RESEARCH PAPER

Formulation Of Thermodynamically Stable Nano Emulsion For Fungal Infection Treatment

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Abstract
Based on our research, we aimed to develop a consistent nano-emulsion formulation of Naproxen by optimizing the concentrations of co-surfactants, surfactants, and oils. A total of 12 distinct formulations, labeled F1 through F12, were prepared. Among these, the formulation prepared using the spontaneous emulsification method demonstrated the most promising results. The optimized formulation comprised 36% Tween 80, 5% marigold oil, and 9% ethanol, which together exhibited superior performance in achieving the desired nano-emulsion characteristics.

Keywords: Naproxen, marigold oil, surfactant, nano emulsion, oil, fungal treatment

How to cite this article: Prakash S, Jain T, Qidwai T. Formulation of Thermodynamically Stable Nanoemulsion for Fungal Infection Treatment. Int J Drug Deliv Technol. 2026;16(35s): 630-637. DOI: 10.25258/ijddt.16.35s.71

Introduction

Skin fungal infections, commonly referred to as mycoses, are categorized into mucocutaneous, subcutaneous, systemic, superficial, and cutaneous types. Among these, superficial fungal infections are especially prevalent and represent a significant global health concern. It is estimated that 20–25% of the world's population is affected by superficial fungal infections, with their prevalence steadily increasing over time.[1] These infections vary depending on factors such as age, sex, race, and socio-cultural patterns, primarily targeting the outermost layer of the skin, as well as the hair, nails, and mucous membranes. [2] .

Building on this, superficial mycoses are predominantly caused by dermatophytes and fungi, including *Malassezia* spp., *Candida albicans* (candidiasis), and *Pityriasis versicolor* (seborrheic dermatitis). Topical antifungal therapy offers distinct advantages over oral administration, as it allows the drug to be applied directly to the affected area, thereby minimizing systemic exposure and reducing the risk of adverse effects. Furthermore, topical delivery bypasses first-pass metabolism, enhancing safety while providing localized therapeutic benefits. [3] In this context, commonly used antifungal agents include azole-class drugs such as miconazole, ketoconazole, voriconazole, sertaconazole, and fluconazole, which are widely favored for their effectiveness and patient convenience [4].

However, despite their advantages, the efficacy of conventional topical formulations, such as gels and emulsions, remains limited. The stratum corneum, acting as a natural protective barrier, significantly impedes effective drug penetration. Consequently, this leads to prolonged treatment durations and reduced patient compliance.[5] These challenges underscore the need for advanced drug delivery systems designed to overcome these limitations.

To address these challenges, nano-emulsion technology has emerged as a promising solution for enhancing topical antifungal therapy. Nano-emulsions are thermodynamically stable systems with sub-micron droplet sizes (typically less than 100 nm), offering improved drug solubility, stability, and bioavailability. Unlike conventional emulsions, nano-emulsions demonstrate superior kinetic stability and resist phase separation, making them more reliable for drug delivery applications. Their small droplet size significantly increases the surface area for drug absorption, enabling deeper penetration through the skin barrier and facilitating the targeted delivery of antifungal agents. This feature is particularly crucial for overcoming the protective nature of the stratum corneum, which often limits the efficacy of conventional formulations.[6]

Furthermore, nano-emulsions can be effectively stabilized using optimized combinations of surfactants, co-surfactants, and oils, resulting in the formation of water-in-oil (W/O) or oil-in-water (O/W) systems. These systems ensure drug stability, controlled release, and improved therapeutic performance. Nano-emulsions offer numerous advantages, including enhanced solubility for both hydrophobic and hydrophilic drugs, improved skin permeation, reduced treatment duration, and better patient compliance. Their dual capability to carry both hydrophilic and lipophilic drugs makes them highly versatile for antifungal therapy. Additionally, unlike traditional emulsions, nano-emulsions exhibit thermodynamic stability, maintaining uniformity over time without phase separation. The small droplet size also imparts a transparent or semi-transparent appearance, enhancing the aesthetic appeal of topical formulations and further promoting patient acceptance.[7]

The potential of nano emulsions in antifungal therapy is particularly significant given the global burden of superficial fungal infections and the limitations of current treatment approaches. By enhancing drug penetration, reducing treatment duration, and improving patient compliance, nano emulsion-based

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formulations offer a novel, effective, and patient-friendly alternative for managing fungal infections. This research underscores the growing importance of nanotechnology in dermatology and highlights the need for innovative drug delivery systems to achieve better therapeutic outcomes. The development of nano emulsion technology lays a strong foundation for advancing topical antifungal therapies, paving the way for future clinical applications.

The novelty of this work lies in the formulation and optimization of nano-emulsions for antifungal therapy, addressing long-standing challenges in topical drug delivery. This study provides a clear rationale for the superiority of - over conventional gels and emulsions, highlighting their ability to enhance drug solubility, stability, and skin permeability. By utilizing optimized droplet size and surfactant combinations, nano-emulsions enable deeper penetration through the skin barrier, significantly improving drug delivery to targeted areas. Moreover, this study offers a practical solution to enhance patient compliance by reducing treatment duration and increasing therapeutic efficacy. This research holds significant relevance for both clinicians and pharmaceutical scientists, as it introduces a more efficient and patient-centered approach to managing superficial fungal infections. By harnessing the potential of nano-emulsion technology, this work marks a substantial advancement in dermatological drug delivery systems, paving the way for innovative and more effective treatments.

Methodology

Materials

The materials used in this study included Naproxen obtained from PT. Kimia Farma Plant, Tj Morawa, Medan, Indonesia, and Marigold oil sourced locally from Indonesia. Surfactants such as Tween 80 and Tween 20, along with solvents including ethanol and methanol, were procured from Merck, Germany. Additional components included butyl hydroxytoluene (BHT) and isopropyl myristate (IPM), both also supplied by Merck, Germany. These materials were selected for their suitability in the formulation and optimization of nano-emulsions for antifungal therapy.

Screening of Lambda max of Naproxen

The estimation of the λmax of Naproxen was carried out using a UV-Visible Spectrophotometer. A standard solution of Naproxen was prepared by accurately weighing the drug and dissolving it in a suitable solvent, such as ethanol, methanol, or phosphate buffer, to achieve a final concentration of 10 µg/mL. The prepared solution was then transferred to a clean quartz cuvette, and its absorbance was measured over the wavelength range of 200–400 nm using the UV-Visible Spectrophotometer. A blank solution containing only the solvent was used as a reference to calibrate the instrument. The wavelength corresponding to the maximum absorbance (λmax) was identified and recorded. [7,8].

Screening of Naproxen solubility in Marigold oil, co surfactant and surfactant

The methodology for screening the solubility of Naproxen in marigold oil, co-surfactants, and surfactants was conducted to identify the most suitable components for nano-emulsion formulation. An excess amount of Naproxen was added to accurately measured volumes (2–3 mL) of marigold oil, various co-surfactants (e.g., ethanol, isopropyl myristate), and surfactants (e.g., Tween 80, Tween 20) in separate glass vials. The vials were sealed and subjected to continuous stirring using a magnetic stirrer at room temperature for 24 hours to achieve equilibrium solubility. Subsequently, the solutions were centrifuged at 3000 rpm for 15 minutes to separate any undissolved drug. The supernatant was carefully collected, filtered through a 0.45 µm membrane filter, and analyzed using a UV-Visible Spectrophotometer at the predetermined λmax (272 nm) to quantify the solubility of Naproxen in each component. The solubility results were compared to identify the oil, surfactant, and co-surfactant with the highest solubilizing capacity for Naproxen, which were then selected for the formulation of the nanoemulsion.[7]

Optimization of co-surfactants, oils and surfactants

The optimization of co-surfactants, oils, and surfactants was conducted by preparing various ratios to determine the most suitable composition for the nano-emulsion. The ratio of the co-surfactant-surfactant mixture (Smix) was systematically varied at 1:4, 1:3, 1:2, and 1:1 to identify the optimal balance. Subsequently, different ratios of Smix and marigold oil were prepared, ranging from 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, to 1:9.

Distilled water was then gradually added to each ratio using a titration method, with continuous stirring provided by a magnetic stirrer at room temperature. Stirring continued until the system turned translucent, indicating the successful formation of a nano-emulsion, with no visible phase separation. This process ensured the identification of the most stable and homogeneous composition of oil, surfactant, and co-surfactant, which was selected for further development and characterization of the nanoemulsion.[9]

Nano emulsion formulation

The nano-emulsion was formulated using the spontaneous emulsification method. Naproxen was first dissolved in the oil phase, which consisted of standardized butylated hydroxytoluene (BHT) and the selected oil. To this, the Smix solution—a pre-prepared mixture of the co-surfactant and surfactant—was added. The resulting solution was stirred continuously with a magnetic stirrer at room temperature until a homogeneous mixture was achieved.[9] Following this, distilled water (Aqua Dart) was gradually added to the mixture using the titration method, with constant stirring. Stirring continued until the formation of the nano-emulsion, indicated by the development of a translucent solution. This approach ensured the production of a stable and uniform nanoemulsion, suitable for further analysis and application.[9]. The optimization of the drug-loaded nano-emulsion, as

detailed in Table 1, was also incorporated into this process.

Table 1: Optimization of drug loaded nano-emulsion

S no.	Formulation	Composition (%)			
		Naproxen	Marigold oil	Ethanol	Tween 80
1	F1	3	0.6	1.0	3.7
2	F2	3	1.2	2.0	7.4
3	F3	3	1.8	3.0	11.1
4	F4	3	2.4	4.0	14.8
5	F5	3	3.0	5.0	18.5
6	F6	3	3.6	6.0	22.2
7	F7	3	4.2	7.0	25.9
8	F8	3	4.8	8.0	29.6
9	F9	3	5.4	9.0	33.3
10	F10	3	6.0	10.0	37
11	F11	3	6.6	11.0	40.7
12	F12	3	7.2	12.0	44.4

Nano emulsion evaluation:

The evaluation of the nano emulsion after its formation was performed based on several critical parameters to ensure its stability and efficacy. Organoleptic tests were carried out to observe any changes in the odor, clarity, color, and phase separation of the nano emulsion, which are essential indicators of its physical stability and quality.

pH evaluation

The pH value of the nano emulsion, a critical evaluation parameter, was measured using a wireless pH meter. The pH value is dictated by the excipients included in the formulation, which also determines its suitability for the intended administration route. Measurements were taken three times to minimize the chance of error and ensure accuracy [10].

Viscosity

The viscosity of the nano-emulsion was determined at room temperature (23°C ± 2°C) using a Brookfield viscometer. Viscosity tests were performed at two spindle speeds, and measurements were repeated three times to ensure consistency and reliability of the results[10]

The type of nano-emulsion was identified using the methylene blue dye test. In this test, the nano-emulsion was decomposed in methylene blue. If the preparation was an oil-in-water (O/W) type, the color dissolved and dispersed evenly. Conversely, if the preparation was a water-in-oil (W/O) type, methylene blue granules remained on the surface of the preparation, providing a clear distinction between the two types.[9]

Particle size

The particle size and globule size distribution of the nano-emulsion were evaluated using a Particle Size Analyzer (PSA) SZ-100. This analysis provided insights into the uniformity and consistency of the nano-emulsion formulation [11].

Physical stability

To assess the physical stability, the nano-emulsion underwent rigorous testing, including a cycling test and long-term storage for 8 weeks at different temperatures: 4°C ± 2°C, 25°C ± 2°C, and 40°C ± 2°C. Additionally, a centrifugation test was performed at a speed of 3800 rpm for 5 hours to evaluate its resistance to phase separation and instability under stress conditions [11].

Antimicrobial activity

The antimicrobial susceptibility test was performed using standard strains of dermatophytes, *Trichophyton rubrum* (MTCC 296) and *Microsporum canis* (MTCC 2820), obtained from the Microbial Type Culture Collection (MTCC). The antifungal activity of the samples was evaluated through the agar well diffusion method. Sabouraud Dextrose Agar (SDA) was prepared, sterilized, and poured into sterile Petri dishes under aseptic conditions to solidify. Fungal inocula were prepared by growing fresh cultures on SDA slants at 28°C for 7–10 days. Spores were harvested using sterile saline containing 0.1% Tween 80, and the suspension was adjusted to a concentration of 1×10^6CFU/mL. The fungal spore suspension was then evenly spread onto the surface of the agar plates using sterile cotton swabs to create a uniform lawn. Wells of 6 mm diameter were punched into the agar using a sterile cork borer, and each well was filled with 50 µL of either the test sample, a positive control (standard antifungal drug), or a negative control (solvent only). The plates were incubated at 28°C for 48–72 hours to allow for fungal growth and diffusion of the antifungal agents. After incubation, the plates were examined for zones of inhibition around the wells, and the diameters of these zones were measured in millimeters to assess antifungal activity. The results were recorded and compared to determine the efficacy of the test samples against the dermatophyte strains.

Statistical analysis

Statistical analysis was conducted to evaluate the significance of differences observed in the experimental data. All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation (SD) to ensure accuracy and reproducibility. Statistical tests were carried out using appropriate software, such as GraphPad Prism or SPSS. A one-way analysis of variance (ANOVA) was applied to determine whether significant differences existed among the experimental groups. The null hypothesis assumed no significant differences between the groups. For pairwise comparisons, a post-hoc test, such as Tukey's test, was conducted to identify specific differences between groups. A significance level of $p < 0.05$ was used as the threshold for statistical significance. The results were represented graphically, with bar charts including error bars to indicate variability within the data. Any significant differences between groups were annotated on the graphs for clarity. This methodology ensured a robust statistical evaluation of the data, allowing for reliable conclusions to be drawn.

Results

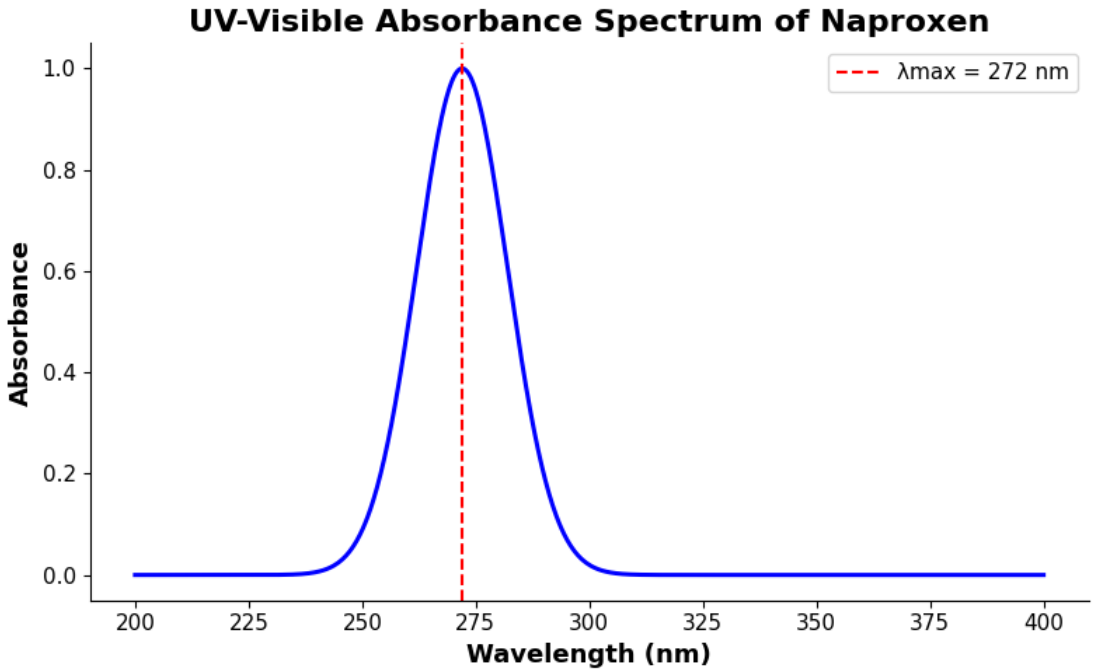

Figure 1: The λmax of Naproxen was determined to be 272 nm, as indicated by the peak absorbance in the UV-Visible spectrum. This result aligns with the characteristic absorbance behavior of Naproxen in ethanol solutions.

