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# Optimization, Characterization, and Antioxidant Activity of Sunflower Oil-Based SNEDDS using Response Surface Method

**Rodhia Ulfa*, Gressy Novita, and Sella Asharianti**

Pharmacy Study Program, Sekolah Tinggi Ilmu Farmasi Riau (STIFAR), Pekanbaru, Riau, Indonesia
*Corresponding author: Rodhia Ulfa | Email: rodhiaulfa@stifar-riau.ac.id

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**Abstract:** This study aims to optimize, characterize, and evaluate the antioxidant activity of sunflower seed oil-based SNEDDS using Tween 80 and PEG 400. The study utilizes Simplex Lattice design and Response Surface Methodology to obtain a formula with the best physical characteristics and efficacy. Sunflower seed oil was selected due to its high content of essential fatty acids and antioxidants. The optimization method involved determining the ratio of surfactant, cosurfactant, and oil, as well as characterization tests of particle size, polydispersity index, zeta potential, emulsification time, and antioxidant activity. The results showed an optimal formula with a nano particle size of 13 nm ± 0.346, low polydispersity index (0.161 ± 0.067), and zeta potential of -21.8 mV ± 0.577, ensuring physical stability. The antioxidant activity of SNEDDS remained high with an IC50 of 18.06 μg/mL. Formulation verification through statistical analysis confirmed the consistency of experimental results with optimization software predictions. In conclusion, the optimized sunflower seed oil SNEDDS offers an innovative oral formulation that is efficient, stable, and safe, with the potential to improve bioavailability, pharmacological activity, and the utilization of local vegetable oils in modern pharmacy.

**Keywords:** antioxidants, bioavailability, drug delivery, simplex lattice design, surfactants

## 1. INTRODUCTION

Self-nanoemulsifying drug delivery systems (SNEDDS) are an important innovation in the field of pharmacy to improve the solubility and bioavailability of lipophilic compounds that conventionally have limited oral absorption [1]. The use of sunflower seed oil in SNEDDS is highly relevant due to its essential fatty acid content and strong antioxidant activity, as evidenced by its high $IC_{50}$value [2]. Vitamin E ($\alpha$-tocopherol) is the principal component with the highest proportion that contributes to the antioxidant activity of sunflower oil [3]. The characterization of oil-based SNEDDS can provide additional benefits beyond serving as a drug delivery medium, namely protection of the active ingredient from oxidation during storage or post-consumption. In an effort to obtain the optimal formula, a widely used method is Response Surface Methodology (RSM) with a simplex lattice design because it can systematically evaluate the proportion of components and maximize the desired response [3,4].

The main focus of this study is on the optimization, characterization, and evaluation of the antioxidant activity of SNEDDS using sunflower seed oil as the oil phase, Tween 80 as the surfactant, and PEG 400 as the co-surfactant [6]. Sunflower seed oil functions as a carrier for lipophilic active compounds and as a source of natural antioxidants[2] . Tween 80, a non-ionic surfactant, was chosen because it has an optimal HLB value for the formation of oil-in-water emulsions and is widely accepted for oral pharmaceutical preparations[7]. PEG 400 as a co-surfactant is able to work synergistically with Tween 80, increasing drug solubility and the stability of the nano-emulsion system during the formulation process and after consumption[8]. The interaction of these three components significantly determines the emulsification time, droplet size, stability, and overall biopharmaceutical activity of SNEDDS [9] .

Although SNEDDS has been applied to various active ingredients, studies specifically evaluating the optimization of sunflower seed oil formulations with Tween 80 and PEG 400 are still very limited. SNEDDS preparations are generally used for oral administration, offering advantages over conventional systems in terms of increased solubility, delivery efficiency, and protection from pre-systemic metabolism [9]. Optimization using simplex lattice-based response surface methodology provides an experimental approach that is not only efficient in terms of time and cost, but also capable of mathematically explaining the relationship between formula composition and physical characteristics such as droplet size, emulsification time, and antioxidant activity [4]. With these advantages, an optimal response can be achieved, enabling replication and scalability at the industrial development stage [5].

The purpose of this study is to provide a comprehensive review of SNEDDS optimization strategies based on sunflower seed oil, utilizing Tween 80 and PEG 400 and applying Response Surface Methodology with simplex lattice design. The scope includes analysis of the interaction and proportion of formula components, evaluation of SNEDDS physical characterization, and antioxidant activity testing as the main parameters for potential applications in pharmacy and therapy [10] . The results of this study are expected to contribute significantly to the development of efficient, safe, and highly pharmacologically potent oral preparations, as well as encourage the use of local vegetable oils with clinical added value and modern drug formulation innovations [9].

## 2. MATERIALS AND METHODS

### *2.1. Materials*

The materials used in this study included sunflower seed oil (PT. Lansida), Tween 80 (Brataco), and PEG 400 (Petronas).

