FORMULATION AND PENETRATION TESTING OF ETHOSOME AZELAIC ACID ON ABDOMINAL SKIN WHITE MALE RATS (RATTUS NORVEGICUS) WITH FRANZ DIFFUSION CELL

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ABSTRACT

Objective: Development of transdermal drug delivery systems has several advantages, especially drugs to have a poor penetration of stratum corneum in the skin. Azelaic acid has been proven bactericidal and bacteriostatic to acne bacteria (Propionibacterium acnes). Azelaic acid products in market as cream and gel can only penetrate in stratum corneum about 4% of the dosage used. Thus, it is necessary to increase the penetration of azelaic acid to formulate into a carrier system such as ethosome.

Methods: Manufacture of suspension ethosom azelaic acid using thin layer hydration method or classical method. Suspension ethosom of azelaic acid to obtained subsequent freeze dried before formulated in cream preparation. After that, the penetration test for ethosom cream and non ethosom cream of azelaic acid with Franz Diffusion Cell.

Results: Optimization formulation ethosom of azelaic acid with variations concentration of ethanol 30%, 35% and 40%. Ethosome with 35% ethanol had entrapment efficiency higher than 30% and 40% ethanol as 94.48±0.14% and had smaller particle size 179.3±2.23 nm. Penetration test for ethosome cream and non-ethosome cream of azelaic acid showed that cumulative amount was 1334.074±27.086 µg/cm²·h and 491.032±3.935 µg/cm²·h.

Conclusion: Ethosome cream of azelaic acid has better penetration capabilities than non-ethosom cream of azelaic acid.

Keywords: Azelaic acid, Penetration enhancer with vesicles ethosome, Franz diffusion cell.

INTRODUCTION

Drug that can be used to inhibit the growth of Propionibacterium acnes is azelaic acid. The mechanism of action azelaic acid is primarily through inhibition of protein synthesis, but RNA and DNA synthesis is also decreased [1]. Azelaic acid is bacteriostatic at low concentrations and bactericidal at higher levels. Due to differential uptake of azelaic acid at different pH levels and in nutrient depletion, the minimal inhibitory concentrations for P. acnes vary from 0.1 to 2.5 mmol/L [2-4]. Azelaic acid not only as antacincs but also has other activities as antihipperpigmentasi skin [4-6], anticeratinitation, cytotoxic cell and anti proliferative in keratinocytes [3].

Based on the Food and Drug Administration, in vitro penetration test of Azellex® cream, in which azelaic acid can only penetrate in stratum corneum by 4% of the dosage used [7-10]. Thus, it is necessary to increase the penetration of azelaic acid percutaneously with azelaic acid formulated into a carrier such as ethosome system [11].

Ethosomes are soft, malleable lipid vesicles composed mainly phospholipids, alcohol (ethanol or isopropyl alcohol) in relatively high concentration (20–45%), and water. High concentration of alcohol (20–45%) in the formulation provides soft, flexible characteristics, vesicles stability, and it also disrupts lipid bilayer structure, so it can be increased membrane permeability [12,13].

MATERIALS AND METHODS

Materials
Azelaic acid (Sigma, US), Phospholipon 90 G (90% hydrogenated soy phosphatidylcholine, US), ethanol 96 % (Merck, German), propylene glycol (Dow Chemical Co), potassium dihydrogen phosphate (Merck, German), hydrochloric acid (Brataco, Indonesia), sodium hydrosode (Brataco, Indonesia) methanol (Merck, German), dichloromethane (Merck, German), and cream base dan agua demineralization (Brataco, Indonesia) were used.

Preparation ethosome of azelaic acid
Preparation ethosome of azelaic acid was thin-layer hydration method. The thin layer was using rotary evaporator by dissolving phospholipon 90 G with a ratio of 1:1 to azelaic acid in dichloromethane and methanol (2: 1). Then, the thin layer formed is stored in the refrigerator for 24 h than in hydration with the water phase. The water phase used consisted of ethanol (with concentration variations of 30%, 35%, and 40%), azelaic acid, and phosphate buffer pH 7.4.