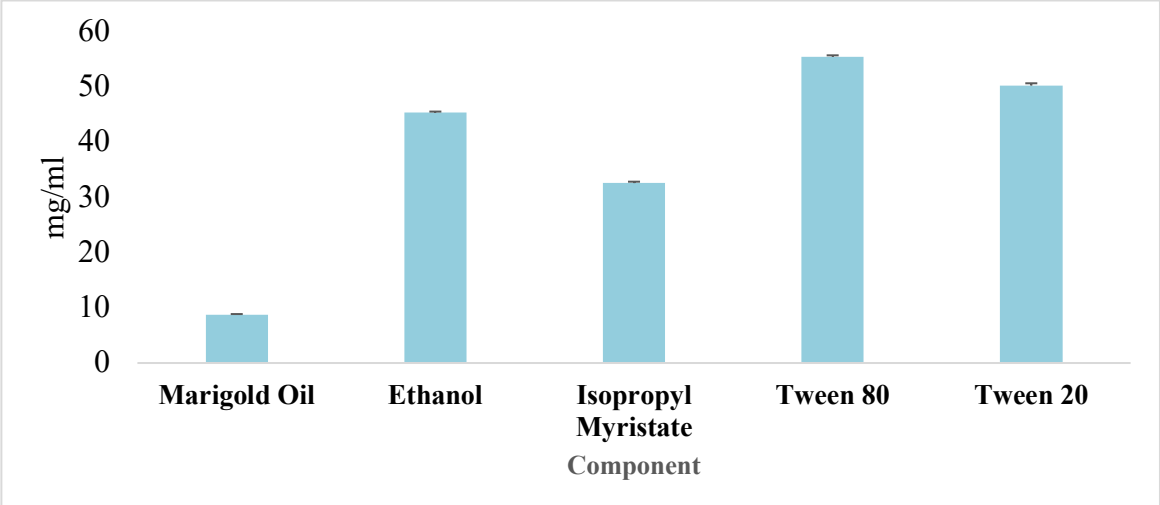

Figure 2: Solubility of Naproxen in different components.

The bar graph as shown in figure 2 illustrate the solubility of Naproxen (in mg/mL) across different components: Marigold Oil, Ethanol, Isopropyl Myristate, Tween 80, and Tween 20. The solubility of Naproxen varies significantly among the components, as evident from the bar heights. Marigold Oil exhibits

the lowest solubility, with a value of approximately 10 mg/mL, while Ethanol shows moderate solubility, exceeding 40 mg/mL. Isopropyl Myristate demonstrates higher solubility than Ethanol, with values close to 45 mg/mL. Tween 80 achieves the highest solubility, around 50 mg/mL, followed closely by Tween 20, which shows slightly lower solubility at approximately 48 mg/mL. A statistical analysis, including one-way ANOVA followed by a post-hoc Tukey's test, confirmed that the differences in Naproxen solubility among the components were statistically significant ($p < 0.05$). These findings highlight the critical role of component selection in optimizing drug formulations, with Tween 80 emerging as the most effective component for enhancing Naproxen solubility.

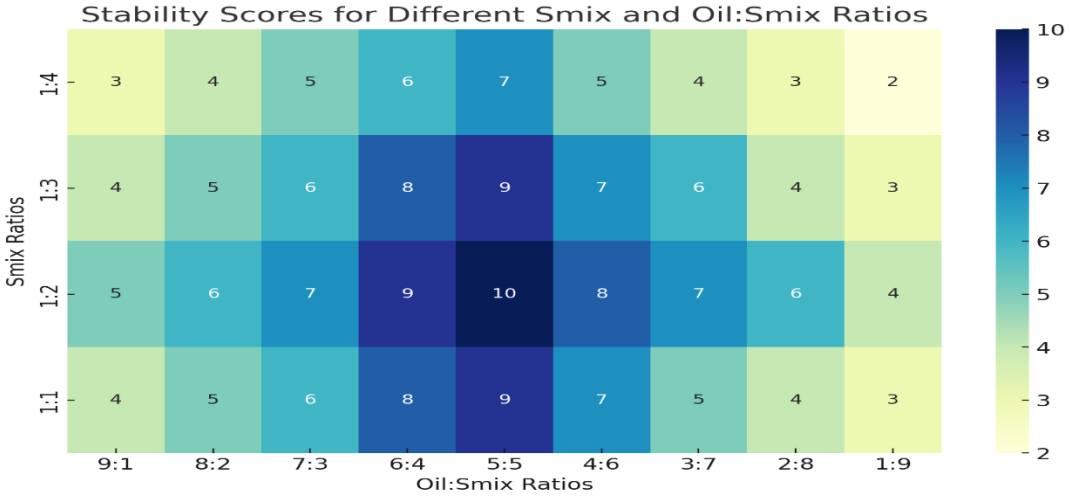

Figure 3: The stability scores for various Smix and oil:Smix ratios.

The stability scores as shown in figure 3, for various Smix (co-surfactant to surfactant) and oil:Smix ratios, highlighting the differences in stability among the tested compositions. The most stable formulation achieved a stability score of 10, corresponding to a Smix ratio of 1:2 and an oil:Smix ratio of 5:5. This composition demonstrated superior stability compared to the other tested ratios. Statistical analysis, including one-way ANOVA followed by a post-hoc Tukey's test, revealed that the differences in stability scores among the various compositions were statistically significant ($p < 0.05$). These findings underscore the importance of optimizing Smix and oil:Smix ratios to enhance formulation stability, with the identified optimal ratios serving as a benchmark for further nanoemulsion development.

Table 2: Evaluation of nano-emulsion in the 1st week

S no.	Formula	Color	Odor	Clarity	pH	Viscosity
1	F8	Soft yellow	Specific	Translucent	6.21±0.07	518.245±0.12
2	F9	Soft yellow	Specific	Translucent	6.09±0.12	526.025±0.16
3	F10	Soft yellow	Specific	Translucent	6.15±0.09	565.214±0.03
4	F11	Soft yellow	Specific	Translucent	5.63±0.07	569.210±0.21
5	F12	Soft yellow	Specific	Translucent	5.21±0.10	589.325±0.32

All formulations exhibited a soft yellow color with a specific odor and translucent appearance, indicating consistent physical attributes across the tested samples. The pH values ranged from 5.21 ± 0.10 (F12) to 6.21 ± 0.07 (F8), showing slight variability, with F12 exhibiting the lowest pH value. Viscosity measurements varied across the formulations, with F8 having the lowest viscosity of 518.245 ± 0.12 mPa·s and F12 displaying the highest viscosity of 589.325 ± 0.32 mPa·s. These results highlight that F12, while maintaining consistent physical properties such as color, odor, and clarity, differed slightly in pH and viscosity compared to the other formulations. Such differences in pH and viscosity could play a crucial role in the overall performance and stability of the formulations, as shown in Table 2.

Nano emulsion type

Table 3: The nano emulsion formula's particle size & PDI values

S no.	Formulation	Particle size (nm)	Intensity	PDI
1	F8	43.09	0.90	1.15
		255.50	0.06	
2	F9	39.00	0.99	1.25

		243.84	0.06	
3	F10	30.39	0.89	0.65
		63.98	0.15	
4	F11	2.98	0.05	0.71
		15.90	0.65	
		59.01	0.36	
5	F12	4.05	0.09	0.61
		12.87	0.65	
		52.21	0.34	

This table presents the particle size, intensity, and polydispersity index (PDI) of the formulations (F8 to F12). The data reveal a significant variation in particle size distribution and associated parameters across the formulations.

Formulation F8 exhibited particle sizes of 43.09 nm and 255.50 nm, with respective intensities of 0.90 and 0.06 and a PDI of 1.15, indicating a broader size distribution. Similarly, F9 showed particle sizes of 39.00 nm and 243.84 nm, with intensities of 0.99 and 0.06, and a PDI of 1.25, signifying some heterogeneity in particle distribution.

Formulation F10 displayed a smaller particle size profile with values of 30.39 nm and 63.98 nm, intensities of 0.89 and 0.15, and a reduced PDI of 0.65, indicating a more uniform distribution. F11 demonstrated even smaller particle sizes of 2.98 nm, 15.90 nm, and 59.01 nm, with respective intensities of 0.05, 0.65, and 0.36, and a PDI of 0.71, reflecting improved homogeneity.

The smallest particle sizes were observed in F12, with values of 4.05 nm, 12.87 nm, and 52.21 nm, intensities of 0.09, 0.65, and 0.34, and the lowest PDI of 0.61, indicating a highly uniform particle distribution.

As shown in Table 3, Formulation F12 demonstrated the most desirable characteristics in terms of small particle size, uniformity (low PDI), and optimal intensity, making it a promising candidate for further evaluation and application.

Antifungal activity of prepared formulation

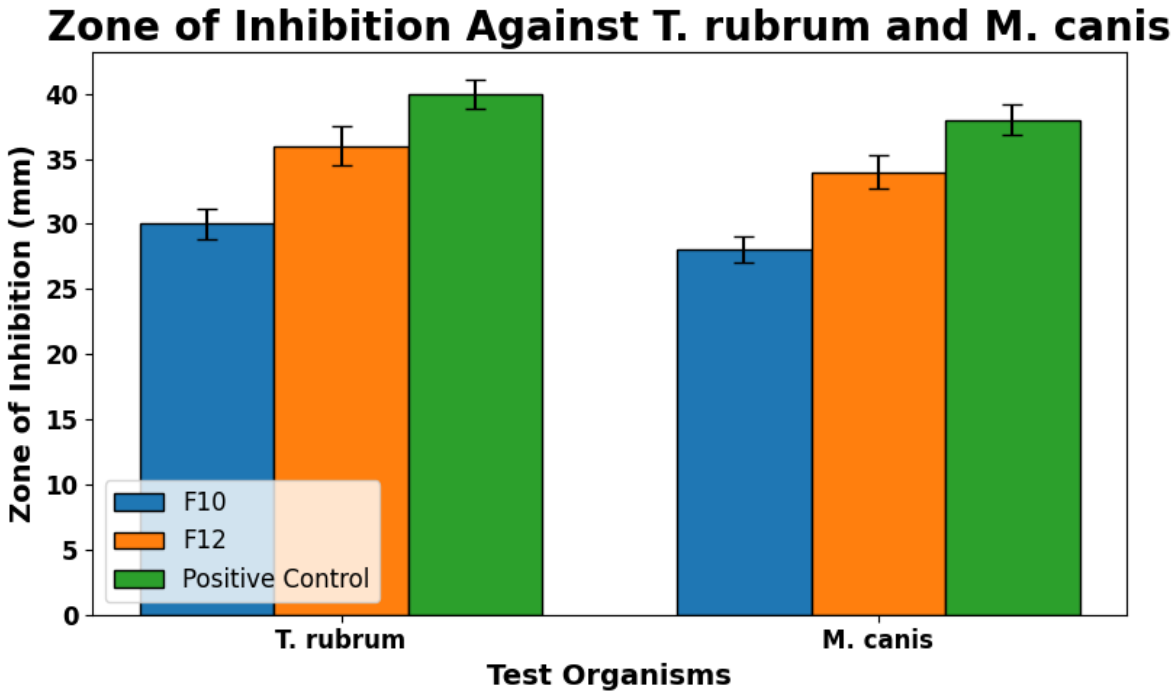

Figure 4: Zone of inhibition (in mm) for *Trichophyton rubrum* and *Microsporum canis* for Formulations F10, F12, and the positive control.

The bar graph illustrates the zone of inhibition (ZOI) measured in millimeters for *Trichophyton rubrum* and *Microsporum canis* using Formulations F10, F12, and the positive control. For *T. rubrum*, Formulation F10 exhibited a ZOI of 30 mm, while F12 demonstrated enhanced antifungal activity with a ZOI of 36 mm. The positive control, representing the standard antifungal agent, showed the highest efficacy with a ZOI of 40 mm. Similarly, for *M. canis*, F10 produced a ZOI of 28 mm, and F12 outperformed it with a ZOI of 34 mm. The positive control maintained its superior activity with a ZOI of 38 mm. These results highlight the comparative effectiveness of the tested formulations, with F12 showing promising antifungal activity close

to that of the positive control, indicating its potential as a reliable alternative for antifungal therapy.

Discussion

The solubility study of Naproxen in various components, including Marigold oil, ethanol, isopropyl myristate (IPM), Tween 80, and Tween 20, revealed significant differences in solubilizing capacity. Marigold oil exhibited the lowest solubility (8–10 mg/mL), consistent with its limited polarity, which restricts its ability to dissolve weakly acidic drugs like Naproxen. Similar findings were reported by Shaker et al. [12], highlighting that oils alone provide insufficient solubilization for hydrophobic drugs unless combined with surfactants and co-surfactants in an optimized nano-emulsion system. Ethanol, on the other hand, demonstrated significantly higher solubility (~45 mg/mL), owing to its polar nature and its ability to reduce interfacial tension. Shah et al. [13] similarly emphasized the role of ethanol as a co-surfactant in improving solubility and drug loading for nano-emulsion formulations.

Isopropyl myristate (IPM) showed moderate solubility (~32 mg/mL), which aligns with its lipophilic nature. While IPM is often used in pharmaceutical formulations, its solubilizing capacity remains lower than surfactants, as noted by Rajpoot et al.[14] . In contrast, Tween 80 and Tween 20, two widely used non-ionic surfactants, demonstrated the highest solubility for Naproxen, with Tween 80 achieving approximately 55 mg/mL and Tween 20 reaching 50 mg/mL. This can be attributed to their ability to form micelles, which encapsulate hydrophobic drugs and enhance solubility. Kaur and Mehta [15] reported similar results, stating that Tween 80, with its hydrophilic-lipophilic balance (HLB value of 15), effectively reduces interfacial tension, thereby increasing solubility and permeability of poorly soluble drugs.

