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Effect of Lecithin's Concentration of Entrapment Vitamin E Acetate Liposomes Using Thin Layers Hydration Method

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Tocopheryl acetate is not oxidized and can penetrate through the skin to the living cells, where about 5% is converted to free tocopherol and provides beneficial antioxidant effects. Lecithin is one of the main components of liposomes vesicles forming. The higher the phospholipid used, the more vesicles are formed, so that drug use is also expected to be optimal. This study aims to determine the effect of increased lecithin concentration on the efficiency of vitamin E application in liposomes made by thin layer hydration method. In this study 3 formulas were prepared using cholesterol and lecithin with a ratio of 20 mg each:100 mg (F I), 20 mg:400 mg (F II) and 20 mg:700 mg (F III). Evaluation of liposomes, among others, the efficiency of adsorption, particle size and morphology. The results showed the formulation of vitamin E in liposome using thin layer hydration method obtained liposomal adsorption efficiency from F I to F III, respectively that is 41,3799%; 47.6162% and 52.7216%. It can be concluded that an increase in lecithin concentration may increase the efficiency of the adsorption in which F III is the best formula for improving the efficiency of adsorption.

Keywords: Liposomes, Vitamin E, Lecithin, Thin-Layer Hydration.

1. INTRODUCTION

Drug delivery system is an important part of the drug development and treatment. Some examples of targeted particulate drug delivery systems, among others, microparticles, nanoparticles, microcapsules, microspheres, micelles, lipoproteins, and liposomes.[1] However, from some of these delivery systems, liposomes can only provide preparation characteristic similar to biological membranes of the body, to localize the drug at the target, and can improve delivery efficiency into the cytoplasm.[2] Almost all the drug characters can be encapsulated in liposomes to make modifications on the preparation.

Liposomes are synthetic analogs of natural membranes, an aqueous vesicle surrounded by a membrane bilayer unilamelar or multilamelar. Liposomes are drug delivery system is unique because it can act as a water-soluble drug carriers or fat soluble.[3,4]

Drug delivery systems such as liposomes, may affect the drug in the body since during the delivery to the workplaces, drugs are often confronted with obstacles and interference thereby reducing the efficacy.[7] Vitamin E is a fat-soluble vitamins (lipophilic), but has difficult to penetrate membrane. Vitamin E is also easily oxidized, so the shelf life becomes a relatively short time. Vitamin E formulated into the liposome system aims to increase the penetration into cells.

In the previous study has been testing the physical characteristics of the combination of salicylic acid liposomes soybean lecithin 200 mg, 400 mg and 600 mg and 20 mg of cholesterol made by the thin layer hydration method. Based on the results obtained liposome vesicle shaped spherical and homogeneous, measuring about 70 nm to 800 nm in combination formula of soy lecithin and cholesterol (600 mg:20 mg).

This method of thin layer hydration is the method most widely used in the manufacture of liposome systems, it is certainty associated with the evaporation of the organic solvent and making a more modest compared to the other methods. Thin-layer hydration method generally produces liposomes with the type of MLV.

The main ingredient of liposom's formulation is phospholipid. Phospholipids are the main elements forming vesicles. Higher concentration of phospholipid used will produce more vesicles are formed, so that entrapment of drug are also expected to be more optimal.[7] More vesicles are formed so that more vitamin E entrapped. But if the concentration of lecithin is too high, it can decrease the efficiency of entrapment.

This research will be used varying concentrations of lecithin from previous studies, using the best cholesterol concentrations than earlier studies to look at the effect of the concentration of lecithin to the entrapment of vitamin E in liposomes made

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Preparation of liposomes using thin layer hydration method and evaluation.

