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Natamycin Nanosuspension For Ophthalmic Drug Delivery System

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ABSTRACT

Natamycin is an antifungal isolated from *Streptomyces natalensis* Sp. in ophthalmic drug delivery system, drug is expected to have prolonged retention time in precornea to increase it bioavailability. One method to prolong the precornea retention time is by preparation mucoadhesive dosage form. The aims of this study is to develop of in situ gelling system formulation consist of natamycin for ophthalmic drug delivery system. In this study, in situ gelling made in the form of nanosuspension using Tween 80, PEG 400, benzalconium chloride and sodium alginate. Evaluation done for morphology and particle size analysis, the value of polidispersity index, and physical stability. Nanosuspension is also evaluated for eye irritation, diffusion and mucoadhesive. The optimum nanosuspension obtained in this study was consist of 8.7% PEG 400, 0.25% of sodium alginate and 10% of Tween 80. The particle size of this suspension was still in the range of nanosuspension the 7th day, then the particles size increased until in the range of micro size which were 1.3-2.6 µm until 84 days. This dosage form was not cause irritation to the eye of tested animal. The amount of natamycin diffused from nanosuspension was 17.08+0.22% after 24th hours.

Keywords: Natamycin, in-situ gel, mucoadhesive, nanosuspension.

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INTRODUCTION

Eye protection is one of the mechanisms that causes low absorption and short duration of therapeutic effect the drug in the eye, so repeated administration need to prolong the effect of the drug. When the drug solution is dripped into the eye, spending tears and blinking process causes a decrease drug concentration in the eye. Naso-lacrimal flow system also causes the pass through naso-lacrimal duct which caused a variety of side effects [1]

Ocular therapeutic effect will be increase significantly if residence time of the drug in the pre-corneal is longer. Some preparations have been developed for ophthalmic use, not only to increase the contact time on the surface, but also decrease drug elimination. Polymer used to form a gel in the eye that would increase the residence time in the pre cornea as delivery systems and bioavailability [1]

Natamycin effective antifungal for various types of yeast infection and filament fungi, including Candida, Aspergillus, Cephalosporium, Fusarium and Penicillium. Topical administration is effective in giving the cornea due to a fungal infection that occurs on the surface of the eye [2]

Sodium alginate is one of the gelling agent used in ophthalmic formulations because biodegradable and non-toxic. The use of sodium alginate is able to prolong the residence time of the drug in pre cornea through gel formation and mucoadhesive properties. This gel formation made by the crosslink from this polymer with calcium ions in tears fluid [3]

The aims of this study is to develop formulation of in situ gelling system consist of natamycin for ophthalmic drug delivery system.

MATERIALS AND METHODS

Chemical materials:

The materials used in this study is natamycin (PT. Cendo Pharmaceutical Industries), Tween 80, polyethylene glycol, sodium alginate, sodium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, potassium chloride, sodium bicarbonate, benzalkonium chloride, simulated tears fluid and deion water.

Instruments:

The instrument used in this study is *ball-mill*, magnetic stirrer (IKA®, RW 20 Digital), ultra turrax (IKA, T25), *probe* sonicator (Misonix, S-4000), evaporator (Heidolph, VV2000), spectrophotometer UV-visible (Beckman, DU720®), particle size analyzer (Delsa™*Nano C Particle Analyzer*, Beckman Coulter), *Scanning Electron Microscopy* (SEM), pH-meter (Mettler Toledo®, S20 dan Beckman®, Φ50), viscometer (Brookfield®, DV-I+ and Hoeppler), micropipette (Scoorex®), *vortex mixer* (IKA®, Genius 3), Texture Analyzer and *climatic chamber*.

Methods:

Formulation of Nanosuspension

Natamycin made using Tween 80, PEG 400, bezalkonium chloride and sodium alginate. Previously, done the optimization of the concentration of sodium alginate gel as a base, then the optimization time with ball-mill as a method to reduce particle size. The particle size natamycin milling results still microsized so we need another process to decrease particle size like combination of ultra Turrax -sonication which the time and speed was optimized.

