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Original Article

OPTIMIZATION OF SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS OF LEMONGRASS (*CYMBOPOGON CITRATUS*) ESSENTIAL OIL

TRI UJILESTARI[1], BAMBANG ARIYADI[2], RONNY MARTIEN[3], ZUPRIZAL[1], NANUNG DANAR DONO[1*]

[1]Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia, [2]Department of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia, [3]Department of Pharmaceutics, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia
Email: nanungdd@ugm.ac.id

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ABSTRACT

Objective: Focus of this study was to optimize and to characterize the self-Nano emulsifying drug delivery system using lemongrass (*Cymbopogon citratus*) essential oil.

Methods: The optimum formulas were analyzed using a D-Optimal mixture experimental design and performed using a Design Expert® Ver. 7.1.5. Formulation variables which include in the design were: oil component X_1 (a mixture of *Cymbopogon citratus* essential oil and virgin coconut oil/VCO), surfactant X_2 (Tween 80), and co-surfactant (PEG 400), while emulsification time in a sec (Y_1) and transmittance in percent (Y_2) as responses.

Results: The optimum formula for SNEDDS in the current study were: *Cymbopogon citratus* essential oil (7.147%), VCO (7.147%), Tween 80 (71.417%), and PEG 400 (14.290%). From the optimizing formula can be shown that the mean of droplet size, polydispersity-index, zeta potential, and viscosity were: 13.17±0.06 nm, 0.17±0.05,-20.90±1.47 mV, 200±0mPa·s (n=3), respectively. Furthermore, the optimized formula has passed the thermodynamic stability test; meanwhile, transmission electron microscopy displayed spherical shape.

Conclusion: The optimized SNEDDS formula was improving solubility of poorly soluble *Cymbopogon citratus* essential oil.

Keywords: *Cymbopogon citratus*, D-optimal mixture design, optimization, self-nanoemulsifying drug delivery system (SNEDDS)

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INTRODUCTION

The productivity of broiler chickens can be optimal when the health condition of the gastrointestinal tract is in good condition [1]. The balanced population between pathogenic and beneficial microflora in the gastrointestinal tract plays an important role in nutrient digestion and absorption [2-4]. *Escherichia coli*, *Salmonella typhimurium*, and *Clostridium perfringens* are the three major pathogens in the gut of poultry [5]. Since the high public concerns on antibiotic resistance, the use of antibiotics as growth promoters in the diets of poultry has been banned. There is an urgent need to explore the alternative for antibiotic growth promoters [6].

Essential oils are potential alternatives for an in-feed antibiotic as they have antibacterial properties [7]. Essential oils are classified as phytobiotics that have pharmacological effects [8]. As shown in lemongrass (*Cymbopogon citratus*) essential oils, this phytobiotic have been found to be effective in inhibiting pathogenic bacteria, such as: *Salmonella typhimurium* [9], *S. enterica* [10], *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* [11], *Klebsiella pneumonia* [12, 13], and also antifungal activity against *Candida albicans* [14].

Essential oils are volatile substances, liquid, soluble in lipid and organic solvents [15], and hydrophobic [16]. Essential oils have antibacterial activity but low aqueous solubility [17]. Self-nanoemulsifying drug delivery system (SNEDDS) is one of the effective strategies to improve oral bioavailability [18], solubility [19] and it can control the drug release [20]. The purpose of the formula optimization study is to determine the variable level from which a strong product with high-quality characteristics can be produced [21].

The best formula to combine oils in water is needed to be generated. The present experiments were carried out in order to find the optimum formula of SNEDDS of *Cymbopogon citratus* essential oil followed by characterization.

MATERIALS AND METHODS

Preparation of SNEDDS

Firstly, surfactant (Tween 80, Kao Indonesia Chemical, Bekasi, Indonesia), co-surfactant (PEG 400, idCHEM Co., Ltd., Kyunggi, South Korea), *Cymbopogon citratus* essential oil (Lansida Group, Yogyakarta, Indonesia) and virgin coconut oil/VCO (Healthy Co, Yogyakarta, Indonesia) were mixed using a magnetic stirrer (IKA® C-MAG HS 7, IKA WORKS Inc., Wilmington, NC, USA). Secondly, ultrasonicator (J. P Selecta, Barcelona, Spain) was utilized to make the SNEDDS formula uniformly disperse. Thirdly, SNEDDS formula was placed into a water bath (Memmert GmbH and Co. KG, Schwabach, Germany) at 45°C for 15 min [22].

