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Isotretinoin self-nano-emulsifying drug delivery system: Preparation, optimization and antibacterial evaluation

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ABSTRACT

Purpose: Isotretinoin (ITN) is a poorly water-soluble drug. The objective of this study was to design a successful liquid self-nanoemulsifying drug delivery system (L-SNEDDS) for ITN to improve its solubility, dissolution rate, and antibacterial activity.

Methods: According to solubility and emulsification studies, castor oil, Cremophor EL, and Transcutol HP were selected as system excipients. A pseudo ternary phase diagram was constructed to reveal the self-emulsification area. The developed SNEDDS were visually assessed, and the droplet size was measured. In vitro release studies and stability studies were conducted. The antimicrobial effectiveness against multiple bacterial strains, including Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), and different accessory gene regulator (Agr) variants were investigated for the optimum ITN-loaded SNEDDS formulation.

Results: Characterization studies showed emulsion homogeneity and stability (%T 95.40–99.20, A graded) with low droplet sizes (31.87 ± 1.23 nm–115.47 ± 0.36 nm). It was found that the developed ITN-SNEDDS provided significantly a higher release rate (>96 % in 1 h) as compared to the raw drug (<10 % in 1 h). The in vitro antimicrobial activities of pure ITN and ITN-loaded SNEDDS demonstrated a remarkable inhibitory effect on bacterial growth with statistically significant findings ($p < 0.0001$) for all tested strains when treated with ITN-SNEDDS as compared to the raw drug.

Conclusion: These outcomes suggested that SNEDDS could be a potential approach for improving solubility, dissolution rates, and antibacterial activity of ITN.

1. Introduction

Acne is a widespread cutaneous disorder with a prevalence of 70–85 % for adolescents (Kanlayavattanakul and Lourith, 2011). It leads to sebum production, keratinization changes, and inflammation under hair follicles by *Propionibacterium acnes*. Additionally, *Staphylococcus aureus* also contributes to opportunistic infections causing acne or atopic dermatitis (Agrawal and Khunger, 2020). Consequently, acne chronically persists and might cause depressing physical and mental implications, such as, permanent scarring lesions, social depression, anxiety and suicidal tendencies (Gupta, 1998).

The most commonly used drugs for acne are benzoyl peroxide, topical antibiotics (clindamycin and erythromycin), and topical retinoids (tretinoin, isotretinoin, retinaldehyde, and retinol β-glucuronide) (Rathi, 2011). Isotretinoin (13-*cis*-retinoic acid) is considered the drug of choice for treatment of severe inflammatory acne conditions and nodulocystic acne, being the exclusive drug available against all pathogenic vital factors of acne (Rathi, 2011; Khalil et al., 2020).

Mostly, isotretinoin (ITN) is administered orally; however, it has some limitations. Orally administered drugs may suffer from poor absorption across the gastrointestinal (GI) lumen, and might be rapidly inactivated by the liver first-pass effect causing the drug to be rapidly cleared from the body. Furthermore, it is unacceptable for some patients who cannot swallow conventional tablets or capsules. However, isotretinoin has poor water solubility and suffers from poor stability by rapid oxidation upon heat or exposure to light (Ramli, 2009). To overcome the disadvantages of these measures, topical lipid-based formulations with biocompatible nontoxic components offer an alternative drug delivery technique and attract innovative research.

Nanoemulsions have also been used for the decontamination of

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radionuclide and photodynamic therapies. Many therapeutic agents, such as, nonsteroidal anti-inflammatory, antioxidants, and lipid drugs were successfully formulated using nanoemulsions as novel nanocarriers for topical applications. Thereby, nanoemulsions have made gains in the dermatology field (Izham et al., 2019). In recent years, SNEDDS (self-nanoemulsifying drug delivery systems) have gained high priority and become more prominent in pharmaceutical industries. The systems have proven to be successful nanocarriers in improving the bioavailability of poorly soluble drugs, especially topical drugs. SNEDDS are isotropic mixtures that comprise a drug, oil, a surfactant and a cosurfactant (Nasr et al., 2016). SNEDDS advantageously present the drug in a dissolved form, and their relatively small droplet size provides a large interfacial area, and this enhances drug absorption and bioavailability by improving drug release and membrane permeation and reducing efflux pumping and presystemic metabolism (Kang et al., 2004). However, several drawbacks related to SNEDDS topical application may arise, such as the potential alteration of cutaneous flora, decreased penetration in the infected area, and the potential of production of allergic contact dermatitis (Bernardi et al., 2011; Chavda, 2013).

The current study was developed to create an optimized SNEDDS formulation of isotretinoin (ITN) using pharmaceutically acceptable, non-toxic, and safe substances to improve its poor bioavailability and antimicrobial activity. This will be done through testing the effect of ITN- loaded SNEDDS against multiple bacterial strains, including *Staphylococcus aureus* (*S.aureus*), methicillin-resistant *Staphylococcus aureus (MRSA),* and different accessory gene regulator variants (Agr).

2. Material and methods

2.1. Materials

Isotretinoin (ITN) (purity > 99.5 %) was obtained by Riyadh Pharma manufacturer (Riyadh, Saudi Arabia). Capryol TM 90 and Transcutol HP (highly purified diethylene glycol monoethyl ether) were purchased from Gattefossé (Lyon, France), and Capmul MCM (propylene glycol monocaprylate type II) was obtained from Abitec (Ohio, USA). Tween® 80, Tween® 20, castor oil and turpentine oil were obtained from Sigma–Aldrich (St. Louis, MO, USA). Propylene glycol (propane-1, 2-diol) was obtained from Winlab (Gemini-house, England). Cremophor EL was supplied from BASF (Ludwigshafen, Germany).

2.1.1. Quantitative determination of isotretinoin (ITN)

UV spectrophotometry was used as a method to analyze isotretinoin (ITN) in different components, as it exhibits simplicity, reproducibility, and provides results within acceptable limits (Ahmad, 2021). The measurements were carried out using the UV–visible Spectrophotometer (Genesys 5, USA). The wavelength for maximum absorbance (λmax) for ITN standard solution in methanol was scanned in 200–400 nm UV regions and standard calibration curves were constructed for ITN in methanol and phosphate buffer (0.5 M, pH 7.8) as shown in Fig. 1 (Tsai, 2013).