Ingredients	Formula I	Formula II	Formula III	Functions
Vitamin E asetate (mg)	50	50	50	Active substance
Lecitin (mg)	100	400	700	Lipid phase
Cholesterol (mg)	20	20	20	Lipid phase
Diethyl ether (ml)	15	15	15	Organic solvent
Phosphate buffer pH 7,4 (ml)	10	10	10	Water phase

Fig. 1. Formula of liposomes vitamin E asetate.

with a thin layer hydration method. Lecithin is used from soybean. Soybean lecithin is a phospholipid from natural materials which have two acyl chain unsaturated.[8] Phospholipids of natural origin with unsaturated acyl chains are more permeable, making it easier to adsorb the active substance.[9] Selection of lecithin/phosphatidylcholine in this study because it is the most widely applied phospholipids in liposomes, have neutral charge, an inert material, and the price is relatively low compared to other types of phospholipids.[10]

2. EXPERIMENTAL DETAILS

The instruments needed were Rotary Evaporator (Eyela n-1000), Spectrophotometer UV-Vis (Shimadzu), Analytical Balance (Mettler Toledo), Particle Size Analyzer (Delsa Max), Sonicator (LAB. Companion), vortex mixer, Microcentrifugation refrigerator (BioLion XC-HR20), SEM (Zeiss EVO MA10), and glass tools.

The materials used in this research were Vitamin E Acetat (BASF), lecithin (Natural Sourching), cholesterol (Kanto chemical), nitrogen, diethyl ether (MERCK), sodium hydroxide (Asahimas chemical), potassium hydrogen phosphate (MERCK), Ethanol Absolute (MERCK), and distilled water.

2.1. Preparation of Maximum Wavelength Vitamin E Asetat in Absolute Ethanol

Standard solution made by dissolved vitamin E acetate in absolute ethanol to obtain a concentration of 0.01% w/v.[11] Spectrophotometer set at a wavelength of 200 nm to 400 nm. This solution was put in a cuvette to be read with a spectrophotometer spectrum, in order to obtain the maximum wavelength of vitamin E acetate is 284 nm. The spectrum obtained is printed.

2.2. Preparation of Calibration Curve Vitamin E Acetate in Absolute Ethanol

Vitamin E acetate solution made with 5 concentrations are 30 ppm, 60 ppm, 90 ppm, 120 ppm and 150 ppm. Spectrophotometer set at a wavelength that has been obtained previously. Each solution of vitamin E acetate absorbance alternately measured by spectrophotometer. Absorbance obtained is then plotted on the calibration curve that describes the relationship of concentration and absorption, so we get the curve equation.

Liposomes vitamin E made using thin layer hydration method. Lecithin, cholesterol and vitamin E was weighed, and all the ingredients are dissolved in 15 ml of diethyl ether. The solution was evaporated with a rotary evaporator to remove the organic solvent at a temperature of 45 °C ± 2 °C with a speed of 150 rpm in a vacuum to form a thin layer. Thin-layer is formed and then purged with nitrogen gas for 1 minute and then allowed to stand for 24 hours. The next stage, a thin layer of the hydrated with phosphate buffer pH 7.4 10 ml at temperature of 60 ± 2 °C until the chipped walls entirely of pumpkins. The results of the hydration process and then put in a vortex for 2 minutes to obtain the final result of the suspension. Then the suspension reduced size vesikelnya using sonicator with a frequency of 40 kHz for 5.[12, 13]

2.3. Determining of Liposomes Morphology Form

The morphology or physical form of the liposomes was determined by using Scanning Electron Microscope (SEM). For the use of SEM, the samples were prepared by means of freeze dry process, it aimed to dry the sample. After the samples were dry, the samples coated with Au metal as a conductor. Further, the samples installed into the specimen room. After that the samples were analyzed. A picture would appear automatically after the specimen was set in the specimen room and the vacuum had been ready. Operating conditions will be optimized, including auto stigmator, auto contrast, auto brightness. If necessary adjust the image manually as well to improve image quality.[14]

2.4. Determination of Liposomes Size Distribution

The particle size distribution was determined by the particle size analyzer (PSA). The tool was on and waited until all the tool parameters were ready for use had been fulfilled. Samples were inserted into the cuvette, and than inserted into the particle size analyzer tool. The tool was set for sample reading for 3 minutes, then running the tool till the results was read on a computer monitor. Then, the data obtained was stored.[3] Determination of particle size distributions made to the liposomes before and after the particle size is reduced using a sonicator.

2.5. Liposomes Purification and Entrapment Efficiency Calculation

The liposomes was purified by multilevel centrifugation with microcentrifugator to separate the entrapped and not entrapped active substances at a speed of 18,000 rpm for 30 minutes at a temperature of 4 °C in a vacuum,[2] then the supernatant and precipitates were separated. Supernatants then were recentrifuged for 30 minutes, the supernatant and precipitates were separated again. Supernatant obtained then was recentrifuged for 15 minutes. Through the centrifugation process a separation between the supernatant and the precipitate was formed.

