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Optimization and evaluation of itraconazole-loaded nanostructured lipid carriers incorporated gel for topical drug delivery

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ABSTRACT: Nanotechnology is a relatively new technology that creates a lot of possibilities for smart drug manufacturing and delivery techniques. Nanostructured lipid carrier (NLC) is a significant advantage over the other drug delivery system. NLC-employed gel (Nanogel), which is composed of hydrophilic polymer network-based nanoparticles, ranges from 10 nm to 1000 nm. Because of its great loading capacity, high stability, extended contact period, and thus extended therapeutic impact, nanogel has been used in topical administration. In this work, Itraconazole has been chosen as an API. With the use of Central Composite Design, NLC has been prepared with two variables. They are combined with solid lipid and liquid lipid, and with the aid of a surfactant and are stabilized in aqueous dispersion. The Hot homogenization technique was adopted for the preparation of the same. The mixture was homogenized for 20 minutes at a speed of 2000-8000 RPM which was afterwards sonicated . The formed NLCs were incorporated into hydrogel using Carbopol 934 P as a gel-forming agent. Particle size was measured with the help of the Dynamic light scattering (DLS) method. The results revealed that the particles' were in nano range (54nm – 488nm). Out of 39 optimized combination found through Design of Expert software, the matched prepared formulation (NF 3) was subjected to in vitro drug diffusion study, it was found that drug diffusion from that formulation steadily increased and remained constant after 5 hours. Drug diffusion was almost 89.15% in 7 hours. Following an ex vivo permeation investigation on goat skin of the same , it was seen that the permeation of Itraconazole through goat abdominal skin from the optimized product was around 51.67% in 7 hours.

KEYWORDS: Nanostructured lipid carrier; Topical delivery system; Hot homogenization; Nanogel; DLS; In vitro drug diffusion; Permeation.

1. INTRODUCTION

Nanotechnology has essentially impacted all technical sectors, including pharmaceutics, over the past 20 years. According to industry estimates, about 40% of lipophilic drug candidates cannot be processed because of solubility and formulation stability problems, although these problems have been resolved by a variety of cutting-edge and new lipophilic drug delivery techniques [1]. The limited physical stability of the dispersions, drug leakage, poor targeting, non-specific clearance by the mononuclear phagocytic system, and challenges with scaling up were major barriers to the development of liposomal formulations. Solid lipid microparticles were created by spray drying, and "Nanopellets for peroral administration" were created by Speiser and colleagues.

The creation of solid lipid nanoparticles (SLNs) at the start of the 1990s combined the benefits of solid particles, emulsions, and liposomes. SLNs, which have a size range of 1 to 1000 nm and are solid, sub-micronic colloidal nanocarriers made of physiological and biodegradable/biocompatible lipids, are effective in incorporating both lipophilic and hydrophilic pharmaceuticals into the lipid matrix in significant amounts. The possibilities for medication delivery using nanocarriers are infinite, and they have recently received attention due to their immense potential [2]. According to the National Nanotechnology Initiative, nanotechnology is the study and application of structures with a size between 1 and 100 nm. Similar to medicine, nanotechnology's overarching goal is to diagnose as precisely and promptly as possible and to cure as effectively and painlessly as possible. Another cutting-edge drug delivery method with high site-specific

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targeting and controlled drug release is composed of polymeric nanoparticles and polymer nanocapsules constructed from non-biodegradable and biodegradable polymers [3].

Nanotechnology is a relatively new technique that provides vast scope for a smart drug delivery and drug manufacture (Nanomedicine) approach that involves the design, synthesis, and characterization of materials or molecules and devices that perform well at the nanoscale. The effectiveness of SLNs as drug delivery systems for a range of administration techniques, including cutaneous, topical, parenteral, and oral delivery, has been well studied. The release characteristics of the SLNs produced after the creation of solid lipids partially crystallize in high-energy modifications with numerous flaws in the crystal lattice are influenced by the crystallinity of the solid lipids. If a polymorphic shift to low-energy modification takes place during storage, the integrated medicine may be expelled from the lipid matrix. There is a higher chance that many commercial goods will be delivered via SLN. However, this system has a **few drawbacks** as well:

- The payload is too low for most medications.
- Expulsion of drugs during storage, and
- High water content in the dispersions of nano lipids.

Professor R.H. Müller (Germany) and Professor M. Gasco (Italy) developed solid lipid nanoparticles (SLNs) in the early 1990s as a novel formulation with several advantages, including the use of biocompatible lipids, the use of few organic solvents during formulation, high in vivo stability, and a broad application spectrum.[4] SLNs are colloidal particles made of solid lipids (at room and body temperatures), surfactants, active ingredients, and water. Nonetheless, SLNs have drawbacks such as low drug loading capacity, unexpected gelation, polymorphic transitions, and drug leakage during storage [4][5][6].

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), in particular, have drawn a lot of attention lately as effective, biodegradable, biocompatible, and non-toxic carriers with a variety of suitable properties for dermal application of cosmetics and medications [7]. A multitude of surfactants work together to stabilise the "solid lipid" core of SLN emulsion spheres, which have an average diameter of 10–1000 nm [8]. In order to prevent the solid lipid matrix from forming into a perfect matrix of solid lipid crystals during storage and to improve the absorption of active ingredients, the liquid lipid component in NLC works to overcome the shortcomings of SLN [9]. NLC has an irregular crystalline structure that allows more space between the lipid chains and the matrix due to the mixture of liquid and solid lipids that make up NLC. [10].

Solid lipid nanoparticles (SLNs) are made of lipids that melt beyond 40 degrees Celsius, allowing them to change the release profile in vivo [11]. In SLNs, the medication is primarily disseminated in molecular form. This configuration introduces numerous flaws into the matrix, allowing for the accommodation of additional medicines. Because NLCs' matrix is made up of a combination of spatially dissimilar lipid molecules typically a mix of solid and liquid lipids, it has more imperfections than SLN, which allows it to hold more drug molecules. Even though the NLC matrix contains liquid lipids, it is solid at a body or room temperature. NLCs combine liquid and solid lipids and control the concentration of the liquid lipid to maintain their solid state. Because of the solid matrix, NLCs, as opposed to emulsions, can more successfully immobilize drugs and prevent the particle from coalescing. NLCs share many of the advantages of SLNs, including low toxicity, biodegradation, drug protection, progressive release, and the avoidance of the use of organic solvents in manufacturing. In NLCs, a mixture of solid and liquid lipids is employed, and because of their structural peculiarities, they do not fit together very well to produce a flawless crystal [12][13]. In recent years, researchers have focused on NLCs as an alternative to SLNs, polymeric nanoparticles, emulsions, microparticles, liposomes, and other nanoparticles [14].

A mixture of solid and liquid lipids is used to create the second-generation lipid composition known as nanostructured lipid carrier (NLC) [15]. Since the lipids used to prepare NLCs to mimic skin and sebum, they are biocompatible and low-risk [16]. A promising DDS that can extend the delivery of liposoluble medicines, increase their stability, and reduce their systemic toxicity is nanostructured lipid carriers (NLC) [17].The traditional mode of administration for the treatment of cutaneous conditions like acne, aging, or other cutaneous diseases like skin inflammation is topical drug delivery [18]. Numerous scientific publications have discussed NLC's capacity to regulate the rate of medication absorption via the epidermis, which prevents undesired active absorption into the bloodstream. The NLCs' smaller size ensures proximity to the Stratum corneum and can improve the active compound's skin penetration. On how nanoparticles penetrate the skin, researchers have agreed [19]. Drug particles concentrate in follicular casts, followed by drug diffusion from nanocarriers into the skin [6]. Skin penetration of NLCs is determined by their composition as well as their physicochemical properties such as size, aggregation, a charge on the particle surface, hydrophobicity, particle solubility in the skin, particle solubilizing properties towards skin lipids, and particle film forming ability [20].

When used as a local therapy, drug delivery to the skin is found to be successful in treating a variety of dermatological conditions, including fungus infections [21].

1.1. Advantages of Nanostructured Lipid Carrier (NLC):

- Greater physical sturdiness.
- An aqueous medium's improved dispersibility.
- High drug entrapment of hydrophilic and lipophilic substances.
- Precise particle sizing.
- A cutting-edge and effective carrier system, especially for lipophilic compounds.
- Skin occlusion has increased.
- Because their lipid components have an approved status or are excipients used in commercially available topical cosmetic or pharmaceutical formulations.
- Drug penetration into the mucosa or skin is increased by the small size of the lipid particles because they ensure tight contact with the stratum corneum.
- Boost the benefit-to-risk ratio.
- Increased skin elasticity and hydration [22].
- These carriers are highly effective systems because of their solid lipid matrices, which are also generally acknowledged to be safe or to have a regulatory acceptable status [23].

