Preparation and Evaluation of Physical Characteristics of Vitamin E Nanoemulsion using virgin coconut Oil (VCO) and olive oil as oil phase with variation Concentration of tween 80 Surfactant

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ABSTRACT:
Vitamin E (alpha-tocopherol) has an anti-aging role and can protect the biologic membrane damage from free radicals. Vitamin E is highly lipophilic and has low stability, therefore to improve its effectiveness it is necessary to select appropriate carriers. Nanoemulsion is a drug delivery system in the form of aqueous oil dispersion stabilized by a combination of surfactants and co-surfactants that having droplet size 20-600 nm. Objective: This research aimed to prepare and to evaluate the physical characteristics of vitamin E nanoemulsion. Preparation of vitamin E nanoemulsion using phase oil of VCO (Virgin Coconut Oil) and Olive Oil. Method: The preparation of vitamin E nanoemulsion was performed by a combination of a low energy emulsification method with a magnetic stirrer and a method with high energy emulsification with a sonicator. The parameters of the nanoemulsion formula include organoleptic, pH, nanoemulsion type, percent transmittance, as well as the size and distribution of droplets. Results: The results showed that the composition of the optimum formula of nanoemulsion vitamin E using VCO as oil phase consists of 2% VCO, 24% tween 80 and 6% PEG 400. The optimum formula was transparent, homogeneous, pH 6.19, nanoemulsion type of oil in water, transmittance percentage 98.7%, droplet size 11.9 nm, polydispersity index 0.023. The composition of the optimum formula of nanoemulsion vitamin E using olive as oil phase consists of 2% olive oil, 18-24% tween 80 and 6% PEG 400. The optimum formula was transparent, homogeneous, pH 6.46 and 6.29, nanoemulsion type of oil in water, transmittance percentage 100%, droplet size 13.2 nm and 10.4 nm, polydispersity index 0.004 and 0.034.

KEYWORDS: Nanoemulsion, Vitamin E, VCO, Olive Oil, Tween 80.

INTRODUCTION:
Antioxidants are important for the body to neutralize free radicals and to avoid interference due to free radicals. One source of antioxidants is vitamin E. Vitamin E has been shown to be beneficial as an anti-aging agent. As an antioxidant, vitamin E has several functions including protecting the lipid membrane in the skin from damage caused by free radicals, strengthening natural antioxidants in the skin and dealing with oxidative stress. Topical use is very important to increase the level of vitamin E in the skin because oral use is not enough to increase the vitamin E concentration.

Topical vitamin E increases vitamin E levels 62-fold in epidermis and 22-fold in the dermis. One of the topical preparations that utilize nanotechnology system is nanoemulsion. Vitamin E nanoemulsion shows anti-aging activity. In addition, the anti-aging activity of nanoemulsion vitamin E is far superior to vitamin E cream. Nanoemulsion is a drug delivery system that formed in water oil dispersion and stabilized by an interfacial film of surfactant and cosurfactant which has 20-600 nm droplet size. Surfactants and cosurfactants are important components in nanoemulsions formulation. In nanoemulsions, surfactants reduce tension interface by absorbing into the droplet surface as a momomolecular film and preventing the joining of oil droplets. Co-surfactant is short chain alcohol which can help reduce the interface tension between the oil phase and the water phase and decreasing the free energy of the
surface. The oil phase can impact on nanoemulsion formation and stabilization. Wooster et al. (2008) showed that a proper combination of surfactant and oil phase properties lead to the formation of stable nanoemulsions with excellent optical clarity. VCO and olive oil are often used in the development of nanoemulsion. Suciati et al. (2014) developed transdermal nanoemulsion formulation for protein vaccine and artin m-adjuvant using VCO as the oil phase. The combination of olive oil and sucrose laurate was a good candidate to prepare nanoemulsion because they have good droplet size and polydispersity index. The previous study used VCO as an oil phase with Tween 80 and PEG 400 as surfactant and cosurfactant showed that nanoemulsions produced have droplet sizes <100 nm [7]. Olive oil has the highest fatty acid content, namely palmitic acid, oleic acid, and linoleic acid. Palmitic acid is used as a carrier and is known not to cause irritation, whereas oleic acid can increase the bioavailability of lipophilic drugs. This study was conducted to prepare and to determine the physical characteristics of vitamin E nanoemulsion by using two different oil phases, VCO and olive oil. Evaluation of physical characteristics was conducted including organoleptic, pH, nanoemulsion type, percent transmittance, as well as the size and distribution of droplets, and physical stability test include centrifugation and heating-cooling cycle tests.

