

Comparison of Vitamin C (Magnesium Ascorbyl Phosphate) Formulation in Nanoemulsion Spray and Cream as Anti-aging

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Abstract : Vitamin C has important role in stimulating the formation of collagen and reducing hyperpigmentation on the skin. However vitamin C has low bioavailability if consumed orally and unstable in form of ascorbic acid. Vitamin C being formulated in a stable nanoemulsion spray and cream with various concentration. Preparations were applied twice a day for 12 weeks on the back hand of 12 volunteers. In every 2 weeks, moisture, evenness, pore and spot of skin was measured by a device called Skin Analyzer Aramo. Nanoemulsion spray containing 10% of vitamin C gave a significant difference in increasing moisture, smoother the skin and reducing hyperpigmentation of back hand skin from volunteers. Different preparations showed a difference in anti-aging activity. Nanoemulsion spray of 10% vitamin C showed better anti-aging activity in increasing moisture, smoother the skin and reducing hyperpigmentation on skin compared to 10% vitamin C in cream preparation. Though in reducing the size of pore, nanoemulsion spray did not significantly different from cream preparation.

Keywords: anti-aging, cream, nanoemulsion spray, vitamin C.

Introduction

Aging is an unavoidable process that definitely occur in every human being, the most visible changes is skin become drier, rough, losing elasticity, hyperpigmentation, and forming wrinkles.¹ A lot of treatment have been done to slower down the aging process, one of the easiest way is applying anti-aging cosmetic products.² Topical or transdermal product avoid the first pass metabolism by the liver and delivery more concentration of the therapeutic agents. Transdermal application are designed to deliver sustained drug amounts, resulting in systemically consistence levels.³ Vitamin C as an antioxidant agents attracted special interest because of their free radical scavenging ability.⁴ Vitamin C is one of the important nutrients as it is necessary for the synthesis of collagen, helps maintain the skin, and reducing hyperpigmentation⁵, but vitamin C can not be synthesized in the body and has low bioavailability if consumed orally.⁶ Vitamin C has problems of poor stability and easily degraded in oxidation pathways and make it difficult to penetrate into the skin.⁷ High concentration of ascorbic acid will cause irritation especially for sensitive skin.⁸

Magnesium ascorbyl phosphate (MAP) is a derivate of vitamin C which is more stable than ascorbic acid.⁶ MAP become the best choice because having a neutral pH and prevent skin exfoliating effect, effective concentration of MAP is only 1-10%.⁹

Smaller particles favor drug penetrate deeper into the skin.¹⁰ Nanoparticles synthesis is currently intensively researched due to its wide variety of potential applications and more economical.¹¹ Developing a nanometer size cosmetic make it easier to penetrate faster and deeper into skin, safer and more efficient. Nanometer is a unit of length in metric system that equal to one billionth of a metre 1×10^{-9} m.¹²

Procedure

Formulation of nanoemulsion spray of vitamin C

One of the methods to prepare nanotechnology product is using a high speed homogenizer, while the aqueous part was being added to the oily part by a continuous stirring using high speed homogenizer.¹³ The formulation components used were listed in Table 1. Magnesium ascorbyl phosphate (MAP), the ester form of vitamin C was dispersed with hydrogenated castor oil until homogen and then added some drops of oleum citri as its perfume (mixture I). Dissolved tween 20, propylene glycol, glycerin until homogen (mixture II). Dissolved sodium metabisulfite and sodium EDTA into aquabidest (mixture III). Combine mixture II into mixture III (mixture IV). Homogenized mixture IV using magnetic stirrer with minimal speed. Added mixture I slowly into mixture IV while stirring until 30 minute. The prepared emulsions were inserted into non visible bottle and sonicated for 1 hour.

