

Self-Nano Emulsifying Drug Delivery System (SNEDDS) of *Curcuma mangga* Val. Essential Oil and The Stability Study

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ABSTRACT

Essential oil of *Curcuma mangga* Val. has been reported to have cytotoxic effect against cancer cell lines. But this oil is unstable in dispensing so that a self nano-emulsifying drug delivery system (SNEDDS) of the oil was conducted to solve the problem and improve its potency. In the study, optimization, verification, characterization, and stability test of the SNEDDS formula were carried out respectively by simplex lattice design (SLD) on Design Expert ver. 10 software, droplet size and Zeta potential determinations using particle size analyzer (PSA) instrument, as well as heating-cooling and freeze-thaw methods. The best SNEDDS formula resulted was Miglyol : Tween 80 : PEG 400 = 16.034% : 68.380% : 15.586%; with transmittance of 84.47±1.05%, droplet size of 15.75 nm, zeta potential of -8.54 mV, polydispersity index (PDI) of 0.188, emulsifying time of 49.67±1.7 seconds in distilled water, 24.33±4.19 seconds in artificial gastric fluid and 21.33±2.87 seconds in artificial intestine fluid. After a freeze-thaw test there was no change on the emulsion's clarity, color, smell, as well as no separation, which means that the formula was stable thermodynamically. The optimum SNEDDS formula resulted has small particle size, better emulsifying time in artificial gastric and intestine fluids, as well as better thermodynamic stability, which in turn will improve the cytotoxic activity of the *Curcuma mangga* Val. rhizome oil toward cancer cells.

Keywords: *Curcuma mangga* Val., Essential oil, Self nano-emulsifying drug delivery system (SNEDDS), Simplex Lattice Design (SLD), Particle Size Analyzer (PSA).

INTRODUCTION

The essential oil of *Curcuma mangga* Val. rhizomes has shown a cytotoxic effect toward some cancer cell-lines (Khasanah, 2002, Nurrokhman, 2004, Verlianara, 2004, Rumiya, *et al.*, 2007, and Astuti, *et al.*, 2014). But the oil is not stable. According to Billia *et al.* (2014), some essential oils have some disadvantage properties such as easy to evaporate, easily decompose against heat, humidity, light and oxygen. These disadvantage properties could cause problems whenever the oil will be administrated/used as is. An appropriate formula which can protect the oil from degradation in order to improve the stability as well the activity and effectiveness is needed. This study is to find out the best nano-emulsion formula for the essential oil of *C. mangga* Val. in order to improve its stability and potency. The self nano-emulsifying drug

delivery system (SNEDDS) formula which has components of: carrier oil, surfactant and cosurfactant was adopted to construct a nano emulsion of the *C. mangga* essential oil.

MATERIAL AND METHODS

C. mangga Val essential oil.

The essential oil of *C. mangga* Val. was isolated by water steamed distillation of sliced clean rhizome in 6 hours. The oil resulted was dried by shaking it with anhydrate CaCl₂ and filtered it into a clean bottle, which then stored in a freezer until it was used in the study.

SNEDDS formulation and optimization.

SNEDDS formula was prepared using Tween 80 as the surfactant, PEG 400 as cosurfactant, and a carrier oil. Different oils of Miglyol (synthetic oil),

virgin coconut oil (VCO) or olive oil were selected as the carrier oil. Best formula was selected from five formulas which were constructed based on the SNEDDS component ratio. The criteria for the selection were least amount of surfactant and best emulsion homogeneity or stability according to visual observation. The best formula obtained was then optimized by Simplex Lattice Design method using Design Expert Ver.10 software.

To find out the optimum formula, two parameters of transmittance and emulsifying time were measured, as well as stability tests of heating-cooling and freeze-thaw tests were conducted on the emulsions. Three different emulsion media of distilled water, artificial gastric fluid (AGF) and artificial intestine fluid (AIF) (Astuti *et al.*, 2018) were used for making the emulsions. To determine the optimum formula the two parameters of the three different nano-emulsions resulted were used as the goal limits.

SNEDDS verification and characterization.

Verification was carried out by measuring the transmittance and emulsifying time of the emulsions. The measurements were then compared to those of the suggested values. The emulsion was also characterized through its organoleptic, pH, % transmittance, droplet size and Zeta potential. The pH was determined using pH meter. The % transmittance was measured by UV Spectrophotometer at 650nm using solution of 100 μ L SNEDDS diluted in distilled water up to 10mL. The droplet size and Zeta potential were determined triplicate from the SNEDDS dilution using particle size analyzer (PSA).

SNEDDS emulsifying time.

