Formulation, Optimization and Evaluation of Nano based Ocular Delivery System Containing Antifungal Drug

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Abstract
Fungal Endophthalmitis is a vision threatening intraocular infection with high morbidity and limited therapy option due to poor ocular drug penetration. Posaconazole is broad spectrum triazole antifungal medication has defensive mechanism on a wide range of Fungal Pathogen, nevertheless its clinical utility is restricted by low aqueous solubility and Optic absorption. The current study was designed to develop and evaluate Posaconazole entrapped SLNs as a potential nanocarrier technology for enhance ocular drug delivery system. PSC Incorporated solid lipid nanoparticles were formulated using biocompatible lipid matrices and surfactants with an Optimized preparation. The formulated nanoparticles were systemically characterized for particle size distribution, Polydispersity index, Zeta potential, Encapsulation efficacy and surface morphology. In vitro drug release studies marked a biphasic release profile, with an initial burst release distinguished by a sustained and controlled release Phase, indicating diffusion mediated drug release kinetics. The optimized SLN formulation demonstrated enhance Physicochemical stability, improve corneal Penetration and reduced ocular irritation compared to conventional formulation. Furthermore, the nano approaches exhibited superior antifungal activity, better therapeutic outcomes. In conclusion, Posaconazole incorporated solid lipid nanoparticles are a promising and effective nanotechnological approach for posterior ocular delivery in treatment of Fungal Endophthalmitis, providing enhanced solubility, prolonged drug release, improved bioavailability and potential better clinical outcomes.

Keywords: Posaconazole, Precirol ATO5, Solid Lipid Nanoparticles, Fungal Endophthalmitis.
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Introduction

The optic organ is one of the most vulnerable elements of the human body. Ocular drug delivery system are now a big problem accompanying to eyes distinctive and convoluted structure. The most Prevalent dose forms in ophthalmic therapy are Eye drops and Eye Ointments.[1] Drug Permeation into the hinder portion of the optic is limited by the eye's natural defense systems, which including blinking, tear production, and Naso lacrimal drainage.[2] As a result, a significant part of the injected medicine is removed from the ocular surface before it reaches the target region. As a result, standard ocular formulations frequently fail to provide adequate treatment of posterior ocular illness such Endophthalmitis.[3]

Endophthalmitis is a serious, potentially blinding illness that affect the rear region of the eye. It is often accompanied with infections like candidemia and fungal keratitis, which can cause inflammation and infection in the deeper ocular tissues.[4] As the infection continues, it may spread to the ocular coats, including the retina, choroid, and vitreous body. This broad involvement of intraocular structures eventually leads to the development of endophthalmitis, which can cause severe visual impairment if not treated swiftly. The vitreous cavity creates an ideal habitat for the growth and proliferation of fungal infections in the eye.[5] Endophthalmitis is classified as either Endogenous or Exogenous depending on where the infection originated. Exogenous Endophthalmitis is caused when bacteria or fungi enter the eye through external sources such as ocular trauma, surgical operations, or penetrating injuries. In contrast, Endogenous endophthalmitis arise when fungal

pathogens are transported through the bloodstream from a systemic infection to the tissue within the eye.[6] If not treated promptly, this infection can cause significant intraocular inflammation and gradual damage to eye tissues. Fungi are prevalent in the environment and are thought to be a significant cause exogenous endophthalmitis.[7] The environment is full of fungi, which are thought to be a major contributor to external endophthalmitis. These infections usually happen when fungal pathogen enter the eye through external source such contact lens use, surgical procedures, contaminated tools or ocular trauma.[8] The risk is significantly increased by environmental exposure to fungal spores, particularly in dusty or agricultural environments. If this organism is not identified and treated very away, they may spread throughout the intraocular tissues of the eye, causing severe inflammation and possibly blindness.[9]

Posaconazole has broad-spectrum antifungal action and has demonstrated promise success in the treatment of severe fungal infections, including endophthalmitis, as a result of its targeted mechanism. Ergosterol depletion and methylated sterol intermediate buildup affect the structural integrity and functional stability of the fungal cell membrane.[10] These changes reduce membrane permeability and key cellular functions, eventually leading to fungal cell malfunction and death, which contributes to posaconazole's powerful antifungal activity.[11] It also act as a triazole derivative, inhibits the enzyme lanosterol 14-α-demethylase (CYP51), which plays a crucial role in ergosterol biosynthesis pathway. This inhibitor hinders the conversion of lanosterol to ergosterol, an essential sterol needed to conserve the structural integrity and activity of the fungal cell membrane.[12] As a result of ergosterol depletion and the accumulation of hazardous sterol intermediates, membrane permeability increases, cellular functions are impeded, and fungal cells eventually die.[13]

PSC lacks a commercially viable ophthalmic formulation and has undesirable physicochemical qualities, its use in treating eye infections is still severely restriction despite its broad-spectrum antifungal activity. Posaconazole is currently utilized off-label in ocular therapy by diluting its oral suspension, accordingly to studies, underscoring the absence of a conventional ocular dose form.[14] Furthermore a number of ocular barriers, such as conjunctival epithelium, sclera, choroidal clearance, and retinal pigment epithelium, which together limit drug penetration into the posterior segment, frequently prevent conventional antifungal treatments from achieving sufficient intraocular drug concentrations.[15] Systemic exposure, inconsistent ocular penetration, and the requirement for high dosages to reach therapeutic levels are among the drawbacks of systemic posaconazole, despite its efficacy in refractory cases of fungal keratitis and endophthalmitis.[16]

Recent research has explored alternative nanocarrier system such as micelles, vesicles, and cationic nanocarriers to improve ocular delivery of Posaconazole; However, these systems primarily focus on anterior segment disease like keratitis and do not adequately address targeted delivery to posterior segment, where endophthalmitis is predominant.[17] Furthermore there has been a dearth of extensive research into solid lipid nanoparticles for posaconazole, primarily aimed at overcoming conjunctival – scleral barriers and improving drug transport to the vitreous and retina. As a result, there is a huge research need in developing a stable, biocompatible SLN based ocular delivery system for posaconazole that can improve drug absorption, reduce systemic exposure, and efficiently target posterior segment infection like Fungal Endophthalmitis.

