ORIGINAL ARTICLE

# Formulation and Evaluation of Self-Nanoemulsifying Drug Delivery System (SNEDDS) Loaded with Usnic Acid for Topical Application

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## ABSTRACT

Usnic acid, a secondary metabolite derived from Usnea sp., exhibits diverse pharmacological activities; however, its therapeutic utility is constrained by poor aqueous solubility and risk of hepatotoxicity upon oral administration. To overcome these limitations, this study aimed to develop a self-nanoemulsifying drug delivery system (SNEDDS) of usnic acid tailored for topical application. The formulation was optimized via a pseudoternary phase diagram and characterized using a particle size analyzer (PSA) and transmission electron microscopy (TEM). The optimized SNEDDS—comprising grapeseed oil, Cremophor RH 40, and polyethylene glycol 400—exhibited rapid emulsification (33.587 ± 1.159 s) and high transmittance (98.408 ± 0.477%). PSA analysis revealed a globule size of 59.6 nm, a polydispersity index (PDI) of 0.193, and a zeta potential of −48.6 mV, aligning with TEM observations that confirmed a spherical morphology. This optimized system was subsequently incorporated into a Carbopol Ultrez 30 hydrogel, yielding a homogeneous formulation with a pH of 5.57 ± 0.012 and a spreadability of 3.6 cm. Upon incorporation into the gel framework, the SNEDDS maintained favorable characteristics, exhibiting a globule size of 72.5 nm nm, a PDI of 0.445, and a zeta potential of -55.3 mV. Finally, in vitro release studies demonstrated that the SNEDDS hydrogel achieved an approximately twofold increase in usnic acid release over 24 h compared with a conventional hydrogel, highlighting its potential as an effective topical delivery system.

**Keywords** Usnic acid; SNEDDS; Carbopol ulterz 30; Hydrogel.

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***Article History:***
Received: 22 Aug 2026
Revised: 28 Aug 2026
Published: 05 Sep 2026

***DOI:***
10.25077/jsfk.13.02.203-213.2026

***Citation:***
Husna N, Fitriani L, Muttadien SE, Noviza D. Formulation and evaluation of Self-Nanoemulsifying Drug Delivery System (SNEDDS) Loaded with Usnic Acid for Topical Application. J Sains Farm Klin. 2026;13(2):203-213



## Introduction

Usnic acid is a secondary metabolite produced by lichens, commonly found in the genus Usnea sp, and it possesses diverse pharmacological activities. Its application, however, is limited by poor aqueous solubility and the potential for hepatotoxicity upon oral administration. Recent medical reports indicate that usnic acid consumption can lead to liver damage, ranging from hepatitis to acute liver failure. At least 21 cases of hepatotoxicity have been reported to the U.S. FDA following the use of supplements containing usnic acid [1]. While the oral use of usnic acid presents a risk of hepatotoxicity, its topical application demonstrates a favorable safety profile and high tolerability. Moreover, its potential applications are extensive, including wound healing and the prevention of respiratory tract infections [2].

Based on the Biopharmaceutical Classification System (BCS), usnic acid is classified as a Class II drug, characterized by high membrane permeability but low solubility. Despite its high permeability, its low aqueous solubility remains a primary obstacle in developing topical formulations [3]. The stratum corneum, with its high lipid and low water content, serves as the main barrier to drug absorption in topical applications [4]. To enhance drug absorption through the skin, nanotechnology approaches can be used, such

as the formulation of Self-Nanoemulsifying Drug Delivery Systems (SNEDDS).

The formulation of SNEDDS for topical applications has shown promising results in several studies. Lalatsa et al. (2020) developed a topical hydrogel incorporating SNEDDS bupavarquon as an alternative treatment for cutaneous leishmaniasis, with the formulation effectively reducing parasite counts and penetrating the skin to achieve therapeutic concentrations at the site of infection [5]. Following this, Ponto et al. (2022) utilized SNEDDS for the natural compound astaxanthin, significantly increasing its penetration into the stratum corneum and hair follicles, with globule sizes around 20 nm and a low polydispersity index of 0.25 [6]. Additionally, Frei et al. (2023) showed that curcumin gels and creams based on SNEDDS provided significant anti-inflammatory benefits, as evidenced by a notable reduction in post-treatment TNF-$\alpha$ levels [7].

In recent years, SNEDDS has become one of the promising drug delivery systems to enhance drug absorption, especially for topical applications [6]. SNEDDS is an isotropic mixture consisting of drug, oil, surfactant, and cosurfactant. This system significantly improves the bioavailability of topical drugs by forming nanosized droplets, thereby increasing the surface area available for contact and drug absorption [8]. Therefore, this study was conducted to develop a topical self-nanoemulsifying drug delivery system (SNEDDS) for usnic acid. The formulated SNEDDS was characterized using a Particle Size Analyzer (PSA) and Transmission Electron Microscopy (TEM). The SNEDDS was then incorporated into a hydrogel, and its in vitro release was assessed using a dialysis bag with a cellulose membrane.