2.1.2. ITN equilibrium solubility in oils and surfactants

The solubility of ITN was assessed in different oils, namely Capryol 90, castor oil, and turpentine oil, as well as in surfactants such as Tween 80 and Cremophor EL, and cosurfactants, including Transcutol HP and propylene glycol (PG). A predetermined quantity of ITN (100 mg) was introduced into 1 mL of various oils, surfactants, and cosurfactants. The mixture was then subjected to vortexing and transferred to a shaker water bath (specifically, a Karl Kolb type D-6.72, located in Bonn, Germany). The shaker water bath was set to operate at a speed of 100 revolutions per minute and maintained at a temperature of 370.5°C for a duration of 72 h, allowing the system to reach equilibrium. Subsequently, the equilibrated samples underwent centrifugation at a speed of 6000 revolutions per minute (rpm) for ten minutes. The liquid portion was subjected to filtration using a syringe filter with a pore size of 0.45 μm. Subsequently, the filtered solution was properly mixed with methanol and the absorbance of ultraviolet (UV) light was measured at a wavelength of 344 nm. This procedure was performed in triplicate according to the shake flask method described by Higuchi and Connors (Higuchi, 1965). The results are illustrated in Table 1.

2.1.3. SNEDDS component selection

The oil and cosurfactant excipients for isotretinoin SNEDDS formulation were selected based on maximum solubility values, and the surfactant material was chosen after conducting the emulsification study. The oil and cosurfactant with highest solubility values were used to conduct the emulsification study, in which the best surfactant among two tested surfactants was chosen according to its ease of emulsification and transparency. Briefly, 300 mg of each surfactant (Tween 80 and Cremophor EL) was mixed with 300 mg of the selected oily phase (castor oil). The mixtures were heated at 50 °C and vortexed for homogenization. 50 mg from each mixture was diluted with 50 ml of distilled water, and the number of flask inversions to yield good homogenous emulsion

Table 1
The equilibrium solubility values of Isotretinoin (ITN) in various oils, surfactants, and cosurfactants at 37 °C are reported in (mg/mL) with standard deviation (SD) indicated.

Excipient Type	Component	Solubility (mg/mL) ± SD
Oil	Capryol 90	17.47 ± 0.58
	Castor oil	30.84 ± 0.12
	Capmul MCM	15.03 ± 0.17
	Turpentine oil	19.24 ± 0.15
Surfactant	Cremophor EL	31.51 ± 0.22
	Tween 80	23.41 ± 0.83
	Tween 20	18.64 ± 0.15
Cosurfactant	Transcutol HP	41.30 ± 0.12
	Propylene glycol	3.37 ± 0.11

*Mean values (n = 3) ± SD.

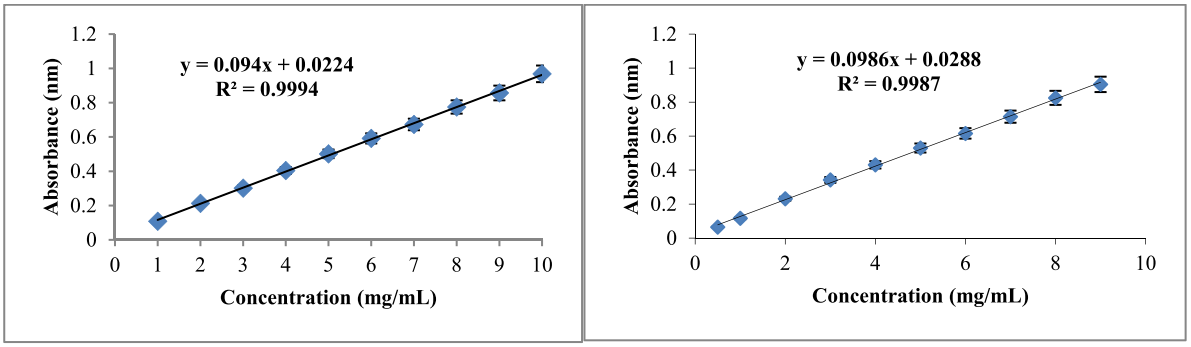

Fig. 1. (a) The linear calibration curve for ITN in methanol. (b) The linear calibration curve of ITN in phosphate buffer (0.2 M, pH 7.8).

was calculated. The percentage of transmittance (% T) of the prepared mixtures was determined using UV spectrophotometer at 638 nm using distilled water as a blank. Correspondingly, each surfactant alone, then mixtures of the selected cosurfactant (100 mg), surfactant (200 mg) and oil (300 mg) were examined in the same manner. The results are illustrated in Table 2.

2.1.4. Pseudo-ternary phase diagram

Selected components were utilized to construct the ternary phase diagram, the oily phase (castor oil), the surfactant phase (Cremophor EL) and the cosurfactant phase (Transcutol). These were mixed at 36 ratios according to the method described by Teaimah et al. and Teail (2022). One gm of each preparation was diluted 200 times with distilled water and stirred at 100 rpm at 37 ± 0.5 °C. Visual observation was carried out to evaluate the spontaneous nanoemulsion formation. Next, the percentage of transmittance (% T) was measured to confirm clarity, and the emulsions were graded according to their transparency percentage as shown in Table 3 (Zhang, 2008). A- and B-graded emulsions were only considered to draw the phase diagram that were identified as clear and translucent emulsions respectively. Fig. 1 shows the phase diagrams drawn using CHEMIX ternary plot software (CHEMIX School Ver. 3.60, Pub. Arne Standnes, Bergen, Norway).

2.1.5. Preparation of ITN-SNEDDS

The SNEDDS formulations were prepared by blending different proportions of oil, surfactant and cosurfactant that were selected from the self-emulsifying region of the ternary phase diagram. Four batches were formulated as shown in Table 4. Initially, 10 mg ITN was added to an accurately weighed amount of oil and cosurfactant in a screw-capped glass vial. The mixture was vortexed until homogeneity was obtained, and was then heated at 50 °C until it became transparent. Finally, the weighed amount of the surfactant was further added to the preconcentrate with vortex mixing to produce the final SNEDDS mixture. The formulations were stored at room temperature for further use.

2.2. Physicochemical evaluation of ITN-loaded SNEDDS

The developed ITN-loaded SNEDDS (F1-F4) were evaluated for various physicochemical parameters, such as percentage of transmittance (% T), droplet size (PS), polydispersity index (PDI), percentage of content, in vitro release and thermodynamic stability.

2.2.1. Visual assessment method

Visual assessment was the main guideline used to assess the self-emulsification efficiency of the formulations. For each SNEDDS formulation, 1 ml was diluted with 200 ml distilled water was stirred constantly at 100 rpm at 37 ± 0. 5 °C. The formulations were visually evaluated for their miscibility, clarity, and appearance. The percentage of transmittance was measured spectrophotometrically at 638 nm using deionized water as a blank. The time required for emulsion droplets to disappear and produce a fine emulsion was recorded.

Table 2
Emulsification efficiency among selected SNEDDS components.