Percent transmittance

SNEDDS formulations were added with a ratio of 1:50 with distilled water and mixed using vortex for 30 min. The percent transmittance was measured using UV-vis spectrophotometer (Genesys 10, Thermo Scientific Inc, Waltham, MA, USA) at 650 nm [21, 22].

Emulsification time

The SNEDDS formula was added drop wise to 500 ml of artificial gastric fluid at 37°C±2°C. The artificial gastric fluid was composed of hydrochloric acid 37%, NaCl (Merck, Germany), and distilled water. Gentle agitation was provided by a magnetic stirrer (Stuart CB162 Hotplate and Magnetic Stirrer, New Jersey, USA) rotating at 100 rpm. The assessment was conducted visually and the time for emulsification was taken [25].

Optimization of SNEDDS

Along with the formula generated in our previous study [26], SNEDDS were optimized using a D-Optimal mixture experimental designed and performed using a Design Expert® Ver. 7.1.5. software (Stat-Ease Inc., Minneapolis, USA) [27] by selecting the percentage of the oil component X1 (a mixture of VCO and Cymbopogon citratus essential oil), the surfactant X2 (Tween 80), and the co-surfactant (PEG 400) as independent variables, while emulsification time in sec (Y1) and transmittance in percent (Y2) as responses. Data were analyzed by one-way ANOVA at 0.05 levels [28].

Characterization of SNEDDS

Measurement of droplet size and zeta potential

The droplet size and zeta potential of the optimum formula were determined by dynamic light scattering techniques using a Zeta

potential/Particle-sizer (Horiba Scientific SZ-100, Horiba, Kyoto, Japan). Liquid SNEDDS (1 ml) was diluted to 100 ml with distilled water and performed in triplicate [29].

Viscosity

The viscosity of optimum formula was measured by Brookfield viscometer (Brookfield engineering laboratories, Stoughton, MA, USA) at 25±0.5˚C, and at 12 rpm. The viscosity of each SNEDDS was performed in triplicate [30].

Thermodynamic stability study

According to the method of [31], the study of the thermodynamic stability was implemented for optimum formula in three steps:

1. Heating-cooling cycle: The optimum formula was stored at 4˚C and 45˚C for 48 h at each temperature using constant climate chamber bath, it was repeated for 6 cycles in triplicate. The formulation that withstands the heating-cooling cycle was continued to the centrifugation test.

2. Centrifugation test: The optimum formula passed heating-cooling cycle were centrifuged at 5000 rpm for 30 min, observed for any sign of creaming, cracking, or separation phase.

3. Freeze-thaw cycle: The optimum formula passed centrifugation test were exposed at-21˚C and 21˚C for 24 h at each temperature, centrifuged at 3000 rpm 5 min, and continued visual observation.

Transmission electron microscopy (TEM)

The micro-morphology of the optimum formula was observed by TEM Joel JEM-100 CX (Joel, Tokyo, Japan). The SNEDDS was diluted with water (1:1000), a sample drop was stained with 2% phosphotungstic acid solution, and placed on a copper grid for 30 s [21].

Table 1: Level of the factors analyzed using D-optimal mixture experimental design

Factors/Independent variables	Level	
	Low	High
X_1 = Quantity of Oil (%)	14.29	20.00
X_2 = Quantity of Tween 80 (%)	60.00	71.43
X_3 = Quantity of PEG 400 (%)	14.29	20.00

Table 2: Percentage of oil, tween 80, and PEG 400 and observed responses

Run	X_1	X_2	X_3	Y_1	Y_2
	Oil (%)	Tween 80 (%)	PEG 400 (%)	Transmittance (%)	Emulsification time (s)
1	17.737	67.973	14.290	99.600	53.480
2	14.607	66.947	18.446	99.700	51.360
3	14.293	71.417	14.290	99.900	42.430
4	20.000	60.005	19.995	99.800	74.400
5	20.000	60.005	19.995	99.100	85.170
6	14.293	71.417	14.290	99.100	49.000
7	16.764	66.028	17.208	99.800	42.890
8	20.000	65.220	14.780	99.800	65.000
9	17.493	62.507	20.000	99.600	70.940
10	17.454	64.168	18.378	99.800	43.170
11	14.986	65.014	20.000	99.600	48.030
12	14.449	68.774	16.777	99.900	53.740
13	14.986	65.014	20.000	99.500	49.330
14	20.000	62.616	17.384	99.600	61.640
15	17.737	67.973	14.290	99.800	55.640
16	20.000	65.220	14.780	99.500	57.800

The optimum formula was replicated three times and verified with a single sample t-test with OpenStat® (Industrial Technology Department, Iowa State University, USA). The optimum formula was selected for characterization.