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Evaluation of Nanosuspension

Evaluations were done includes organoleptic, pH, viscosity, concentration, morphology (SEM), particle size and polydispersity index, stability test at room temperature and climatic chamber, mucoadhesive test, diffusion and irritation test.

RESULTS AND DISCUSSION

Formulation of Nanosuspension

The optimization of concentration of sodium alginate as gelling agent made by mixing 0.125, 0.25%, 0.5%, 0.75% and 1% into a solution containing surfactants and benzalconium chloride, and then observed visually separation and viscosity for 28 days. The results showed that sodium alginate 0.25% is the optimal concentration for in situ gel formation, not separation and has a viscosity that is suitable for the eye is 15-20 cP[1]

Then, the optimization of grinding time had been done using -mill at a steady speed and time variations 0.5-6 hours. The powder then dispersed in cosurfactant (PEG 400) 8.7%, surfactant (Tween 80) 10%, 0.01% benzalkonium chloride and sodium alginate 0.25%. The results of particle size analysis shown in Figure 1.

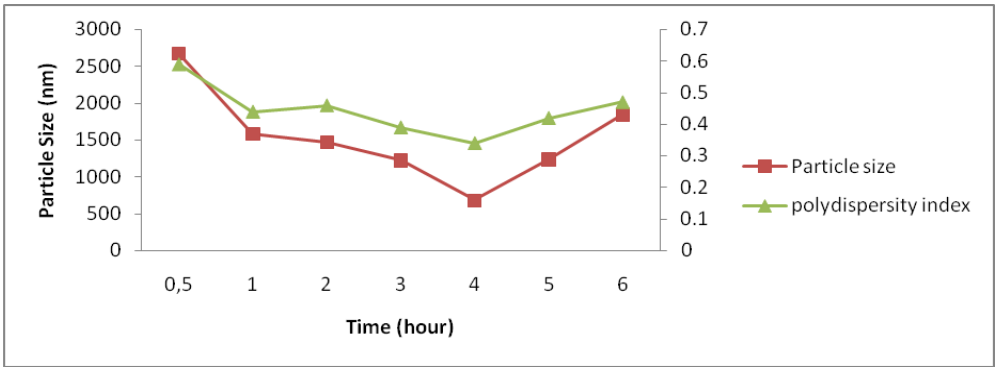
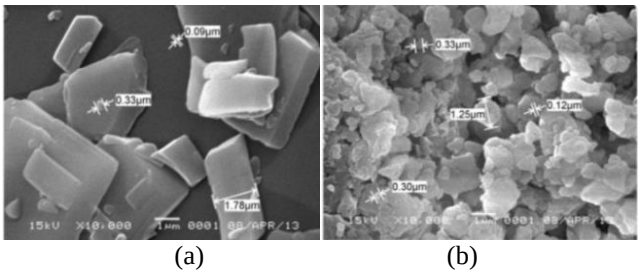


Figure 1. The effect of milling time on particle size of natamycin nanosuspension

On the optimization of the grinding process obtained optimum time is 4 hours to produce nanosuspension with particle size is about 694.2 nm. When the grinding time is increased to 5 and 6 hours, the size of the particles produced 1435.0 nm after milled for 5 hours and 1821.5 nm after milled for 6 hours. This is because the aggregation back natamycin powder that has been refined for stronger cohesion between particles of natamycin. Natamycin powder particle size before and after grinding four hours can be seen in Figure 2.



(a) (b)

Figure 2. SEM of Natamycin: a. before milling b. after milling for 4 hour

The particle size measuring 4 hours milling results > 1µm, so the particle size must be decrease again with a combination of ultra turax-sonication. Optimization with ultra Turrax done with variations of speed and different times, i.e 12,500 rpm for 15, 30 and 60 minutes, and 350 rpm for 15, 30 and 60 minutes. At 12500 rpm for 15, 30, 60 minutes of the obtained particle size > 4000 nm, whereas at 350 rpm for 15 and 30 minutes the obtained particle size is 2671.0 $\pm$ 85.40 nm and 1209.0 $\pm$ 48.09 nm in a row, but at 350 rpm for 60 minutes the particle of suspension obtained nano size is 685.87 $\pm$ 14.43 nm. The results of optimization of sonication time can be seen in Table 1.

