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Original Article

# DEVELOPMENT AND CHARACTERIZATION OF COMPRITOL ATO® BASE IN NANOSTRUCTURED LIPID CARRIERS FORMULATION WITH THE PROBE SONICATION METHOD

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**ABSTRACT**

**Objective:** Vitamin E acetate has antioxidant activity that can prevent premature aging of the skin, but it is highly lipophilic (log p±12.2) and potentially degraded. To overcome the permeability and stability problems, the vitamin E acetate needs to be developed in Nanostructured Lipid Carrier (NLC), a nano-delivery system based on solid lipid and liquid lipid that is stabilized by surfactants as a colloidal system.

**Methods:** The formulation of vitamin E acetate into NLC was carried out using hot homogenization method and then sonicated using a probe sonicator. The materials used were vitamin E acetate 2%, Compritol® 2-6%, Myritol® 1%, and Plantacare® 1-3%. The results of the NLC were then characterized by measuring the particle size, zeta potential, polydispersity index, entrapment efficiency, and its morphology.

**Results:** The results of characterization showed that NLC of vitamin E acetate has 280-375 nm particle size, the zeta potential was-23 mV to-28 mV, the polydispersity index was<0.5, the entrapment efficiency was 92-97%, and the morphological results was in the form of a spherical shape.

**Conclusion:** The results show that the Nanosturctured Lipid Carriers of vitamin E acetate shows good results.

**Keywords:** Vitamin E acetate, Compritol ATO®, NLC, Probe sonication


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## INTRODUCTION

Skin aging is a skin problem characterized by wrinkles, fine lines, rough texture, and uneven pigmentation [1]. The premature aging process can be prevented by protecting the skin from UV exposure, maintaining skin moisture and its cleanliness, and improving diet as well. One of the efforts that can be taken to prevent premature aging is by using antioxidants, one of which is vitamin E acetate. The antioxidant value of this vitamin is ($IC_{50}$ = 20.5±0.2 g/ml) [2]. Vitamin E acetate is able to bind free radicals, improve skin elasticity, and prevent premature aging. However, according to MSDS (Material Safety Data Sheet), vitamin E acetate is not a water-soluble vitamin; hence it leads to to inefficient performance in the manufacture of the drug and cosmetic preparations; also, vitamin E has low solubility which causes small penetration of drugs in the body [3]. Nanoparticle technology is the latest drug delivery system that changes a particle into a nanometer scale with a size of 10-1000 nm. Changing the compound into nano size makes the particle size smaller, thus having a higher comparison value between the surface area and the volume when compared to similar particles in the larger size. A compound with a nanoparticle size is more reactive because more surface atoms can make contact with other materials directly. Its solubility can be increased and at the same time offering the ability to penetrate the lipid membrane of the cell [4]. A solid lipid nanoparticle (SLN) and nanostructured lipid carriers (NLC) have lipophilic bioactive trapped. A SLN is formed by a solid lipid-based core, whereas a NLC is formed from a mixture of solid lipid and liquid lipid. SLN and NLC has been proposed to address the need for active delivery to overcome skin aging problems. SLN and NLC replace liquid lipids (oils) from oil/water emulsions (O/W) using solid lipids or without an oil mixture so that the lipid particles become solid at room temperature and body temperature [5]. NLC is a conducting system that can retain water due to its occlusive nature. When compared with SLN, NLC has better properties thus more preferred. NLC is a modified version of SLN. Vitamin E acetate has log P value of 12.2 and is highly lipophilic, low solubility, and potential to be degraded. The lipid mixture of NLC has a slower polymorphic transition and a low crystallinity index so that it can improve the efficiency of encapsulation, drug loading, and the physical stability of the vitamin. In addition, it can improve the chemical stability and bioavailability of the vitamin as well as protect the incorporated bioactive compound from degradation [6].

