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Original Article

FORMULATION GEL SELF NANOEMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS) ALPHA-BISABOLOL AS ANTIOXIDANT

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ABSTRACT

Objective: Alpha-Bisabolol is monocyclic sesquiterpene alcohol obtained from the distillation of chamomile flowers (Matricaria chamomilla), that has many uses in the pharmaceutical industry, one of them is an antioxidant. Alpha-Bisabolol was formulated into Self Nanoemulsifying Drug Delivery System (SNEDDS) to protect it from oxidation reactions in skin metabolism and to help penetrate it to the dermis layer where it works. The purpose of this study is to develop the use of Alpha-Bisabolol as an antioxidant that is formulated into SNEDDS gel dosage form. Optimization of SNEDDS was carried out on four formulas with a different comparison of surfactant and use of percent transmittance as the parameter.

Methods: Optimum SNEDDS is then tested for characterization, including dispersibility test, particle size, polydispersity index (PDI), zeta potential, and freeze-thaw cycle. The result for SNEDDS optimization shows that formula 4 produces the highest percent transmittance of 99.44±0.04%.

Results: Optimum formula that was characterized resulting clear nanoemulsion with an emulsification time 29.64±0.09 second, with the particle size of 16.74 nm, PDI of 0.121, and zeta potential of-18.7 mV also shows good stability after freeze-thaw cycle test for three cycles. Optimum SNEDDS then formulate to gel form. Based on the result of the physical and chemical quality evaluation test for Alpha-Bisabolol SNEDDS gel, it was found that SNEDDS gel was homogeneous with a clear yellowish appearance, odorless with semi-solid form, had viscosity 180±2 cP with plastic thixotropy flow properties, spreadability 2937.7691±2.40 mm^2 and pH 5.55±0.015.

Conclusion: It can be concluded that Alpha-Bisabolol and SNEDDS Alpha-Bisabolol gel have antioxidant activity with IC_{50} of 123.78 g/ml and 371.44 g/ml, respectively.

Keywords: Alpha-Bisabolol, Self-nano emulsifying drug delivery system (SNEDDS), Gel, Antioxidant

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INTRODUCTION

Indonesia is a tropical country that always gets sun exposure throughout the year. Sunlight on the earth's surface contains ultraviolet radiation (UVR), which can have benefits or adverse effects on human health. UVA and UVB rays contribute to the formation of free radicals that can degrade collagen and elastin fibers, thereby triggering premature aging [1]. Aging is characterized by a progressive decline in function over time. Skin aging is a complex process that is influenced by intrinsic and extrinsic factors. One way that can be used to prevent premature aging is to use antioxidants [2].

Alpha-Bisabolol has been widely used as a raw material in dermatological and cosmetic formulations such as after-shave cream, hand and body lotions, deodorants, lipsticks, sun-care, and baby care products and can be used as an antispasmodic, anti-inflammatory, hypoallergenic, and antioxidant [3]. Skin is the first barrier between the body and exogenous substances. The enzymatic activity in skin is increased in the deeper layers of the skin. Accordingly, substances that can pass through the highly lipophilic stratum corneum must remain resistant to metabolic degradation after entering the deeper layers of the skin [4].

Self Nanoemulsifying Drug Delivery System (SNEDDS) is a nanoemulsion preconcentrate or anhydrous form of a nanoemulsion. The SNEDDS system makes the drug or active ingredient more stable in the long term and increases the solubility of the drug, thereby increasing its bioavailability in the body [5]. In this study, Alpha-Bisabolol was formulated in the form of a SNEDDS gel to protect against oxidation reactions in skin metabolism and help penetrate to the dermis layer.

MATERIALS AND METHODS

Materials

Alpha-Bisabolol was purchased from Shanghai Leasun Chemical Co., Ltd., China; Alkamuls® CRH 40 was purchased from Solvay, Singapore; Polyethyleneglycol 400 was purchased from Arrow Fine Chemicals, India; Carbomer 940 was purchased from Tinphy, Hongkong; propylene glycol was purchased from Dow Chemicals, USA; Glydant ® Plus Liquid was purchased from Lonza, Switzerland; Triethanolamine was purchase from Merck, Germany, distilled water.

Instruments

Particle size analyzer (Malvern), magnetic stirrer (IKA, C-MAG HS 7), analytical balance (Kern), UV-Vis spectrophotometer (Shimadzu, UV 1800), Sonicator (GT Sonic), oven (Memmert), water bath (Memmert, W 600), pH meter (Eutech Instrument pH, 510), viscometer (Brookfield, DV-II+Pro), glassware (pyrex) and other supporting equipment.