Table 1. Nanoemulsion spray

No	Components	F1 (%)	F2 (%)	F3 (%)	F4 (%)
1	Magnesium ascorbyl phosphate	-	3	6	10
2	Hydrogenated castor oil	5	5	5	5
3	Tween 20	2	2	2	2
4	Glycerin	3	3	3	3
5	Propylene glycol	5	5	5	5
6	Sodium EDTA	0.1	0.1	0.1	0.1
7	Natrium metabisulfite	0.1	0.1	0.1	0.1
8	Oleum Citri	0.1	0.1	0.1	0.1
9	Aquabidest ad	100 ml	100 ml	100 ml	100 ml

Formulation of cream vitamin C

The formulation components used were listed in Table 2. Components were divided into 2 groups: water soluble (aqueous phase) and oil soluble (oily phase) components. Oil soluble components such as vaseline, stearic acid, glyceryl monostearate, and cetyl alcohol were melted in china dish using waterbath at 70°-75°C. All aqueous soluble ingredients (sodium metabisulfite, propylene glycol, sodium EDTA, and TEA) were dissolved in hot water. Formerly prepared oily phase being crushed homogenously in hot and dry mortar. Added aqueous phase drop by drop with a constant crushing until resulting a cream form. Finally, added vitamin C (magnesium ascorbyl phosphate) which already dissolved in aquabidest. Added oleum citri as odor enhancer into cream preparation.

Table 2. Cream formulation

No	Components	F1 (%)	F2 (%)	F3 (%)	F4 (%)
1	Magnesium ascorbyl phosphate	-	3	6	10
2	Vaseline	5	5	5	5
3	Cetyl alcohol	3	3	3	3
4	Stearic acid	3	3	3	3
5	Glyceryl monostearate	3	3	3	3
6	Propylene glycol	5	5	5	5
7	TEA	1	1	1	1
8	Sodium EDTA	0.1	0.1	0.1	0.1
9	Sodium metabisulfite	0.1	0.1	0.1	0.1
10	Oleum Citri	0.1	0.1	0.1	0.1
11	Aquabidest ad	100 g	100 g	100 g	100 g

Evaluation of Nanoemulsion Spray and Cream

The prepared nanoemulsion spray and cream were stored in a non visible bottle and kept in room temperature for 12 weeks.

Determination of physical appearance

The color, odor, and homogeneity were being observed since preparations were made and during storage until 12 weeks.

Determination of pH

The pH of nanoemulsion and cream were found by immersing pH meter into a 10% diluted nanoemulsion and cream. The determinations were carried out in triplicate and the average pH is recorded until 12 weeks of storage. Results were shown in Table 4.

Determination of nanoemulsion size

Size of particle in nanoemulsion preparations were observed by a device called Particle Size Analyzer (PSA).¹⁴ Measurement in this device was the average distribution of particles in a sample. Results can be shown in Table 5.

Anti-Aging Test using Skin Analyzer

Anti-aging activity was done on 12 volunteers which divided into 4 groups: blank group, vitamin C 3%, 6% and 10%. Each group consisted of 3 volunteers, right hand for nanoemulsion spray application and left hand for cream application. All preparations were applied twice a day (morning and night) for 12 weeks on the back hand of 12 volunteers. Before started the treatment, all volunteers hand have been checked for the pre-treatment condition. In every 2 weeks, moisture, evenness, pore, and spot of skin were measured by Skin Analyzer Aramo. The result between nanoemulsion spray and cream were compared and analyzed by statistic.

Results and Discussion

All of the nanoemulsion spray and cream preparations were stable until 12 weeks of storage. There was shown by no color and odor changed on both nanoemulsion spray and cream, no sedimentation formed on nanoemulsion and cream still homogen during storage.

