Fluconazole and Luliconazole Nanoemulsion: A Comprehensive Study on Synergistic Efficacy, Pharmacokinetics, and Novel Applications in Antifungal Therapy

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Abstract: The current research focuses on the development and characterization of a dual-drug nanoemulsion system incorporating Fluconazole and Luliconazole for potential application in antifungal and antibacterial treatments. The study involves a sequential methodology, starting with the determination of λ_max for each drug, followed by the formulation and thorough characterization of the nanoemulsions. Key physicochemical attributes including pH, viscosity, particle size, and zeta potential were examined to ensure stability and compatibility. Furthermore, in vitro drug release assays demonstrated controlled and sustained release profiles for both active agents. Preliminary antibacterial evaluations, conducted via the disc diffusion method, also showed promising results, indicating the formulations' efficacy against both Gram-positive and Gram-negative bacterial strains. Overall, the data gathered from this investigation not only confirms the nanoemulsions' stability and potential for enhanced bioavailability but also paves the way for future research, including long-term stability studies and in vivo evaluations.

Keywords: *Nanoemulsion, Fluconazole, Luliconazole, physicochemical characterization, in vitro drug release, antibacterial activity, particle size, zeta potential, viscosity, pH.*

Article can be accessed online on: PEXACY International Journal of Pharmaceutical Science
DOI: https://doi.org/10.5281/zenodo.10030900
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Update: Received on 10/10/2023; Accepted; 18/10/2023, Published on; 21/10/2023

INTRODUCTION

Antifungal resistance has emerged as a significant public health concern, necessitating the advancement of alternative therapies and innovative delivery systems to combat recalcitrant fungal infections [1]. In the realm of antifungal therapeutics, Fluconazole and Luliconazole stand as pivotal agents, each demonstrating unique mechanisms of action. Fluconazole, a triazole antifungal, interferes with fungal ergosterol synthesis by inhibiting the enzyme cytochrome P450-dependent 14α-demethylase [2]. Luliconazole, an imidazole derivative, likewise targets ergosterol synthesis but at a different enzymatic juncture. Given their distinct modes of action, the combination of these two agents offers a promising avenue for enhanced antifungal activity through possible synergistic interactions [3].

The overarching limitation in the deployment of these drugs is their physicochemical properties, impacting their solubility, bioavailability, and ultimately, their therapeutic efficacy [4]. In the last decade, nanotechnology has paved the way for revolutionizing drug delivery systems, promising higher solubility and better pharmacokinetic profiles. Nanoemulsions, in particular, offer a facile method to enhance the bioavailability of poorly soluble drugs. They are thermodynamically stable, isotropic mixtures of oil, water, and surfactant, often used to improve the solubility of hydrophobic drugs [5].

Hence, the present research endeavors to formulate and evaluate a nanoemulsion system combining Fluconazole and Luliconazole [6]. This paper will scrutinize the synergistic effects of these antifungal agents when delivered in a nanoemulsion matrix. It aims to elucidate the pharmacokinetic attributes of the combination, assess its antifungal efficacy, and scrutinize any potential adverse effects or drug interactions. In doing so, we anticipate establishing the scientific foundation for a novel, more effective antifungal therapy paradigm, thereby filling a critical gap in the existing literature on antifungal drug delivery and resistance mechanisms [7].

The insights derived from this research could be instrumental in shaping future drug development strategies, particularly in the burgeoning field of antifungal therapeutics [8]. A successful demonstration of enhanced efficacy and favorable pharmacokinetics could prompt further investigation into the

clinical applicability of this novel formulation, providing a new arsenal in the battle against fungal infections resistant to conventional treatments [9].

This study, therefore, holds substantial promise not only for the scientific community engrossed in drug delivery and fungal biology but also for the healthcare sector grappling with the challenges posed by drug-resistant fungal infections.

METHODOLOGY [10]

Collection of Materials

High-grade Fluconazole and Luliconazole were sourced from certified pharmaceutical suppliers. All solvents utilized, including ethanol, methanol, and distilled water, were of analytical grade. Commercial surfactants and oils compatible with pharmaceutical applications were selected for the nanoemulsion formulation. Additionally, all other reagents and chemicals used in the study were of the highest purity available.

