INTRODUCTION

Tetrahydrocurcumin (THC) is an active metabolite of curcumin, a yellow dye found in turmeric (Curcuma longa L.). Compared to the other curcuminoids, THC shows a stronger antioxidant and anti-inflammation activity [1]. The docking analysis showed that THC might be able to inhibit amyloid precursor protein (APP) so that it has the potential to be used in the treatment of Alzheimer’s disease [2]. Although THC has potent activity, the bioavailability of THC is limited due to its poor water solubility and absorption. Several approaches to increase the solubility of THC have been carried out. These approaches include THC formulations into solid dispersions and inclusion complexes [3, 4]. Both of these formulations succeeded in increasing the solubility of THC, but each had limitations. Solid dispersion causes the active substance to be in an amorphous state so that its solubility in water increases. However, crystallization can occur during the manufacturing process in the presence of mechanical stress or during storage due to temperature and humidity stresses, whereas inclusion complexes have limited drug loading.

A promising strategy to enhance drug solubility and absorption is a self-nanoemulsifying drug delivery system (SNEDDS). This system is an isotropic mixture of oil, surfactant, and co-surfactant. When dispersing the SNEDDS containing an hydrophobic drug to water, the drug in oil nanodroplet will quickly spread and emulsified, which causes an increase in the drug solubility, dissolution, and absorption. The SNEDDS formulation was reported to have succeeded in increasing the dissolution of rivaroxaban, curcumin, duloxetine technical grade was kindly provided by Gattefosse, France. Kolliphor and Myritol (technical grade, PT BASF Indonesia) and methanol (spectrophotometric grade, Merck) were used.

METHODS

Preliminary studies

Five hundred milligrams of THC were accurately weighed, added to 1 ml of a vehicle in a microtube, vortexed for 5 min and stirred for 24 h. After stirring, the microtube was incubated at room temperature for 24 h, and then centrifuged at 6000 rpm for 10 min. The supernatant was diluted in methanol, and the soluble THC in the vehicle was determined by Ultraviolet (UV)-visible spectrophotometric method. The best oil, surfactant, and co-surfactant in dissolving THC were selected for further study.

The compatibility between SNEDDS components performed by vortexing the mixture of selected surfactant Kolliphor:Labrasol (1:3), co-surfactant (PEG400), and PEG400 at a various ratio of 10%–90% of each component. After allowed for 24 h, the mixture was observed for any separation.

Construction of the pseudoternary phase diagram

The mixtures from the compatibility study were studied for the self-nanoemulsifying properties. A 100 µl of the mixture were introduced into a microtube, vortexed for 5 min and stirred for 24 h. After stirring, the microtube was incubated at room temperature for 24 h, and then centrifuged at 6000 rpm for 10 min. The supernatant was diluted in methanol, and the soluble THC in the vehicle was determined by Ultraviolet (UV)-visible spectrophotometric method. The best oil, surfactant, and co-surfactant in dissolving THC were selected for further study.
to 100 ml of water under magnetic stirring at 50 rpm. The time interval from the introducing to changes in the emulsion clarity was measured and noted as emulsification time. The percentage transmittance of the emulsion formed then was measured using visible spectrophotometry at A of 650 nm. The emulsification time ≤ 2 min and percentage transmittance ≥ 80% without any phase separation was considered to have self-nanoemulsifying properties. The points of composition where the nanoemulsion formed spontaneously were marked as a black triangle.

**Experimental design**

The experimental design for SNEDDS containing THC in this study used three factors (independent variables), i.e., the oil, surfactant, and cosurfactant. Three responses (dependent variables) were tested, i.e., emulsification time, percentage transmittance, and dissolution efficiency at minute 15 (DFE). The highest and lowest percentage of oil, surfactant, and cosurfactant which showed self-nanoemulsification properties was used as the high and low levels of the design constraint of D-optimal design.

**Preparation of SNEDDS containing THC**

The SNEDDS blanks were made by mixing the oil, surfactant, and cosurfactant based on the predetermined runs made by the Design-Expert 7.0 software. One milliliter of SNEDDS blanks was added with 97.45 mg of THC, vortexed and sonicated until the THC completely dissolved. The SNEDDS were tested for the emulsification time, percentage transmittance, and DFE as the responses.

