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OPTIMIZING NOVASOMES: IMPACT OF OLEIC ACID AND CO-SURFACTANT RATIO ON POSACONAZOLE DELIVERY: IN VITRO & EX VIVO PHARMACOKINETIC STUDY

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ABSTRACT
Background: Posaconazole, currently available as a solid oral dosage form, possesses erratic pharmacokinetics that complicate dosing regimens and increase the risk of adverse effects and drug interactions. Hence, innovative strategies, especially topical ones, are necessary to enhance the therapeutic profile and improve patient outcomes. **Methodology:** The regular 2^3 factorial design and the concentrations of Oleic acid: Span 80 (2:1) ratio, Cholesterol, and Tween 80 as independent variables were used for the formulation development. The preliminary effect was determined by dependent variables like vesicle size and entrapment efficiency; then the final optimized batch was loaded in 3 % w/w Carbopol gel. **Result and Discussion:** The optimized batch's vesicle size and entrapment efficiency were 193.34±14.84 nm and 90.03±0.11 %, respectively. These results were found statistically significant (ANOVA) in the trial version of Stat-Ease 360®. Other evaluation parameters like zeta potential and pH of all the formulations were also significant ($p<0.005$). **Conclusion:** The optimized batch (NF7), when loaded with gel (NF7G-3), showed sustained release of Posaconazole up to 7 h in an *In vitro* diffusion study facilitated 87.14±0.11 % release confirming non-Fickian or Anomalous diffusion, interpreted from Korsmeyer Peppas (K_{KP}) model. With the results from *In vitro* and *Ex vivo* pharmacokinetic release, the Posaconazole-loaded NF7G-3 Novasomal gel can exhibit potential formulation for the topical treatment of fungal infections. It will help significantly mitigate the negative effects of Posaconazole.

INTRODUCTION

Fungal infections pose a significant threat to public health, with their incidence steadily rising in recent years. Contributing factors such as the widespread use of immunosuppressive therapies, increased prevalence of immunocompromised individuals, and the emergence of drug-resistant fungal strains have heightened concerns regarding managing and treating these infections [1]. Because of the rise in fungal infections and the

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inability or delayed curative action of existing medications, it's important to adopt a novel approach that overcomes the present obstacles [2]. As per the data analyzed- Sphingosomes, Ethosomes, Niosomes, Bilosomes, Cubosomes, and Ufosomes or Ufasomes are the choices of researchers as Vesicular Drug Delivery Systems (VDDS) in the therapy of different fungal diseases because of certain advantages associated with these types of VDDS, such as improved bioavailability, improved drug pharmacokinetics, improved encapsulation, and stability, etc.[3]. Apart from these VDDS, another VDDS called Novasome, formulated by the fusion of Niosomes and Ufasomes, could be the better choice for managing fungal diseases[4]. Systemic and topical antifungal medications are part of the treatment for fungal infections. From both, topically applied antifungal drugs are typically preferred due to the hazards associated with conventional systemic therapy, such as particular organic toxic effects, interactions among drugs, and numerous other issues [5]. The researchers have tested several approaches by formulating the VDDS with some anti-fungal drugs such as Itraconazole [6], Terconazole [5], and Fluconazole [7], indicating that azoles persist as the most frequently used anti-fungal agents in medical practice [8]. Among the arsenal of antifungal agents, Posaconazole (hereafter referred to as PSZ) has emerged as a potent and broad-spectrum option, exhibiting efficacy against a wide range of fungal pathogens, including yeasts and molds. However, despite its efficacy, PSZ faces several challenges that limit its therapeutic potential. Chief among these challenges is its poor aqueous solubility, which can lead to erratic absorption [9] and variable bioavailability, particularly in patients with compromised gastrointestinal function. Moreover, the erratic pharmacokinetics of PSZ further complicate dosing regimens, necessitating frequent administration and potentially increasing the risk of adverse effects and drug interactions [9,10]. These limitations underscore the need for innovative drug delivery strategies to enhance PSZ's therapeutic profile and improve patient outcomes.

In recent years, vesicular systems originating from lipid bases, which may include Liposomes and Niosomes, gained traction as promising drug delivery Vehicles for poorly water-soluble drugs. Among these lipid-based carriers, Novasomes represent a novel and versatile platform that offers several advantages, including improved stability, biocompatibility, and controlled release properties [5–8]. The use of this approach represents the fusion of basic structural similarity with Liposomes, as well as the use of non-ionic surfactants (Niosomes). The use of unsaturated fatty acids acts with the drug delivery performed for permeation enhancement and possesses a sustained released absorption through the skin [11–14]. The formulation of PSZ-loaded Novasomes presents an innovative approach to addressing the challenges associated with conventional PSZ formulations. By encapsulating PSZ within the unsaturated fatty acid lipid bilayers of Novasomes, it is possible to improve its solubility, enhance its stability, and prolong its action. Additionally, the flexible composition of Novasomes allows for optimizing drug encapsulation efficiency and release kinetics, enabling sustained drug release and targeted delivery to sites of infection.

This investigation aimed to create and assess a topical gel based on Novasomes that contains PSZ to improve drug delivery and treatment effectiveness for fungal infections. Hence, the same goals were set to reduce side effects by formulating a topical formulation with an enhanced pharmacokinetic release profile of existing PSZ and formulating a more effective option by optimizing this formulation.