Crosslinked hydrogel materials called nanogels can release pharmaceuticals under controlled circumstances because they have the properties of both hydrogel and nanoparticles. Nanogels are water soluble and include both of these properties. Thus, these carriers provide a polymeric nanotechnological approach with exceptional qualities like high drug loading capacity, high stability, responsiveness to a wide range of environmental stimuli, whereby they may contract or expand in response to a change in pH or temperature, resulting in the release of the drug under specific circumstances [24]. Nanogels are hydrogels that are made up of nanometer-sized particles [25][26]. It is composed of Hydrogels which are three-dimensional networks of polymers with hydrophilic groups. A gel is in a semisolid state. Its characteristics are determined by the interaction between the liquid component and the solid-state polymer. Due to their lack of greasiness and ease of removal from the skin, topical gel formulations are better suited for medication delivery than creams and ointments.

The triazole group antifungal Itraconazole (ITZ) has a wide range of actions. Because of its high patient tolerance, ITZ is widely used to treat fungal diseases. Itraconazole is mainly preferred for the treatment of topical infections like tinea pedis, and candidiasis. It is practically insoluble in water having oral bioavailability of Itraconazole is 55%. Itraconazole also can suppress chronic respiration, interact with phospholipids, inhibit mycelial conversion, inhibit purine uptake, and inhibit triglyceride and/or phospholipid biosynthesis [27]. It prevents cytochrome P-450-dependent enzymes from working properly, impairing the production of ergosterol. It has been used to treat aspergillosis, cryptococcal meningitis, blastomycosis, and histoplasmosis. Its average molecular weight is 705.633. Through the suppression of the enzyme cytochrome P450 14-demethylase, itraconazole is a highly selective inhibitor of fungal cytochrome P-450 sterol C-14-demethylation. This enzyme is necessary for the formation of the fungal cell wall because it changes lanosterol into ergosterol. The increase of 14 -methyl sterols in fungi coincides with the eventual loss of natural sterols, which may contribute to fluconazole's fungistatic effects. Demethylation in mammalian cells is significantly less responsive to fluconazole inhibition. In vitro tests show that itraconazole is effective against *Candida* spp. and *Cryptococcus neoformans*. For systemic and intracranial fungal infections caused by *Cryptococcus neoformans* as well as for systemic infections caused by *Candida albicans*, fungistatic activity has also been shown in normal and immunocompromised animal models.

1.2. Properties of nanogels:

i. **Biocompatibility and degradability:** Due to the great biocompatibility and biodegradability of nanogel-based drug delivery systems, this sector is now very promising [28].
ii. **Higher drug loading capacity:** The functional group that is present in the polymeric unit affects nanogels' ability to load more drugs. These functional groups have a significant impact on the ability to transport and release medicines, and some functional groups may be able to combine with medications or antibodies to serve as targets. These pendent functional groups of polymeric chains help to create hydrogen bonds or van der Waals forces of interactions within the gel network, which

helps to increase the effectiveness of drug delivery. Additionally, increased loading is caused by the presence of functional groups at the drug or protein molecule interface [28].

iii. **Solubility:** Nanogels can solubilize hydrophobic medications and diagnostic agents in the gel's center or network [28]**.**

iv. **Electromobility:** It is possible to create nanogels without using energy or abrasive processes like sonication or homogenization, which are essential for enclosing biomacromolecules [28].

v. **Colloidal stability:** In comparison to surfactant micelles, nanogels or polymeric micellar nanogel systems show superior stability, lower critical micelle concentrations, slower rates of dissociation, and longer retention of loaded drugs [28].

1.3. Method of praparation of nanogel:

i. **High-pressure homogenization:** A dependable and effective method for producing Nanostructured Lipid Carrier, SLN. High shear stress and pressure (100–2000 bars) are applied to the lipid. It varies from 5 to 10. It is possible to homogenize substances at high pressure. It can be of two types: a) Hot homogenization technique and b) Cold homogenization technique [29][30][31].

ii. **Microemulsion technique:** Microemulsion serves as the starting point for the development of nanoparticles that are the required size. The hot microemulsion is then gently mechanically mixed with water in the range of 1:25-1:50 to disperse it in a cold aqueous media. The oil droplets quickly recrystallize as a result of this dispersion in the cold aqueous solution. Lecithin, biliary salts, and alcohols like butanol are examples of surfactants and co-surfactants. Because of its regulatory implications, excipients like butanol are less frequently used [29][32].

iii. **High shear homogenization:** This process involves melt of materials, addition of phospholipids, aqueous media. Molten material was then mechanically stirred or ultrasonically dispersed at a higher temperature. Ultrasonic energy is used to decrease the particle size [29][30].

iv. **Double emulsion technique:** This strategy is being employed primarily to create NLC for hydrophilic pharmaceuticals in order to overcome the challenge of hydrophilic moiety escape in the aqueous phase. A w/o/w type emulsion is created by transferring the solution of drug in molten lipid and then sonicating it to disperse it into an aqueous emulsifier solution. In the end, NLCs will be gathered via solvent evaporation and ultra-filtration [32].

v. **Membrane contactor technique:** Lipids are melted above their melting point and then poured into a pressurized container. To form tiny droplets, lipid melt is transported through a ceramic membrane that is porous. Under continual agitation, the aqueous solution moves tangentially inside the membrane. This innovative technique's advantages are the industrial flexibility and monitoring of particle diameter through appropriate configured dimensions [30][29].

vi. **Phase inversion – temperature method:** Surfactants (non-ionic, polyoxyethylated) are used in this method. They have temperature dependency. Due to the hydration of the hydrophilic groups at low temperatures, these surfactants have high hydrophilic-lipophilic balance (HLB) values. However, when temperature increaes, the hydrophilic-lipophilic balance value decreases as a result of the ethoxy groups' drying. W/O-type emulsion forms at high temperature than the temperature of phase inversion, and vice versa. To create NLC, hot liquid was subsequently diluted with cold water. This innovative technique enables the incorporation of thermolabile medicines without the use of any organic solvent [33][34].

vii. **Supercritical fluid method:** Supercritical fluids have been used in a broad variety of processes, including chromatography, green chemical reactions, and extraction. This method has recently been investigated to produce nanoparticles. This fluid contains gas/liquid which persist at high temperature & pressure. The resulting dispersion is then atomized and sprayed within a sealed chamber, where the gas decompresses and evaporates, forming nanostructured lipid carriers. Oil in water emulsion has been created by researchers. The solvent has been completely removed, which causes NLCs to precipitate. It was also mentioned that the NLCs produced had consistent particle sizes. The SCF process has many advantages, such as not using organic solvents [35].

viii. **Hot melt extrusion technology:** This process has been formerly employed to create pipes, tubes, and plastic bags. However, since the 1980s, there has been an increase in interest in using HME in the pharmaceutical business, and it is now utilized for the production of implants, tablets, and capsules, among other products. This process approaches various advantages, such as the absence of the solvent, which eliminates requirement for drying. Additionally, it is employed to increase the bioavailability and solubility of hydrophobic medicines. It is also a cost-effective technology because it requires less time for manufacturing [36].

ix. **Solvent Injection Method:** This method has a similar fundamental idea. This method's benefits include simple handling and a quick production cycle without the need for technologically complex machinery (such a high-pressure homogenizer). The usage of organic solvents, however, is the principal drawback [34].