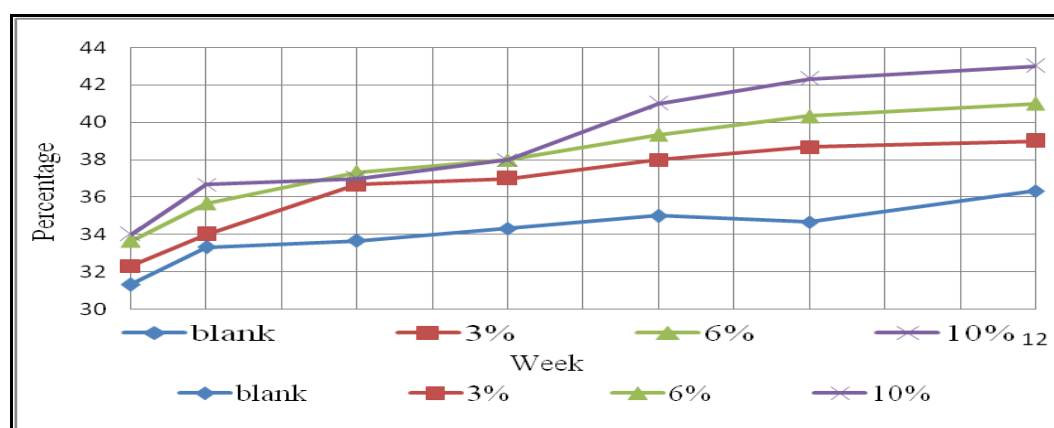
Average pH of all nanoemulsion before storage was 6.70- 6.97. After 12 weeks of storage, pH of nanoemulsion decreased to 6.50- 6.90. Although showing a decreased pH, all of the nanoemulsion were in the acceptable pH range for cosmetic product which is pH 5-8.¹⁵

Particle size of nanoemulsion spray containing vitamin C was determined by Particle Size Analyzer. Nanoemulsion containing 3% vitamin C had the smallest particle size since it was made and after 12 weeks storage which was 13.49 nm and 17.79 nm. The higher the concentration of vitamin C in a preparation showed the bigger particle size. In determining the particle size of a sample, the greatest intensity from the table indicate the most exact size of the sample. Smaller intensity indicate the size of impurity in a sample. It can be concluded that, particle size before nanoemulsions being stored were 13.49 nm (3% vitamin C); 19.53 nm (6% vitamin C); 19.50 nm (10% vitamin C). After 12 weeks storage, nanoemulsion become larger in size which were 17.79 nm (3% vitamin C); 20.34 nm (6% vitamin C); and 24.69 nm (10% vitamin C). Enlargement of the particle size could be caused by adding and mixing the non nanometer size's ingredients. One of the instability of nanoemulsion is flocculation which is merging of particles because electrostatic repellence. However increasing of particle sizes was not visible like the common instability such as separation phase of emulsion, sedimentation of big particles, formation of lipid layer or changing in consistency.¹⁶ Result can be shown in table below.

Before storage			After 12 weeks storage		
Formula	Particle size	Intensity	Formula	Particle size	Intensity
3%	13.49 nm	0.91	3%	17.79 nm	0.75
	489.91 nm	0.09		134.93 nm	0.25
6%	19.53 nm	0.65	6%	20.34 nm	0.60
	107,18 nm	0.35		323.68 nm	0.40
10%	19.50 nm	0.71	10%	24.69 nm	0.65
	325.93 nm	0.29		324.68 nm	0.35