## MATERIALS AND METHODS

### Materials

The ingredients used were vitamin E acetate (dl-alpha tocopheryl acetate) 99.4% as the active ingredient (DSM Nutritional Products Asia Pacific Pte. Ltd, Singapore), Glyceryl Behenate or Compritol ATO®(Gattefose, Germany), Captrilic Triglyceride or Myritol®(BASF Indonesia), and Lauryl Glucoside or Plantacare®(Evonik Industries, Singapore), Aquadest, Methanol PA (PT Merapi Utama Pharma).

### Methods

#### Preparation of vitamin E acetate

The active ingredient used was vitamin E acetate purchased from one of the chemical industries, namely DSM Nutritional Products Asia Pacific Pte. Ltd, Singapore. The inspection on the active ingredients was carried out by checking the data completeness of the Certificate of Analysis (CoA) obtained at the time of purchasing the material.

#### FT-IR (Fourier transform infra-red) testing

The samples to be tested (solid lipid; the mixture of solid lipid and liquid lipid; and the mixture of active ingredients and solid lipid) were grounded into a fine powder and put into the FTIR tool. Then the sample was read using Agilent Cary 630 FTIR Spectrometer instrument [7].

#### Differential scanning calorimetry (DSC) testing

A total of 5-10 mg of sample (solid lipid of Compritol® and vitamin E acetate) was placed in an aluminum plate on a DSC instrument with a Thermal Analysis License (USA). The sample was then pressed and put into DSC device along with the reference. The initial temperature, final temperature, rate were set for the sample. The sample was then heated at the temperature of 30-300 °C with a heating rate of 10 $^0$C/min. The resulting data is a thermogram with thermal parameters, namely: initial temperature, offset, maximum peak [7].

**Preparation of NLC of vitamin E acetate**

Vitamin E acetate NLC was formulated using hot homogenization method followed by an ultrasonication probe. The solid lipid (Compritol®) was added to the active substance (vitamin E acetate) and the result was melted and mixed using a magnetic stirrer for 15 min. Afterwards the lipid phase and the aqueous phase were heated simultaneously. The next step taken was reducing the particle size using an ultrasonication probe for 15 min and at an amplitude of 60% and temperature at 70 °C. The final result of NLC with a cloudy to milky white color and without any phase separation was chosen to be processed further [7].

**Characterization of vitamin E acetate**

The parameters used in the NLC characterization of vitamin E acetate consisted of particle size, polydispersity index, and zeta potential. Measurements were performed using a Malvern ZSP Zetasizer (UK) to measure the particle size at room temperature (25 °C). The next step was taken 10 drops from the sample of the NLC adapter to be added to water up to 10 ml and put into a disposable cuvette to ensure the formation of NLC vitamin E acetate.

The morphological form of NLC vitamin E acetate was formed using a transmission electron microscope (JEM-1400 Flash Electron Microscope JEOL Ltd.). A total of 1 g NLC vitamin E acetate oil phase was dispersed into 5 ml of deionized water before being analyzed. The mixture was then stirred and dripped into the test object. Grid at 400 mesh dropped into 10 ml of uranyl acetate on the grid; then the remaining droplets were then cleaned again using filter paper and left for 30 min to make it dry before inserting it into the TEM tool for image capture [8].

**Entrapment efficiency (EE)**

The calculation of entrapment efficiency was carried out by putting 1 ml of NLC vitamin E acetate into a Vivaspin filter tube (Vivaspin®, Goettingen, Germany) with a membrane filter having 5 kDa cutting molecular weight. The next step was to centrifuge it at a speed of 14,500 rpm for 3 h. Afterwards the supernatant was taken and diluted, then measured by UV spectrophotometric method (Shimadzu UV-1800) at a maximum wavelength to obtain an unabsorbed (unbound) level of vitamin E acetate [9].