Alpha-bisabolol SNEDDS optimization

Alpha-Bisabolol was mixed with Virgin Coconut Oil, added Alkamuls® CRH 40 and PEG 400, then homogenized with a magnetic stirrer for 10 min followed by sonication for 15 min and conditioned on a water bath at 40 °C for 10 min. The results of SNEDDS Alpha-Bisabolol were measured transmittance percent at 650 nm wavelength and determined the optimum SNEDDS formula [6].

Dispersibility test

A much of 0.1 ml of optimum SNEDDS was dissolved in 10 ml of distilled water. Then it was homogenized with a magnetic stirrer at room temperature and the time to reach emulsification was calculated using a stopwatch [6].

Freeze-thaw cycle tests

The optimum SNEDDS formulation was stored at temperatures between 4 °C and 45 °C for not less than 48 h at each temperature and carried out in 3 cycles.

Particle size, polydispersity index, and zeta potential analysis

Particle size and polydispersity index were determined using Zetasizer. The sample was diluted in distilled water with a 1:100 ratio. Zeta potential was determined by Dynamic Light Scattering

using a Particle Size Analyzer. The sample was diluted in distilled water with a 1:100 ratio [7].

Gel preparation

Carbomer 940 was dispersed in water and left for 24 h. Then stir thoroughly to prevent lumps from forming. Added TEA little by little to form a gel base. Glydant® Plus Liquid and sodium metabisulfite are mixed with propylene glycol, then added to the gel base. SNEDDS Alpha-Bisabolol is added little by little into the gel base while stirring until a homogeneous gel is formed.

Evaluation of physical and chemical quality parameters for gel preparations

Evaluation was carried out after the gel preparation was formed at room temperature. The tests include organoleptic evaluation, homogeneity, viscosity and flow properties, spreadability, and pH test [8].

Table 1: Optimization of SNEDDS alpha-bisabolol formula comparison

Ingredient	Formula			
	F1	F2	F3	F4
Alpha-Bisabolol	0.1	0.1	0.1	0.1
Virgin Coconut Oil	0.5	0.5	0.5	0.5
Alkamuls® CRH 40	5	6	7	8
PEG 400	1	1	1	1

Note: Formula made in 100 grams

Table 2: Formula of SNEDDS alpha-bisabolol gel

Ingredients	Formula (% w/w)	
	Blank	Gel
SNEDDS Alpha-Bisabolol	-	80
Carbomer 940	1	1
TEA	1	1
Propylene glycol	5	5
Glydant® Plus Liquid	0.2	0.2
Sodium Metabisulfite	0.1	0.1
Distilled water ad	100	100

Antioxidant activity test of SNEDDS alpha-bisabolol. Preparation of 0.4 mmol DPPH solution

4.0 mg of DPPH (BM = 394.32) was weighed and dissolved in 25.0 ml of pro analyses methanol and homogenized. The solution is placed in a dark bottle.

Blank solution preparation

1 ml of 0.4 mmol DPPH solution was put into a test tube that had been calibrated at 5.0 ml and then pro analyses methanol was added to the mark (5 ml) and then homogenized.

Determination of maximum absorbance wavelength of DPPH solution

1 ml of 0.4 mmol DPPH solution was put into a test tube that had been calibrated at 5.0 ml and then added pro-analytical methanol to the mark and homogenized. The solution was left for 30 min in a dark place and the absorbance was measured at a wavelength of 400-800 nm.

Determination of the operating time

1 ml of 0.4 mmol DPPH solution was added with 6 ppm standard solution of vitamin C to the mark of the 5 ml volumetric flask. The solution was read for absorbance at the maximum wavelength with a span of 5 min for 1 h.

Alpha-bisabolol test solution preparation

An amount of 10 mg of Alpha-Bisabolol was weighed and dissolved in pro-analytical methanol to 10 ml (1000 ppm). This solution is the main solution. Then 200 µl; 300 µl; 400 µl; 500 µl and 600 µl of the main solution were added into a 5 ml test tube to obtain a concentration of 40 ppm; 60 ppm; 80 ppm; 100 ppm and 120 ppm.

Preparation of SNEDDS alpha-bisabolol gel test solution

Equivalent of 10 mg of Alpha-Bisabolol dissolved with pro-analytical methanol in a 10.0 ml volumetric flask so that the concentration of the main solution was 1000 ppm. Then 600 µl; 700 µl; 800 µl; 900 µl and 1000 µl of the main solution were added into a 5 ml test tube to obtain a concentration of 120 ppm; 140 ppm; 160 ppm; 180 ppm and 200 ppm.