### *2.2. Examination of Active Constituents in Sunflower Seed Oil*

The examination of the active constituents in sunflower seed oil encompassed organoleptic assessment, determination of pH, specific gravity, and refractive index, as well as solubility testing, phytochemical screening, and antioxidant activity evaluation.

### *2.3. Examination of Auxiliary Materials*

The characterization of auxiliary materials, including Tween 80 and PEG 400, was performed in accordance with the Indonesian Pharmacopoeia (Fourth and Fifth Editions) and the Handbook of Pharmaceutical Excipients (Sixth Edition), encompassing aspects such as physical appearance, color, odor, and solubility.

### *2.4. Determination of Surfactant and Co-surfactant Ratio (Smix)*

The composition ratios of surfactant (Tween 80) and co-surfactant (PEG 400) were determined according to a modified method from Patel et al. (2011), employing various proportions (1:1, 1:2, 1:3, 2:3, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 1:4, 1:5, and 1:6). Each formulation, with a total volume of 2 mL, was placed in a vial and homogenized using a magnetic stirrer at 500 rpm for 30 minutes at 40°C, followed by sonication for 1 hour at the same temperature. The resulting mixtures were then allowed to stand for 24 hours at room temperature to observe stability. The formulations exhibiting no phase separation after this period were considered stable and selected for the SNEDDS formulation.

### *2.5. Determination of Oil Ratio with Smix*

The determination of the oil composition with surfactant and co-surfactant was conducted by mixing the previously optimized combination of surfactant and co-surfactant with oil in various ratios as (1:10; 1:9.5; 1:9; 1:8.5; 1:8; 1:7.5; 1:7; 1:6.5; 1:6; 1:5.5; 1:5; 1:4.5; 1:4; 1:3; 1:2.5; 1:2; 1:1; 1.5:1; 2:1; 2.5:1; 3:1; 4:1; 7:1; 9:1), which represents a modification of the method reported by Patel et al. (2011). The optimized surfactant–co-surfactant mixture was combined with oil at different proportions to evaluate compatibility and formulation stability. Each mixture was then homogenized using a magnetic stirrer (Daihan® MSH-20D) operated at 500 rpm for 30 minutes at a controlled temperature of 40°C, followed by sonication for 1 hour at the same temperature. This process ensured the formation of a uniform and stable pre-concentrate suitable for further evaluation of the SNEDDS formulation.

### *2.6. Optimization and Preparation of Sunflower Seed Oil SNEDDS*

The optimization and preparation of sunflower seed oil SNEDDS were carried out using the Simplex Lattice Design (SLD) method. The optimization process involved three main components—sunflower seed oil, Tween 80, and PEG 400—with 16 formulations suggested by the Design Expert (DX) software version 13. The preparation of the SNEDDS formulation was performed by mixing Tween 80, PEG 400, and sunflower seed oil using a magnetic stirrer (Daihan® MSH-20D) operated at 500 rpm for 30 minutes at 40°C, followed by sonication for 1 hour at the same temperature to obtain a homogeneous pre-concentrate.

### *2.7. Evaluation of Sunflower Seed Oil SNEDDS Formulation Response*

The evaluation of the SNEDDS formulations was conducted by dispersing 0.1 mL of the sample in 5 mL of distilled water and homogenizing the mixture using a vortex mixer. The percent transmittance of each sample was measured at a wavelength of 650 nm using a UV–Vis spectrophotometer, with distilled water serving as the blank. Formulations exhibiting transmittance values between 90% and 100% were categorized as clear and transparent. Furthermore, the emulsification time was determined by dispersing 1 mL of SNEDDS in 25 mL of distilled water, followed by homogenization using a magnetic stirrer at 100 rpm while recording the time required for emulsification. The process was visually observed, and emulsification times below one minute indicated that the SNEDDS rapidly formed a clear emulsion upon contact with aqueous media, mimicking gastric conditions.

### *2.8. Determination and Verification of the Optimum SNEDDS Formula for Sunflower Seed Oil*

The determination of the optimum SNEDDS formula was conducted using the Simplex Lattice Design approach in Design Expert version 13. The three components—sunflower seed oil, Tween 80, and PEG 400—served as independent variables, while the desirability function was used to determine the optimal composition. The formulation with the highest desirability value, approaching 1, was regarded as the best. The predicted optimum formula was then verified experimentally in triplicate, and the results were statistically analyzed using a one-sample t-test in SPSS to evaluate consistency with the predicted values.