### Materials and Methods:

#### Chemicals and reagents:
Vitamin E was obtained from Laboratory of Pharmacy, Faculty of Pharmacy, Halu Oleo University, VCO (Virgin Coconut Oil) (Brataco®), Olive Oil (Brataco®), tween 80 (Brataco®), PEG 400 (Brataco®), methylparaben (Brataco®), propylparaben (Brataco®), oleum rose Brataco®), and distilled water (Brataco®).

#### Instrumentations:
Cup glass (Pyrex®), Erlenmeyer flask (Pyrex®), glass stirrer, measuring flask (Pyrex®), drop pipette, test tube, volume pipette (Pyrex®), micro pipette (Pyrex®), centrifugator (Boeco S-8®), pH meter (HANNA®), Stirer (Stuart CB 162), Spectrophotometer (Perkin Elmer®), Particle Size Analyzer (Horiba Scientific®), Stopwatch, glass bottle, vial bottle, hotplate stirrer (Heidolph MR2002).

#### Nanoemulsion Preparation:
The following table is a modified formula of vitamin E nanoemulsion.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Materials</th>
<th>Formula I (% v/v)</th>
<th>Formula II (% v/v)</th>
<th>Formula III (% v/v)</th>
<th>Formula IV (% v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vitamin E</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Oil phase</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Tween 80</td>
<td>6</td>
<td>12</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>PEG 400</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Distilled water</td>
<td>85</td>
<td>79</td>
<td>73</td>
<td>67</td>
</tr>
</tbody>
</table>

Nanoemulsion is started by preparing all the ingredients short-chain the modified monomolecular in Table 1. Tween 80 and PEG 400 were put in a test tube and mixed by using vortex for 1 minute at 1000 rpm. Then, the test tube is inserted into a glass beaker inside the sonicator for 20 minutes. The oil phase is added and homogenized by using vortex for 5 minutes at 1800 rpm, stored in a water bath for 15 minutes and sonicated for 30 minutes. Vitamin E is added and homogenized with vortex mixer 5 minutes at 2500 rpm, and sonicated for 40 minutes. The mixture is poured into Beaker glass and mixed using magnetic stirrer on top of the hot plate stirrer for 10 minutes while adding distilled water gradually.

### Vitamin E Nanoemulsion Characterization:

#### Organoleptic Test:
The organoleptic test is carried out by observing color, odor, clarity, homogeneity, and phase separation.

#### pH Measurement:
The pH measurement is by pH Meter and carried out at room temperature.

#### Nanoemulsion Type Test:
The dilution method is used to obtain the nanoemulsion type. This test is carried out by dissolving the sample into the distilled water (1: 100) and the oil phase (1: 100). If the sample dissolves completely in distilled water, the type of nanoemulsion is classified as oil type in water (o/w), whereas if the sample dissolves completely in the oil phase, the type of nanoemulsion belongs to the type of water in oil (w/o).

#### Percentage of Transmittance:
Three milliliters of the sample was put into the cuvette and measured its percent transmittance at 650 nm by using Visible Spectrophotometer and distilled water is used as the blank.

#### Droplet Size Test:
Droplet size is measured by using a particle size analyzer with the type of dynamic light scattering.
RESULTS:
Nanoemulsion Preparation:
The results of the four formulas of nanoemulsion vitamin E can be seen in Figure 1.

Characterization of Vitamin E Nanoemulsion:
Nanoemulsion is said to be good and stable if it has a clear appearance, no phase separation, o/w nanoemulsion type, range pH value is 4.5-6.5, and the percent transmittance is close to 100%. Therefore, physical characterization was carried out after 24 hours at room temperature which included organoleptic examination, pH, nanoemulsion type, and percent transmittance. Physical characterization of vitamin E nanoemulsion using VCO and Olive oil can be seen in Table 2 and Table 3.