Oil/Surfactants/Cosurfactant blends	No. of flask inversions	Transparency (%)
Tween 80	17	95.5 %
Cremophor EL	14	99.1 %
Castor oil / Tween 80	18	70.6 %
Castor oil / Cremophor EL	11	88.6 %
Castor oil / Tween 80/ Transcutol HP	7	80.1 %
Castor oil / Cremophor EL / Transcutol HP	4	95.2 %

Table 3
Visual grading of emulsions according to appearance and transparency (T %).

Appearance	Transparency %	Emulsion Grade
Clear bluish Translucent bluish	> 95 80–90	A B
White turbid	36–80	C
No emulsification	0–35	D

Table 4
The composition of selected Isotretinoin self-nano-emulsifying formulations (ITN-SNEDDS).

Formulation	Castor Oil (wt/wt %)	Cremophor EL (wt/wt %)	Transcutol HP (wt/wt %)
F1	10	20	70
F2	20	40	40
F3	30	60	10
F4	20	60	20

2.2.2. Droplet size (PS) and polydispersity index (PDI)

The droplet size is a crucial factor to assess emulsions performance in terms of physical stability as well as rate and extent of drug release. The polydispersity index (PDI) is another essential factor for indicating the uniformity of size distribution. Droplet size and polydispersity index (PDI) of the selected SNEDDS formulations were measured by laser light diffraction analysis technique using Malvern Zetasizer (Model ZEN3600, Malvern, United Kingdom). The formulations were diluted with distilled water at a ratio of 1:1000 v/v with agitation for one minute. Next, samples were transferred into cuvettes and ten readings were recorded for each sample. Each experiment was done in triplicate.

2.2.3. Percentage of content

The drug content of ITN-loaded SNEDDS formulations, each containing 10 mg of medication, was evaluated. In summary, a quantity of 0.1 g of each sample was diluted with 25 ml of methanol, subjected to vortex, and thereafter examined using a UV spectrophotometer at a wavelength of 344 nm. Methanol was employed as a reference solution blank for comparison. The amount of the drug was calculated using the standard calibration curves previously prepared for the drug in methanol.

2.2.4. In vitro release studies

The in vitro release of ITN from the selected SNEDDS formulations was tested according to the FDA specifications using the United States Pharmacopeia (USP) dissolution apparatus type II (model: ERWEKA dissolution rate testing apparatus DT 600, Heusenstamm, Germany). The paddle stirrer speed was adjusted at 75 rpm, and the dissolution was performed for one gram formula equivalent to 10 mg ITN. The test was carried out in 900 ml of 0.05 M phosphate buffer (pH = 7.8) for 1 h at 737 ± 0. 5 °C. Samples were withdrawn at a predetermined time intervals of 5, 10, 15, 30, 45 and 60 min, filtered using a 0.45 μm syringe filter, replaced with a fresh buffer and analyzed by UV at 344 nm. Each experiment was carried out in triplicate.

2.2.5. Thermodynamic stability

Kinetic stability of prepared nanosized emulsions was investigated by subjecting formulations to a three-phase stress condition: heating–cooling, centrifugation and freeze thaw cycles. The initial test phase consisted of three heating–cooling cycles conducted within a temperature range of 4 °C (refrigerator) to 50 °C (oven), with a duration of 48 h at each temperature for storage. The formulations that had successfully undergone the initial testing phase were subsequently exposed to the phase 2 evaluation. During this stage, the formulations were put to centrifugation at a speed of 3500 revolutions per minute (rpm) and a temperature of 25 °C for a duration of 30 min. Formulations

that did not exhibit phase separation were subjected to a phase 3 evaluation, which involved subjecting the formulations to three freeze/thaw cycles, with temperatures ranging from −21C° (freeze) to + 25 °C (thaw), for a minimum duration of 48 h.

2.2.6. In vitro antimicrobial activity of pure ITN and ITN-loaded SNEDDS bacterial isolates

For this study five different types of Staphylococcus aureus strains (Staphylococcus aureus, methicillin resistant Staphylococcus aureus, NE1532, NE95 & NE873) were used as shown in Table 5. Staphylococcus aureus bacteria was used as the control organism.

2.3. Culture and media conditions

Strains of wild-type *Staphylococcus aureus*, *MRSA* (methicillin-resistant Staphylococcus aureus), and different QS mutants (AgrA, AgrB and AgrC) were cultured in tryptic soy broth (TSB) and incubated at 37 °C for 18–24 hr. The solutions were adjusted to 0.5 McFarland standard and left overnight with continuous shaking at 200 rpm.

2.3.1. Determination of the antibacterial inhibition zone

The in vitro antimicrobial activity of the optimized SNEDDS formulation (F2) was evaluated through inhibition zone observation (Balouiri et al., 2016). The agar plate surface was inoculated through spreading a volume of the bacterial suspension over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer, and a volume of 100 µL of the prepared isotretinoin-loaded SNEDDS compared with pure isotretinoin solution at different concentrations (2.5, 5, 10 mg/ml) was introduced into the well. Agar plates were incubated at 37 °C for 18 h depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

2.3.2. Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MICs) of pure ITN and ITN-loaded SNEDDS were determined by the broth dilution method using bio screen C system (Growth Curves USA, Piscataway, NJ, USA). This method involved the measurement of the turbidity of a solution, which reflected the bacterial growth in different concentrations of the added antimicrobial agent (Islam et al., 2008).

3. Results

3.1. UV determination of ITN in methanol and phosphate buffer (0.5 M, pH 7.8)

The maximum wavelength (λmax) was detected at 344 nm for ITN and this wavelength was used for quantitative analysis of ITN in SNEDDS. For Calibration curve construction 0.1 mg/ml stock solution of ITN was subjected to serial dilutions in both methanol and the buffer medium. The calibration curves showed linearity and the regression correlation coefficient (r^2) was found within the limit (Fig. 2).

Table 5
Description of Staphylococcus aureus strains in this study.

Strain Name	Abbreviation	Description
Staphylococcus aureus	S. aureus	Wild-type
Methicillin-resistant Staphylococcus aureus	MRSA	Methicillin-resistant Staphylococcus aureus
NE1532	AgrA	4 P16 AgrA accessory gene regulator protein A SAUSA300_1992
NE95	AgrB	1 O21 AgrB accessory gene regulator protein B SAUSA300_1989
NE873	AgrC	3 B17 AgrC accessory gene regulator protein C SAUSA300_1991

3.2. Solubility study

As shown in Table 1, the results suggested that among tested oils ITN solubility was higher in castor oil (30.84 ± 0.12 mg/ml as compared to the Capryol 90, Capmul MCM and turpentine oil with solubility values of 17.47 ± 0.58 mg/ml, 15.03 ± 0.17 mg/ml and19.24 ± 0.15 mg/ml, respectively. However, as a surfactant, Cremophor EL (31.51 ± 0.22 mg/ml) showed better solubilizing potential for ITN than Tween 80 (23.41 ± 0.83 mg/ml) and Tween 20 (18.64 ± 0.15 mg/ml). While, Transcutol HP (41.30 0.12 mg/ml) showed higher ITN solubility as a cosurfactant when compared to propylene glycol (PG) (3.37 ± 0.11 mg/ml).