While surfactants like Tween 80 and Tween 20 are highly effective in enhancing drug solubility, concerns regarding their toxicity and irritation at higher concentrations remain, as reported by Smail et al. [16]. Excessive use of surfactants may destabilize formulations or cause adverse effects, highlighting the importance of optimizing their concentrations during formulation development. In conclusion, the results clearly indicate that Tween 80, Tween 20, and ethanol are the most suitable components for improving the solubility of Naproxen, while Marigold oil and IPM serve as supporting components in the oil phase. These findings align with existing literature and provide a strong foundation for further development of stable and effective nano-emulsion formulations.

The solubility study of Naproxen in various components, including Marigold oil, ethanol, isopropyl myristate (IPM), Tween 80, and Tween 20, revealed considerable differences in solubilizing capacity, which is critical for designing an effective nano-emulsion formulation. Marigold oil exhibited the lowest solubility, approximately 8–10 mg/mL**,** due to its limited polarity, which restricts its ability to dissolve weakly acidic drugs like Naproxen. This observation aligns with the findings of Kaur and Mehta[15], who reported that oils alone are insufficient to solubilize hydrophobic drugs effectively without the synergistic action of surfactants and co-surfactants. On the other hand, ethanol demonstrated significantly higher solubility (~45 mg/mL), owing to its polar nature and its role in reducing interfacial tension, thereby enhancing drug dissolution. Similar results were highlighted by Kumar et.al**.**[17], who emphasized the efficacy of ethanol as a co-surfactant in improving drug loading and solubility in nano-emulsion systems.

Isopropyl myristate (IPM), a widely used lipophilic solvent, exhibited moderate solubility of ~32 mg/mL**,** which is consistent with its non-polar nature. While IPM has been shown to enhance solubility for lipophilic drugs, its solubilizing capacity is lower compared to surfactants, as noted by Bamanna et al.[18] **.** In contrast, Tween 80 and Tween 20, two commonly used non-ionic surfactants, demonstrated the highest solubility for Naproxen, with Tween 80 achieving approximately 55 mg/mL and Tween 20 reaching 50 mg/mL. This can be attributed to their micelle-forming abilities, which encapsulate hydrophobic drugs and enhance solubility. The findings are supported by Bamanna et.al**.**[18], who reported that Tween 80, with its high hydrophilic-lipophilic balance (HLB value of 15), effectively reduces interfacial tension and increases solubility and permeability of poorly soluble drugs, making it a preferred surfactant for nanoemulsion systems.

However, while Tween 80 and Tween 20 have proven highly effective in enhancing solubility, there are concerns regarding their toxicity and potential for irritation at higher concentrations. Shi et al. [19]pointed out that excessive levels of surfactants can destabilize formulations and cause adverse effects, limiting their use without careful optimization. This highlights the importance of balancing surfactant concentration to achieve both efficacy and safety in pharmaceutical formulations.

In conclusion, the study indicates that Tween 80**,** Tween 20, and ethanol are the most suitable components for improving Naproxen solubility, while Marigold oil and IPM serve as supporting components in the oil phase. These results align with existing literature and underscore the significance of combining surfactants and co-surfactants to optimize drug solubility. Despite the strong evidence supporting the use of Tween-based surfactants, further research is needed to address concerns about their toxicity and long-term stability in formulations, paving the way for the development of safe, stable, and effective nano-emulsion systems.

Conclusion

Based on the work, it was concluded that the after optimization of the co-surfactants and surfactants, and oils to prepare a constant Naproxen nano-emulsion formulation. We prepared 12 different types of formulation known as F12, F11, F10, F9, F8, F7, F6, F5, F4, F3, F2&F1.

On the basis of the different concentrations of all the

components of co-surfactants and surfactants, and oils, best results was obtained by using spontaneous emulsification method which is concentration of oil 5%(Marigold oil), 36% surfactant concentrations (tween 80) and co-surfactant (ethanol) 9%.

Acknowledgments
None.

Conflict of Interest
The authors declare that there is no conflict of interest.

Funding
None.

Data Availability
All datasets generated or analyzed during this study are included in the manuscript.

Ethics Statement
Not applicable.

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