



Preparation of Spray Nanoemulsion and Cream Containing Vitamin E as Anti-Aging Product Tested in Vitro and in Vivo Method

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Abstract : Skin is a layer covering body and protect body from negative effect and environment factor. Some studies indicated that aging resulted by sun-light exposure and many other factors including free radical. Thus, it requires anti-aging product capable to resist and even to repair any disorder in skin caused by free radical. One of anti-oxidant required in one's body is vitamin E (*tocopherol*). The objective of this study was to formulate special preparation in spray nanoemulsion and cream containing vitamin E as *anti-aging* tested *in vitro* and *in vivo* method. This experiment used vitamin E (*alfa tocopherol*) as anti-aging component function and compare effectiveness of anti-aging made in preparation of spray nano-emulsion and cream containing vitamin E. Testing on preparation of spray nanoemulsion and cream vitamin E on skin and effectiveness anti-aging using skin analyzer applied on skin back of hand. Treatment took for 12 weeks using/applying each preparation twice daily. Parameters tested included moisture, avens, pore and total spot.

The result showed that vitamin E can be formulated in spray nanoemulsion and cream containing vitamin E with homogenous outcome, no irritation, pH ranged 6.7-7.16, stable during storage for 12 weeks, spray nanoemulsion has nanoparticle sized of 186,26-338,93 nm, spray nanoemulsion also had been shown penetrating into skin better than any preparation in cream and preparation spray nano-emulsion capable to show its effectiveness as a *anti-aging* product after treatment for 12 weeks than a preparation in cream. The results of this study suggest and indicated that be higher concentration of vitamin E and be smaller particle size it may produce effectiveness excellent as anti-aging.

Keyword : *aging, spray nanoemulsion, cream, vitamin E.*

Introduction

Skin is an organ to cover body and having the main function as a protection from various interferences and outside stimulation or environment^{1,2}. The skin aging process can be divided into intrinsic aging and extrinsic aging. Ultraviolet (UV) irradiation causes premature aging which is called as photoaging. It is caused by intrinsic process superimposed with degenerative changes to solar radiation^{3,4}. Premature aging may happen to everyone, especially in Indonesia with tropical climate so that the result of total exposure from the sun and its possibility to expose with high potency for free radical. The Sun-light is one of factors for premature aging⁵. Anti-oxidant is a substance that is important for body to neutralize free radicals and to avoid disorder due to free radicals^{6,7}. The free radical scavenging activities such as hydroxyl, DPPH and anti-peroxidation^{8,9}.

Nanotechnology is a potentially the most important engineering revolution since the industrial age. Nanotechnology is science and technology conducted at the nanoscale. So far nanotechnology resulted in variants of formulations like nanoparticles, nanocapsules, nanospheres, nanosuspensions, nanocrystal, nanocrythosome and nanoemulsion. Nanotechnology is defined as creation and manipulation of materials at nanoscale level to create products^{10,11,12}. Nanoemulsion drug delivery system are the alternative for the bioavailability enhancement of poorly soluble^{13,14,15}.

Anti-oxidant compound in food play an important role as a health protecting factor¹⁶. One of good anti-oxidants is vitamin E that is called *alfa tocopherol*¹⁷. This research used ester form of vitamin E as tocopherol acetate^{18,19}. Based on the statement above, in this study it was evaluated that good penetration using Vitamin E as active substance provide it present as preparation in particle of smaller size of particle, in this preparation with *spray* nano-emulsion containing vitamin E²⁰. Vitamin E has potential as a supplement as well as a source of antioxidants in pharmaceutical preparations such as emulsions, creams, ointments and gels²¹. The aim of this study was to formulate a *spray* preparation nano-emulsion containing vitamin E compared with cream Vitamin E as anti-aging²².

