RESEARCH ARTICLE

Creation and Assessment of an Ocular Film Based on Solid Lipid Nanoparticles of Fluconazole for the Treatment of Fungal Infection

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ABSTRACT

This work focuses on the integration of the lipophilic medication fluconazole into solid lipids, particularly stearic acid, and stabilized with Tween 80. Two approaches, namely the microemulsion method and ultrasonication, were evaluated for producing fluconazole-loaded solid lipid nanoparticles (SLNs). The formulation included Carbopol 934, which facilitates controlled drug release. It was found that higher concentrations of Carbopol 934 enhanced the sustained release of the drug by creating robust matrices due to its cross-linked nature. Particle size analysis revealed that the SLNs maintained an ideal size of less than 400 nm, exhibited a favorable polydispersity index (PDI), and displayed a negative zeta potential, all of which contribute to a longer precorneal residence time. Ultrasound-produced nanoparticles (FUSLN-4) significantly improved drug delivery to the cornea. X-ray diffraction and calorimetry confirmed reduced drug crystallinity within these nanoparticles, leading to enhanced drug release (up to 12 hours) and corneal penetration. Importantly, this method maintained corneal moisture. FUSLN-4 outperformed a microemulsion formulation and showed excellent stability (two years at room temperature), offering a promising solution to the challenges of ophthalmic drug delivery.

Keywords: Fluconazole, Ocular Film, Solid Lipid Nanoparticles, Fungal Infection

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INTRODUCTION

Third generation triazole antifungal medication fluconazole (FLZ) exhibits broad range action against both superficial and systemic fungal infections [1, 2]. Fungal cytochrome P-450 is inhibited, which stops the fungal membrane from producing essential components like ergosterol [3]. Compared to human enzymes, fungal species have a higher inhibitory effect on cytochrome P-450 enzymes, which enhances the safety profile of triazoles [4, 5]. The molecular weight of FLZ is 306.3 da, and it has a weak base pKa value of 3.7. It is also weakly soluble in water (5 mg/mL at 37 °C) [6, 7]. It comes in oral and parenteral dosing forms, and both can have major adverse effects such vomiting, diarrhea, upset stomach, feeling ill, rash, and decreased red blood cell counts. Furthermore, individuals on triazoles may experience hepatotoxicity [8].

Solid lipid nanoparticles, or SLNs for short, are colloidal lipid-based nanocarriers composed of a solid lipid core encircled by a surfactant layer. SLNs are typically 10–1000 nm in diameter. The chemical makeup of formulation components, formulation composition, preparation methods, and preparation parameters all affect the structural features and kinds of SLNs. Enhancing absorption and increasing safety are the primary benefits of utilizing SLNs to deliver active chemicals. Moreover, the potential of SLNs as lipid-based nanocarriers for various applications is demonstrated by their capacity to maintain the release of the active ingredient and stimulate the associated biological activity.

The eye is a vital organ since it is the sensory component of the visual system that creates the feeling of sight. As a result, the eye is protected from damaging external substances by a number of natural processes, including blinking and tear film turnover. These defense mechanisms are advantageous, but because medications are quickly removed from the cornea, they complicate ocular drug delivery. Because of this, the majority of conventional formulations—including eye drops and ointments—need to be administered repeatedly, which can decrease patient acceptance and raise the likelihood of treatment failure because of the high likelihood of missed doses. To further avoid microbiological contamination, eye drop solutions need extra preservatives because they are unstable. Higher medication stability and reduced toxicity from preservatives are two benefits of formulating the solid lipid nanoparticle-loaded ocular film. By extending the drug's contact duration with the cornea and raising intraocular bioavailability, novel drug delivery technologies like ocular film have been created to get over the main drawbacks of traditional ocular drug delivery methods [9, 10].