## METHODS

### Materials

Usnic acid was obtained from Tokyo Chemical Industry (Japan); methanol pro analysis from Merck (Germany); and distilled water from Kafarma (Indonesia). Various oils were utilized, including olive oil, sunflower oil, castor oil, and avocado oil supplied by Lantabura International (Indonesia); palm kernel oil from Soon Soon Oilmills (Malaysia); grapeseed oil and jojoba oil obtained from Merck (Germany). Tween 80 and propylene glycol from PT. Brataco Chemica (Indonesia); Cremophor® RH 40 and Cremophor® EL were sourced from Merck (Germany); polyethylene glycol 400 was sourced from Sigma-Aldrich (USA); Carbopol Ultrez 30 was sourced from Merck (Germany); glycerin and triethanolamine were purchase from PT. Brataco Chemica (Indonesia); phosphate buffer at pH 7.4 from Sigma-Aldrich (USA); and hydrochloric acid from Merck (USA).

### Determination of SNEDDS Component

#### Solubility Test of Usnic Acid in Various Oils, Surfactants, and Cosurfactants

The solubility of usnic acid was tested in various oils (olive oil, sunflower oil, castor oil, avocado oil, palm kernel oil, grapeseed oil and jojoba oil), surfactants, and cosurfactants (Cremophor® RH 40, Cremophor® EL, Tween 80, Polyethylene glycol 400 and propylenglycol). A quantity of 5 mg usnic acid was added to 2 mL of each oil, surfactant, and cosurfactant in separate vials. The mixtures were vortexed for 2 minutes and then placed in an orbital shaker water bath (Memmert®) at 100 rpm and 37±0.5°C for 72 hours. Samples were then centrifuged (Benchmark LC-8 Series, USA) at 4000 rpm for 10 minutes. The supernatant was filtered through a 0.45 µm filter paper. The filtrate was diluted with methanol pro-analysis and analyzed using a UV-Vis spectrophotometer (Thermo Scientific®) with the wavelength 288 nm [10]. The oil with the highest usnic acid solubility was selected for further study.

### Determination of Surfactant for SNEDDS Formulation

Various surfactants were tested for their emulsification ability in the selected oil. Each surfactant (500 µL) was mixed with 500 µL of oil and heated at 50°C for 2 minutes. Then, 100 µL of each mixture was transferred into a 50 mL volumetric flask and diluted with distilled water up to the mark. The resulting emulsions were left to stand for 2 hours, after which the percentage transmittance was measured using a UV-Vis spectrophotometer (Thermo Scientific®) at a wavelength of 650 nm with distilled water as the blank. Each test was performed in triplicate [8]. The surfactant with the highest percentage transmittance (%T) was selected to

proceed for cosurfactant determination.

### Determination of Cosurfactant for SNEDDS Formulation

Each mixture consisting of 200 µL cosurfactant, 400 µL selected surfactant, and 600 µL selected oil phase was evaluated using the same method as in the surfactant determination [8]. The cosurfactant with the highest percentage transmittance (%T) was selected as the cosurfactant component for the SNEDDS formulation.

### Optimization of SNEDDS Base Formula Using Ternary Phase Diagram

A series of SNEDDS base mixtures was systematically prepared using varying proportions of oil (20–60%), surfactant (30–60%), and cosurfactant (0–40%), with the total concentration of components in each formula totaling 100% w/w. The components were mixed by simple manual stirring using a glass stirring rod until a homogeneous mixture was obtained. SNEDDS base formulations that produced a clear visual appearance (%transmittance > 95%) after dilution with distilled water were identified on the ternary phase diagram, where each axis represents oil, surfactant, and cosurfactant [11].

### Preparation of SNEDDS loaded with Usnic Acid

Based on the identification of the self-emulsification region in the ternary phase diagram, one optimal base formula was selected and incorporated with usnic acid SNEDDS. Usnic acid at a concentration of 0.2% was dissolved in the oil phase, followed by the addition of surfactant and cosurfactant. The mixture was stirred using a magnetic stirrer (IKA, Germany) at 100 rpm to produce the final usnic acid SNEDDS formulation [10].

### Characterization of Usnic Acid SNEDDS

### Determination of Globule Size, Polydispersity Index, and Zeta Potential

0.1 mL of usnic acid SNEDDS was diluted with 10 mL of distilled water. The globule size, polydispersity index (PDI), and zeta potential were measured using a Particle Size Analyzer (PSA) (Horiba® SZ 100, Japan) [12].

