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Formulation And Characterization Posaconazole Nanoemulgel by Using Natural Oils For Fungal Infections

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ABSTRACT:

Nanoemulgel (NEG) pharmaceutical formulations are gaining popularity because of their ability to serve both as a nanoemulsion and as a gel. These products are well-known for their ease of use, spreadability, controlled release, and ability to hydrate dry skin. Natural essential oils have been shown to promote the cutaneous permeability of topical formulations, enhancing medication safety and efficacy. Herein, we developed NEG for the enhanced permeation of posaconazole against candidiasis using Mustard oil (Mustard oil −NEG), using the gelling agents carbopol 943 and hydroxypropyl methylcellulose (HPMC). We tested various excipients to increase the solubility of posaconazole and formulate a nanoemulsion (NE). We measured the NE droplet particle size, shape, entrapment efficiency, and drug release. Furthermore, the

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physicochemical properties of the optimized nanoemulsion formulation were characterized by techniques such as Fourier transform infrared (FT-IR) spectroscopy and X-ray diffraction (XRD) analysis. The NEs were loaded into gels to form NEGs. NEGs were characterized for drug content, homogeneity, rheology, spreadability, and antifungal activity against *Candida albicans*, both *in vitro* and *in vivo*. Optimized posaconazole NEG preparations consisted of either 15% Mustard oil or 20% Arachis oil. Droplet sizes in the optimized NEs were <100 nm, and the polydispersity indexes were 0.24 and 0.26. The percentages of posaconazole released after 24 h from the clove-oil− NEG and eucalyptus-oil−NEGs were 91 ± 4.5 and 89 ± 7%, respectively. Scanning electron microscopy (SEM) showed that the NEGs had a smooth, uniform, and consistent shape and internal structural organization. The drug contents in the clove-oil−NEG and eucalyptus-oil−NEG were 98.5 ± 2.2 and 98.8 ± 3.4%, respectively. Permeation values of posaconazole from clove-oil−NEG and eucalyptus-oil−NEG were 117 ± 7 and 108.34 ± 6 μg cm^{-2}, respectively. The posaconazole NEG formulations also had higher levels of fungal growth inhibition than a marketed formulation. Finally, *in vivo* studies showed that the NEGs do not irritate the skin. Posaconazole NEG with either 15% Mustard oil or 20% Arachis oil is stable with better efficacy than posaconazole alone due to excellent dispersion, drug dissolution, and permeability and thus might be recommended for the effective and safe treatment of candidiasis.

Introduction:

Trichophyton rubrum (tinea pedis) is a fungal infection of the skin affecting millions of people worldwide. It is a keratinolytic filamentous fungal infection that invades and feeds on keratinized tissues. It is well-established as a public health concern, affecting human health and quality of life. The infection often recurs due to development of resistance to conventional treatment.[1] Minor cutaneous or subcutaneous infections have the potential to become invasive and occasionally fatal. The most prevalent type of mycosis dermatophytosis frequently affects the skin, nails, and hair. Epidemiological studies have estimated that approximately 25% of the world's population is affected by dermatophytosis, leading to one million deaths annually and severe fungal illnesses affecting one billion people. Complications in treating dermatophytosis include the deficient number of antifungals that are effective against dermatophytes and the evolution of drug resistance to these substances. The variability at the inter- and intraregional levels in view of antifungal drug responsiveness has been addressed due to resistance in many

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countries worldwide, particularly in the United States, Europe, Asia, and Australia. Resistance can emerge through various processes, including overexpression of active efflux pumps, the expression of multidrug-resistant genes, and changes in the enzyme structure and chemistry. Addition- ally, one issue with the usage of azoles is emergence of resistance to *Candida glabrata* isolates, prominently reported worldwide.

The primary medical treatment of dermatophytosis consists of topical antifungal therapy in localized and naive dermatophyte infections.4 Systemic therapies are also indicated for more extensive infections. Even though dermatophyte infections are rarely life-threatening, their chronic nature and propensity for relapse necessitate prolonged therapy, which increases toxicity risks and the emergence of drug resistance. Resistance to azoles has been reported infrequently until now.5 One of the biggest problems with the therapeutic use of azole antifungals is that they are known to have systemic and ocular toxicity.6 Besides common reversible side effects with posaconazole, severe adverse events are also reported in users, such as anaphylaxis, hepatotoxicity, endocrine dysregulation, and prolongation of the QTc interval. Posaconazole therefore comes with strong warnings in the United States, advising against using it unless other antifungal drugs are ineffective or intolerable. As a result, it is not used in many countries. Posaconazole is commercialized as a shampoo, solution, and lotion for cutaneous fungal infections. There are many studies indicating that the addition of oils from various sources enhances the antifungal spectrum and efficacy of antifungal agents including posaconazole against resistant species. These oil sources include Schinus lentiscifolius Marchand,7 Otacanthus azureus (Linden) Ronse,8 Carum copticum, Thymus vulgaris, 9 Hirtellina lobelii DC,10 Cryptocarya aschersoniana, Schinus terebinthifolia, Cinnamomum amoenum, 11 Allium sativum, 12 Melaleuca alternifolia, 13 Agastache rugosa, 14 Ligusticum chuanxiong, 15 Pancalieri, 16 and M. alternifolia. 17,18 Essential oils may be synergistic with antifungal agents by enhancing permeability, neutralizing free radicals, and increasing anti-inflammatory effects.19 Topical treatments such as cream and ointment have many drawbacks, including a lower spreading coefficient, lower penetration into the stratum corneum, and less patient compliance due to stickiness or the need to rub the product. Gels have the limitation of being unable to distribute hydrophobic medicines.20 An emulgel made from selected oils and emulsifiers could be a better topical formulation, solving drug solubility issues by making the drug available in a form that can penetrate the stratum corneum. In this scenario, a lower drug