Emulsifying time test of SNEDDS formula was conducted in three different fluids of CO₂ free distilled water, artificial gastric fluid (AGF) with pH 1.2 and artificial intestine fluid (AIF) with pH 7. On each fluid, a 0.5mL SNEDDS was dispersed in a 250 ml flask, at 37 \pm 2 $^{\circ}$ C on a magnetic stirrer with 50 rpm rotation. The emulsifying time was started as the SNEDDS contact the fluid, and was stopped when nano-emulsion was completely formed (Astuti *et al.*, 2018; Beandrade, 2018; Khan *et al.*, 2015).

RESULT AND DISCUSSION

Five tentative compositions of SNEDDS formulas (Table I.A), while the formula stability using three different oils (Table I.B). Based on formula stability observation (Table I.B.), Miglyol was

the best carrier oil. So that Miglyol was selected as the *C. mangga* oil carrier. Combination 1:2:1 had already resulted isotropic mixture, therefore the SNEDDS formula optimization could be conducted within combinations of 1:2:1 (Table I.B.). The lower and upper limits of Miglyol, Tween 80 and PEG 400 were respectively 15-25, 60-70, and 15-25, which were optimized by Simplex Lattice Design (SLD) method using Design Expert Ver.10 software.

The SLD method resulted in 14 SNEDDS combinations to be prepared for measuring the transmittance and emulsifying time (Table II.A). These measurement values were then uploaded back to the program to find the optimum formula. The formula was then determined statistically using ANOVA upon the transmittance and emulsifying time data, and resulted equation models (Table II.B).

All the responses showed p values of <0.05 (Table II.B.); which means that any proportion change will significantly change the responses. The higher transmittance response value the better the SNEDDS formula. Positive value of the coefficient in the equation means positive synergy of the component in the emulsion in giving responses; while negative value coefficient means the component giving negative synergy (Huang *et al.*, 2004). The increasing transmittance response of the SNEDDS was supported by Tween 80 (B) and PEG 400 (C); while the Miglyol (A) reduced the transmittance response (Table II.B). This is in line with the fact that increasing oil concentration will decrease the transmittance due to its larger droplet size (Wulandari *et al.*, 2016). Different from the transmittance response; the lesser the value of emulsifying time the faster the time for emulsifying, which means the better the SNEDDS formula. Other responses (out of emulsifying response) were due to the component interactions. Interaction between Miglyol and PEG 400 (AC) gave negative values and causing decreasing emulsifying time and faster impact to those three-emulsifying responses (Table II.B). However, the interaction between Tween 80-PEG 400 (BC) gave negative coefficients (faster emulsifying time) only in AGF and AIF media, but not in distilled water.

The relationship between the transmittance and surfactant (Tween 80) - co-surfactant (PEG 400) of the SNEDDS formula is shown as a contour plot (Figure 1.A). The higher of % transmittance of the formula the yellow-brighter was shown in the figure. It was showed that the higher the surfactant and co-surfactant, the higher the transmittance (Figure 1.A).

Table I. SNEDDS component ratio and formula stability

Table 1.	Formula	Carrier oil	Surfactant (Tween 80)	Cosurfactant (PEG 400)		
A. Component ratio of SNEDDS	I	1	1	1		
	II	1	2	1		
	III	1	3	1		
	IV	1	4	1		
	V	1	5	1		
B. SNEDDS formula stability in 24 hours	Miglyol	Oil : Tween 80 : PEG 400	1 : 1 : 1	Unstable		
			1 : 2 : 1	Stable		
			1 : 3 : 1	Stable		
			1 : 4 : 1	Stable		
			1 : 5 : 1	Stable		
	VCO	Oil : Tween 80 : PEG 400	1 : 1 : 1	Unstable		
			1 : 2 : 1	Unstable		
			1 : 3 : 1	Stable		
			1 : 4 : 1	Stable		
			1 : 5 : 1	Stable		
			Olive oil	Oil : Tween 80 : PEG 400	1 : 1 : 1	Unstable
					1 : 2 : 1	Unstable
					1 : 3 : 1	Unstable
					1 : 4 : 1	Unstable
					1 : 5 : 1	Unstable

This because the surfactant and co-surfactant reduced the water surface-tension and causing it more flexible and causing the system produced much smaller (nano size) oil droplets; so that the UV beam could easily went through the solution and increased its transmittance.

The relationship between the emulsifying time and surfactant (Tween 80) - co-surfactant (PEG 400) of the formula is shown as contour plots (Figure 1. B, C and D). The faster the formula producing nano-emulsion, the dark-bluish was shown in the figure. The yellow-reddish color expressed the slowest time of emulsifying (Figure 1. B, C and D). The faster the emulsifying time, the easier the SNEDDS forming oil in water (O/W) nano-emulsion. In this case the same reason was occurred (Figure 1.A).

