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Formulation and Evaluation of Ketoconazole Nano-suspension

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Abstract : The use of antifungals that are widely used by the public is ketoconazole. Ketoconazole is included in the Biopharmaceutical Classification System (BCS) II category, where the drug is poorly soluble in water but has high permeability. In this study, ketoconazole was prepared in a nanosuspension formulation increase solubility. The method for preparing 2% ketoconazole nanosuspension used a sonicator bath with variations of tween 80 (F1 8%, F2 10%, F3 12%) and transcutol as surfactant and co-surfactant. The results showed that the particle size of the ketoconazole nanosuspension F1 was 102 ± 4.3 nm, F2 was 105 ± 3.2 nm, and F3 was 90 ± 2.4 nm on day 0. Testing on day 28 showed an increase in particle size in F1 115 ± 3.1 nm, F2 129±4.5nm, F3 97±2.4nm ($p>0.05$). In testing the morphology of the nanosuspension using Scanning Electron Microscopy (SEM), The results showed that the irregular spheres were scattered. Organoleptic testing showed clear and homogeneous preparations. pH testing for 28 days showed a pH range of 5.6-6 which is included in the physiological skin pH range ($p>0.50$). Nanosuspension preparations can be said to meet the physical evaluation requirements.

Keywords: BCS II, Ketoconazole, Nanosuspension, SEM, Permeability

1. INTRODUCTION

The fungal disease of the skin is a disease that is still commonly found in Indonesia [3]. Fungus treatment that is often used by the community is the azole group, one of the drugs is ketoconazole 2%. Azole antifungals have a broad spectrum that can be well tolerated by the body. Ketoconazole can inhibit the growth or death of fungal cells by causing irregularities in the fungal cytoplasmic membrane [6]. Ketoconazole is included in the Biopharmaceutical Classification System (BCS) class II, which is a class of drugs that are poorly soluble in water, have high permeability [2]. Ketoconazole preparations on the market are in the form of creams and tablets. Absorption of ketoconazole orally is not maximized due to low solubility and side effects. Drug ingredients that are poorly soluble in water show imperfect absorption [11].

Nanotechnology is a form of new technique in the world of health that can optimize a drug delivery system [7]. One of the nanotechnology that has been developed for drug delivery is nanosuspension [13]. Nanosuspension can help increase the solubility and bioavailability of class II BCS drugs. Nanosuspension is a colloidal dispersion system that contains drugs with a particle size of 10-1000 nm dispersed with surfactants or polymeric materials as stabilizers [12]. In a previous [14] study regarding the manufacture of Paracetamol suistained release nanosuspension preparations, the particle size distribution of formulation was obtained in narrow ranges from 145nm to 86 nm and the average particle size was found to be 95±8 nm. At 15 minutes,

54.081% of drug was released from the pure drug in water, 29.050% of drug released from NS1, 27.250 % drug released from NS2 and 14.725% of drug released from NS1.

In this study, ketoconazole nanosuspension was prepared using a sonicator bath to reduce particle size. The preparation of this preparation requires consideration in the selection of stabilizers in the form of surfactants, polymers, or a combination of both to increase solubility by reducing the surface tension of a drug [9]. This study used a non-ionic surfactant, namely tween 80 which is non-toxic and non-irritant, and co-surfactant in the form of transcutol. This study aims to evaluate the variation of tween 80 in ketoconazole nanosuspension preparations related to particle size, polydispersity index, organoleptic, SEM, pH, and room temperature stability.

2. MATERIALS AND METHODS

2.1. Materials

The tools used in this study include magnetic stirrer (SH-2), sonicator bath (WL-963), Particle Size Analyzer (PSA) (LS 13-320), pH meter (Atc), SEM (FEI-S50), analytical balance (M5-M214A), and other glassware. The materials used in this study included Ketoconazole (Dexa Medica), tween 80 (MagLab), Transcutol (AvenaLab), 96% ethanol (Onemed), and distilled water (MagLab).

2.2. The Nanosuspension Preparation

In the process of making nanosuspension preparations, a modified procedure was carried out from research conducted by Dzakwan [4]. The design of the nanosuspension spray preparation formula:

Table 1. Ketoconazole Nanosuspension Formula Design

Ingredients	Formula 1	Formula II	Formula III
Ketoconazole	2%	2%	2%
Tween 80	8%	10%	12%
Transcutol	2%	2%	2%
Aquadest	ad 100%	ad 100%	ad 100%

The process of making 30 ml of nanosuspension spray preparations, the ingredients are weighed according to the concentration of each ingredient. The ketoconazole and tween 80 materials were put into a beaker and mixed using a magnetic stirrer for 15 minutes. Then the transcutol was put into the mixture in the beaker glass, then stirred again with a magnetic stirrer for 15 minutes until all the ingredients were mixed homogeneously. Furthermore, the mixture was reduced in particle size using a sonicator bath frequency 50 Hz with temperature 25-30℃ for 60 minutes. Then, aAfter the nanosuspension is formed, visually the preparation is transparent, up to 30 ml of aquadest is added to the nanosuspension preparation and stirred using a magnetic stirrer for approximately 15 minutes until it is homogeneous.