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dose may produce more pharmacological action. Emulgels are a mixture of gel and emulsion, especially recommended for topical drug delivery of hydrophobic drugs. There are multiple advantages to these formulations, such as their ease of application, removal, and spreading, their long shelf life, and less greasy texture.21 Other advantages of emulgel formulation include their faster release of active pharmaceutical ingredients compared to creams in a more gradual and consistent manner than gels.22,23 An emulgel composed of multiple phases may be composed of a vast range of different components.24 These are highly concentrated, with oil droplets tightly packed and occupying more than 74% of the internal phase volume fraction.25 These characteristics give these emulsions a semisolid texture, easing topical application.26 It has been proven that these microgels have important roles in protecting bioactive agents, controlling the release of active molecules, templates for other particles, and filled hydrogel beads.27, 28 The emulsion is converted into a gel by the addition of a gelling agent that increases the mucoadhesive property, prolonging the contact period of the medication over the skin.29,30 Posaconazole formulation can improve efficacy through better skin permeation and skin hydration. It is also retained on the skin, giving a longer duration of action to improve patient compliance. Addition of the permeability enhancers Arachis oil and Mustard oil disrupts lipids in the stratus corneum, interacts with intercellular proteins, and improves absorption. This increases the penetration of posaconazole and is therefore synergistic in the formulation, enhancing the antifungal properties.31 Herein, we improved the permeation and efficacy of posaconazole by preparing it as an NEG using Arachis oil and Mustard oil. The nanoemulsion was characterized for droplet size, surface charge, and drug release percentage, as well as by transmission electron microscopy. Organoleptic properties of posaconazole NEGs were also investigated to determine physicochemical characteristics, homogeneity, viscosity, pH, spreadability, drug content, and ex vivo permeation. The antifungal activity of in vitro posaconazole NEGs against Candida albicans and their skin irritation potential in vivo were measured. Overall, posaconazole NEGs have improved pharmaceutical properties in treating fungal infections.

RESULTS

Identification of Drug, Calibration Plot, Melting Point, and Solubility Study:

To identify the most suitable solvent for posaconazole, we determined its solubility in a number of different solvents. We found decreasing solubility in the following order: Soybean oil >

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Mustard oil > Tween 20 > PEG 200 > span 80 > transcutol > labrasol > Cotton seed oil > olive oil > castor oil > paraffin oil > water. A calibration curve obtained with ethanol had linearity in the range of 0−25 µg mL−1. A regression of this calibration curve yielded a regression coefficient of r 2 = 0.997, slope of 0.0273, and intercept of 0.0166 (Figure 1). [8]Based on these solubility profiles of the drug in different solvents, Mustard oil and Soybean oil were selected for further use in NE formation due to having the highest solubility (Figure 2). Drug solubilities in Tween 20 (66 ± 4.5 mg mL−1) and span 80 (56 ± 5 mg mL−1) were used as a surfactant and a cosurfactant in the NE formulation. Posaconazole was identified on the basis of its appearance, and it appeared as a white, odorless powder, and the melting point was recorded at 146 °C using a melting point apparatus.[9]

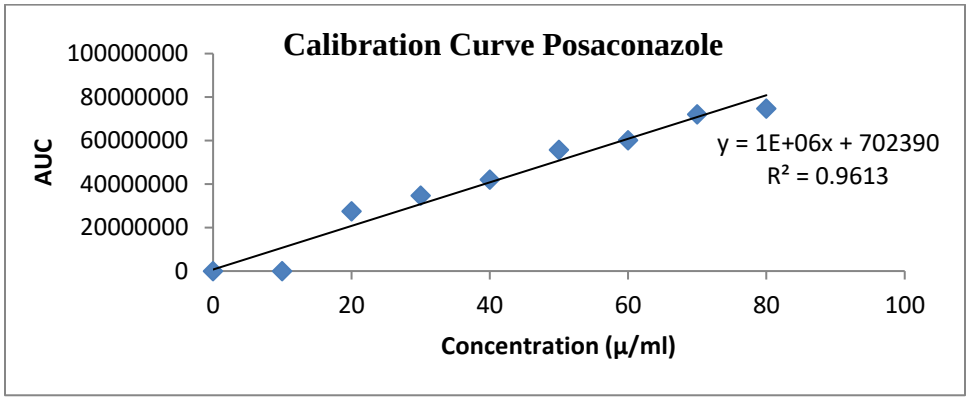

Figure 1. Calibration plot of posaconazole in phosphate-buffered saline (PBS).

