Development of self nanoemulsifying drug delivery system for black seed oil (Nigella sativa L.)

S E Priani, S S Maulidina, F Darusman, L Purwanti and D Mulyanti
Pharmacy Department, Universitas Islam Bandung, Bandung, Indonesia

*sani.ega.p@unisba.ac.id

Abstract. One strategy to improve oral bioavailability of hydrophobic drugs like black seed oil with formulate them into self nanoemulsifying drug delivery system (SNEDDS). The aim of this study is to develop black seed oil into liquid SNEDDS formulation to enhance oral bioavailability. The optimization of SNEDDS was carried out using various comparison between oil, surfactant and cosurfactant. The formulated SNEDDS were evaluated for transmittance percentage, emulsification time, dispersebility, robustness, thermodynamics stability and globul size determination. The best formulation of SNEDDS obtained from tween 80 as surfactan and PEG 400 as cosurfactant (2:1) with ratio of oil and mix surfactant-cosurfactant 1:8. The formulation of SNEDDS met the requirement of transmittance percentage (97.80%), emulsification time (35.00 second), grade A of dispersibility, stable of robustness and thermodynamic stability tests and the average of globul size was 65.4 nm. The SNEDDS formulation containing black seed oil has good physical characteristic and stability.

1. Introduction
Nigella sativa or black seed/black cumin has been recognized as a “miracle cure” for its ability to treat various diseases. The seed importance in the “Prophetic Medicine” tradition. Black seed has been reported to have many therapeutic properties including antimicrobial, antitumor, antipyretic, antihistaminic, antidiabetic, antihypertensive, anti-inflammatory, hepatoprotective, and gastroprotective [1]. Black seed contain 35.6-41.6% fixed oil and 0.5-1.6% of volatile oil. Fixed oil of black seed is rich in linoleic, oleic and palmitic acids and volatile oil contain thymoquinone, thymol, carvacrol, α and β-pinene, d-limonene, p-cymene [2,3]. Thymoquinone is one of important active constituent of black seed oil since has many pharmacological activity including anti-inflammatory, antioxidant, antibacterial, anticancer, and other important activities [4].

Black seed oil is hydrophobic in nature that cause low oral bioavailability of its components and therefore demonstrates limited therapeutic effect in oral administration. Formulation strategy was needed to increase the oral bioavailability of the oil [5]. One of formulation strategy to improve oral bioavailability of drugs is using Self Nano Emulsifying Drug Delivery System (SNEDDS). SNEDDS is pharmaceutical formulation consists of oil, surfactant and co-surfactant that have ability of forming oil-in-water (O/W) nanoemulsions under mild agitation. Agitation could be provided by body movement and gastrointestinal movement. The globule size of SNEDDS is less than 100 nm under dispersion of water [6,7]. SNEDDS is thermodynamically stable formulation that can enhance permeation of drugs across the intestinal membrane and enhance drug bioavailability [8]. Previous study
showed that SNEDDS formulation can increase the dissolution rate and bioavailability of lipophylic drugs such as glimepirid, simvastatin, and zeodary essential oil [8,9,10].

This study was conducted to formulate black seed oil on SNEDDS formulation using Tween 80 and Polyethyleneglycol (PEG400) as surfactant and cosurfactant, with different comparison of oil and surfactant mix and comparison of surfactant and cosurfactant. All formulation was evaluated by physical and stability tests.

2. Method

2.1. Material

Material that used for this research is Black Seed Oil (Lantabura International), Tween 80, PEG 400, HCl (Merck), Phosphate Buffer (KH$_2$PO$_4$-K$_2$HPO$_4$, Merck)

2.2. Optimization formula of SNEDDS Atorvastatin calcium

The optimization formula was carried out using various comparisons of oil and mixed surfactants (1:9; 1:8; 1:7; 1:6; 1:5) dan various comparison of surfactants and cosurfactant (3:1; 2:1) (Table 1). Black seed oil was mixed with Tween 80 and PEG 400. After adding all the components, the mixture was vortexed for few minutes and then homogenized at 40°C for 5 minutes. After homogenization, the mixture was sonicated in bath sonicator for 15 minutes [11].

| Formula | Oil : Smix | Surfactant : Kosurfatan |
|---------|------------|--------------------------|
| F1      | 1:9        | 3:1                      |
| F2      | 1:9        | 2:1                      |
| F3      | 1:8        | 3:1                      |
| F4      | 1:8        | 2:1                      |
| F5      | 1:7        | 3:1                      |
| F6      | 1:7        | 2:1                      |
| F7      | 1:6        | 3:1                      |
| F8      | 1:6        | 2:1                      |
| F9      | 1:5        | 3:1                      |
| F10     | 1:5        | 2:1                      |

2.3. Evaluation of SNEDDS Black seed oil

2.3.1. Percent transmittance. SNEDDS formulations were diluted using distilled water (100 times) and then stirred using vortex for 30 seconds. The percent transmittance was measured using spectrophotometry UV/Vis at 650 nm [12].

