

## Improving The Penetration of Sodium Ascorbyl Phosphate Using Niosome Span 80 System in Form of Gel in In Vitro

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**Abstract:** To determine the stability and the penetration of sodium ascorbyl phosphate using the niosome span 80 system in the form of gel in in vitro.

**Methods:** The concentration of span 80 was varied into three formulas: Formula 1 (100 $\mu$ mol), Formula 2 (200 $\mu$ mol) and Formula 3 (300 $\mu$ mol). The production of niosome with thin layer hydration method and the test which was conducted including entrapment efficiency, gel stability test and diffusion test in in vitro. Testing the efficiency of entrapment used dialysis method. The niosome suspension was formulated in the form of gel using basic gel of viscolam MAC 10 8%. The stability test was done for 28 days including organoleptic, pH, and determination of the gel concentrate as well as diffusion test using snake skin shed membrane for 8 hours.

**Results:** The entrapment efficiency showed that the optimal concentration of span 80 in formula 1 (100 $\mu$ mol) of 98.7665% was  $\pm$  0.0587. The NAF niosome gel has the most excellent stability compared to NAF gel without niosome in organoleptic, pH and the determination of concentrate. The diffusion test results for 8 hours showed NAF niosome gel can diffuse at 82.6565% was  $\pm$  0.0378.

**Conclusion:** It can be concluded from this study that the NAF by using niosome span 80 system in form of gel can improve the stability and penetration in in vitro using the snake skin shed.

**Keywords:** niosome, sodium ascorbyl phosphate, span 80, penetration.

### Introduction

Sodium ascorbyl phosphate (NAF) is the derivative of vitamin C, commonly used in cosmetic field as an antioxidant. NAF has -4 partition coefficient and hydrophilic compound which makes it difficult to penetrate on the stratum corneum layer<sup>1,2</sup>. Therefore, it is needed an appropriate carrier system to deliver the compound passing through the stratum corneum layer. Carrier system which can be chosen is niosome system. The most used surfactant in niosome system is a class of nonionic surfactants, such as sorbitan monooleate (span 80) with HLB 4.3 which can be used as a composer of niosome system.<sup>3</sup> Niosome can be used as a carrier of medicine which is hydrophilic or lipophilic.<sup>4</sup> Niosome can ensnare medicine so that it can diffuse penetrating membrane lipid bilayer through stratum corneum layer<sup>5</sup>. In the use of span 80 as composer of niosome which can improve the efficiency of drug entrapment and can increase the stability of the medicine<sup>3</sup>. In addition, span 80 can increase the penetration of the medicine into the skin<sup>6</sup>.

In this study, niosome NAF can be formulated in a gel formulation as a carrier. Gel formulation has the advantages compared to the other formulation which are not sticky, easy to clean, do not leave scars and the evaporation of the water causes cool and comfortable effect after being used.<sup>7</sup> This study aims to determine the

concentration of span 80 which is able to ensnare NAF in niosome system optimally so it was expected that it can improve the penetration of NAF niosome in *in vitro*.

## Materials and Methods

The used materials were sodium ascorbyl phosphate (BASF), sorbitan monoleate (Sigma Aldrich), cholesterol (Sigma Aldrich), chloroform, viscolam mac 10, DMDM hydantoin, trietanilamin (TEA), distilled water, dialysis tubing cellulose membrane type D9777-100 FT batch # 3110 with a cut-off of 12000 and the membrane of snake skin removal (*phyton molurus*). The used tools in this study were the glass tools (Iwaki Pyrex®), micropipette (Ecopipette®), Rotavapor (Heidolph type of Hei-VAP), UV-Vis spectrophotometer (2450 Shimadzu type), magnetic stirrer (Rexim RSH-1DR), Microscope (Zeiss star Prima) and camera (AxioCam with J image), *flow through* diffusion-type, a peristaltic pump (Waston marlow 323), round-bottom flask (Iwaki Pyrex®), vacuum, heater (Cimarec), and sonication of bath type (krisbow®).