Materials and Method

The equipments used in this study included Particle size analyzer (PSA), Scanning Electron Microscope (SEM), High Performance Liquid Chromatography (HPLC) (Shimadzu LC 20AD), pH Meter (Hanna Instrument), spray bottle, skin analyzer (AramoHuvivis), magnetic stirrer, magnetic bar, thermostat, glass wares, mortar and pestle, analytical balance and sonicator. All chemicals used were tocopherol acetate (Vitamin E), glycerin, Hydrogenated Castor Oil, Tween 20, Natrium EDTA, Sodium metabisulfite, perfume, propylene glycol, triethanol amine (TEA), vaseline, cetyl alcohol, stearic acid, glyceryl monostearate, distilled water.

Procedure

A spray formulated nano-emulsion Vitamin E and Cream Vitamin E was prepared with various concentrations (0%) as blank, 1%, 3% and 5% Vitamin E, each preparation was added additional substances such as surfactant with the same concentration in each tested material.

Preparation of Emulsion and Vitamin E nano-emulsion

Tween 20 was mixed with Tocopherol acetate (Vitamin E), then dissolved and added Hydrogenated castor oil as oil phase. The water phase was composed of aquadest (distilled water) and glycerine. The oil phase and water phase was mixed slowly using magnetic stirrer and stirred for 60 minutes^{23,24}. Oleum rosae was added to formula as perfume. The emulsion of vitamin E was sonicated using sonicator for 30 minutes^{25,26}.

Preparation and Formulation of Cream Containing Vitamin E

All material was provided and weighed, and then separated into two layers that were oil phase and water phase. Oil phase consisted of vaseline, stearic acid, glycerine monostearate, and cetyl alcohol were melted on heater at temperature of 70°-75° C, and then vitamin E was added into the melted formula²⁷. The water phase was composed of aquadest, propylene glycol, sodium acetate and TEA and dissolved with heater. The water phase and oil phase was milled in hot mortar with stirring slowly on a temperature of $\pm 70^{\circ}\text{C}$ until cream mass formed, then added oleum rosae as perfume sufficiently²⁸.

Stability Evaluation

The formula was stored in a transparent spray bottle and kept it at room temperature for 12 weeks. During storage, several examinations were done, and observed such as organoleptic, particle size in nano-emulsion and pH determination^{28,29,30}.

Anti-Aging Test using Skin Analyzer

The test of anti-aging activity was done with 12 volunteers that have been divided in each different vitamin E concentration. All volunteers's hand skin condition was pretested with various test parameters including moisture, evenness, pore, and spot number using skin analyzer and moisture checker. Nano-emulsion and cream vitamin E had been applied twice daily at night and mornings for 12 weeks. The change of skin

condition was measured each week for 12 weeks using skin analyzer. The result was evaluated statistically using one way of anova .

Results and Discussion

The pH values of Preparation

The pH was measured to know the acidity of all prepared cosmetics. According to a regulation in SNI 16-4399-1996 that pH of skin moisturizer product should be ranged between 4.5-8.0^{5,29}. If the cosmetic product has pH very high or low it may cause an irritated skin^{31,32,33}.

Characteristics of nano-emulsion and cream containing Vitamin E

Based on data from the result of observation and stability test for 12 weeks indicated that the nano-emulsion prepared spray and cream vitamin E have a good stability for 12 weeks. Stability of a prepared pharmacy can be seen by any change of color, it's smelt and pH during storage^{29,34}. The characteristics of vitamin E Spray and Cream can be seen in Table 1.

Table 1. Appearance of spray and cream vitamin E

Formula (spray)	Appearance			Formula (cream)	Appearance		
	Colour	Smell	Consistency		Colour	Smell	Consistency
F1	White	Rose	Emulsion	F1	White	Rose	Semi solid
F2	White	Rose	Emulsion	F2	White	Rose	Semi solid
F3	White	Rose	Emulsion	F3	White	Rose	Semi solid
F4	White	Rose	Emulsion	F4	White	Rose	Semi solid

Note: F1 (Blank), F2 = 1%, F3 = 3%, F4 = 5% Vitamin E

Homogeneity of Prepared Cream

The aim of homogeneity test was to show the materials distribution in the formula. Based on the homogeneity observation of cream formula had been shown not any coarse grains found on object glass^{28,30,35}. so that should be concluded this formula was homogenous as presented in Figure 1.