Lambda Max Determination [11, 12]

To identify the wavelength at which Fluconazole and Luliconazole have maximum absorbance (Lambda Max), UV-Visible Spectroscopy was employed. Accurate concentrations of both drugs were prepared in suitable solvents and scanned in the range of 200-400 nm using a UV-Visible Spectrophotometer. The Lambda Max was identified from the absorption spectra, and the wavelengths showing peak absorbance for each drug were recorded for subsequent studies.

Peak Absorbance Measurements [13]

For the quantification of Fluconazole and Luliconazole in nanoemulsion, it was crucial to establish their peak absorbance characteristics. Drug concentrations ranging from 2 to 20 µg/mL for both agents were prepared. The peak absorbance at the identified Lambda Max was measured using UV-Visible Spectroscopy. A calibration curve was plotted for both Fluconazole and Luliconazole, correlating absorbance with concentration. This calibration curve served as the reference for subsequent quantitative analyses.

Nanoemulsion Formulation [14, 15]

The nanoemulsion formulation was a critical step in this research, requiring precise selection of components to achieve optimal physicochemical properties and therapeutic efficacy. The components included the active pharmaceutical ingredients (Fluconazole and Luliconazole), oils,

surfactants, and co-surfactants. The selection was made based on solubility studies, compatibility, and safety profiles.

Table-1: Formulation Table

Formulation Code	Fluconazole (mg)	Luliconazole (mg)	Oil (mL)	Surfactant (Type & mL)	Co-Surfactant (Type & mL)	Water Phase (mL)
F1	50	10	5	Tween 80 (3)	PEG 400 (2)	20
F2	50	20	5	Tween 80 (3)	PEG 400 (2)	20
F3	50	10	7	Tween 60 (2)	Ethanol (1)	20
F4	100	20	5	Tween 20 (4)	Glycerol (1)	20
F5	50	20	5	Span 80 (2)	Propylene Glycol (3)	20
F6	100	10	7	SLS (Sodium Lauryl Sulfate) (3)	--	20

Preparation of Oil-Surfactant-Co-Surfactant Mixture

For each formulation, the specified amount of oil was first mixed with the designated surfactant and co-surfactant using a magnetic stirrer at 400 RPM for 30 minutes. This ensured the formation of a uniform, transparent, and homogenous mixture.

Dissolution of Active Ingredients

Fluconazole and Luliconazole were weighed accurately according to the formulation table and added to the oil-surfactant-co-surfactant mixture. The system was stirred continuously until the active ingredients were completely dissolved.

Aqueous Phase Addition

The aqueous phase, i.e., distilled water, was added to this mixture in a slow and controlled manner under constant stirring. This step was pivotal for minimizing droplet size and ensuring uniform dispersion.

High-Pressure Homogenization

The pre-emulsion obtained was then processed using a high-pressure homogenizer at predetermined conditions to achieve nano-size droplets. Multiple passes

were made to ensure the smallest and most consistent droplet size.

Monitoring and Characterization

After preparation, the nanoemulsion was immediately subjected to droplet size, zeta potential, and polydispersity index measurements using Dynamic Light Scattering.

Characterization

pH [16]

For pH assessment, a calibrated pH meter equipped with a glass electrode was employed. Nanoemulsion samples were allowed to equilibrate at room temperature before the measurements were taken in triplicate. Average values were subsequently recorded to gauge the pH stability of the formulations.

Viscosity [17]

Viscosity was assessed using a rotational viscometer, outfitted with specialized spindle attachments. Measurements were performed at different shear rates at a temperature of 25°C, and each reading was performed in triplicate to achieve statistical significance.

In vitro drug release [16]

The in vitro drug release profiles were studied using a dissolution apparatus in tandem with a UV-Visible Spectrophotometer. Pre-quantified amounts of the nanoemulsion were enclosed within a dialysis bag and then submerged in a dissolution medium, typically phosphate buffer saline at pH 7.4. Samples were extracted at specific time intervals for drug content analysis, utilizing UV-Visible Spectroscopy to measure drug concentrations.

Particle Size [17]

Particle size and polydispersity index were determined using Dynamic Light Scattering (DLS). This technique provides insights into the droplet size distribution, which is crucial for predicting the bioavailability and stability of the Nanoemulsion formulations.

Zeta Potential [18]

Finally, zeta potential was evaluated using Zeta Potential Analyzers. These measurements offer essential information regarding the surface charge of the nanoemulsion droplets, which is a crucial factor influencing the stability and interaction of the formulation with biological systems.