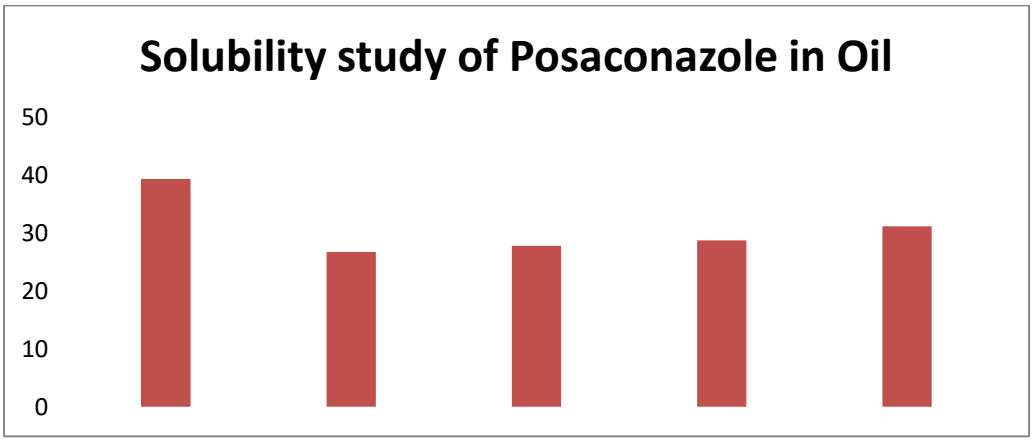

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Figure 2. Solubility of posaconazole in different solvents. Data are expressed as mean ± standard deviation (SD) (n = 3)

Pseudoternary Phase Diagram.

A pseudoternary phase diagram was constructed for the %oil, %Smix, and %water components. The most stable NE region was found for an Smix ratio of 3:1 (Figure 3A,B). At other ratios of Smix, unstable NE was produced due to opacity, turbidity, or phase separation during freeze−thaw cycles, centrifugation, and dilution test or storage. For an Smix ratio of 3:1, with a high surfactant concentration, improved thermodynamic stability of the NE occurs, probably due to a lowering of the interfacial tension, resulting in small globules possessing surface charge. [10] The optimized formulation comprised 20% Soybean oil, with an Smix ratio of 3:1 (45%) and 35% water. A second preparation of NE comprised 15% Mustard oil, with an Smix ratio of 3:1 (35%) and 50% water [11](Table 1).

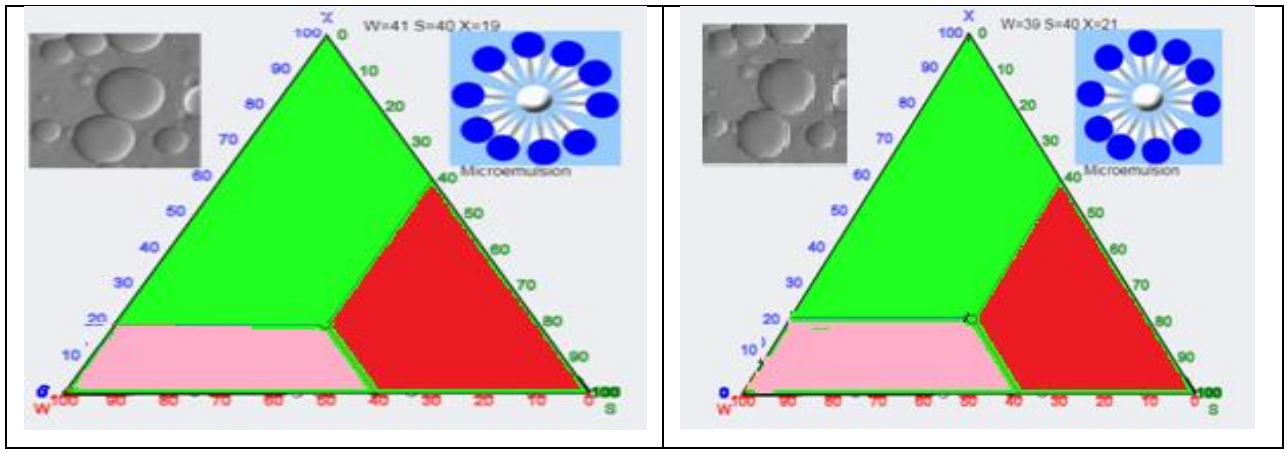

Figure 3: Pseudoternary phase diagram showing apices of the triangle of % Soybean oil, % Smix (3:1), and % water (A); and % Mustard oil, % Smix (3:1), and % water (B). Black dot point indicates the NE region.