Material and Methods

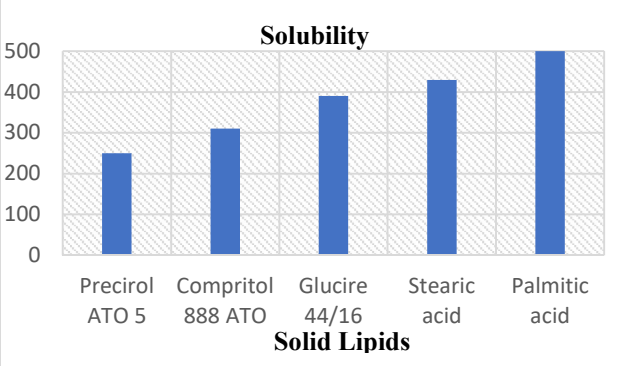
Posaconazole provided from Sun Pharmaceutical Pvt Ltd. in Baroda, India as free gift sample. Glyceryl Palmitostearate solid lipid (melting point 50°–60°C) were obtained as a gift from procured from Gattefosse India Pvt Ltd. Mumbai India, the reagents employed in research were analytical grade Span20 (Sorbitan monolaurate HLB: - 8.6) and Tween 80 (Polysorbate 80 HLB: - 15.0), Acetone of HPLC grade was purchased from Fisher scientific, Germany.

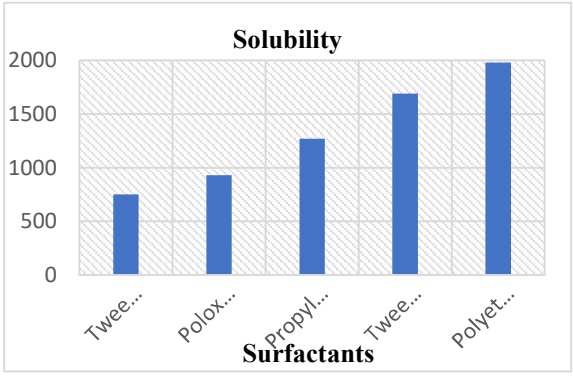
Determination of Posaconazole lipid Solubility

The solubility of Posaconazole in various solid lipids was examined using melting behavior and drug-lipid compatibility. A modified partitioning approach was used to determine the distribution of posaconazole between an aqueous phase and various solid lipids with goal of discovering a lipid with greater solubilization capacity. The lipids considered are Gelucire® 44/16, Compritol® 888 ATO, Precirol® ATO 5, and stearic acid. A known amount of PSC was gradually added to 500 mg of each solid lipid in wide-mouth screw capped bottles with continuous mixing and vertexing. The mixtures were then heated above the respective melting points of the lipids to obtain molten systems with continuous mixing ensuring homogeneous molten phase.[18] The addition of Posaconazole was continued until a clear, pale-yellow solution was formed, indicating solubilization limit at given temperature & concentration. UV Visible spectrophotometry was used to measure the amount of drug solubilize at 262nm. The solubility of PSC in each lipid was determined in triplicate and validated for selection of Precirol® ATO 5 due to its superior

solubility at 500 mg concentration test conducted as discussed ensuring higher PSC entrapment capacity.

Sr No.	PSC (mg)	Solid Lipid	IUPAC Nomenclature	Melting Point
1	20	Compritol 888 ATO	Glyceryl Dibehenate NF	65-77°C
2	20	Precirol ATO5	Glyceryl Distearate NF	50-60°C
3	20	Glucire 44/16	Lauryl Polyoxyl-32 Glyceride NF	42.5-47.5°C
4	20	Stearic acid	Octadecanoic acid	69-71°C
5	20	Palmitic Acid	N-Hexadecenoic acid	61-62.5°C





Lipids Required to Soluble 20mg Drug
Surfactants to soluble 20mg Drug

Preparation of Posaconazole SLNs: -

A modified solvent diffusion evaporation method was used to create Posaconazole loaded SLNs. To summarize, the organic phase was developed by dissolving Posaconazole and Glyceryl palmitostearate in acetone solvent at 75°C. After dissolving span 20 and polysorbate 80 in distill water and heating it to the same temperature as the organic phase, the aqueous phase was created. The organic phase was then added dropwise to the heated aqueous phase and homogenized at 10,000 rpm with a Remi Homogenizer. After complete addition, heating was turned off, and the resulting emulsion was constantly agitated to allow all of the acetone to evaporate. Posaconazole loaded SLN was further sonicated before being centrifuged at 15,000 rpm for 30 minutes. Finally, SLNs were effectively produced.[19]

Composition of SLNs:

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Posaconazole(mg)	20	20	20	20	20	20	20	20	20
Precirol ATO 5 (mg)	150	250	350	200	300	400	250	350	450
Span20 : Tween80 (mg)	400	-	600	400	500	600	-	500	-
Acetone (ml)	10	10	10	10	10	10	10	10	10
Distilled Water (ml)	50	50	50	50	50	50	50	50	50

Preparation of Standard curve of Posaconazole:

Posaconazole drug standard stock solution was made by Precisely measuring 10 mg of the drug, dissolving it in 10ml methanol while stirring continuously until the drug was fully soluble and then adding methanol to make up the volume to 100ml to achieve a concentration of 100µg/ml. By transferring appropriate aliquots of 0.5,1.0,1.5,2.0,2.5 ml from this

stock solution into various 10ml volumetric flasks and diluting them to the appropriate level with methanol, the final concentrations of 5,10,15,20 and 25µg/ml were obtained. Using methanol as blank, the produced solution was determined throughout a wavelength range of 200-800nm. Posaconazole's maximum absorption wavelength was determined to be 262nm.[20]

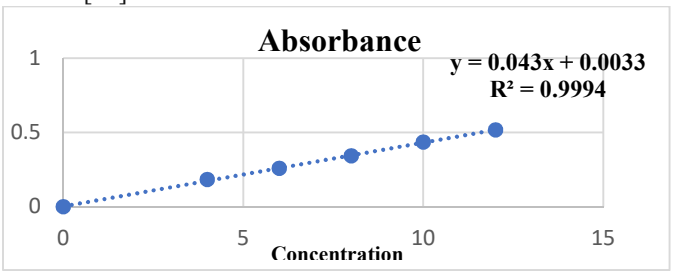


Figure 1 Calibration Curve of Drug in Methanol

Characterization of SLNs:

1. **Particle Size, Polydispersity Index and Zeta Potential:**

The SLN's particle size (z -average diameter) and polydispersity index (PI) was determined by dynamic light scattering on a Malvern Zetasizer Nano ZS (Malvern Instruments, UK) at 25℃ 11. The same equipment was used to measure the Zetasizer potential (ZP) of nanoparticle dispersions. Before measurement, the nanoparticle dispersion was dilute with ultrapure water to achieve the desired scattering intensity. To quantify particle size, the Polydispersity index nanoparticle dispersion was poured into a cuvette, which was placed in the instruments cuvette holder and analyzed using the Zetasizer software. The Zeta potential was measured using a folded capillary cuvette. All measurements were performed in triplicate. [21]

2. **Determination of Entrapment Efficiency:**

The centrifugation method used to determine the entrapment effectiveness of SLNs dispersion. The SLN formulation containing 20mg of Posaconazole was centrifuged at 15,000 rpm for 30 minutes in a chilled centrifuge to separate the free medication from the Nanoparticles. Following centrifugation, the clear supernatant was carefully collected. The resulting supernatant was diluted with methanol and filtered to determine the concentration of unentrapped (free) medication. The sample's absorbance was measured at 262nm with a UV Spectrophotometer. [22]

The entrapment efficiency was then computed using the following formula:

Entrapment efficiency = Wt. of drug incorporated/Wt. of drug initially taken × 100

3. **Posaconazole In vitro drug Release studies:**

The in vitro release of Posaconazole from the optimized SLN formulation under simulated ocular circumstances was evaluated using a dialysis bag diffusion method. The dissolution study was conducted in 150ml of artificial tear fluid (pH 7.4), which was made up of sodium chloride, Sodium Bicarbonate, calcium chloride dihydrate, and purified water. The medium was kept at 37 ± 0.5°C and agitated at 50 RPM.

A dialysis bag with a molecular weight cut-off (MWCO) of 12kDa was filled with a determined amount (3 ml) of the formulation and immersed in the dissolution medium. Over the course of ten hours, aliquots (5ml) were removed and replaced with an equivalent volume of fresh medium at predetermined intervals to maintain sink conditions. Following the removal of the sample and their filtration via a 0.45µm membrane filter, the drug concentration and cumulative percentage drug release were determined using a validated HPLC method. The pure drug's release profile was also assessed under the same circumstances for comparison. Every experiment was carried out in six replicates, and mean values were used to express the findings. [23]

Transmission Electron Microscopy (TEM):

TEM is a high-resolution analytical process used to measure the particle size of solid lipid nanoparticles and asses their morphological properties, such as Shape and surface of structure. A carbon coated copper grid serves as supporting substrate, allowing the SLN formulation to disperse uniformly and remain stable throughout imagining. Furthermore, using appropriate staining agents improves image contrast, allowing for the detection of particle aggregation and overlapping within the system. Therefore Instrument (JEM -1230, JEOL, Tokyo, Japan) operating at 70 kV was used to examine the morphology of Posaconazole loaded SLNs. The samples were stained with 1%w/v EDTA improve contrast and diluted 50 times with the original dispersion liquid for Analysis.[24]

4. **Powder X-Ray Diffraction Analysis:**

The proposed ocular formulation's crystalline properties were assessed using powder X-Ray diffraction (PXRD) analysis. An XPERT-PRO multipurpose X-ray diffractometer (PANalytical, Netherlands) was used to record the sample's diffraction patterns. The Powder X-Ray Diffractometer measuring program was operate to conduct the analysis under controlled circumstances. The X-Ray source was Ni-Filtered CuKα radiation. 45kV of voltage and 40 mA of current were used to run the device. Samples were scanned between 5° and 40° in terms of diffraction angle (2θ). The degree of crystallinity and potential structural alteration in the formulation were evaluated using the generated diffraction patterns. The Examination also assisted in determining whether the medication has changed from a crystalline phase to an amorphous state.[25]

5. **Differential Scanning Calorimetry Analysis:**

Differential Scanning Calorimeter analysis was performed using a DSC TA-60 Calorimeter (Shimadzu, Tokyo, Japan) to evaluate the materials thermal behavior. Aluminum pans containing carefully weighed samples (3-5mg) were heated from 40℃ to 200℃ at a rate of 10℃ per minute. The analysis was performed in an inert nitrogen atmosphere at a flow rate of 35ml/min using empty alumina pan as the reference.[26]