3.3. Selection of the surfactant

In this study, the two surfactants (Cremophor EL and Tween 80) were assessed for their emulsification ability. The results showed that Cremophor EL alone exhibited higher emulsification efficiency (Transparency 99.1 %, 14 flask inversions) when compared to Tween 80 (Transparency 95.5 %, 17 flask inversions). Upon addition of the oily phase, Cremophor EL also showed better emulsification (Transparency 80.6 %, 11 flask inversions) as compared to Tween 80 (Transparency 70.6 %, 18 flask inversions). Addition of Transcutol HP as a cosurfactant improved the dispersibility of the formulation. However, the Transparency was 95.2 % under 3 flask inversions with Cremophor EL, while it was 80.1 % under 4 flask inversions when Tween 80 was utilized as a surfactant. The results suggested the selection of Cremophor EL as a surfactant. Based on solubility and emulsification results, the SNEDDS formulations were developed using castor oil, Cremophor EL, and Transcutol HP as an oil, surfactant and cosurfactant respectively.

3.4. The ternary phase diagram

Fig. 2 shows the pseudo ternary phase diagram constructed to choose the proper proportions of oil/surfactant/cosurfactant for ITN SNEDDS formulations. The shaded area representing clear (A) and translucent (B) emulsions were found to be stable after 48 h of storage. It was noted that the resultant emulsions with 10–30 % oil and 20–60 % surfactant were found to be clear and were considered for formula selection.

3.5. Physicochemical evaluation of ITN-Loaded SNEDDS

3.5.1. Visual assessment and self-emulsification

Visual assessment of the four SNEDDS formulations confirmed emulsion homogeneity and miscibility (Table 6); the four emulsions (F1-F4) provided fine bluish/transparent (A-graded) systems with no visible particulates indicating the ability of the utilized surfactant to promote self-emulsification at oil contents of 10 %-30 % (w/w). Clarity was confirmed by the measured percentage of transmittance that was near 100 %. Emulsification time ranged from 10 − 40 sec for the tested formulations. It was observed that rapid emulsification was contributed to formulations with low oil content of 10–20 % w/w and high cosurfactant content 50–70 % w/w that produce mixtures of low viscosities (Teail, 2022).

Emulsification time, percentage of transmittance (% T), Droplet Size (PS), Polydispersity Index (PDI), Dispersibility Grade, Percentage Content of ITN-SNEDDS

3.5.2. Droplet size (PS) and polydispersity index (PDI) measurement

The mean droplet size of the diluted SNEDDS formulations was found to be in the nano size range (<200 nm). The formulation with the lowest oil and highest cosurfactant content produced droplets of the lowest size (F1, 31.87 ± 23) upon dilution with water (Table 5). However, droplet sizes increased upon increasing the oil concentration. The formulation F3 contained 30 % w/w oil and was found to have a mean droplet size of 115.47 ± 0.36 nm. While, formulations F2 and F4 had droplets with

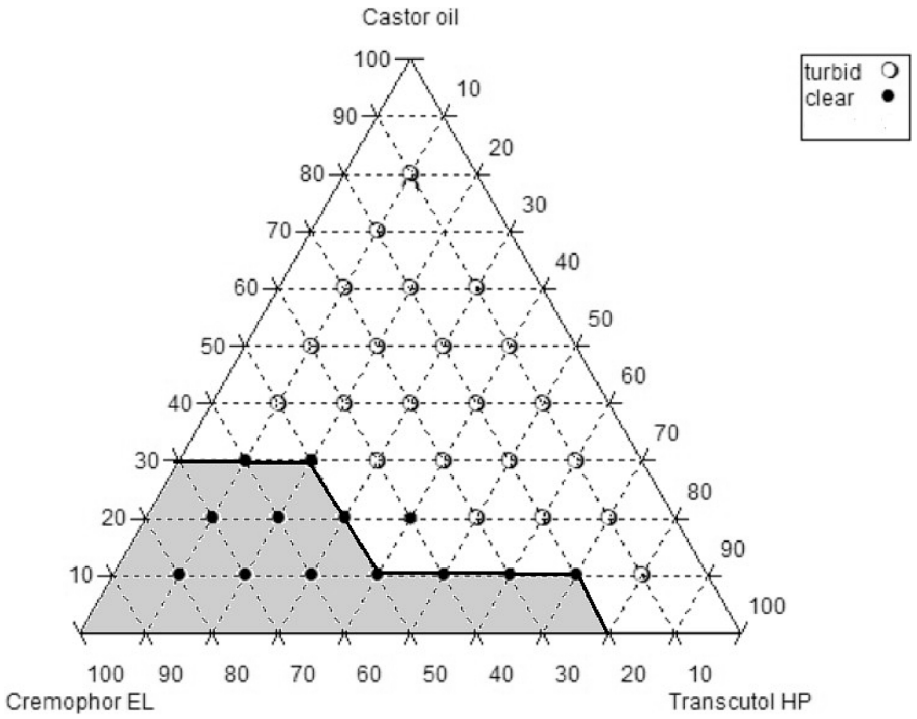

Fig. 2. Pseudo-ternary phase diagram of ITN SNEDDS (castor oil/Cremophor EL/ Transcutol HP). The shaded area represents the region of clear and translucent emulsions.

Table 6

Formulation No.	Emulsification Time (sec ± SD)	Particle Size (nm ± SD)	PDI & Emulsion Grade	Drug Content (%)
F1	10.64 ± 0.81	31.87 ± 1.23	0.19 A	98.51 ± 0.56
F2	16.40 ± 0.46	64.36 ± 0.23	0.22 A	99.38 ± 1.03
F3	42.38 ± 1.16	115.47 ± 0.36	0.21 A	93.67 ± 0.34
F4	38.99 ± 1.45	57.05 ± 0.25	0.18 A	101.27 ± 0.92

Abbreviations: % T, percentage of transmittance; PDI, Polydispersity index.

sizes of 64.36 ± 0.23 nm and 57.05 ± 0.25 nm, respectively. In addition, the four formulations showed low Polydispersity values of 0.22 and less indicating the good uniformity of size distribution (Madan et al., 2014).

3.5.3. *Percentage of drug content determination*

The drug content of the four tested SNEDDS formulations was calculated using the calibration curve of ITN prepared in methanol. Despite the difference in formulation compositions, the essays for drug content among the formulations (F1-F4) were found to be in the range of 98.51 % and 101.27 % (Table 5). This complies with the USP guidelines acceptance range (±15 %).

3.5.4. *In vitro release study*

Fig. 3 showed the in vitro release profiles of plain ITN and the tested

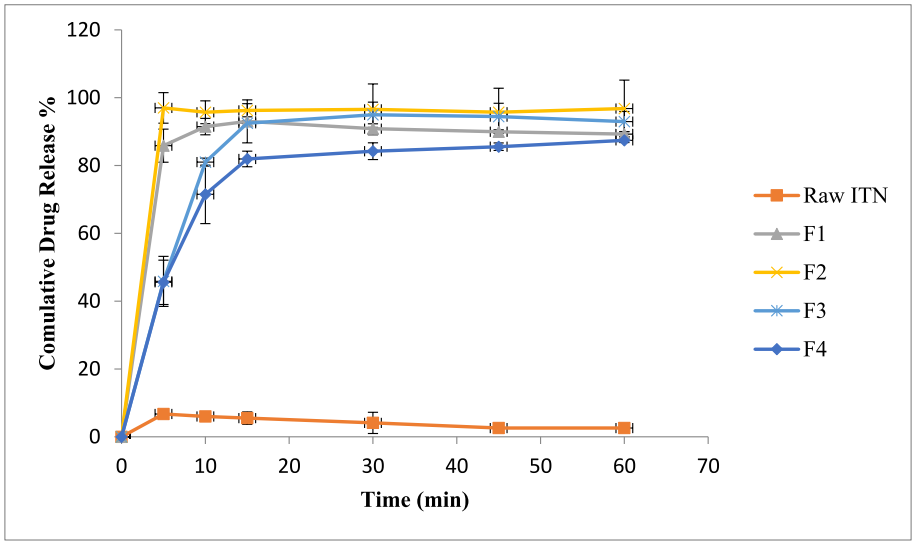

Fig. 3. In vitro release profiles of raw ITN and SNEDDS formulations (F1-F4). Abbreviations: ITN: Isotretinoin, F1-F4; self-nanoemulsifying formulations.

SNEDDS formulations (F1-F4) performed in the 0.2 M phosphate buffer (pH = 7.8). Since ITN is insoluble in water, it exhibited only 10 % release within ten minutes to observe occurrence of precipitation over time. The release of ITN from the four liquid SNEDDS formulations showed more than 50 % within ten minutes and reached more than 80 % after 30 min. Among the four SNEDDS formulations, F2 showed the best release behavior with the highest cumulative percentage of release of 96 % after five minutes. Accordingly, the dissolution profiles in all SNEDDS formulations ensured a significant increase in the release of ITN ($p < 0.05$). The overall dissolution study results showed that F2 formulation had a comparably higher ITN release as compared to the other representative formulations and the raw drug powder. Consequently, the F2 formulation was selected for further microbiological studies.

3.5.5. *Thermodynamic stability study*

The oil content in the formulation played an important role in its stability. It was noticed that formulations with 10–20 % of oil could pass the stability study (F1, F2 & F4), while, F3 that contained 30 % of oil failed to pass the study due to observed phase separation and drug precipitation.

3.5.6. *In vitro antimicrobial activity & determination of inhibition zone*

In vitro antimicrobial assessment involved several assays to determine the inhibition zone and the efficacy of ITN and ITN-loaded SNEDDS. The well diffusion assay results depicted in Fig. 4 showed superior diffusion and inhibition zone by the ITN-loaded SNEDDS formulation compared to pure ITN due to the lipophilic nature of the drug, highlighting the formulation's enhanced delivery capabilities.

The bacterial growth curves for SA, MRSA, and Agr mutants treated with varying ITN concentrations (2.5, 5, 10 mg/ml) are shown in Fig. 5. The ITN-loaded SNEDDS formulation demonstrated a more potent antimicrobial effect, with a higher inhibitory impact at a concentration of 5 mg/ml compared to pure ITN.

Fig. 5. Bacterial growth curves after the exposure of bacterial cultures SA, MRSA and Agr mutants to pure ITN and ITN-loaded SNEDDS (2.5, 5 & 10 mg/ml).

Furthermore, the MIC determination revealed that both ITN and SNEDDS possessed an MIC of 5 mg/ml for SA and MRSA, suggesting similar inhibitory effects on these strains. However, when examining the Agr variants (Agr A, B, and C), ITN exhibited an MIC of 5 mg/ml, whereas SNEDDS demonstrated a reduced MIC of 2.5 mg/ml, emphasizing the formulation's increased antimicrobial potency against Agr-regulated bacterial isolates.

Statistical analysis of optical density measurements highlighted significant differences between SNEDDS and ITN treatments across all bacterial strains, with p-values indicating statistical significance ($\alpha = 0.05$). These results suggest that the SNEDDS formulation enhances the antimicrobial activity of ITN, potentially improving therapeutic outcomes for bacterial infections (Table 7, 8).

4. Discussion

The substantial involvement of *Propionibacterium acnes (P. acnes)* in causing acne vulgaris is well documented. This bacterium plays a role in causing acne by initiating immunological responses, changing the sebum composition, and encouraging the growth of inflammatory mediators in the pilosebaceous unit. Any treatment plan for acne must address its severity by targeting the activity of *P. acnes* (McLaughlin et al., 2019). Isotretinoin is known for its diverse effects on severe acne and is also involved in decreasing the presence of *P. acnes* via changing the skin's microenvironment. However, the complete efficacy of isotretinoin is sometimes restricted by its inadequate solubility and bioavailability (Paichitrojjana and Paichitrojjana, 2023). Improving these pharmacokinetic features using a self-nanoemulsifying drug delivery system (SNEDDS) could potentially enhance the inhibitory effect on *P. acnes*, resulting in a more effective acne treatment regimen (Hosny et al., 2020). Our study has established a foundation by examining the increased solubility and dissolution of isotretinoin in SNEDDS.

Self-emulsifying drug delivery systems offer an immense potential for improving solubility and permeability through various mechanisms, such as, bypassing the first-pass effect, inhibiting efflux transporters and promoting drug absorption through the lymphatic system (Midha et al., 2015). Self-nanoemulsifying drug delivery systems (SNEDDS) are monophasic blends composed of a mixture of an oil, surfactant and a

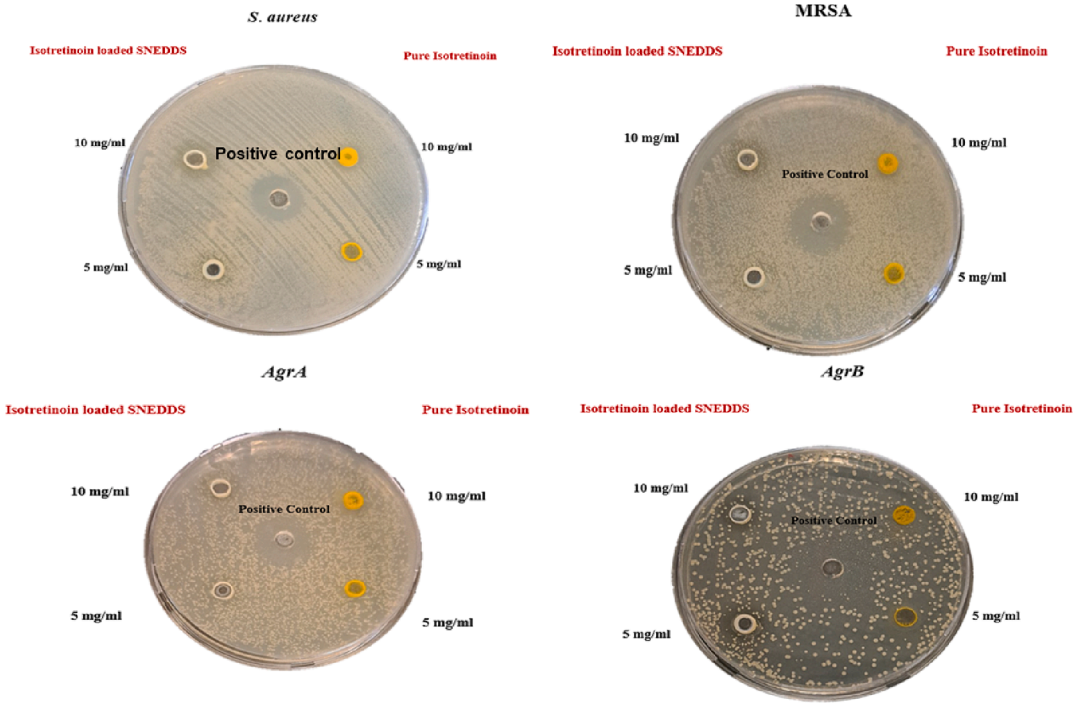

Fig. 4. In vitro determination of inhibition zone using the well diffusion method in ITN-SNEDDS (5 mg/ml, 10 mg/ml).

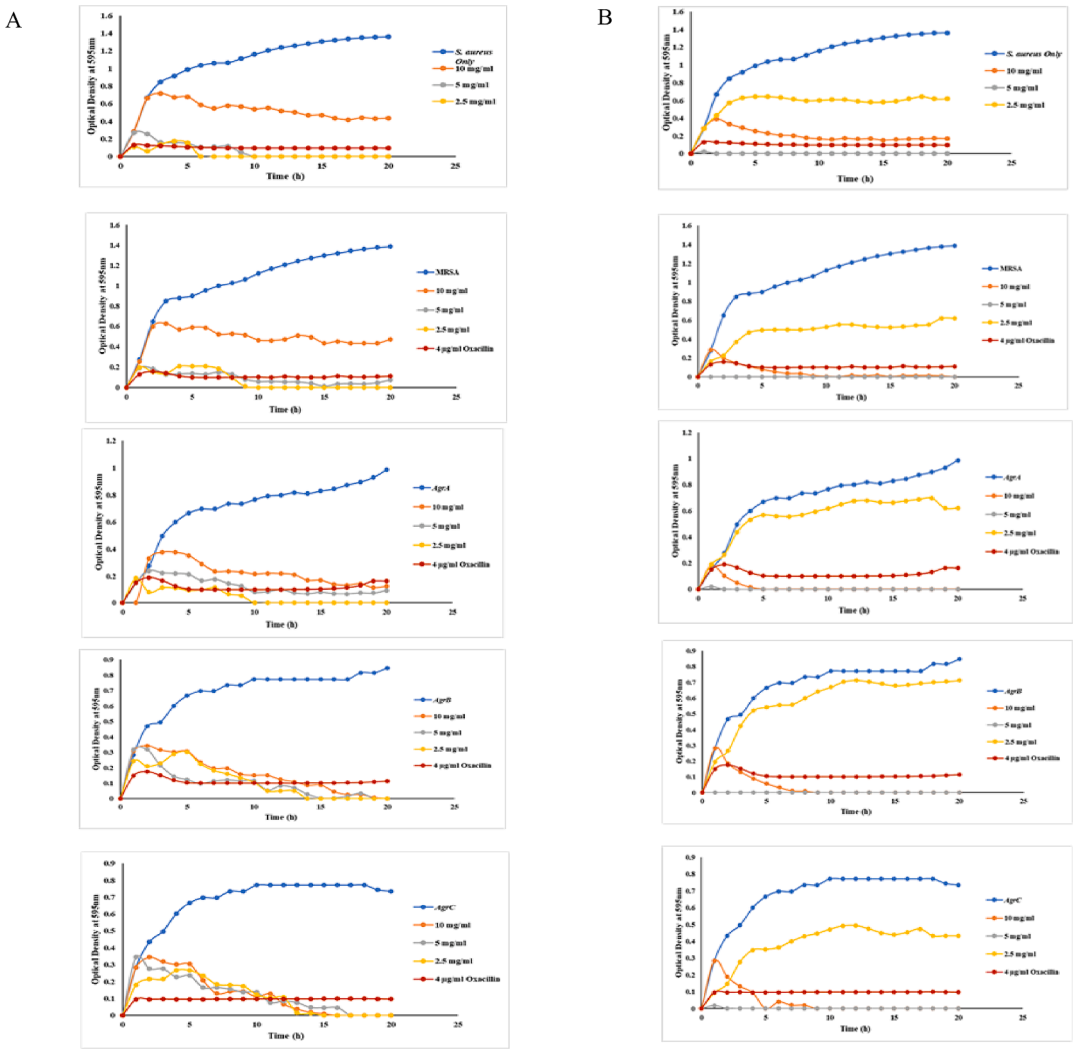

Fig. 5. Bacterial growth curves after the exposure of bacterial cultures SA, MRSA, and Agr mutants to pure ITN and ITN-loaded SNEDDS (2.5, 5 & 10 mg/ml).

Table 7
MIC (mg/ml) Values of Pure ITN and SNEDDS against Various Bacterial Isolates.

Bacterial isolates	Pure ITN	ITN-loaded SNEDDS
S. aureus	5 mg/ml	5 mg/ml
MRSA	5 mg/ml	5 mg/ml
Agr A	5 mg/ml	2.5 mg/ml
Agr B	5 mg/ml	2.5 mg/ml
Agr C	5 mg/ml	2.5 mg/ml

Table 8
Statistical Analysis of Bacterial Growth Inhibition by ITN and SNEDDs.