2.3. The Evaluation of Ketokonazole Nanosuspension :

2.3.1 The Organoleptic Test

The organoleptic test of the nanosuspension was carried out by visually observing the shape, aroma, and color of the nanosuspension preparation [16].

2.3.2 The Particle Size Test and Polydispersion Index

Particle size testing was carried out with a PSA tool by means of 1 ml of nanosuspension preparation added with 10 aquadest then put into the cuvette up to the mark, then read with the PSA tool. The results obtained are in the form of particle size values and polydispersity index graphs [6].

2.3.3 The Particle Morphology Test

The morphology of the nanosuspension particles was measured using an SEM tool by preparing a platinum plate and placing the sample on its surface. Then the plate is inserted into the SEM tool for approximately 15 minutes. SEM tools that are connected to a computer and observed at a voltage of 7 kV at a magnification of 40,000 times [6].

2.3.4 The pH test

The pH test was carried out using a pH meter beginning with calibrating the electrode with a standard pH buffer of 4.7 and 9. After the pH display was appropriate and stable, the electrode was dipped into the preparation. The pH value will appear on the screen when the number is stable. The pH value required for topical preparations on the skin is 4.5-6.5 [15].

2.3.5 The Stability Test

Testing the stability of the ketoconazole nanosuspension preparations was carried out by placing the preparations at room temperature conditions and evaluating organoleptic, particle size, and pH on days 0, 14, and 28 [14]. In the stability test, the nanosuspension preparations were tested for organoleptic, particle size, polydisspecificity index, and pH stored at room temperature.

2.3.6 Data analysis

The research data were analyzed descriptively and statistically. Statistical testing using the one-way ANOVA method. The value of $p > 0.05$ indicates that there is no difference in the effect of the variables between the formulas. While the value of $p < 0.05$ indicates that there is a difference due to the influence of variables in the study, namely variations in the concentration of tween 80 surfactant.

3. RESULTS AND DISCUSSION

In this study, ketoconazole was obtained from PT. Dexa Medica Palembang as a test sample. Evaluation of the preparations carried out included organoleptic tests, particle size and PI tests, pH tests, particle morphology tests, and stability tests. Optimization of the preparation of ketoconazole nanosuspension preparations with varying concentrations of tween 80. Tween 80 is a non-ionic surfactant, non-toxic, non-irritating to the skin, and safe for topical application. The process of reducing the size of ketoconazole particles using a sonicator bath. The sonicator device utilizes sonic vibrations (sound) to break the particles down to nano size. Ultrasonic waves when in a liquid medium will cause acoustic cavitation which causes the particles to disintegrate due to sound [17]. The particles in the nanosuspension have a large enough surface area so that the surface free energy is also high. This causes the particles in the nanosuspension to tend to interact with each other so that agglomeration or aggregation can occur [1]. In this study, the stabilization of nanosuspensions used tween 80 with a mechanism of steric hindrance, in which the particles in the nanosuspension preparations would be covered by a stabilizer so that interactions between particles could be minimized [20]. The organoleptic test results obtained are as follows.

Table 2. Organoleptic Test Results

No	Formula	Observation		
		Form	**Colour**	**Homogenity**
1	F1	dispersed	slightly turbid	homogeneous
2	F2	dispersed	slightly turbid	homogeneous
3	F3	dispersed	transparent	homogeneous

This organoleptic test was carried out on day 0. The size of the nanosuspension particles can be seen visually, showing relatively clear and transparent dispersion results [12]. Optimization of nanosuspension preparations was carried out by comparing nanosuspension preparations F1, F2, and F3, where in the three formulas there were differences in Tween 80 concentration. From the test results, it was found that the F3 formula produced a clear solution because it was influenced by the high concentration of tween 80, which was 12%. Whereas for F1 and F2 the results obtained were slightly turbid preparations, and when left to stand for 1 week experienced fine particle settling which could still be dispersed when shaken. This could be due to the fact that the concentration of tween 80 to stabilize the particles is still not optimal [6].

Figure 1. Optimization Results of Making Ketoconazaol NanosuspensionF1(left), F2(middle), F3 (right**)**

Tests for particle size and distribution were carried out to see whether the nanosuspension preparations entered the range of less than 1000 nm [12]. Following are the results of testing the particle size and polydispersity index. The polydispersity index value of below 0.5 indicating that nanosuspensions of ketokonazole have the homogeneous dispersion system, so it could have a better stability.