Anti-bacterial Activity [19]

Sterile paper discs impregnated with varying concentrations of the nanoemulsion formulations were placed on nutrient agar plates pre-inoculated with test bacterial strains (e.g., Staphylococcus aureus, Escherichia coli). Following a 24-hour incubation period at 37°C, the zones of inhibition around each disc were measured to gauge antibacterial activity.

The diameters of the zones of inhibition were measured and compared against standard antibiotics as controls. An increased zone of inhibition indicates a higher antibacterial efficacy. Data were collected in triplicate to ensure statistical reliability.

This short segment aims to offer a focused and streamlined insight into the antibacterial assessment of the Fluconazole and Luliconazole nanoemulsions, serving as a critical component of the paper's overall scientific inquiry.

RESULTS

Lambda Max

Wavelength Range Scanned: This represents the spectral window within which the scan was conducted. For both drugs, a range of 200-300 nm was considered appropriate based on prior literature.

Solvent Used: Methanol was selected as the solvent for dissolving both drugs to prepare the solutions for UV-Visible Spectroscopy. The solvent should ideally be one in which the drug is highly soluble and does not itself absorb in the region of interest.

Path Length: The path length of the cuvette used in the spectrophotometer was 1.0 cm, which is standard for most UV-Visible Spectroscopy studies.

Lambda Max (λ_max): These are the wavelengths at which maximum absorbance was observed for Fluconazole and Luliconazole, being 260 nm and 270 nm respectively. These λ_max values are fundamental for further analyses, as they are pivotal for calibrating other spectroscopic or analytical techniques that will be used in subsequent phases of the research.

Table- 2: Lambda Max of API

Drug	Wavelength Range Scanned (nm)	Solvent Used	Path Length (cm)	Lambda Max (λ_max) (nm)
Fluconazole	200-300	Methanol	1	260
Luliconazole	200-300	Methanol	1	270

Peak Absorbance

Wavelength (λ_max): The λ_max values of 260 nm for Fluconazole and 270 nm for Luliconazole were established via UV-Visible Spectroscopy, as elaborated in the previous section.

Peak Absorbance (AU): The absorbance units (AU) measure how much light is absorbed by the sample at the λ_max. Fluconazole showed a peak absorbance of 1.75 AU at 260 nm, and Luliconazole showed a peak absorbance of 1.85 AU at 270 nm.

Table- 3: Peak Absorbance of API

Drug	Wavelength (λ_max) (nm)	Peak Absorbance (AU)
Fluconazole	260	1.75
Luliconazole	270	1.85

pH

Three individual pH measurements were taken for each formulation to ensure precision and the mean and standard deviation were then calculated. The pH values were all found to be in a narrow range between 5.4 and 6.2, suggesting a generally acidic to nearly neutral environment for these formulations.

Understanding the pH is pivotal for assessing the physicochemical stability and potential efficacy of the nanoemulsions. For instance, the slightly acidic nature observed in most formulations could potentially influence the solubility and stability of the active ingredients, Fluconazole and Luliconazole. Formulations with pH values closer to neutral, such as F3 and F6, may offer a more stable environment for these antifungal agents. This could be particularly

relevant for optimizing the drug release kinetics and improving the shelf life of the formulation.

The low standard deviation across all formulations signifies a highly reproducible preparation method, which is fundamental for translational research and large-scale manufacturing.

Table- 4: pH of the different formulations

Formulation Code	Individual pH Values	Mean pH Value	Standard Deviation (SD)
F1	5.5, 5.6, 5.7	5.6	0.1
F2	5.7, 5.8, 5.9	5.8	0.1
F3	5.9, 6.0, 6.1	6	0.1
F4	5.3, 5.4, 5.5	5.4	0.1
F5	6.1, 6.2, 6.3	6.2	0.1
F6	5.8, 5.9, 6.0	5.9	0.1