Novasomes use various methods to increase PSZ's penetration through the skin. Oleic acid alters the lipid structure of the skin, increasing permeability, and they fuse with the stratum corneum, releasing PSZ straight into deeper layers. Because Novasomes increase skin moisture, PSZ dispersion is aided by the stratum corneum swelling and opening of intercellular channels. Their sustained release mechanism sustains a concentration gradient for deeper penetration over time, and their nanoscale size and tailored surface charge assist travel through the skin. Surfactants such as Span 80 and Tween 80 further improve spreadability by lowering surface tension, and the lipid components rearrange skin lipids to open up new channels for PSZ. By combining these techniques, PSZ is effectively delivered and made bioavailable via the skin barrier.

MATERIALS AND METHODS

Materials

PSZ was accepted as a kind donation from M/s. Everest Organic Limited, Hyderabad. All other chemicals such as Oleic acid, Cholesterol, Span 80, Tween 80, Methanol, and Chloroform were used of analytical grade and received from the central chemical store of Yashwantrao Bhonsale College of Pharmacy, Sawantwadi.

Methods

Designing of Experiments

In this research, we utilize a trial version of Stat-Ease 360® (distributed by Stat-Ease, Inc., Minneapolis, MN; a subsequent of Design Expert®). We practiced the regular 3-factor 2-level (2^3) method for experimental design. A 3-factor, 2-level design provides a well-rounded method of designing experiments, enabling the investigation of nonlinear effects, incorporating intermediate factor levels, and improving modeling accuracy. To sum up, it practically balances information gain and resource efficiency.

Based on the previous literature review, three factors, including vesicle size (VS), entrapment efficiency (EE), and zeta potential (ZP), were considered for the study. The effect of these three factors depends on the concertation of independent variables such as the concentration of Oleic acid, Cholesterol, Span 80, and Tween 80.

The components that were selected to create the Novasome formulation—Span 80, Tween 80, Cholesterol, and Oleic acid—all significantly impact the composition and operation of lipid-based drug delivery systems. Oleic acid's concentration is carefully calibrated to balance membrane fluidity and stability. It functions as a fluidizing agent, augmenting the lipid bilayer's flexibility and permeability. The lipid bilayer is stabilized by Cholesterol, added at a concentration that preserves structural integrity without interfering with medication release.

A non-ionic surfactant called Span 80 influences the size and surface charge of the vesicles by helping to emulsify and stabilize them. To attain the desired vesicle size and homogeneity, its concentration is adjusted. Together with Span 80, Tween 80 is a non-ionic surfactant that stabilizes the emulsion and enhances the homogeneity and dispersity of the formulation. To guarantee the ideal surfactant balance for stable Novasome formation, the concentration of Tween 80 is adjusted. The concentrations are determined through preliminary experiments, literature review, and systematic optimization studies, guided by performance metrics of the batches from literature reviews such as vesicle size, encapsulation efficiency, stability, and release profile. These variables will perform on two levels, low and high, as shown in Table 1. As per the implication from Stat-Ease 360® software, a total of 08 runs were planned.

Table 1: Considered factors with their subsequent levels

Factors*	Independent variables conc. (mg)	(-1) Low level	(+1) High level
A	Oleic acid	100	200
B	Cholesterol	25	50
C	Tween 80 (1:1 ratio)	50	100

*Where, dependent variables will be- PS, EE, and ZP

Development Novasomes loaded with PSZ

Novasomes loaded with PSZ were prepared using a universally used thin-film hydration approach [3,12,13] with slight modification. Each batch is compiled with the ingredients per Table 2 and 100 mg of PSZ. Firstly, Oleic acid and Span 80 were mixed (in a ratio of 2:1), and then a solution (1:1-Methanol: Chloroform) containing PSZ and Cholesterol was added. The resultant mixture, with the application of a vacuum, is then transferred to the dried round bottom flask of a rotatory evaporator (Make-Superfit, Ambala). The solvent is allowed to evaporate by maintaining temp. 30±2°C at 10 mm Hg pressure using optimum speed to get the thin film. After completely removing the organic solvent (mixture of Methanol: Chloroform), the thin film was hydrated using phosphate buffer solution (pH 7.4) mixed with Tween 80 as Co-surfactant to get thick, uniform whitish dispersion of Novasomes. Afterward, the depression was sonicated (Make-Labman LMUC-3) for separation of any agglomeration, subsequently reducing the vesicle size.

CHARACTERIZATION OF PURE PSZ AND PSZ-LOADED NOVASOMES

Characterization of purity PSZ

The received sample of PSZ was identified for its purity by performing a DSC- Differential scanning calorimetric carried out at the Department of Pharmaceutical Chemistry, KLE University-Belagum using DSC-60 (Make- Shimadzu, Japan). With slight modification, the sample, which weighed about 5 mg, was enclosed into the pan (made up of aluminum) and heated up at a degree of 10^0C/min from 32–300^0C range of temperature [15].

XRD study of pure PSZ

The recognition of the crystalline or amorphous nature is evaluated by P-XRD that is X-ray powder diffraction at ICT, Jalna. An X-ray diffractometer (Make- Bruker D8 ADVANCE, Bruker) and the radiation source was copper. The scan range was 0 to 89.6242 degrees with a diffraction angle of 2 Theta. A 40

kV-voltage with 40 mA- current was used to calculate the pattern XRD.

Compatibility study

The findings of the compatibilities in the research are important because they demonstrate that the ingredients in the Novasomes formulation do not adversely interact with PSZ, preserving its stability and effectiveness. These results show that the medication maintains its therapeutic qualities and chemical stability in the formulation. Stability ensures that a medication does not lose effectiveness over time, whereas activity and a regular release profile preserve its efficacy. As a result, these findings confirm that itraconazole may be consistently delivered by the Novasomes formulation to successfully treat fungal infections without sacrificing its efficacy or safety.

