

Development and characterization of self-nanoemulsifying drug delivery system (SNEDDS) formulation for enhancing dissolution of fenofibric acid

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ABSTRACT: Fenofibric acid is an active form of fenofibrate and a poorly water-soluble drug. The study aims to formulate and characterize self-nanoemulsifying drug delivery system (SNEDDS) to improve the dissolution of fenofibric acid. The selection of oil, surfactant, and cosurfactant for preliminary screening was determined by the solubility study of fenofibric acid in various vehicles. The composition of selected oil, surfactant, and cosurfactant was glyceryl monolinoleate (Maisine® CC), Kolliphor® RH 40, and Transcutol® HP, PEG 400 respectively. The study results showed that the best SNEDDS formulation was Fd with a ratio of oil, surfactant, and cosurfactant of 10:70:20 % v/v/v (Maisine® CC: Kolliphor® RH 40: Transcutol® HP). It produced good self-nanoemulsifying drug delivery system such as the droplet size was 15.30 nm, 99.27 % transmittance, thermodynamically stable, self-emulsification time was 18.84 seconds, the morphology of the formed nanoemulsion droplets was observed as sphere-shaped droplets and 1.6 fold increase in the dissolution of fenofibric acid.

KEYWORDS: Fenofibric acid; dissolution; long-chain triglyceride; Maisine® CC; SNEDDS.

1. INTRODUCTION

Drug solubility is the rate-limiting step of dissolution for a poorly water-soluble drug. Based on the classification of the Biopharmaceutical Classification System (BCS), drugs that belong to class II and IV have low solubility in water [1, 2]. Recently, more than 40% of new drugs in the market have low solubility in water. Therefore, considerable effort has been made in increasing drug solubility leading to the improvement of its oral bioavailability [3, 4].

Several techniques which can be used to improve solubility and dissolution of drugs include lipid-based formulations, such as liposomes [5, 6], solid lipid nanoparticles [7, 8], nanostructured lipid carriers [9, 10], micro/nanoemulsion [11, 12], and self-nanoemulsifying drug delivery systems (SNEDDS) [13-19]. Lipid-based formulation increases the absorption of a poorly water-soluble drug from the gastrointestinal tract by 1) increasing drug solubilization (accelerate the dissolution process) 2) by recruiting intestinal lymphatic drug transport (reduced first-pass metabolism), and 3) by interacting with enterocytes-based transport processes [20].

SNEDDS consisted of an isotropic mixture of oil, surfactant, and cosurfactant, which upon dilution with aqueous phase of the gastrointestinal tract (GIT) spontaneously forms an oil/water (o/w) nanoemulsion. SNEDDS formulation has the potential to be used for delivery of lipophilic drugs (Log P >2) that are usually associated with low solubility in water, and also have a low bioavailability [21, 22]. In addition, hydrophobic drugs can be dissolved in these systems, and maintained in a dissolved state, thus, enabling them to be administrated as a unit dosage form per-oral administration [15, 18]. SNEDDS can be presented in the form of a pre-concentrated solution. Therefore, the dissolution step required for solid crystalline compounds can be avoided. The advantages of SNEDDS compared to conventional emulsions or other lipid carriers are that they require reduced amounts of energy for their preparation and physical stability upon storage [15, 18].

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Fenofibric acid (2-[4-(4-chloro benzoyl) phenoxy]-2-methyl propanoic acid) in Figure 1 is a BCS class II drug whose formulation can be developed using the SNEDDS formulation technology. Fenofibric acid exhibits low aqueous solubility. The poor aqueous solubility of fenofibric acid can lead to poor absorption via the oral delivery route and its bioavailability to be unpredictable [23], therefore, the required approach to improve the solubility, dissolution, and oral bioavailability of fenofibric acid with a simple, easy, and efficient technique.

Fenofibric acid is an anti-hyperlipidemia drug which is an active form of fenofibrate [23, 24]. There is no use of fenofibric acid in cholesterol therapy in Indonesia because this drug has not been approved yet to be marketed in Indonesia. Currently, only a few studies on the development of fenofibric acid formulations have been reported. The methods used to improve the dissolution of fenofibric acid were solid dispersion techniques [23, 26].