1.4. Applications of Nanogel:

Nanogel can be applied in the following areas such as

- Topical delivery.
- Oral delivery.
- Parenteral delivery.
- Ocular delivery.
- Drug delivery to brain.
- Pulmonary delivery.
- Chemotherapy.
- Gene delivery, etc.[37]

2. RESULTS

2.1. Compatibility Study of the materials:

Drug Excipient interaction is studied before the development of the formulations to check the compatibility of the materials.. Itraconazole, Excipients, and Excipients mixed with the drug were mixed separately with IR grade KBr in the ratio of 1:100 and corresponding pellets were prepared by applying 5.5 metric ton pressure in a hydraulic press. Excipients were Stearic Acid, Olive oil, Tween 80, and Carbopol 934 P. The pellets were scanned over a wave number range of 4000-400 cm^{-1} in FTIR Spectroscopy [12]. The peaks in the spectra of a particular compound do not deviate significantly from those of the mixture. In the spectrum of the combination, the drug Itraconazole's distinct peaks were visible (1023 cm^{-1} for R-COOH where C-O Stretch is present, 1091 cm^{-1} for R-O-R where C-O stretch is present, 769 cm^{-1} for R-Cl where C-Cl stretch is present, etc.) were observed in the spectra of the mixture (1414 cm^{-1} for C-C in the ring where Ar C-C Stretch is present, 751 cm^{-1} for R-Cl where C-Cl stretch is present, etc.). The combination can be used to move forward with nanogel formulations, according to a correct analysis of the results. It has been represented in Figure 1.

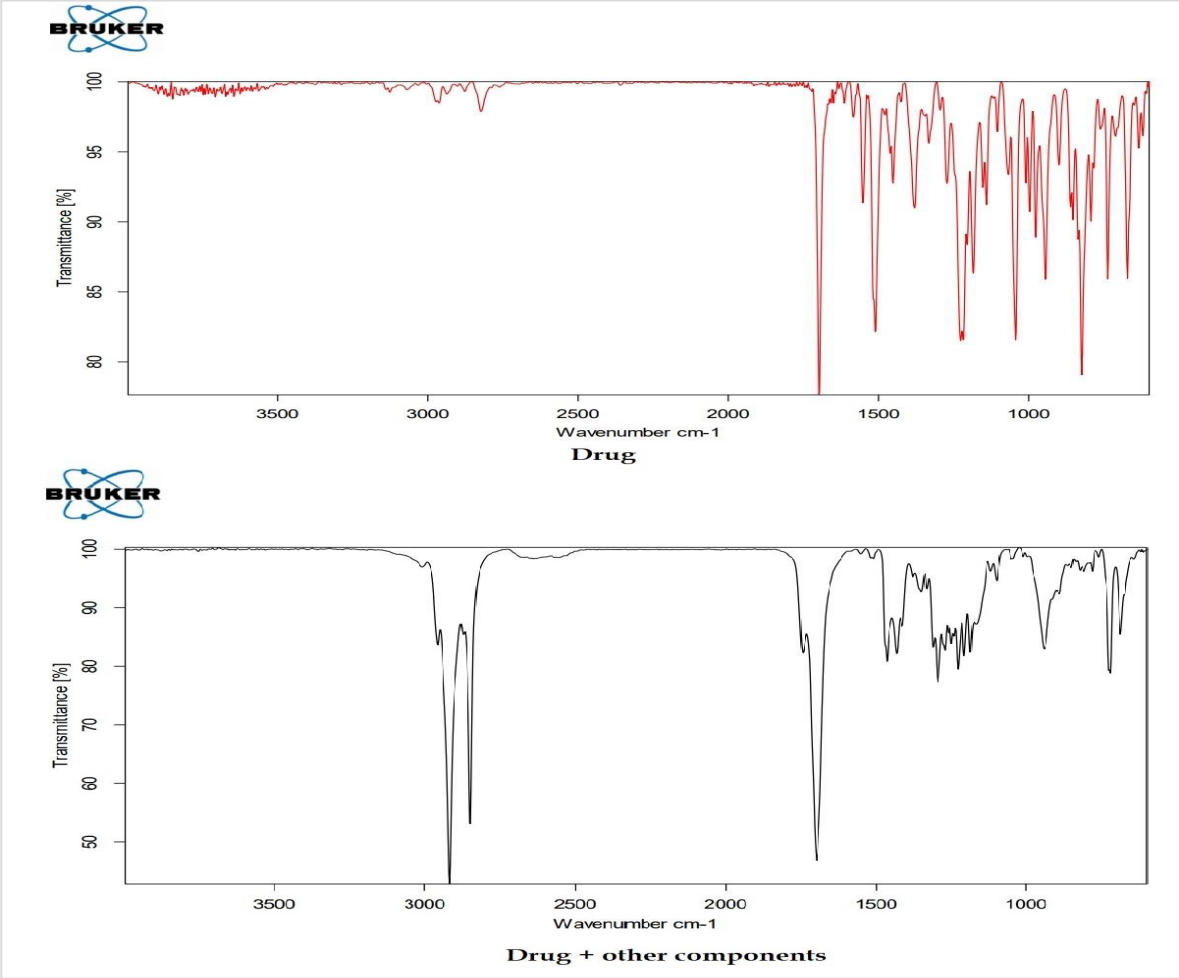

Figure 1. Compatibility study of drug & drug and component mix

2.2. Evaluation of Itraconazole-loaded Nanogel:

2.2.1. Physical appearance: After developing the products (Nanostructured Lipid Carriers), it has been shown that NF1, NF3, and NF10 are more suitable than the rest for preparing nanogel considering their homogeneity, texture, and state.

2.2.2. pH Determination: pH of NLC incorporated gel (Nanogel) formulation was found to be 6.91 to 7.47. pH of NF1 formulation was found to be 7.34, pH of NF2 was found to be 7.28, pH of NF3 was found to be 7.41, pH of NF7 was found to be 6.97, pH of NF8 formulation was found to be 7.24, pH of NF9 formulation was found to be 7.47, pH of NF10 formulation was found to be 6.91, pH of NF11 was found to be 7.15, and pH of NF13 formulation was found to be 7.23.

2.2.3. Determination of Particle Size: NLC Formulations were created using Face Centered Central Composite Design (α=1) in Design-Expert Software (Version 7.1.5). Homogenization Speed (RPM) and Liquid Lipid (mL) were chosen as independent variables in this design and were set at high and low levels. The low level of liquid lipid (in mL) was 2.00, and the high level was 5.00. However, the low level and high levels of homogenization speed (RPM) respectively were 2000.00 and 8000.00. 13 formulations have been discovered.

In this instance, the particle size was discovered to be 78.16 to 488.6 nm. Out of 9 formulations obtained from Face Centered Central Composite Design, it has been observed that NF3 and NF10 showed particle size within 100 nm, & NF9 showed particle size of 488.6 nm. Whereas the rest showed particle size in the range of 252 to 355.1 nm. The dynamic light scattering image of formulated NLC preparation has been mentioned in Figure 2.

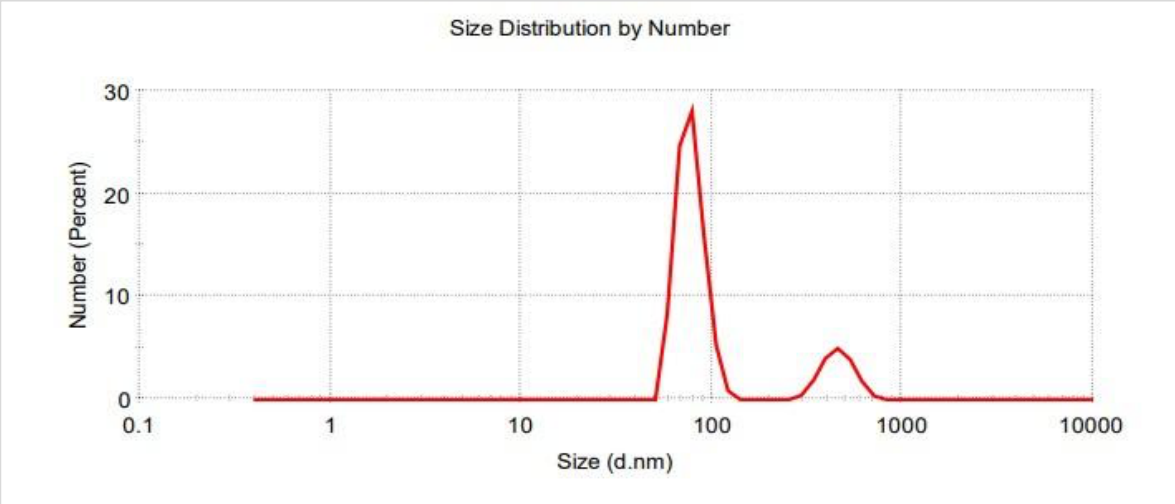

Figure 2. Dynamic light scattering (DLS) image of formulated NF3

2.2.4. SEM analysis: The SEM images of the nanogel formulations are shown in Figure 3. The photo depicts a sizable number of particles that indicate how the ITZ-loaded NLC is distributed throughout the hydrogel to produce the ideal nanogel. That the particles are in the nano range (72.62 nm – 176.4 nm) is the case.