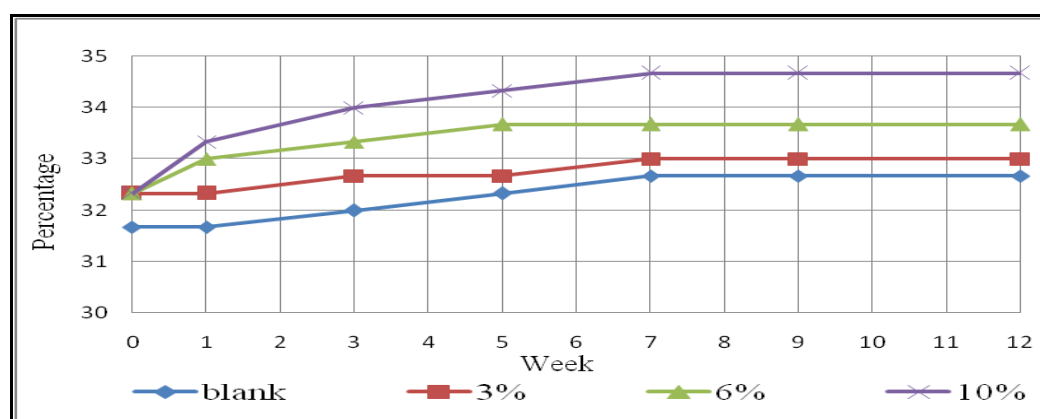
Anti-aging Activity

Moisture



Nanoemulsion

All volunteers had a normal moisture which was on range 30-44 before treatment and after 12 weeks. But on each measurement in every 2 weeks, moisture of their back hand were increased significantly ($p < 0.05$) by using nanoemulsion containing 3%, 6%, and 10% vitamin C and even nanoemulsion without vitamin C.



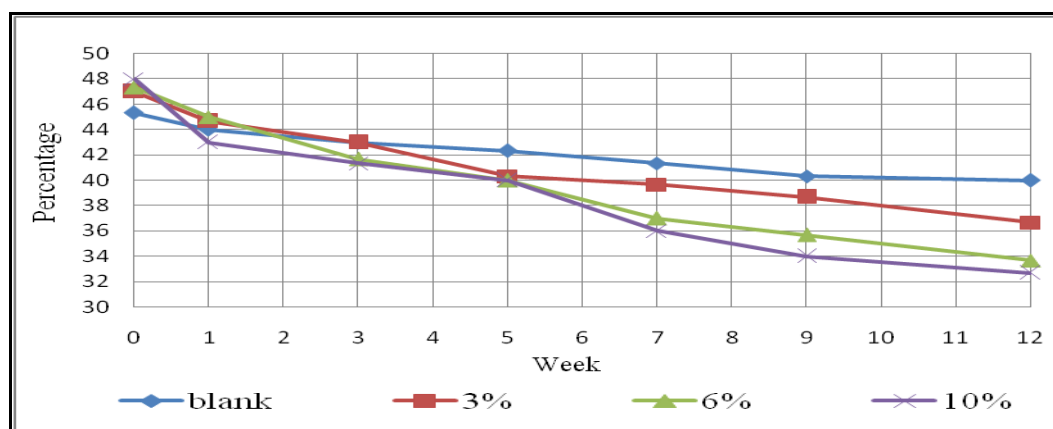
Cream

Usage of cream preparation showed increasing in moisture of volunteers' back hand, however the percentage was not higher than treatment using nanoemulsion spray. There was a significant difference between cream without vitamin C with cream containing 6% and 10% of vitamin C also between 3% and 10% vitamin C in cream preparations.

Compared using Anova statistic, nanoemulsion containing vitamin C showed a significant difference to cream containing vitamin C in increasing moisture of skin (significance < 0.05).

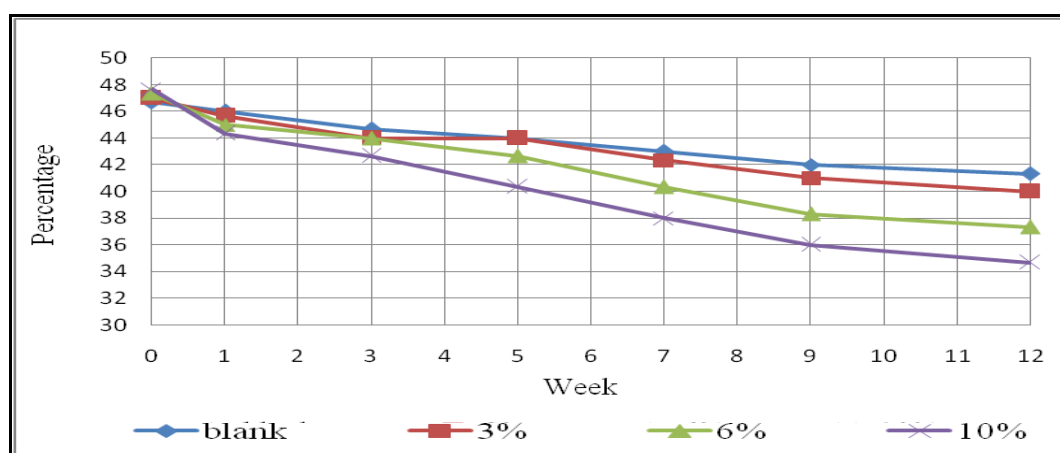
Skin has to maintain its epidermal water to keep a good work of its function. Wrinkles were formed when skin was lack of water and reduction of elasticity.¹⁵