In this study, SNEDDS containing fenofibric acid was formulated and characterized to enhance the dissolution of fenofibric acid.

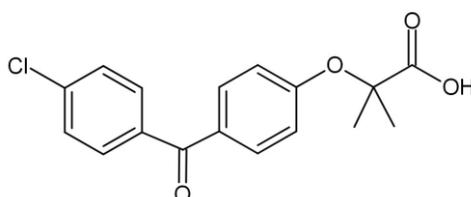


Figure 1. Chemical structure of fenofibric acid.

2. RESULTS AND DISCUSSION

2.1. Solubility study of fenofibric acid in oils, surfactants, and cosurfactants

The selection of oil, surfactant, and cosurfactant was done based on the solubility of fenofibric acid in each vehicle. The solubility test done in oil showed that Maisine® CC (MCC) was the chosen oil due to the highest solubilization for fenofibric acid (7,79mg/ml \pm 0.69 mg/ml). Other types of oils, namely soybean oil, virgin coconut oil, olive oil, sunflower oil, rice bran oil, oleic acid, and glyceryl monooleate have lower solubility of fenofibric acid than MCC. The result is shown in Table 1. MCC (glyceryl monolinoleate) is a winterized oil composed of long-chain (C₁₈) mono-, di-, and triglycerides and corn glycerides. Oil component in SNEDDS formulation play an important role in drug solubility enhancement and drug transport mechanisms [28].

The selected surfactant was Kolliphor® RH 40 (KRH 40), which can solubilize of fenofibric acid as much as 69.22 mg/ml. The main constituent of KRH 40 is glycerol polyethylene glycol hydroxy-stearate, which along with fatty acid glycerol polyglycol esters, forms the hydrophobic part of the product. The hydrophilic part consists of polyethylene glycols and glycerol ethoxylate. KRH 40 has a hydrophilic-lipophilic balance (HLB) between 14 and 16 [29]. The selection of surfactants not only refers to the solubility of fenofibric acid but also depends on good emulsification capabilities. The solubility value of fenofibric acid in Tween 20, Tween 80, Kolliphor® HS 15 (KHS 15), and KRH 40 was not significantly different, in the range of 62.85 - 69.76 mg/ml. However, KRH 40 was exhibited as the best emulsification ability among others. High HLB-value surfactants have high hydrophilicity to aid droplet formation and provide a large surface area that will promote the rapid spread of formulations in water media. Surfactants play an important role in reducing surface free energy thus, it will reduce the size of the emulsion globules formed upon SNEDDS diluted with water [14, 27, 28].

The selected cosurfactants were Transcutol® HP (THP) and polyethylene glycol 400 (PEG 400). Cosurfactants have a role in assisting fenofibric acid dissolve in the system and reduce the bending stress of the interface. They also allow an interfacial film with sufficient elasticity to assume different curvatures required to form a nanoemulsion over a wide range of compositions [28, 30].

2.2. Construction of pseudo-ternary phase diagram

SNEDDS formulations without containing fenofibric acid that produced nanoemulsion (globule size <100 nm) upon dilution with distilled water were constructed by a pseudo-ternary diagram. For this reason, the pseudo-ternary diagrams were constructed to identify self-nanoemulsifying regions and to select suitable concentrations of oil, surfactant, and cosurfactant for SNEDDS formulations [27, 30]. As a result, the concentration of ingredients that produced nanoemulsions for oil were of 10%–30%, surfactants 20%–80%, and cosurfactants were of 10%–70% (Figure 2). The globule size was found to be increased by increasing oil

concentration, whereas the globule size would be decreased by increasing surfactant concentration and by decreasing cosurfactant concentration. These results were similar to the report by Kim et al., 2018 and Kanwal et al., 2019 [16, 17]. According to the pseudo-ternary diagram obtained up to eight of SNEDDS formulations containing fenofibric acid were to be characterized (Table 2). The concentration of ingredients that produced nanoemulsions after load fenofibric acid were oil 10%–20%, surfactants 50%–80%, and cosurfactants were of 10%–30%.