Supernatant part then was diluted the concentration. Dilution was done by adding a phosphate buffer pH 7.0 in a 10 ml flask until the absorbance obtained from the solutions was in the range of 0.15 to 0.85. After dilution was completed, then the absorbance was measured alternately by UV-Vis spectrophotometer at a wavelength of 258.4. Precipitate part was dissolved in absolute ethanol and then inserted in the sonicator for 10 minutes,[15] it aimed to pull out the entrapped vitamin E acetate from liposomes vesicles, then the concentration was diluted with a solvent concentration phosphate buffer pH 7.0 in a 10 ml flask. The absorption obtained was inserted into the equation of the calibration curve to obtain the concentration of vitamin E acetate entrapped within liposomes. The entrapment efficiency was calculated as:[16]

$$\% \text{ EP} = (xj/xt) \times 100\% \quad (1)$$

EP: entrapment efficiency, xj: entrapped drugs, xt: total drug.

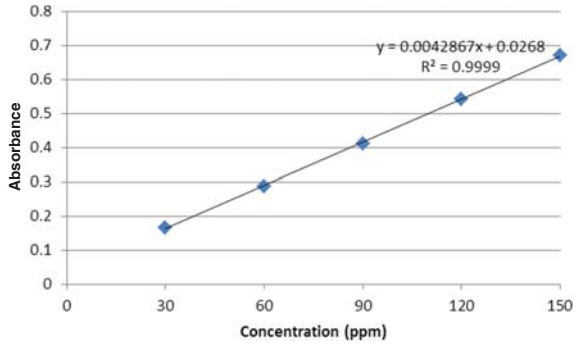

Fig. 2. Calibrating curve of liposom vitamin E acetate in absolute ethanol.

Entrapment ability of vitamin E in liposomes is measured by comparing the concentration of vitamin E trapped to the concentration of total vitamin E. The total concentration of vitamin E obtained from the concentration of trapped vitamin E plus vitamin E concentrations are free.

3. RESULTS AND DISCUSSION

The results of the determination of vitamin E acetate maximum wavelength of 0.01% w/v in absolute ethanol showed maximum absorption at a wavelength of 284.80 nm with the absorbance of 0.5331. This indicates that the active substance used is vitamin E acetate. After the determination of vitamin E Acetate wavelength, the calibration curve of Vitamin E acetate in ethanol absolute is determined. The solution is prepared by 5 different concentrations i.e., 30 ppm, 60 ppm, 90 ppm,120 ppm and 150 ppm. Absorbance is measured using a UV-VIS spectrophotometer at a wavelength of 284.8 nm and the absorbance obtained sequentially by 0.166; 0.287; 0.411; 0.541; and 0.670. Based on the relationship between concentration and absorbance, then a linear line with a correlation coefficient (r) close to 1 is 0.9999 and the linear regression equation $y = 0.0268 + 0.0042867x$ are obtained.

In this study, vitamin E was formulated in a liposome nanoparticle systems. Each formula is made 3 batches, each batch is made by 40 ml. Each formula uses a different lecithin concentrations, it aims to see the effect of the concentration of lecithin to the efficiency of the adsorption generated. Selected lecithin concentration of results orientation and previous research. Of the three different concentrations of lecithin 100 mg, 400 mg and 700 mg, the concentration which resulted in the entrapment efficiency percentage is best to use lecithin 700 mg. This represents an increase of efficiency entrapment occurred long with an increase in lecithin is used.

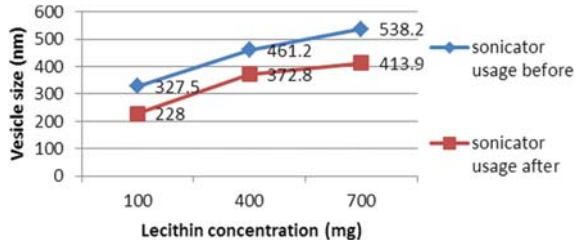

Fig. 3. Graph entrapment efficiency liposome of vitamin E acetate.