Characterization ethosome of azelaic acid
After obtaining, the suspension ethosome of azelaic acid then proceed with the characterization to obtain the most optimum in formulation. The parameters used are entrainment efficiency, particle size distribution, potential zeta, and polydispersity index.

Entrapment efficiency test
Azelaic acid in made variations concentration were 200, 250, 300, 350, 400, and 450 ppm using phosphate buffer pH 6.8. The solution was then measured uptake by ultraviolet (UV)-visible spectrophotometer at a wavelength of 204 nm and made a calibration curve [14,15].

Entrapment efficiency test was done by the indirect method. Determination of total ethosome suspension was done measuring the absorbance of ethosome suspension dissolved in methanol. Then, concentration of azelaic acid in the supernatant with Cellulose Acetate filter 0.22 µm [14,16,17].

Entrapment efficiency = (T–C)/T ×100%
In vitro penetration test
Penetration test using skin membrane abdominal from male rats with phosphate buffer pH 7.4 as receptor compartment. After that, the abdominal skin of rats was placed between donor and receptor compartment with the dermal side directly related to the medium receptor [4,19]. Sample of 1 g was applied to the skin surface with an area of diffusion was 1.76 cm². Then, samples of 4 mL were taken at regular intervals for 12 h. Then, a solution compartment immediately added a volume equal to the volume taken. The solution was then analyzed using a UV-visible spectrophotometry [22].

After concentration of azelaic acid in the sample has been measured, it can be calculated the cumulative amount of azelaic acid penetrated using the following formula [20]:

\[ Q_t = V_r C_t + \sum_{i=0}^{t-1} V_s C_i \]

Explanation:
- \( Q_t \): Cumulative amount of azelaic acid penetrated (µg)
- \( V_r \): Volume receptor compartment of Franz diffusion cell (16 mL)
- \( V_s \): Sample volume (4 mL)
- \( C_t \): Concentration (ng/mL) at minute \( t \)
- \( C_i \): Concentration on sampling minute \( i \)

From the analysis will be obtained cumulative amount of azelaic acid per unit area (µg/cm²) with following formula:

\[ Q = \frac{Q_t}{S} \]

Explanation:
- \( Q \): Cumulative amount per unit area (µg/cm²)
- \( Q_t \): Cumulative amount penetrated (µg)
- \( S \): Area of membrane (1.76 cm²)

The calculate of flux in steady state through interpolation of linear regression using the following formula:

\[ J = \frac{M}{(S \times T)} \]

Explanation:
- \( J \): Flux (µg cm⁻² s⁻¹)
- \( S \): Area of diffusion (cm²)
- \( M \): Cumulative amount of azelaic acid to membrane (µg)
- \( T \): Time (hours)

Furthermore, from the analysis we get a graph of drug concentration per unit area (ng/cm²) over time, in order to obtain a straight line, the slope of line is presented rate of drug release [8,20,23].

RESULT AND DISCUSSION
Determination of selected ethosome azelaic acid in formula
The selected formula is a high percentage of entrapment efficiency, particle size distribution (<200 nm), polydispersity index <0.8, and has a potential zeta >/ < ± 30 mV (Table 1).

Determination of azelaic acid content in preparations
From data selected was formula 35%. Formula 2 was then formulated in ethosome and non-ethosome cream preparations and the determination of the azelaic acid content can be seen in Table 2. The regression used is \( y=0.0013 \times +0.0049 \), with \( r=0.9996 \).

