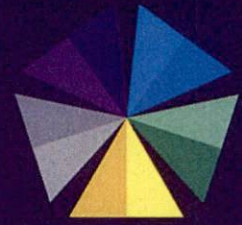


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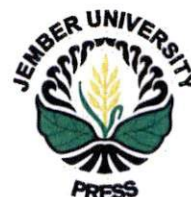
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*Membangun Generasi
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CHARACTERIZATION AND THE RELEASE TEST OF ANTI-AGING TRETINOIN IN NANOEMULSION USING OLIVE OIL

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INTRODUCTION

People are more and more desire a youthful and attractive appearance. Aging of the skin can be the result of a normal process that involves a lower rate of metabolic activities and is called chronologic aging. Photoaging is the consequence of chronic sun exposure and manifests clinically with fine and coarse wrinkling, roughness, dryness, laxity, shallowness, pigmentary mottling, telangiectasia, and in some cases with preneoplastic and neoplastic changes (Stefanaki et al., 2005). Tretinoin is a compound that is able to show an effect of prevention and treatment of wrinkles due to photo aging, acne, and inflammation of the skin. It is all-trans retinol is oxidized through retinal to its most active metabolite (Kligman *et. al*, 1986). The effectivity of an active ingredient is determined among others by the ability to reach the site of action. To reach the site of action the active ingredients used orally or topically must first dissolve, release then absorbed or penetrates through the membrane and then diffuses into the site of action. Tretinoin practically insoluble to increase it used nanoemulsion delivery as a vehicle. Solubility is one of the factors of drug absorption. Higher solubility of drug caused higher amount of drug to absorb. Smaller droplet of emulsion caused rapid drug release. In this study tretinoin loaded in nanoemulsion using olive oil than it characterized and test of tretinoin release compared with tretinoin in emulsion. Olive oil used as oil phase in nanoemulsion as known it widely used in cosmetic.

MATERIAL AND METHODS

Research Materials

Tretinoin (PT. Cortico Mulia), olive oil, Tween 80 (Sigma Aldrich), Span 80 (Sigma Aldrich), ethanol 96 % (E-Merck), NaH₂PO₄ (E-Merck) and Na₂HPO₄ (E-Merck), aquademineralisata (PT Brataco).

Research Instruments

Stirrer plate (Dragon Lab MS-Pro), ultrasonic (Branson 3510), shaker machine (Wine shake), pH meter (Eutech Instruments pH 700), particle analyzer (Delsa Nano C), Franz diffusion cell with cellophane membranes, spectrophotometer (Shimadzu UV-1800), Transmission Electron

Microscopy (TEM-type JOEL JEM-1400), light microscope.

Methods

Nanoemulsion preparation:

The formula of nanoemulsion type O/W (Erawati et al., 2014) modified consists of olive oil; Span 80-Tween 80-ethanol 96%; and a solution of phosphate buffer pH 6.0 ± 0.5 (with ratio = 1; 9; 27.5). Olive oil, Span 80, Tween 80, ethanol 96%, and tretinoin mixed in a 100 ml glass beaker, stirred with a magnetic stirrer 600 rpm each for 5 minutes. Then added with a solution of phosphate buffer pH 6.0 ± 0.5 (dripped slowly) while stirring with a magnetic stirrer 1000 rpm for 10 minutes to form a clear nanoemulsion system. The formula of tretinoin in nanoemulsion and emulsion presented in Table 1. The tretinoin nanoemulsion characterized includes; pH, droplet morphology by TEM-type JOEL JEM-1400, droplet size and polydispersity index by particle analyzer Delsa Nano C.