Table 2. Physical characterization of vitamin E nanoemulsion using VCO as oil phase

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Characterization</th>
<th>Formula Ia</th>
<th>Formula IIa</th>
<th>Formula IIIa</th>
<th>Formula IVa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colour</td>
<td>Opaque</td>
<td>Opaque</td>
<td>Transparent</td>
<td>Transparent</td>
</tr>
<tr>
<td>2.</td>
<td>Odor</td>
<td>Distinctive</td>
<td>Distinctive</td>
<td>Distinctive</td>
<td>Distinctive</td>
</tr>
<tr>
<td>3.</td>
<td>Clarity</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>4.</td>
<td>Homogeneity</td>
<td>Non homogene</td>
<td>Non homogene</td>
<td>Homogeneous</td>
<td>Homogeneous</td>
</tr>
<tr>
<td>5.</td>
<td>Phase</td>
<td>Two-phase</td>
<td>Two-phase</td>
<td>One-phase</td>
<td>One-phase</td>
</tr>
<tr>
<td>7.</td>
<td>Nanoemulsion type</td>
<td>O/W</td>
<td>O/W</td>
<td>O/W</td>
<td>O/W</td>
</tr>
<tr>
<td>8.</td>
<td>Percent of transmittance</td>
<td>0.56%</td>
<td>0.76%</td>
<td>95.73%</td>
<td>98.7%</td>
</tr>
</tbody>
</table>

Table 3. Physical characterization of vitamin E nanoemulsion using Olive Oil as oil phase

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Characterization</th>
<th>Formula Ia</th>
<th>Formula IIa</th>
<th>Formula IIIa</th>
<th>Formula IVa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colour</td>
<td>Opaque</td>
<td>Yellowish</td>
<td>Transparent</td>
<td>Transparent</td>
</tr>
<tr>
<td>2.</td>
<td>Odor</td>
<td>Distinctive</td>
<td>Distinctive</td>
<td>Distinctive</td>
<td>Distinctive</td>
</tr>
<tr>
<td>3.</td>
<td>Clarity</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>4.</td>
<td>Homogeneity</td>
<td>Non homogene</td>
<td>Non homogene</td>
<td>Homogeneous</td>
<td>Homogeneous</td>
</tr>
<tr>
<td>5.</td>
<td>Phase</td>
<td>Two-phase</td>
<td>Two-phase</td>
<td>One-phase</td>
<td>One-phase</td>
</tr>
<tr>
<td>6.</td>
<td>pH</td>
<td>6.57</td>
<td>6.48</td>
<td>6.46</td>
<td>6.29</td>
</tr>
<tr>
<td>7.</td>
<td>Nanoemulsion type</td>
<td>O/W</td>
<td>O/W</td>
<td>O/W</td>
<td>O/W</td>
</tr>
<tr>
<td>8.</td>
<td>Percent of transmittance</td>
<td>0.2%</td>
<td>49.7%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

The Size Distribution of Nanoemulsion:
Physical characterization exhibited that formula IIIb, IVa and IVb are good nanoemulsions. Testing the size and size distribution of droplets is only conducted in formulas IVa, IIIb and IVb which qualify as nanoemulsion based on previous tests while for formula IIIa droplets are not tested for size and distribution because it does not qualify as nanoemulsion based on transmittance testing. The mean of droplet and distribution size of nanoemulsion formed from nanoemulsion vitamin E was determined using the SZ-100 Nano Model Horiba particle size analyzer. The average results of the size and distribution droplet nanoemulsion can be seen in Figure 2.
DISCUSSION:
Nanoemulsion Preparation:
The disadvantage of nanoemulsion preparation spontaneously is more amounts of surfactants needed to produce droplet sizes <200nm. Therefore, this study, the combination methods are used for preparing vitamin E nanoemulsion by using low energy (magnetic stirrer) and a high energy method (sonicator). Optimization of preparation nanoemulsion vitamin E methods was obtained through the orientation in the composition of surfactants and cosurfactants, time and speed of stirring with vortex, magnetic stirrer, and sonicator. The concentration of vitamin E used in nanoemulsions is 1%. Referring to Mukul et al. (2014) the recommended concentration for topical use of vitamin E is 1% - 5%. In addition, according to Iskandar et al (2016), Vitamin E with 1% has shown anti-aging activity. Vitamin E has strong antioxidant with 21.7μg/mL for IC50. The proper use of the oil phase can increase the solubility of lipophilic active ingredients. Drug solubility in oil will affect nanoemulsion ability to keep the drug in its dissolved form. The oils phase used in this study were Virgin Coconut Oil (VCO) and olive oil. Olive oil has a long triglyceride chain that is often used in making nanoemulsions. Olive oil is also safe to use, not easily oxidized and has a good dissolving capacity. The long-chain fatty acids contained in olive oil consist mainly of oleic acid (C18) ranging from 55 to 83%. These fatty acids can increase the bioavailability of the lipophilic drug. While VCO (Virgin Coconut Oil) is not easily oxidized and has a good dissolution capacity. In addition, VCO has the ability to prevent Ostwald ripening and can produce droplet size <100nm. Surfactantsand cosurfactants used were tween 80 and PEG 400 which had low toxicity and did not irritate the skin.