Table 3. Particle Size Test Results and Polydispersity Index (n-3)

Formula	**Particle Size**		**Polydispersity Index**	
	0 Day	**28th day**	**0 day**	**28th day**
1	106±2.11	115±1.11	0.3±0.04	0.3±0.04
2	102±1.24	129±3.44	0.3±0.05	0.3±0.05
3	95±2.67	97±2.14	0.1±0.045	0.1±0.04

The measurement results showed changes in particle size from day 0 and day 28. Factors causing changes in particle size could be because the particles are unstable during storage and experience particle deposition resulting in agglomeration [4]. The changes in particle size up to day 28 were also visible as there was a change in color which became visually slightly cloudy. The polydispersity index value obtained for each formula, on the 0th and 28th day tests obtained a value less than 0.5 which indicates a uniform distribution of particles [8]. Statistical tests also showed no significant difference in the results of the particle size and polydidercity index tests on the 0th and 28th days for each sample ($p>0.05$).

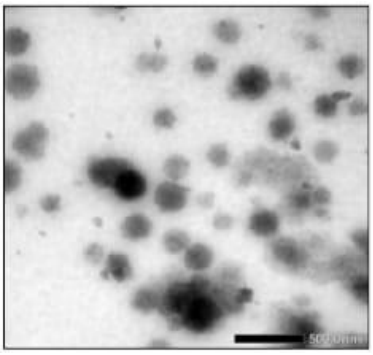

Figure 2. The Results of Particle Morphology Test of Ketoconazole Nanosuspension F3 (magnification 10000x)

In testing the morphology of the nanosuspension particles using the SEM tool, shown in the Figure 2, it can be seen that the particles are almost spherical in shape which are irregular. The formula used in this test is formula 3 because the particle size is the smallest among the other formulas. SEM testing of this nanosuspension wanted to see the shape resulting from the nano manufacturing process. Based on the results obtained, some particles tend to combine which may result in agglomeration or settling of large particles for a certain period of time [18]. This is possible visually it may not be visible because the color is clear, but microscopically it becomes visible. Thus, these events can be considered in determining the concentration of surfactants and co-surfactants used [9].

The results of the nanosuspension pH test showed a pH value in the range of 5.6-6. The pH value shows that all the formulas made meet the requirements for the pH range criteria that are tolerated by the skin, namely 4.5-6.5 [1]. The increase in pH in each formula can be caused by an increase in tween concentration, where the pH of tween is 5.5-7.2. The degree of acidity or pH is a value that is used to see the level of acidity or alkalinity of a substance, solution, or object.

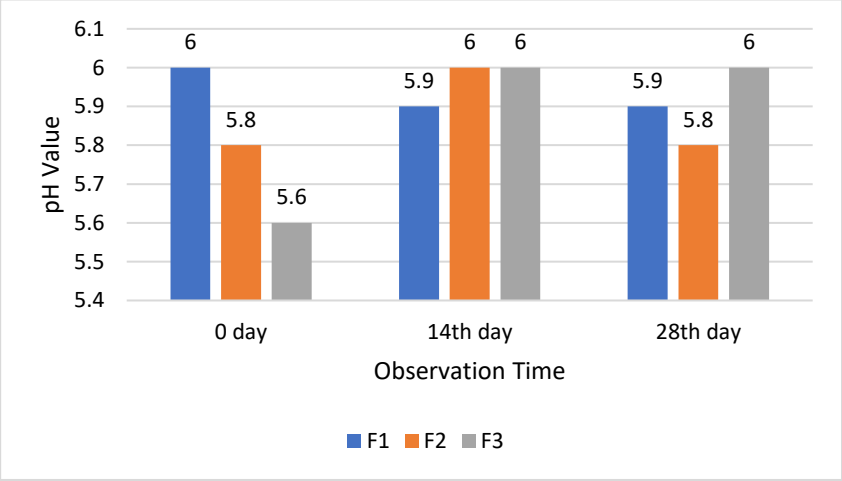

Figure 3. The Results of pH testing of ketoconazole nanosuspension preparations

The pH value of the nanosuspension preparation tends to be acidic because of the acidic nature of kecoconazole [10]. The ketoconazole nanosuspension was tested for 28 days to see if there was a change in pH during storage at room temperature. The results of the statistical tests showed that there was no significant difference from the results of the tests on days 0, 14 and 28 on the pH value of each preparation ($p>0.05$).

4. CONCLUSIONS

Based on the results of the research that has been done, it was found that of the 3 formulas for the ketoconazole nanosuspension, the particle size entered the nano range, namely 95-129 nm, the pH entered the range, namely 5.6-6, the particle morphology test showed irregular spherical particle shapes, and visually the appearance of the nanosuspension is clear and there is no precipitate. The test was carried out for 28 days and each preparation was tested. There is no significant difference from any results evaluated based on statistical tests.

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