Table 1. Formula of Tretinoin in Nanoemulsion and Emulsion

Materials	Concentration (%)	
	nanoemulsion	emulsion
Tretinoin	0.1	0.1
Olive oil	2.66	1,66
Span 80	1.92	14,17
Tween 80	18.66	6,40
Ethanol 96%	3.42	-
Phosphate buffer solution pH 6.0 ± 0.5	ad 100	ad 100

Emulsion preparation:

Emulsion begins with making the aqueous phase in a beaker glass by mixing Tween 80 and buffer solution pH 6.0 ± 0.5 is stirred using a magnetic stirrer for 5 minutes at 1000 rpm. Furthermore, in another beaker glass made oil phase by mixing olive oil, Span 80 and tretinoin stirred at 1000 rpm

for 5 minutes. Then, stirring constantly added the water phase to the oil phase.

Release test.

Membrane Preparation;

A cellophane membrane cut to size, then immersed in aquademineralisata for ± 12 hours. A moment before use, the membrane is drained until no water is dripping, and then mounted on the surface of the receptor compartment of Franz diffusion cell.

Measurement of tretinoin release;

Receptor compartment of Franz diffusion cell filled with phosphate buffer medium of pH 6.0 ± 0.2 up to full. Then, 2 ml of tretinoin nanoemulsion inserted into the donor compartment. Experimental temperature is set and maintained at a temperature of 32 ± 2°C. Magnetic stirrer rotated at a speed of 100 rpm. Samples (1 ml) were taken within a certain time interval, i.e. at 0, 5, 10, 15, 30, 45 minutes, and then 1, 1.5, 2, 3, 4, 6, 8, 10, 12 hours. Immediately after sampling medium was replaced with phosphate buffer pH 6.0 ± 0.2 with a volume of samples taken. Subsequently, samples were taken observed with UV-Vis spectrophotometer tretinoin concentration in the sample is calculated using the standard curve regression equation, then correction to the measured concentration using the equation Wurster as follows:

$$C_n = C'n + \frac{a}{b} \sum_{s=1}^{N-1} C_s$$

which:

- C_n*: real concentration after correction (ppm)
- C'n*: concentration readable (calculated from the absorption spectrophotometer, ppm)
- C_s*: concentration of the sample before
- a*: volume of sample taken
- b*: volume of media

Determination of tretinoin cumulative amount released per unit membrane area (µg/cm²) was calculated from the concentration obtained each time (µg/ml) which had been corrected with the equation Wurster. Furthermore, multiplied by the number of medium and divided by the membrane surface area. The results obtained by the cumulative number of tretinoin released per unit time. The release profile of tretinoin, is done by making a curve relations between the cumulative number of tretinoin released (µg/cm²) versus time (minutes). The release rate (Flux) of tretinoin in nanoemulsion was obtained from the slope of the

regression equation in the steady state than compare with tretinoin in emulsion.

RESULT AND DISCUSSION

The characteristics of tretinoin nanoemulsion and emulsion include pH, droplet size, polidispersity index and droplet morphology presented in Table 2 and Figure 1 & 2. The pH value of both nanoemulsion and emulsion tretinoin are in the range of pH skin, it is expected that will not cause irritation when used.

Table 2. Characteristics of Tretinoin Nanoemulsion and Emulsion

Characteristic	Nanoemulsion	Emulsion
pH	6.29 ± 0.01	6.21 ± 0.015
Droplet size	85.53 ± 6.28 nm	11.70 ± 2.51 µm
Polidispersity Index	0.676 ± 0.05	-

Tretinoin nanoemulsin droplet size about 85.53 ± 6.28 nm, it's smaller than tretioin emulsion droplet size was 11.70 ± 2.51 µm (Table 1). Droplet morphology of tretinoin nanoemulsion by TEM type JOEL JEM 1400 (Figure 1) and tretinoin emulsion by light microscope (Figure 2) both are appear spherical.