**Formulation optimization**

The emulsification time and percentage transmittance measurement were performed as previously described. The dissolution test was performed at 50 rpm, 37±0.5°C, and artificial gastric fluid (AGF) as a dissolution medium. Five hundred milliliters of SNEDDS were filled into the capsule size "0" were introduced to the medium. Two milliliters of each sample were taken at intervals of 5, 10, 15, 20, 40, and 60 min. Each sample was replaced with the same amount and temperature of the AGF. The absorbance was measured using a UV-visible spectrophotometer at a wavelength of 281 nm.

The model and effects of the various combinations on the responses were predicted by the equation and contour plots. The optimum formulation was determined by desirability value. The predictive responses of the optimum formulation were verified by comparing them with the experimental responses. The optimum formulation then characterized for the nanoemulsion droplet size, zeta potential, and dissolution efficiency.

**Dissolution testing of the optimum formulation**

The dissolution testing was performed in accordance with the DFE testing described in the previous part of the formulation optimization. The dissolution profile of the capsule containing the optimum formulation was obtained with an average release of PGV-0 concentration versus time of three replications for 60 min. The SNEDDS THC dissolution profile was compared to THC unmodified.

**RESULTS AND DISCUSSION**

**Preliminary studies**

The preliminary studies were carried out to select the SNEDDS components that had a high THC dissolution capacity so that SNEDDS can load a lot of THC. This could to reduce the dose and minimize side effects. The SNEDDS must also be easily nano-emulsified spontaneously when in contact with the gastrointestinal fluid so it could to increased dissolution, absorption, and bioavailability.

The solubility test result is presented in Fig. 1. The THC showed higher solubility in Labrasil compared with oleic acid. Among the surfactants, the mixture of Kolliphor:Labrasol (1:3) showed the highest capacity to dissolve the THC. It also showed a good compatibility without any separation.

**Construction of the pseudoternary phase diagram**

The composition area that was predicted to formed nanoemulsion when the SNEDDS mixed with the water can be seen as the grey area in Fig. 2. In the range of 10–20% Labrafil showed the self-nanoemulsifying properties with all the concentration of the mix surf and PEG400 (10–80%). While in the range of 20–30% of Labrafil, the self-nanoemulsifying properties only showed when it mixed with 30–80% mix surf and 10–50% PEG400. The high and low levels for the D-optimal design constraints were set as follows 10 and 30% for oil (Labrafil), 10 and 80% for both surfactants (Kolliphor:Labrasol 1:3), and cosurfactants (PEG 400).

**Experimental design**

D-optimal design was applied to optimize the SNEDDS of THC more efficient and effective than the trial and error technique. With order to quadratic model fit, in this study D-optimal design requires 12 experimental runs to analyze the experimental error and significance of quadratic fit.

**Formulation optimization**

The D-optimal design and the responses are presented in Table 1. Determination of the optimum formula based on transmittance parameters and emulsification time in aquades, AGF, and AIF media.

![Fig. 1. The tetrahydrocurcumin solubility capacity of various vehicles](image1)

![Fig. 2. Pseudoternary phase diagram of Labrafil, Kolliphor: Labrasol (1:3) and PEG400](image2)
Optimization is done using the help of Design-Expert software version 7.1.3 (Stat Ease Inc., Minneapolis). The determination of the optimum formulation is based on the desirability value obtained from the software’s calculations. The closer to one the value of desirability indicates that the formula can achieve the optimum formula according to the desired variables.

A successful SNEDDS formulation must be rapidly distributed as nanodroplets in the gastrointestinal fluid. Therefore, the emulsification time determination becomes important [9].

The emulsification time of SNEDDS containing THC is showed in Table 1. The results showed that all the runs formed nanoemulsion spontaneously with the time required was less than 1 minute. According to Khedkar et al. (2015), the oil-surfactant-cosurfactant system which in less than 2 minutes forms a clear/translucent emulsion is recommended for SNEDDS formuations. If the emulsification time is more than 2 min and produces a cloudy emulsion system, it is not recommended for SNEDDS formulations [9].