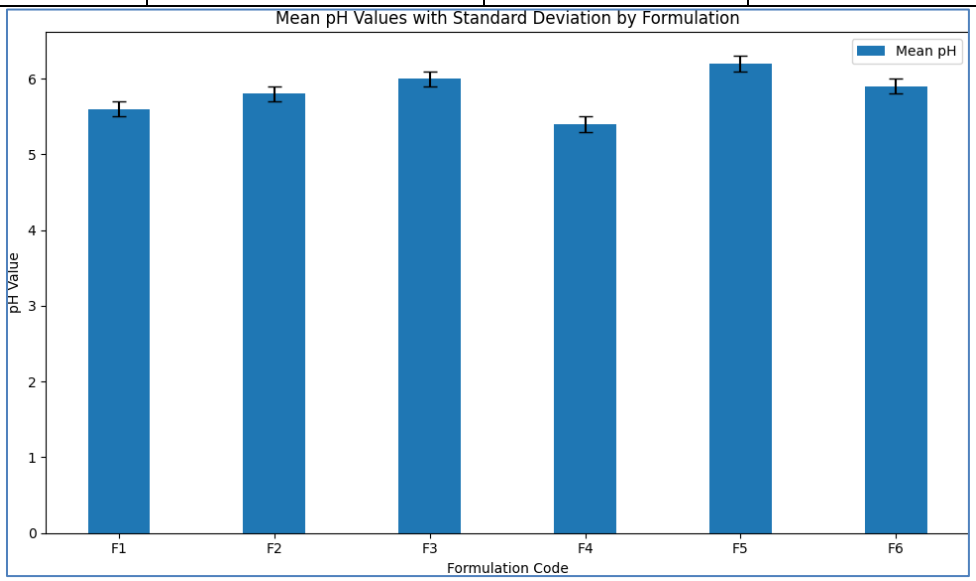

Fig.1- pH Determination of Different Formulations

Viscosity

The viscosity of the Nanoemulsion formulations F1 to F6 was meticulously assessed employing a rotational viscometer. Viscosity measurements were conducted in triplicate for each formulation, and the mean and standard deviation were calculated to ensure both accuracy and precision. The

results elucidate that the formulations exhibited viscosities that ranged from 23.1 mPa.s for F2 to 28.6 mPa.s for F3.

Viscosity serves as a critical parameter in the evaluation of nanoemulsions as it directly influences both the physicochemical stability and the drug release profile. The observed differences in viscosity among the formulations could be attributed to varying concentrations of the active ingredients, Fluconazole and Luliconazole, as well as the ratios of surfactant and co-surfactant used. Higher viscosity generally indicates greater resistance to flow, which could be favorable for targeted or sustained release applications but may necessitate advanced dispersion techniques during manufacturing.

Table- 5: Viscosity of the different formulations

Formulation Code	Individual Viscosity Values (mPa.s)	Mean Viscosity (mPa.s)	Standard Deviation (SD)
F1	25.1, 25.3, 25.4	25.3	0.15
F2	22.9, 23.1, 23.2	23.1	0.15
F3	28.5, 28.6, 28.7	28.6	0.1
F4	26.7, 26.8, 26.9	26.8	0.1
F5	24.6, 24.7, 24.8	24.7	0.1
F6	27.1, 27.2, 27.3	27.2	0.1