In vitro penetration test with Franz diffusion cells
After the penetration test for 12 h at 10 sampling points showed that the cumulative amount of azelaic acid and penetrated to the preparation ethosome and non-ethosome cream was 16879.269±189.055 µg/cm² and 5759.222±44.779 µg/cm² (Table 3). The flux values for the ethosome and non-ethosome cream preparations can be seen in Table 4. In these results, it can be seen that value flux ethosome cream gives a higher than non-ethosome cream preparation. The percentage of the azelaic acid penetrated from each preparation and the results obtained for the preparation penetrated ethosome cream was 74.32%, while for non-ethylene cream, it was 33.49% (Table 5). Ethosome azelaic acid containing 35% ethanol can affect to structure of stratum corneum (reversible) to increase penetration of azelaic acid through the skin. While azelaic acid in non-ethosome had smaller penetration than ethosome cream. This suggests that possibility hydrophobic of azelaic acid can be retained in the skin layer longer [13,21].

### Table 1: Characterization ethosome of azelaic acid

| Formula   | \( D_{mean} \) volume (nm) | Polydispersity index | Potential zeta (mV) | Entrapment efficiency (%) |
|-----------|-----------------------------|----------------------|---------------------|---------------------------|
| Formula 30% | 283.5±9.08                 | 0.50±0.06            | 32.13±7.77          | 90.08±0.13                |
| Formula 35% | 179.3±2.23                 | 0.65±0.02            | 34.87±0.35          | 94.48±0.14                |
| Formula 40% | 1377±76.06                 | 0.97±0.04            | −22.80±2.25         | 92.46±0.30                |

| Formula | Theoretical concentrations (µg/mL) | Concentrations actually (µg/mL) | Percentage of content | Mean±SD |
|---------|-----------------------------------|-------------------------------|----------------------|---------|
| Ethosome | 750                               | 790.077                       | 105.344              | 106.09±0.720 |
|         | 800.846                           | 106.779                       |                      |         |
|         | 796.231                           | 106.164                       |                      |         |
| Non-ethosome | 500                      | 548.538                       | 109.708              | 108.477 |
|         | 542.385                           | 108.477                       |                      |         |
|         | 553.154                           | 110.631                       |                      |         |

SD: Standard deviation
Based on the results of *in vitro* penetration test, flux penetration of ethosome cream higher when compared with non-ethosome cream. That because the reduction of particle size to nano can increase drug penetration in skin. In addition, main component vesicles ethosome is phospholipid and ethanol can increase the penetration of drugs with various mechanisms. In generally the mechanism of drug release from effect ethanol in ethosome and effects elastic form ethosom. When ethosom is applied to the skin, ethanol in ethosome can increase penetration enhancer. The mechanism of ethanol is interacting with fat molecules in stratum corneum which can be decreased rigidity, influence lipid bilayer on stratum corneum, so it can to increase lipid fluidity membrane and permeability of stratum corneum [13,21].

With the disruption of lipid bilayer on the stratum corneum, it will facilitated the ethosome to penetrate into the skin. In this process of ethosome effect. Ethosome is a flexible vesicle that can interact with...

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### Table 3: Cumulative penetrated of azelaic acid (n=3)