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Tabel 1 Particle size in variation sonication time

Sonication time (minute)	Particle size after formulated in suspension (nm)	Polydispercity index
15	751,43±27,67	0,37±0,02
30	660,20±84,57	0,34±0,01
45	800,27±72,34	0,36±0,03

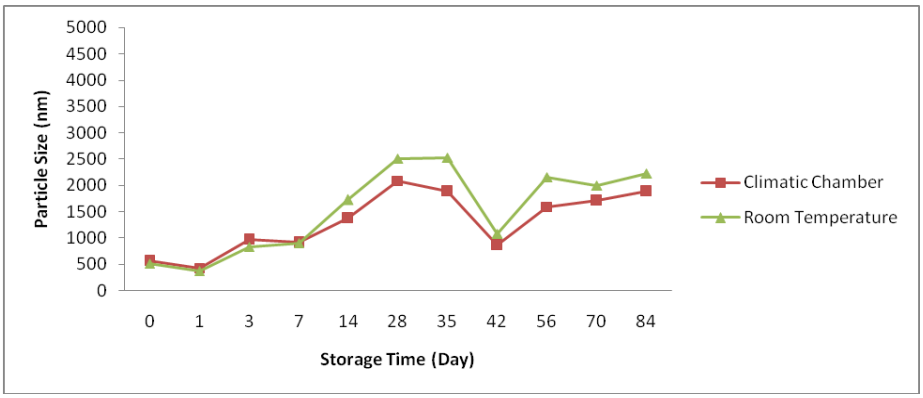
Evaluation of Nanosuspension

Evaluation for this preparation include the visual appearance, pH, particle size, polydispersity index and stability at room temperature (25 ± 20^{0}C, RH 70 ± 5%) and climatic chamber (temperature 40 ± 20^{0}C, RH 75 ± 5%) .

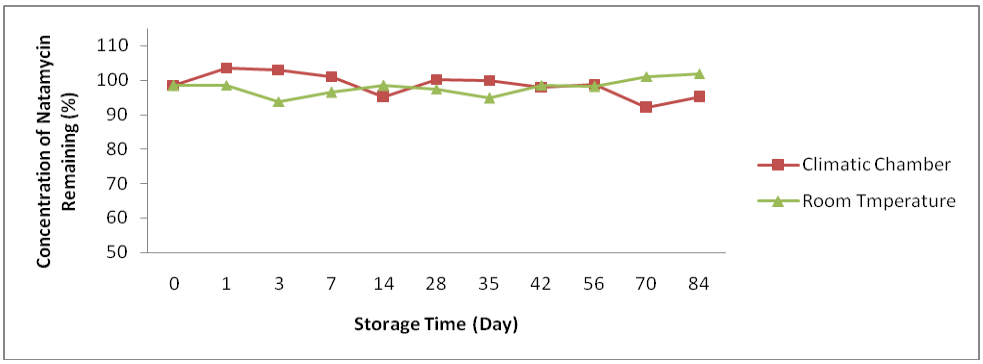
Nanosuspension preparations stored in the climatic chamber changes its color to be dark brown, while stored at room temperature remained stable colour. This changes occured because the concentration of natamycin in that preparation was decreased with the remaining 94.99% concentration due to decomposition natamycin in this preparation because has pH 5.5 [2].

Stability Test Nanosuspension

Stability tests performed include: particle size, concentration and pH shown in Figure 3.



(a)



(b)