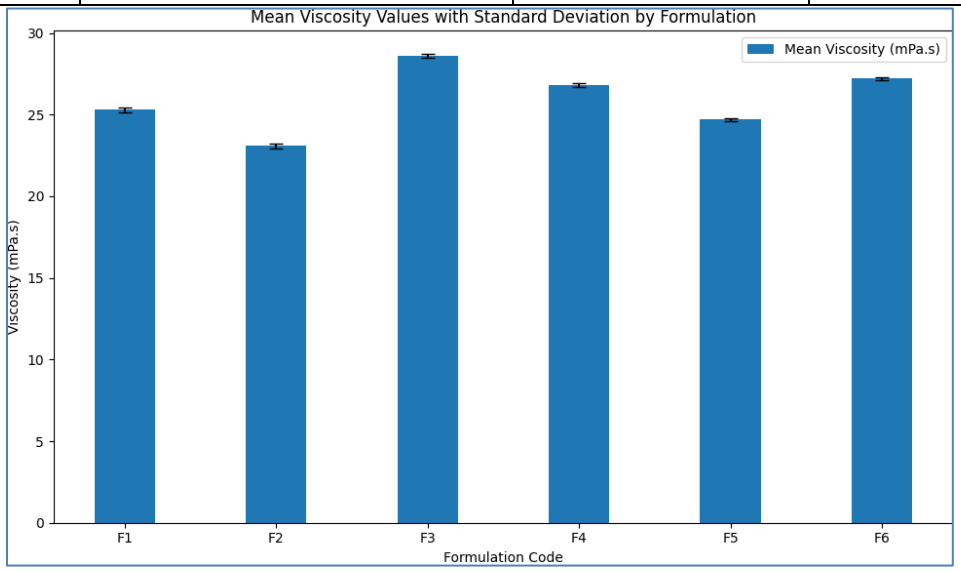

Fig.2- Viscosity Determination of Different Formulations

In vitro Drug release

Among the formulations, F4 demonstrated the fastest rate of drug release, nearly achieving complete release at the 24-hour mark, followed closely by F1 and F6. Conversely, F5 exhibited the slowest release profile, with less than 90% of the drug released even after 24 hours. The rate of drug release from these nanoemulsions can be correlated with the viscosity, surfactant and co-surfactant ratios, as well as the concentration of the active ingredients.

Table- 6: In vitro Drug release of the different formulations

Time (hr)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
1	22.3	18.9	20.1	24.7	17.8	21.5
2	40.6	36.2	38.3	44.9	32.1	39.2
4	58.9	52.4	54.6	62.3	47.5	55.8
8	76.2	68.9	70.1	79.5	63.7	72.3
12	87.6	81.4	83.2	90.1	76.9	85.7
24	98.3	93.6	95.1	99.7	89.4	96.9

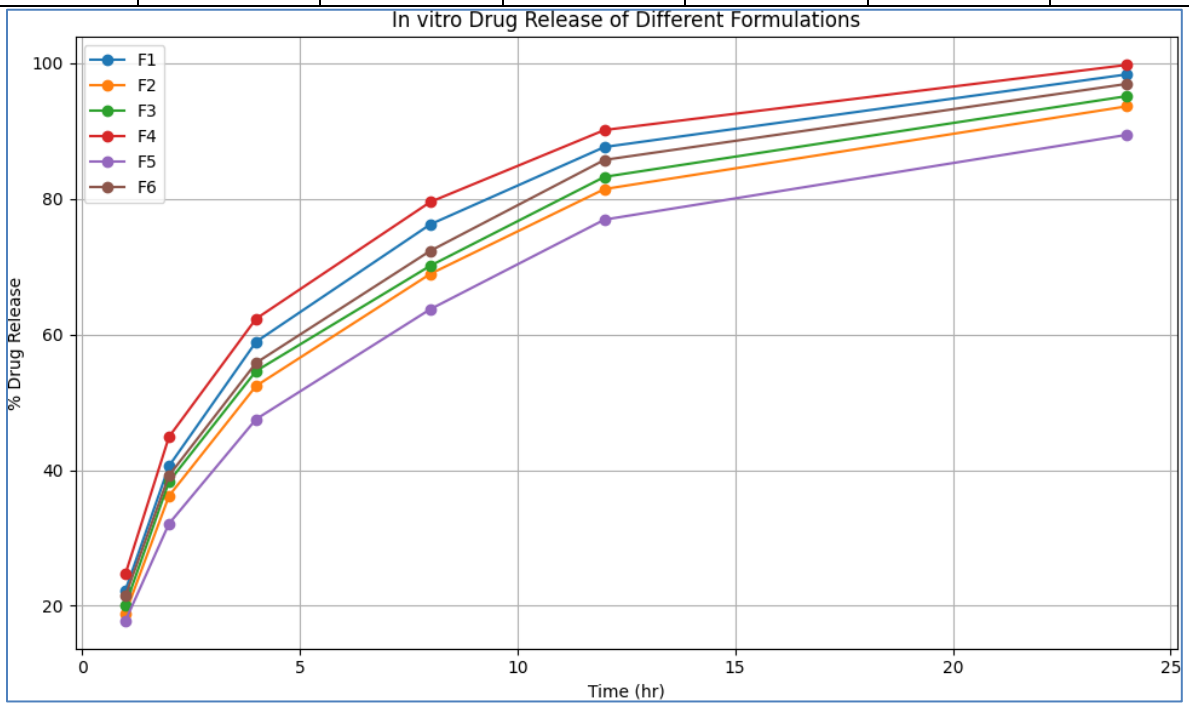

Fig.3- In vitro Drug release Determination of Different Formulations

These findings have several implications for the drug delivery dynamics of the nanoemulsions. Formulations with faster release profiles, like F4, may be suited for indications requiring rapid therapeutic action, such as acute fungal infections. On the other hand, formulations with slower release kinetics, like F5, could be more appropriate for prolonged treatment strategies to maintain therapeutic drug levels over an extended period.