The suggested equation for the emulsification time of the SNEDDS was a 2-factor interaction [2FI] (equation 1).

\[
Y_\text{e} = 1.86 \times 10^{-3}(A) - 1.81 \times 10^{-3}(B) - 4.6 \times 10^{-4}(C) - 1.1 \times 10^{-5}(A)(B) - 6.24 \times 10^{-5}(A)(C) + 2.93 \times 10^{-5}(B)(C)
\]

(Eq. 1)

Where \(Y_e\) = emulsification time; \(A\) = Labrafil; \(B\) = Kolliphor:Labrasol (1:3); \(C\) = PEG400

Equation 1 illustrates that increasing the proportion of Labrafil (A) results in increased the emulsification time. The Labrafil had the greatest effect on the emulsification time. Labrafil has hydrophilic-lipophilic balance (HLB) of 4; indicate a dominant lipophilic affinity [10], so it is not easily dispersed in water. Otherwise, an increase in mix surf and co-surfactant proportion caused a decrease in the emulsification time. Kolliphor (HLB 13.5) and Labrasol (HLB 14) have high hydrophilicity, lead it to quickly be dispersed in water [11,12]. PEG400 is a liquid hydrophilic polymer that often used as cosolvent, so it is also easily dispersed in water [13]. The interaction of Labrafil-mix surf and Labrafil-PEG400 reduced the emulsification time. The surfactant molecule decreases the interfacial tension by positioning itself in the oil-water interface so that the oil droplets become more easily dispersed in the water. The more surfactant molecules that interact with oil, the less time it takes for the emulsion to occur. The presence of the PEG400 in oil-water interface increases the fluidity of the interfacial film and facilitates the formation of emulsion [14], while the interaction of mix surf:PEG400 increased the emulsification time. Hydrogen bonds can be formed between surfactants and polyalcohols such as PEG400. Too much interaction between surfactants and cosurfactants causes the interaction energy to decrease due to the self-association mechanism of intermolecular hydrogen bonds, which reduces the ability of OH alcohol groups to form hydrogen bonds with water. As a result, the dispersed ability will decrease [15].

The influence of factors on the emulsification time is shown in Fig. 3. The increase of Labrafil from 10% to 30% decreased the emulsification time from 0.22 to 0.13 min.

A good SNEDDS is it forms nanodroplets when dispersed into water. The size of the oil droplet affects the clarity of the emulsion. If the globule size of emulsion system is very small, the light passes through, so the beam of light will be continued so that the solution looks transparent and the resulting transmittance is greater. Aquades do not have particles that resist the transmission of light so that they will pass on the light passing through them without light scattering and have a transmittance value of 100%. The closer to 100%, it is estimated that the emulsion droplets have reached nanometer size [16].

The estimated percentage transmittance for each run composition is calculated by Equation 2.

\[
Y_\text{a} = +0.043(A) + 0.64(B) + 0.11(C) − 6.21 \times 10^{-3}(A)(B) + 9.57 \times 10^{-3}(A)(C)−5.18 \times 10^{-3}(B)(C)
\]

(Eq. 2)

![Effect of self-nanoemulsifying drug delivery system components on emulsification time](image)

**Table 1:** The actual design of self-nanoemulsifying drug delivery system tetrahydrocurcumin with the response values

| Standard | Run | Factor | Response (Y) |
|----------|-----|--------|--------------|
|          |     | A (%)  | B (%)        | C (%)        | Emulsification time (min) | Transmittance (%) | DE_{50} (%) |
| 12       | 1   | 30     | 10           | 10           | 0.256±0.02               | 57.23±0.03       | 10.37±0.02 |
| 10       | 2   | 10     | 10           | 10           | 0.232±0.04               | 52.41±0.13       | 10.9±0.03  |
| 3        | 3   | 30     | 80           | 80           | 0.127±0.01               | 84.40±0.03       | 17.88±0.05 |
| 2        | 4   | 10     | 10           | 10           | 0.245±0.03               | 51.43±0.11       | 9.41±0.02  |
| 11       | 5   | 10     | 10           | 10           | 0.244±0.03               | 52.32±0.10       | 7.58±0.02  |
| 5        | 6   | 30     | 80           | 10           | 0.140±0.01               | 80.03±0.04       | 42.30±0.01 |
| 9        | 7   | 10     | 80           | 80           | 0.220±0.02               | 72.63±0.13       | 6.91±0.02  |
| 4        | 8   | 30     | 10           | 80           | 0.125±0.02               | 75.93±0.24       | 12.08±0.03 |
| 1        | 9   | 10     | 10           | 10           | 0.267±0.04               | 53.23±0.10       | 9.40±0.02  |
| 7        | 10  | 10     | 10           | 80           | 0.177±0.02               | 66.54±0.10       | 16.85±0.04 |
| 6        | 11  | 10     | 80           | 10           | 0.120±0.01               | 92.74±1.33       | 49.46±0.12 |
| 8        | 12  | 10     | 10           | 10           | 0.218±0.02               | 52.51±0.46       | 8.08±0.02  |