RESULTS AND DISCUSSION

Optimization Formula of SNEDDS

Table 3: The statistical parameter of each response using an experimental design

Response	*F* value	Probability>*F*	Significance
Y = Emulsification time			
Model	9.32	0.0031	Significant
Lack of fit	4.66	0.0535	Not significant
Y = Transmittance			
Model	0.15	0.8596	Not significant
Lack of fit	0.24	0.9652	Not significant

Emulsification time

The probability of the model was less than 0.05, and the lack of fit was more than 0.05 (table 3). This result indicated that the chosen model can describe the relationship between the variables. Fig. 1 showed that the residual is normally distributed around the line and nothing stands out. The relationship between variables was illustrated using a special model cubic (fig. 2).

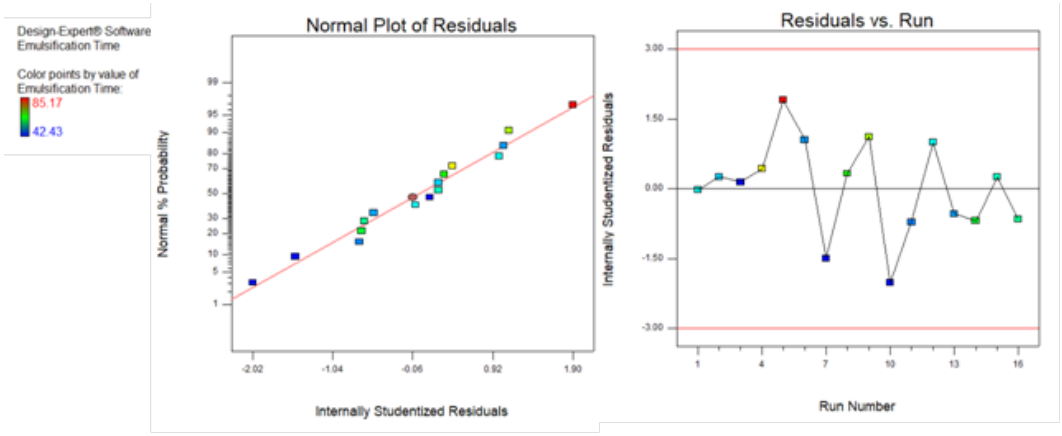

Fig. 1: The normal probability plot residual of emulsification time

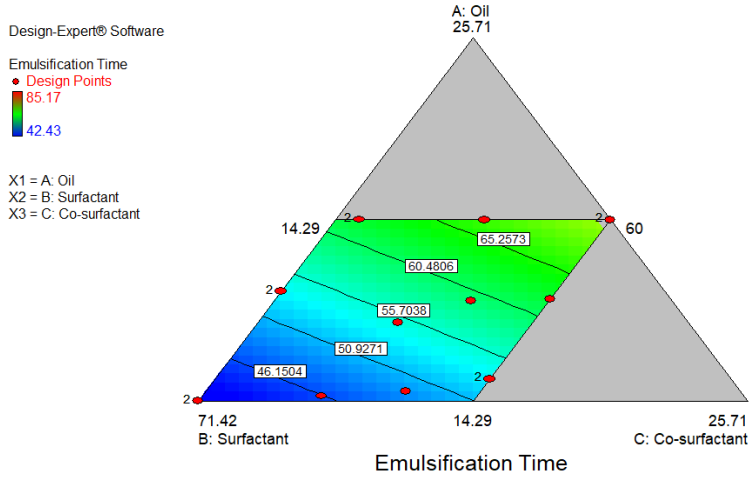

Fig. 2: Special model cubic of emulsification time of the mixture study result

Emulsification time used as a response is a way to determine the ability of surfactant and co-surfactant components to emulsify oil components [32]. Selection of the optimum formula based upon "trading off" from the minimization of emulsification time. Emulsification time is one of the important keys to estimating emulsification efficiency [33].