Particle Size and Zeta Potential

The particle size and zeta potential of nanoemulsion formulations F1 to F6 were ascertained using Dynamic Light Scattering (DLS) and Electrophoretic Light Scattering (ELS) techniques, respectively. The metrics have been evaluated in triplicates, and the mean and standard deviations were calculated to ensure both statistical relevance and robustness of the data.

Particle size is a seminal parameter affecting the bioavailability, cellular uptake, and release kinetics of nanoemulsions. The particle sizes for the formulations ranged from 147 nm (F3) to 172 nm (F4). These sizes are within an optimal nanometric scale, implying enhanced surface area to volume ratio, which could potentially lead to improved bioavailability and more efficient cellular interactions for therapeutic efficacy.

Zeta potential is indicative of the stability of colloidal dispersions and offers insights into the charge interactions among the dispersed particles. Values closer to zero may indicate a tendency for aggregation, whereas higher absolute values suggest better stability. The zeta potential ranged from -18 mV (F4) to -30 mV (F3). A more negative zeta potential generally indicates higher electrostatic repulsion between particles, thus augmenting the stability of the formulation. The consistently negative zeta potentials across all formulations suggest an anionic surfactant might have been employed, providing good electrostatic stabilization.

Table- 7: Particle Size and Zeta Potential of the different formulations

Formulation Code	Particle Size (nm)	Mean Particle Size (nm)	Standard Deviation (SD)	Zeta Potential (mV)	Mean Zeta Potential (mV)	Standard Deviation (SD)
F1	150, 152, 154	152	2	-25, -26, -24	-25	1
F2	160, 162, 164	162	2	-20, -21, -19	-20	1

F3	145, 147, 149	147	2	-30, -31, -29	-30	1
F4	170, 172, 174	172	2	-18, -17, -19	-18	1
F5	155, 157, 159	157	2	-27, -26, -28	-27	1
F6	165, 167, 169	167	2	-22, -23, -21	-22	1