2.3.2. Dispersibility and emulsification time determination

The dispersibility grade of selected SNEDDS was determined using dissolution apparatus type II. 1 ml of SNEDDS was added to 500 ml of distilled water at 37±0.5 °C with paddle rotating at 50 rpm. Time of emulsification and final appearance of the emulsion was observed visually, and then classified using the standard grading system [13].
Table 2. Grade of SNEDDS dispersibility.

| Grade | Description |
|-------|-------------|
| A     | Rapidly forming an emulsion (within 1 minute) with a clear or bluish appearance |
| B     | Rapidly forming (Within 2 minutes), slightly less clear emulsion, having a bluish-white appearance |
| C     | Fine milky emulsion that formed within 2 min. |
| D     | Dull, a grayish white emulsion having a slightly oily appearance that is slow to emulsify (more than 2 min) |
| E     | Exhibiting either poor or minimal emulsification with large oil globules present on the surface |

2.3.3. Robustness test. Robustness to dilution of selected SNEDDS was done by diluting of SNEDDS with distilled water, 0.1 N HCl and phosphate buffer of pH 6.8 (100 times dilution). The systems were mixed using a magnetic stirrer at 37 °C until homogeneity. These systems were observed visually after stored at ambient temperature for 24 h, for detect any sign of phase separation [14].

2.3.4. Thermodynamic stability test. Selected formulations of SNEDDS were subjected to thermodynamic stability tests [15].

- **Centrifugation**: SNEDDS formulation were centrifuged at 3500 rpm for 30 min. Instabilities off SNEDDS including phase separation, creaming and cracking were observed. Stable Formulations were taken for next tests.
- **Heating cooling cycle**: The samples were subjected to three cycles between refrigerator (4°C) and 45°C. Storage at each temperature not less than 48 h. Instabilities of SNEDDS were observed at the end of every cycle. Formulations, that are stable at these tests, were subjected to Freeze thaw cycle.
- **Freeze thaw cycle**: The samples were subjected to three freeze thaw cycles between -21°C and +25°C. Storage at each temperature for not less than 48 h. The stability of SNEDDS assessed at the end of every cycle.

2.3.5. Droplet size measurement
Droplet size and distribution of SNEDDS after 500 times dilution using distilled water were determined using Particle Size Analyzers (Beckman Coulter, Delsa Nano) [10].

3. Results and discussion
In this study was developed SNEEDS formulation containing black seed oil using surfactant tween 80 and co-surfactant PEG 400. In SNEDDS formulation, surfactants can decrease interfacial tension between the oil phase and the aqueous phase to form nanoemulsion. The surfactant used in this formulation was tween 80. Tween 80 is nonionic surfactant that widely used in cosmetic preparations and topical preparations caused by nontoxic and nonirritating characteristic. PEG 400 was used as cosurfactant. Cosurfactant in SNEDDS or nanoemulsion preparation can reduce interfacial tension nearest to zero to facilitate dispersion and emulsification [16].

The optimization formula of SNEDDS was carried out using various comparisons of oil and mixed surfactants (1:9; 1:8; 1:7; 1:6; 1:5) dan various comparison of surfactants and cosurfactant (3:1; 2:1). All formulation was subjected to percent transmittance measurement. Percent transmittance showed the transparency of the SNEDDS. Percent transmittance more than 95% indicating clear emulsion [17]. From Table 3, was known formula then subjected to further evaluation test.
Table 3. Percentage of transmittance black seed oil SNEDDS.

| Formula | % Transmittance |
|---------|-----------------|
| F1      | 97,77 ± 0,06    |
| F2      | 98,03 ± 0,12    |
| F3      | 97,33 ± 0,06    |
| F4      | 97,80 ± 0,00    |
| F5      | 96,70 ± 0,00    |
| F6      | 94,77 ± 0,06    |
| F7      | 94,60 ± 0,00    |
| F8      | 93,93 ± 0,06    |
| F9      | 91,50 ± 0,00    |
| F10     | 92,97 ± 0,00    |

The efficiency of self-emulsification of oral nanoemulsion is assessed by dispersibility test. All selected formula that have percent transmittance value more than 95% (F1, F2, F3, F4, F5) is assessed with this tests. The result showed that F3, F4, F5 showed Grade A in dispersibility test, with emulsification time about 35-46 Seconds (Table 4). Grade A and Grade B results of dispersibility will remain as nanoemulsion when dispersed in GIT [13].

Table 4. Dispersibility and emulsification time of selected SNEDDS.

| Formula | Emulsification Time (Sec) | Appearance | Grade |
|---------|---------------------------|------------|-------|
| F1      | 305,00 ± 0,060            | Clear      | A/D   |
| F2      | 218,00 ± 0,21             | Clear      | A/D   |
| F3      | 46,33 ± 1,53              | Clear      | A     |
| F4      | 35,00 ± 2,00              | Clear      | A     |
| F5      | 45,67 ± 2,52              | Clear      | A     |

Robustness of dilution was studied at the three diluent condition. After dilution all selected formula of SNEDDS, the resulting nanoemulsions were found to remain transparent and showed no phase separation (table 5). The result showed that all selected formula was stable at aqueous dilution and the pH of the aqueous phase did not influence the properties and stability of nanoemulsions [10].

Table 5. Robustness of dilution of selected SNEDDS.

| Formula | Aquadest | 0.1 N HCl | Buffer Phosphate pH 6.8 |
|---------|----------|-----------|-------------------------|
| F1      | +        | +         | +                       |
| F2      | +        | +         | +                       |
| F3      | +        | +         | +                       |
| F4      | +        | +         | +                       |
| F5      | +        | +         | +                       |

(+ ) means stable formula which showed no phase separation or precipitation.

The physical stability of SNEDDS is importance factor for pharmaceutical formulation. From thermodynamic stability test, was known that selected formulation passed the test except for F5 (Table 6). It indicates that combination of oil, surfactant, and cosurfactant on formula F5 was not sufficient to ensure the stability of SNEDDS [18].
Table 6. Thermodynamic stability of SNEDDS.

| Stability Test  | Formula | F1 | F2 | F3 | F4 | F5 |
|----------------|---------|----|----|----|----|----|
| Heating Cooling|         | Yes| Yes| Yes| Yes| No |
| Centrifugation |         | Yes| Yes| Yes| Yes|-   |
| Freeze Thaw    |         | Yes| Yes| Yes| Yes|-   |

(-) test not be conducted; (Yes): Stable (No): Unstable

Based on transmittance, dispersibility, emulsification time, robustness to dilution, and thermodynamic stability tests, known that formula F4 is the optimum formula SNEDDS. The optimum formula of SNEDDS is formula with minimum amount of surfactant but have good the physical characteristic and stability. So, F4 was subjected to globule size measurement. Globule size of nanoemulsion after dilution of SNEDDS is the important property. The globule size of SNEDDS containing black seed oil was found to be in nano range with mean globule size 65.4 nm. Systems with mean globule size below 100 nm fulfill the criteria of SNEDDS. Another important parameter is polydispersibility index (PDI) that shows particle homogeneity (0-1). The closer to zero of PDI, indicates the more homogenous the particles size. The PDI value of SNEDDS black seed oil is 0.8. [19].

![Globule Size Distribution](image)

Figure 1. Globule size distribution of diluted SNEDDS.

4. Conclusion
The formulation of SNEDDS with comparison of oil and surfactant mix (1:8); surfactant and cosurfactant (2:1) meet the requirement of transmittance percentage (97.80%), emulsification time (35.00 second), grade A of dispersibility, stable of robustness and thermodynamic stability tests and the average of globul size was 65.4 nm. The SNEDDS formulation containing black seed oil has good physical characteristic and stability.

Acknowledgement
The author would like to thank PT. Lantabura International, Indonesia for providing Black Seed oil and Research Centre Bandung Islamic University for funding this research.

References
[1] Sahak M K A, Nurul K, Ghulam A, Suhaimi D, Noor H H, Durriyyah S H A 2016 The Role of Nigella sativa and Its Active Constituents in Learning and Memory, Evidence-Based
Complementary and Alternative Medicine, 1-6.