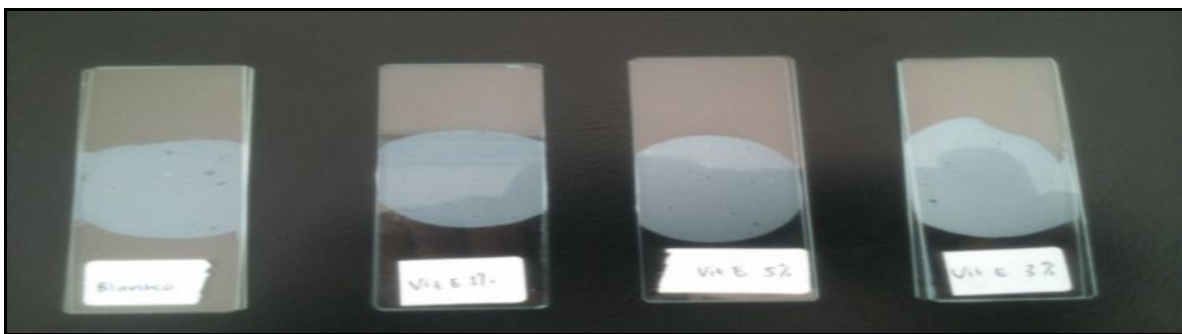


Figure 1. Homogeneity of prepared cream

Particle Size of nano-emulsion prepared spray (by Particle Size Analyzer)

The result of particle size measured by Particle Size Analyzer (PSA) had been shown in Table 2.

Table 2. Spray Nanoemulsion Particle size

No	Formula	Before (initially)		After 12 weeks	
		Size (nm)	Intensity (%)	Size (nm)	Intensity (%)
1	F2 (1%)	183.72	0.54	187.32	0.52
2	F3(3%)	186.26	0.53	308.17	0.91
3	F4(5%)	186.26	0.57	338.93	0.93

Based on the result that found it was that particle size of spray nano-emulsion had been changed after 12 weeks storage, but still persistent in nano size. That storage condition caused oxidation process and influence of surfactant components^{36,37}.

The result was estimated that nano-emulsion *spray* was stable after 12 weeks stored. The vitamin E concentration caused formula size particle had been changed but still within acceptable range of 2-500 nm size³⁸.

The Anti-Aging Activity

The anti-aging activity test using skin analyzer (Aramo) to measure parameter including moisture, evenness of skin, size of pore, and the spot total^{39,40}. The anti-aging activity was started by measuring initial condition of back skin of hand each volunteers. The data obtained on each *anti-aging* parameter was analyzed statistically with ANOVA .

Moisture

The moisture in back hand of each volunteers was measured using moisture checker in Skin analyzer Aramo device. The result has been done and plotted to obtain a graphic indicated that early condition in back hand of each volunteers was close to approach dehydration (0 – 32). The result was measured by skin analyzer for 12 weeks had been shown to increase moisture caused by nano-emulsion spray and vitamin E cream. The result is presented in Figure 2 and 3.

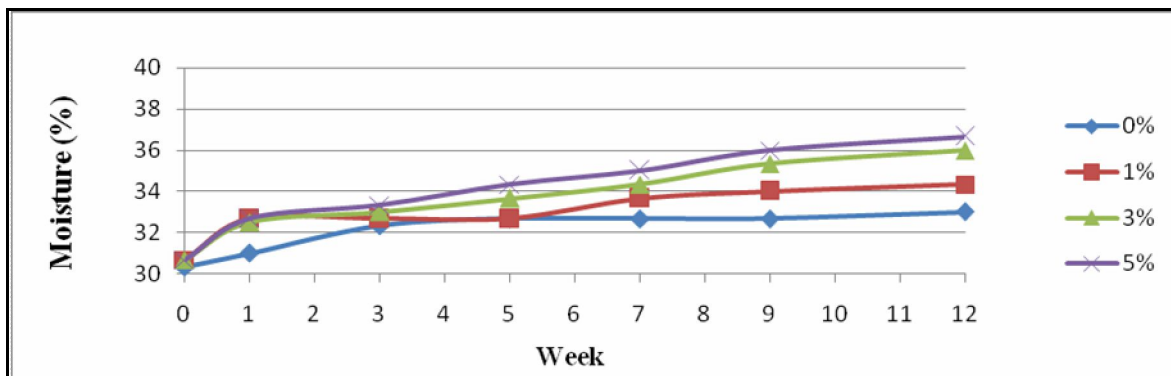


Figure 2.The effect of spray nanoemulsi containing vitamin E 0 % (blank), 1%, 3% and 5% on moisture for 12 weeks.