Positive control preparation

10 mg of Vitamin C dissolved in methanol pro-analysis to 10 ml to obtain a main solution of 1000 ppm. Then 20 µl, 25 µl, 30 µl, 35 µl and 40 µl of the main solution added into a 5.0 ml volumetric flask to obtain concentrations of 4 ppm, 5 ppm, 6 ppm, 7 ppm, and 8 ppm. Add 1.0 ml of DPPH solution (0.4 mmol) to each volumetric flask and add pro-analytical methanol to the 5.0 ml mark. It was homogenized and the flask was covered with aluminum foil. Then incubated at 37 °C for 30 min. For further use in the measurement of antioxidant activity.

Calculation of % free radical reduction

The data obtained from the antioxidant activity test were used to calculate the % reduction of free radicals with the formula Eq 1:

$$\text{Free radical scavenging: } \frac{\text{Blank Absorbance} - \text{Sample Absorbance}}{\text{Blank Absorbance}} \times 100\ \% \quad \ldots\ldots \text{(Eq. 1)}$$

Data analysis

Data was analyzed by t-test using SPSS to compare the antioxidant activity of Alpha-Bisabolol and SNEDDS Alpha-Bisabolol gel.

RESULTS AND DISCUSSION

The result of determining the maximum wavelength of the DPPH solution which has been measured at a wavelength between 400-800 nm is 515 nm. The results of determining the operating time of DPPH solution with antioxidants to obtain a constant reaction result for 60 min at a maximum wavelength of 515 nm is for 55 min. The results of the antioxidant activity of vitamin C as a positive control and Alpha-Bisabolol are presented in table 3 and table 4 with five series concentrations.

Table 3: Antioxidant activity of vitamin C

Linear regression	R^2	IC_{50}	Mean IC_{50}	SD
y = 8.082x+24.006	R^2 = 0.9954	3.22	3.32	0.09
y = 8.542x+21.116	R^2 = 0.9972	3.38		
y = 8.672x+20.72	R^2 = 0.9973	3.38		

Table 4: Antioxidant activity of alpha-bisabolol

Linear regresion	R^2	IC50	Mean IC50	SD
y = 0.3257x+9.634	R^2 = 0.9963	123.94	123.79	0.85
y = 0.3233x+10.276	R^2 = 0.9964	122.87		
y = 0.3188x+10.29	R^2 = 0.9949	124.56		

SNEDDS alpha-bisabolol optimization result

SNEDDS formula optimization was carried out to determine the best formula that could be used and further characterization was tested. The parameter used in the optimization of the SNEDDS formula is the percent transmittance. One of the parameters used in isotropic is the clarity of the resulting nanoemulsion; therefore, the percent transmittance was measured to see the level of clarity of SNEDDS dispersed in aqueous media as measured by UV-Vis spectrophotometer at a 650 nm wavelength using distilled water as blank. The size of the dispersed phase greatly affects the appearance of the formed nanoemulsion. The optimization results can be seen in table 5.

Table 5: Results of optimization of SNEDDS alpha-bisabolol formula

Formula	Transmittance (%)			Average (%)
	1	2	3	
Blank	100	100	100	100
1	98.52	98.55	98.52	98.53±0.01
2	99.32	99.33	99.35	99.33±0.01
3	99.39	99.38	99.41	99.39±0.01
4	99.41	99.43	99.49	99.44±0.04

Date was given in mean+SD, n=3

From the results of the percent transmittance test of the four formulas, it can be seen that increasing the concentration of surfactants can increase the percent transmittance of the SNEDDS formula. This can be caused by the greater concentration of surfactant used, the greater its ability to cover the oil so that the higher the transmittance percentage value is obtained. In addition, the molecular structure of the surfactant used in this case, Alkamuls® CRH 40 has a branched alkyl structure which promotes the penetration of the surfactant into the oil, resulting in a spontaneous nanoemulsion with a clear visual appearance [9]. Based on these results, it can be concluded that the fourth formula is the optimum SNEDDS Alpha-Bisabolol formula.