### The Production of Niosome

The production of niosome with thin layer hydration classical method. NAF, span and cholesterol (in various comparison according to Table 1)<sup>8</sup>.

The formula was varied by three formulas which were made with thin layer hydration method. Span 80 and cholesterol were dissolved in chloroform until soluble and put into 100 mL round bottom flask as well as place on a rotary evaporator at a speed of 150 rpm with temperature of  $30 \pm 2$  °C. Thin layer niosome was hydrated with NAF solution until film layer hydrated film layer to form niosome suspension and particle size reduction was done by using a bath-type sonicator for 15 minutes.

**Table 1 Span 80 Comparison**

Materials	F I	F II	F III
NAF (mg/ml)	10	10	10
Span 80 (μmol)	100	200	300
Cholesterol (μmol)	20	40	60

### Entrapment Efficiency Testing

After the production of niosome, niosome entangled NAF was measured the entrapment efficiency by separation method using dialysis membrane. Niosome suspension was inserted 2 mL into dialysis membrane type D9777-100FT batch # 3110 with cut off 12000. In the medium receivers 50 mL of distilled water was used. The entrapment efficiency testing was conducted for 4 hours and measured the NAF level which was not caught on the receiving medium at a wavelength of 259.00 nm using UV spectrophotometer. The replication was done three times for each formula. The entrapment efficiency could be calculated as follows:

$$EP = \frac{Q_t - Q_c}{Q_t} \times 100\%$$

Description:

EP = entrapment efficiency

Qt = NAF level which was added in the formula

Qc = NAF level in the receiver medium (not absorbed)<sup>10</sup>

### Vesicles Morphological observation

Morphological observation was done using an optical microscope (Zeiss star Primo) and camera (AxioCam with image J) with 100x magnification.

### Formulation Gel

After making a gel base, gel base which had good viscosity which was 8%. NAF niosome gel formulation was made by NAF niosome and DMDM hydantoin into the gel base. Weighed NAF niosome

which is equivalent to 1% NAF based on the efficiency entrapment percentage and DMDM hydantoin as a preservative. NAF gel was made by NAF and DMDM hyantoin which were dissolved into 1 mL water and added into the gel base and stirred until homogeneous.<sup>11</sup> table 3

**Table 3 NAF niosome gel formula and NAF gel**

Ingredients	Formulation I	Formulation II
NAF Niosome (%)	1	-
NAF (%)	-	1
Dmdm hidantoin (g)	0,06	0,06
Gel base (g)	Add 10	Add 10

Description :

Formulation I: NAF niosome gel formulation

Formulation II: NAF gel formulation

### Formulation Gel Stability Test

The investigation of gel formulation stability was done at a temperature ( $28 \pm 2$  °C) on days 0, 1, 3, 7, 14, 21, 28 for organoleptic, pH and NAF level measurement in the gel formulation to see the stability of the formulations during storage for 4 weeks.

The evaluation of NAF niosome gel formulation organoleptic and NAF gel were done by assessing changes in color, texture, smell and growth of microorganisms. The determination of the pH of the formulation was done by using pH meter and NAF level establishment were performed during the storage by weighing 100 mg gel diluted with 10 mL distilled water and stirred by using magnetic stirrer.

The assay is determined by measuring the absorbance at a wavelength of 259.00 nm with a UV spectrophotometer.

### In Vitro Penetration Test with Shed Snake Skin Membrane

Diffusion test was done in in vitro using diffusion cell *flow-through* type and shed snake skin membrane. The used liquid receiver (receptor compartment) was pH 7.4 phosphate buffer with temperature of  $37 \pm 0.5$  °C as much 50 mL, 200 mg weighed gel formulation and placed on snake skin membrane, the receiver fluid was flown through the bottom of skin membrane with a peristaltic pump.