### Emulsification Time

0.1 mL of usnic acid SNEDDS was diluted with 10 mL of distilled water. The mixture was stirred using a magnetic stirrer (IKA, Germany) at 100 rpm. The time required to form a homogeneous nanoemulsion was recorded with a stopwatch [12]. Visual observation was conducted using the grading system listed in the reference [13].

### Percent Transmittance

0.1 mL of usnic acid SNEDDS was diluted with 10 mL of distilled water. The percent transmittance was measured using a UV-Vis spectrophotometer (Thermo Scientific®) at a wavelength of 650 nm, with distilled water as the blank [12].

### Robustness to Dilution Test

0.1 mL of usnic acid SNEDDS was diluted with distilled water at ratios of 1:10, 1:50, and 1:100, and also diluted with phosphate buffers at pH 5.5 and 7.4 at the same ratios (1:10, 1:50, and 1:100). The mixtures were stirred with a magnetic stirrer (IKA, Germany) at 100 rpm and 37°C. Then, the samples were stored at room temperature for 24 hours and visually observed for any signs of phase separation [14].

### Morphology Analysis

Morphological analysis was performed by diluting the usnic acid SNEDDS at a 1:100 rasio with distilled water to form a nanoemulsion. One drop of the sample was placed on a copper grid, stained with 1% phosphotungstic acid, and dried for 15 minutes before observation using transmission electron microscopy (TEM) [15].

### Drug Loading Efficiency

The content of usnic acid in the SNEDDS formulation was determined using a UV-Vis spectrophotometer (Thermo Scientific®) with the wavelength 288 nm. A quantity of 10 mg of usnic acid SNEDDS was dissolved in 10 mL of methanol. The absorbance of the solution was measured at the maximum wavelength of usnic acid. The blank solution was prepared using the same procedure but without adding usnic acid [8]. Drug Loading Efficiency was calculated using the following equation:

$$\% \text{ Drug Loading} = \frac{\text{Amount of drug in formulation}}{\text{Total weight of formulation}} \times 100\%$$

### Preparation of Hydrogel Formulation

The hydrogel was prepared using 0.5% Carbopol Ultrez 30 as the gelling agent. Carbopol Ultrez 30 was first wetted with glycerin and then disperse in water in a baker glass. The dispersion was allowed overnight at room temperature. Subsequenly, trietanolamine was added dropwise to facilitate gel formation, while stirring was maintainded using a stirrer at room temperature until homogeneous hydrogel was obtained. The amount of usnic acid dispersed in the hydrogel was 0.05% (w/w), equivalent to the amount of usnic acid in 25% (w/w) usnic acid SNEDDS. The formulations of the usnic acid hydrogel and usnic acid SNEDDS hydrogel are presented in the **Table 1**.

**Table 1.** Formulations of usnic acid SNEDDS (F1) and usnic acid hydrogel (F2)

| | F1 (% b/b) | F2 (% b/b) |
|---|---|---|
| Usnic acid | - | 0.05 |
| Usnic acid - SNEDDS equivalent as usnic acid 0.05% | 25 | - |
| Carbopol Ultrez 30 | 0.5 | 0.5 |
| Glycerin | 5 | 5 |
| Triethanolamine (TEA) | 0.26 | 0.34 |
| Distilled water add | 100 | 100 |

### Evaluation of Hydrogel Formulation

### Organoleptic Test

The organoleptic test was performed by visually observing the appearance, including shape, color, and odor.

### Homogeneity

Homogeneity was assessed visually. 0.2 grams of hydrogel sampel was spread on a glass slide and observed for texture. The hydrogel preparation must show a homogeneous composition without coarse granules [16].

### pH Measurement

The pH of the hydrogel was measured using a semi-solid pH meter (Hanna HI 5211, USA) calibrated with buffer solutions at pH 4, pH 7, and pH 10 [16].

### Spreadability Test

The spreadability test was conducted by placing 0.5 grams of hydrogel on a transparent glass plate lined with graph paper, then covered with another glass slide. A specific weight (1, 3, 5, and 10 grams) was applied on top for 1 minute. The spread diameter was measured after the weight was removed [17].

### Determination of Globule Size, Polydispersity Index, and Zeta Potential

The usnic acid SNEDDS hydrogel was diluted 1:100 with distilled water. The globule size, polydispersity index, and zeta potential were measured using a PSA (Horiba® SZ 100, Japan) [5].