6. **Release Kinetics:**

To study the drug release behavior of solid lipid nanoparticles (SLNs), many kinetic models are used, including zero-order, first-order, and Higuchi models. The Korsmeyer-Peppas model best fits the drug

release mechanism, with higher regression coefficient (R^2) values. The drug release mechanism in this model is described by the release exponent (n) while the time dependent release profile is defined by the release rate constant (k). The Korsmeyer-Peppas equation is especially effective for studying systems with unclear release mechanisms or numerous processes, such as diffusion and erosion. As a result, this model is well suited to assessing the drug release behavior of ocular formulations, which frequently contain complex release events.[27]

Results and Discussion

Selection of Lipid for SLNs:

Precirol ATO 5 was chosen as lipid matrix for the production of Posaconazole loaded solid lipid nanoparticles (SLNs) due to its high drug-lipid partition coefficient. This lipid had a significant affinity for Posaconazole indicating effective drug incorporation and entrapment. Furthermore, its high biocompatibility and capacity to offer controlled and prolong drug release make it an ideal candidate for the production of stable Nano formulation.[28]

Particle Size & Zeta Potential :

Formulation	Particle Size (Nm)	PDI (± SD, n=3)	ZP (± SD, n=3)	EE (%)
F1	191± 0.08	0.092 ± 0.03	-24.43 ± 0.061	89.95
F2	227± 0.17	0.143±0.01	-21.70 ± 0.012	87.21
F3	254± 0.23	0.079± 0.95	-23.12 ± 0.036	86.99
F4	275± 0.09	0.127± 0.05	-20.18 ± 0.019	85.35
F5	265± 0.34	0.093± 0.02	-18.27 ± 0.043	84.58
F6	236± 0.58	0.101± 0.14	-21.11 ± 0.075	88.01
F7	271± 0.21	0.151± 0.08	-22.95 ± 0.021	83.28
F8	280± 0.02	0.091± 0.16	-24.18 ± 0.005	81.93
F9	288± 0.41	0.089± 0.07	-24.49 ± 0.033	84.66

Experimental Design:

Table 1 Formulation Variables and their levels in Box -Behnken Design:[29]

Independent Variables	**Levels**		
	Low	**Medium**	**High**
Temperature	60°c	70°c	80°c
HLB Value of Surfactant	12	15	17
Drug: Lipid Ratio (w/w)	0.1	0.15	0.2
Studies Response	**Goals**		
Particle Size	Minimize		
Polydispersity Index	Minimize		
Drug Entrapment Efficiency	Maximize		

Formulation	**Temperature**	**HLB Value**	**Drug: Lipid (w/w)**	**Particle Size (Nm)**	**Polydispersity Index (%)**	**Drug Entrapment Efficiency (%)**
F1	70	14.5	0.125	191	93.36	89.95
F2	60	14.5	0.15	227	91.22	87.21
F3	70	17	0.1	254	88.01	86.99
F4	70	17	0.15	275	85.94	85.35
F5	60	17	0.125	265	87.12	84.58
F6	60	14.5	0.1	236	90.05	88.01
F7	70	12	0.15	271	84.19	83.28
F8	80	17	0.125	280	82.98	81.93

F9	80	14.5	0.15	288	82.07	84.66

Statistical Analysis:
The statistical result of Optimized Formulations was obtained by using one-way ANOVA, Turkey-Kramer multiple comparison tests, and the student's t-test for stability and group comparison. Using Design Expert software version 11, a student t-test was used to compare groups with a p-value of <0.005. All results are shown as mean ± SD.[30]

Table 2 ANNOVA TEST FOR PARTICLE SIZE

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	8692.75	9	965.86	11.44	0.0348	**significant**
A-Temperature	2145.12	1	2145.12	25.41	0.0151	
B-Three HLB Value	378.12	1	378.12	4.48	0.1246	
C-Drug: Lipid	924.50	1	924.50	10.95	0.0454	
AB	121.00	1	121.00	1.43	0.3172	
AC	462.25	1	462.25	5.48	0.1012	
BC	90.25	1	90.25	1.07	0.3772	
A^2	2642.29	1	2642.29	31.30	0.0113	
B^2	3749.14	1	3749.14	44.41	0.0069	
C^2	1575.00	1	1575.00	18.66	0.0229	
Residual	253.25	3	84.42			
Cor Total	8946.00	12				

The Model F value of 11.44 indicates the model is significant. There is only 3.48% chances that an F value these large will occur owing to noise.

Table 3 ANNOVA TEST FOR POLYDISPERSITY INDEX

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0059	8	0.0007	10.25	0.0408	significant
A-Temperature	0.0009	1	0.0009	12.81	0.0373	
B-HLB Value	0.0006	1	0.0006	8.98	0.0578	
C-Drug: Lipid Ratio	0.0024	1	0.0024	32.99	0.0105	
AB	0.0007	1	0.0007	9.01	0.0576	
AC	0.0010	1	0.0010	13.75	0.0341	
BC	0.0003	1	0.0003	3.77	0.1474	
A^2	4.500E-06	1	4.500E-06	0.0624	0.8189	
B^2	0.0000	1	0.0000	0.6928	0.4663	
C^2	0.0000	0				
Residual	0.0002	3	0.0001			
Cor Total	0.0061	11				

The **Model F-value** of 10.25 implies the model is significant. There is only a 4.08% chance that an F-value this large could occur due to noise.