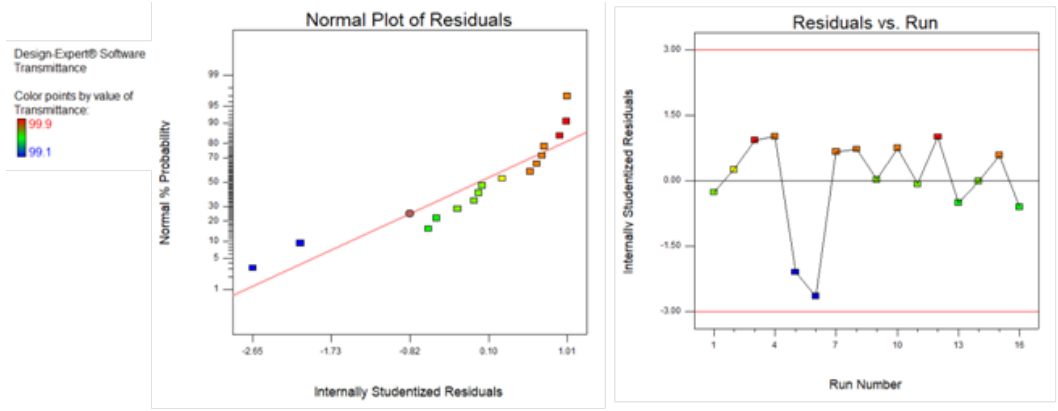

Fig. 3: The normal probability plot residual of transmittance

Percent transmittance

The probability of the model and the lack of fit were both more than 0.05 (table 3). The data indicated the chosen model can describe the relationship between the variables. Fig. 3 showed that the residual was normally distributed around the line and no one stands out. The relationship between variables was illustrated using a special model cubic (fig. 4).

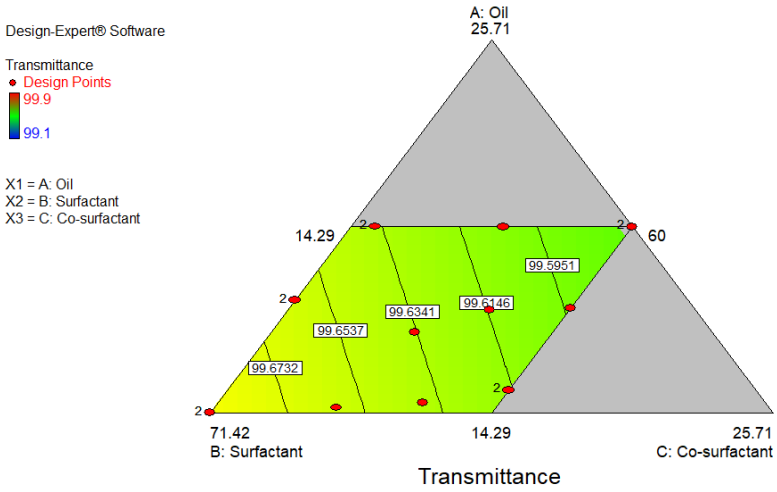

Fig. 4: Special model cubic of transmittance of the mixture study result

The optimization formula aimed to find products that have high-quality characteristics that will be produced [34]. Transmittance must be maximized to produce good quality products. The transmittance value of all treatments is above 99 percent, indicating the efficiency of self-nanoemulsion [35]. A good nanoemulsion has a transmittance value close to 100 percent that indicated a clear nanoemulsion [36].

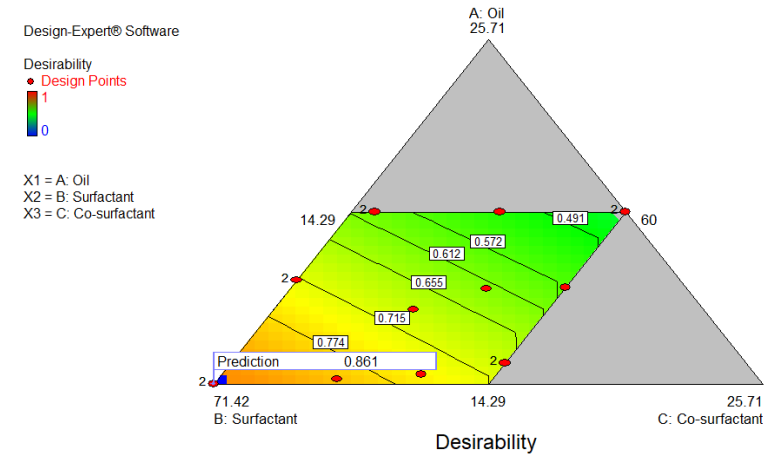

Fig. 5: Desirability

The optimized formula that selected was the one that have a desirability value of close to 1 [37]. Fig. 5 showed the desirability which used to predict the optimum formula. The first solution found through numerical optimization and desirability value was 0.861. The composition of optimum formula consists of an oil component (14.293%), Tween 80 (71.417%), and PEG 400 (14.290%).

Table 4: Predicted value and actual data of the optimized SNEDDS

Response	Actual data±SD	Predicted value	*P*-value
Emulsification time	42.98±1.33	41.384	0.172
Transmittance	99.73±0.12	99.693	0.604

*Actual data are mean±SD, n=3 [SD: Standard deviation], the actual data and predicted value are shown in table 4. The *P*-value of emulsification time and transmittance was higher than 0.05, that indicated no significant difference between actual data and the predicted value.