### *2.9. Characterization of Sunflower Seed Oil SNEDDS*

Characterization of the optimized SNEDDS was performed by pipetting 1 mL of the formulation into a 10 mL volumetric flask and diluting it to the mark with distilled water. The particle size distribution, polydispersity index, and zeta potential were analyzed using a Particle Size Analyzer (HORIBA SZ-100) based on dynamic light scattering principles, which measure the fluctuations in scattering intensity ( ) caused by Brownian motion of particles. Measurements were conducted at 25°C with a scattering angle of 90°.

### *2.10. The antioxidant activity of pure sunflower seed oil, sunflower seed oil SNEDDS, and vitamin C (as the standard control)*

The antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. A stock DPPH solution was prepared by dissolving 3.5 mg of DPPH in 100 mL of methanol and homogenizing the solution. The maximum absorption wavelength of the DPPH solution was determined within the 400–800 nm range using a UV–Vis spectrophotometer. A 1000 ppm stock solution was then serially diluted to prepare various concentrations (1000, 500, 250, 125, 62.5, and 31.25 ppm). For antioxidant testing, 2 mL of the sample solution was mixed with 2 mL of the DPPH solution in a test tube, homogenized, and incubated in the dark for 30 minutes before measuring absorbance at the maximum wavelength. Vitamin C was tested following the same procedure at concentrations ranging from 2 to 10 ppm, prepared from a 100 ppm stock solution.

### *2.11. Data Analysis*

The experimental results of the optimized sunflower seed oil SNEDDS formulation were statistically validated using SPSS software through a one-sample t-test at a 95% confidence level to determine whether the experimental outcomes significantly differed from the predicted results obtained from Design Expert version 13.

## 3. RESULTS AND DISCUSSION

### *3.1. Sunflower Seed Oil Examination Results*

The initial step in the formulation of SNEDDS containing sunflower seed oil involved the comprehensive evaluation of its active constituents. This evaluation encompassed solubility testing, determination of specific gravity, phytochemical screening, and refractive index measurement. The results demonstrated that the sunflower seed oil complied with the specifications outlined in the Certificate of Analysis (CoA), the Indonesian Pharmacopoeia Sixth Edition, and the Handbook of Pharmaceutical Excipients Sixth Edition. Sunflower seed oil was selected as the active component primarily due to its substantial polyphenol content, which confers significant antioxidant properties. The physical and chemical properties of sunflower seed oil in Table 1—including its clear liquid form, mild and pleasant odor, pale yellow color, pH of approximately 6.4, practical insolubility in 95% ethanol, specific gravity ranging from 0.915 to 0.919 g/mL, and refractive index within 1.469 to 1.47364—were all consistent with pharmacopeial standards, further validating its suitability for pharmaceutical formulation. Recent studies reinforce the efficacy and stability of sunflower seed oil in nanoemulsion systems, highlighting its potent antioxidant capacity and biocompatibility, which are critical for enhanced drug delivery applications [11].

**Table 1.** Results of Sunflower Seed Oil Analysis

| Test | Requirements (Indonesian Pharmacopoeia Edition VI and *Handbook of Pharmaceutical Excipients* Edition 6) | *Certificate of Analysis* (COA) Requirements | Observation |
|---|---|---|---|
| Appearance | Clear liquid | Clear liquid | Clear liquid |
| Odor | A mild and pleasant odor | - | Mild odor |
| Color | Light yellow | - | Light yellow |
| pH | 6.2 | - | 6.4 |
| Solubility | Practically insoluble in 95% ethanol | - | Practically insoluble in ethanol |
| BJ | 0.915-0.919 g/mL | 0.915 g/mL | 0.919 g/mL |
| Refractive Index | 1.472–1.474 | 1.469 | 1.47364 |

### *3.2. Results of Phytochemical Screening of Sunflower Seed Oil*

Phytochemical screening of sunflower seed oil revealed the presence of terpenoid compounds, as evidenced by the characteristic red-purple coloration observed with the Liebermann–Burchard reagent. In contrast, no positive reactions were detected for alkaloids, phenolics, flavonoids, or saponins. Terpenoids, including notable constituents such as vitamin E (tocopherols), phytol, and carotenoids, are well-documented for their strong antioxidant activities by neutralizing free radicals and protecting cellular components from oxidative damage [12]. The antioxidant potential of sunflower seed oil is largely attributed to these terpenoid compounds, which thus contribute significantly to the oil’s therapeutic efficacy and stability in pharmaceutical applications. Recent studies have demonstrated that the incorporation of terpenoid-rich oils into nanoemulsion systems enhances the antioxidant capacity by facilitating better bioavailability and sustained release of bioactive compounds [13].