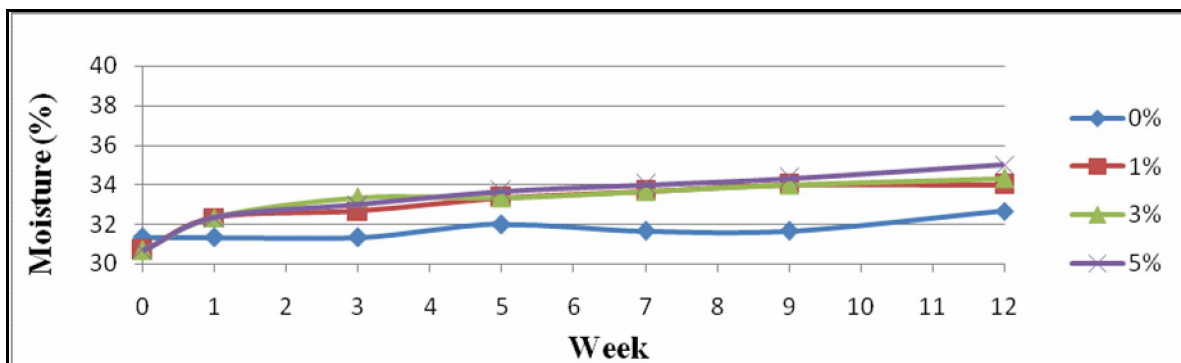


Figure 3.The effect of cream containing vitamin E 0 % (blank), 1%, 3% and 5% on moisture for 12 weeks.

Based on Figure 2 and 3 can be seen the result is a rising percentage of water content each formula every weeks measured. The result of statistical analysis by Anova showed that nano-emulsion spray 5% containing vitamin E is significantly different ($p \geq 0.05$) with all of nano-emulsion spray and cream vitamin E formula of every concentration.

The result of test suggested that the various concentration of vitamin E is influencing and profitable to skin healthy⁴¹. Vitamin E was usually used in purpose against dryness of the skin as anti-aging in sun-screen product³⁹. The best sun-screen products should contain at least 1% Vitamin E. This study proved that Vitamin E resolved dryness and gave natural moisture on skin^{4,19}.

Evenness

Evenness skin of back hand of those volunteers was measured using device *skin analyzer* magnification lens 60% (normal lens) with a blue sensor. The result of statistical analysis also indicated that prepared nanospray group of 3% and 5% generated decreasing evenness percentage approaching almost the same as that of the condition a week backward, but if compared both groups was higher decreased percentage occurred on a prepared nano-emulsion spray group containing 5% Vitamin E compared to a prepared nano-emulsion spray group containing Vitamin E 3%. According to statistical analysis was found to be significantly different ($p \geq 0.05$) from week-1 through week-12 on a prepared nano-emulsion spray containing Vitamin E 3% with 5%. The result can be seen in Figure 4 and 5.

The function of Vitamin E on skin is beneficial to restore structure of skin, to prevent aging, strengthen skin and got evenness on skin surface. In other research showed that Vitamin E is the best anti-oxidant component and evenness the skin, due to the nature of Vitamin E that dissolved in fatty material of skin and helpful to penetrate directly^{4,31}.