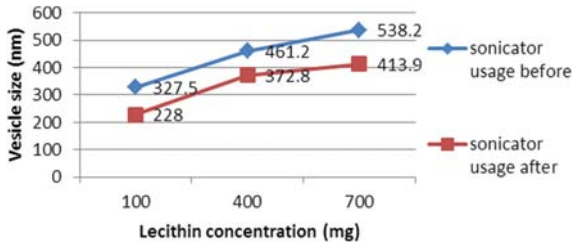

Fig. 4. Comparison graph vesicle size liposomes before and after use sonicator evaluation of morphology.

3.1. Particle Size Distribution

The results of the measurement of particle size distribution before sonicated has a range of about 300 nm to 600 nm. The greater the concentration of lecithin showed the resulting particle size increases. Increasing the particle size of the liposomes along with increasing concentrations of lecithin used. Once the particle size is reduced using a sonicator for 5 minutes with the power of 40 kHz shows a decrease in particle size to produce a range of 200 to 500 nm. The data show results in the form of vesicles, liposomes LUV (Large Unilamellar vesicles). The reduced size of liposome particles by using a sonicator a physical effect on the formation of small bubbles of gases while in hidrofoik part of the lipid bilayer. The presence of these gases can break down fragments of the liposome membrane and forming smaller aggregates.

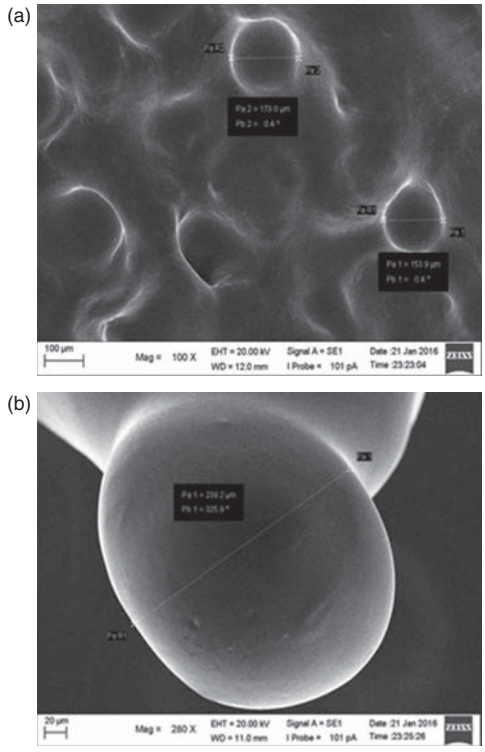

Fig. 5. The results SEM morphology vitamin E acetate liposome with a magnification of 100 (a) and 280 times (b).

The results obtained from testing the morphology using SEM to digital zoom 100 times indicate the formation of vesicles liposomes. At 280 times magnification can be seen the formation of an intact liposome vesicles and spherical. From the readings vitamin E liposome vesicle morphology using SEM looks much improved vesicle size of the particle measurement results using PSA (Particle Size Analyzer). This may be caused by the magnification of the size of the vesicles due to the process of freeze dried and the use of contrast agents is gold that can affect the size of the vesicles of liposomes.[17]

3.2. The Entrapment Efficiency

Based on analysis of the one-way ANOVA, gained significance $0.000 < 0.05$, it can be concluded that the entrapment efficiency data has different variants or differences that are affected by the concentration of lecithin. The entrapment efficiency analysis followed by Tukey's test, the conclusion increase lecithin concentration of 300 mg in each formula has significant influence on the adsorption efficiency.

Results of ANOVA one way of the data size of the vesicles after application sonicator shows there are significant differences between the size of the vesicles at a concentration of lecithin 100 mg of the size of the vesicles at a concentration of lecithin, 400 mg and 700 mg, but there was no significant difference between the size of the vesicles at a concentration of lecithin 400 mg of the size of the vesicles at a concentration of 700 mg lecithin.

4. CONCLUSION

Based on the results of this study concluded that the increased concentration of lecithin which is one of the main components making up the liposomes enhance the adsorption efficiency. The results show that the entrapment efficiency percentage consecutively is $41.3799\% \pm 1.06$; $47{,}6162\% \pm 0.78$; and $52{,}7216\% \pm 1.33$, while the vesicles size obtained after using sonicator is 228 ± 18.09 nm; 372.8 ± 15.91 nm; and 413.9 ± 13.96 nm.

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