Table 1. The solubility of fenofibric acid in oils, surfactants, and cosurfactants (n=3).

Component	Solubility (mg/ml)
Oil	
Soya oil	0.70 ± 0.32
Virgin coconut oil	0.95 ± 0.27
Olive oil	0.52 ± 0.05
Sunflower oil	0.78 ± 0.04
Rice bran oil	1.05 ± 0.29
MCC	7.79 ± 0.69
Oleic acid	0.34 ± 0.08
Glyceryl monooleate	0.29 ± 0.13
Surfactant	
Tween 20	62.85 ± 1.28
Tween 80	66.92 ± 1.23
KRH 40	69.22 ± 0.58
KHS 15	69.76 ± 0.20
Cosurfactant	
Propylene glycol	20.88 ± 0.56
Glycerin	8.76 ± 0.52
PEG 400	70.89 ± 1.60
THP	88.18 ± 0.78

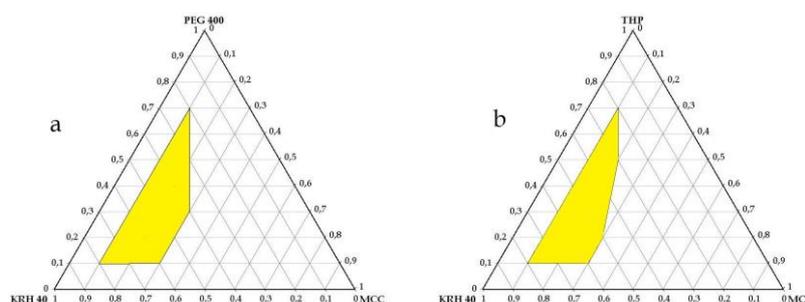


Figure 2. Pseudo-ternary diagram of SNEDDS formulations, (a) MCC-KRH 40-PEG 400, (b) MCC-KRH 40-THP.

2.3. Characterization of fenofibric acid-SNEDDS

2.3.1. Solubility of fenofibric acid in SNEDDS formulation

The highest solubility of fenofibric acid in the SNEDDS formulation was 60.92 ± 2.15 mg/ml (Fd). While the smallest amount of fenofibric acid that can dissolve in SNEDDS was 42.20 ± 5.56 mg/ml (Fc). These results indicated that the solubility of fenofibric acid in SNEDDS was influenced by the ratio of oil, surfactants, and cosurfactants in SNEDDS. The use of THP as a cosurfactant showed better improvement of solubility of fenofibric acid in SNEDDS formulation compared to that of PEG 400. All of the solubility data is shown in Table 3. The high solubility of a drug in SNEDDS formulation reduces the potential for drug precipitation while SNEDDS is diluted with water [27].

Table 2. Formulations of fenofibric acid-SNEDDS.

Formulation	Oil (%)	Surfactant (%)	Cosurfactant (%)
	MCC	KRH 40	PEG 400
Fa	10	70	20
Fb	10	80	10
Fc	20	70	10
Formulation	MCC	KRH 40	THP
	Fd	10	70
Fe	10	80	10
Ff	20	50	30
Fg	20	60	20
Fh	20	70	10

Table 3. Characterization of fenofibric acid-SNEDDS (n=3)

Formulation	Solubility (mg/ml)	Globule size (nm)	Polydispersity index	Zeta potential (mV)	Percentage of transmittance (%)
Fa	42.54 ± 5.93	17.90 ± 1.90	0.29 ± 0.32	-20.57 ± 0.75	99.17 ± 0.15
Fb	42.70 ± 0.86	14.07 ± 2.50	0.09 ± 0.08	-18.70 ± 0.45	99.30 ± 0.10
Fc	42.20 ± 5.56	41.13 ± 1.05	0.35 ± 0.01	-19.77 ± 0.77	95.10 ± 0.10
Fd	60.92 ± 2.15	15.30 ± 4.32	0.09 ± 0.09	-17.50 ± 1.55	99.27 ± 0.05
Fe	52.59 ± 4.61	14.67 ± 1.28	0.11 ± 0.05	-19.40 ± 1.05	99.57 ± 0.05
Ff	59.34 ± 5.61	50.70 ± 1.15	0.39 ± 0.01	-19.47 ± 0.81	89.85 ± 0.10
Fg	46.96 ± 0.62	41.13 ± 1.75	0.36 ± 0.04	-17.13 ± 0.05	95.10 ± 0.26
Fh	45.06 ± 5.86	40.23 ± 7.11	0.37 ± 0.10	-20.87 ± 0.95	95.57 ± 0.15

2.3.2. Globule size, polydispersity index, and zeta potential

The globule size and the polydispersity index of all fenofibric acid-SNEDDS (Fa-Fh) formulations, after being diluted 100 times [14, 18, 31] with distilled water, were <100 nm which ranged from 14.07 ± 2.50 nm to 50.70 ± 1.15 nm; whereas, the values of the polydispersity index of all formulations were <0.4, which ranges from 0.09 ± 0.09 to 0.39 ± 0.01. The polydispersity index varies from 0.0–1.0. The closer to zero the polydispersity index value, the more the particles are homogenous [30]. These results indicate that fenofibric acid-SNEDDS formulations have a homogenous droplet population and uniform nanoemulsions. Determination of the zeta potential value aimed to see the distance between the globule particles and to indicate the potential stability of the colloidal system [30]. The farther the distance between globule particle charges, the more stable an emulsion will be because it prevents coalescence. The critical zeta potential values for stable emulsion were more positive than +30 mV or more negative than -30 mV. All formulations of fenofibric acid-SNEDDS (Fa-Fh) obtained from this study have zeta potential values ranging from -17.13 mV to -20.87 mV. It was indicated that the value of zeta potential was lower than the values reported in the literature. This might be attributed to the presence of KRH 40 and THP which sterically stabilize the system by forming a coat around their surface [30]. These results are in alignment with the results reported by Nasr et al., 2016 and Kaseem et al., 2016. The negative zeta potential values were due to the presence of fatty acid and nonionic surfactant in SNEDDS formulations [15]. The zeta potential value of all formulations can be seen in Table 3.

2.3.3. Percentage of transmittance

The percent transmittance value indicates the level of clarity of an emulsion. If the transmittance value of an emulsion is closer to 100%, the emulsion reflects in the range of nano-size globules. All formulations (Fa-Fh) showed percentages of transmittance ranging from approximately 89-99%. This result indicated that the globule size of all formulation at size <100 nm (Table 3).

2.3.4. Self-emulsification time

The time of self-emulsification showed the time required of SNEDDS formulation to form a homogenous mixture upon dilution under mild agitation. It has been reported that the mechanism of self-emulsification involves the erosion of a fine cloud of small droplets from the surface of large droplets, rather than the progressive reduction in droplet size [31, 32]. The emulsification time of fenofibric acid-SNEDDS ranged from 18.84 ± 1.06 to 142.82 ± 2.43 seconds (Table 4). These results showed that the higher the amount of oil, the longer the emulsification time; conversely, the higher the amount of cosurfactant, the faster the emulsification time. Basalious et al., 2010 reported a similar result, in which rapid emulsification is correlated with lower content of oil and higher content of cosurfactant [33]. The reason is the increase in interfacial tension between the larger volume of oil and aqueous phase with a net decrease in surfactant system, which the emulsification times longer [31]. The formula of Fd was given the fastest emulsification time compared to other formulations.

Table 4. Characterization of fenofibric acid-SNEDDS after dilution 100 fold (n=3).

Formulation	Self emulsification time (second)	Robustness for dilution			Physical stability accelerated	
		50x	100x	250x	Heating-cooling	Centrifugation
Fa	44.17 ± 1.27	√	√	√	√	√
Fb	44.84 ± 1.38	√	√	√	√	√
Fc	142.82 ± 2.43	√	√	√	√	√
Fd	18.84 ± 1.06	√	√	√	√	√
Fe	38.05 ± 1.71	√	√	√	√	√
Ff	23.82 ± 5.61	√	√	√	√	√
Fg	79.24 ± 4.30	√	√	√	√	√
Fh	119.47 ± 5.79	√	√	√	√	√

√ : Stable and globule size in nano-range (<100 nm)

2.3.5. Robustness to dilution

Robustness to dilution aims to show whether SNEDDS will continue to form stable nanoemulsions after being diluted with distilled water. From the observations, it was found that all formulations were still in the form of nanoemulsions (<100 nm), and no sign of phase separation showed, after 50, 100, and 250 times dilutions respectively. The result is shown in Table 4.

2.3.6. Accelerated physical stability test

Accelerated stability testing included heating-cooling and centrifuge methods were used to assess the stability of SNEDDS. The test was carried out to observe whether SNEDDS was still stable after being stored in extreme conditions. The result showed that all formulations were stable marked by the absence of separation and the size of the globule particles after being diluted with distilled water was still in the nano range (<100 nm). The result can be seen in Table 4.

2.3.7. In vitro dissolution test

In vitro dissolution test of fenofibric acid-SNEDDS was carried out to determine the dissolution of fenofibric acid from SNEDDS formulations. All SNEDDS formulations revealed a higher dissolution percentage compared to the pure fenofibric acid. Within the initial 30 minutes, the formulation of Fa, Fb, Fd, Fe, Ff, Fg can dissolve and release up to 86.01% to 97.77% of fenofibric acid whereas the pure drug can only release 56.26% of fenofibric acid. After 60 minutes, all formulations dissolved and released about approximately 99%, while the dissolution percentage of the pure drug was only 59.03%. This means that the preparation of fenofibric acid in the form of SNEDDS can improve its solubility significantly. The percentage of dissolution of each formulation are shown in Figure 3. The higher the surfactant concentration in the formulation, the more the increase in percent dissolution of fenofibric acid. The higher the concentration of oil, the slower the dissolution of fenofibric acid. Besides, the dissolution also was related to the emulsification time in each formulation. Formulations that have a faster emulsification time lead to the higher dissolution of fenofibric acid especially within the initial ten minutes. In line to this, the emulsification time of Fc and Fh

were longer than other formulations, which may be attributed to the lower percentage of dissolution. Fenofibric acid in SNEDDS formulations was in the dissolved state when contacted with the dissolution media. The fenofibric acid was immediately transferred to the dissolution media, thus, it enhanced drug dissolution rate and extent [18, 30]. Similar observations were reported by Senapati et al., 2016, Syukri et al., 2018, and Nasr et al., 2016, that showed that the release of the drug (efavirenz, andrographolide, and olmesartan medoxomil) from selected SNEDDS was found to be significantly higher as compared with the pure drug [14, 19, 30]. Based on the evaluation of all characteristics, it was found that the best formulation was Fd.

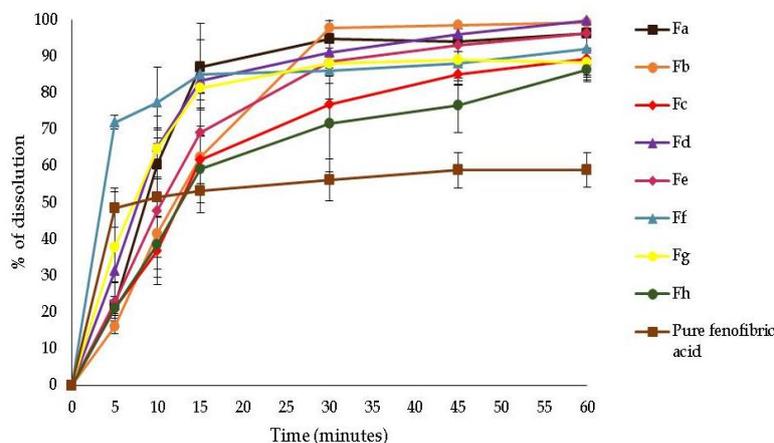


Figure 3. Profil of dissolution of fenofibric acid-SNEDDS formulations (n=3).

2.3.8. Surface morphology determination of fenofibric acid-SNEDDS

Investigation of surface morphology of fenofibric acid-SNEDDS globules after being diluted with distilled water was conducted by using Transmission Electron Microscope (TEM, Jeol 1010, Tokyo, Japan). The shapes of the globules were spherical with a diameter of <100 nm. The size of globules was uniform and there was a clear boundary that was seen around globule particles. The existence of a boundary line around globule particles was a barrier that prevented coalescence (Figure 4). These results were similar to the report by Salem et al., 2018 [27].

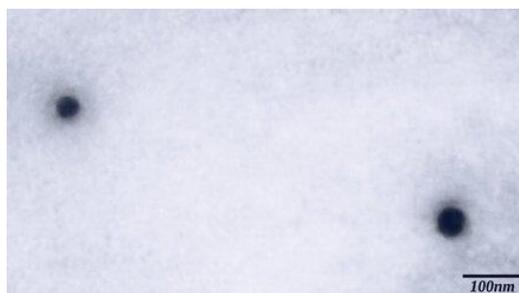


Figure 4. TEM photograph of fenofibric acid-SNEDDS formulation (Fd), magnification 50,000 x.

2.3.9. Fourier-transform infrared spectroscopy (FT-IR).

FT-IR analysis was carried out to identify possible interactions of SNEDDS components with fenofibric acid. The fenofibric acid spectrum showed the characteristics of wide peaks at wavenumber $2,991\text{ cm}^{-1}$ (O-H strain), indicating the presence of carboxylic acid groups in the structure of fenofibric acid. The peaks at $1,701\text{ cm}^{-1}$ (strain C=O) and $1,645\text{ cm}^{-1}$ (symmetrical strain C-C=C) showed the functional groups of alkenes and ketones. The peaks at $1,595\text{ cm}^{-1}$ and $1,500\text{ cm}^{-1}$ (symmetrical strain C-C=C) showed aromatic rings, and the vibrations at 758 cm^{-1} and 671 cm^{-1} showed alkyl halide (C-Cl) groups. The small peaks of fenofibric acid appeared in the Fd spectrum with wavenumbers at around $1,645\text{ cm}^{-1}$ showed C=C aromatic strains ($1,675\text{--}1,500\text{ cm}^{-1}$), and strains C-Cl at wavenumbers around 671 cm^{-1} . Furthermore, the appearance of an extra peak in the spectrum of Fd at 2857 cm^{-1} and 2924 cm^{-1} of -CH stretching showed the contribution of surfactant [14]. FT-IR analysis showed that fenofibric acid did not interact chemically with components in SNEDDS formulation (Figure 5).

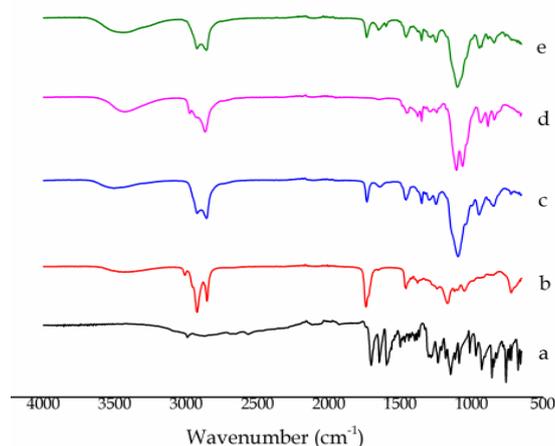


Figure 5. FT-IR spectra of (a) fenofibric acid, (b) MCC, (c) KRH 40, (d) THP, (e) fenofibric acid-SNEDDS (Fd).