*mean±SD (n=3), DE_{50}: Dissolution efficiency at minute 15
Where \( Y_2 = \% \text{transmittance}; A = \text{Labrafil}; B = \text{mix surf (Kolliphor:Labrasol 1:3)}; C = \text{PEG400} \)

Equation 2 illustrates the 2FI model. The coefficients of the main effect (A, B, and C) were positive with the highest coefficient of B. This means increasing the concentration of Labrafil, mix surf, or PEG400 resulting in increased percentage transmittance. The mix surf was the most influential component to increasing percentage transmittance. It can be explained by the deposition of surfactant as a monolayer at the oil/water interface above its critical micelle concentration causing a reduction in both the interfacial dilution modulus and the interfacial tension. As a result, the droplet is damaged to smaller and uniform droplets. The beam of light passes through without a lot of scattering, and the transmittance is greater [15].

Visually, the effect of the component ratio on the percentage transmittance is shown in Fig. 4. An increase in the percentage of mix surf from 10% to 80% showed non-linear percentage transmittance increase from 58.4 nm to 82.65%.

The last parameter in this optimization is dissolution. It is important for THC in SNEDDS to dissolve both in molecular and micellar form to be ready to absorbed in the gastrointestinal tract. \( \text{DE}_{15} \) could be estimated following Equation 3.

\[
Y_3 = -0.19 (A) + 0.58 (B) + 0.1 (C) + 1.64 \times 10^{-3} (A)(B) + 3.35 \times 10^{-3} (A)(C) - 8.08 \times 10^{-3} (B)(C) \quad \text{(Eq. 3)}
\]

Where \( Y_3 = \text{DE}_{15}; A = \text{Labrafil}; B = \text{Kolliphor:Labrasol (1:3)}; C = \text{PEG400} \).

As seen in Equation 3, the 2FI model applied for the dissolution efficiency of THC at 15 min. Equation 3 contains positive coefficients at main effect B and C and interaction effects of (A)(B) and (A)(C). The positive effect of surfactant and cosurfactant to the dissolution properties is similar to previous reports [17,18].

The effect of SNEDDS component on \( \text{DE}_{15} \) is presented in Fig. 5. As seen in Fig. 5, in the range of 10–80% mix surf, the \( \text{DE}_{15} \) increased from 14.4% to 45.5%.

Overlay plot from the optimization with criteria was emulsification time < 2 min, percentage transmittance ≥ 80% and \( \text{DE}_{15} \) ≥ 40%, shown in Fig. 6. The yellow area shows the area that meets the criteria.

Simultaneous optimization for all responses is done with using the desirability function. This is calculated by combining individual desirability using geometric averages. A function desirability gives a number between 0 and 1, with 0 representing the value completely unwanted and 1 represents the ideal response value.

To determine the optimum formulation quantitatively, the desirability function was applied with the criteria of emulsification time to be minimized, percentage transmittance, and \( \text{DE}_{15} \) to be maximized. The desirability value is shown in Fig. 7. The maximum desirability was achieved at composition: Labrafil
THC nanoemulsion was quite good, could be seen in the absence of surfactant and cosurfactant in SNEDDS increasing the solubility of THC and stabilizing oil droplets containing THC longer in the liquid bulk. The right composition had also produced nano-sized droplets that have higher solubility.

CONCLUSION

D-optimal mixture design with desirability function was effective in optimizing the SNEDDS THC. It also generated valid equations and models to describe the effects of the SNEDDS formulation factors on the responses of emulsification time, % transmittance, and DE15. The SNEDDS formulation significantly enhanced the dissolution of THC solubility.

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