The compatibility of PSZ with selected excipients was performed by analyzing the sample, mixed with a 1:1 ratio closed in glass vials, and analyzed after 15 days by keeping it at room temperature. The sample proceeded for FTIR, carried out at ICT, Jalna, using an IR spectrophotometer (Jasco FTIR-4100). The IR absorption spectrum of Posaconazole was taken from 400 cm^{-1} to 4500 cm^{-1} using a KBr disc [12]. Similarly, an XRD study observed a crystalline nature when the PSZ was combined with solid excipients. These findings confirm that PSZ may be consistently delivered by the Novasomes formulation to successfully treat fungal infections without sacrificing its efficacy or safety.

Determination of VS and ZP

Vesicle size (VS) with zeta potential (ZP) is determined at the School of Nanoscience and Technology, located at the campus of Shivaji University, Kolhapur, by using an instrument Litesizer 500 (Make Anton Par, GmbH, Austria). For the same, dilution is made with distilled water, and at 25°C with an angle of scattering 90° [6]. Statistical analysis of vesical size is done by selecting factors B, BC, C, A, and AB from right to left so that the t-value of the Pareto chart will cross the t-value limit. As per the literature, factor AC also influences VS. Hence it has also been selected to validate the hypothesis [16] as shown in Figure 3 and Figure 4.

Determination of EE

The entrapment efficiency (EE) was determined[6] with some alteration. For the separation of the oil-water phase, PSZ-loaded vesicles (2ml) were kept for centrifugation, supernatant liquid was discarded, and the remaining content was proceeded for direct vesicle lysis using Methanol as solvent. The solution is then filtered using Whatman filter paper with a pore size of 2.5 µm and washed out the residue. Dilution was made up to 10 ml of resultant solution, and the absorbance was measured spectrophotometrically with an instrument UV-1900 (Make-Shimadzu, Japan) with wavelength 262 nm. For statistical analysis, in a half-normal plot, factors B, AB, C, AC, and A from right to left such that the t-value of the Pareto chart will cross the t-value limit, as shown in Figure 3 and Figure 4. Entrapment efficiency is calculated using the following formula [16,17]

$$EE(\%) = \frac{Quantity\ of\ drug\ loaded - Quatity\ of\ drug\ after\ lysis}{Quantity\ of\ drug\ loaded} X100 \quad (1)$$

pH Determination

The pH of Novasomal dispersion was measured by directly immersing the electrode. Before measuring the pH, the digital pH meter (Make-Systronic pH 802) was calibrated by solution pH 3.0, 7.0, and 10.0. The meter was then immersed in the pH electrode in the dispersion and waited for 1-2 minutes until the reading was stable.

Optimization of Formula

After running one-way ANOVA in Stat-Ease 360® software, the optimal formulation was selected from the prepared batches based on vesicle size, entrapment efficiency, and zeta potential. The Optimized Novasomal formulation was then loaded into the gel base prepared from Carbopol 940P.

Preparation of PSZ-loaded Novasomal gel

Firstly, three concertation of Carbopol 940P grade were targeted to prepare NF7G-1 with 1% w/w, NF7G-2 with 2% w/w, and NF7G-3 with 3% w/w as the gel base, with 0.1 % w/w sodium benzoate will serve as preservative[6][16]. Triethanolamine was used to adjust the gel's pH. The prepared gel base was observed physically regarding its consistency and viscosity. It was decided to load the optimized Novasomal formulation, NF7, in gel base-3 (NF7G-3). Simultaneously, the plain gel of PSZ (1% w/w) was prepared with the same concertation of Co-surfactants for the comparative study.

Characterization of NF7G-3

pH and Viscosity Determination

A digital pH meter (Make-Systronic pH 802) was used for the pH determination of NF7G-3 [6]. The Viscosity of NF7G-3 was

measured by an Ametek Brookfield viscometer (Model-LV DVE, Ametek Brookfield Engineering Labs., Inc. USA). The prepared NF7G-3 was placed in an appropriate container and set beneath the viscometer, which was brought down to submerge the spindle up to the spindle shaft's immersion mark. 20 rpm and the use of spindle number 64 was employed for plain drug-loaded gel and 100 rpm for NF7G-3 gel, based on the reading of trial-and-error runs to determine the viscosity [16].

NF7G-3 Drug Content Determination

The drug content is revealed with minor alternation, where the 1 gm gel was collected from different regions across the container and diluted by methanol. It was then filtered to remove traces of gelling agent, if any, by using Whatman filter paper with a pore size of 2.5 µm with Methanol washing, and then the volume of the filtrate was adjusted to 10 ml. After suitable dilution. The sample is analyzed spectrophotometrically with an instrument UV-1900 (Make-Shimadzu, Japan) with a wavelength of 262 nm using the following formula;

$$DC(\%) = \frac{Concetation\ obtained\ after\ analysis}{Theoritocal\ concentration}\ X\ 100\ \ldots..\ (2)$$

Diffusion Study by In vitro Method

An in vitro diffusion study was performed using a Franz diffusion cell (Make-Local) with a volume capacity of 40 ml. The receiver compartment was loaded with phosphate buffer (pH 7.4) solution while the donor compartment comprised NF7G-3 equivalent to 2 mg of PSZ placed on the eggshell membrane [18]. An assembly was maintained at the temperature of 37±0.5^{0}C. The study was carried out up to 7 h, where, at the predetermined time interval, 1 ml sample was removed and analyzed at 262 nm spectrophotometrically after dilution. By replacing the same quantity of buffer solution after the removal of the aliquot, the sink condition was maintained [16]. The same process has been repeated for NF7 dispersion and PSZ-loaded plain gel.