3. CONCLUSION

In this study, fenofibric acid was successfully formulated and characterized in SNEDDS formulation to increase the dissolution of fenofibric acid. SNEDDS formulations were constructed based on the pseudo-ternary diagram, physical properties, and the dissolution test. From this study, it was concluded that the selection of the right type and concentration of oil, surfactant, and cosurfactant can determine the success of SNEDDS formulations, characterization, and dissolution of fenofibric acid. The increase in the dissolution of fenofibric acid in SNEDDS formulations is influenced by the globule size, self-emulsification time, stability, and the solubility of the drug in the system. The drug once dissolved in the SNEDDS formulation will be emulsified in nano-size droplets quickly when in contact with GIT liquid. Thus, it will provide a large interfacial surface area for drug release, which in turn can affect the process of absorption of fenofibric acid into the blood. Finally, SNEDDS formulation represented an interesting approach to enhancing the dissolution of fenofibric acid.

4. MATERIALS AND METHODS

4.1. Materials

Fenofibric acid was purchased from BOC Sciences (New York, USA), MCC (glyceryl monolinoleate) and THP (diethylene glycol monoethyl ether) were kindly gifted from Gattefossé (Lyon, France), KRH 40 (PEG-40 hydrogenated castor oil), KHS 15 (PEG [15] hydroxy stearate) and glyceryl monooleate were kindly gifted from Badische Anilin und Soda Fabrik (BASF) (Ludwigshafen, Germany). Tween 20 (polysorbate 20), Tween 80 (polysorbate 80), propylene glycol, and glycerin were purchased from PT. Brataco Chemica (Bandung, Indonesia). Virgin coconut oil, soya oil, sunflower oil, olive oil, and oleic acid were purchased from Bandung, Indonesia. Polyethylene glycol 400 (PEG 400), potassium dihydrogen phosphate (KH₂PO₄), sodium hydroxide (NaOH), and methanol were purchased from Merck (Darmstadt, Germany). All solvents used were analytical grade.

4.2. Solubility study of fenofibric acid in oils, surfactants, and cosurfactants

The solubility of fenofibric acid was determined in various oils, i.e., soya oil, virgin coconut oil, olive oil, sunflower oil, rice bran oil, MCC, oleic acid, and glyceryl monooleate. An excess of fenofibric acid was added to 1 ml of each vehicle in glass tubes. The suspension was mixed using a vortex mixer for 2 min and equilibrated at 25 °C in a water bath shaker at 100 rpm for 72 h. After equilibrium, the tubes were centrifuged at 5,000 rpm for 20 min. The supernatants were solubilized with methanol and then the amount of fenofibric acid dissolved in each vehicle was analyzed via ultraviolet (UV) spectrophotometer validated method (UV-Vis, Beckman DU 720) at a maximum wavelength of 300 nm. The validated UV spectrophotometer showed that $r = 0.999$ (with 5-17.5 µg/ml of calibration curve concentrations range), precision = 1.61% and 0.15%, accuracy = 96.90% and 100.87%. Determination of solubility of fenofibric acid in surfactants (Tween 20, Tween 80, KHS 15, KRH 40), and cosurfactants (propylene glycol, glycerin, PEG 400, THP) were conducted in the same procedure as described above [14].

4.3. Construction of pseudo-ternary phase diagram

The selected oil, surfactant, and cosurfactant were MCC, KRH 40, and THP, PEG 400, respectively. All ingredients were mixed in the ratio of oil: Smix (surfactant and cosurfactant) was 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The composition of SNEDDS with ratios of oil: Smix that produced nanoemulsion (globule size <100 nm) was plotted on a pseudo-ternary diagram using ProSim software (Labege, France) [27].

4.4. Preparation of fenofibric acid-SNEDDS

The oil, surfactant, and cosurfactant were mixed into a vial and stirred by using vortex-mixer at 3,000 rpm for 1 min. Then, 35 mg of fenofibric acid was added to the mixture, and stirring is continued for 2 min. Thereafter, the mixture was stirred using a magnetic stirrer at 500 rpm at 40 °C for 30 min until a clear solution was obtained.

4.5. Characterization of fenofibric acid-SNEDDS

4.5.1. Study of fenofibric acid solubility in SNEDDS formulations

About 200 mg of fenofibric acid is added to 1 ml of each SNEDDS formulation (Table 2). The drug suspension was mixed using a vortex mixer for 2 min and equilibrated at 25°C in a water bath shaker at 100 rpm for 72 h. After equilibrium is achieved, the tubes are centrifuged at 5,000 rpm for 20 min. The supernatants were solubilized with methanol and analyzed for fenofibric acid with a spectrophotometer (UV-Vis, Beckman DU 720) at a maximum wavelength of 300 nm.

4.5.2. Globule size analyze, polydispersity index and zeta potential

About 0.1 ml of SNEDDS was diluted in 10 ml of distilled water. The droplet size and polydispersity index were measured using a Delsa™ Nano Particle Analyzer (Beckman Coulter, USA), while zeta potential measurements were performed using a Malvern® Zetasizer Nano ZSP (England).

4.5.3. Percentage of transmittance

About 0.1 ml of SNEDDS was diluted with 10 ml of distilled water, then the clarity of the nanoemulsion was measured as the percentage of transmittance at λ max of 500 nm, using distilled water as the blank sample on a UV-Vis spectrophotometer (Beckman DU 720) [14].

4.5.4. Self-emulsification time

Self-emulsification time was determined by diluting of 0.1 ml of fenofibric acid-SNEDDS with 10 ml distilled water at 37 °C and mixing gently using a magnetic stirrer with a rotation speed of 100 rpm. The time required to self-emulsify completely to form a homogenous nanoemulsion was recorded by a stopwatch.

4.5.5. Robustness for dilution

As much as 0.1 ml of SNEDDS was diluted with distilled water for 50, 100, 250, and 1000 times. The diluted systems were mixed using a magnetic stirrer to complete homogeneity. The droplet size and any signs of phase separation were also observed visually.

4.5.6. Accelerated physical stability test

The heating-cooling cycle method was employed to study the stability of SNEDDS. Six heating-cooling cycles were carried out at 4 °C and 40 °C for 48 h. All formulations were observed for separation, particle size globules, creaming, and coalescence after dilution with distilled water [14]. Another stability test was done by using the centrifugation method. All formulations were centrifuged at a speed of 5,000 rpm for 30 minutes and observed for separation [14].

4.5.7. In vitro dissolution test

In vitro dissolution testing of fenofibric acid-SNEDDS was performed using USP dissolution apparatus I (Hanson Virtual Instruments SR8 Plus) in 900 ml of simulated intestinal fluid media (SIFsp, pH 6.8 without enzyme) at a rotation speed of 100 rpm temperature of 37 ± 0.5 °C [19, 23]. Formulation of Fa-Fh was placed in hard gelatin capsules [13, 14, 19, 30] prior to beginning of the dissolution test. Then 5 ml of samples were taken at interval times of 5, 10, 15, 30, 45, and 60 min. Immediately after each sampling, 5 ml of new dissolution media was added to replace the volume of the sample drawn. The amount of fenofibric acid dissolved in the medium was analyzed by using a UV-Vis spectrophotometer at a maximum wavelength of 298 nm.

4.5.8. Surface morphology determination of fenofibric acid-SNEDDS

The fenofibric acid-SNEDDS were diluted 100 times with distilled water to form a nanoemulsion. A drop of nanoemulsion was applied to a copper grid and left for 10 min to allow the particles to bind to collodion and the excess was removed. A drop of 2% of the uranyl acetate solution was then applied. The excess was removed and the samples were air-dried for 1 min, then observed under TEM (Jeol 1010, Tokyo, Japan).

4.5.9. Fourier-transform infrared spectroscopy (FT-IR)

The Fourier-transform infrared (FT-IR) spectrum of each sample was performed using IR Agilent Cary 630 (USA) and analyzed within a wavelength of 500–4,000 cm^{-1} with a resolution of 2 cm^{-1} . Afterwards, the peak of the obtained spectrum was compared. The intensity and shift of the vibration peak were observed.

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