### Determination of In Vitro Release Profile

The release profile of the hydrogel preparations was determined using a cellulose membrane dialysis bag and analyzed with a UV-Vis spectrophotometer (Thermo Scientific®). One gram of each hydrogel formula was weighed and placed into a cellulose membrane dialysis bag with a molecular weight cutoff of 10–14 kDa, which had been pre-hydrated with phosphate buffer at pH 7.4. Both ends of the dialysis bag were then tied with thread. The dialysis bag containing the sample was placed into a beaker containing 50 mL of phosphate buffer solution at pH 7.4. The medium temperature was maintained at 37±1°C with stirring at 250 rpm using a magnetic stirrer (IKA, Germany). Sampling was performed at 30 minute intervals and at 1, 2, 3, 4, 6, and 24 hours. Each 5 mL sample taken was replaced with an equal volume of phosphate buffer pH 7.4 [18]. The release data were subsequently analyzed using SPSS 29 with a T-test to assess significance of differences in drug release percentage between the usnic acid hydrogel and usnic acid SNEDDS hydrogel formulations.

## RESULT AND DISCUSSION

The selection of appropriate excipients plays a crucial role in the successful development of SNEDDS formulations. The solubility of the drug in the excipients

is a

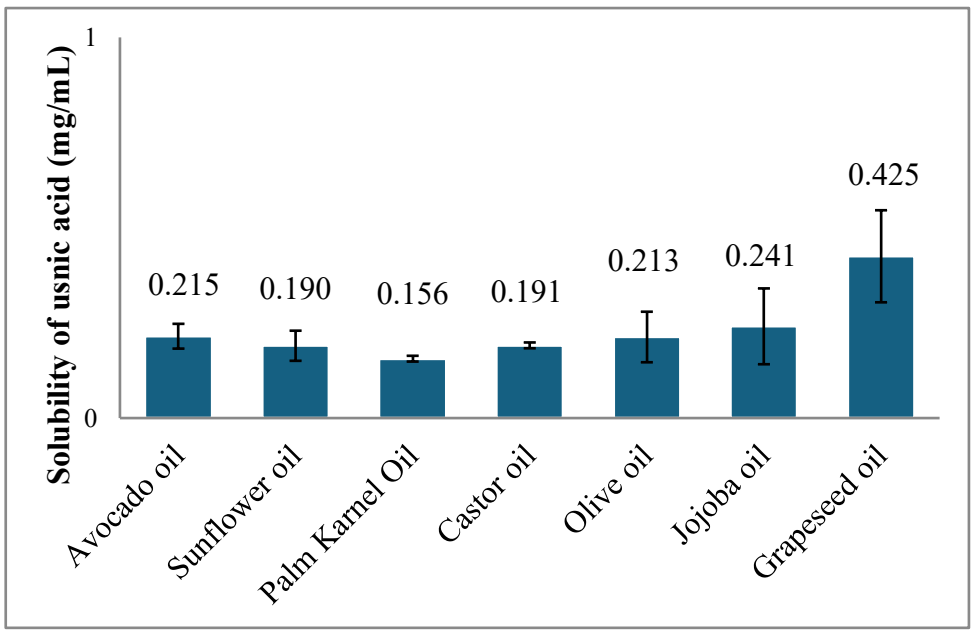


**Figure 1**. Solubility of usnic acid in oils

**Table 2.** Emulsification efficiency of surfactants

| Surfactant | %Transmittance |
|---|---|
| Cremophor RH 40 | 63.930 ± 1.844 |
| Cremophor EL | 60.731 ± 1.146 |
| Tween 80 | 52,.75 ± 0.035 |

n = 3

critical factor in the stability of the SNEDDS formulation, as drug precipitation often occurs prior to situ solubilization [19]. In developing the usnic acid SNEDDS formulation for topical application, seven different natural oils were tested as potential oil phases. The oils added to the SNEDDS formulation to increase the solubility of the lipophilic active compound usnic acid, and act as carriers that facilitate drug penetration through the outer layer of the skin. Furthermore, the interaction between these oils and the lipids in the stratum corneum can increase membrane fluidity, thereby promoting easier drug diffusion [14].

Based on the solubility results in **Figure 1**, grapeseed oil exhibited the highest capacity to dissolve usnic acid, reaching a concentration of 0.425 mg/mL. Its high content of unsaturated fatty acids, approximately 82%, with linoleic acid as the primary component, made it an ideal candidate [20]. Consequently, grapeseed oil was chosen as the SNEDDS oil phase for usnic acid and was used in all subsequent experiments.

Three surfactants were tested to assess the solubility of usnic acid, Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil), Cremophor EL (polyoxyl 35 castor oil), and Tween 80 (polysorbate 80). As shown in **Figure 2**, usnic acid exhibited the highest solubility in Cremophor RH 40. This is because the polyoxyethylene castor oil surfactant is more hydrophilic, allowing it to dissolve hydrophilic substances more effectively [21]. In addition to solubility, choosing a surfactant requires considering not only its solubilizing capacity but also its emulsification efficiency. The surfactant was selected based on its emulsification efficiency with grape seed oil, as indicated by the % transmittance of the resulting mixtures. As shown in **Table 2**, Cremophor RH 40 exhibited the highest % transmittance, indicating that the mixture of grape seed oil and Cremophor RH 40 had the highest emulsification efficiency. Therefore, Cremophor RH 40 was selected as the surfactant for further formulation development.