Permeation Study by Ex vivo Method

The goat skin (abdominal area) was selected for an ex vivo diffusion study as per literature, acquired from a local slaughterhouse, and free from any evidence of damage or lesions. Then, to eliminate any traces of dirt, the skin was rinsed using buffer and trimmed by using scissors as well, and excess fat was removed [16]. Before placing the NF7G-3 gel on a prepared skin specimen, the receiver compartment was filled with phosphate buffer (pH 7.4). Thereafter, the skin is fitted between the donor-receiver compartment; afterward, an optimized NF7G-3 equivalent to 2 mg of PSZ is applied, and a phosphate buffer with approximately equal amounts of gel is added to initiate the passage for flow. Further evaluation was replicated, as mentioned above, in the section diffusion study using the *in vitro* method. In the present investigation, the steady-state flux (J_{max}) was calculated to evaluate the drug diffusion measurement and investigate the *ex* vivo permeation of NF7G-3 gel across the membrane [16].

RESULTS AND DISCUSSION

Pure PSZ Characterization

Curiously, two distinct endothermal events were arising between 100°C to 150°C, while the melting point of pure PSZ was 170.1±0.5°C. The presence of the earliest peak is concerned with a nematic-like phase transition, whereas the subsequent peak at 172.26°C possesses a sharp trough correlated with the melting point of pure PSZ, as shown in Figure 1. This confirms the purity of the obtained PSZ molecule.

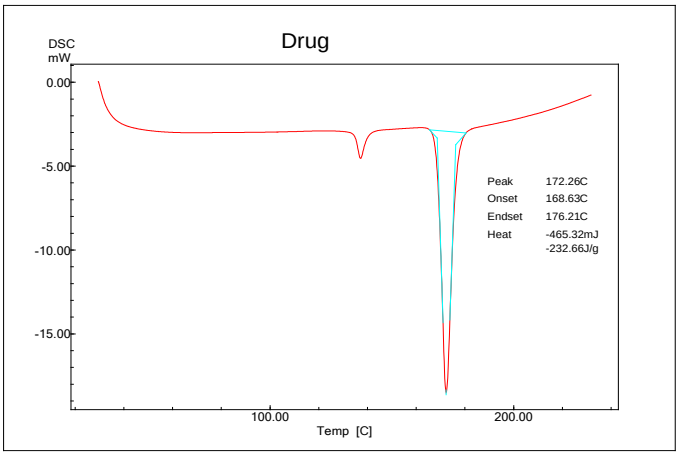

Figure 1: DSC thermograph of Pure Posaconazole

XRD study of pure PSZ

Figure 2 shows the XRD Pattern of the pure drug. The result shows characteristics peaks at 10.00, 11.050, 12.800, 15.200, 15.600, 17.600 (high intensity), 25.200, 26.000, 27.200, 29.200, 43.210, and 43.610, which confirms the crystalline nature of Posaconazole.

Compatibility study

The major peaks (in cm^{-1}) for pure PSZ for IR studies were found at, 2836.77, 1683, 1016.30, 1370.17, 1231.33, 1134.90, 1087.66 correlates with frequencies of major functional groups such as Stretching (O-H), 3 amides stretching (C=O), Fluro compound(I-F), Aromatic amine Stretching (C-N), Alkyl-aryl

ether stretching (C-O), Stretching (C-O), Stretching 2 alcohol. A similar pattern of frequencies was found at 2836.77, 1683, 1016.30, 1370.17, 1231.33, 1134.90, and 1087.66 representing the same functional group can be observed in Figure 2 [19]. No significant changes were observed from most peaks obtained in XRD studies compared to pure PSZ [20]. These two results highlight that the selected drug PSZ is compatible with the selected excipients.