Table 4 ANNOVA TEST FOR DRUG ENTRAPPMENT EFFICIENCY

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	80.22	9	8.91	37.63	0.0063	**significant**
A-Temperature	2.57	1	2.57	10.83	0.0461	
B-Three HLB Value	6.23	1	6.23	26.30	0.0144	
C-Drug: Lipid	5.80	1	5.80	24.47	0.0158	
AB	3.26	1	3.26	13.75	0.0341	

AC	1.28	1	1.28	5.39	0.1029	
BC	0.0272	1	0.0272	0.1149	0.7569	
A^2	17.25	1	17.25	72.84	0.0034	
B^2	48.26	1	48.26	203.74	0.0007	
C^2	0.2092	1	0.2092	0.8830	0.4167	
Residual	0.7106	3	0.2369			
Cor Total	80.93	12				

The Model F value of 37.63 indicates the model is significant. There is only 0.63% Probability that a F value these large could occur due to noise.

Optimization and Characterization of PSC SLNs:

Posaconazole loaded SLNs were created utilizing the solvent evaporation-diffusion method. It was discovered that raising the surfactant concentration resulted in reduction in particle size while improving entrapment efficiency. The impact could be ascribed to surfactant's strong influence on vesicle formation at greater concentrations, which is most likely related to osmotic pressure phenomena. SLNs are a unique drug delivery technology because they can encapsulate both hydrophilic and hydrophobic molecules while providing a high drug loading capacity. They improve intracellular uptake by allowing the medication to disperse at controlled rates. Vesicular lipids serve as simplified models for biological membranes and cells. Because of their structural similarities to bio membranes, these systems are ideal for investigating the origin, function, and evolution of cells, as well as present biosystems.

To maximize medication absorption and hence bioavailability, it is critical to consider characteristics such as minimum particle size, low PDI, Maximum entrapment efficiency and optimum zeta Potential.[31]

Particle size & PDI:

In addition to mean particle size (nm), the polydispersity index (PDI) of the optimized formulation is critical for determining particle size distribution and homogeneity. These two important characteristics for solid lipid nanoparticles have a major impact on drug release rate, biodistribution, and total bioavailability. Particles larger than 100-150nm are more likely to be detected and swallowed by phagocytic cells, or they may remain in tissue for extended period of time. Posaconazole loaded solid lipid nanoparticles ranged in particle size from 191.55 ± 1.84 nm to 280.47 ± 0.06 nm, making them suitable for Ocular medication administration. The constructed mathematical model, based on the formulation variables, can be used to anticipate reactions at different values of each factor. A positive coefficient in the model represents a direct association between the independent and dependent variables. As a result, as the lipid concentration (Factor A) in the dispersion increase, so does the particle size.

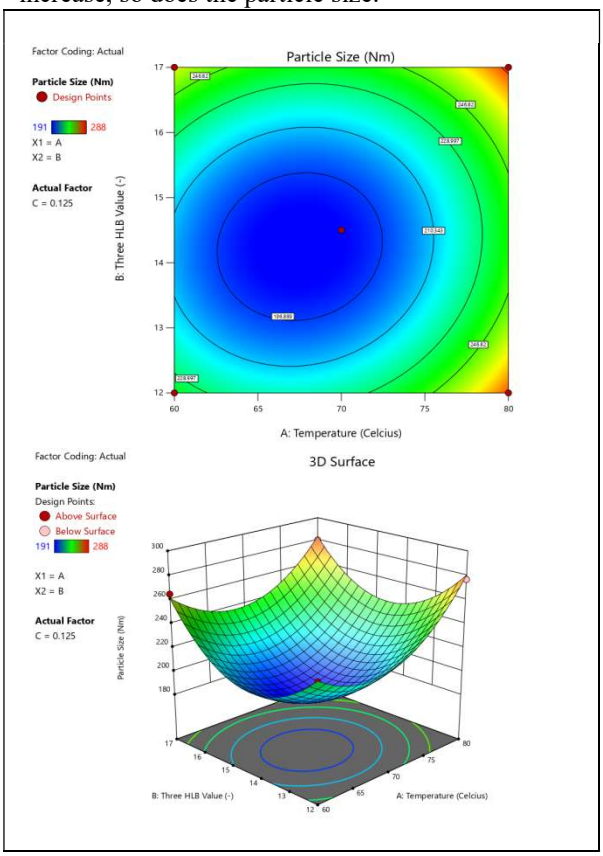


Figure 1 Counter (A) and 3D Surface Plot (B) of Particle size against Independent Factor

Batch F3 had the largest particle size of Posaconazole loaded SLNs (280.47 ± 0.06 nm) at high (+1) and low (-1) amounts of factors A and B, respectively. Among the formulation factors, lipid content had the strongest positive effect on particle size and PDI. Specifically, increasing the concentration of Glyceryl Palmitostearate (A) from 200mg to 400mg while maintaining the surfactant content (B) resulted in considerable increase in particle size and PDI. Furthermore, simultaneous increases in lipid and surfactant concentration (interaction term AB) resulted in moderate increase in particle size, indicating a synergistic effect between these factors.

The observed increase in vesicle size with higher lipid concentration (A) is due to increased particle aggregation and viscosity of the lipid phase. Increased viscosity slows the rate of diffusion of the non-polar phase into the polar phase during nanoparticle formation, favoring the production of bigger particles

or lipid aggregates. The surfactant dosage decreased vesicle size in solid lipid nanoparticles while slightly increasing the Polydispersity index. An increase in surfactant concentration during formulation reduces the interfacial tension between the organic and aqueous phases, allowing for the production of smaller particles. Surfactant molecules also provide steric stability at the particle surface, preventing smaller particles form aggregating and coalescing into bigger ones, resulting in greater dispersion uniformity.