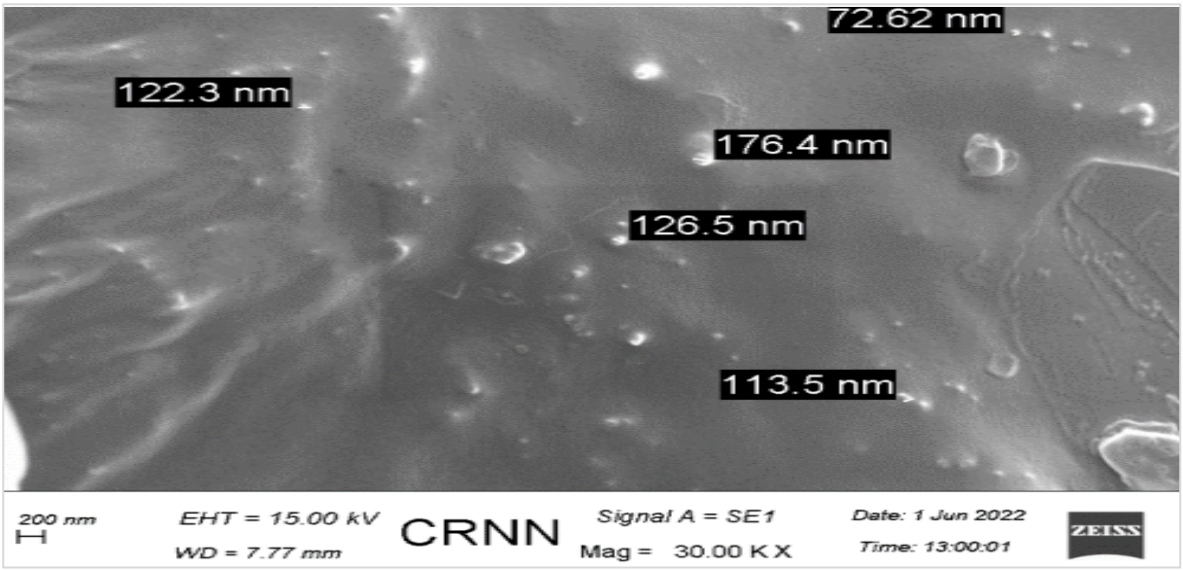

Figure 3. SEM image of prepared nanogel formulation

2.2.5. Determination of Spreadability Coefficient: The Spreadability Coefficient (gm. cm/sec) of different NLC formulations has been measured. It has been found that particle size is inversely proportional to the spreadability coefficient of nanogel formulation. The more the particle size, the less the spreadability coefficient. Itraconazole-loaded Nanogel compositions had spreadability coefficients ranging from 0.256 to 0.442 gm. cm/sec.

2.2.6. Determination of % Entrapment Efficiency: Entrapment efficiency (%EE) of the NLC formulations was determined to be between 44% to 88.5%. It has been found that particle size is directly proportional to the Entrapment Efficiency of NLC formulation. The more the particle size, the more the Entrapment Efficiency. The characterization of Itraconazole-loaded nanogel formulations has been mentioned in Table 1.

Table 1. Characterization of Itraconazole-loaded nanogel

Formulation Number	Particle Size (nm)	Spreadability (gm. cm/sec)	% Entrapment Efficiency
NF1	252	0.429	49.26
NF2	246.8	0.442	81.6
NF3	78.16	0.395	57.23
NF7	283	0.279	79
NF8	354	0.317	62
NF9	488.6	0.256	88.5
NF10	54.33	0.319	81.52
NF11	367	0.334	44
NF13	355.1	0.339	84.41

2.2.7. Optimization of the Formulation: Face-Centered Central Composite design approach was used to optimize Itraconazole-loaded nanogel formulations. To check for errors in the results, the design displayed 13 formulation compositions with five common compositions. The software was modified to incorporate the data on particle size, Spreadability, and Entrapment Efficiency to interpret the findings. Predicted values, polynomial equations, contour graphs, and 3D response surface graphs were all generated by the software to assess the effects of independent factors on dependent components. When the anticipated value produced by the program was compared to the actual particle size and entrapment efficiency, the result was found to be closer to the predicted value. Table 2 shows a statistical model summary of the regression analysis for particle size Spreadability coefficient and entrapment effectiveness. All responses were found to fit best with a quadratic model having the highest correlation coefficient (R^2). The chosen independent variable had an individual as well as a combined impact on the dependent variables, making the quadratic response optimal for optimization. For both replies, it was discovered that the expected R^2 and the adjusted R^2 were in good agreement. The F value displayed a value larger than 4, and the ANOVA result was determined to be significant. The ANOVA of the quadratic model for responses of developed Itraconazole-loaded nanogel has been given in Table 3. To assess the software's accuracy, a percentage prediction calculation was made. The polynomial equation's positive sign encourages the interaction of independent variables with the dependent variables, whereas its negative value denotes the opposite relationship. The tested independent variable was determined to be suitable for the optimization since the total combined desirability was found to be closer to unity.

Table 2. Statistical model summary of regression analysis results for response Particle Size, Spreadability, and % Entrapment Efficiency

Model	R^2	Adjusted R^2	Predicted R^2	SD	% CV	Remark
Particle Size						
Linear	0.0154	- 0.1815	- 1.0332	161.03	74.99	
2FI	0.0506	- 0.2659	- 3.3752	166.68	77.62	
Quadratic	0.8771	0.7892	- 0.2517	68.01	31.67	Suggested
Spreadability						
Linear	0.2485	0.0982	- 0.7588	0.055	15.13	
2FI	0.2586	0.0114	- 3.3936	0.057	15.84	
Quadratic	0.7775	0.6186	- 0.9532	0.035	9.84	Suggested
% Entrapment Efficiency						
Linear	0.5892	0.5071	0.2704	10.46	15.88	
2FI	0.6602	0.5470	0.4116	10.03	15.22	
Quadratic	0.8609	0.7615	0.0364	7.27	11.04	Suggested

Table 3. ANOVA of Quadratic model for responses of developed Itraconazole loaded Nanogel

ANOVA Results	Particle Size (nm)	Spreadability (gm.cm/sec)	% Entrapment Efficiency
Regression			
Some of square	2.310E+005	0.031	2292.57
Degree of Freedom	5	5	5
Mean Square	46195.21	6.162E-003	458.51
F – Value	9.99	4.89	8.66
P	0.0044	0.0303	0.0066
Influence	Significant	Significant	Significant
Lack of fit - test			
Some of square	32378.08	8.817E-003	370.45
Degree of Freedom	3	3	3
Mean Square	10792.69	2.939E-003	123.48
Residual			
Some of square	32378.08	8.817E-003	370.45
Degree of Freedom	7	7	7
Mean Square	4625.44	1.260E-003	52.92

i. Effect of independent variables on Particle Size:

Particle sizes for several batches were found to range from 54.33 nm to 488.6 nm. There was a significant change in particle size due to the Liquid Lipid's variable content and homogenization speed. The polynomial Equation (1), and 3D response plot (Figure 4) demonstrate that the following independent variables influence particle size:

$$\text{Particle Size} = +1327.80732 - 277.45046*A - 0.29492*B + 0.010700*AB + 31.57816*A^2 + 2.48929E\text{-}005*B^2 \quad .....(1)$$

Where, A = Liquid Lipid, and B = Homogenization Speed.

Particle Size was negatively impacted by both liquid lipid and homogenization speed. To attain the minimum size, the optimal lipid content and homogenization speed both were maintained. When the liquid lipid and the homogenization speed were decreased that time particle size was increased.

The effects of both liquid lipid and homogenization speed on particle size were negative. Equation (1) also demonstrated the combined impact of homogenization speed and liquid lipid on particle size. This mixture had a beneficial impact on particle size. As a result of equation (1), it is possible to conclude that liquid lipid and homogenization speed both had a greater influence (Positive Impact) on particle size.