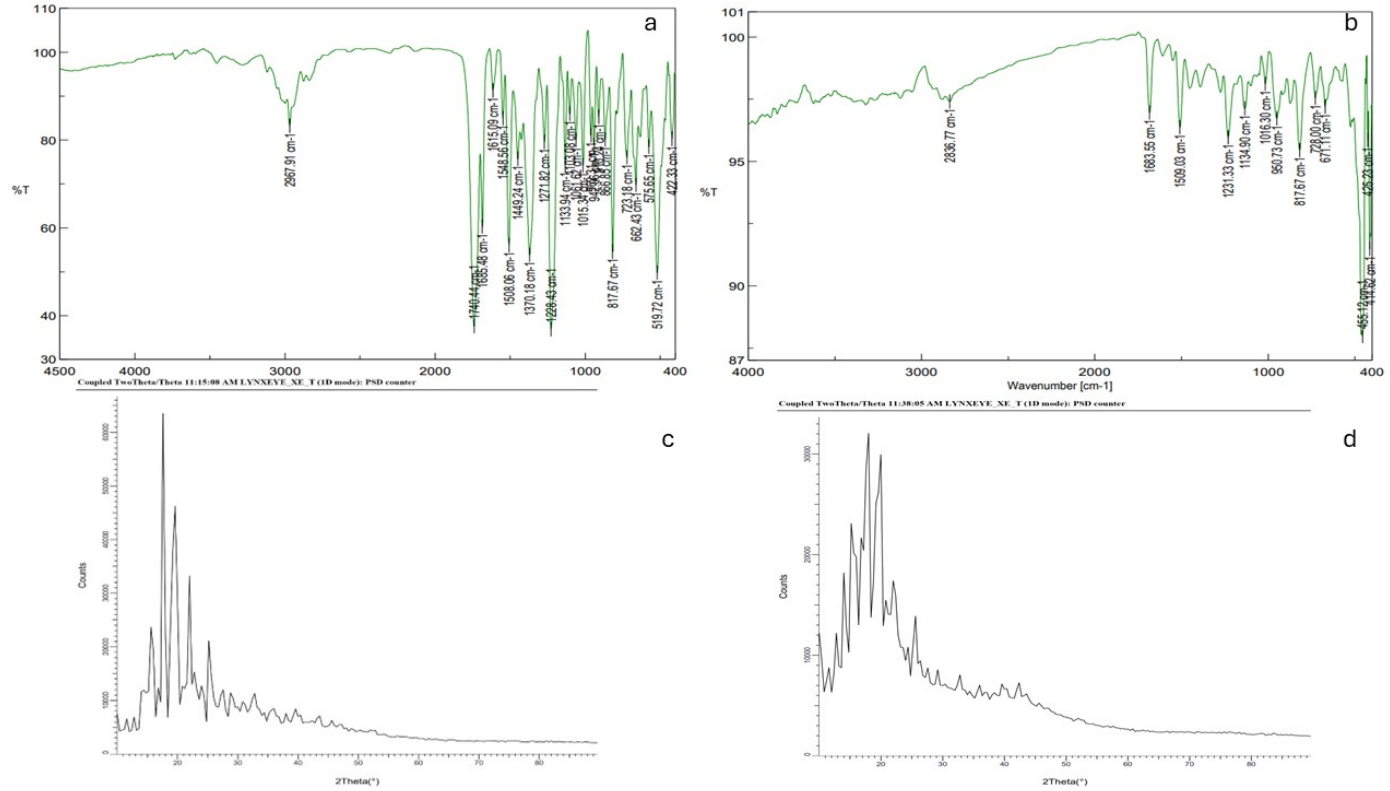

Figure 2: (a) FTIR of pure PSZ, (b) FTIR of PSZ with excipients, (c) XRD diffractogram of pure PSZ, (d) XRD diffractogram of pure PSZ with excipients

Table 2. Compositions of Novasomal formulation and the summary of responses (n=3) for respective batches

Formulation ID#	Factor A	Factor B	Factor C	Response 1-EE (%)	Response 2-VS (nm)	Response 3-ZP (mV)
NF1	-1	-1	-1	31.22±0.79	64.87±12.81	-23.56±1.1
NF2	1	-1	-1	53.09±0.08	146.32±13.99	-29.18±0.4
NF3	-1	1	-1	77.83±1.93	437.8±18.37	-25.8±0.8
NF4	1	1	-1	54.07±0.07	427.45±21.77	-38.00±1.6
NF5	-1	-1	1	42.33±0.57	73.78±31.50	-24.23±1.1
NF6	1	-1	1	86.07±0.82	180.94±25.80	-35.8±1.8
NF7	-1	1	1	90.03±0.11	193.34±14.84	-32.83±0.7
NF8	1	1	1	85.35±0.14	218.84±16.42	-48.03±1.0

All values are expressed in Mean±SD

#100 mg of PSZ is added in every formulation, and the ratio of Oleic acid: Span 80 (2:1) is kept constant in the hydrophobic phase.

Optimization of the designing of experiments

The nanoscale vesicle size, high entrapment efficiency, stability, and suitable surface charge of the improved Novasome formula for PSZ are essential for efficient skin administration. A significant dosage of PSZ is guaranteed to be available at the target site due to the high entrapment effectiveness (more than 70%) and tiny vesicle size (usually preferred less than 200 nm), which enable improved penetration into the stratum corneum.

PSZ therapeutic levels are sustained over time by stability and a regulated release profile, which improves efficacy and lowers application frequency. Optimized surface charge (in the range of ± 30mV) and lipid composition improve adhesion and fusion with the skin, facilitating better drug penetration and retention. Components like Oleic acid and surfactants enhance skin hydration and reduce surface tension, further aiding PSZ delivery. These optimized characteristics collectively contribute to PSZ's improved bioavailability and therapeutic outcomes.

Utilization of a trial version of Stat-Ease 360® software allowed for optimizing the impact of independent variables. A total of 08 possible combinations of Novasome formulations were prepared, as shown in Table 2, and the results underwent analysis of variance testing (ANOVA). We computed regression polynomials for the dependent variables (VS, EE, and ZP). This research aimed to determine the effect of independent variables on dependent variables (VS and EE). Hence, only these variables (responses) are discussed in detail.

Impact on VS of selected variables

The VS of formulation NF1 to NF8 was found in the range of 64.87±12.81 nm to 437.8±18.37 nm. The 3D response surface plot as shown in Figure 3, confirms the influence of independent variables Oleic acid (A), Cholesterol (B), and Tween 80 (C), which affects the vesicle size. The quadratic equation for the same is shown as follows:

VS = 217.9175 + 25.47 (A) + 101.44 (B) - 51.1925 (C) - 21.6825 (AB) + 7.695 (AC) - 62.075 (BC) ….. (3)

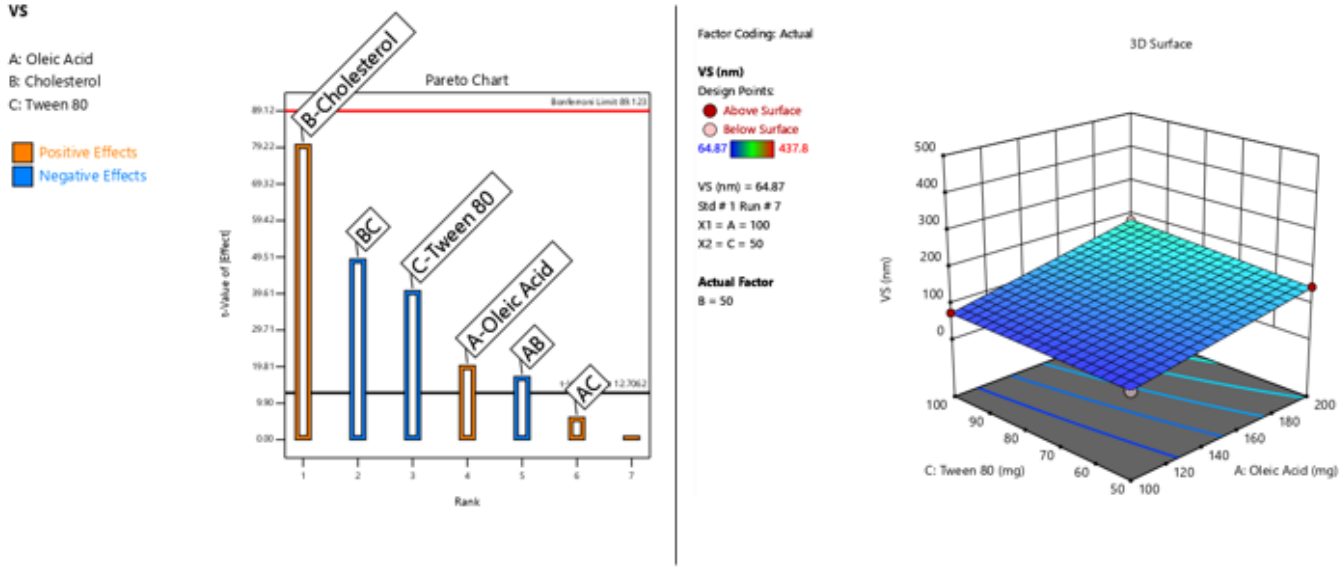

Figure 3: VS Pareto chart for selected variables and its 3D response plot

The positive value of factor A (Oleic acid) and Cholesterol (B) indicates that an increase in concertation of these factors could increase the vesicle size, while the negative value of factor C (Co-surfactant) indicates the negative impact on VS. Alone of these factors couldn't give desirable vesicle size hence to be used in combination [6,16–18], at the optimum concertation of PSZ: Oleic acid: Co-surfactants in the ratio 1:1:1 give the vesicle size about 193.34±14.84 nm, which is in the appropriate range for better penetration [11]. The vesicle size depends on the ratio of Oleic acid to Co-surfactant. The concertation of Cholesterol shows a negative effect on vesicle size; it could correlate with the fact that a decrease in the concentration of Cholesterol could result in the leakage of the drug molecule, leaving behind the macromolecular size Oleic acid dispersion in phosphate buffer solution. Our study also correlates with the fact-finding by Kandadi Prabhakar *et al*. that, in combination with Tween 80, Oleic acid is responsible for decreasing the vesicle size.

Impact on EE of selected variables

The entrapment efficiency of formulation NF1 to NF8 was 31.22±0.79 % to 90.03±0.11 %. The EE is also dependent on the factors Oleic acid (A), Cholesterol (B), and Tween 80 (C) along with the proper combination. The selection of factors for ANOVA is shown in Figure 4, along with its 3D response. The quadratic equation for the same is shown as follows:

EE = 64.99875 + 4.64625 (A) + 11.82125 (B) + 10.94625 (C) - 11.75625 (AB) + 5.11875 (AC) ….. (4)