MATERIAL AND METHODS

Methods for Preparation of Nanoparticles

Ultrasonication Method

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Table 1: Preparation of solid lipid nanoparticles containing fluconazole (FUSLN and FMSLN)

Batches	Drug(%w/v)	Stearic acid (%w/v)	Tween80 (ml)	Carbopol 934 (mg)	DCM (ml)	W
1-FUSLN	0.04	0.5	3	25	3	q.s.
2-FUSLN	0.04	0.5	3	50	3	q.s.
3-FUSLN	0.04	0.5	3	75	3	q.s.
4-FUSLN	0.04	0.5	3	100	3	q.s.
5-FUSLN	0.04	0.5	3	125	3	q.s.
6-FMSLN	0.04	0.5	3	150	3	q.s.
7-FMSLN	0.04	0.5	3	175	3	q.s.
8-FMSLN	0.04	0.5	3	200	3	q.s.
9-FMSLN	0.04	0.5	3	225	3	q.s.
10-FMSLN	0.04	0.5	3	250	3	q.s.

Table 2: Composition of Drug-loaded SLNPs Incorporated Ophthalmic films

Ingredients	F1	F2	F3	F4	F5	F6
Optimized drug Loaded SLNPs	5.76	5.76	5.76	5.76	5.76	5.76
PVA(gm)	0.3	0.4				
EC(gm)			0.30	0.4		
Chitosan(gm)					0.3	0.30
Chloroform(ml)			15	15		
Distilled water(ml)	16	16				
Distilled water+ acetic acid(ml)					16+0.8	16+0.8
Poly ethylene glycol 400%W/V	25%	25%	25%	25%	25%	25%

To soften the lipid side chains, stearic acid is heated in a porcelain dish for at least 30 minutes at amplitude of 45%. Once softened, the required quantity of Tween 80 is added, and the mixture is then subjected to ultrasonication at a frequency of 0.5. After this sonication process, the blend is diluted 80 mL chilled DW and stirred continuously for 20 minutes. This ultrasonication process facilitates the dissolution of the drug into a stable solid lipid nanoparticle (SLN) solution.

Microemulsion Technique

Stearic acid is gently warmed in a porcelain dish to soften its lipid side chains before adding the necessary amount of Tween 80. The drug is then dissolved in the lipid phase using dichloromethane. The mixture is heated to 70°C for 15 minutes while gradually incorporating the lipid phase with continuous stirring. After heating, the oil-in-water microemulsion is carefully dripped into cold water (at 2-4°C) in a beaker, while maintaining a stirring speed of 2000 rpm for four hours. Following this, the solid lipid nanoparticle (SLN) dispersion undergoes ultrasonic treatment with a probe sonicator (PCI Analytics, Mumbai, India) at a frequency of 0.5 for 30 minutes at amplitude of 45%. This procedure results in a stable suspension of drug-loaded SLNs.

Lyophilization with Mannitol

Mannitol is added as a cryoprotectant to the aqueous SLN dispersion at the appropriate concentration (w/v %). This combined solution is then subjected to lyophilization for 24 hours to ensure its physical stability and ability to be redispersed. Initially, the mixture is pre-frozen at -74°C under a pressure of 0.02 mm Hg, after which it is placed into an adapter. This adapter is then inserted into a freeze-dryer to create a free-flowing powder of drug-loaded SLNs.

Methods for Preparation of Films

Spin Coating

The spine coating is utilized for experiments in a small lab, and a flat substrate can serve as a thin, uniform coating. This is regarded as a recommended technique for the procedure of creating a thin film solution.

Solvent Casting Technique

The solvent casting technique employs solvents like acetic acid, distilled water, and chloroform, along with retarding polymers such as PVA, EC and chitosan. Poly ethylene glycol (400 a plasticizer in this process **(Table 1)**. To prepare the casting solution, the desired concentration of polymer is mixed in an appropriate solvent for 30 minutes using a magnetic stirrer.

Physicochemical Characterization of Drug-loaded SLN

Measurements of Particle Size, Polydispersity Index, and Zeta Potential

Photon correlation spectroscopy (PCS) is employed to assess the particle size and polydispersity index (PDI) of solid lipid nanoparticles (SLNs) using the Zetasizer Nano ZS-90 from Malvern Instruments Ltd., based in Worcestershire, UK. Before the analysis, each sample of the SLN formulation is diluted with double-distilled water. Zeta potential measurements are also carried out using the same Zetasizer Nano ZS-90, in conjunction with a laser Doppler anemometer to assess the electrophoretic mobility of the particles. Each measurement consists of three individual analyses.