To find out the optimum SNEDDS formula, transmittance limit of 86.1% and emulsifying time of 60 seconds were assigned to the SLD program as the goal limits for all the three different nano-emulsions. There were 4 formulas suggested by the SLD program (Table 3.A), which one of them would be selected as the optimum SNEDDS formula. Formula 1 with the best desirability value of 0.904

was selected as the optimum SNEDDS formula with composition of Miglyol: Tween 80:PEG 400 = 16.034%:68.380%:15.586% (Table 3.A).

Optimum SNEDDS verification, characterization, and stability

The optimum SNEDDS verification of transmittance and emulsifying time were shown in Table 3.B. It was found that the observation values were much better than the prediction values, either the transmittance or emulsifying time in all fluids. The optimum SNEDDS formula characterizations are shown in Figure 2. The droplet size was 15.75 nm (Figure 2A), which was considered as small size droplet, because the nano-emulsion must have droplet size 20 - 200 nm (Aboofazeli, 2010). The droplet size is an important factor in drug absorption and delivery (Senapati *et al.*, 2017). This droplet size is influenced by shear and surfactant effects, as well as the relative viscosity between oil (dispersed phase) and water (continuous phase) (Singh *et al.*, 2017).

The droplet uniformity is measured by polydispersity index (PDI). The PDI value of optimum SNEDDS was 0.188 (Figure 2A),

Table II. SNEDDS formulas and the response models and equations

	Formula No.	Composition (% w/w)			Transmittance (%)	Emulsifying time (seconds)		
		Miglyol	Tween 80	PEG 400		DW	AGF	AIF
A. 14 SNEDDS formulas (resulted from Design Expert Ver.10 program), and the Transmittance and emulsifying time.	1	20	60	20	82.5	64	23	20
	2	16.67	66.67	16.67	81.2	58	31	35
	3	21.67	61.67	16.67	67.4	144	124	124
	4	15	70	15	82.3	42	42	32
	5	25	60	15	71.4	215	155	121
	6	18.33	63.33	18.33	71.5	74	54	38
	7	15	65	20	86.1	70	20	23
	8	15	60	25	81.4	54	15	18
	9	15	60	25	82.4	31	22	15
	10	16.67	61.67	21.67	68.5	34	18	16
	11	25	60	15	71.8	238	154	162
	12	20	65	25	71.5	130	213	207
	13	15	70	15	81.6	36	22	28
	14	20	65	15	77.5	143	200	210

	Response	Model	Equation	p value
B. Model and equation of SNEDDS formulas' responses	Transmittance	Linear	$Y = -17.50A + 100.09B + 92.02C$	< 0.05
	Emulsifying in Distilled water	Quadratic	$229.14A + 38.00B + 41.47C + 2.79AB - 303.36AC + 73.79BC$	< 0.05
	Emulsifying in AGF	Special Quadratic	$154.12A + 31.62B + 18.12C + 451.48AB - 258.55AC - 25.55BC + 715.76A^2BC - 4810.24AB^2C + 905.05ABC^2$	< 0.05
	Emulsifying in AIF	Special Quadratic	$141.54A + 30.04B + 16.54C + 491.17AB - 235.49AC - 0.49BC + 652.92A^2BC - 5053.08AB^2C + 163.86ABC^2$	< 0.05

Note:DW = distilled water; AGF = artificial gastric fluid; AIF = artificial intestine fluid; Y = Response, A = Miglyol; B = Tween 80; C = PEG 400.

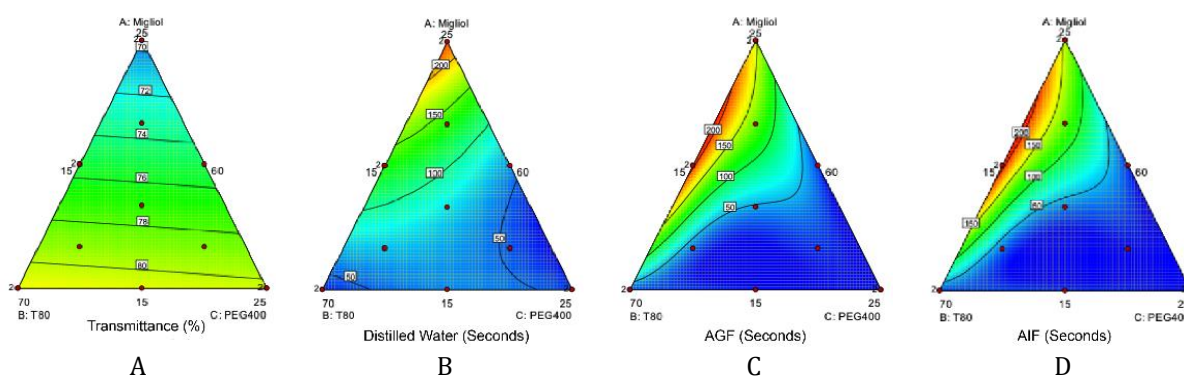


Figure 1A. Contour plot of transmittance (%) of SNEDDS in distilled water, B. Emulsifying times of SNEDDS in media of distilled water, C. artificial gastric fluid (AGF), and D. artificial intestine fluid (AIF).