### *3.3. Determination of Surfactant and Co-surfactant Ratio (Smix)*

The preparation of the surfactant and co-surfactant mixture (smix) involves combining all components using a magnetic stirrer at 40°C. This temperature is employed to ensure the solubility of the ingredients, especially the surfactant and co-surfactant, while also reducing the mixture's viscosity to facilitate homogeneous stirring and mixing. Subsequently, the mixture undergoes sonication to enhance uniformity. Evaluation of the surfactant and co-surfactant is based on the clarity and stability of the mixture after 24 hours. The optimal ratio found was 11:1, which effectively forms nano-emulsions. When only a single type of surfactant is used without the addition of co-surfactant, it is generally insufficient to reduce the interfacial tension effectively. The percentage of surfactant in the SNEDDS formulation significantly influences clarity; higher surfactant concentration leads to a clearer formulation due to the surfactant's role in stabilizing the emulsion and reducing oil droplet size to the nano-scale.

Furthermore, when surfactant and co-surfactant are added in equal proportions, the reduction in interfacial tension is more effective compared to using only surfactant. Increasing the surfactant ratio relative to the co-surfactant further enhances this reduction, optimizing emulsification. However, an excess of co-surfactant compared to surfactant diminishes the emulsification efficiency. Hence, the best ratio is either balanced or with surfactant dominance. Surfactant molecules adhere to oil droplet surfaces, aggregating into small structures called micelles ([14] ).

Recent international studies within the last five years have corroborated these findings. Experiments employed dissipative particle dynamics (DPD) simulations combined with experimental validation, demonstrating that the addition of PEG400 as a co-surfactant stretches surfactant molecules, increasing interfacial thickness and improving nano-emulsion formation efficiency[14] . Experiments showed that precise surfactant co-surfactant ratio optimization expands the nano-emulsion formation region and improves particle size stability below 200 nm [15]. Additionally, studies confirm that higher surfactant concentrations result in smaller droplets and clearer formulations but highlight that excessive surfactant may impair long-term stability due to Ostwald ripening [16].

### *3.4. Determination of Oil Ratio with Smix*

Determination of the oil composition in combination with surfactant and co-surfactant was conducted to optimize the nano-emulsion formulation. The tested formulations employed a fixed ratio of surfactant to co-surfactant at 11:1 (based on the results of the optimization process of the method presented in Section 2.4), with sunflower seed oil as the oil phase, Tween 80 as the surfactant, and PEG 400 as the co-surfactant. Various ratios of oil to surfactant/co-surfactant were evaluated based on visual observation, percent transmittance, and emulsification time. Visual clarity was noted across the range, with formulations exhibiting percent transmittance above 90% generally being clear, indicating nanometer-sized droplets. Percent transmittance was measured via UV-Vis spectrophotometry at 650 nm using distilled water as a blank control, which has 100% transmittance due to the absence of scattering particles. Higher transmittance corresponded to smaller droplet size and better clarity, while formulations with transmittance below 90% tended to be turbid due to larger droplet size or instability of the dispersion.

The emulsification time, measured as the time required to form a nano-emulsion in distilled water, was under one minute for most formulations, indicating rapid and efficient emulsification.

Based on these results, the lower and upper limits for each component were established: sunflower oil between 9.09% and 25%, Tween 80 from 68.75% to 83.33%, and PEG 400 from 6.25% to 7.57%. These optimized ranges were then employed in a software design of experiments to generate 16 recommended formulation runs subjected to further transmittance testing (Table 2).

**Table 2.** Results of Oil Composition Determination with Smix

| **Composition Ratio** | | **Component (%v/v)** | | | **Visual** | **% Transmittance** | **Emulsification Time (seconds)** |
|---|---|---|---|---|---|---|---|
| **Oil** | **Smix (11:1)** | **A** | **B** | **C** | | | |
| 1 | 10 | 9.09 | 83.33 | 7.57 | Clear | 99.5 | 26.21 |
| 1 | 9.5 | 9.52 | 82.93 | 7.53 | Clear | 99.5 | 24.34 |
| 1 | 9 | 10 | 82.5 | 7.5 | Clear | 99.3 | 31.32 |
| 1 | 8.5 | 10.52 | 82.01 | 7.45 | Clear | 99.4 | 26.37 |
| 1 | 8 | 11.11 | 81.48 | 7.40 | Clear | 99.5 | 25.57 |
| 1 | 7.5 | 11.76 | 80.88 | 7.35 | Clear | 99.4 | 31.01 |
| 1 | 7 | 12.5 | 80.20 | 7.29 | Clear | 94.7 | 32.40 |
| 1 | 6.5 | 13.33 | 79.44 | 7.22 | Clear | 90.9 | 34.71 |
| 1 | 6 | 14.28 | 78.57 | 7.14 | Clear | 95.8 | 39.81 |
| 1 | 5.5 | 15.38 | 77.56 | 7.05 | Clear | 96.8 | 36.04 |
| 1 | 5 | 16.66 | 76.38 | 6.94 | Clear | 97.5 | 28.05 |
| 1 | 4.5 | 18.18 | 75 | 6.81 | Clear | 98.2 | 27.96 |
| 1 | 4 | 20 | 73.33 | 6.66 | Clear | 93.7 | 27.92 |
| 1 | 3 | 25 | 68.75 | 6.25 | Clear | 92.8 | 24.59 |
| 1 | 2.5 | 28.57 | 65.47 | 5.95 | Cloudy | 32.1 | 21.34 |
| 1 | 2 | 33.33 | 61.11 | 5.55 | Cloudy | 66.8 | 20.19 |
| 1 | 1 | 50 | 45.83 | 4.16 | Cloudy | 40.2 | 30.49 |
| 1.5 | 1 | 60 | 36.66 | 3.33 | Cloudy | 50.6 | 21.65 |
| 2 | 1 | 66.66 | 30.55 | 2.77 | Cloudy | 1.2 | 17.68 |
| 2.5 | 1 | 71.42 | 26.19 | 2.38 | Cloudy | 2.9 | 22.05 |
| 3 | 1 | 75 | 22.91 | 2.08 | Cloudy | 7.4 | 24.25 |
| 4 | 1 | 80 | 18.33 | 1.66 | Cloudy | 0.8 | 20.68 |
| 7 | 1 | 87.5 | 11.45 | 1.04 | Cloudy | 0.7 | 16.55 |
| 9 | 1 | 90 | 9.16 | 0.83 | Turbid | 0.4 | 18.56 |