Evenness



Nanoemulsion

All of the back hand of volunteers had a normal evenness which was 32-50. The higher the percentage, showed the rougher of the skin.¹⁷ After using nanoemulsion, evenness of volunteers' skin showed better result by decline percentage of its roughness. Nanoemulsion with 10% of vitamin C showed greatest decline. Each preparation showed a significant difference except between 6% and 10% vitamin C nanoemulsion did not show a significant difference ($p > 0.05$). Vitamin C able to smoother the skin by catalyze hydroxyproline and hydroxylisine enzyme which take part in producing collagen.⁶

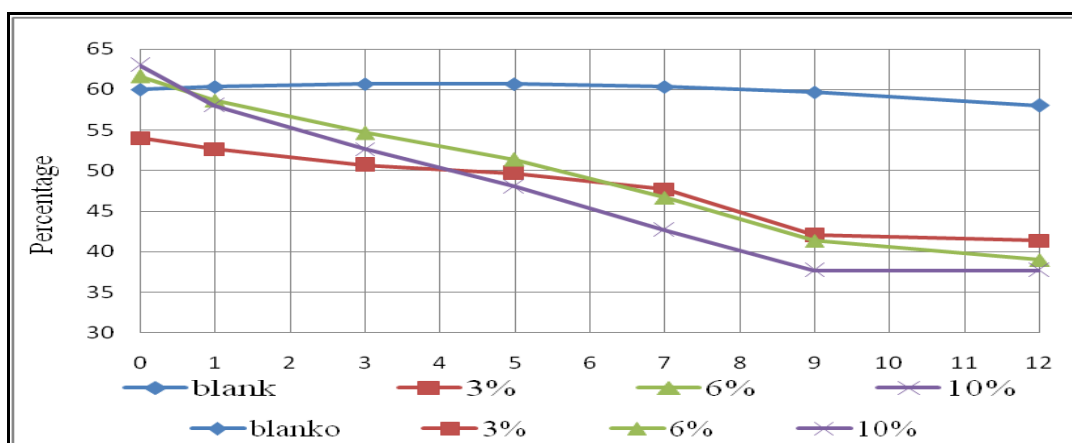


Cream

Before using cream, evenness of skin were above 45 (normal 32-51). Smooth skin indicated by percentage below 32. After using cream, skin become smoother, but based on statistic test, there was no significant difference between cream without vitamin C and cream containing 3% of vitamin C.