For the selection of the cosurfactant, the emulsification efficiency of the oil phase, surfactant, and cosurfactant mixture was evaluated based on the percentage transmittance (% transmittance). As shown in **Table 3**, the mixture of grape seed oil (oil phase), Cremophor RH 40 (surfactant), and PEG 400 (cosurfactant) exhibited a higher % transmittance than

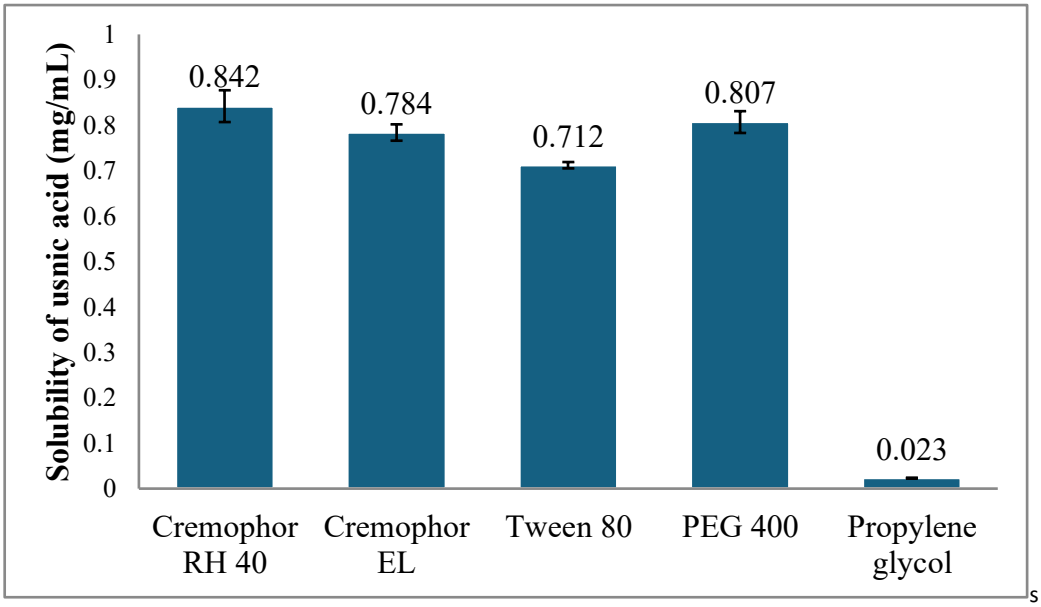


**Figure 2**. Solubility of usnic acid in surfactants and cosurfactants

**Table 3.** Emulsification efficiency of cosurfactants

| Cosurfactant | %Transmittance |
|---|---|
| PEG 400 | 59.018 ± 1.123 |
| Propylene glycol | 14.755 ± 3.344 |

n = 3

**Table 4.** Characterization of usnic acid SNEDDS for globule size, polydispersity index, and zeta potential

| | SNEDDS base | Usnic acid SNEDDS |
|---|---|---|
| Globule Size (nm) | 67.6 | 59.6 |
| PDI | 0.390 | 0.193 |
| Zeta Potensial (mV) | -49.8 | -48.6 |

the mixture containing propylene glycol. This may be attributed to the HLB value of PEG 400 (9.7), indicating a more lipophilic character compared with propylene glycol, which has a higher HLB value of 11.5. This increased hydrophobicity enables PEG 400 to more effectively support Cremophor RH 40 in lowering the surface tension of grapeseed oil [22], and it was selected as a cosurfactant.

Based on the results of solubility and emulsification efficiency tests, grapeseed oil, Cremophor RH 40, and PEG 400 were chosen as the oil, surfactant, and osurfactant components for the usnic acid SNEDDS.