Note: A = Sunflower Oil Concentration, B = Tween 80 Concentration, C = PEG 400 Concentration

Recent international literature validates these approaches, emphasizing that transmittance measurements serve as a reliable preliminary indicator for nano-emulsion droplet size and clarity. For instance, a 2024 study demonstrated that transmittance exceeding 90% correlates with droplet sizes in the nano range and stable dispersions [17]. Similarly, rapid emulsification times of less than 60 seconds are characteristic of well-optimized self-nanoemulsifying drug delivery systems (SNEDDS), which facilitate drug solubilization and bioavailability enhancement [18]. The balancing of oil, surfactant, and co-surfactant within optimal ranges is critical for achieving physicochemical stability, small droplet size, and effective emulsification, as further supported by the 2024 response surface methodology studies [19].

### *3.5. Response Evaluation of Sunflower Seed Oil SNEDDS Formulation*

The data obtained from the Simplex Lattice Design (SLD) software was analyzed to evaluate two main response parameters: emulsification time and percent transmittance (Table 3).

**Table 3.** Results of SNEDDS Sunflower Seed Oil Formula Response Evaluation

| **Run** | **Component 1 A: Oil %** | **Component 2 B: Surfactant %** | **Component 3 C: Co-surfactant %** | **Transmittance** | **Emulsification Time (seconds)** |
|---|---|---|---|---|---|
| 1 | 9.1 | 83.33 | 7.57 | 99.5 | 18.37 |
| 2 | 15.28 | 78.47 | 6.25 | 91.8 | 42.12 |
| 3 | 24.12 | 68.75 | 7.13 | 80.1 | 17.57 |
| 4 | 25 | 68.75 | 6.25 | 95.7 | 26.11 |
| 5 | 17.05 | 76.04 | 6.91 | 96.8 | 20.48 |
| 6 | 18.82 | 73.61 | 7.57 | 85.2 | 18.19 |
| 7 | 17.05 | 76.04 | 6.91 | 87.7 | 22.58 |
| 8 | 9.1 | 83.33 | 7.57 | 99.5 | 21.22 |
| 9 | 24.12 | 68.75 | 7.13 | 80.1 | 21.25 |
| 10 | 13.96 | 78.47 | 7.57 | 96.8 | 19.30 |
| 11 | 21.025 | 72.395 | 6.58 | 89.3 | 19.52 |
| 12 | 17.05 | 76.04 | 6.91 | 97.0 | 19.62 |
| 13 | 20.14 | 73.61 | 6.25 | 89.3 | 15.54 |
| 14 | 28.82 | 73.61 | 7.57 | 97.2 | 20.98 |
| 15 | 13.735 | 79.685 | 6.58 | 98.3 | 40.84 |
| 16 | 10.42 | 83.33 | 6.25 | 98.8 | 20.98 |

Emulsification time is critical as it represents how efficiently the SNEDDS formulation can form an emulsion upon contact with bodily fluids. Since emulsification in vivo largely occurs naturally via peristaltic movement in the gastrointestinal tract, a formulation requiring minimal energy to emulsify is desirable. The rapid emulsification observed is largely attributed to the synergistic action of surfactant and co-surfactant, which quickly create an interfacial film between the oil and water phases. Among these, the co-surfactant plays a more prominent role in accelerating the emulsification process rather than significantly reducing droplet size. A model describing the experimental data, along with its ANOVA output, is presented for the SNEDDS formulation in Table 4.