The entrapment efficiency (EE) was calculated using the equation:

$$\% \text{ EE} = \frac{\text{Total drugs taken} - \text{free drugs}}{\text{Total drugs taken}} \times 100\ \%$$

**RESULTS**

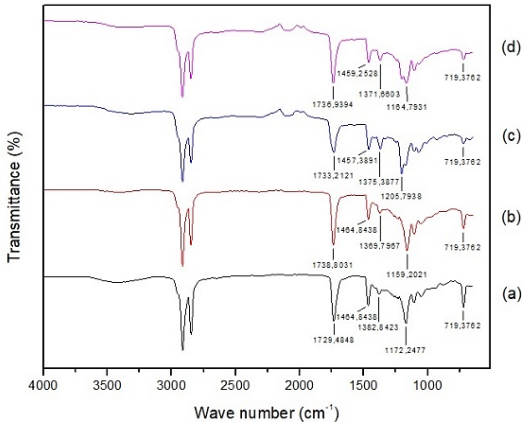


**Fig. 1: FTIR absorption spectra of (a) Compritol®; (b) mixture of Compritol® and Myritol®; (c) mixture of Compritol® and vitamin E acetate; (d) mixture of Compritol®, Myritol®, and vitamin E acetate**

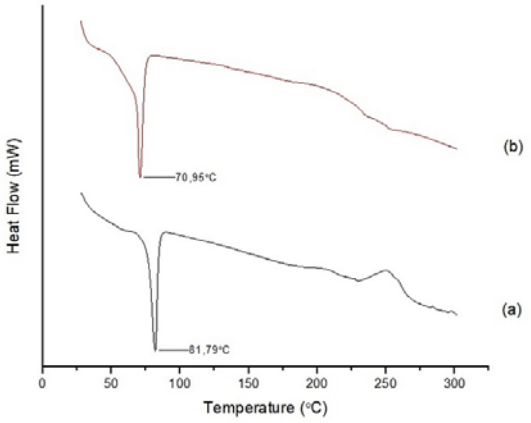


**Fig. 2: DSC thermograms of (a) Compritol®; (b) a mixture of Compritol® and vitamin E acetate**

**Table 1: Formulation and characterization of vitamin E acetate NLC**

| Formulation | | | | | Particle size | PdI | ZP | Entrapment |
|---|---|---|---|---|---|---|---|---|
| Code | Vit E (%) | MYR (%) | CMP (%) | PLA (%) | (nm) | | (mV) | Efficiency (%) |
| F1 | 2 | 1 | 6 | 1 | 318.60±5.93 | 0.41±0.02 | -26.53±0.31 | 93.79±1.07 |
| F2 | 2 | 1 | 2 | 3 | 765.23±16.62 | 1.0±0.04 | -23.77±0.40 | 97.60±0.75 |
| F3 | 2 | 1 | 4 | 2 | 280.20±9.34 | 0.43±0.02 | -28.80±0.61 | 95.99±0.02 |
| F4 | 2 | 1 | 5 | 1.5 | 375.63±4.18 | 0.13±0.04 | -24.17±0.25 | 92.34±0.78 |
| F5 | 2 | 1 | 3 | 2.5 | 287.13±7.46 | 0.44±0.02 | -28.13±2.19 | 97.06±1.16 |

Vit E: Vitamin E acetate, CMP: Compritol®, MYR: Myritol®, PLA: Plantacare®, PdI: Polydispersity Index, ZP: Zeta Potential