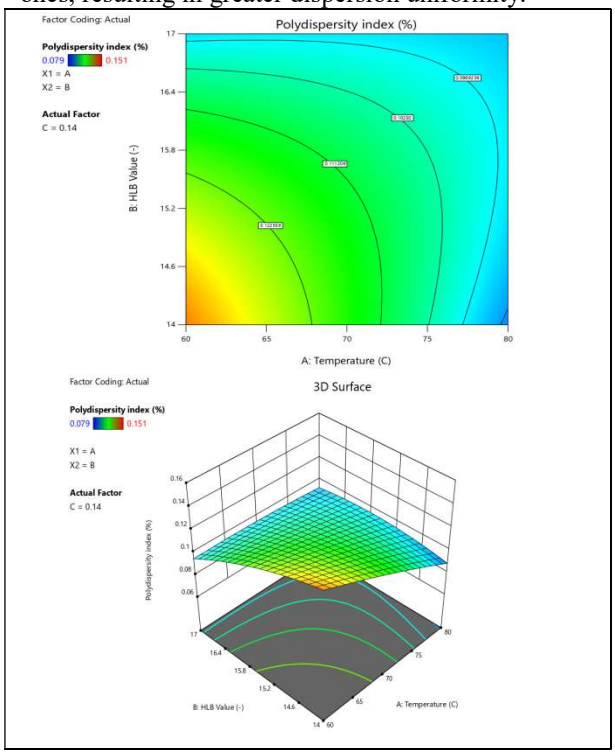


Figure 2 Counter (C) and 3D Surface Plot (D) of Polydispersity Index against Independent Factors

Drug Entrapment Efficiency:

Critical quality attributes (CQAs) were evaluated to determine the formulation process's efficiency and reproducibility. The entrapment efficiency (EE%) of the produced solid lipid nanoparticles varied from 80.5 ± 3.18% to 93.8 ± 0.44%. The statistical analysis of the model equation revealed that both lipid concentration (factor A) and surfactant concentration (factor B) had a negative effect on EE%. Batch F9 showed increased drug entrapment at lower lipid (250mg) and surfactant (0.25%) levels, with an EE% of 80.8 ± 1.06%. These data imply that high lipid and surfactant concentrations can diminish encapsulation efficiency, either due to drug partitioning into the external phase or structural changes in the nanoparticulate system.

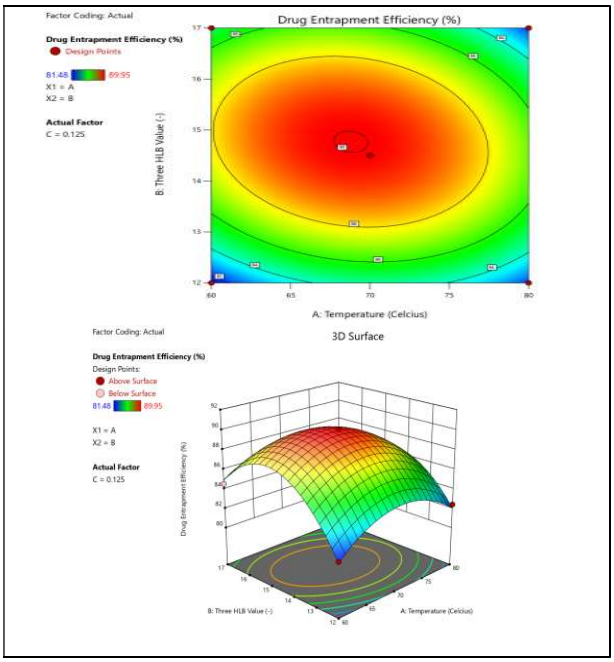


Figure 3 Counter (E) and 3D Surface Plot (F) of % Drug Entrapment Efficiency

Fourier Transform Infrared Spectroscopy:

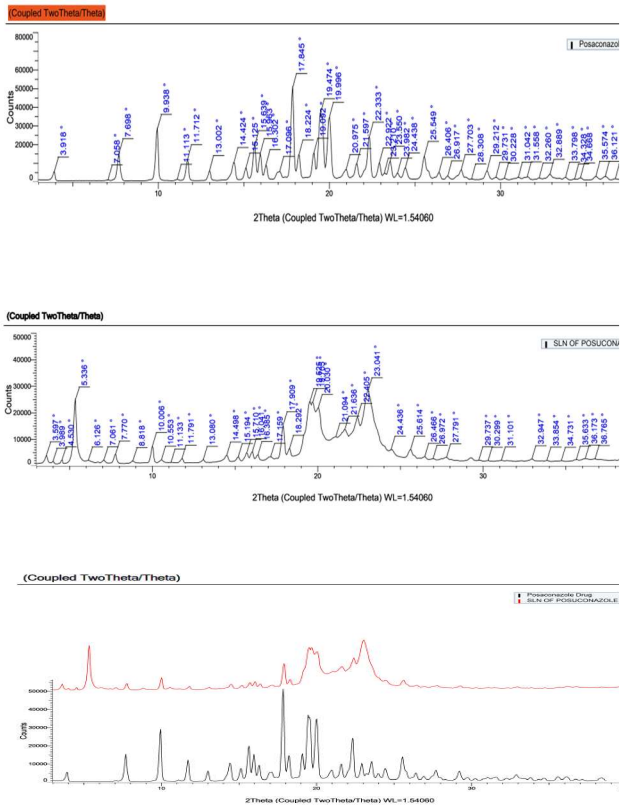
FTIR was used to evaluate functional groups and look into possible molecular interactions between the drug and excipients. FTIR analysis generates characteristic absorption bands that correspond to various vibrational modes chemical bonds, acting as a molecular fingerprint for identifying structural features. This method is especially useful for discovering potential physicochemical interactions, such as hydrogen bonding or chemical incompatibilities between formulation and components. FTIR studies was used to analyzed the functional groups and confirm the molecular interactions between the Drug and Excipients. FTIR analysis generates unique absorption bands that correspond to specific bond vibrations, acting as a fingerprint for identifying structural features and detecting potential chemical interactions.