Determination of Entrapped Drug

The efficiency of capture (%) is determined using the subsequent formula:

Efficiency of capture (%) = (actual load/theoretical load) × 100

Theoretical loading of drugs (%) is computed using the following formula:

Loading theoretically (%) = (total drug) / (total drug + excipients total)

For the analysis, 50 mg of lyophilized SLN powder is combined with a methanol: distilled water solution (1:4) and taken to centrifugation at 12,000 rpm for 30 min to isolate the drug. The free drug concentration in supernatant is quantified by measuring the absorbance at designated wavelengths utilizing a UV-Visible spectrophotometer (Sytronics, Mumbai, India). To establish the total drug within the SLN, the lyophilized powder will be sonicated in dichloromethane, filtered using a 0. 2 µm syringe filter, and evaluated with the spectrophotometer. The actual loading of the drug (%) will be calculated as:

(%) Actual loading = (Total drug − Free drug) / (Mg of lyophilized powder) × 100

FTIR Analysis

The composition of the FTIR solid lipid nanoparticles (SLNs) is analysed using a BRUKER spectroscopy

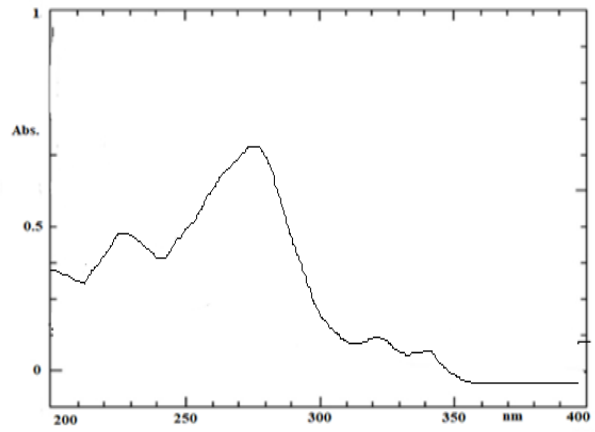

Fig. 1: Fluconazole UV Spectra

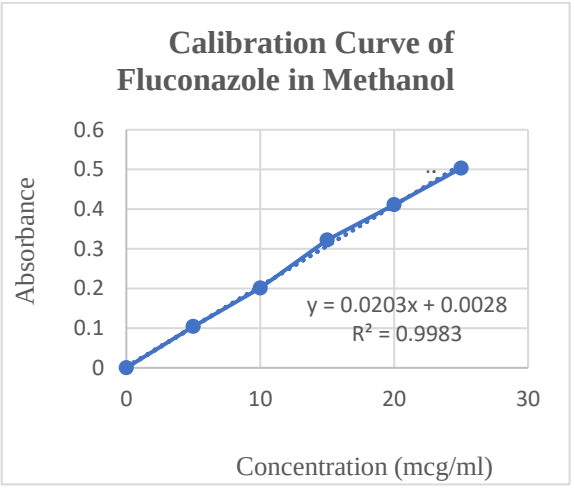

Fig. 2: Calibration curve of Fluconazole in Methanol

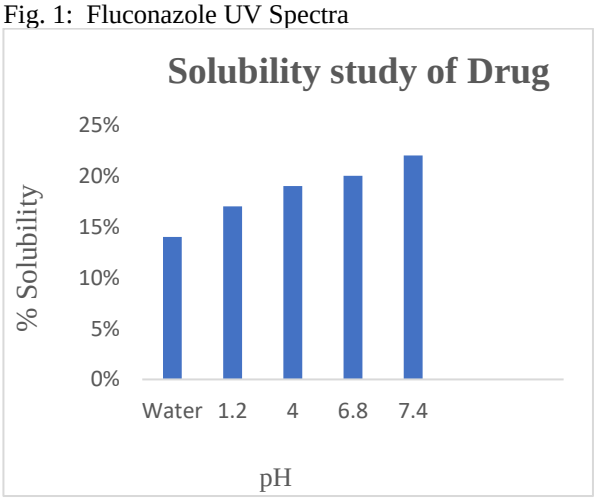

Figure 3: Solubility of Fluconazole in aqueous solutions and buffer systems

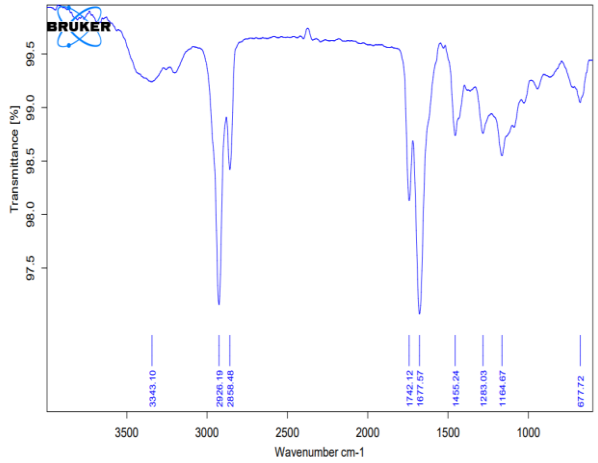

Fig. 4: Fluconazole FTIR spectra

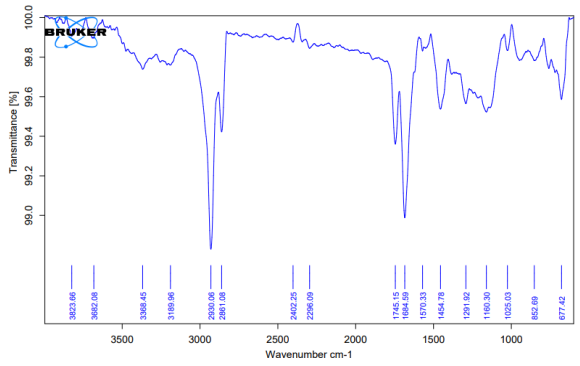

Fig. 5: Chitosan FTIR Spectra

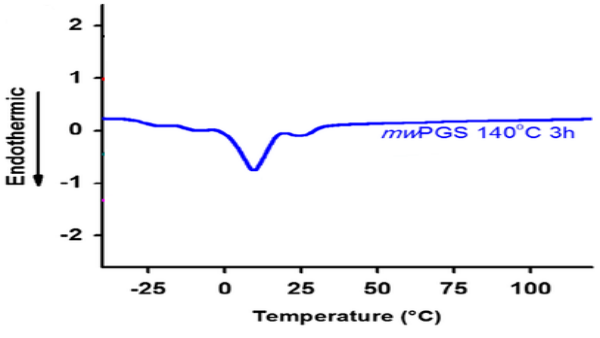

Fig. 6: DSC thermo gram of Fluconazole

Table 3: Parameters found in calibration curve

Sr. no.	Parameter	Outcomes
1	Wavelength	265 nm
2	equation of Regression	y = 0.0203x – 0.0028