Bacterial strain	Mean	SD	Df	P-value
SA + ITN SA + SNEDDs	0.278 0.442	0.222 0.330	T = 4.136 DF = 38	P-value <0.0001
MRSA + ITN MRSA + SNEDDs	0.135 0.359	0.169 0.232	T = 7.501 DF= 38	P-value< 0.0001
Agr A + ITN Agr A+ SNEDDs	0.196 0.471	0.115 0.341	T = 4.267 DF- 38	P-value <0.0001
Agr B + ITN Agr B+ SNEDDs	0.225 0.625	0.152 0.164	T = 8.494 DF = 38	P-value < 0.0001
Agr C + ITN Agr C+ SNEDDs	0.162 0.312	0.099 0.174	T = 6.735 DF= 38	P-value < 0.0001

SD: Standard deviation, Df: Degree of freedom.

cosurfactant that emulsify spontaneously to provide the drug in a solubilized form (Date et al., 2010).The main mechanism by which lipid-based formulations improve the bioavailability of poorly water soluble drugs involves solubilization of drugs; thus, solubility is an important criterion to keep the drug in a solubilized form preventing precipitation to achieve a successful formulation with good stability and improve bioavailability (Kadian and Nanda, 2023). In this study, solubility results revealed that ITN was highly soluble in castor oil, Cremophor EL and Transcutol HP. It could be seen that higher solubilizing capacity of SNEDDS components along with the good miscibility reflected the clarity and stability of the resultant formulations.

Two nonionic surfactants were compared (Cremophor EL and Tween 80) to reveal that Cremophor EL exhibited higher emulsification efficiency with castor oil under gentle stirring (Transparency of 88.6 %, 11 flask inversions). To the contrary, Tween 80 showed poor emulsification properties with the engaged oil requiring higher flask inversions (Transparency of 70.6 %, 18 flask inversions). Predictably, the properties of a surfactant, such as hydrophile-lipophile balance (HLB), number and affinity for the oil phase can impact the emulsification process (Rahman and Mujahid, 2018). Better emulsification of Cremophor EL was primarily due to the difference in the nature of the hydrophobic tail group; Cremophor EL is not hydrogenated and has a lower degree of ethoxylation, while Tween 80 is derived from polyethoxylated Sorbitan and oleic acid (Zeng et al., 2017; Zeeb, 2014). The addition of Transcutol HP as a cosurfactant improved the dispersibility of the formulation and

showed good emulsification with both tested surfactants and a maximum transparency of 99.2 % with only 4 flask inversions. This might be attributed to the fact that short chain alcohols could appreciably impact the interfacial fluidity within emulsion droplets by decreasing its surface tension, thickness and viscosity (Jiajia and McClements, 2013). Based on the solubility and emulsification study results, ITN SNEDDS formulations were developed employing varying concentrations of castor oil (10–30 %), Cremophor EL (20–60 %) and Transcutol HP (20–70 %) as oil, surfactant and cosurfactant, respectively.

The constructed pseudo-ternary phase diagram revealed the self-emulsification area, in which it was noticed that the turbidity of the formulation increased upon utilizing higher concentrations of oil. This was explained by the increase of the interfacial tension area where the concentration of the surfactant might have been insufficient. The visual observation indicated formulation homogeneity through the high rate of emulsification (<1 min), the percentage of transmittance closer to 100 %, and the hazy transparent appearance. On the other hand, the droplet size is an important factor in self-emulsification characteristics, because it determines the rate and extent of drug release as well as absorption. Smaller droplet sizes provide larger interfacial surface area, and a faster and complete rate of drug absorption (Balata et al., 2016). Droplet size batches and PDI measurements revealed mono-dispersed emulsions in the nano size range. A direct correlation between the oil content and droplet size was noticed along with increasing surfactant/cosurfactant proportion.

The dissolution studies revealed a significant improvement in the cumulative percentage of ITN release from SNEDDS systems as compared to the plain drug powder. There was no marginal difference in drug release for the SNEDDS formulations. However, F2 had comparably the highest drug release (96 % after five minutes) as compared to the other representative formulations; this might be attributed to the component synergism achieved in the formulation, and the very short emulsification time (less than 20 sec). It was evident that optimum percentages of SNEDDS components caused a spontaneous emulsion formation of the oily drug solution in the form of nanodroplets that once contacted with the dissolution media induced a higher surface area and faster rate of drug release (Rashid and Ahmad, 2018).

These findings are similar to previous studies performed by Chavda et al. that tested isotretinoin release from a solidified SNEDDS formula with 102.83 % within 60 min and 100 % release of liquid SNEDDS within 20 min ensuring the improvement of ITN in vitro release profile (https://doi.org/10.1155/2013/108569]). SNEDDS must undergo in situ solubilization and form a thermodynamically stable emulsion with no signs of phase separation, cracking, or creaming. However, upon storage, the drug may precipitate from emulsions with high oil content (>30 %). Thus, stability of a formulation is essential to ensure the efficacy of the formulation and keep the drug in a solubilized form on long term storage. In regard to stability and release profiles, F2 SNEDDS formulation was considered the optimum formulation to be investigated in further anti-bacterial assays.

The results of the anti-bacterial assays highlight the strong influence of self-nanoemulsifying drug delivery systems (SNEDDS) on the proliferation of bacteria, as indicated by the notably higher differences in optical density as compared to the control group subjected to ITN only. The significant variations in optical density suggest that SNEDDS may have a wider and more powerful impact on inhibiting the growth of bacteria, irrespective of the particular bacterial strain. The consistent response observed among different strains indicates that SNEDDS has the potential to be a versatile and successful approach for addressing bacterial infections.

Inhibiting bacterial growth was significantly different between isotretinoin (ITN)-loaded SNEDDS and ITN. In agar diffusion tests against *Staphylococcus aureus* and methicillin-resistant strains, SNEDDS with ITN demonstrated dramatically higher antibacterial efficiency than ITN alone. In broth dilution trials, SNEDDS reduced bacterial growth more than ITN and had lower minimum inhibitory concentrations (MICs). The statistical analysis highlighted the observed differences, demonstrating that SNEDDS is more antimicrobial. ITN-loaded SNEDDS are more effective because nanoemulsion technology increases ITN solubility and bioavailability, which improves medication delivery to bacterial cells (Baloch et al., 2019). SNEDDS components may break bacterial membranes and increase medication penetration, furthering its antibacterial activity.