The SNEDDS formulation was optimized using a ternary phase diagram to identify the self-emulsification region and optimal proportions of oil, surfactant, and cosurfactant. As shown in **Figure 3**, optimal nanoemulsion formation occurred at 22–40% oil, 28–58% surfactant, and 10–40% cosurfactant. These findings are consistent with Ashfaq et al. (2022), who reported that spontaneous emulsification requires a Smix concentration of 45–75%, with efficiency improving above 60%. Their study also demonstrated that increasing surfactant concentration from 30% to 60% reduced globule size, whereas further increases to 70%

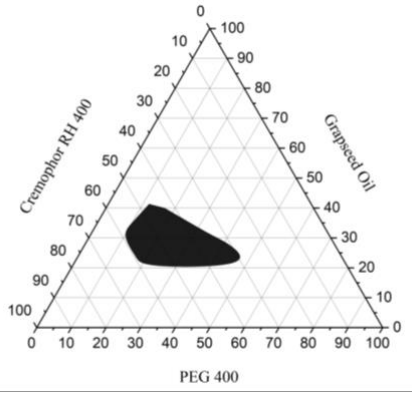


**Figure 3**. Optimization results area of usnic acid SNEDDS base

**Table 6**. Results of robustness to dilution test of usnic acid SNEDDS

| Dilution | Aquadest | Phosphate Buffer pH 5,5 | Phosphate Buffer pH 7,4 |
|---|---|---|---|
| 1 : 10 | √ | √ | √ |
| 1 : 50 | √ | √ | √ |
| 1 : 100 | √ | √ | √ |

*(√) The formula remains stable without any phase separation or precipitation

**Table 5.** Characterization of usnic acid SNEDDS for emulsification time and %Transmittance

| Organoleptic | Emulsification Time (seconds) | %Transmittance |
|---|---|---|
| Clear yellow | 33.587 ± 1.159 | 98.408 ± 0.477 |

n = 3

resulted in larger globules [11].

**Table 4** summarizes the characteristics of the optimized SNEDDS and usnic acid-loaded SNEDDS. Both formulations exhibited nanosized globules (<200 nm), with mean sizes of 67.6 and 59.6 nm, respectively. The low polydispersity (0.390 and 0.193) indicates relatively narrow and homogeneous globule size distributions. The zeta potentials were −49.8 and −48.6 mV, respectively, indicating good stability and dispersion [23], which may be attributed to the presence of free fatty acids in the oil phase. [12].

Emulsification time assessment is employed to quantify the rate at which a SNEDDS formulation generates a nanoemulsion. The data presented in **Table 5** categorizes the usnic acid SNEDDS formulation as Grade A, as it produced a clear nanoemulsion within less than one minute. This observation is corroborated by Nasr et al. (2016), who developed an olmesartan medoxomil SNEDDS using Cremophor RH 40, which demonstrated a similar emulsification time of under one minute. Their findings further emphasize that emulsification time is predominantly influenced by the formulation's composition and the relative proportions of oil, surfactant, and co-surfactant. The results indicate that increasing surfactant concentration enhances the spontaneity of the emulsification process, thereby reducing emulsification time [8].

The transmittance test is conducted to evaluate the optical clarity of the SNEDDS formulation following a 1:100 dilution with distilled water. The percent transmittance for the usnic acid SNEDDS, as reported in **Table 5**, was 98.408%, indicating a uniform dispersion resulting in a clear solution. However, a high transmittance value alone does not definitively confirm the formation of a nanoemulsion. Therefore, assessment of globule size is essential to verify that the usnic acid SNEDDS formulation qualifies as a nanoemulsion.

The robustness to dilution test was conducted to evaluate the stability of the SNEDDS formulation upon dispersion in different media: distilled water, phosphate buffer at pH 5.5 (mimicking the skin's surface pH), and phosphate buffer at pH 7.4 (simulating the pH prior to epidermal penetration). Following dilution, the usnic acid SNEDDS formulation yielded a clear and transparent nanoemulsion with no evidence of phase separation after 24 hours of storage at room temperature, as summarized in **Table 6**. These findings demonstrate that the formulation maintains stability against both dilution and pH variation, underscoring its suitability for topical administration.

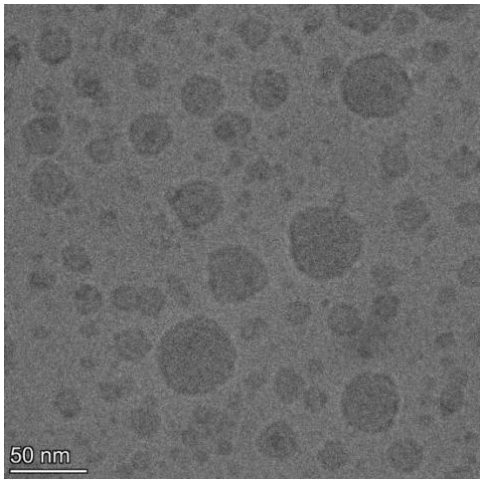


**Figure 4**. TEM Image of Vusnic acid SNEDDS

**Table 7**. The organoleptic and homogeneity analysis of the hydrogel formulation

| Formulation | Organoleptic | Homogeneity |
|---|---|---|
| F1 | Semi-solid, pale yellow color, and odorless | Homogeneous |
| F2 | Semi-solid, deep yellow color, and characteristic aroma of grapeseed oil | Homogeneous |

The morphological characteristics of the nanoemulsion were examined using Transmission Electron Microscopy (TEM). TEM analysis of the usnic acid SNEDDS, as depicted in **Figure 4**, demonstrates a uniform dispersion of discrete globules without any signs of aggregation following dilution in distilled water. The nanodroplets are confirmed to be spherical, and monodisperse, with a size distribution predominantly below 200 nm, which conforms to the stringent physicochemical criteria defining nanoemulsions [8].