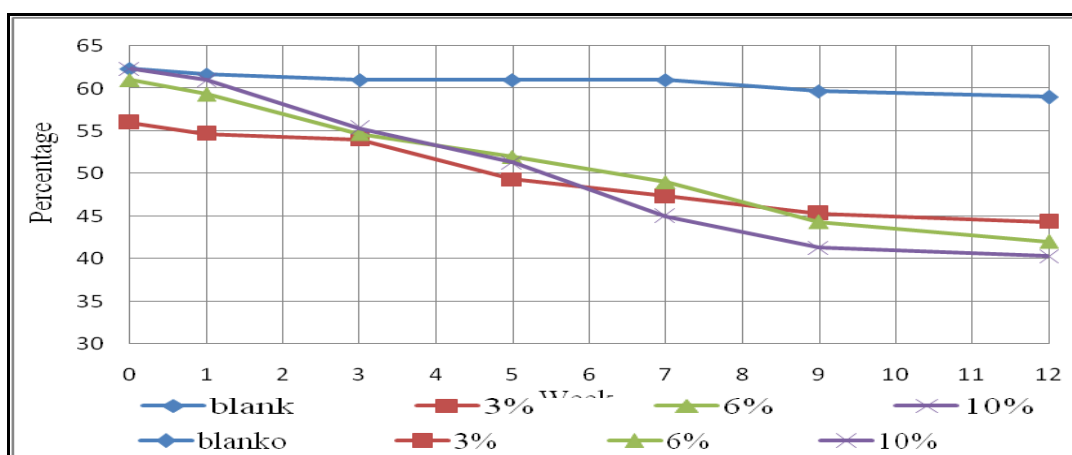
The same amount of vitamin C in nanoemulsion and cream showed significant difference to smoother the skin. Low concentration of vitamin C in nanoemulsion able to smooth the skin, in a meanwhile cream needed higher concentration to attain the similar result. Hydrophilic compound makes it difficult to penetrate on the stratum corneum layer which is lypophilic.¹⁸ Difusion of hydrophilic compound need development of technology such as nanoparticles technique.¹⁹

Pore



Nanoemulsion

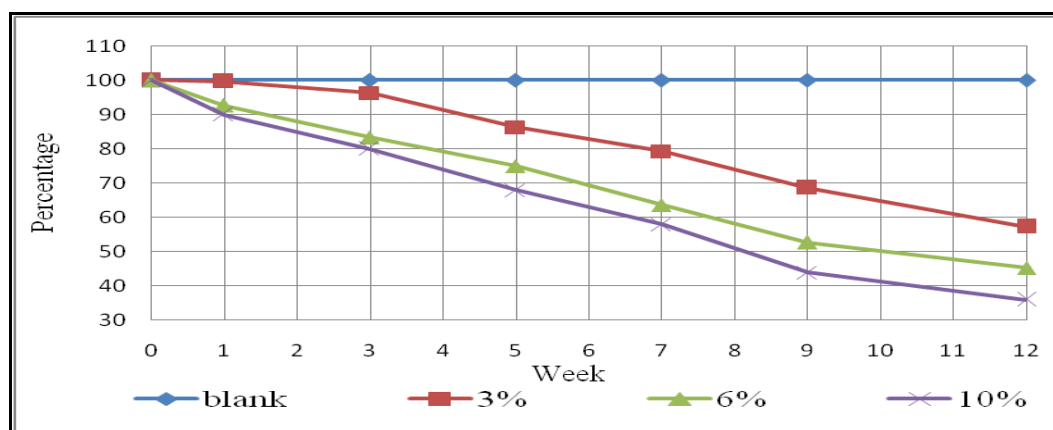
Pore of volunteers' skin were so extensive which were above 40 (average 20-39). After treatment by nanoemulsion, the size of pore were reduced. Blank nanoemulsion showed 2% of decreased. Nanoemulsion containing 3%, 6%, and 10% vitamin C showed decreased 12.67%; 22.67%; and 25.67% respectively. After 12 weeks of usage, nanoemulsion containing 6% and 10% vitamin C apparently able to decrease pore's size until below 40.