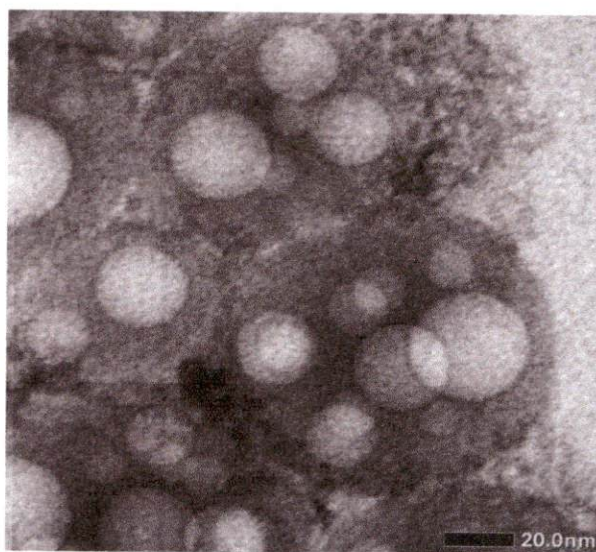


Figure 1. Droplet morphology of tretinoin in nanoemulsion by TEM type JEM 1400 bar length 20 nm.

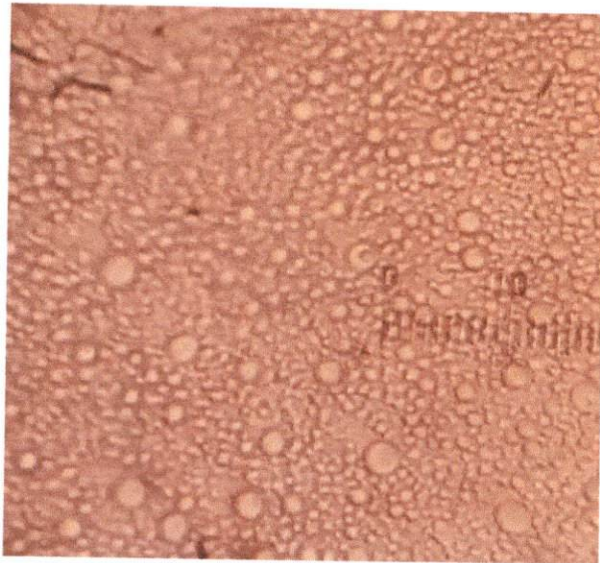


Figure 2. Droplet morphology of tretinoin in emulsion by light microscope scale 10 μm.

Release profile of tretinoin in nanoemulsion and in emulsion presented in Figure 3. The release-rate (Flux) of tretinoin in nanoemulsion and emulsion (Figure 4) was 0.049 ± 0.002 and 0.038 ± 0.003 μg/cm².menit respectively. The result of statistical analysis by independent T-test ($\alpha = 0.05$) known significant figure was $0.003 < 0.05$ so that release-rate (Flux) tretinoin in nanoemulsion higher than it's in emulsion. It can cause by droplet size of tretinoin-nanoemulsion smaller than droplet size of tretinoin-emulsion.

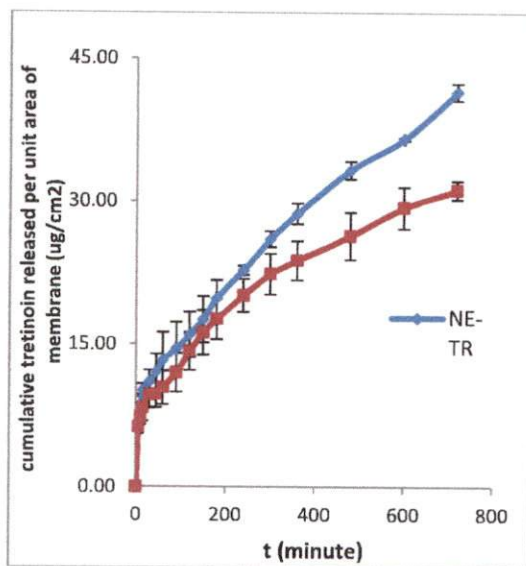


Figure 3. Release profile of tretinoin in nanoemulsion (NE-TR) and in emulsion (E-TR)