At 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> hour, the 3 ml receiver fluid was taken and replaced with 7.4 phosphate daparr with the same volume. The absorbance of samples were measured by UV spectrophotometer at a maximum wavelength of 257.80 nm. The replication was done 3 times.

The diffused percentage amount could be calculated as follows:

$$\% \text{ Diffusion} = \frac{KA}{KF}$$

KA was the NAF level in the receptor compartment and KF was added formula.

### The Data Analysis

The data analysis was done using SPSS. This experiment was *One Way* ANOVA and Independent-Sample T Test.

## Results and Discussion

### The Production of Niosome

The first stage in this research was the production of niosome by using the thin layer hydration classical method. This method was the most common method used to produce niosome because the formulation method

was easier, quick processing and availability of laboratory equipment. The main composer materials were monooleate sorbitan (Span 80) and cholesterol. Span 80 had long alkyl chain which was expected that NAF could be entangled in the core vesicles and could form thicker vesicles to protect NAF from oxidation and photolysis while cholesterol had the function for stabilizing and preventing the leakage of the vesicles. Cholesterol fills lipid molecules ranks on the double lipid layer of the vesicle.<sup>4</sup>The result of white milk formula 1 suspension with a distinctive odor span 80. Formula 2 looked like solid milk-white suspension and white milk formulation 3 looked more concentrated and viscous because of the increasing number of span 80 which was added.

### Entrapment Efficiency Testing

Niosome entrapment efficiency in this study used the method of separation which was dialysis. The entrapment percentage was calculated from receiver fluid which was assumed that NAF were not entangled in the receiver fluid. The niosome entrapment result in F1 was  $98.7665\% \pm 0.0587$  and F2 was  $98.9575\% \pm 0.0085$ , and F3 was  $98.9096\% \pm 0.1835$ .

One way ANOVA analysis for trapping efficiency of the experimental results showed that the concentration of span 80 had a value of  $p > 0.05$  which meant that changes in the concentration of span 80 was used in the formula did not have significant effect on the niosome system. The concentration span of 80 (100  $\mu\text{mol}$ ) as a formula that gave optimal trapping efficiency.

### Vesicles Morphological Observation

The result of light microscopy (Figure 1) showed round vesicles with varying sizes about 0.88 to 4.87  $\mu\text{m}$ .

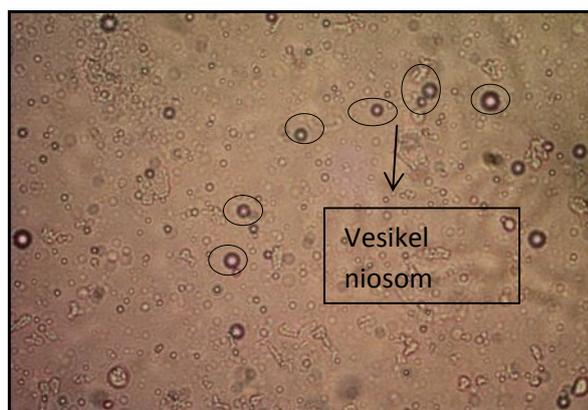


Figure 1. The niosome vesicle result magnification 100x

### The production of gel formulation

The use of MAC 10 viscolam as a base gel, because it could form a soft layer film and give a cooling sensation when it was applied to the skin so it could improve the convenience of NAF niosome gel formulation users. The concentration of the base gel has the best viscosity which was 8%. The goal in using NAF niosome gel formulation was to increase the penetration of medical ingredients into the skin and had a good dispersive power when it was applied to the skin.

### The Stability Test of Gel Formulation

The test was done to see the changes in the two gels which were stored in a period of 28 days. During the period of storage time, the observation was done at 0, 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>th</sup>, 28<sup>th</sup> days which could be seen in Table 5.