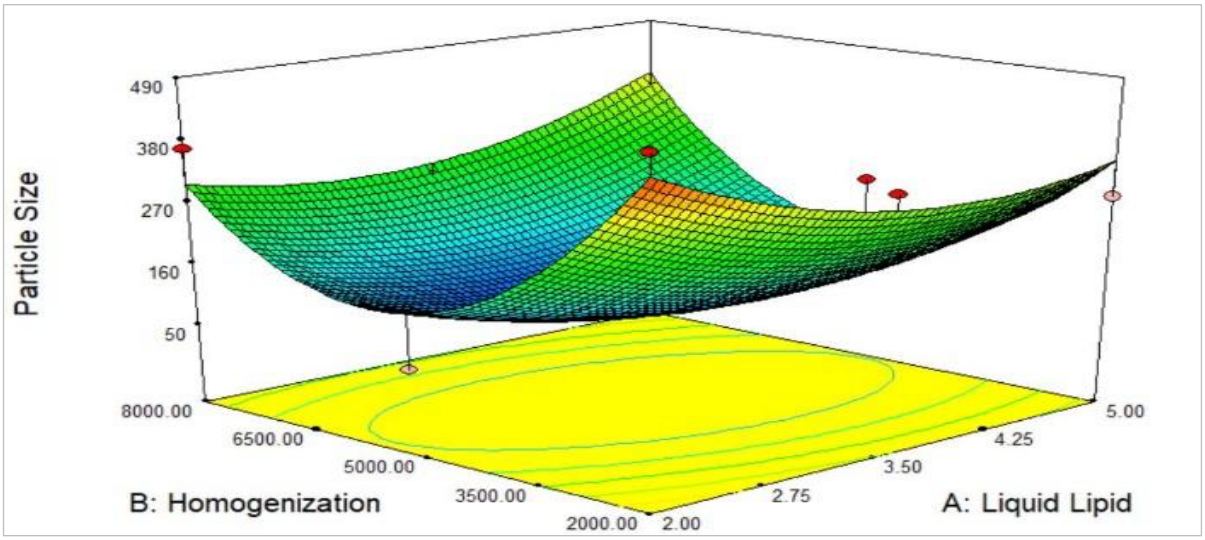

Figure 4. 3D Response surface plot showing the effect of independent on particle size

ii. Effect of independent variables on Spreadability coefficient:

Spreadability coefficients for several batches were found to range from 0.256 gm. cm/sec to 0.442 gm. cm/sec. There was a significant change in the Spreadability coefficient due to the Liquid Lipid's variable content and homogenization speed. The polynomial Equation (2), and 3D response plot (Figure 5) demonstrate that the following independent variables influence the Spreadability coefficient:

$$\text{Spreadability} = -0.14047 + 0.18020*A + 7.06015E\text{-}005*B - 2.22222E\text{-}006*AB - 0.022107*A^2 - 5.13793E\text{-}009*B^2 \quad ..(2)$$

Where, A = Liquid Lipid, and B = Homogenization Speed.

The effects of both liquid lipid and homogenization speed on the spreadability coefficient were positive. Equation (2) also demonstrated the combined impact of homogenization speed and liquid lipid on the spreadability coefficient. This mixture had a negative impact on the spreadability coefficient. As a result of equation (2), it can be concluded that liquid lipid and homogenization speed both had a negative impact on the spreadability coefficient.

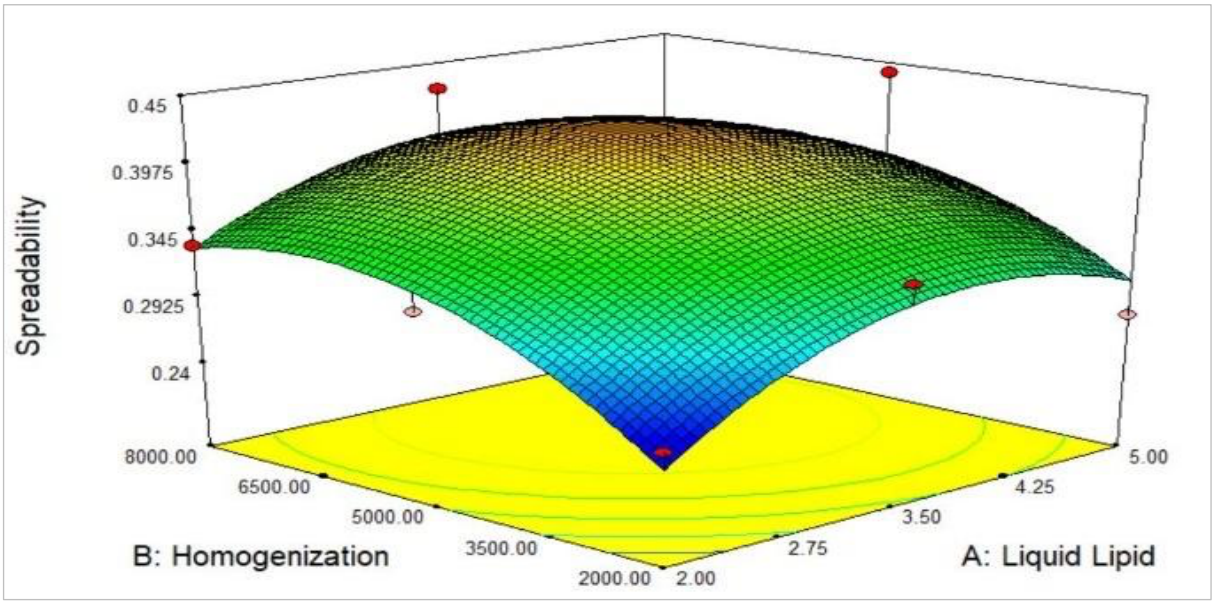

Figure 5. 3D Response surface plot showing the effect of independent on spreadability coefficient

iii. Effect of independent variables on Entrapment Efficiency (EE):

Entrapment Efficiency for several batches was found to range from 44% to 88.5%. There was a significant change in Entrapment Efficiency due to the Liquid Lipid's variable content and homogenization speed. The polynomial Equation (3), and 3D response plot (Figure 6) demonstrate that the following independent variables influence Entrapment Efficiency:

$$EE = +179.30255 - 48.15023*A - 9.16437E\text{-}003*B + 1.52778E003*AB + 5.92352*A^2 - 1.55230E\text{-}007*B^2 \quad .....(3)$$

Where, A = Liquid Lipid, and B = Homogenization Speed.

The effects of both liquid lipid and homogenization speed on EE were negative. Equation (3) also demonstrated the combined impact of homogenization speed and liquid lipid on % EE. This mixture had a beneficial impact (Positive Impact) on EE. As a result of equation (3), it is possible to conclude that liquid lipid and homogenization speed both had a greater influence on EE.

The "Numerical Optimization" method yielded 39 options. Desirability is 1.000 for every single option. Table 4 displays the "Solutions Obtained Through Numerical Optimization."

Table 4. Solution obtained from numerical optimization

Solution Number	Liquid Lipid (mL)	Homogenization Speed (RPM)	Desirability
1	3.5	5000	1
2	3.5	8000	1
3	5	5000	1
4	5	8000	1
5	2	2000	1
6	3.2	3200	1
7	2.3	7400	1
8	4.85	2750	1
9	2.3	5600	1
10	4.8221	4719.2	1
11	3.5912	7391	1
12	2.4818	3669.8	1
13	4.1069	2783.6	1
14	2.4572	4654.4	1
15	2.5286	5933.6	1
16	2.5994	2273	1
17	2.8904	2028.2	1
18	3.5384	5253.2	1
19	4.8449	3719	1
20	4.9589	2538.8	1
21	3.8888	5498	1
22	2.3951	4272.2	1
23	2.2883	5927.6	1
24	2.2715	3309.8	1
25	4.16	4892	1
26	2.3945	6006.8	1
27	2.6807	6067.4	1
28	3.6284	7917.2	1
29	4.9088	3786.8	1
30	2.9651	3123.8	1
31	3.1571	2505.2	1
32	3.7106	4884.2	1
33	4.4195	6033.2	1
34	2.0015	4250.6	1
35	2.7896	6737.6	1
36	3.3815	6398.6	1
37	3.3647	7899.8	1
38	4.6412	5307.2	1
39	3.5	5000	1

39 optimized formulations were supplied by Design-Expert Software (Version 7.1.5). Among them, formulations with a liquid lipid quantity equivalent to 2.00 mL were found to be excluded from the optimization list. As a result, those formulas were abandoned. The Face-Centered Central Composite Design model was used in 13 runs by the Design - Expert Software, with 5 formulations being replicated. As a result, 9 combinations were considered. Six of these 39 formulations were included in the optimization list by the program. NF3 (Run 3) (which has been repeated in the Numerical Optimization list) has been considered an optimized product, and further studies have been conducted on this product, depending on the particle size.