The drug and excipients were discovered to be chemically compatible, as demonstrated by the lack of any major shifts, disappearances or the creation of new distinctive peaks. Minor variations in peak intensity were attributed to physical mixing and possible hydrogen bonding interactions rather than chemical alterations. These data validate the drug's compatibility with the chosen excipients and the retention of molecular stability inside the formulations. [32]

X-Ray Diffractometer Studies:

Powder XRD is a non-destructive analytical technique used to reveal structural properties of materials at the

atomic and molecular levels. The sample's crystalline nature was determined by its diffraction pattern, which shows well defined, distinct, and non-overlapping peaks that corresponded to the material's intrinsic lattice organization. This approach allows for precise characterization of interior structural characteristics on a length scale of about 0.1-100nm. Furthermore, Powder XRD provides vital insights into the degree of crystallinity, Phase identification, and molecular organization within the material system, making it an essential tool in solid state characterization.[33]



Transmission Electron Microscopy (TEM):

TEM was used to study the nanoparticle's size, shape & surface properties. The posaconazole loaded solid lipid nanoparticles were consistent in both size and shape, exhibiting primarily as nanosized, spherical particles. The structural architecture was made up of drug enriched lipid core surrounded by a surfactant shells.

Detailed TEM investigation indicated that the Posaconazole loaded nanoparticles had a well-defined spherical geometry, smooth surface morphology, and were uniformly disseminated with no apparent aggregation. The particle size measured using TEM was quite similar to the values obtained using dynamic light scattering, which typically ranged between 50 to 100 nm depending on the formulation parameters. Furthermore, High resolution TEM micrographs confirmed the nanoscale dimensions and homogeneity of the synthesized formulation, showing the successful formulation of a stable and uniformly dispersed nanoparticulate system. [34]

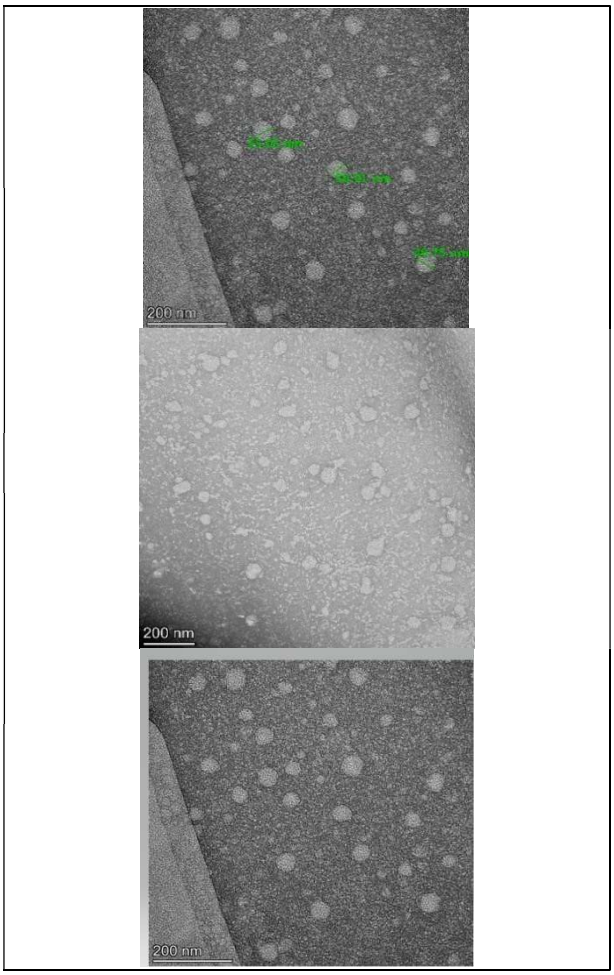


Figure 4 TEM Image of Posaconazole SLNs

Differential Scanning Calorimetry Analysis (DSC)

DSC was used to examine the thermal behavior and crystallinity of pure Posaconazole, the lipid matrix, and the resulting solid lipid nanoparticles. The thermogram of pure Posaconazole showed a clear and well-defined endothermic peak in the range of 166-170 °C, which corresponded to its characteristic melting point and confirmed its highly crystalline structure. In contrast, the DSC thermogram of the blank lipid, Precirol ATO5, showed a distinct melting endotherm in its typical temperature range of 55- 70 °C, indicating a semi-crystalline lipid structure.

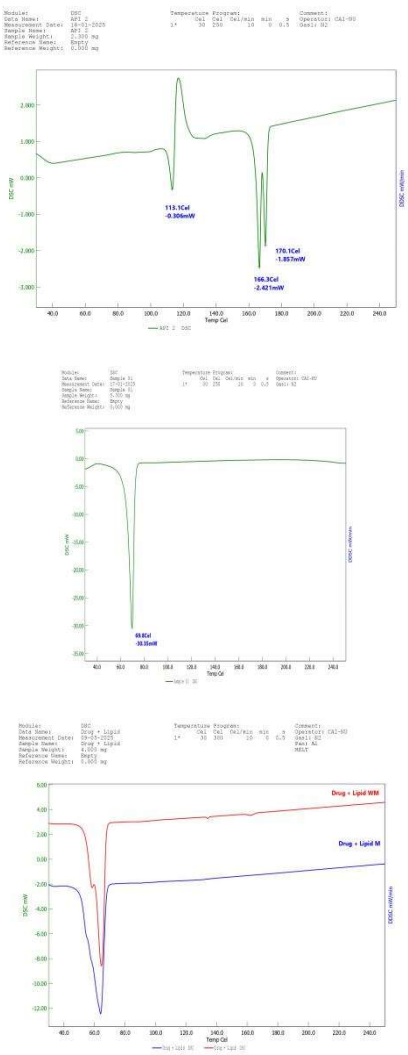


Figure 5 DSC Analysis Posaconazole SLNs

Notably, the thermal profile of the Posaconazole loaded SLNs showed considerable changes. Posaconazole's characteristic melting endotherm was either moved, widened or missing in the SLN thermogram. The result suggested that during the creation of nanoparticles, the drug was either molecularly dispersed across the lipid matrix or change into amorphous or less organized state. The attenuation or elimination of the drug's melting peak implies a decrease in crystallinity and supports the encapsulation of Posaconazole inside the lipid matrix, indicating the successful development of a stable nanoparticulate system.