Characterization of SNEDDS

Table 5: Droplet size, PI, zeta potential, and viscosity of the optimum formulation

Replication	Droplet size (nm)	Polydispersity index (PI)	Zeta potential (mV)	Viscosity (mPa·s)
R1	13.2	0.219	-22.6	200
R2	13.2	0.118	-20.0	200
R3	13.1	0.179	-20.1	200
mean±SD	13.17±0.06	0.17±0.05	-20.90±1.47	200±0

* SD: Standard deviation, n=3

Droplet size

The droplet size of nanoemulsion was found to be 13.17±0.06 with polydispersity-index (PI) 0.17±0.05 (table 5). Droplet size is a crucial factor to evaluate the system of nanoemulsion [38]. Smaller particle size causes better absorption at gastrointestinal tract [39]. The optimum formula shows a PI value lower than 0.5 which indicate uniformity droplet size distribution [40].

Zeta potential

The zeta potential of the optimum formulation was -20.90±1.47 mV as shown in table 5. The fatty acid content in the formula may cause the negative charge of the zeta potential [41].

Viscosity measurement

The viscosity of the optimum formulation is 200±0 mPa·s (table 5). The concentration of surfactant and carrier oil used in SNEDDS is related to the value of viscosity [18]. The viscosity is directly proportional to the oil concentration [42].

Thermodynamic stability

The optimum SNEDDS formula did not change visible appearance which showed good stability at various storage conditions (table 6). Nanoemulsion is more stable than an emulsion, SNEDDS that will form nanoemulsion must be thermodynamically stable with the characteristic of not experiencing precipitation, cracking, or creaming [43].

Table 6: Results for thermodynamic stability studies

Replication	Heating-cooling cycle	Centrifugation test	Freeze-thaw cycle
R1	√	√	√
R2	√	√	√
R3	√	√	√

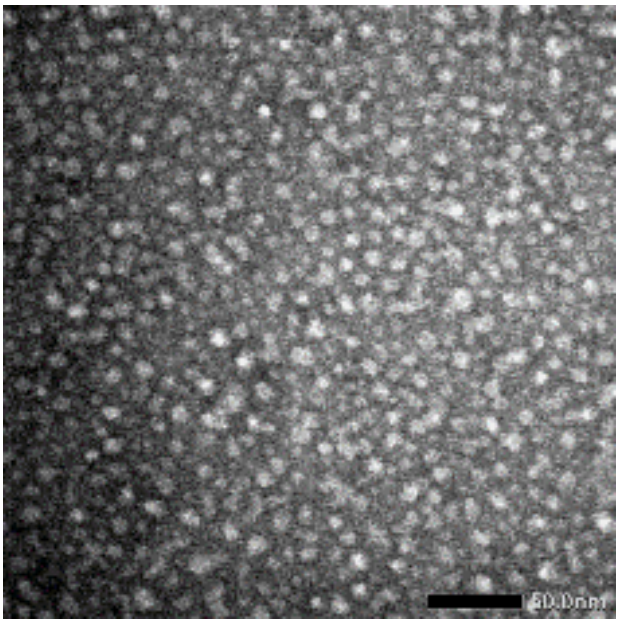

Fig. 6: TEM of optimized SNEDDS formulation (80,000×; dilution 1000-fold with water)

Transmission electron microscopy (TEM)

The TEM of the optimum formula is presented in fig. 6. After dilution, all droplets demonstrated spherical shape and almost same size, represented a successful formation of SNEDDS. The TEM of the optimized formulation shows no coalescence signs indicating that the selected formula has good quality [44].

CONCLUSION

The optimization of *Cymbopogon citratus* SNEDDS using D-Optimal mixture experimental design was performed. The optimum formula for SNEDDS was a mixture of Tween 80, PEG 400, VCO, and *Cymbopogon citratus* essential oil at a ratio of 71.417: 14.290: 7.147: 7.147 (%). From the optimizing formula can be shown that the mean of droplet size, PI, zeta potential, and viscosity were: 13.17±0.06 nm, 0.17±0.05,-20.90±1.47 mV, 200±0 mPa·s, respectively. Furthermore, the optimized formula was passed the thermodynamic stability test, meanwhile team displayed spherical shape. The optimized SNEDDS formula was improving solubility of poorly soluble *Cymbopogon citratus* essential oil.

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AUTHOR CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

All the authors declared none

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