S.No	Formulations	Amphiphilic Region %	Smix %	Aqueous Region %
1	**Arachis oil**	**25**	**45**	**30**
2	Mustard oil	**20**	**35**	**45**

Table 1: Optimized NE Formulation Composition

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Determination of Globule Size:

The average sizes of the lipid droplets in the optimised formulations of nanoemulsions (NEs) containing 20% Arachis oil and 15% Mustard oil were measured to be 67 ± 3.4 nm and 78 ± 5.2 nm, respectively, as seen in Figure 4A and Figure 4B. The polydispersity indices (PDI) of the NEs were 0.24 and 0.26, respectively. As per the International Union of Pure and Applied Chemistry (IUPAC), the term "polydispersity" pertains to the degree of heterogeneity in the distribution of particle sizes within a given sample. The PDI value often falls between the range of 0.01 to 0.7. On the other hand, a Polydispersity Index (PDI) score of 0.05 is often considered to indicate a high level of monodispersity. A PDI value over 0.7 indicates a higher degree of particle dispersion, which may render it unsuitable for size analysis with the Malvern Zetasizer. Insights into the stability of the nanoelectrospray (NE) were obtained by the measurement of the droplet surface charge, quantified as the potential. The voltage readings obtained were 19 mV when using a 20% concentration of Arachis oil and 22 mV when using a 15% concentration of Mustard oil. [12] Transmission electron microscopy (TEM) was employed to conduct a detailed examination of the size of the NE globules (Figure 5A,B). The globules exhibited a spherical shape, uniformity, homogeneity, and a state of dispersion. The dimensions of the globules observed by transmission electron microscopy (TEM) were found to be similar to those observed using the Malvern Zetasizer.

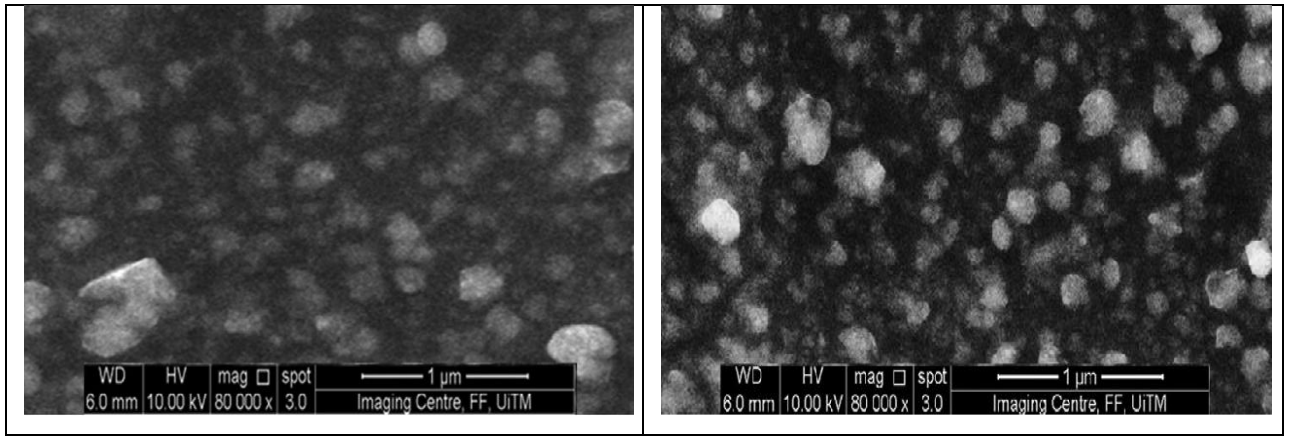

Figure 4: (A) Intensity-based size distribution curve obtained using the dynamic light scattering (DLS) technique of 20% Soybean-oil−NE. (B) Intensitybased size distribution curve of the nanoemulsion containing 15% Sunflower-oil−NE.

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Drug Gel Content: Posaconazole was analyzed at 225 nm using a UV−visible pectrophotometer, and the drug content was obtained as a percentage. Spectrophotometric analysis was performed for a standard curve of the drug. The curve was plotted between the concentration (μg mL−1) and absorbance. The total drug content of posaconazole in the formulations was estimated from a reference standard curve (y = 0.0273x + 0.0166, r 2 = 0.997) (Figure 1). The drug contents in the optimized NEG formulations containing 20% Arachis oil and 15% Mustard oil were 98.5± 2.2 and 98.8± 3.4%, respectively. [13]

Determination of Viscosity: The viscosity results of blank and drug-loaded preparations are listed in Table 6. All formulations (including blank and drug loaded) appeared to be less viscous, which is a characteristic hallmark of NE demonstrating Newtonian type flow pattern.32 According to Tukey -HSD tests, the viscosity results of all NE formulations (blank NE, DIF-loaded and DIF-IC-loaded NE formulations) were divided into four distinct subsets. Drug-loaded NEs expressed higher viscosity than the blank NE formulae. The pH values for all nanoemulgel formulations ranged from 5.63–6.42 (Table 7). The pH, viscosity and conductivity values of all nanoemulgel formulations generated statistically significant results ($p < 0.05$). [14]

Homogeneity and Spreadability: The NEG homogeneity was inspected visually by placing it in a settled position in a container. It was of uniform consistency and homogeneous, with no aggregates.17 The spreadability values of 20% Soybean-oil−NEG and 15% Sunflower-oil−NEG were between 4.12 ± 0.23 and 4.32 ± 0.17 cm. [15]

X-ray Diffraction (XRD): Posaconazole had a strong peak at 2 angles of 17.5, 19.8, 23.2, and 27.1°, demonstrating the substance's crystallinity.33 Both formulations had much less crystallinity, indicating that the medication had changed into an amorphous state that was highly soluble and capable of being dissolved and absorbed into the systemic circulation. [16]

Scanning Electron Microscopy (SEM): The surface topography of the NEGs was assessed using an EVO LS 10 transmission electron microscope (TEM) manufactured by Carl Zeiss in Brighton, Germany. The nanoemulgels formulated with Arachis oil and Mustard oil exhibited consistent, dense, and homogeneous structures without any discernible pores. [17]

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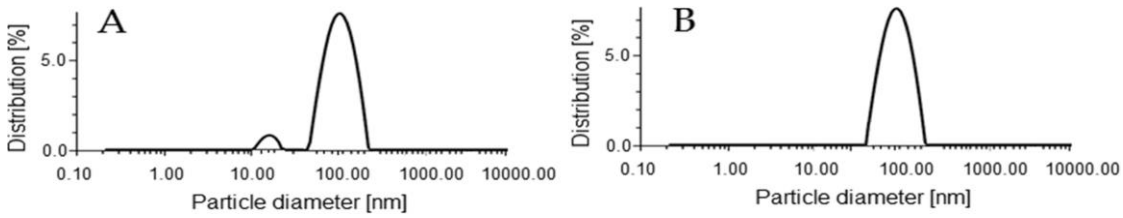

Figure 5. TEM size analysis of NE droplets. (A) TEM of 20% Soybean-oil−NE; and (B) TEM of 15% Sunflower-oil−NE.

Fourier Transform Infrared (FT-IR) Analysis: Fourier Transform Infrared (FT-IR) spectra of posaconazole and NEGs loaded with posaconazole. The drug exhibits an infrared (IR) absorption peak at 1646.12 cm-1, which can be attributed to the vibrational characteristics of a carbonyl group (stretch CO). Another IR absorption peak is observed at 1505 cm-1, which corresponds to the stretching vibration of an aliphatic ether group (C-O). Additionally, a peak at 1245.12 cm-1 is observed, indicating the stretching vibration of a cyclic ether group (C-O). Another notable peak is observed at 1200 cm-1, which can be attributed to the presence of a tertiary amine. Lastly, an IR absorption peak is observed at 810 cm-1, indicating the stretching vibration of the C-Cl bond. Sunflower. [18]

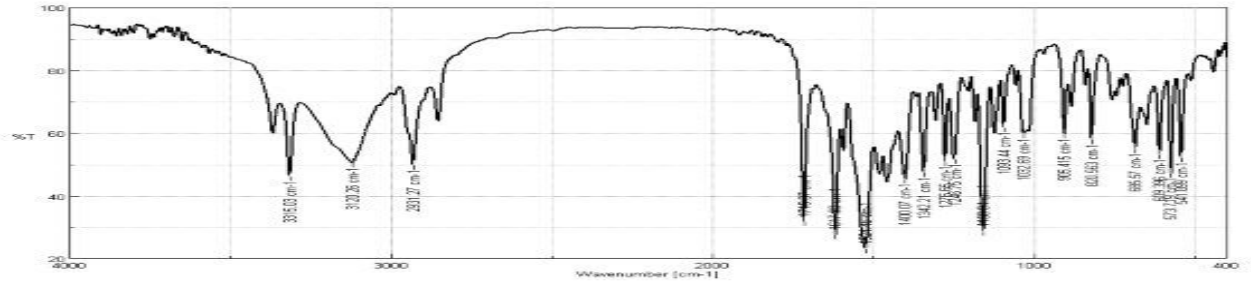

Figure 8. (A) FT-IR spectra of posaconazole

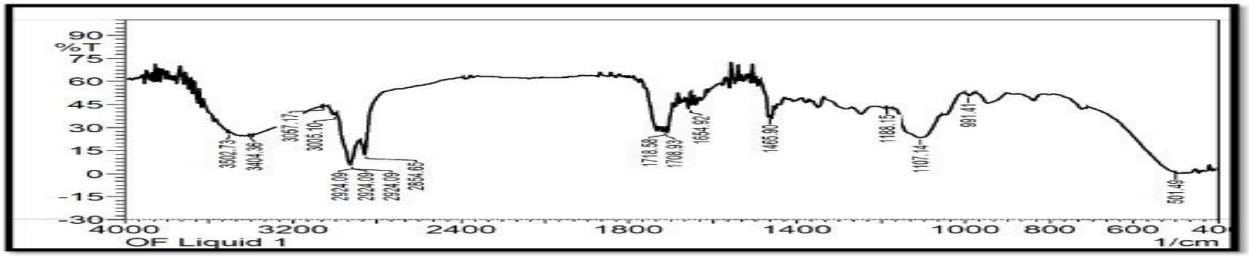

Figure 8. (B) FT-IR spectra Arachis oil -containing

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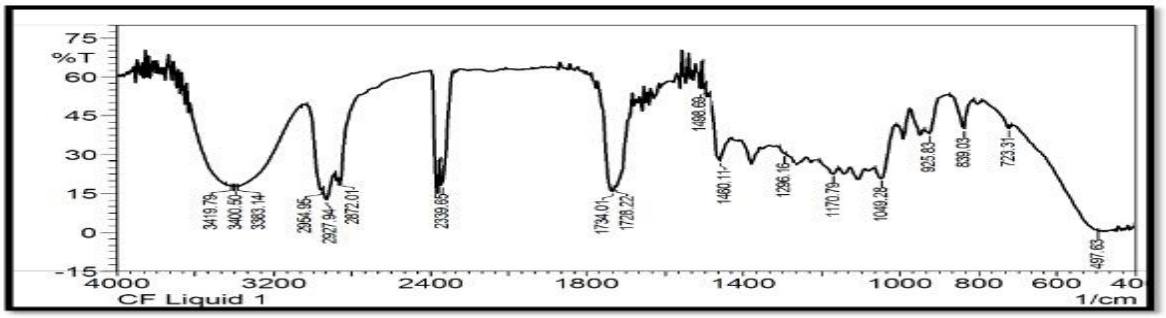

Figure 8. (C) FT-IR spectra Mustard oil -containing NEG.