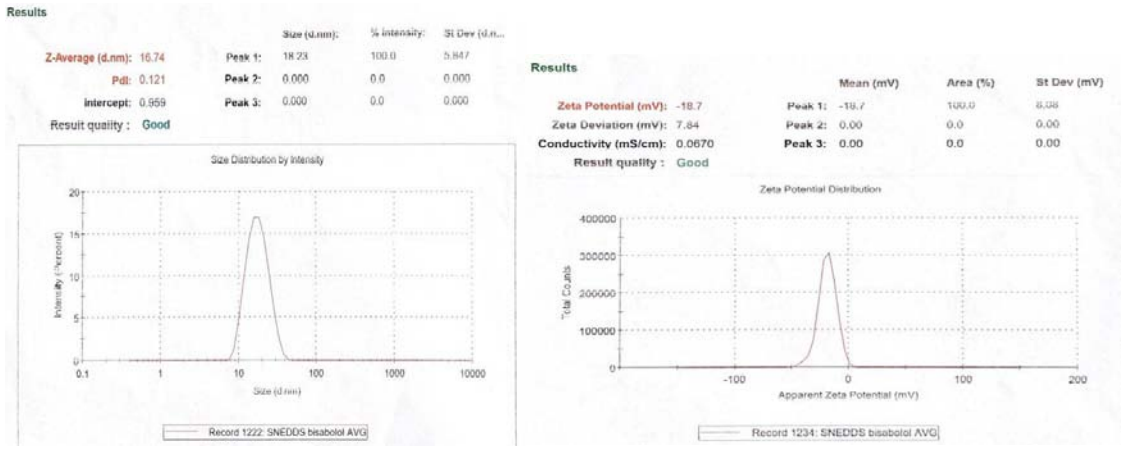
Result of optimum alpha-bisabolol SNEDDS characterization.

The result of the dispersibility test was shown in table 6.

Table 6: Results of dispersibility test

Formula	Emulsification time (s)			Average (s)
	1	2	3	
4	29.74	29.56	29.62	29.64±0.09

Date was given in mean+SD, n=3



(a) **(b)**

Fig. 1: Result of (a) Particle size analysis and (b) Zeta potential analysis

Nanoemultion has a clear appearance with an emulsification time of less than 1 minute. Based on these results, SNEDDS Alpha-Bisabolol is included in group 1 because it is able to form an emulsion system in less than 1 minute with a clear appearance [10].

Particle size, polydispersity index, and zeta potential

Particle size and polydispersity index tests were carried out to determine particle size and particle distribution in SNEDDS Alpha-Bisabolol. The results of the particle size and polydispersity index of SNEDDS Alpha-Bisabolol can be seen in fig. 1.

The particle size test results showed that SNEDDS Alpha-Bisabolol had a particle size of 16.74 nm; this result met the requirements for SNEDDS particle size, which was less than 100 nm in water dispersion. This result may be influenced by the surfactant's ability to penetrate into the oil. In addition, these results may also be influenced by sonication. The cavitation effect of ultrasonic waves on the sonication process can cause particles to have diameters in the nanoscale. Polydispersity index value obtained 0.121. These results indicate that the particles in SNEDDS are distributed homogeneously, thereby preventing aggregation.

A zeta potential value greater than+30 mV or less than-30 mV indicates a stable nanoparticle system. The zeta potential result of SNEDDS Alpha-Bisabolol showed a value of-18.7 mV. A negative zeta potential value indicates that the surface charge of the globule is negative. The result of zeta potential which is not greater than+30 mV or less than-30 mV is due to the use of nonionic Alkamuls® CRH 40 and PEG 400, which can reduce the zeta potential value [11].

Freeze-thaw cycle

Freeze-thaw cycle test was carried out to see the stability of the resulting Alpha-Bisabolol SNEDDS. Stable SNEDDS was characterized by the absence of separation during the freeze-thaw cycle test. Based on the tests that have been carried out for 3 cycles, stable SNEDDS results are obtained.

Physical and chemical quality parameters of gel evaluation test result

Based on the organoleptic test can be shown that blank formula resulting clear, odorless with semi-solid dosage form gel while SNEDDS Alpha-Bisabolol gel resulting clear yellowish, odorless with semi-solid dosage form gel**.** The homogeneity test was carried out to see whether the components in the gel formula were completely mixed or not. After the homogeneity test was carried out with three replications, a homogeneous gel was formed for each blank formula and SNEDDS Alpha-Bisabolol gel. Based on the viscosity test that has been carried out using spindle no. 6 with RPM 2.5, the viscosity is obtained as follows in fig. 2.

Flow properties

Based on the results of the flow properties test that has been carried out, it was obtained that blank formula and SNEDDS Alpha-Bisabolol gel had thixotropic plastic flow properties (fig. 3) [12].

Spreadability

Based on tests that have been carried out, each blank formula of gel and SNEDDS Alpha-Bisabolol gel has a spreadability value of 2062.5238±4.19 mm^2 and 2937.7691±2.40 mm^2.

pH test

Gel use on the skin must have a pH according to the skin's pH range of 4.5-6.5. The results of the pH test can be seen in table 7.

SNEDDS alpha-bisabolol gel antioxidant activity test results

The antioxidant activity of SNEDDS Alpha-Bisabolol gel can be seen in table 8.