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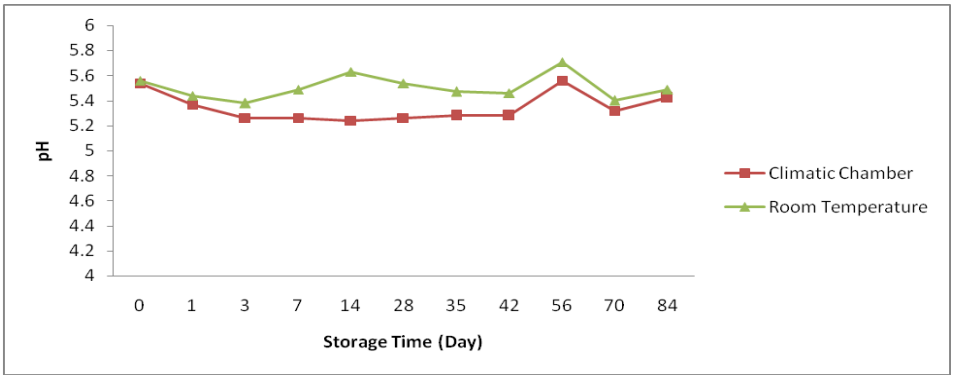

(c)

Fig 3. Results of Stability Test (a) particle size (b) concentration and (c) pH

The particle sizes were observed during stability test were compared statistically by an analysis of variance (ANOVA) with the following hypotheses:

H_0 = there is no difference between the particle size of samples stored at room temperature and climatic chamber
H_1 = there is a difference between the particle size of samples stored at room temperature and climatic chamber

Table 2. Statistical analysis of particle size in ANOVA

Time	Particle size (nm)	
	climatic chamber	suhu kamar
0	554,20	516,83
1	424,13	370,87
3	961,93	829,30
7	908,70	905,63
14	1372,63	1724,40
28	2083,97	2510,20
35	1883,40	2523,77
42	855,97	1079,53
56	1595,37	2160,17
70	1703,73	1998,30
84	1884,57	2225,70

Statistical analysis with 95% confidence level ($\alpha = 0.05$) showed that of F_{hit} (2,32) $<F_{table}$ (4.35) so H_0 accepted and H_1 accepted, This mean that there is no difference between the particle size of samples stored in the climatic chamber and room temperature.

During the stability test at a temperature of 40^0C, concentration of natamycin in the preparation changed, remained 94.99% and visible to the color change becomes dark brown. To determine the self-life this preparation cannot be done from the accelerated test, but should the long-term test. This is common for natamycin preparation at pH of about 5.5, causing decomposition of natamycin and rising temperatures will increase the speed of decomposition. pH nanosuspension relatively stable during storage with the value of 5.24 to 5.71 and still in range of ionization constants natamycin at 4.6 and 8.36 [4].

Mucoadhesive force was done to determine the force required to separate the preparation of the cornea. The test was done using the Texture Analyzer modified. Corneal goat soaked 12 hours with artificial

tears and then attached to the bottom and dropped with this preparation, this cornea was close with the other cornea in the inverted position. The mucoadhesive force yield is 11.14 ± 0.93 N / cm^2.

Diffusion test was performed using a goat corneal because this cornea has similar shape, size and structure of the human cornea [9]. From Figure 6 shows that the levels of natamycin that diffuses until the 24th hour of the preparation nanosuspension is 17.08 ± 0.22%.

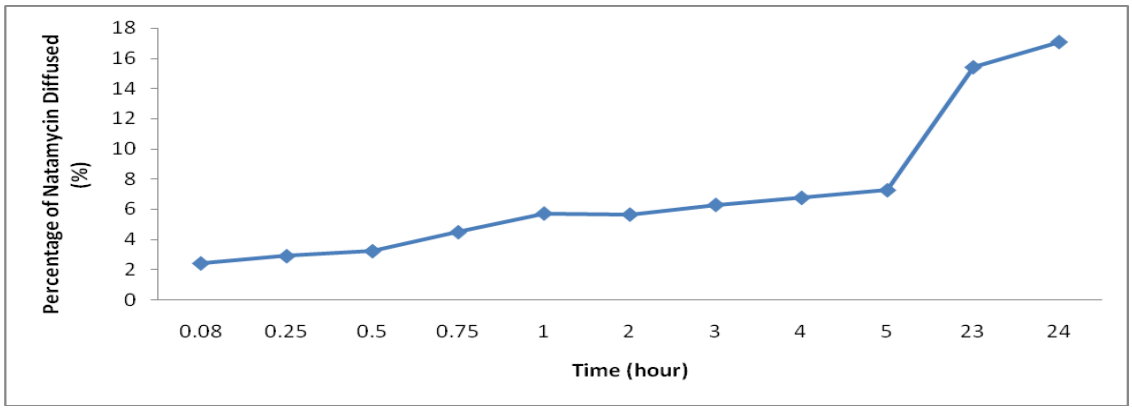

Figure 4 Graph of diffusion test on nanosuspension preparation

Natamycin nanosuspension is very difficult to penetrate the cornea because its solubility in non-polar solvents are very small compared to their solubility in the polar solvent (methanol). While the diffusion test is only using artificial tears media that is not a good medium for dissolving natamycin.