Drug Release and Kinetic Profile: Percentage drug release from the nanoemulsion was compared to a pure drug. The percentage cumulative posaconazole release from retention of posaconazole may be ascribed to the poor permeation and solubility of keto-gel. No significant differences were observed between the two formulations when comparing drug retention in the different skin layers ($p > 0.05$). [19]

Formulation Code	**Zero-order kinetic**		**First Order Kinetics**		**Higuchi Model**		**Korsmeyer-Peppas Model**			**Hixson Crowel Model**	
	R2	**K_0(h^{-1})**	**R2**	**K_1(h^{-1})**	**R2**	**K_H(h-1/2)**	**R2**	**N**	**K_K**	**R2**	**K_{HC}(h-1/3)**
Arachis oil	0.988	0.200	0.827	-0.004	0.952	4.786	0.919	0.368	10.186	0.927	0.008
Mustard oil	0.998	0.195	0.859	-0.003	0.971	4.690	0.956	0.405	8.394	0.953	0.007

Table 3. Kinetic Model Fitting of posaconazole Release from Arachis oil and Mustard oil-Containing NE

In Vivo Skin Irritation: The use of a topical gel has the potential to induce irritation in both the epidermal and dermal layers of the skin. The binding of surfactants and polymers to skin tissue and proteins, such as keratin, can lead to the occurrence of acute irritation or poisoning.

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Consequently, an investigation was conducted to assess the possible adverse or toxic effects of our formulations on the skin of representative animal models. The animals were regularly monitored for dermal irritation, namely erythema (redness) or edoema (swelling). [20]

Histology of Treated Skin: Skin micrographs of the 20% Soybean-oil−NEG-treated group, the 0.7% formalin-treated group, and the 15% Sunflower-oil−NEG-treated group are shown in Figure 12A−C. Histology of formulation-treated animal skin shows the normal layer of epidermis, the subcutaneous layer, the dermis, and blood vessels. Therefore, the developed formulation was deemed to be safe for topical application due to its excellent tolerance to the skin. On the other hand, formalin-treated skin showed irritation produced on the epidermal and dermal layers with an irritation score of 2.5 (moderate erythema). [21]

In Vitro Antifungal Activity: When the antifungal zones of inhibition were examined, it became clear that the formulations that had been developed were more effective in inhibiting fungal growth. Both the Arachis oil and the Mustard oil had zones of inhibition that were similar in size, measuring 52.9 7 and 48.75 5.8 mm, respectively. The designed product's decreased zone of inhibition, which was 34.9 4 mm in size. Due to the presence of Soybean and Mustard oil in the placebo formulation, a zone of inhibition of 10 mm x 4 mm was seen. [22]

DISCUSSION

The issue of irregular drug solubility, leading to variable absorption and unclear pharmacokinetics, poses challenges for lipophilic therapeutic candidates. Various strategies have been documented in the scientific literature to improve the solubility of lipophilic medications. These approaches encompass solid dispersion, drug delivery through nanocarriers, complexation, and physicochemical modification. Oral administration of drugs carries the potential for unpredictability and limited bioavailability due to many clinical factors, including significant first-pass metabolism, enzymatic degradation, and systemic toxicity. [23] By employing a topical administration method as a viable alternative to oral delivery, the aforementioned concerns can be mitigated. One of the main challenges that a topical treatment may face is to its ability to traverse a protective, stratified skin barrier, therefore impeding its penetration into the dermis and other interconnected components of the skin. [24] The achievement of cutaneous disruption required for drug release and permeation via the topical administration route can be facilitated by

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the use of various techniques such as penetration enhancers, ultrasonic mediation, microneedles, and other related methodologies. A nanoemulsion, which is a type of colloidal preparation, consists of a heterogeneous mixture of oil and water. In this mixture, either the oil or water might act as the dispersion medium or the dispersed phase. In this particular formulation, the use of an emulsifier is employed to effectively diminish surface tension and provide stability within the solution. When comparing this system to a suspension or an emulsion, it exhibits greater durability and thermodynamic stability. Nevertheless, the practicality of using nanoemulsions in topical applications was limited due to their reduced viscosity and inadequate spreadability. [25] However, this challenge may be addressed by transforming the nanoemulsion into a suitable gelling agent, commonly referred to as nanoemulgel. The use of nanoemulgel has been shown to improve the retention and skin penetration of medicine, while also providing protection against enzymatic degradation and hydrolysis. Nonetheless, it also has notable advantages such as enhanced drug loading capacity, increased drug diffusion properties, biocompatibility, and decreased irritation. The present investigation elucidated the utilisation of Hydroxypropyl methylcellulose (HPMC), a gelling agent derived from carbopol 943, in conjunction with nanoemulsion gels (NEGs) containing posaconazole. The NEGs were formulated using Arachis oil (Soybean-oilNEG) and Mustard oil (Mustard oil NEG) as the foundation. In order to develop a robust, reliable, and effective pharmaceutical formulation, an extensive preformulation investigation was conducted on the active pharmaceutical ingredients (APIs) and their physical-chemical properties. To provide optimal conditions for therapeutically beneficial delivery systems, it is essential to determine the physicochemical properties of the medicine prior to its incorporation into the formulation. This phase entails the characterization of the physical, chemical, and mechanical properties of a pharmaceutical compound, both independently and in combination with excipients. These research provide crucial principles for the development of stable, dependable, and secure pharmaceutical formulations. The solubility of an active pharmaceutical ingredient (API) is a critical determinant in the accurate prediction of its bioavailability, dissolving characteristics, and ultimately, the efficacy of the therapeutic treatment. The process of drug development poses challenges due to the presence of poorly soluble medicines, which hinder the processes of absorption and breakdown. [26] The primary aim of preformulation research is the identification and selection of appropriate excipients based on the solubility characteristics of the pharmaceutical compound. Excipients possessing high

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solubility facilitate precise dissolution, enhance bioavailability, reduce dosing intervals, and enhance patient adherence. The existing body of literature encompasses several approaches to enhance solubility, such as the formation of salts, utilisation of cosolvents, development of solid dispersions, implementation of size reduction techniques, application of physical and chemical modifications, and incorporation of surfactants. [27] The occlusive impact of the solvent in topical administrations is contingent upon the interplay between the solubility of the excipients and their ability to penetrate the skin. The solvent solubility of the medicine must be adequate to accommodate the dosage of the drug without causing precipitation and ensuring its physical stability. The solubility of posaconazole in water is rather low. When immersed in water, the substance has the potential to undergo destabilisation, leading to potential injury or degradation. Hence, the augmentation of solubility has the potential to significantly enhance the therapeutic effectiveness and absorption at the specific site of action. Various combinations of surfactants and cosurfactants were evaluated in the NE formulations. Furthermore, we conducted a series of experiments with various Smix ratios. Among these ratios, the 3:1 ratio proved to be the most appropriate as it effectively targeted the region with the greatest NE (non-equilibrium) area on the pseudoternary phase diagram. [28] The optimal droplet size for percutaneous absorption is the following. The absorption of particles bigger than 200 nm poses a challenge for several layers of the skin. The presence of these tiny values indicates a uniform distribution of globules and a consistent preparation process. The presence of surface charges on globules can create a repulsive force between them, impeding the aggregation of globules to a sufficient extent that stable non-equilibrium (NE) structures are not formed. The negative surface charge of the NE is likely attributed to the presence of hydroxyl ions in the medium. Furthermore, the incorporation of a polymeric gel imposes constraints on the movement of globules, so conferring stability to the gel system. [29] The size distribution of the posaconazole PLGA nanoparticles mentioned before was found to be similar. One potential consequence is that bigger dispersed particles have the capacity to cause skin irritation or lead to undesirable abnormalities. Furthermore, there is a potential for the drug's absorption via the skin and subsequent release to be compromised, resulting in a diminished efficacy of the formulation. In contrast, diminutive particles have enhanced solubility and absorption rates. Hence, it is imperative that the active component is evenly distributed throughout the whole vehicle. Particle sizes below 100 nm, which are crucial for facilitating the profound penetration of topical medications into the skin, fall within the range

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deemed acceptable in the existing body of literature. The nanoemulgel was synthesised by the amalgamation of a nano emulsion with a gel matrix. The homogenous dispersion of drugs within the formulation may contribute to the drug content, while the little drug loss observed during processing suggests that the medication exhibits favourable stability in carbopol and HPMC-based gelling agents.[30] Carbopol, a cross-linked polyacrylic polymer that is soluble in water, has been extensively employed as a gelling agent in topical applications for a considerable period of time. Furthermore, it is utilised in several pharmacological and biological contexts as a stabiliser, suspending agent, and emulsifier. Triethanolamine is included into the gel in order to adjust the pH of the produced solution, which is intended to mimic the pH of human skin, either by neutralising or raising it. The preservation of the acid-base balance, reduction of cutaneous irritation, and enhancement of absorption are critical factors in maintaining the stability, safety, and efficacy of the active ingredient in topical dosage forms. Consequently, it is imperative to ensure the appropriate pH levels when formulating topical delivery to the skin. It is recommended that the pH level of skincare products should closely approximate the pH range of the skin, which typically falls between 4 and 6. The gel's viscosity, which has an impact on the dispersion and flow of medication following application and perhaps influences patient adherence, plays a critical role in drug release. Increased viscosity might potentially lead to a decelerated release of drugs, while conversely, it may impede the drainage of drugs when topically administered [31]. Furthermore, the crucial attributes of topically applied medicines encompass spreadability and gel adherence. The viscosity measurements (in Poes) of the gel indicate the presence of thixotropic behaviour within the range of shear rates from 0 to 250 (s1), whereby the viscosity of the gel decreases with an increase in shear rate. The rheological characteristics of gels can be influenced by several variables, including the kind of gelling agents, the synthesis processes employed, the molecular weight of the polymer, and the pattern of cross-linking. The rheological findings indicate that the gels formed exhibit a high degree of dispersibility with the application of light stress. The gels that were produced had a uniform, uninterrupted, and impermeable composition, as seen by the scanning electron microscopy (SEM) images of the gels. The formation exhibited unaltered IR absorption peaks, each of which has individual characteristics. Except for the amorphous condition of the drug in the formulation, it was observed that posaconazole exhibited compatibility with the excipients and did not exhibit any interactions with them. This was supported by the observation that the intensity of the peaks

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reduced in the formulations including Arachis oil and Mustard oil. The spectroscopic examination of the posaconazole-Pluronic F127 system and the infrared spectra of posaconazole-loaded nanoemulsion gels (NEGs) exhibit notable similarities. [31] The utilisation of X-ray diffraction (XRD) as a method for investigating polymorphism, which refers to the extent of crystal structure present in both the analyte and excipients within the test sample, is a valuable technique. The stability of the tested sample has been confirmed via the utilisation of X-ray diffraction (XRD) analysis. The alteration in the physical structure of a molecule has adverse effects on its solubility profile, drug dissolution, absorption, and systemic availability. [32] The X-ray diffraction (XRD) analysis revealed a significant reduction in the intensity of the prominent XRD peaks for both the 20% Soybean-oil–NEG and 15% Sunflower–NEG samples, as seen in Figure 9A–C. This observation indicates the transition of the drug from a crystalline form to an amorphous state. The formulations containing 15% Mustard oil and 20% Arachis oil exhibited an initial abrupt release pattern. For example, the formulations exhibited an initial quick release of the medication, which was afterwards followed by a consistent and moderate release for the remaining period, suggesting a controlled release pattern. The initial burst release of a medication confers advantages by facilitating the attainment of a therapeutic concentration in the early stages, followed by the sustained release of the drug to maintain a steady state over an extended duration. [33] The process of nanoprecipitation facilitates the acceleration of drug release from NE (nanoemulsion) by inducing the partitioning of the oil component into the surfactant layer, followed by its subsequent transfer into the aqueous phase. Negative ions (NEGs) have the ability to penetrate the skin layers more effectively and release their therapeutic components upon topical application due to the liberation of oil globules from the gel matrix.. 45. When considering skin compatibility and efficacy as a penetration booster, natural oil emerges as a preferable alternative to synthetic oil. The results obtained from the aforementioned experimental studies reveal the collective impacts of PEG 200, Arachis oil, Mustard oil, and a permeation enhancer. In addition, the application of nanoemulsion droplets resulted in the modification of the skin's structure through the alteration of the lipid or protein bilayer. This modification led to an increase in the fluidity of the skin and facilitated the diffusion of drugs through the cutaneous layer. The use of posaconazole nanoemulsion gels (NEGs) with Sunflower and Arachis oil leads to enhanced spreadability, stability, and skin hydration. Consequently, this promotes improved penetration into the skin. [34] The dual functionality of the formulation serves to both hydrate the

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skin and enhance the transportation of therapeutic agents to the underlying tissues, such as the dermis, muscles, and capillaries. The natural oils employed in this study are non-toxic, possess moisturising properties, facilitate penetration, and serve to prevent the loss of electrolytes and fluids, hence retaining skin tonicity. The inadequate flux seen in the pure posaconazole gel formulation can be attributed to deficiencies in drug solubility, dissolution, and penetration. [35] Shahid et al. developed a cationic nanoemulsion containing posaconazole for the purpose of topical application in the treatment of cutaneous fungal infections. According to the researchers, the scientists observed a maximum drug penetration of 95.34 ± 1.22 g cm2. Additionally, they recorded a nanoemulsion size of 239 nm. A zone of inhibition of 44.2 mm by 1.4 mm was seen in the presence of the Candida krusei fungal strain. The investigation on in vivo skin irritation found that the internally developed formulation had a high level of skin tolerance due to its biocompatibility. The results of our investigation on skin irritation align with the findings of Shahid et al. on acute dermal irritation[36]. The previous work on posaconazole-loaded polylactic nanoparticles against C. albicans and other species reported an encapsulation efficiency of only 45% for posaconazole. Furthermore, the study did not investigate the skin adhesiveness of the nanoparticles. The nanoemulgel containing luliconazole was prepared in advance for the treatment of fungal infections. The size of the dispersed globules of the optimum drug-loaded nanoemulsion was reported to be 17 ± 3.67 nm. The luliconazole-NEGs exhibited a maximum zone of inhibition of 6 mm in diameter against Candida albicans.[37] A transferosomal gel containing Posaconazole was discovered, characterised by vesicles with an average size of 126.9 ± 5.45 nm and an entrapment efficiency of 82.6%. Cumulative release of the drug amounted to 97%. The diameter of the zone of inhibition seen in the transferosomal gel was approximately 38 mm. The enhanced antifungal activity seen in our current experiment may be attributed to the incorporation of posaconazole into natural oils containing NE, followed by its transfer into the nanoemulgel for better retention. [38]

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