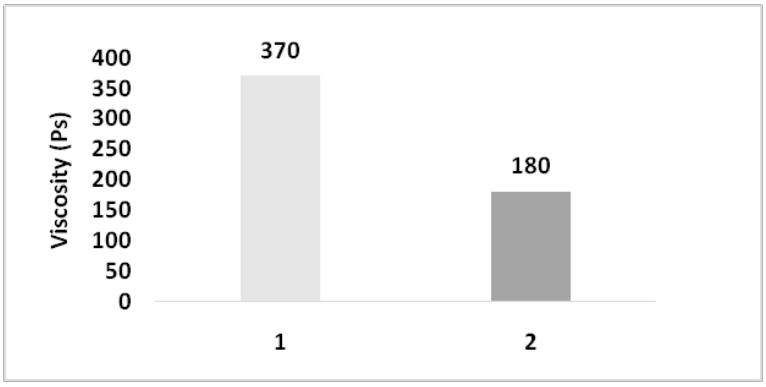


Fig. 2: Comparison of Viscosity between (1) blank formula and (2) SNEDDS alpha-bisabolol gel formula

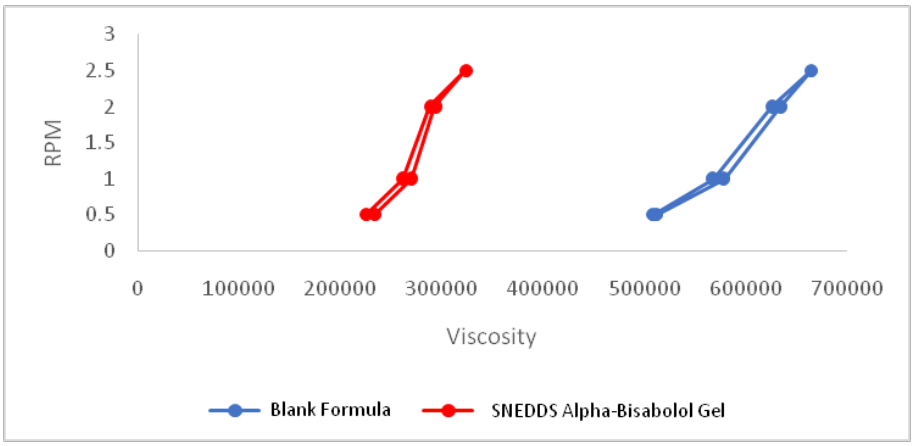


Fig. 3: Flow properties of blank formula and SNEDDS alpha-bisabolol gel formula

Table 7: Results of pH test

Formula	Replication			Average±SD
	1	2	3	
Blank Formula	6.43	6.45	6.42	6.43±0.015
SNEDDS Alpha-Bisabolol Gel	5.57	5.55	5.54	5.55±0.015

Date was given in mean+SD, n=3

Table 8: Antioxidant activity of SNEDDS alpha-bisabolol gel

Linear regression	R^2	IC_{50}	Mean IC_{50}	SD
y = 0.1368x-0.892	R^2 = 0.9723	372.02	371.44	0.65
y = 0.1375x-1.09	R^2 = 0.9750	371.56		
y = 0.1376x-1.014	R^2 = 0.9754	370.74		

Based on the tests that have been carried out, the IC50 value of SNEDDS Alpha-Bisabolol gel was 371.44±0.65 g/ml, which means that the antioxidant activity of SNEDDS is weak [13]. Based on t-test using SPSS can be seen that the antioxidant activity of Alpha-Bisabolol and SNEDDS Alpha-Bisabolol gel had a meaningful difference which was marked by a signification value less than 0.05.

CONCLUSION

The optimum SNEDDS Alpha-Bisabolol was obtained with the ratio formula between oil, surfactant, and cosurfactant of 0.5:8:1 with a transmittance of 99.44±0.04%. Based on the SNEDDS Alpha-Bisabolol optimum characterization, a clear nanoemulsion was produced with an emulsification time of 29.64±0.09 seconds, a particle size of 16.74 nm, polydispersity index of 0.121, the zeta potential of-18.7 mV, and thermodynamically stability. Based on the results of the evaluation of the physical and chemical quality of the SNEDDS Alpha-Bisabolol gel, it was found that the SNEDDS Alpha-Bisabolol gel was homogeneous with a clear yellowish appearance, odorless in semi-solid form, had a viscosity of 180±2 Ps with plastic thixotropic flow properties, the ability to spread of 2937.7691±2.40 mm^2 and pH 5.55±0.015 and SNEDDS Alpha-Bisabolol gel with IC_{50} of 371.44 g/ml.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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