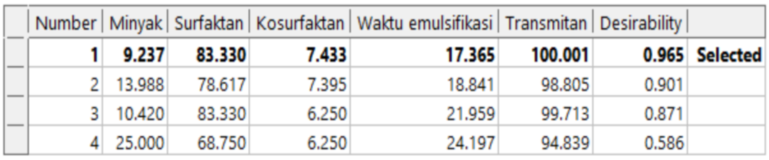
**Table 4.** ANOVA of the fitted equation for transmittance and Emulsification Time of SNEDDS

| | Transmittance | | | | Emulsification Time | | | |
|---|---|---|---|---|---|---|---|---|
| | Sum of squares | Mean square | F value | p value | Sum of squares | Mean square | F value | p value |
| Model (significant) | 520.60 | 86.77 | 5.40 | 0.0126 | 54.98 | 27.49 | 9.09 | 0.0034 |
| Residual | 144.51 | 16.06 | | | 39.32 | 3.02 | | |
| Lack of fit (not significant) | 16.06 | 4.01 | 0.1563 | 0.9518 | 19.28 | 2.41 | 0.6011 | 0.7514 |
| Pure error | 128.45 | 25.69 | | | 20.04 | 4.01 | | |
| Std deviation | 4.01 | | | | 1.74 | | | |
| Predicted $R^2$ | 0.2932 | | | | 0.3791 | | | |
| Adjusted $R^2$ | 0.6379 | | | | 0.5189 | | | |
| $R^2$ | 0.7827 | | | | 0.5830 | | | |
| Adeq precision | 7.2598 | | | | 7.5688 | | | |

The relationship between percent transmittance and emulsification time showed that formulations exhibiting high transmittance values close to 100% generally correspond to shorter emulsification times and smaller droplet sizes, indicating efficient and spontaneous nano-emulsion formation. These findings are consistent with current pharmaceutical sciences literature, which underscores that high transmittance reflects optical clarity attributed to nanoscale droplet dimensions, while short emulsification time correlates with better dispersion kinetics essential for enhanced oral bioavailability.

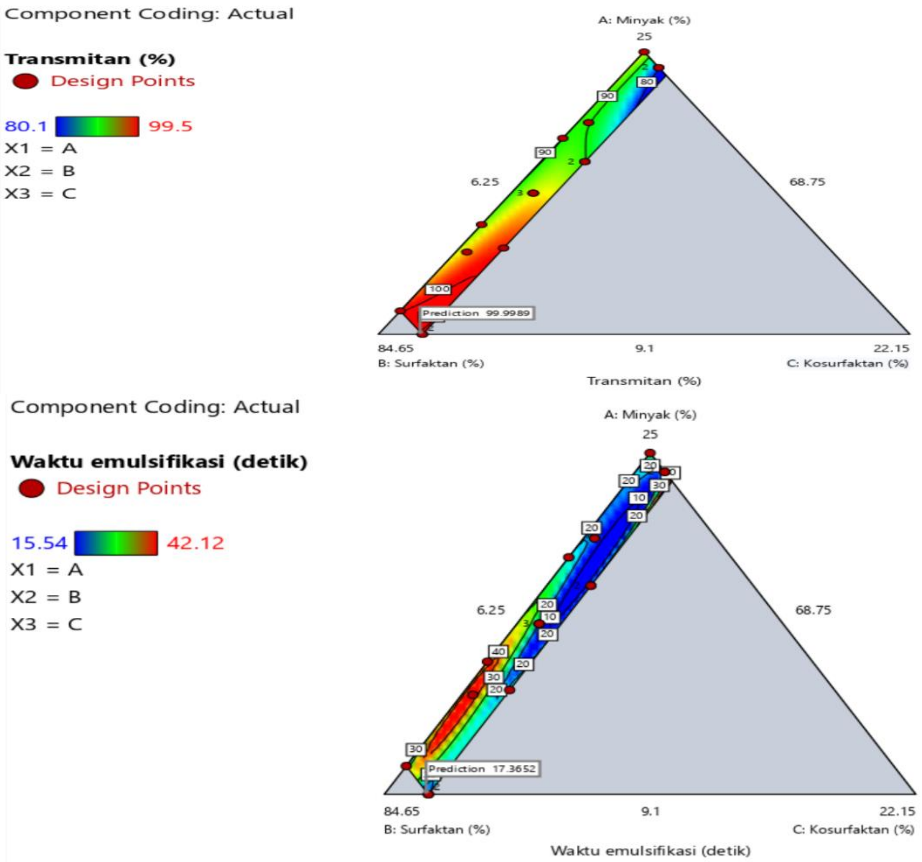
Recent advances within the past five years support these observations. For example, studies have demonstrated that the co-surfactant aids in lowering interfacial tension more rapidly, thereby facilitating the spontaneous formation of stable nano-emulsions within seconds to a few minutes [20]. Another study emphasized that optimum surfactant co-surfactant ratios significantly reduce emulsification time, improving drug dissolution rates in oral delivery systems [21]. Furthermore, modelling approaches such as those applied in design of experiments (DOE) frameworks, similar to the SLD used here, confirm the importance of balancing formulation components to achieve ideal physical characteristics, emulsification efficiency, and therapeutic performance [5].