The usnic acid content within the SNEDDS formulation was quantitatively analyzed using a UV-Vis Spectrophotometer. The results demonstrated a drug loading efficiency of 97.422% ± 1.678%, indicating a uniform distribution of the active compound throughout the formulation. These findings align with those reported by Nasr et al. (2016), who observed drug loading efficiencies for an olmesartan medoxomil SNEDDS ranging from 92.37% ± 0.75% to 99.09% ± 0.56% [8].

The optimized usnic acid SNEDDS formulation was subsequently incorporated into a hydrogel matrix utilizing Carbopol Ultrez 30 as the gelling agent. As presented in **Table 7**, both the usnic acid hydrogel and the usnic acid SNEDDS hydrogel exhibited a characteristic semi-solid, gel-like consistency. odor attributable to the grapeseed oil component. Both the usnic acid hydrogel and the usnic acid SNEDDS hydrogel demonstrated homogeneity, with no observable rough particles. This finding confirms the uniform dispersion of usnic acid and its SNEDDS formulation within the hydrogel matrix.

For optimal skin compatibility, topical hydrogel formulations should maintain a pH between 4.5 and 6.5. As presented in **Table 8**, the usnic acid hydrogel and the usnic acid SNEDDS hydrogel exhibited pH values of 5.573 ± 0.006 and 5.577 ± 0.012, respectively. These measurements fall within the skin's physiological pH range, suggesting that the formulations are likely to be well tolerated and have minimal potential to cause cutaneous irritation upon topical application [24].

The spreadability test is a critical parameter for evaluating topical drug delivery systems, reflecting the ease with which a formulation can be applied to the skin surface. According to the data presented in **Table 9**, the usnic acid SNEDDS hydrogel exhibited higher spreadability compared to the usnic acid hydrogel. This improvement can be attributed to the incorporation of Cremophor RH 40 and PEG 400, which act synergistically to reduce the viscosity of Carbopol Ultrez 30, thereby facilitating easier dispersion. These findings corroborate the observations of Al Haushey (2024), who demonstrated that adding solvents such as PEG 400, glycerin, and propylene glycol effectively reduce the viscosity of Carbopol-based gels, thereby enhancing their spreadability [25].

The interaction between usnic acid SNEDDS and the hydrogel matrix may affect emulsification behavior

**Table 8**. The pH of hydrogel formula

| Formulation | pH |
|---|---|
| F1 | 5.573 ± 0.006 |
| F2 | 5.577 ± 0.012 |

n = 3

**Table 9**. Spreadability test of the hydrogel formula

| Formulation | Spreadability (cm) | | | |
|---|---|---|---|---|
| | 1 gram | 3 gram | 5 gram | 7 gram |
| F1 | 3.2 | 3.4 | 3.5 | 3.9 |
| F2 | 3.2 | 3.5 | 3.7 | 4.0 |