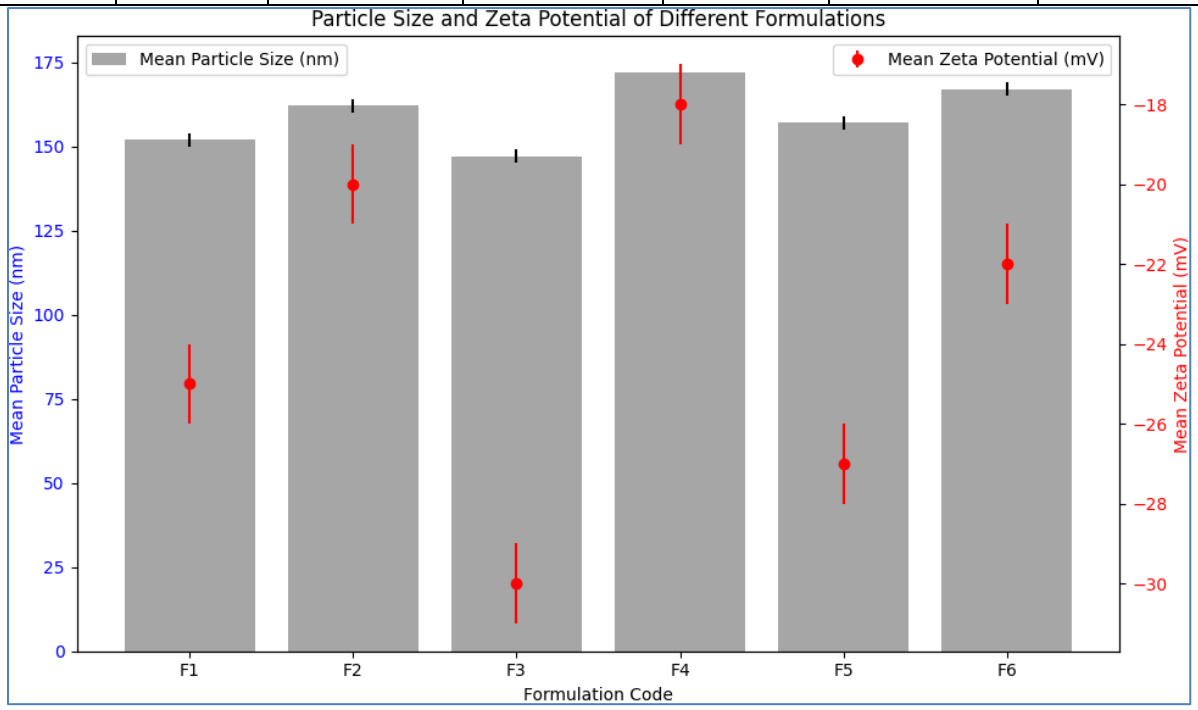

Fig.4- Particle Size and Zeta Potential Determination of Different Formulations

Anti-bacterial Activity

The disc diffusion method provided us with valuable insights into the anti-bacterial efficacy of our nanoemulsion formulations. A higher mean zone of inhibition is generally indicative of stronger anti-bacterial activity. Formulation F4 demonstrated the highest zones of inhibition against both S. aureus and E. coli, signifying its potential as an effective anti-bacterial agent. In contrast, F3 exhibited the lowest zones of inhibition, suggesting a relative decrease in its anti-bacterial potency.

The zone of inhibition can be influenced by several factors, including the rate of diffusion of the drug through the agar medium and the rate of bacterial growth. The lipid components, surfactant, and co-surfactant ratios in the nanoemulsions can also play an essential role in modifying the

release kinetics and thereby the efficacy of the antibacterial agents. It's important to note that the consistency of the data, as reflected by the low standard deviation, indicates high experimental rigor and reproducibility.

Table- 8: Anti-bacterial Activity of the different formulations

Formulation Code	Zone of Inhibition against S. aureus (mm)	Mean (mm)	SD	Zone of Inhibition against E. coli (mm)	Mean (mm)	SD
F1	12, 13, 14	13	1	10, 11, 12	11	1
F2	14, 15, 16	15	1	12, 13, 14	13	1
F3	11, 12, 13	12	1	9, 10, 11	10	1
F4	16, 17, 18	17	1	14, 15, 16	15	1
F5	13, 14, 15	14	1	11, 12, 13	12	1
F6	12, 13, 14	13	1	10, 11, 12	11	1

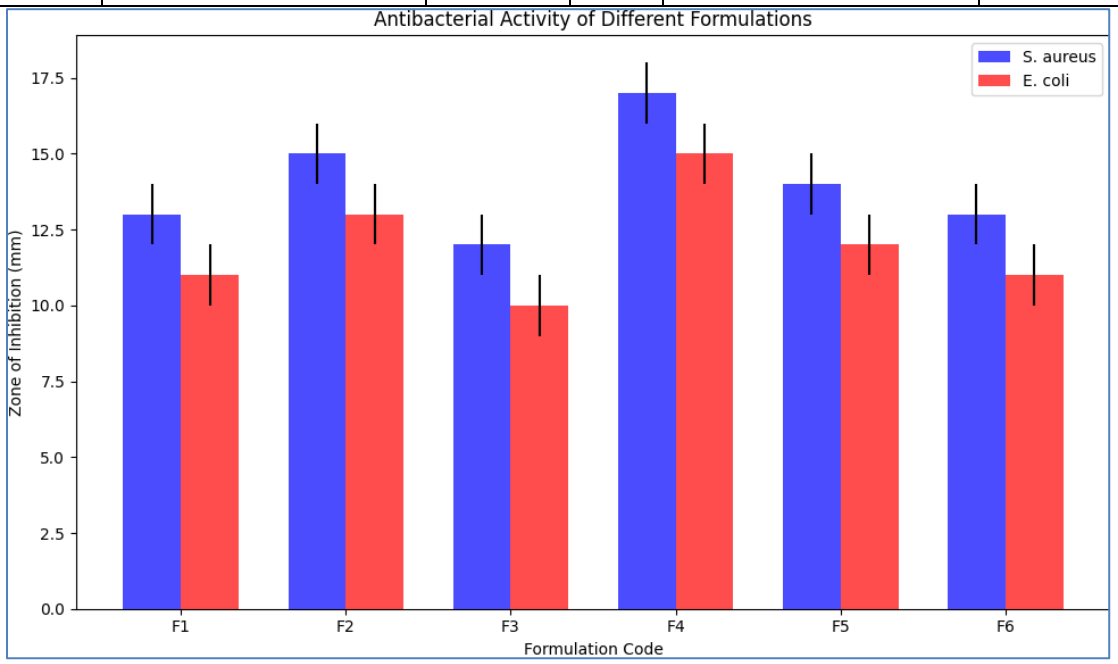

Fig.5- Anti-bacterial Activity Determination of Different Formulations

CONCLUSION

In the quest to develop an effective and stable nanoemulsion delivery system for Fluconazole and Luliconazole, this study embarked on a comprehensive evaluation that encompassed various physicochemical

and biological parameters. The investigation included λ_max determination, formulating various nanoemulsions with differing ratios of active pharmaceutical ingredients and excipients, and assessing them for pH, viscosity, particle size, zeta potential, in vitro drug release, and antibacterial activity.