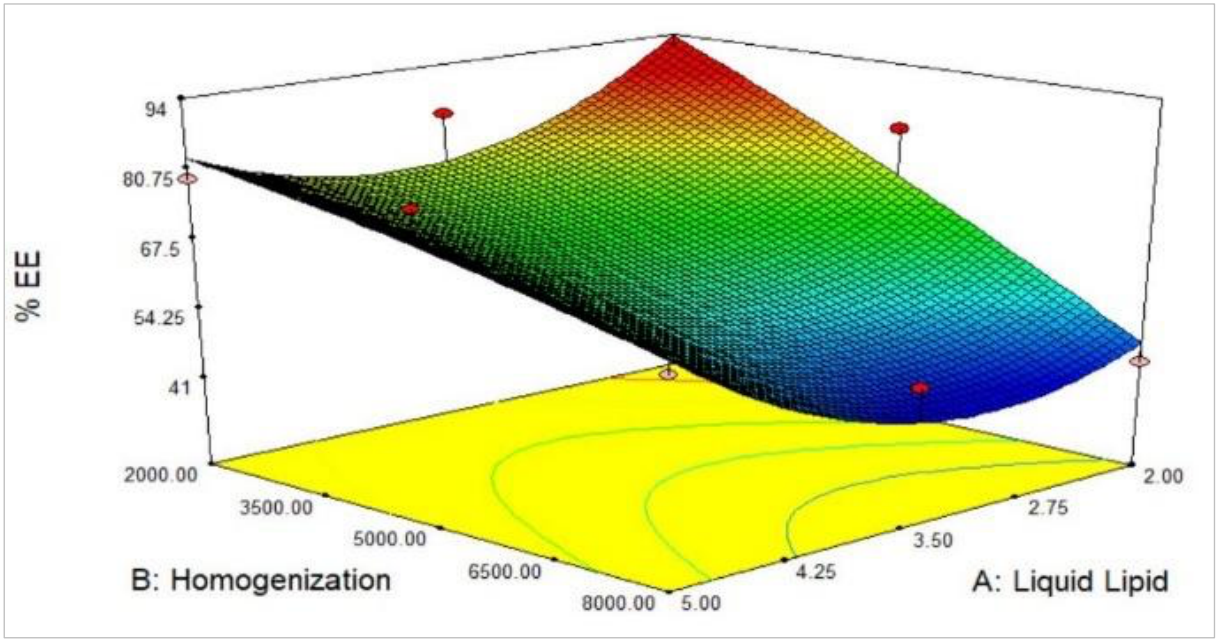

Figure 6. 3D Response surface plot showing the effect of independent on entrapment efficency

iv. In vitro drug diffusion study using dialysis membrane:

The in vitro drug diffusion profile of Itraconazole from optimized Formulation (NF3) in pH 7.41, was reported in Figure 7. The in vitro drug diffusion research lasted 7 hours. The drug diffusion from the optimized formulation gradually increased and kept a constant rate after 5 hours. Itraconazole diffused at an 89.15% rate in 7 hours.

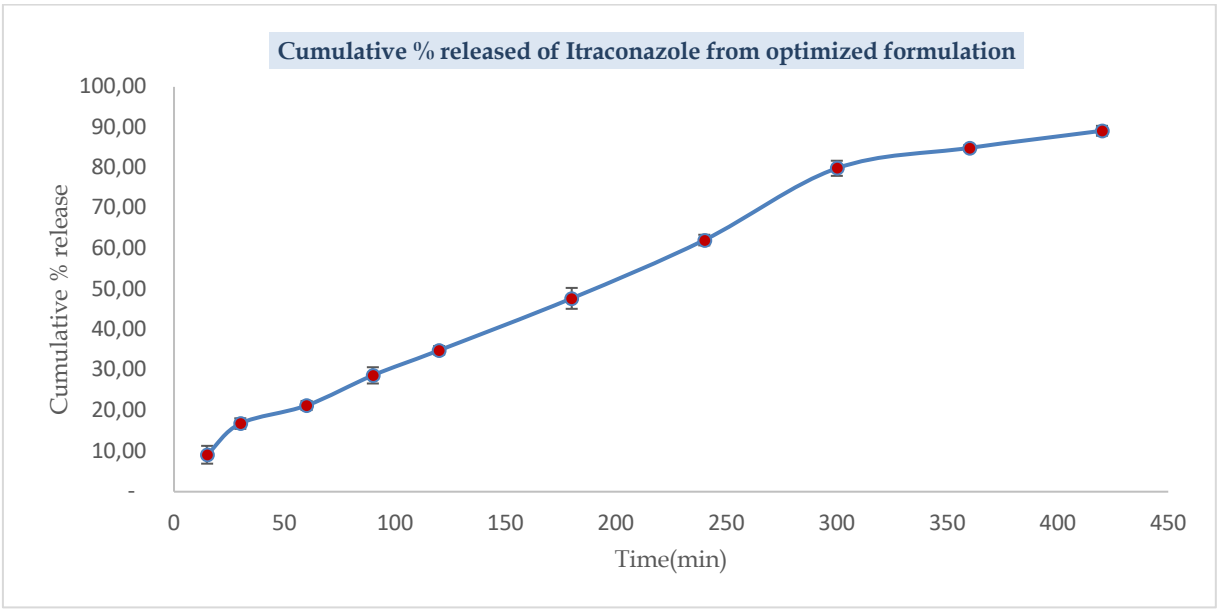

Figure 7. Cumulative % Release of Itraconazole from Optimized Formulation (NF3)

v. Ex vivo permeation study using abdominal skin:

Following an in vitro drug diffusion study using a dialysis membrane, an ex vivo experiment was carried out to confirm the correlations between the studies. The findings were sufficient to proceed. Adult healthy goat abdominal skin was chosen for this study (drug permeation rates). Skin permeability is described in the following order: mouse > rat > guinea pig > rabbit > monkey > dog > goat >sheep > pig > human being.[37] It was discovered that Itraconazole permeation through goat abdomen skin from the optimized product is approximately 51.67% in 7 hours. Zero-order kinetics could be used to describe drug permeation. The permeability coefficient was calculated using **P = K. Vr/S**, where, **S** means effective surface area of the goat abdominal skin, **Vr** stands for the volume of the receiver chamber, **K** is the Zero order constant, and **P** means the Permeability Coefficient. It was found to be 0.733 cm/min. Figure 8 represents the cumulative % of permeation of Itraconazole from NLC-incorporated topical gel.

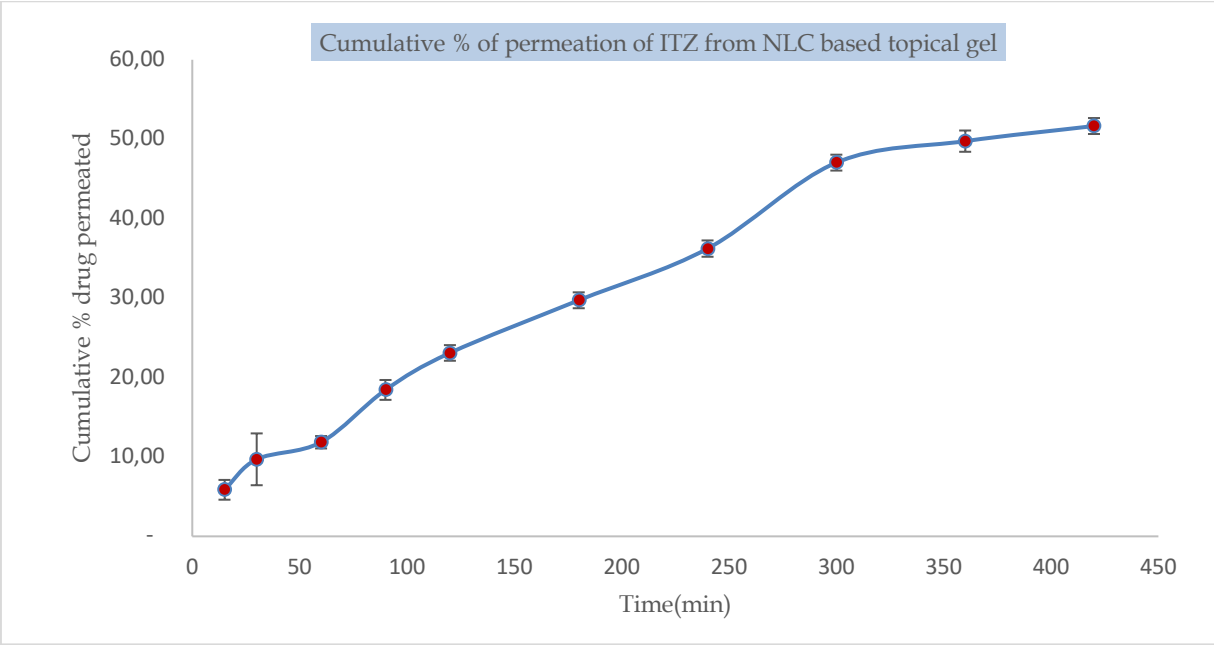

Figure 8. Cumulative % of permeation of ITZ from NLC incorporated gel for topical drug delivery