**Table 5. The result of organoleptic checking**

Observation	Formula	The duration of observation (Days)						
		0	1	3	7	14	21	28
Color	NAF Gel	+	+	+	+	+	++	++
	NAF niosome Gel	+	+	+	+	+	++	++
Smell	NAF Gel	+	+	+	+	+	+	+
	NAF niosome Gel	+	+	+	+	+	+	+
Texture	NAF Gel	+	+	+	+	+	+	+
	NAF niosome Gel	+	+	+	+	+	+	+
Microbial growth	NAF Gel	+	+	+	+	+	+	+
	NAF niosome Gel	+	+	+	+	+	+	+

Description :

Smell = + (typical odor), ++ (acid smell)

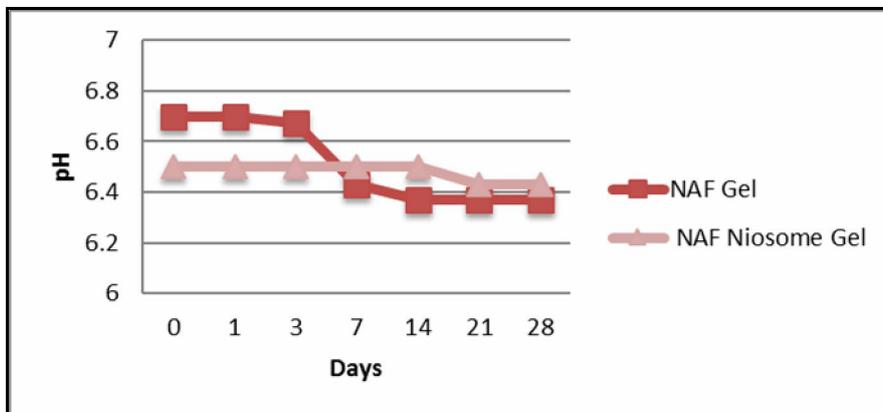
Color = + (clear / transparent) (white milk), ++ (light yellow),

Texture = + (viscous), ++ (less viscous)

Microbial growth = + (no microbial growth), ++ (microbial growth)

The NAF gel results showed there was change in the clear color into light yellow and NAF niosome gel formulations experienced the change of a milky white color slightly yellow during storage at day 21<sup>st</sup> and 28<sup>th</sup>. NAF Niosome gel formulation and NAF gel did not undergo the change in smell and the change in texture as well as did not undergo microbial growth during storage for 28 days. Discoloration was caused by the presence of oxidized NAF portion. Based on the results, it could be concluded that the NAF gel formulation which was made with niosome system was more stable than the NAF gel without niosome.

The PH testing aimed to determine the decrease or increase in the pH value which was suitable and acceptable to the skin during storage of 28 days could be seen in figure 2



**Figure 2. The pH gel testing graph**

The PH test results on the graph showed that the NAF niosome gel formulation had pH value of 6.7 and NAF gel had a pH value of 6.5 so that the value was in accordance with the physiological pH of the skin during 28 days of storage. Theoretically skin pH range was 4.0 to 6.8<sup>12</sup>. NAF gel formulations was made niosome system did not undergo a sizeable decrease while NAF gel showed a sizeable drop in pH. From the results of these data, NAF niosome gel was more stable in maintaining pH compared to NAF gel.

The measurement of gel formulation dosage level was conducted to determine the decrease or increase of active substance level which was contained in the gel formulation during the storage for 28 days. NAF gel formulations was made niosome system experienced the decrease in the level lower compared to NAF gel without niosome. That was because the niosome system could protect NAF from the oxidation process or degradation so it could improve the stability of the gel formulation which could be seen in figure 3.

Based on the results of statistical analysis using Independent T test, it was obtained the measurement results of gel formulation dosage levels which showed significant differences between the NAF niosome gel and NAF gel with  $p < 0.05$ . This showed that the NAF niosome gel formulation was more stable in storage for 28 days than NAF gel without niosome.