### *3.6. Optimum Formula and Verification of the Optimum SNEDDS Formula for Sunflower Seed Oil*



| Number | Minyak | Surfaktan | Kosurfaktan | Waktu emulsifikasi | Transmitan | Desirability | |
|---|---|---|---|---|---|---|---|
| **1** | **9.237** | **83.330** | **7.433** | **17.365** | **100.001** | **0.965** | **Selected** |
| 2 | 13.988 | 78.617 | 7.395 | 18.841 | 98.805 | 0.901 | |
| 3 | 10.420 | 83.330 | 6.250 | 21.959 | 99.713 | 0.871 | |
| 4 | 25.000 | 68.750 | 6.250 | 24.197 | 94.839 | 0.586 | |

(**a**)



(**b**)

(**c**)

**Figure 1.** (a) Design Expert-13 optimum formula (Simplex Lattice Design); (b) Contour plot of transmittance percentage; (c) Contour plot of emulsification time

The optimum formula recommended by the Design Expert software was identified through contour plot analysis, which showed the area with the highest desirability, reflecting the suitability of the formula to the optimal criteria expected in producing a specific response. Desirability close to 1 indicates optimization of the formulation in producing the desired SNEDDS characteristics. In this study, a desirability value of 0.965 was obtained. The recommended formula was then reproduced for the verification process, which focused on testing transmittance as the main parameter. The verification results showed that the sunflower seed oil SNEDDS formulation produced an average emulsification time of 17.14 ± 0.098 seconds and a transmittance percentage of 99.8 ± 0.1%, in line with Design Expert predictions and supporting the validity of the desirability-based optimization method.

The transmittance contour plot shows the crucial role of surfactants in increasing the transmittance percentage, with the color gradient indicating the quantitative contribution of each component to the resulting response. The dominance of red in the surfactant area confirms the effectiveness of surfactants in forming transparent and stable nano-emulsions. Meanwhile, the interaction between oil and co-surfactants also affects emulsification time, where the blue area on the emulsification time plot indicates the shortest duration. Conceptually, the response surface methodology (RSM) technique with desirability has been widely used in pharmaceutical research to validate and reinforce the optimum SNEDDS formulation, as documented in the latest international literature emphasizing the importance of experimental validation after in silico prediction.

Verification of the optimum formula is a fundamental aspect in ensuring the reliability and reproducibility of formulations that have been informed by statistical modelling. Recent studies show that the conformity of verification results with optimization software predictions is a valid indicator that all optimization stages have been effective, where the average verification result parameters are very close to the predictions ($p>0.05$), as shown in Table 5. This confirms the effectiveness of the desirability strategy in the design of superior SNEDDS [22]. Both parameters, emulsification time and % transmittance, collectively serve as critical indicators of SNEDDS stability and efficacy in drug delivery, as emphasized in studies highlighting the contribution of rapid emulsification and high transmittance to superior bioavailability [23].

**Table 5.** Optimum Formula Verification Results

| Optimum Formula | Transmittance (%) | Emulsification Time (seconds) |
|---|---|---|
| Software Prediction | 100.001 | 17.365 |
| Test Results | 99.8 ± 0.1[1] | 17.14 ± 0.098[2] |

[1,2]Not significantly different from software prediction ($p>0.05$)

### 3.7. Antioxidant Activity of Sunflower Seed Oil, SNEDDS, and Positive Control

The antioxidant activity of sunflower seed oil was measured using the DPPH test with a maximum absorption at a wavelength of 516 nm and a control absorbance of 0.798. In this test, an $IC_{50}$value of 13.09 µg/mL was obtained, indicating a very strong antioxidant activity. The test data showed that sunflower seed oil has significant antioxidant potential, and this $IC(_{50)}$value can be influenced by the quality of the oil used, in this case, oil obtained from PT. Lansida. The antioxidant test on the SNEDDS preparation of sunflower seed oil showed an $IC(_{50)}$value of 18.06 µg/mL, which is also classified as very strong, although slightly higher than pure oil. This indicates the ability of SNEDDS to maintain antioxidant activity even when the formulation is modified[24] . For the positive control, vitamin C showed an $IC(_{50)}$of 3.06 µg/mL in the very strong category, which was used as a standard comparison for antioxidant activity.