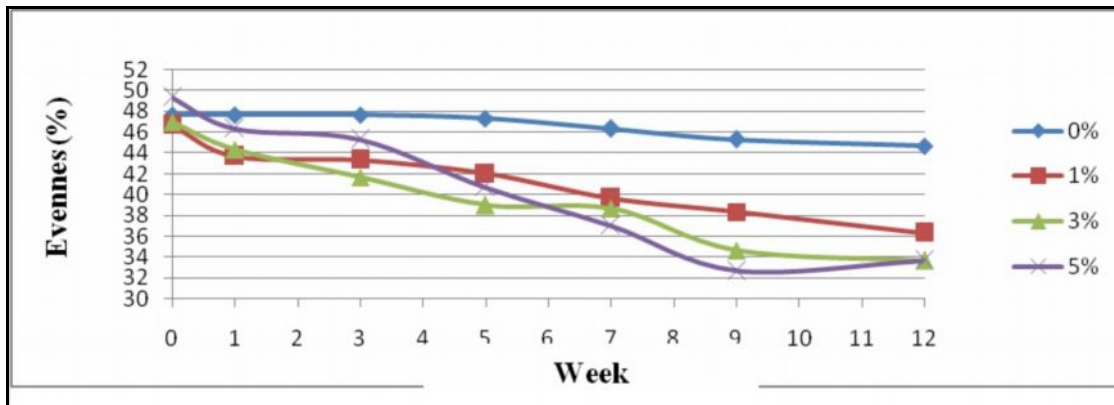


Figure 4. The effect of spray nanoemulsion containing vitamin E 0 % (blank), 1%, 3% dan 5% on skin evenness for 12 weeks

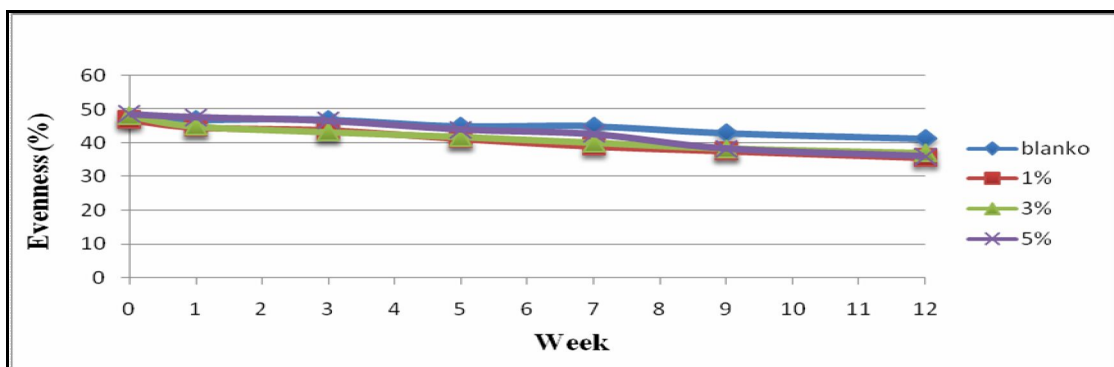


Figure 5. The effect of cream containing vitamin E 0 % (blank), 1%, 3% dan 5% on evenness for 12 weeks.

Pore

The existence of skin pore of back hand the volunteers was measured using the same skin analyzer device automatically its pore size in analysis included in reading⁴¹.

The result presented is nano-emulsion spray group of Vitamin E 5% have decreasing compared with cream of Vitamin E 5% group since early week until week 12 presented in Figure 6 and 7. The graphic presented indicate that is a significantly difference ($p \leq 0.05$) between nano-emulsion spray group and cream of Vitamin E. The result showed that in statistical analysis of Anova indicate significantly different ($p \leq 0.05$) between nano-emulsion spray group of vitamin E 3% and 5% with all of groups.