Mechanism of Drug Release Kinetics:

PSC in vitro release profile form solid lipid nanoparticles was assessed utilizing the dialysis bag diffusion method. To summarize, precisely measured quantity of the lyophilized PSC -Solid Lipid Nanoparticles formulation was dispersed in Phosphate buffer (pH 7.4) and transported to a dialysis membrane having a MWCO of 12-14 KDa. The Dialysis bag was immersed in 100-200 ml of release medium at 37 ± 0.5 °C and stirred continuously at 100rpm to imitate physiological condition. To maintain sink conditions throughout the investigation, predefined aliquots (2-5) of the dissolution medium were taken at frequent intervals (0.5, 1, 2, 4, 6, 8, 12, 24 and 48 hours) and promptly replaced with an equal volume of fresh buffer. A UV- Visible Spectrophotometer with a maximum absorption wavelength of 262 nm was used to evaluate the samples drug content.

Posaconazole loaded solid lipid nanoparticles demonstrated a distinctive biphasic release pattern in vitro, with an initial burst release phase lasting 2-4 hours, followed by a protected and regulated release lasting 2-4 hours followed by a protracted and regulate release lasting 48-72 hours. The initial fast release may be due to the desorption of drug molecules adsorbed or weakly attached to the particle surface, whereas the succeeding sustained phase is caused by drug diffusion from the lipid core matrix.

In comparison, the pure drug suspension had a substantially quicker release profile, releasing around 78.03 – 90.21% of the Posaconazole within 8-12 hours. Posaconazole's weak water solubility and crystalline structure cause poor dissolution control, as seen by its fast release.

The Higuchi model (R^2 = 0.9903) best fit the release data indicating that drug release from the SLN is primarily controlled by diffusion via the lipid matrix. Furthermore, research using the Korsmeyer-Peppas model produce a release exponent (n) ranging from 0.45-0.65, indicating anomalous transport. This study indicates that the release mechanism is controlled by a combination of drug diffusion and gradual lipid matrix erosion or structural relaxation.[35]

Ocular Irritation Studies:

The HET- CAM Test involved incubating Fertilized hen's eggs at 37 ± 0.5 °C and 60-70% relative humidity for 9-10 days. Prior of the experiment, the eggshell surface was sterilized with 70% ethanol. A small aperture was then formed at the eggs to aspirate portion of the albumin, thereby facilitating the segregation of the chorioallantoic membrane from the inner shell membrane. A window was then aseptically opened in the eggshell to expose the chorioallantoic membrane while causing as possible minimal disruption to the underlying vascular network. A Predetermined amount about 0.1-0.3 ml of the test formulation was sophistically applied onto the surface of the chorioallantoic membrane. Simultaneously appropriate negative and positive controls were included to evaluate the experimental conditions. The CAM was systemically watched throughout a 300

seconds period for the development of vascular reaction including Bleeding, Cell lysis and coagulation and the onset time for each endpoint was accurately recorded. The Observed reaction duration was then used to produce an irritation score using a standard scoring method, enabling the test chemical to be classified based on irritation potential.[36]

HET CAM Study of Optimized Formulation

Group	Hemorrhage (S)	Lysis (S)	Coagulation (S)	Irritation Score (S)	Classification
Negative Control	Not Observed	Not Observed	Not Observed	0.00	Non-Irritant
Optimized SLN Formulation	Not Observed	Not Observed	Not Observed	0.00	Non-Irritant
Positive Control	25±2	45±3	80±4	12.5±0.4	Severe Irritant

Conclusion:

The present study successfully developed and characterized Posaconazole-loaded SLNs with desirable physicochemical and biopharmaceutical properties. PXRD and DSC analyses confirmed the molecular dispersion/amorphization of the drug within the lipid matrix, while particle size, PDI, and zeta potential results indicated good colloidal stability. In-vitro release studies demonstrated a biphasic pattern consisting of an initial burst followed by a prolonged sustained release, with kinetic modeling suggesting a diffusion-controlled (Higuchi-type) mechanism coupled with anomalous transport**.** Such a release profile is expected to enhance oral bioavailability, maintain therapeutic drug levels for extended periods, and potentially reduce dosing frequency. Overall, the findings point to SLNs as a potential Nano carrier system for posaconazole, capable of increasing its solubility, dissolution rate, and therapeutic effectiveness in antifungal therapy. Further in-vivo pharmacokinetic and pharmacodynamics studies are required to validate these findings and prove the formulation's therapeutic relevance.

AUTHOR'S CONTRIBUTION

Author 1: Kruti Bhatt (Ph.D Scholar, LJ Institute of Pharmacy) contributed equally to the work. Her roles included literature review, data collection and initial drafting of the manuscript.

Author 2: Dr. Shreeraj Shah (Corresponding Author) Director, Professor, and Head of the Department of Pharmaceutical Technology, L.J. Institute of Pharmacy, LJK University. My contribution in this manuscript was overall supervision, project administration, final manuscript editing, and approval for submission.

Author 3: Shruti Rawal (Assistant Professor) Institute of Pharmacy Nirma University. She contributed to the data validation, literature review.

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