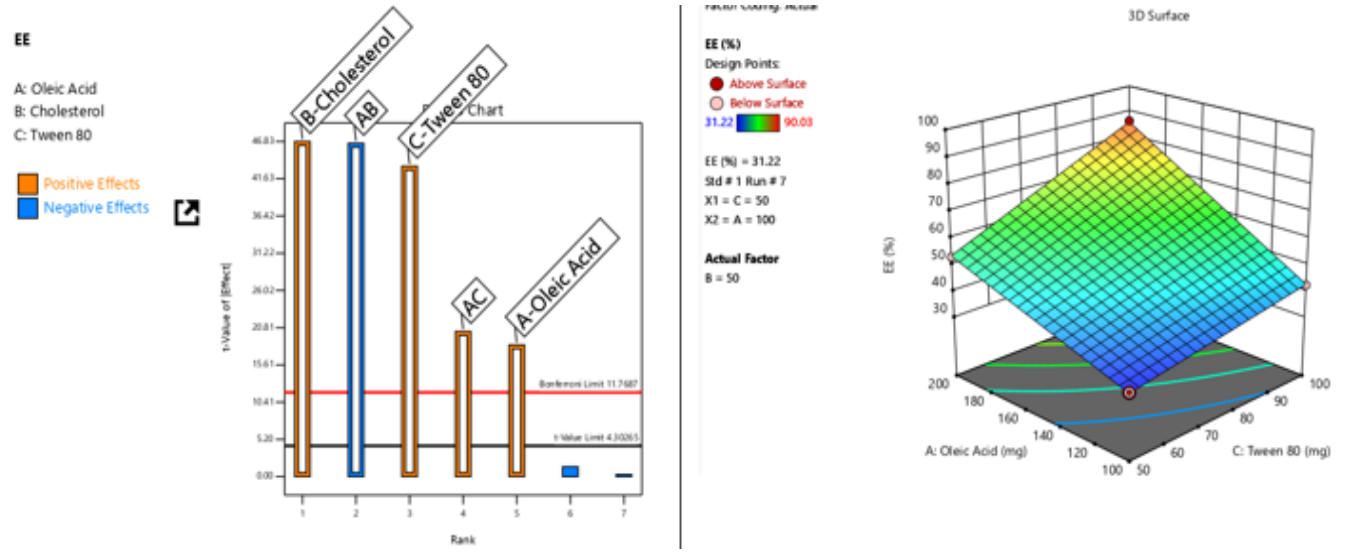

Figure 4: EE Pareto chart for selected variables and its 3D response plot

The positive values of factor A (Oleic acid), cholesterol (B), and co-surfactants (C), along with a combination of AC, have a positive influence on EE. Using only a combination of AB could negatively affect EE. This is because, physically, Oleic acid (A) is in a liquid state; the role of cholesterol (B) in the formulation of vesicular delivery is that it prevents the leakage of the drug from the vesicular bilayer. After hydration with phosphate buffer, the Oleic acid forms an oil-in-water dispersion called a nanoemulsion. To stabilize this, a co-surfactant in optimum concentration is needed that may reduce the interfacial tension and form a uniform dispersion of vesicles in a phosphate buffer solution. The absence of a Co-surfactant disturbs the vesicular bilayer and may result in leakage of the drug; hence, entrapment efficiency could be decreased [21]. As per sufficient clinical evidence obtained by Shaimaa Mosallam *et al.*, the entrapment efficiency of Oleic acid was found to be 99.45 ± 0.78%, which is highly correlating to our findings, i.e., 90.03±0.11 % [5] in addition, Radwa M. A. Abd-Elal claims that an increase in ratio beyond 1:1 decreases the entrapment efficiency because the dispersion media may contain mixed micelles, which raises the drug's solubility.

With slight modification in the method, we have added a 1:1 ratio of Tween-80 as a Co-surfactant with Oleic acid, which significantly balances the micelles and may attract the unentrapped traces of drug molecules, enhancing entrapment efficiency. It also supports the study done by Kaur N. et al., who obtained the maximum entrapment efficiency at the ratio of 4:1 and correlating to our study, as well as the clinical efficiency of formulation proven by Shaimaa Mosallam *et al.* [5].

Effect on ZP of selected variables

The ZP of the formulation was determined in the range of -48.03±1.0 to -23.56±1.1 mV for the prepared batches. The optimization for zeta potential was also done in the same way (data not shown), and the results were found significant ($p < 0.05$). As per the literature review, ±30 mV or higher values of ZP signify the stability of formulations; it is expected that zeta potential values must fall between. The optimized batch NF7 had a negative zeta potential of -32.83±0.7 mV as well, and all values are within the permissible range, indicating stability of the formulation and correlating the fact that surfactants often have a minor negative charge caused by ionization of free fatty acids [16].

Effect on pH of selected variables

The measurement of pH values falls in the range from 6.92±0.03 to 7.5±0.02, which is in the permissible range for topical preparation and can be considered an optimum [22-24]. From the data analyzed, batch NF7 was considered the optimum batch with PS 193.34+14.84 nm, EE about 90.03+0.11%, and ZP -32.83+0.7 mV because all values were found in the permissible range. Hence, the batch was selected as the optimum to be loaded into the gel base to improve its stability.

Study of PSZ-loaded Novasomal Gel

pH, viscosity, and drug content

The pH of prepared NF7G-3 is around 7.19+0.23 (n=3). A slight shifting of the pH may be because of the free carboxylic group present in the final formulation derived from Oleic acid. But the pH is in the acceptable range [22,23]. The viscosity (n=2) of the

drug-loaded gel was calculated as 6295±49.49 cp, while for NF7G-3, it is around 5889±4.42[16]. The content (n=3) was found to be 98.54±1.27 %.

Diffusion study by In vitro Method

Gel NF7G-3 exhibited 87.14±0.11% diffusion of the drug throughout the 7 h study. However, NF7 Novasomal dispersion diffused about 51.25±0.81 % of the drug throughout 4.5 h, while the plain drug-loaded gel diffused only 37.45±0.99 % within 5h, as shown in Figure 5. With this study, the PSZ-loaded Novasomal gel was diffused quite sustainably. This happened due to the use of permeation enhancers, which not only help with penetration but also aid as a depot intended for the slow delivery of dermally employed formulation in a controlled manner [25]. The release of the entrapped drug correlated with the information that the lipophilic drug PSZ, having a 4.66 log P value, was mainly integrated amongst the fatty acid chain from the vesicle's bilayers, which are responsible for the release of the drug [16]. With the research we have conducted, we have applied various mathematical modeling as shown in Table 3, and found that the Korsmeyer Peppas model is best suited for our study (1.0<n>0.5), indicating drug release follows diffusion and then dissolution that is Anomalous (non-Fickian) diffusion [26].

Permeation Study by Ex vivo Method

Ex vivo skin diffusion tests are frequently employed to simulate the human topical absorption process. Goat skin was used for *ex vivo* permeation study in this respect, and the findings are shown in Figure 5 and Table 4. The NF7G-3 gel formulation noticeably improved PSZ permeation. Transdermal flux and penetration of PSZ occurred in the following order: NF7G-3>NF7>plain gel. There can be a few possible explanations for this observation, which include formulation of vesicular drug delivery (Novasomes) technology may aid in drug penetration into the membrane, as well as Tween 80 was commonly used as a solubilizing agent in the formulation of Novsomes, which increased the diffusion of the drug [16].