Characterization of Vitamin E Nanoemulsion:
The organoleptic test shows that formula Ia, Ib and formula IIIa, IIIb are almost the same in color, odor, clarity, homogeneity, and phase (appearing to separate or creaming phase). According to Ansel (1985), creaming on nanoemulsion can occur due to several things, one of which is the droplet size of the dispersed phase as and the rate of separation increases with increasing size of the dispersed droplets. Formulas IIIa, IIIb and IVa, and IVb have the same physical appearance in color, odor, clarity, homogeneity, and phase (no phase separation occurs). Organoleptically formulas III and IV are accepted to be qualified as nanoemulsion. Acidity test was carried out to see whether the nanoemulsion pH was in accordance with the pH of the skin (ranges from 4-6.5) so that it can minimize the risk of irritation. Both formulas I of VCO and Olive Oil as the oil phase exceed 6.57 for pH values. While other formulas are in the skin pH range and are considered safe when applied to the skin. Nanoemulsion shows that all the formulas vitamin E of nanoemulsion are oil in water (o/w) because the surfactant used is tween 80 which has HLB 15 values. According to Ansel (1985) to form the oil-in-water emulsion system, the emulsifier used has 8-18 HLB values. Percent transmittance was measured using a Visible spectrophotometer with distilled water as the blank at 650 nm. Percent of transmittance shows the clarity level of nanoemulsion formed. The higher the percent transmittance or close to 100%, the smaller the size of the droplet formed and considered as transparent nature. The average percent of transmittance for formula Ia is 0.56%, formula IIa is 0.76%, Ib formula is 0.2% and formula IIb is 49.7% which means the size of the emulsion droplet formed is still large so the nanoemulsion produced is not transparent or clear. Transmitting percentage of formula IIIa is 95.73% and formula IVa is 98.7%, which is close to 100%. Formula IIIa is and formula IVa is 100% which means the nanoemulsion system formed is transparent or clear.

The Size Distribution of Nanoemulsion:
The diameter of nanoemulsion droplets formed in FIIIb was 13.2±12.2 nm, FIVa was 11.9 nm±0.7 nm and the nanoemulsion diameter formed in FIVb was 10.4±9.9 nm. These results proved that the three formulas made had reached the good nanoemulsion formed (<200nm) [4]. Droplet size with <90 nm can increase the stability...
of nanoemulsion against sedimentation. Each droplet collection is called polydispersion. The polydispersity index of the nanoemulsion system describes the droplet size distribution and the physical stability of a dispersion system. The low polydispersity index value indicates that the formed dispersion system is more stable for the long term. While high polydispersity indicates that the droplet size distribution is not uniform. This is because the droplets aggregate form groups (grouped together) so that they are unevenly dispersed (polydisperse), and cause a decrease of the nanoemulsion stability. The good index value is between 0 (uniform size distribution) to 0.5 (wide size distribution). The polydispersity index value of Formula III, IV, and V are 0.004; 0.023; and 0.034 respectively. This shows that the three formulas produce a uniform level of droplet size distribution.

CONCLUSION:
We successfully developed nanoemulsion of vitamin E by using VCO and olive oil as oil phase with concentration of Tween 80 were 18-24% with physical characteristic of nanoemulsion was transparent, clear, homogenous, one phase, O/W types, and 98.7% and 100% for percent of transmittance, the size distribution were from 10.4-13.2 nm, the polydispersity index value of those three formulas is 0.004; 0.023; and 0.034 respectively that exhibits dispersion system is uniform. The physical characterization of vitamin E nanoemulsion was qualified for physical Characterization of Vitamin E Nanoemulsion.

REFERENCES: