International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.9, No.2, pp 81-88, 2016

Formulation Vitamin C Using Niosomes System Span 80 In Gel For Increase Stability And Penetration In Vitro

Pratiwi Apridamayanti*, Nina Listiyana, Rise Desnita

Of Pharmacy, Faculty of Medicine, University of Tanjungpura, Pontianak
Jl. Prof. Dr. H. Hadari Nawawi 78124, Indonesia

Abstract: **Abstract:** The purpose of this research is to create drug delivery systems that may improve stability and penetration of Vitamin C by the system niosomes Span 80.
Methods: Span 80 was varied in three concentrations, the formula I (100 mol), formula II (200 mol) and formula III (300 mol). Niosomes manufacture is done using classic methods Hydration Thin Layer. Tests performed include testing the efficiency of entrapment, niosomes morphological observation, stability test and diffusion test *in vitro*. The entrapment efficiency testing was conducted using a dialysis membrane. Niosomes formulated in a gel preparation by using gel base of 8 % HPMC and for the a comparison gel formulation of vitamin C without niosomes was used. Niosomes morphological observation was performed using a light microscope. Test of preparation stability included organoleptic observation, pH testing, and the assay. Diffusion test in vitro used *Franz* diffusion cells.
Results: The results showed the most optimum entrapment efficiency Span 80 concentration was optimum formula I (100 mol) of 99,1243% ± 0,0255. The formed niosomes had measurement of 0,3 to 4 lm. The stability test results for 28 days demonstrated that niosomes gel dosage vitamin C had better stability compared to gel dosage of vitamin C in which the gel niosomes vitamin C had higher levels of 82,7716% ± 5,1312 while the gel vitamin C had higher levels of 71,8330% ± 3,0261. Diffusion test results for 8 hours showed that there was no significant difference between niosomes gel dosage of vitamin C.
Conclusion: The system niosomes Span 80 which can increase the stability of vitamin C.
Keywords: Vitamin C, niosomes, span 80, stability and penetration.

Preliminary

Adult drug substance development is not only in terms of pharmacological activity but also in term of the drug substance stability. The stability of the drug substance is a phase that determines whether it is good or not if the medical ingredient is made into a preparation. In a broad sense stability is defined as the durability of a product during storage and its use for which it still has the same properties and characteristics as at the time of manufacture [(1-2)].

Vitamin C or *L-ascorbic acid* is a vitamin-shaped white or slightly yellow powder, no odor, has a characteristic that is acidic, hydrophilic, or water-soluble [(3)]. Vitamin C has problems of poor stability and easily degraded in oxidation pathways, as well as the constraints that have difficulty penetrating into the skin. One way that can be done to overcome these problems is by using a new drug delivery system that is niosomes[(4-5)].

Niosomes vesicles is a system that can be used as a drug carrier lipophilic, hydrophilic and ampifilik. Niosomes have formed vesicles with a bilayer structure both unilamelar and multilamelar composed of a nonionic surfactant and cholesterol. Niosomes is currently reported to increase drug stability, and increase the penetration of the adsorbed compound across the skin [(6-8)].

Sorbitan monolaurate or Span 80 is a nonionic surfactant that is frequently used as a constituent niosomes. Span 80 has a HLB value of 4.3. The sorbitan monooleate is insoluble in water and soluble in oil, and also stable at high temperatures and toxic [(9)]. In this study, vitamin C will be formulated using a system niosomes in gel dosage form. Gel preparations have an advantage compared with other preparations that provide prolonged contact on the skin, a good spread of drugs, easy to use, and easy to clean.

Niosomes are made using classical methods Hydration Thin Layer. Niosomes obtained will be characterized, will be used to make gel formulation, then be tested for the stability and the penetration using *Franz* diffusion cells. Preparation of vitamin C with niosomes system is expected to improve the stability and penetration in vitro.

Research Methodology

Tools and materials

The tools used in this study were a *vacuum rotary evaporator (Heidolph®),* 100 mL round- bottom flask (Iwaki *Pyrex®),* a set of glasses (Iwaki *Pyrex®), glass* objects, a light microscope (Zeiss Primo Star *AxioCamERc 5s),* desiccator, Sonicator *(Krisbow®),* UV-Vis spectrophotometer (Shimadzu type 2450), analytical balance *(Precisa®),*pH meter *(Hanna pHep®* type *H198107),* pH indicator *(MColorpHast* [TM),] a peristaltic pump *(Watson Marlow 323),* micro pipette *(Ecopipette®), magnetic stirrer (AS ONE Rexim RSH 1-DR), heater (Baby IQ* [TM),] Vortex, and *Franz* diffusion cell types *flow trough.*

The materials used included vitamin C (Aland Jiangsu, *Nutraceutical* CO, LTD *Batch* No. HSA12060002), Sorbitan Monooleate *(Sigma Aldrich),* cholesterol *(Sigma Aldrich),* chloroform, Aquadest, Hydroxy Propyl Methyl cellulose (HPMC 4000), DMDM hydantoin, *cellulose membrane dialysis tubing D9777-100 FT type cut-off* of 12,000, and a removable membrane snakeskin *(Python morulus).*

Manufacture Niosomes

Niosomes were prepared by the method of Thin Layer Classic hydration. Span 80 and cholesterol dissolved in 5 mL of chloroform in a round bottom flask of 100 mL, then it was rotavapored at room temperature (30 ° ± 2 ° C) for 20 minutes at a speed of 150 rpm in a vacuum to remove the organic solvent and to form a layer of thin-layer on pumpkin wall. Next, bottom flask was removed and then inserted into a desiccator and then was vacuumed for 15 minutes and then left to stand for 24 hours. The thin layer was then hydrated with 5 mL of vitamin C at room temperature were carried out at the rotary at a speed of 150 rpm at room temperature (30 ° ± 2 ° C) until all of the thin layers on the walls of the flask forming a homogeneous suspension niosomes. Niosomes particle size was reduced using a *bath-type* sonicator for 15 minutes.

Table 1. Comparison of Vitamin C, Span 80 and Cholesterol

Material	Formula I	Formula II	Formula III
Vitamin C (mg / mL)	10	10	10
Span 80 (mol)	100	200	300
Cholesterol (mol)	20	40	60

Determination Method of Determination of entrapment efficiency

Determination of entrapment efficiency of vitamin C was made by the method of separation using dialysis membranes with a *cut-off* of 12,000. This method was done by inserting a solution of 2 mL of sample into *cellulose membrane dialysis tubing.* Was used as 200 mL of distilled water the medium of the recipient

used which were stirred with a *magnetic stirerr* with a speed of 220 rpm. The measurement of unabsorbed vitamin C level was performed spektrofotometer by UV at a wavelength of 263.1 nm.

Morphological observation Niosomes

Niosomes morphology were observed using light microscope Primo Star Zeiss type *AxioCamERc 5s*. Observation by light microscopy was done by dripping niosomes on the glass object which was then observed.

Gel formulation preparation

Niosomes gel preparation of vitamin C and vitamin C gel was made by mixing the gel base that had been optimized. At niosomes gel dosage of vitamin C, vitamin C niosomes number was entered into a gel dosage of vitamin C is equivalent to 1% calculated based on the percent esifiensi niosomes the adsorption of vitamin C. Preparation of gel vitamin C made by dissolving vitamin C with distilled water while stirring until homogenous. Then vitamin C has been dissolved is added to the gel base.

Table 2. Formula Gel preparations Niosomes Vitamin C and Vitamin C Gel preparations

Material	Vitamin C gel Niosomes	Gel Vitamin C
Niosomes vitamin C (mg)	10	-
Vitamin C (mg)	-	10
DMDM Hydantoin (g)	0.06	0.06
Base Gel (g)	add 10	add 10

Stability Test

Stability test against niosomes gel dosage of vitamin C and vitamin C gel was made by doing observations and measurements every day for 28 days, on days 0, 1, 3, 7, 14, 21, and 28. The test conducted included organoleptic test, the measurement of pH and the determination of levels of vitamin C in the preparation of the gel.

Organoleptic testing preparations included color, odor, consistency and growth of microbes. Gel preparations organoleptic test carried out before and after the storage conditions at 30 ° C ± 2 º together. PH measurements of each preparation were done by dipping into the gel preparation pH meter. Determination of vitamin C in the preparation of gels was made with each gel niosomes vitamin C and vitamin C gel weighed 0.1 grams, then diluted with distilled water in a flask add 10 mL then stirrer with a speed of 500 rpm until homogeneous. The uptake of the solution was measured by spektrofotometer UV at a wavelength of 263,1 nm and the levels were calculated by using the calibration curve equation. In the process of preparation and measurement of absorption, the soil sample solution should be avoided from light.

Diffusion Test

Diffusion test performed in vitro using *Franz* diffusion cell types *flow through.* Removable membrane snakeskin *(Python morulus)* which has been prepared placed between two parts of Franz diffusion cell with removable membrane snake skin facing the donor compartment. Was used phosphate buffer pH 7,4 50 mL as a donor compartement. The preparation gel weighed as much as 0,2 grams then applied to the surface membrane of snake skin, the receptor solution would then be fed from the bottom of the snake skin membrane using a peristaltic pump at a speed of 64 rpm. On the hour to 0.5, 1, 2, 3, 4, 5, 6, 7, and 8. Samples were taken as many as 5 mL of solution of the receptor compartment and replaced with phosphate buffer pH 7.4 with the same volume. It then assayed by UV at a wavelength of 265,6 nm.

Results and Discussion

Manufacture Niosomes

Niosomes manufacture was done by using classic methods Hydration Thin Layer. Thin Layer Selection hydration method due to the manufacturing process of this method was faster, easier and simpler to use. The main components were a nonionic surfactant niosomes manufacture and cholesterol. In this study, the nonionic surfactant used was sorbitan monooleate or better known as Span 80. Span 80 was used because it has a long alkyl chain which was expected to form a vesicle that was thicker to protect the active substance that was very easily oxidized. The concentration of surfactant used for manufacturing niosomes mol was the range of 100 - 300 mol. On the other hend cholesterol was used to prevent leakage of the vesicles by filling out the ranks of the double lipid molecules formed in vesicles [(10)]. Niosomes produced had distinctive smell like the smell of Span 80 beside, it was it creamy white and has an acidic pH is 3,5. Determination formula to be selected and used was based on the percentage of the highest efficiency of entrapment.

Determination of entrapment efficiency of Vitamin C

Determination of entrapment efficiency of vitamin C was conducted using a dialysis membrane. The principle of the method of dialysis was determining the levels of vitamin C that was not entangled in niosomes. Vitamin C that was not entangled would go out through the pores of the dialysis membrane with a *cut-off* of 12,000 which then was assayed using a UV spectrophotometer. Data results obtained in the determination of entrapment efficiency can be seen in Table 3.

Table 3. Percentage of entrapment efficiency *(± SD, n = 3)*

Formula	Span 80: Cholesterol (mol)	Drug entrapment efficiency (%)
A	100: 20	99,1243 ± 0,0255
B	200: 40	99,1218 ± 0,1386
C	300: 60	99,0163 ± 0,1807

Based on the table above, it can be seen that the percentage of entrapment optimum efficiency was on the formula A with a concentration of Span 80 and cholesterol (100:20 mol) of 99,1243%. The results showed that the percentage of drug entrapment efficiency after 3 times replication in each formula was relatively the same or not much different. The result of statistical data was acquisitioned and then analyzed using *One-Way ANOVA* in SPSS to determine niosomes formula that produced optimum drug entrapment. The analysis results obtained on the three niosomes formula was there was not a significant difference. It can be seen to be significant at p values > 0,05 is 0,555.

Morphological observation Niosomes

Niosomes resulting vesicles have a size between 0,3 to 4 lm. Niosomes morphological observations can be seen in Figure 1.

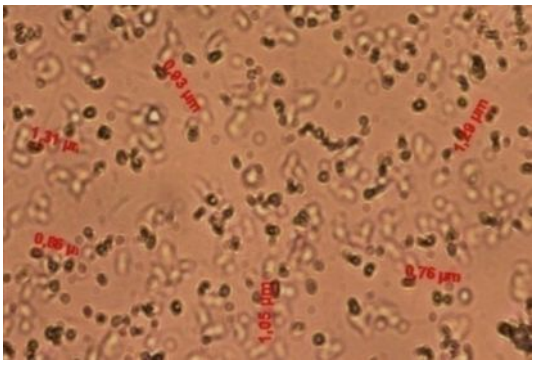

Figure 1. The observation of morphology Niosomes (magnification 10x)

Gel formulation preparation

Gel dosage of vitamin C and vitamin C niosomes gel were made using a base gel with a concentration of 8 %. Preparation of a gel vitamin C was made by dissolving vitamin C with distilled water stirred until homogeneous. Then, it was mixed with a gel base and then added with *DMDM Hydantoin*. *DMDM Hydantoin* is used as a preservative commonly used in cosmetic preparation and the most effective against microbes because it has antimicrobial activity of broad spectrum [12]. *DMDM Hydantoin* is also a preservative that is compatible with the surfactant is a nonionic.

Stability Test

Organoleptic observations made include changes in color, odor, consistency, and the growth of microbes. The observation of organoleptic was very important observation to be done due to the purpose of seeing see if there is a change during storage for 28 days. Data organoleptic observations can be seen in Table 4.

Table 4. Data Observations organoleptic 28 Days

Stability Test	Formula	Days to-						
		0	1	3	7	14	21	28
Color	GV	+++	+++	+++	+++	++	++	+
	GN-V	+++	+++	+++	+++	+++	++	+
Odor	GV	+++	+++	+++	+++	+++	+++	+++
	GN-V	++	++	++	++	++	++	++
Consistency	GV	++	++	++	++	++	+	+
	GN-V	++	++	++	++	++	++	++
Microbial growth	GV	+++	+++	+++	+++	+++	+++	+++
	GN-V	+++	+++	+++	+++	+++	+++	+++

Description :
GV : Gel vitamin C
G-NV : Gel vitamin C niosomes
Color : (+) Yellow, (++) slightly yellow, (+++) clear
Odour : (+) sour smell, (++) distinctive smell Span, (+++) no odor
Consistency : (+) is not thick, (++) thick, (+++) very viscous
Microbial growth: (+) no, (++) there is little, (+++) no

Based on these data it can be seen that there was a difference of two gels were made. In observation of the color between formula niosomes gel dosage of vitamin C and vitamin C gel, there was a change in the color at day 21 to 28. The color change that occurs was dosage change color to yellow. This could occur because some of the active substance had been oxidized. On observations up to 28 days there was no change in the smell of the gel formulation niosomes vitamin C and vitamin C. Observation of consistency gel preparation could be seen in formula niosomes gel dosage of vitamin C did not change. On the other hand, the gel dosage vitamin C which on day 21 turned into a little melted or not lumpy. Organoleptic observations after 28 days, there were the microbes that grew on both the gel preparation.

PH test is a test conducted to determine the chemical stability. PH observations for 28 days can be seen in Table 5. Based on these data we can see there had been a decrease in pH between niosomes gel dosage of vitamin C with gel preparations of vitamin C. Vitamin C gel preparation pH changed from day 0 to 28 ie of pH 5 to 4,1, while the gel preparation niosomes changed pH from pH 5 to 4,4. The observation pH gel formulation niosomes vitamin C and gel formulation of vitamin C, both still in the pH range of the skin, but it can be concluded that the gel preparation niosomes vitamin C is a preparation which more was stable due to changes in pH smaller than the gel formulation of vitamin C after test for 28 days.

Table 5. Results of Measurement of pH For 28 days *(± SD, n = 3)*

Days to-	Formula	
	Gel vitamin C	Niosomes gel vitamin C
0	5,1 ± 0,0577	5,0 ± 0,0577
1	4,9 ± 0,1000	5,0 ± 0,0577
3	4,9 ± 0,1155	4,9 ± 0,1155
7	4,4 ± 0,0000	4,9 ± 0,1528
14	4,3 ± 0,1155	4,6 ± 0,1732
21	4,1 ± 0,1155	4,4 ± 0,1155
28	4,1 ± 0,1155	4,4 ± 0,1155

The assay was conducted to determine levels of vitamin C in the preparations made and very important. Assay results obtained shown that the levels of vitamin C in preparation niosomes gel gel dosage of vitamin C and vitamin C decreased levels at any time during the 28 days. This can occur because of the nature of vitamin C which is very easily oxidized and unstable. Gel formulation made with niosomes system capable of protecting the active substance from the oxidation process so as to maintain the stability of vitamin C. But not in gel dosage of vitamin C are not protected by niosomes vesicle system, causing instability in the active substance, causing a decrease in the levels. Data from the assay were analyzed by SPSS using test *Independent-Sample T-Test*. Test *T-Test* aims to compare between vitamin C preparations niosomes gel and gel dosage of vitamin C. Based on the analysis results obtained showed that the gel preparation niosomes gel dosage of vitamin C with vitamin C after a stability test on the 28th day has a significance value of $p < 0,05$ is 0,034, indicating that the two preparations are significantly different gel where the gel niosomes vitamin C had higher levels of 82,7716% ± 5,1312 while the gel vitamin C had higher levels of 71,8330% ± 3,0261. It can be concluded that the gel preparation niosomes vitamin C levels have better stability compared to gel dosage of vitamin C.

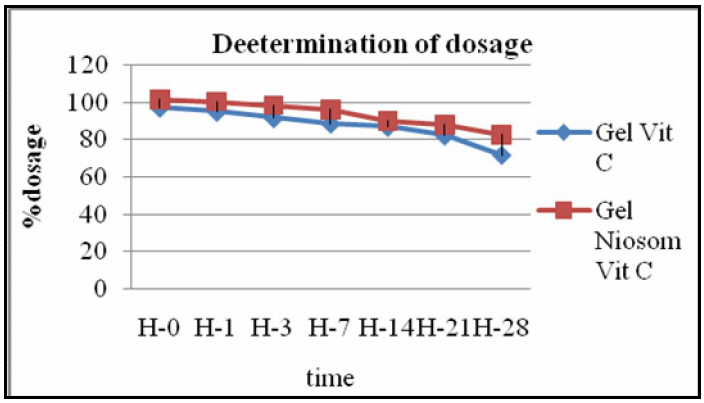

Figure 2. Graph Results Determination of Levels of preparations Niosomes Gel Vitamin C and Gel Vitamin C.

Diffusion Test

Diffusion test performed *in vitro* using *Franz* diffusion cell types *flow through*. This test was conducted to determine levels of vitamin C in a gel formulation of vitamin C and vitamin C gel niosomes which penetrated through the skin. Snake skin removable membrane used for the membrane can describe the structure of the stratum corneum similar to human skin. Also, snakes can release the skin on a regular basis so as to provide a removable repeated skin and snake skin can be obtained without harming the animal [(13)].

Based on the test results performed diffusion can be seen in Figure 3 that gel dosage vitamin C has a diffusion percent higher than the gel niosomes vitamin C. The low percentage of gel diffusion of niosomes vitamin C can be occured due to several factors: active substances are trapped in the vesicle niosomes proved difficult to penetrate the membrane so that the active substance which penetrated into fewer and possibly

niosomes resulting vesicles have a large size so that the active substance which penetrated into fewer. Results diffusion test data were analyzed using *Independent test-Sample T-Test*. *T-Test* test was aimed to compare between the gel and gel niosomes vitamin C vitamin C. Based on these tests, it showed that the significant value of gel vitamin C and vitamin C is a gel niosomes p> 0,05 is 0,560, which means on both the gel perfomed did not differ significantly where the dosage of vitamin C gel diffused niosomes of 18.6368% while the gel dosage of vitamin C of 24,3445%.

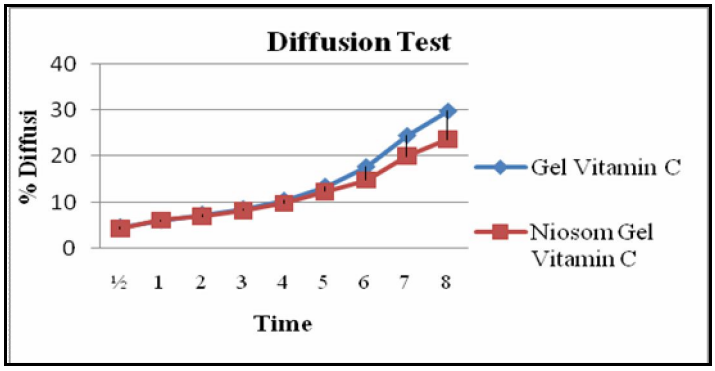

Figure 3. Graph diffusion Test Results

Conclusion

Based on research that has been done, it can be concluded that the concentration span 80 that can adsorb vitamin C in optimum niosomes system is 100 mol which gives percent entrapment of 99,1243% ± 0,0255. The preparation gel with niosomes system can improve the stability of vitamin C significantly by organoleptic testing, pH and the levels, compared to the gel dosage vitamin C without system nisom where gel niosomes vitamin C had higher levels of 82,7716% ± 5,1312 while the gel vitamin C had higher levels of 71,8330% ± 3,0261**.** Test diffusion for 8 hours showed that no significant differences where niosomes gel dosage of vitamin C.

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