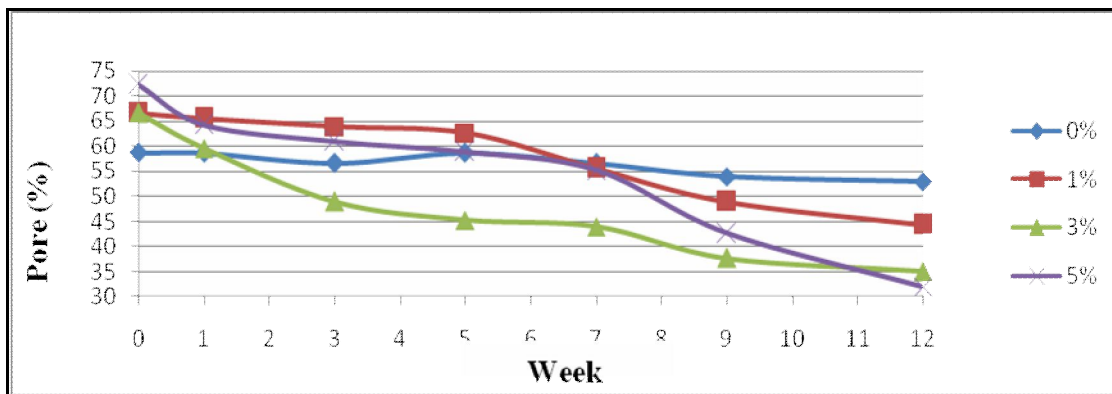


Figure 6. The effect of spray nanoemulsion containing vitamin E 0 % (blank), 1%, 3% and 5% on pores for 12 weeks.

Pores may enlarge if exposure to sun-light, and accumulate died skin cell which may cause and trigger emerging pimples and influence on pore size^{39,40}. Vitamin E (*tocopherol*) can clean out died skin cell and stimulate formation new cell as well as neutralize or scavage free radical that may risky in skin damaged, so it may decrease pores of skin^{40,42}.

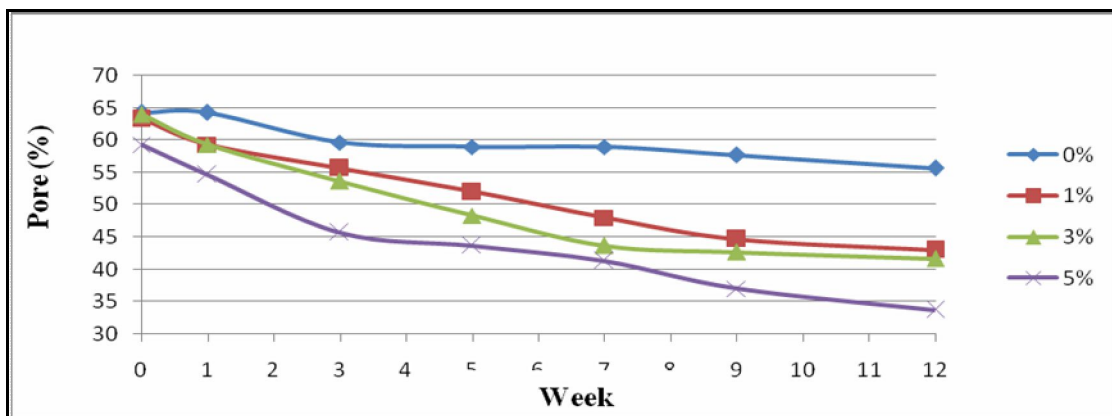


Figure 7. The effect of cream containing vitamin E 0 % (blank), 1%, 3% dan 5% for 12 weeks on pores.

Spot

Skin spot on back hand of volunteers was measured using skin analyzer device with magnifying lens at 60x (polarizing lens) in orange censor. The figure showed that initial skin condition of back-hand in all volunteers present many spots on skin (40 – 100).

The result of statistical analysis showed that there is significantly different ($p \geq 0.05$) between before and after treatment nano-emulsion spray group and cream of Vitamin E group. The result in statistical analysis found that occurrence is significantly different ($p \geq 0.05$) between a nano-emulsion prepared *spray* group 5% with all other group either nano-emulsion spray group and cream of vitamin E, which means that nano-

emulsion *spray* 5% become the best formula in reducing percentage total spots compared to other groups. The result can be seen in Figure 8 and 9.

Vitamin E could naturally keep skin health and also protect skin and hence as sun-screen, and this study proved that Vitamin E is also beneficial to generate of skin and natural moisturizer mainly on skin after exposure to sun-light, as well as to reduce any spots and also to keep skin healthy^{40,42}.