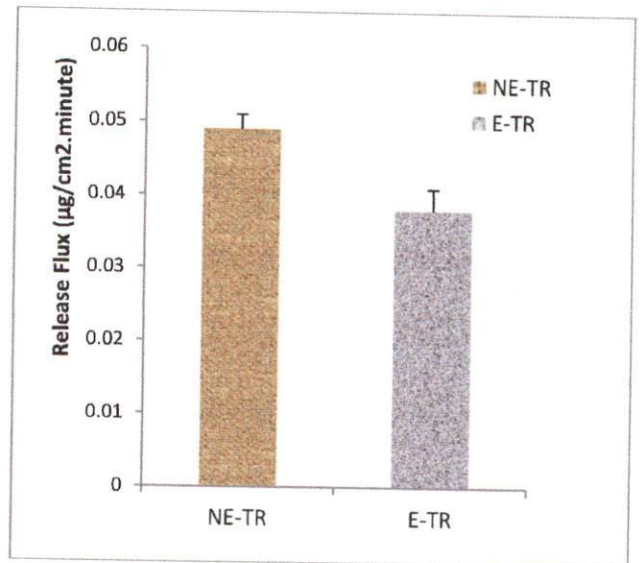


Figure 4. Release Flux of tretinoin in nanoemulsion (NE-TR) and in emulsion (E-TR)

CONCLUSION

Tretinoin in nanoemulsi that have been made have characteristics as desired, the droplet size below 500 nm, and spherical droplet known more easily penetrate into the skin (Hoeller, 2008), and the appropriate pH value of system are expected to not irritate the skin. The release-rate (flux) of tretinoin in nanoemulsion higher than it's in emulsion.

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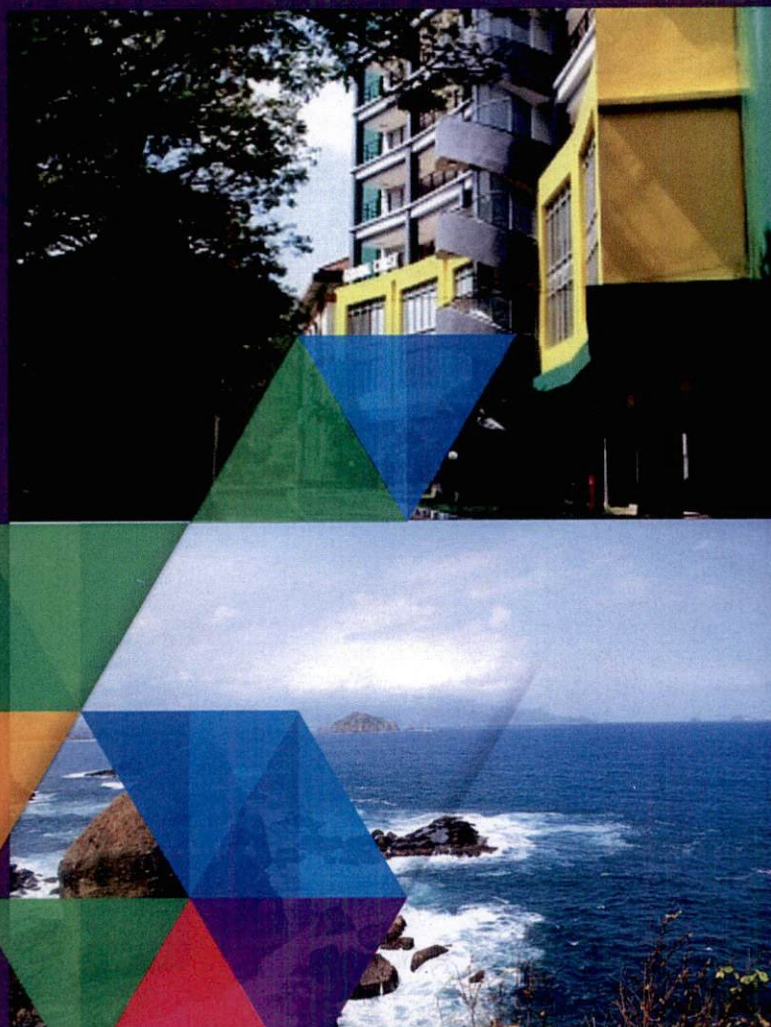
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