3. DISCUSSION

As was already mentioned, NLC which had been loaded with Itraconazole was used for this study. The study's objective was to assess the effectiveness of Itraconazole-loaded Nanogel when administered topically. First, an FTIR study was used to determine whether the medicine and excipients were compatible. It is possible to get the conclusion that this combination can be used to move forward with nanogel formulations by evaluating the FTIR spectra. The formulations were made using Design-Expert software (Version 7.1.5) with a face-centered Central composite Design to optimize the formulation based on particle size, spreadability coefficient, and entrapment efficiency. To examine and analyze the inaccuracy, the software gave 13 formulations with 5 repeats. The particle size of the Itraconazole-loaded NLC formulations was measured to be in the range of 54.33 nm to 488.6 nm, and the percent entrapment efficiency was determined to be in the range of 44% to 88.5%. Itraconazole-loaded Nanogel formulations were observed to spread at a rate between 0.256 to 0.442 gm. cm/sec. The findings were analyzed using ANOVA. The influence of Independent Variables (Factor) on the response parameter particle size, spreadability coefficient, and percent entrapment efficiency was described using polynomial equations and the software's generated 3D response surface graphs. Based on the values of the response parameter, the software predicted 39 optimal formulations.

From among the formulations, one with a small particle size was picked for further investigation. The surface morphology and particle size distribution of the formulation could be confirmed by examining the SEM image. The optimized formulation's particle size was determined by doing a SEM analysis, and it ranged from 72.64 to 176.4 nm. The ex-vivo penetration of the improved formulation via goat abdominal skin and the in vitro drug diffusion utilizing a dialysis membrane were both examined using a Franz diffusion cell. The 7-hour in vitro drug diffusion investigation was conducted. After 5 hours, it was seen that the drug diffusion

from the improved formulation steadily picked up and remained constant. Itraconazole's diffusion rate in 7 hours was 89.15%. An ex-vivo experiment was performed following an in vitro drug diffusion investigation utilizing a dialysis membrane to verify the correlations between the experiments. The findings were sufficient to move forward. Itraconazole from the optimized product has been reported to permeate into goat abdominal skin by about 51.67% in 7 hours. Zero-order kinetics may be used to explain drug penetration. Using **P = K. Vr/S** the permeability coefficient was computed. S is the goat abdominal skin's effective surface area, Vr is the receiver chamber's volume, K is the zero-order constant, and P is the permeability coefficient. It was measured at 0.733 cm/min.

We can therefore conclude from the results of the present research that the NLC formulations effectively deliver Itraconazole through the skin. By conducting these tests, the formulation can be improved for use in topical drug delivery in the future.

4. CONCLUSION

In this research, the Face-Centered Central Composite design was used to develop and optimize the Itraconazole-loaded Nanogel formulation concerning three response parameters: particle size, entrapment efficiency, and spreadability. The optimized product was then tested for optimal drug diffusion through the dialysis membrane and drug permeation through the epidermis. SEM analysis was used to corroborate the morphology (surface texture, particle size, and shape) of the formulation. According to this research, a potentially effective system for the topical delivery of antifungal medication is a nanogel that has been loaded with itraconazole.

5. MATERIALS AND METHODS

Itraconazole was gifted by Macleods Pharmaceutical Ltd. (Andheri East, Mumbai, India). Stearic acid and Carbopol 934 P were purchased from Loba Chemie Pvt Ltd. (Mumbai, Maharashtra, India) and Olive oil of Bertolli was purchased locally. Tween80 was procured from Merck Specialities Private Limited (Shiv Sagar Estate 'A', Mumbai: 400018). Triethanolamine of Merck Life Science Private Limited (Mumbai, India). Double Distilled Water was prepared in the laboratory of Guru Nanak Institute of Pharmaceutical Science & Technology (Sodepur, Kolkata).

5.1. Compatibility Study of the materials:

In this work, FTIR has been chosen to study the compatibility between the drug and other materials. KBR pellets were prepared and scanned to get FTIR spectra of the drug (Itraconazole), materials (Stearic acid, Olive oil, Carbopol 934 P, etc.) individually, and of the mixture (Nanostructured Lipid Carrier). FTIR spectrophotometer (Perkin Elmer Spectrum Two) was used to obtain the spectra. The sample was prepared by being mixed with potassium bromide and then put in the sample vessel. The spectrum was scanned over a frequency range of 4000 – 400 cm^{-1}[12].

5.2. Method of Preparation of Nanogel:

5.2.1. Design of Experiment for the preparation of Nanostructured Lipid Carriers (NLCs): In this work, NLCs were formulated with different variables, with the help of **"Design - Expert Software** (Version 7.1.5)". Chosen for the creation of the Nanostructured Lipid Carrier was **Central Composite Design**. The choice for Liquid Lipid's low and high values was **2.00** and **5.00**, respectively. Similarly to this, **2000.00** and **8000.00** were chosen as the low and high values for homogenization speed.[38][39] The coded values of independent variables have been mentioned in Table 5.

Table 5. Coded values of independent variables

Independent Variables	Unit	Low Actual (-1 level)	High Actual (+1 Level)	-alpha	+alpha
Liquid Lipid	mL	2.00	5.00	2.00	5.00
Homogenization Speed	RPM	2000.00	8000.00	2000.00	8000.00

According to the Face-centered Central Composite Design (CCD), 13 runs have been found and mentioned in Table 6, and among 13 runs, it has been found that 5 runs are repeated, hence the total number of runs has been 9.

Table 6. Face centered central composite design

Run	Factor I (Liquid lipid in mL)	Factor II (Homogenization Speed in RPM)
1	3.50	8000.00
2	5.00	5000.00
3	3.50	5000.00
4	3.50	5000.00
5	3.50	5000.00
6	3.50	5000.00
7	5.00	2000.00
8	5.00	8000.00
9	2.00	2000.00
10	2.00	5000.00
11	2.00	8000.00
12	3.50	5000.00
13	3.50	2000.00

5.2.2. Method of Preparation of Nanostructured Lipid Carrier (NLC): The lipid mixture has been prepared by mixing stearic acid and olive oil, by continuous stirring in a hot condition (60-70^{o}C), with the help of a magnetic stirrer (REMI 1MLH) of REMI ELEKTROTECHNIK LTD. (Vasai, India). An aqueous form of Itraconazole was mixed with the lipid phase in a hot condition in the presence of a surfactant (Tween 80). The mixture was placed in a beaker for homogenization ((REMI MOTOR of REMI ELEKTROTECHNIK LTD., Vasai, India) for 15-20 minutes at 2000 - 8000 RPM. The hot condition was maintained throughout the process. The mixture was transferred to the homogenizer tube for further homogenization. The mixture was sonicated for 30 minutes using Ultrasonic Sonicator (LABMAN SCIENTIFIC INSTRUMENTS PRIVATE LIMITED, Chennai: 600118, India) and kept for 48 hours at room temperature. Then the formulations were lyophilized using a lyophilizer (OPTICS TECHNOLOGY, DELHI: 34, INDIA).[40][15][41] The Composition of different formulations has given in Table 7 and diagrammatically represented in Figure 9.

5.2.3. Method of Preparation of Nanogel: The prepared NLC (approximately 10% w/v) was added to the hydrogel[42] created by carbopol 934 P that was dissolved in Double Distilled Water at a concentration of 1.25 (w/v) to form the hydrogel. It was combined while being stirred with a magnetic stirrer. The formulation was stabilized by the addition of 0.15 mL of Tween 80. To get the desired consistency of the final product, 1 - 2 drops of triethanolamine were added to the mass at the end.