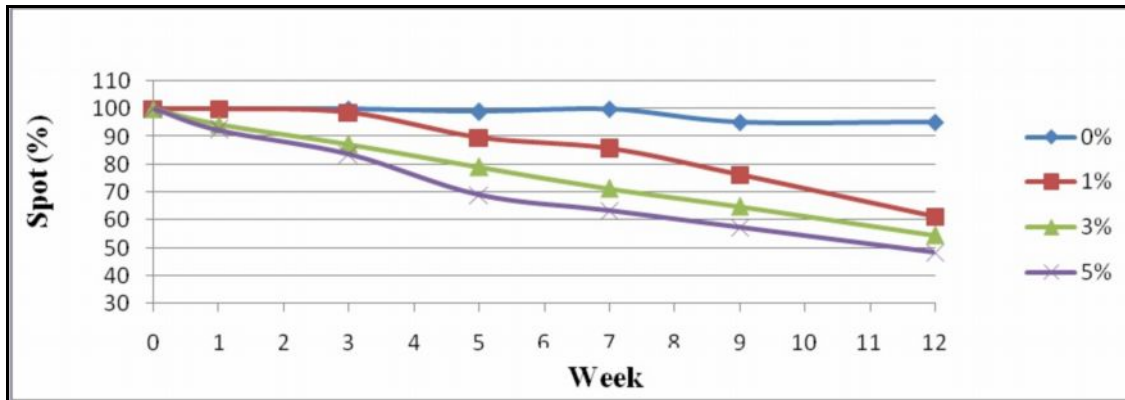


Figure 8. The effect of spray nanoemulsion containing vitamin E 0 % (blank), 1%, 3% dan 5% for 12 weeks on spot

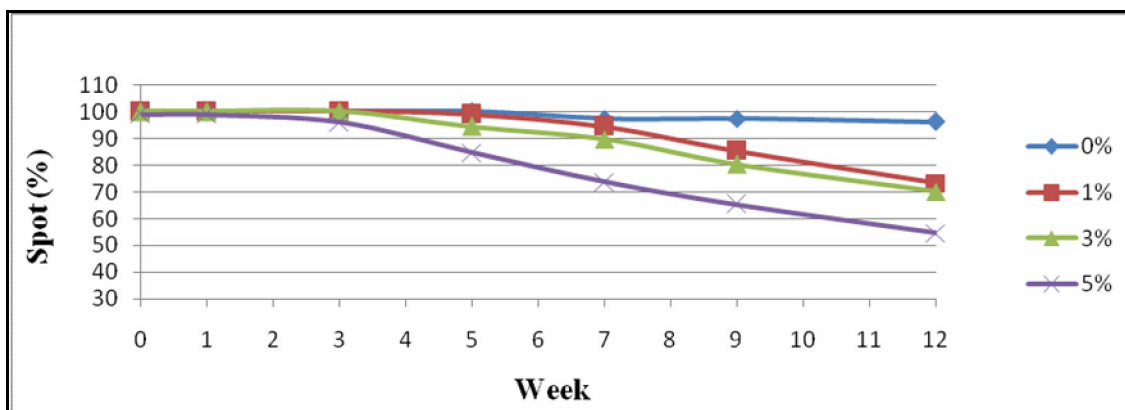


Figure 9. Graph the results of measurements of spot cream containing vitamin E 0 % (blank), 1%, 3% dan 5% for 12 weeks.

Discussion

Vitamin E can be formulated into a nano-emulsion spray and cream and found to be in reliable stability. Vitamin E in any nano-emulsion spray proved to be helpful and function as anti-aging better than cream of Vitamin E. Activity of anti-aging of vitamin E in a nano-emulsion spray and cream are increasing on dependent concentration of vitamin E. The particle size is also significantly influence to the effectiveness of formula, that the smaller the size of particle the easier the formula to penetrate to the skin surface, so that may improve penetration of preparation into skin. The nano-emulsion spray vitamin E 5% proved to become the most beneficial formula in generating effectiveness as anti-aging product.

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