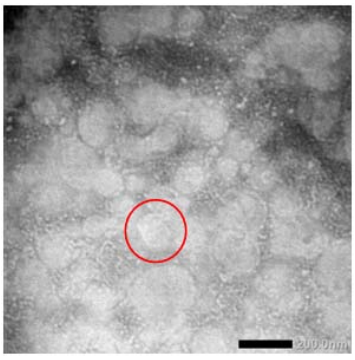

**Fig. 3: TEM photograph imaging result of vitamin E acetate NLC**

**DISCUSSION**

FT-IR analysis was a qualitative identification that involves an infrared spectrum. It provided information about the compatibility and interactions of substances used in the formula of NLC [10]. The test included the analysis of Compritol® solid lipid mixed with Myritol® liquid lipid; Compritol® solid lipid mixed with vitamin E acetate; and vitamin E acetate mixed with the Compritol® and Myritol® lipids pair mixture. The FT-IR results was seen in the wave range of 500-1500$^{-1}$, indicating that there was no significant change between the characteristic peaks of the active ingredient of vitamin E acetate NLC when in the solid lipid and when in the lipids pair [7]. These results indicated that the tested substances were compatible, and the absence of new peaks in the spectrum provides that there was no interaction between vitamin E acetate and Compritol®. It also indicated that the mixture did not form new molecular groups (fig. 1).

The DSC was performed to find out the endothermic point of the single ingredient and the mixture used in the formulation of NLC vitamin E acetate. The test was performed on a single Compritol® solid lipid and on the mixture of vitamin E acetate and Compritol® solid lipid. Using a sample of 10 mg of Compritol®; mixture of Compritol® and Myritol®; mixture of Compritol® and vitamin E acetate; and mixture of Compritol®, Myritol®, and vitamin E acetate, it was found that the endothermic peak of a single substance was the presence at a temperature of 81.79 °C and the endothermic peak of a mixed substance at a temperature of 70.95 °C as presented in fig. 2. From the DSC thermogram, it can be seen that the endothermic curve with a lower melting point shifted in the mixture of Compritol® solid lipid and NLC of vitamin E acetate at temperature of 70.95 °C (compared to the single ingredient Compritol® curve with the temperature of 81.79 °C). This result indicates that the addition of active ingredients is influential in shifting the melting point to be lower compared to the melting point of the single lipid (Compritol®) [11].

The formulation of vitamin E acetate into NLC mixed with Compritol® and Myritol® to using Plantacare surfactant is successfully produced. The characterization parameters were particle size, polydispersity index, zeta potential, and efficiency entrapment. Based on the measurement results, the Compritol® with surfactant formulas of F1, F3, F4 and F5 had good characterization results where the particle size range was (280-375 nm) while the F2 surfactant had a particle size value that did not meet the requirements (>500 nm) [12]. It happened due to the effect of solid lipid concentration and the surfactant concentration used before and also affected by the ability of the surfactant to reduce surface tension. The greater the concentration of solid lipid used leads the greater the particle size produced. The agglomeration formed because the surface area of the solid lipid was not covered by the surfactant [13]. The polydispersity index showed the distribution and homogeneity results of the particle sizes. The results from F1, F3, F4 and F5 formulas showed good characterization results below 0.5, indicating the NLC of vitamin E acetate had a good size distribution. As for the F2 formula, it had polydispersity index values that did not meet the requirements because of the effect of the concentration of the solid lipid and surfactants used. A zeta potential characterization describes repulsion between particles that can potentially cause particle aggregation. The zeta potential of the formula with surfactant Plantacare showed good characterization when it was in the range of (-23)-(-28 mV) indicating that the NLC of vitamin E acetate formula had good stability result [14, 15]. The data showed that all formulas had good efficiency entrapment results (92-97)% (table 1).

The Transmission Electron Microscopy (TEM) imaging result showed that the NLC of vitamin E acetate mixed with Compritol® solid lipid had formed a spherical shape with the size below 500 nm. This result showed a good particle distribution and was correlated with the particle size testing (fig. 3).

## CONCLUSION

The Nanostructured Lipid Carrier of vitamin E acetate formulas can be developed using the Compritol ATO® solid lipid and Plantacare surfactant. The NLC of vitamin E acetate in surfactant formulas of F1, F3, F4, and F5 have good characterization results where the Plantacare particle size parameters is (287-375) nm, polydispersity index is (<0.5), zeta potential is (-23 mV to-28 mV), and entrapment efficiency is (92-97) %. The results from TEM imaging in order to evaluate the morphological shape of the NLC shows a spherical shape.

## FUNDING

Nil

## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICT OF INTERESTS

Declared none

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