Table 3: Outcomes of *In vitro* drug diffusion data modeling and fitting to several mathematical models

Models	Parameter	Batches		
		PSZ loaded gel	NF7	NF7G-3
Zero-order	K_0	7.372	11.957	14.847
	R^2	0.9613	0.9658	0.9532
First Order	K_1	0.086	0.155	0.254
	R^2	0.9529	0.9819	0.9360
Higuchi Model	K_H	0.8308	0.8999	0.8760
	R^2	13.523	21.091	31.510
Korsmeyer Peppas model	K_{KP}	7.302	14.577	18.990
	R^2	0.9613	0.9788	0.9662
	n	1.007	0.837	0.845

Table 4: Outcomes of *Ex vivo* permeation study (flux determination)

Batches	Flux (µg/cm²/h)	Partition coefficient (Kp) (cm²/h)
PSZ loaded gel	3.79±0.06	$2.83 \times 10^{-3} \pm 2.95 \times 10^{-5}$
NF7	5.63±0.05	$2.81 \times 10^{-3} \pm 2.65 \times 10^{-5}$
NF7G-3	6.64±0.01	$3.32 \times 10^{-3} \pm 5.06 \times 10^{-6}$

All values are expressed in Mean±SD

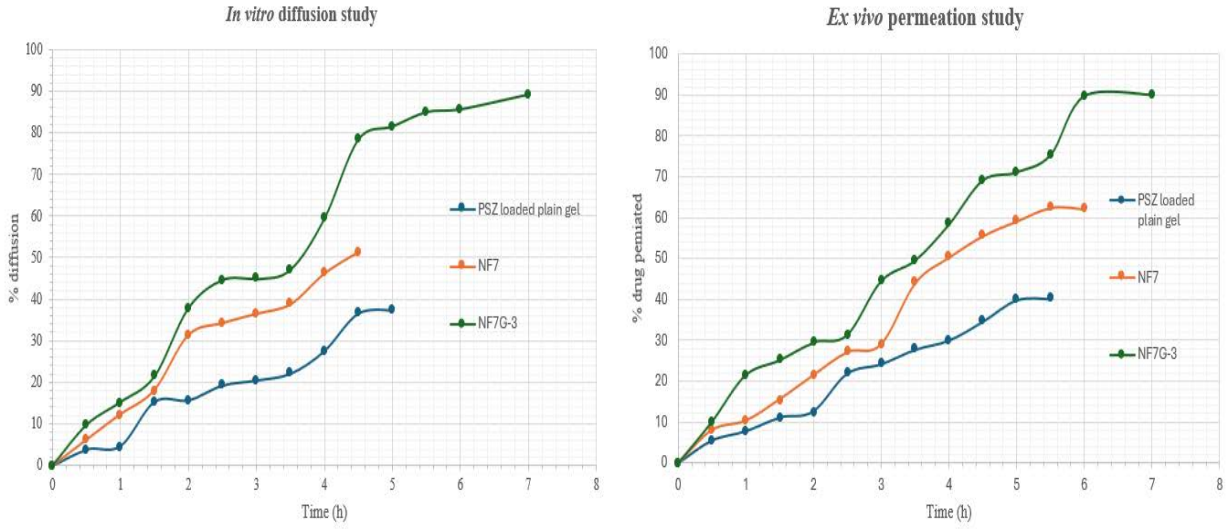

Figure 5: *In vitro* diffusion and *Ex vivo* permeation study (7 hour study)

CONCLUSION

The study examined the effects of three independent factors on the vesicle size (VS), entrapment efficiency (EE), and zeta potential (ZP) of different formulations containing oleic acid (A), cholesterol (B), and tween 80 (C). The diameters of the vesicles varied from 64.87 nm to 437.8 nm; Tween 80 reduced the size, while oleic acid and cholesterol increased it. All three criteria, especially when combined, had a beneficial influence on the entrapment efficiency, which ranged from 31.22% to 90.03%. Stable formulations were indicated by zeta potential values ranging from -48.03 mV to -23.56 mV. Batch NF7 was chosen for additional gel research due to its good pH, viscosity, drug content, and ideal VS, EE, and ZP values. In contrast to other formulations, the NF7G-3 gel showed prolonged drug diffusion and improved penetration. The 7 h release study of NF7G-3, compared with the simple gel made with PSZ alone, revealed a longer PSZ release. This investigation concludes that adding Co-surfactant and Oleic acid-unsaturated fatty acid as carriers may have contributed to PSZ's permeability across the skin layers. According to the study, an improved PSZ-loaded Novasomal gel may be a superb option and a promising carrier for topical fungal infection treatment that can effectively transport poorly soluble medications like PSZ. By trailing all analyzed data, we could conclude that the ratio of Drug: Oleic acid & Span 80 (2:1): Tween 80 exhibits desired results in the ratio 1:1:1. With this prepared formulation NF7G-3, it could be possible to treat the widely spreading fungal infection by topical application of PSZ-loaded topical Novasomal gel (as no marketed formulation available), with proven *In vitro* as well as *Ex vivo* pharmacokinetic release and can minimize the adverse effect of PSZ. Hence, with these positive results of *In vitro* diffusion and *Ex vivo* permeation, we have planned to perform an *In vivo* study to determine the efficiency of prepared formulation against fungal infections like Acne or Psoriasis or Candidiasis as a subsequent part of this study.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

Prashant Pingale examined and interpreted the experiment's design and associated data. Tushar Rukari conducted the laboratory tests and documented the observations, while Chandrashekhar D. Upasani oversaw the work and helped with the final manuscript drafting. Every author read and approved the final manuscript.

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