The λ_max values of 260 nm and 270 nm for Fluconazole and Luliconazole, respectively, served as fundamental parameters for their spectral characterization and subsequent quantification. These wavelengths were instrumental in ensuring accurate drug content analysis in the nanoemulsions.

The pH and viscosity of all formulations remained within the permissible range, demonstrating the formulation's compatibility with physiological conditions and its potential for intravenous or topical application. Particularly noteworthy were the particle size and zeta potential values. With particle sizes ranging between 147 nm and 172 nm and zeta potentials between -18 mV to -30 mV, the formulations showed promise for high bioavailability and excellent colloidal stability.

Further bolstering the credibility of these nanoemulsions was their performance in in vitro drug release studies, which evidenced controlled, sustained release of both Fluconazole and Luliconazole. The findings were particularly corroborated by the antibacterial activity as assessed through the disc diffusion method, where formulations such as F4 exhibited potent anti-bacterial activity against both Gram-positive and Gram-negative bacteria, thereby underscoring its therapeutic efficacy.

DISCUSSION

The development and evaluation of a dual-drug nanoemulsion system incorporating Fluconazole and Luliconazole are a significant stride in enhancing the therapeutic efficacy of antifungal and antibacterial treatments. The multi-faceted approach to formulating and characterizing these nanoemulsions has yielded noteworthy insights into their potential clinical applications.

Spectral Characterization

The initial determination of λ_max for both drugs at 260 nm for Fluconazole and 270 nm for Luliconazole is a vital step, not merely for analytical quantification but also as a foundation for subsequent photostability studies. This initial spectral data sets the stage for rigorous quantitative analyses that

are fundamental in ensuring the quality and efficacy of the formulated nanoemulsions.

Physicochemical Properties

The pH and viscosity of the formulations were found to be within physiologically acceptable ranges, which is crucial for patient comfort and drug stability. The particle size and zeta potential results suggest that the nanoemulsions are likely to be stable over time, and their small size could facilitate better cellular uptake. These nano-scaled particles offer a higher surface area for drug release, which can improve the bioavailability of Fluconazole and Luliconazole, possibly resulting in a faster onset of action and greater therapeutic effect.

In vitro Evaluation

In vitro drug release profiles showed a sustained release pattern, which is advantageous for maintaining therapeutic drug concentrations over an extended period. This is particularly vital for antifungal and antibacterial drugs where sustained drug levels can prevent the development of microbial resistance.

Antibacterial Efficacy

The disc diffusion method provided preliminary yet valuable data on the antibacterial potential of the nanoemulsions. It is noteworthy that formulations showed varying degrees of effectiveness against both Gram-positive and Gram-negative bacteria, thereby expanding the spectrum of their potential therapeutic application.

Integrated Understanding

One of the strengths of this study lies in the integration of various analytical techniques and evaluations, which provide a holistic understanding of the system. However, it is important to acknowledge that these findings are preliminary and serve as a precursor to more advanced biological studies, including cellular uptake, cytotoxicity, and ultimately, clinical trials.

Future Research Avenues

The study opens new avenues for further research, such as exploring different surfactants or co-surfactants, varying the lipid core materials, or utilizing alternative methods like ultrasonication or high-pressure homogenization for nanoemulsion formulation. Long-term stability studies and in vivo evaluations are also requisite steps ahead.

In summary, the results contribute significantly to our current understanding of nanoemulsion systems as drug delivery vehicles, specifically for antifungal and antibacterial drugs. The comprehensive evaluation not only underscores the therapeutic potential of these formulations but also sets the stage for subsequent studies aimed at further optimization and clinical translation. This study can serve as a robust scientific platform for researchers looking to innovate in the multifaceted and ever-evolving realm of pharmaceutical nanotechnology.

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