5.3. Evaluation of Itraconazole-loaded Nanogel:

5.3.1. Physical appearance: Visual observations were used to evaluate the nanogel compositions' physical characteristics and uniformity [15].

5.3.2. Determination of pH: Using pH meter, the pH of the nanogel formulations was identified.

Table 7. Composition of different NLC (Nanostructured Lipid Carrier) formulations

Formulation Number	Amount of Stearic Acid (gm)	Amount of Olive Oil (mL)	Amount of Itraconazole (gm)	Amount of Double Distilled Water (mL)	Amount of Tween 80 (mL)	Homogenization Speed (RPM)
NF1	6.50	3.50	0.01	10.00	0.3	8000.00
NF2	5.00	5.00	0.01	10.00	0.3	5000.00
NF3	6.50	3.50	0.01	10.00	0.3	5000.00
NF7	5.00	5.00	0.01	10.00	0.3	2000.00
NF8	5.00	5.00	0.01	10.00	0.3	8000.00
NF9	8.00	2.00	0.01	10.00	0.3	2000.00
NF10	8.00	2.00	0.01	10.00	0.3	5000.00
NF11	8.00	2.00	0.01	10.00	0.3	8000.00

NF13	6.50	3.50	0.01	10.00	0.3	2000.00

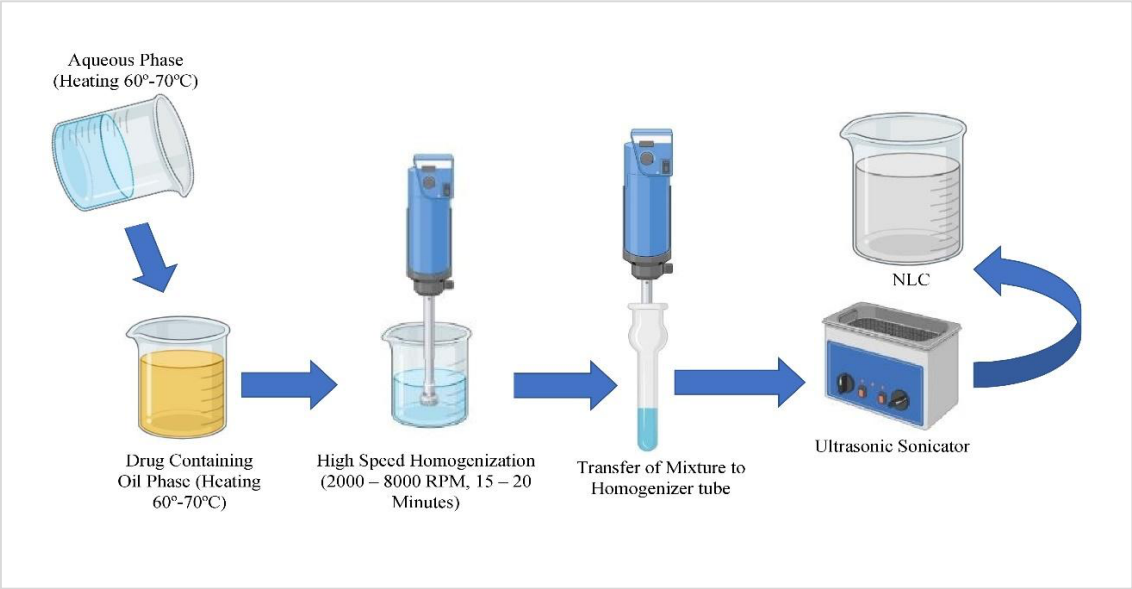

Figure 9. Preparation of nanostructured lipid carrier (NLC)

5.3.3. Determination of Particle Size: Using a Zetasizer (Version 7.11) equipment, Dynamic Light Scattering (DLS) was employed to detect the particle size of the NLC formulations at 25°C. The NLC formulations were dissolved in methanol and 100-fold diluted with it before the particle size and polydispersity index were assessed.[12][43]

5.3.4. SEM analysis: Scanning electron microscopy was used to verify the particle size and shape. After being lyophilized, the NLC-loaded hydrogel was examined in a SEM (ZEISS EVO 18, CARL ZEISS MICROSCOPY, PENTA FET X 3). The nanogel particles ranged in size from 54 to 489 nm in diameter.

5.3.5. Spreadability Coefficient: Spreadability determinant equipment was used to calculate the Spreadability Coefficient. It is made up of a wooden block with a pulley attached to one end. The slip and drag properties of nanogel were used to calculate the spreadability coefficient. The hardwood block was fastened with a glass side. The glass slide was covered with a little amount of nanogel and sandwiched between two slides. 20 gm of weight was measured and put into the pulley-attached pan. The amount of time (in Seconds) needed for the slide to travel 5 cm was recorded. A higher spreadability coefficient is indicated by a shorter interval.[44]

5.3.6. Determination of Entrapment Efficiency: Using a cooling centrifuge made by REMI ELEKTROTECHNIK LTD., Vasai, India, 1 gm of drug-loaded Nanostructured Lipid Carrier (NLC) was combined with 5 mL of methanol before being cold centrifuged (5°- 8°C) at 12,000 RPM for 20 minutes. The supernatant fluid was collected and then filtered through filter paper. The filtered substance was then put into a petri dish and given time to dry. Upon drying, 10 mL of 0.1 (N) HCl was added to the petri dish and stirred using a glass rod. To determine the concentration of the medication entrapped, the material is then once again filtered and spectrophotometrically measured at 262 nm using a UV Spectrophotometer (Jasco V-630 Spectrophotometer). The same steps were taken with blank formulation, whose value was deducted from the first outcome. The formula below was used to compute the percentage of EE.[45][46]

$$\% \, \boldsymbol{EE} = \frac{\boldsymbol{Experimental\ Drug\ Content}}{\boldsymbol{Theoretical\ Drug\ Content}} \times \mathbf{100}$$

5.3.7. Optimization of the Formulation: With the aid of "Design - Expert Software, (Version 7.1.5)" the formulations were improved regarding the response, such as particle size, spreadability, and % EE. The model was initially examined using the ANOVA and R-squared test. Finally, from the model

graph, the 3D response curve and perturbation plot were derived. The software then performed numerical and graphical optimization.[39]

5.3.8. In vitro drug diffusion study: It was carried out utilizing a Franz Diffusion Cell apparatus with a receptor compartment capacity of 45 ml and a cross-sectional area of 0.785 cm^2 through dialysis membrane 50 (Hi-Media). The membrane was covered with the manufactured Nanogel. Once mended, it was placed in the donor chamber. for the membrane to face the receiving compartment. Phosphate Buffer pH 7.4 was poured into the receiver compartment. Magnetic beads were used to agitate the receptor solution continually while keeping the temperature at 32±0.5°C. The medicine was examined after the samples were taken at various intervals. A fresh buffer solution with a pH of 7.4 was substituted for 5 ml of the receptor solution in its stead. Drug content was examined in the samples using a UV-visible spectrophotometer at 262 nm.[47][48][49] The slope has been found from y= 0.0271x where the value of the regression coefficient (R^2) was found to be 0.9988.

5.4. Ex vivo permeation study using goat abdominal skin:

5.4.1. Preparation of skin: A fresh goat's abdominal skin was used in the permeation test; it was obtained from a slaughter house for the test. The skin was hydrated for an hour in a phosphate buffer at pH 7.4.[37]

5.4.2. Ex vivo permeation study: It was executed by Franz During the ex vivo skin permeation investigation, a diffusion cell with a 45 ml capacity was used. On the goat's abdominal skin towards the donor compartment, 1 gm of nanogel was applied. The skin was then applied to the receiver compartment of the diffusion cell that contained a pH 7.4 phosphate buffer. Magnetic beads were used to agitate the receptor solution continually while keeping the temperature at 32±0.5°C. The medicine was examined after the samples were taken at various intervals. A fresh buffer solution with a pH of 7.4 was substituted for 5 ml of the receptor solution in its stead. Drug content was examined in the samples using a UV-visible spectrophotometer at 262 nm.

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Author contributions: Concept – G.R.B.; Design – G.P., P.D.; Supervision – G.R.B.; Resources – S.P[1]., G.P., P.D., S.P[2].; Literature Search – G.P., P.D.; Writing – G.P., G.R.B.; Critical Reviews – G.R.B.
Conflict of interest statement: The authors declared no conflict of interest.

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