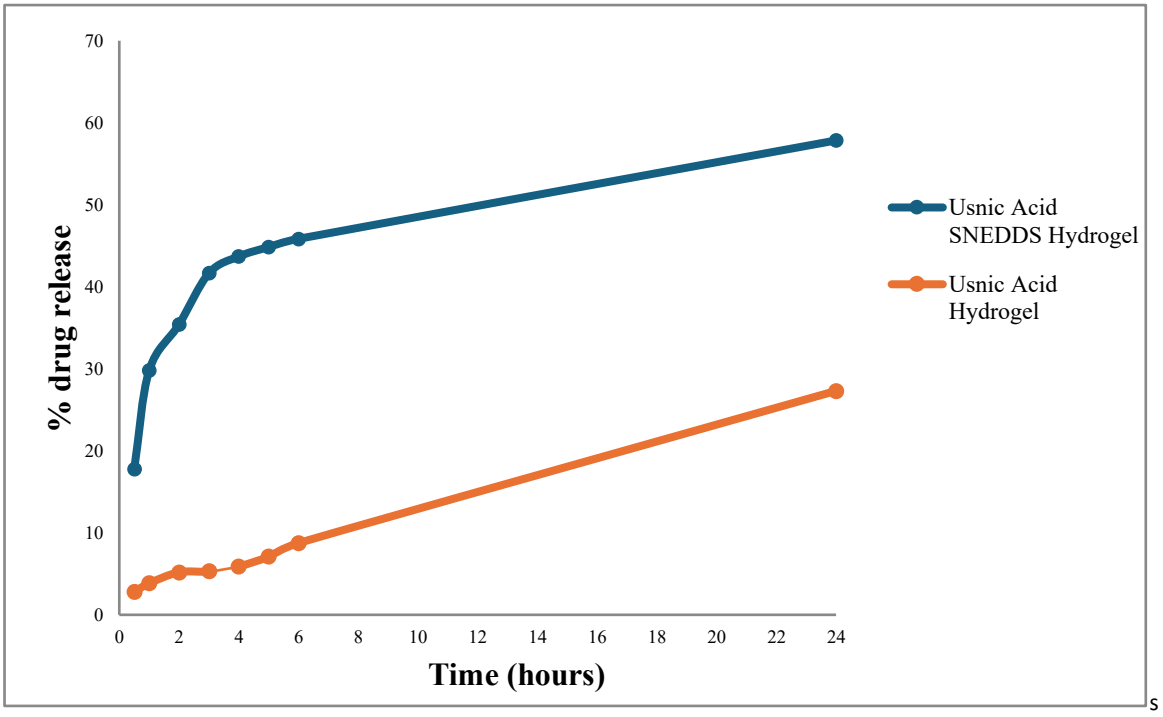


**Figure 5**. Drug release percentages of usnic acid SNEDDS hydrogel and usnic acid hydrogel in pH 7.4 phosphate buffer

**Table 10**. The globule size, PDI, and zeta potential of usnic acid SNEDDS hydrogel

| Ukuran Globu (nm) | PDI | Zeta Potensial (mV) |
|---|---|---|
| 72.5 | 0.445 | -55.3 |

and globule size distribution [5]. As shown in **Table 10**, incorporation into the hydrogel increased the mean globule size from 55.456 to 72.5 nm, while maintaining the nanometric range. This increase may result from polymer–droplet interactions or adsorption, promoting partial aggregation or coalescence 55.456 to 72.5 nm, while maintaining the nanometric range. This increase may result from polymer–droplet interactions or adsorption, promoting partial aggregation or coalescence [26]. Despite this size increase, the formulation exhibited favorable colloidal stability, as evidenced by a PDI of 0.567 and a strongly negative zeta potential of -55.871 mV, indicating a relatively low propensity for aggregation or flocculation.

The release profiles showed that the usnic acid SNEDDS hydrogel significantly enhanced drug release, achieving a cumulative release of 57.841% ± 7.166% after 24 h, compared with 27.305% ± 2.456% for the plain usnic acid hydrogel. This improvement is attributed to nanoscale droplets and the presence of surfactants and co-surfactant, which enhance drug solubilization, dispersion, and diffusion across biological membranes compared with the non-nanoformulated hydrogel [27]. Further studies should investigate the release mechanism using kinetic models and determine whether the enhanced in vitro release translates into improved ex vivo skin permeation and drug deposition. Subsenquent investigations of skin retention and in vivo efficacy and safety further establish the potential of SNEDDS hydrogel as a topical delivery system.

The SNEDDS hydrogel exhibited an initial burst release, with >30% of usnic acid released within 2–3 h, likely driven by the high concentration gradient between the donor and receptor phases [28]. Thereafter, the release rate gradually decreased as the concentration gradient diminished, with diffusion from the hydrogel matrix becoming the predominant mechanism and resulting in a sustained release profile.

In contrast, the plain usnic acid hydrogel exhibited a slower, near-linear release, with <30% of the drug released after 24 h. This limited release is likely due to the poor aqueous solubility of usnic acid, which may

promote aggregation or crystallization within the hydrogel matrix, thereby restricting drug diffusion [29].

Statistical analysis confirmed a significant difference in cumulative drug release at 24 h between the two formulations ($p < 0.05$).

## CONCLUSION

Usnic acid can be incorporated into an SNEDDS formulation using grapeseed oil as the oil phase, Cremophor RH 40 as the surfactant, and PEG 400 as the cosurfactant. Drug release study indicates that the SNEDDS hydrogel releases usnic acid at twice the amount compared to the usnic acid hydrogel over 24 hours. Consenquently, the favorable physicochemical characteristics and enhances release profile suggest that the SNEDDS formulation show promising potential for topical delivery of usnic acid.

## CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

## ACKNOWLEDGEMENT

The authors gratefully acknowledge Universitas Andalas for funding this research under the Undergraduate Thesis Research Scheme (Batch I), Fiscal Year 2025, in accordance with Research Contract No. 209/UN16.19/PT.01.03/PSS/2025.

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