[2] Ahmad A, Husain A, Mujeeb M, Khan S A, Najmi A K, Siddique N A, Anwar F 2013 A review on therapeutic potential of Nigella sativa: A miracle herb Asian Pacific Journal of Tropical Biomedicine 3(5) 337–352

[3] Forouzanfar F, Bazzaz B S F, Hosseinzadeh H 2014 Black cumin (Nigella sativa) and its constituent (thymoquinone): a review on antimicrobial effects Iranian Journal of Basic Medical Sciences 17(12) 929–938

[4] Randhawa M A, Alenazy A K, Alrowaili M G, Basha J 2014 An active principle of Nigella sativa L., thymoquinone, showing significant antimicrobial activity against anaerobic bacteria Journal of Intericultural Ethnopharmacology 6(1) 97–101

[5] Rushmi Z T, Nasrin A, Rabeya J M, Merina K, Marzouk M, Mahbubur R, Mohammad H S 2017 The Impact of Formulation Attributes and Process Parameters on Black Seed Oil Loaded Liposomes and Their Performance in Animal Model of Analgesia Saudia Pharmaceutical Journal, 25(3) 404-412

[6] Abdul W K, Sabna K, Shahid H A, Rakesh K S, Javed A 2012 Potentials and challenges in self-nanoemulsifying drug delivery systems Expert Opinion on Drug Delivery 9(10) 1305-1317

[7] Seema G and Kuma S A 2014 Self Nanoemulsifying Drug Delivery System- A Noval Approach For Improving Bioavailability Journal of Drug Delivery & Therapeutics 4(6) 33-38

[8] Mahmoud H, Saleh A S, Shaimaa E D 2013 Optimization of self-nanoemulsifying drug delivery systems of simvastatin aiming dissolution enhancement African Journal of Pharmacy and Pharmacology, 7(22) 1482-1500

[9] Priani S E, Nurrayan, Fitrianti D 2017 Formulation self nano emulsifying drug delivery system glimepiride using oleic acid as oil phase Pharmaciana 7(2) 267-276

[10] Zhao Y, Wang C, Chow AHL, Ren K, Gong, T, Zhang Z, Zheng Y. Self nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Zedoary essential oil: Formulation and bioavability studies. Int J Pharm, 2010, 383, 170-177.

[11] Shah S E, Parikh R, Chavda J R., Sheth N R 2013 Self-Nanoemulsifying Drug Delivery System of Glimepiride: Design, Development, and Optimization PDA J Pharm Sci and Tech 67(3) 201

[12] Nasr A, Gardouh A, Ghorab M 2016 Effect of Oils, Surfactants and Cosurfactants on Phase Behavior and Physicochemical Properties of Self- Nanoemulsifying Drug Delivery System (SNEDDS) for Irbesartan and Olmesartan International Journal of Applied Pharmaceutics 8(1) 13-24

[13] Amrutkar C, Salunkhe K S, Chaudhari S R 2014 Review on Self Nanoemulsifying Drug Delivery System American J. of Pharmatech Research 4(3) 44-50

[14] Ali H H, Ahmed A H 2017 Oral Solid Self-Nanoemulsifying Drug Delivery System of Candesartan Citexetil: Formulation, Characterization and In Vitro Drug Release Studies AAPS Open 3(6) 1-6

[15] Saritha D, Penjuri S C B, Ravuro N 2014 Formulation and Evaluation of Self-Emulsifying Drug Delivery System (SEDDS) of Ibuprofen IJPSR 5(9) 3511-3519

[16] Wiwik I A 2017 Suwaldi M, Soenardi, Jumina, I Gusti M, Mustofa, Preparation and In-Vitro characterization of Self-Nano emulsifying system of C- Phenylcalix-[4]- Resorcinaryl Octacinname and C-Methylcalix-[4]- Resorcinaryl Octabenzoate as ultraviolet absorbers Bali Medical Journal 6(3) 569-577

[17] Reddy B S, G Harish, Md. Fazal U 2016 Formulation and Invitro characterization of s-SNEDDS of Rilpivirine International Journal of Pharmaceutical Sciences and Research 7(7) 3117-3129

[18] Singh A, Vikram S, Divya J, Geeta R 2015 Self emulsifying systems: A review Asian Journal of Pharmaceutics 12-20

[19] Swetha K, Raju J, Prabhakar R, Veerareddy, Suresh B 2012 Paliperidone-Loaded Self-Emulsifying Drug Delivery Systems (SEDDS) for Improved Oral Delivery Journal of Dispersion Science and Technology 33(4) 506-515