The results showed that the formulation inhibited *S. aureus* and *MRSA,* and significantly reduced Agr variants, which affect S. aureus virulence and antibiotic resistance. This suggests that the SNEDDS formulation may help treat certain bacterial infections, especially when traditional antibiotics fail due to resistance. (Zorzi et al., 2021).

Previous study on cephalexin (CEP)-loaded SNEDDS on improving poorly soluble medicines' antibacterial activity has significant similarities to our study. The formulation had a prolonged release profile and outperformed the raw drug dispersion against Gram-positive and Gram-negative microorganisms in the CEP-SNEDDS investigation. This consistency between the two investigations shows that SNEDDS can improve antibiotic administration and efficacy (Zafar et al., 2022).

Nano-formulations like SNEDDS, liposomes, polymeric micelles, nanogels, and metal nanoparticles can help solve the problem of antimicrobial resistance, which has arisen due to the overuse of antibiotics in medical, agricultural, and animal production. These nanostructures improve antimicrobial drug solubility, absorption, and efficacy, giving a promising solution to bacterial resistance (Zong et al., 2022).

Another study that supports our findings, is Hussain and colleagues published, the formation of SNEDDS for rifampicin (RIF) and testing its antibacterial effect. Solubility, stability at acidic pH, and bioavailability are difficulties with oral RIF treatment, according to the study, The use of nanoemulsions improveed drug solubility and bioavailability and therapeutic efficacy (Hussain et al., 2020).

Research using lipid-based nanoparticles like SNEDDS to prolong medication release is effective. When exposed to gastrointestinal fluids, a nanoemulsion forms, encapsulating medication molecules in the oily core or interface for slow release. SNEDDS components like Cremophor EL and Transcutol HP may also disrupt bacterial membranes, boosting the drug's antibacterial effects.These effects were demonstrated in formation of SNEDDS for ciprofloxacin (CIP), showing its potential to deliver controlled and sustained drug release, improving treatment efficacy and reducing dose frequency (Arshad et al., 2022).

CIP-loaded SNEDDS release CIP sustainably by choosing excipients and improving formulation. Upon hydration, polymers like amidated pluronic in SNEDDS form a gel-like structure that inhibits drug diffusion (Anwer et al., 2021).

This study's observations are consistent with the outcomes reported in previous investigations conducted within the realm of antimicrobial and medication delivery research (Anwer et al., 2021). Numerous studies have consistently documented comparable results in examining the antimicrobial efficacy of innovative medication delivery methods. One example of a study conducted by Zong et al. revealed that lipid-based nanocarriers, similar to SNEDDS, displayed enhanced antibacterial efficacy against several bacterial strains in comparison to conventional medication formulations (Zong et al., 2022). The observed consistent pattern indicates that lipid-based medication delivery systems feature intrinsic characteristics that augment their effectiveness in impeding bacterial multiplication. Additionally, the utilization of nanoscale emulsions for antibiotic delivery was investigated in a study conducted by Razdan et al. The researchers observed that the formation of these emulsions resulted in notable enhancements in antibacterial efficacy against a range of bacterial species. The increased bactericidal or bacteriostatic capabilities exhibited by these nanoemulsions align with the findings reported in our study, underscoring the potential of medication delivery techniques based on nanotechnology in combating bacterial infections (Razdan et al., 2022).

Our findings have major clinical implications for treating acne and

other bacterial infections. The improved antibacterial efficiency of ITN loaded SNEDDS suggests better acne treatment results. ITN's solubility and bioavailability improve with SNEDDS, improving medication delivery to the infection site and antibacterial activities. This may speed acne healing, minimize inflammation, and lessen scarring. ITN-loaded SNEDDS also work better against antibiotic-resistant bacteria like methicillin-resistant *Staphylococcus aureus*, which could help dermatology and other professions combat antibiotic resistance (Patra et al., 2018). Also, support existing literature that nanotechnology-based drug delivery methods can significantly reduce the complexity of bacterial infections and antibiotic resistance (Rashid and Ahmad, 2018; Sinha et al., 2014).

A complete assessment of the isotretinoin-loaded SNEDDS formulation against *Propionibacterium acnes* is needed to confirm its acne therapy efficacy. The current work highlights isotretinoin's improved solubility and antibacterial activity in SNEDDS, but testing its direct effect on *P. acnes*, the acne-causing bacterium, is crucial. Next, in vitro and in vivo models should test isotretinoin's inhibitory concentration on *P. acnes* and the formulation's capacity to enter the skin and minimize bacterial colonization. SNEDDS' effects *on P. acnes'* virulence factors and skin microenvironment should also be examined. Investigation into these delivery platforms may lead to more efficient and adaptive antibacterial drugs. This suggests more research is needed to understand their mechanisms and assess their therapeutic potential. This work offers significant potential for the development of novel antimicrobial drugs with a wider spectrum of efficacy, addressing the critical issue of antibiotic resistance in microbiology and medicine.

5. Conclusions

- Enhanced Bioavailability: The ITN-SNEDDS formulations successfully improved the solubility and dissolution rate of isotretinoin, addressing its poor bioavailability.
- Antimicrobial Activity: The formulations demonstrated significant antimicrobial activity against various bacterial strains, including antibiotic-resistant ones, highlighting their potential in treating bacterial infections.
- Topical Formulation Potential: Further research is needed to explore ITN-SNEDDS in topical formulations and assess its skin permeation properties.
- Optimal Component Ratios: Formulation F2 showed the best drug release profile, emphasizing the importance of optimizing SNEDDS component ratios.
- Stability Considerations: Formulations with lower oil content (10–20 %) exhibited better stability, indicating a need to balance oil content for maintaining stability.
- Acne Treatment Implications: The antimicrobial activity and potential for enhanced skin permeation suggest that ITN-SNEDDS could be a promising approach for acne treatment, especially for antibiotic-resistant *P. acnes* strains.
- Addressing Antibiotic Resistance: The study contributes to efforts in developing novel drug delivery systems like SNEDDS to overcome antibiotic resistance challenges in dermatology and infectious diseases.
- Further ex vivo permeation studies are required to confirm the enhanced cutaneous permeation of ITN-SNEDDS formulation. Additionally, in vivo safety and hepatoprotective activity in the formulation in comparison to the commercially marketed product is required. Further clinical studies must explore scalability, reproducibility, and commercial viability of the formulation.

CRediT authorship contribution statement

Rihaf Alfaraj: Funding acquisition, Methodology, Writing – original draft. **Sandra Hababah:** Formal analysis, Methodology, Writing – review & editing. **Esra K. Eltayb:** . **Fulwah Y. Alqahtani:** Conceptualization, Supervision, Validation. **Fadilah S. Aleanizy:** Conceptualization, Project administration, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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