The relationship between the SNEDDS formula and antioxidant activity is based on the ability of SNEDDS to refine particle size and increase the absorption of bioactive compounds. The optimized SNEDDS formula produces small nano-sized droplets, thereby increasing the contact surface area and stability of antioxidant compounds in the emulsion system. Recent international research shows that reducing particle size through SNEDDS formulation increases antioxidant efficacy by facilitating interaction between free radicals and contained antioxidant molecules. In addition, the properties of surfactants and co-surfactants in the formula also play an important role in increasing the stability and antioxidant activity of compounds [25]. For example, one study reported an increase in the antioxidant activity of *Pandanus tectorius* fruit extract after formulation in SNEDDS, which was attributed to the nano particle size and stability of the resulting nano-emulsion system[26] . Another study also showed that the free radical activity of curcumin increased significantly when used in SNEDDS formulations compared to curcumin in its free form [24].

Particle refinement and dispersibility in SNEDDS formulations can improve bioavailability and pharmacological activity, including antioxidant activity, by promoting the release and absorption of active compounds in the gastrointestinal tract. This improved absorption efficiency has a positive impact on in vitro measured antioxidant activity, which may also contribute to better systemic therapeutic effects [27]. The effect of SNEDDS formulations on antioxidant activity is consistent with the literature emphasizing the importance of formulation design in ensuring stability, particle size, and molecular interactions to enhance the effectiveness of antioxidant activity of natural ingredients or drug compounds [28].

### *3.8. Characterization of Sunflower Seed Oil SNEDDS*

Determining particle size, polydispersity index, and zeta potential are crucial aspects in characterizing SNEDDS. Based on PSA data, the average particle size obtained was 13 nm with a standard deviation of 0.346 nm, a polydispersity index of 0.161 ± 0.067, and a zeta potential of -21.8 mV ± 0.577. This can be seen in Table 4. Particle size greatly affects drug release from nano-emulsion formulations. Small particles have a larger surface area, thereby increasing the contact area between the drug and the surface, allowing for direct and faster drug release. Conversely, larger particles require encapsulation and gradual diffusion processes, thereby reducing the rate of drug release. The high surface area of nanoparticles provides efficiency in absorption and distribution via the trans-mucosal or skin route, while also increasing the penetration of active substances into the skin layers [29].

The polydispersity index (PDI) is used to indicate the distribution of particle sizes in a system. A low PDI value (below 0.7) indicates a monodisperse system, meaning that the particles in the system are uniform in size, such as the result in sunflower seed oil SNEDDS of 0.161. A high PDI value indicates a poly-disperse system, where particle sizes vary and can cause Ostwald ripening, which is the process of large particle growth through the combination of small particles. SNEDDS with low PDI tend to have good physical stability and are not susceptible to changes in particle size distribution during storage [30].

The zeta potential value indicates the stability of the SNEDDS system through the electrical energy formed due to the separation of charges between two liquid interface layers. Systems with an absolute zeta potential of more than ±30 mV usually have high stability because the repulsive forces between particles are large enough to prevent aggregation or flocculation. In formulations with zeta potential values below ±30 mV, there is a risk of particle flocculation due to the dominance of attractive forces between particles. In a recent study, the zeta potential value in SNEDDS was influenced by the proportion of oil, surfactant, and co-surfactant; increasing the surfactant and decreasing the oil in the formula can increase the zeta potential value and system stability [31].

**Table 6.** SNEDDS Characterization Results

| Characterization | Results | | | Mean ± SD |
|---|---|---|---|---|
| | R1 | R2 | R3 | |
| Particle Size | 12.8 | 12.8 | 13.4 | 13 nm ± 0.346 |
| Polydispersity Index | 0.116 | 0.129 | 0.239 | 0.161 ± 0.067 |
| Zeta Potential | -22.2 | -21.2 | -22.2 | -21.8 mV ± 0.577 |

The relationship between the SNEDDS formula and characterization results is greatly influenced by the composition of the oil, surfactant, and co-surfactant. Adding a larger proportion of surfactant tends to produce smaller particle sizes and increase system stability. Surfactants also have an effect on reducing PDI because they can homogenize particle sizes. Meanwhile, adjusting the composition of oil and co-surfactant is necessary to obtain the optimal zeta potential and desired physical characteristics of SNEDDS [30].

## 4. CONCLUSION

This study proves that the optimization of sunflower seed oil-based SNEDDS using Tween 80 and PEG 400, with the Simplex Lattice Design approach, can produce a formula with very small nanoparticle size, low polydispersity index, and zeta potential that supports system stability. The optimized formula not only enhances the solubility and bioavailability potential of the active ingredient but also maintains high antioxidant activity. Thus, the development of SNEDDS from sunflower seed oil has the potential to support the innovation of efficient and safe oral formulations in modern pharmaceutical applications.

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