Table III. Four SNEDDS formulas and verification of the optimum formula

	Formula	Miglyol	Tween 80	PEG 400	Desirability
A. Four SNEDDS formulas	1	16.034	68.380	15.586	0.904
	2	15.819	69.181	15.000	0.812
	3	22.040	60.073	17.887	0.665
	4	15.000	68.750	16.250	0.421
B. Verification of optimum SNEDDS formula		Transmittance (%)	Emulsifying time (seconds) in:		
			DW	AGF	AIF
	Prediction	80.039	60.000	60.000	60.807
Observation	84.47 ± 1.05	49.67 ± 1.70	24.33 ± 4.19	21.33 ± 2.87	

Note: DW = distilled water, AGF = artificial gastric fluid, AIF = artificial intestine fluid

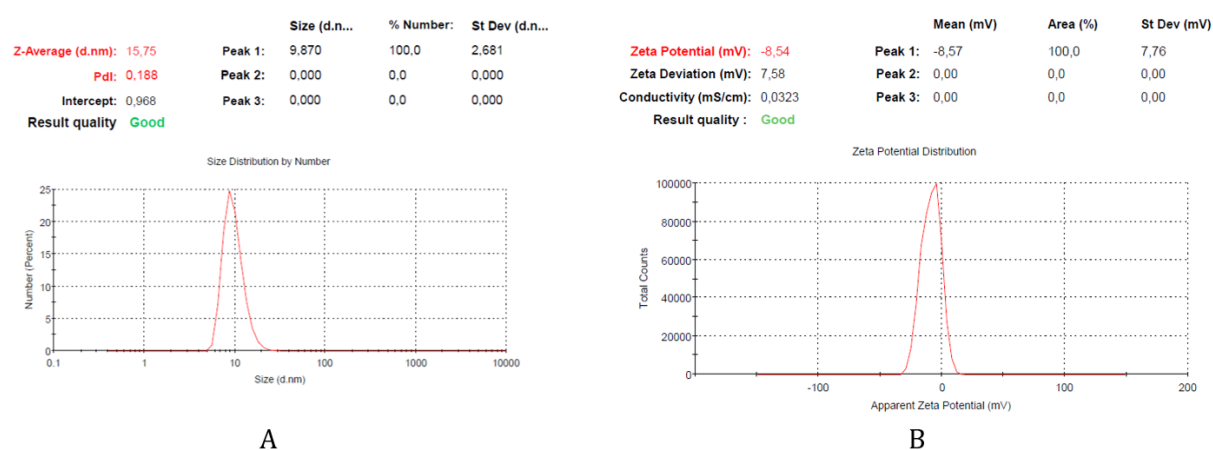


Figure 2. Optimum SNEDDS formula characterizations: A. Average droplet size and polydispersity index (PDI), B. Zeta potential

which means uniform and monodisperse as it was <0.2 (Singh *et al.*, 2017). Zeta potential value showing the charge difference between the medium and the particle carrier, as well as the repulsion strength between the medium and the dispersed particle (Chabib *et al.*, 2016). The higher the zeta potential the stronger the repulsion and the better emulsion stability because it decreases the possibility of the particles to aggregate, flocculate, coalesce and coagulate (Khan *et al.*, 2014, Chabib *et al.*, 2016, Ke *et al.*, 2016). The optimum SNEDDS zeta potential was - 8.54 mV which is stable but not in a long time since the value of 8.54 < 30 (no matter of positive or negative values) (Singh *et al.*, 2017).

There was no change on the emulsion’s clarity, color, smell, as well as no separation after the heating-cooling and freeze-thaw tests. This

showed that the optimum SNEDDS formula was stable thermodynamically.

CONCLUSION

The optimum SNEDDS formula for *C. mangga* Val. oil was composed by Miglyol:Tween 80:PEG 400 = 16.034%:68.380%:15.586%; with 84.47±1.05% transmittance, 15.75 nm droplet size, -8.54 mV Zeta potential, 0.188 PDI, 49.67±1.7 seconds of emulsifying time in distilled water, 24.33±4.19 seconds in AGF and 21.33±2.87 seconds in AIF. The optimum SNEDDS formula was stable thermodynamically. Due to the tiny particle size, better AFG and AIF emulsifying time and thermodynamic stability; this optimum SNEDDS formula will hopefully improve the cytotoxic activity of the *Curcuma mangga* Val. oil against cancer cell.

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