Cream

On the pre treatment measurement, pore of volunteers were extensive. After applied cream preparations for 12 weeks, size of pore were reduced but still in an extensive category. Genetic factor also take part in determining size of pore on skin.²⁰ Group using 10% of vitamin C showed the greatest decrease in size of pore, but still there was no significant difference between group using both nanoemulsion and cream containing vitamin C. Physical activity which increasing body temperature will open the pore of skin, while sunlight and age factor will worsen it.²¹

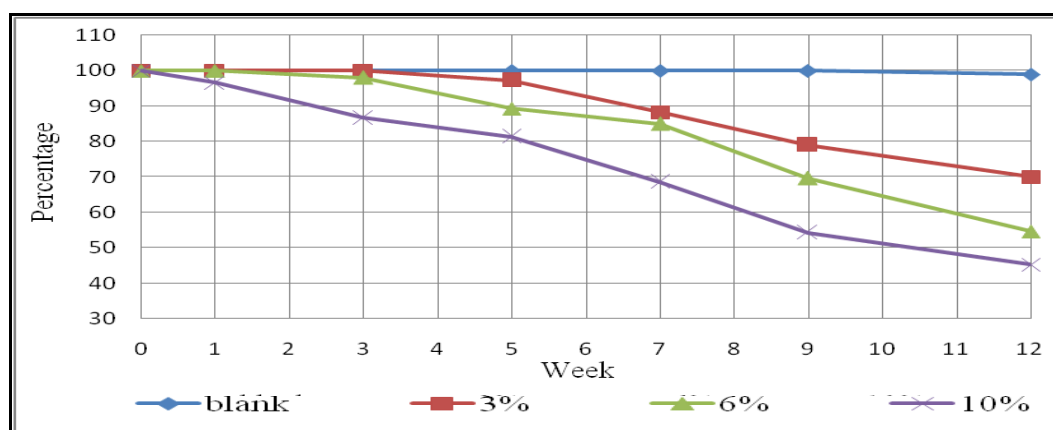
Spot



Nanoemulsion

Amount of spot on volunteers' skin were in maximum percentage of 100. In a routine usage of nanoemulsion containing vitamin C, amount of spot on their back hand were decreased, although until the last day of treatment there was no one had little spot on their skin. Decline only existed until medium category which was 20-39. This can be caused by outdoor activity that volunteers did and exposed on sunlight was not totally stop. However, statistic test showed that usage of vitamin C nanoemulsion decreased the amount of spot significantly ($p < 0.05$). Nanoemulsion without vitamin C (blank nanoemulsion) did not decrease any percentage of spot until 12 weeks' treatment. This result proved that vitamin C has a good activity in reducing hyperpigmentation on skin.

Accumulation of excessive epidermal pigmentation leads to various dermatological disorders, such as melasma, freckling, and age spots. Tyrosinase inhibitors in cosmetics are used to prevent hyperpigmentation through the inhibition of enzymatic oxidation.²² Vitamin C and its derivative able to reduce pigmentation by inhibiting tyrosinase to oxidize of DOPA into melanine. Vitamin C concentration in skin will decrease after exposed to sunlight, so it need a continuous application for normalize the hyperpigmentation.²³



Cream

Cream containing 3% of vitamin C start to reduced amount of spot after 5 weeks treatment. Based on statistic test, all cream preparations showed a significant difference to each group ($p < 0.05$). More than 3% vitamin C added to moisturizer cream able to reduce hyperpigmentation on female volunteers after 6 months treatment.²⁴

Nanoemulsion containing vitamin C proved that it was faster to reduce amount of spot on skin compared to cream preparation. Usage of 10% vitamin C nanoemulsion after 12 weeks able to decrease 64% spot while cream of 10% vitamin C decreased 54.67%. Nanoemulsion preparation showed faster and greater

effect in reducing hyperpigmentation. Smaller particles were needed to elevate the penetration of active ingredients in topical application.¹⁵

Conclusion

Vitamin C could be formulated in a stable nanoemulsion spray and cream until 12 weeks of storage. Different preparations and different concentration of vitamin C showed a difference in anti-aging activity. Nanoemulsion spray of 10% vitamin C showed the best anti-aging activity in increasing moisture, smoother the skin and reducing hyperpigmentation on skin compared to 10% vitamin C in cream preparation. In reducing the size of pore, nanoemulsion spray did not significantly different from cream preparation. In other words, cream containing vitamin C effective to reduce size of pore.

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