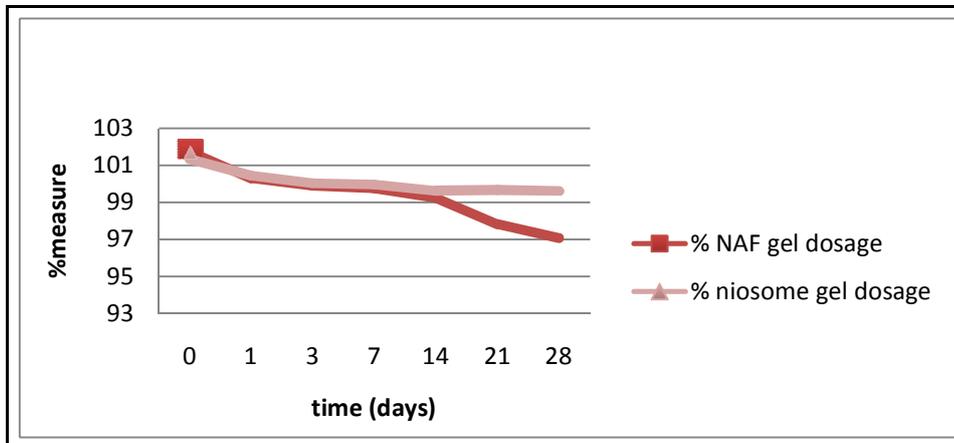


Figure 3. The result of dosage measurement graph

#### Franz Cell Diffusion Test With Shed Snake Skin Membrane

The diffusion test results showed that the NAF gel formulation was made with the NAF niosome system which could diffuse higher than NAF gel that could be seen in Figure 4.

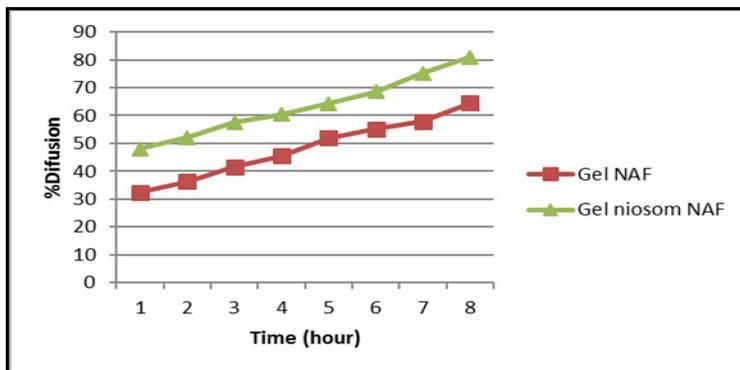


Figure 4. The rate of diffusion profile in the gel formulation

Total percentage of NAF diffusion gel for 8 hours was  $63.0093\% \pm 0.120$  while the number of NAF niosome gel diffusion percentage for 8 hours was  $82.6565\% \pm 0.0378$ . The data were analyzed statistically using Independent T test showed significance value of  $p < 0.05$ . It showed that two formulas were significantly different. There was NAF niosome system which was dispersed in gel formulation which might increase the penetration through the stratum corneum compared to NAF gel formulation without niosom.

#### Conclusion

Based on research that has been done, it could be concluded that the optimum formula which was obtained by entrapment efficiency with dialysis method was formula 1 (100  $\mu\text{mol}$ ) with optimum entrapment efficiency value that was  $98.7665 \pm 0.0587$ . The gel formulation stability test for 28 days showed NAF niosome gel NAF gel was more stable than the NAF gel that was seen from the organoleptic test, the measurement of pH and concentration measurement. NAF niosome gel formulation diffusion test for 8 hours showed NAF was  $82.6575\% \pm 0.0378$  while the NAF gel diffused by  $63.0093\% \pm 0.120$ . NAF which was created in the span 80 niosome system could improve penetration greater than NAF gel formulation without niosome.

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