Corneal membrane can be passed by a 100-200 nm-sized particles through paraselular, while these preparations have particle size > 600 nm and therefore can not pass through tight junctions of the cornea, causing the amount of drug that can pass through the cornea very little[5].

Suspension preparation made by one factory in Indonesia and nanosuspension preparations made by one of the other country. Generally dosage existing conventional dosage form and not a mucoadhesive preparation so that the residence time in pre cornea less time than mucoadhesive gel formulation in sodium alginate as a based. Preparations with sodium alginate gel base capable of increasing the residence time of the drug in pre cornea through its mucoadhesive properties, that occurred from cross link between the calcium ion (Ca^{2+}) with the alginate monomers to form egg-box structure [3].

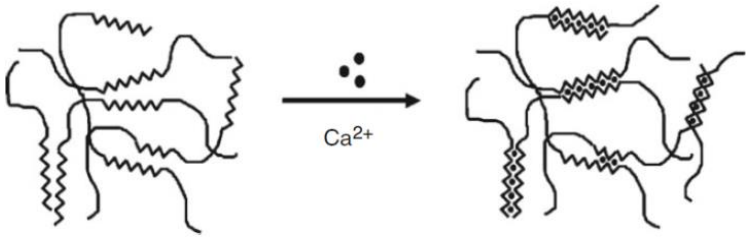

Figure 5. Formation of egg-box structure from the interaction of sodium alginate with calcium ions [3]

The formation of these structures that cause changes in the form of a liquid suspension into a gel when it interacts with calcium ions in the fluid of the eye.

The next evaluation is irritation test to test animals for 72 hours and the result that the eyes of test animals was unchanged during the test and there was no difference between the right eye (with treatment) with the left eye as a negative control (no treatment). [6]

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Table 2 Total Scores for Ocular Irritation [7]

Parameter	Time after treatment (hour)			
	1	24	48	72
CORNEA				
E = Degree of Opacity	0	0	0	0
F = Area of Cornea Involved	0	0	0	0
Score (E x F) x 5	0	0	0	0
IRIS (D)	0	0	0	0
Score (D x 5)	0	0	0	0
KONJUNGTIVA				
A= Redness	0	0	0	0
B = Chemosis	0	0	0	0
C = Discharge	0	0	0	0
Score (A + B + C) x 2	0	0	0	0
Total Score	0	0	0	0

Score for each section then summed and given criteria according this table:

Table 3 Classification of irritation [12]

Maximum Mean Score	Classification of Irritation
0,0 - 0,5	Non-irritant
0,6 - 2,5	Practically non-irritant
2,6 -15,0	Minimal Irritant
15,1 - 25,0	Mild Irritant
25,1 - 50,0	Moderate Irritant
50,1 - 80,0	Severe Irritant
80,1 - 100,0	Very Severe Irritant
100,1 – 110	Extremely Severe Irritant

Irritation test results showed a total score of zero for the second test animal because it is not irritation for 72 hours of observation, it means that this preparation safe for used.

CONCLUSIONS

The optimum formula of nanosuspension using 8.7% PEG as co-surfactant, 0.25% sodium alginate and 10% Tween 80 yield nanosuspension with particle size 681.33±10.22 nm. This particle size is stable less from 1 µm during storage until 7 days, but this size increase in range 1,3-2,6 µm until day 84th. This preparation has mucoadhesive force 11.14±0.93 N/cm^2 . I*n vitro diffusion test showed that until* 24th hour, amount of natamycin which penetrated into cornea is 17,08±0,22%. Irritation test result that this preparation not irritant.

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