranes. Small particles, usually below 200 nm, also reduce the risk of irritation and improve the efficiency of active substance delivery (11).

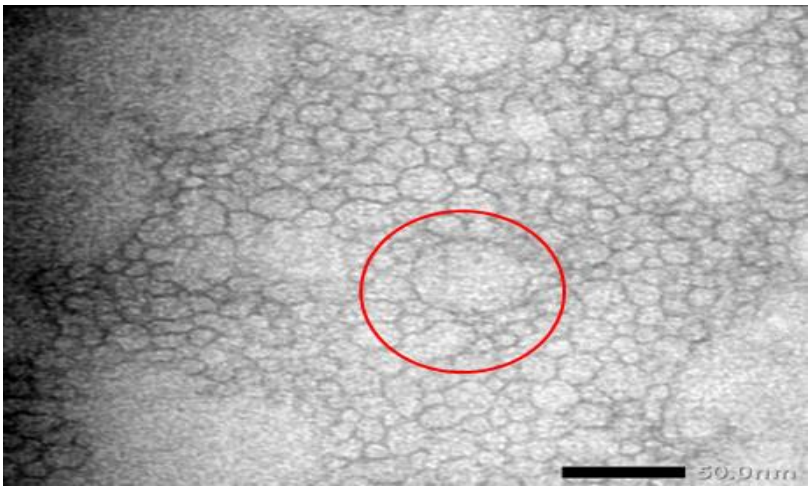
Measuring the distribution of particle size in the formulation provides information about the homogeneity of the particle. Low polydispersion index indicates a uniform distribution in size, which is important for the physical stability and consistent performance of the NLC. The polydispersion index of NLC tretinoin in all F1-F5 formulas has a result (<0.5), this indicates that the preparation has good globular distribution and homogeneity (8).

Assessing the surface charge of the NLC particle through the zeta potential provides an overview of the physical stability of the nanoparticle suspension. The characterization of zeta potential describes the repulsion between particles that has the potential to cause particle aggregation. The results of all NLC Tretinoin formulas have a zeta potential value of -7 mV to -19.3 mV, this is due to the presence of non-ionic surfactants that work by applying steric resistance to NLC particles to prevent aggregation (8).

Measuring the percentage of active ingredients successfully incorporated into the NLC shows the efficiency of the formulation. High entrapment efficiency ensures effective dosage and reduces the waste of the active ingredient, essential for therapeutic effectiveness. The determination of efficiency entrapment shows that the entire formula has a good efficiency entrapment result (>80%), this shows the ability to absorb solid lipids used well (8). (Table 1 and table 2).

Observing the physical shape and surface structure of NLC particles provides information about its physical properties and biological behavior. Particles with uniform morphology, such as round or spherical shapes, tend to be more stable and have more controlled active release profiles. The results of morphology figure 3 show that NLC Tretinoin has a spherical shape, particle distribution and homogeneous rate. This shows the success of the NLC formula using apifil with the probe sonication method in the preparation of Tretinoin NLCs and is in line with the results of the polydispersity index characterization (12).

Figure 3. Morphological Analysis of NLC Tretinoin TEM



5. Conclusion

Tretinoin has been successfully formulated in NLC and has good stability. All formulas have particle sizes showing 96-141 nm, the polydispersity index of all formulas shows 0,12 – 0,44, zeta potential has values <-20 mV and efficiency entrapment values show >80%. Morphological testing shows spherical and homogeneously distributed shapes. The research's novelty is that the formulation of tretinoin in NLC can produce good characterizations such as particle size, polydispersity index, zeta potential, entrapment efficiency and a spherical morphology that suggests that tretinoins in the NLC have indications of success in dealing with the problem of the side effects of tretinoin.

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