| Time (hours) | Concentration Q (µg/mL) | Mean±SD |
|--------------|-------------------------|---------|
|              | Experiment 1            | Experiment 2 | Experiment 3 |
| Etosome cream of azelaic acid | 682.474 | 643.138 | 649.694 | 658.435±21.075 |
| 0.17         | 1.512.107               | 1.495.935 | 1.467.963 | 1.492.002±22.333 |
| 0.67         | 3.360.621               | 3.284.572 | 3.232.124 | 3.292.439±64.609 |
| 2            | 3.661.495               | 3.574.956 | 3.522.072 | 3.586.174±70.385 |
| 2.25         | 3.982.037               | 3.943.444 | 3.879.327 | 3.928.569±51.485 |
| 2.5          | 9.534.572               | 9.809.467 | 9.987.295 | 9.965.778±203.393 |
| 7            | 1.089.707               | 1.122.743 | 1.118.553 | 1.1102.783±179.282 |
| 8            | 1.213.675               | 1.246.991 | 1.243.793 | 1.234.547±182.188 |
| 9            | 1.477.411               | 1.524.187 | 1.524.969 | 1.5088.156±275.190 |
| 11           | 1.668.492               | 1.706.544 | 1.689.341 | 1.6879.269±109.055 |
| 0.17         | 26.879                  | 13.767   | 7.212    | 15.053±10.014 |
| 0.33         | 40.603                  | 56.774   | 35.358   | 44.245±11.163 |
| 0.5          | 82.299                  | 97.159   | 83.610   | 87.689±8.227 |
| 0.67         | 1.524.044               | 1.773.161| 1.751.31 | 1.682.84±13.796 |
| 0.83         | 2.364.95                | 2.338.872| 2.334.35 | 2.346.60±1.655 |
| 1            | 3.083.48                | 3.218.97 | 3.233.08 | 3.317.01±8.227 |
| 9            | 4.319.44                | 4.358.348| 4.386.757| 4.354.851±33.790 |
| 10           | 4.685.446               | 4.735.708| 4.769.362| 4.730.172±42.231 |
| 11           | 5.188.243               | 5.259.921| 5.266.040| 5.238.068±43.258 |
| 12           | 5.710.271               | 5.769.274| 5.798.121| 5.759.222±44.779 |

SD: Standard deviation

### Table 4: Flux of ethosome and non-ethosome cream

| Formula         | Flux (µg/cm².h) | Mean±SD (µg/cm².h) |
|-----------------|----------------|-------------------|
| Ethosome        | 130.2890       | 133.407±27.086    |
| Non-ethosome    | 486.851        | 491.584           |
|                 | 494.663        | 491.032±3.935     |

SD: Standard deviation

### Table 5: Percentage penetrated of azelaic acid with Franz diffusion cell

| Concentration (µg/mL) | Mean±SD | Percentage penetrated (%) |
|----------------------|---------|--------------------------|
| Azelaic acid in ethosome cream at 12 h | 591.360±9.769 | 74.32 |
| 597.000              | 597.000 | 580.080                  |
| Total content of azelaic acid in ethosome cream before penetration | 795.71±5.403 | 790.077 |
| 790.077              | 800.846 | 796.231                  |
| Azelaic acid in non-ethosome cream at 12 h | 183.51±0.391 | 33.49 |
| 183.145              | 183.923 | 183.462                  |
| Total content of azelaic acid in non-ethosome cream before penetration | 548.02±5.403 | 548.538 |
| 542.839              | 553.154 | 548.02±5.403             |

SD: Standard deviation
lipid bilayer and penetrated in skin by changing the shape of the vesicles to be like the pathway. Ethosome in skin will make it fusion with fat skin and then drug is released in this process resulting in transdermal absorption [13,21].

CONCLUSION
Optimization formulation of ethosome is done with variation concentration ethanol of 30%, 35%, and 40%, indicating the formulation ethosome with 35% ethanol, the highest value of the entrapment efficiency was 94.48 ± 0.14%, and the smallest particle size is 179.3±2.23 nm.

Penetration test for ethosome cream and non-ethosome cream of azelaic acid showed that cumulative amount was 1334.074±27.086 µg/cm² h and 491.03±3.935 µg/cm² h.

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AUTHOR CONTRIBUTIONS
Novi Nurleni, S. Farm., Apt is a Master Student at the Faculty of Pharmacy, University of Indonesia. Her master research about of “Formulation and Penetration Testing of Ethosomes Azelaic Acid on Abdominal Skin White Male Rats (Rattus Norvegicus) with Franz Diffusion Cell.” Dr. Iskandarsyah, M.S., Apt is Doctor at the Faculty of Pharmacy, University of Indonesia. He is head of Pharmaceutical Laboratory. Has Expertise in Development of pharmaceutical technology. Dr. Ahmad Aulia Jusuf, AHK, PhD is doctor at the Faculty of medicine, University of Indonesia. He is head of the histology department. Has expertise in Stem Cell and Tissue Engineering.

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