3	Correlation coefficient	R^2 = 0.9983

instrument along with a potassium bromide (KBr) disc. This KBr disc, which includes a freeze-dried sample, is prepared by mixing 5 mg of SLN with 100 mg of KBr for the drying process. The spectroscopy readings are recorded across a wavelength range of 400 to 4000 cm.

Electron Microscopy

The shape and size of the drug-carrying solid lipid nanoparticles (SLNs) were analyzed using transmission electron microscopy (TEM). TEM images were obtained digitally after staining samples with 2% phosphotungstic acid using a Hitachi H7500 TEM. A PANalytical XPERT-PRO diffractometer determined the SLNs' crystallinity via powder X-ray diffraction (PXRD), using CuKα radiation. Thermal properties were assessed using differential scanning calorimetry (DSC) with a TA-60 DSC calorimeter, heating samples from 40°C to 200°C at 10°C/min under a nitrogen atmosphere. Finally, in vitro drug release was assessed with a modified USP Apparatus 1 at 37°C, utilizing a dialysis membrane (12000-14000 Da MWCO) in a synthetic tear solution (pH 7.4) agitated at 100 rpm. The drug quantity was determined by measuring absorption at the relevant wavelength using an ultraviolet light spectroscope (System, Mumbai, India). The experimental conditions were consistently maintained throughout the study. The release data was graphically analysed by plotting the percentage of drug released against time.

Formulation of Drug-loaded SLN Incorporated Ophthalmic Films

Six ophthalmic films incorporating SLN will be developed utilizing the solvent casting method. The casting solvents used will include acetic acid mixed with distilled water; chloroform combined with distilled water, and will feature retarding polymers such as chitosan, ethyl cellulose, and PVA. The formulation will also include the plasticizer PEG 400. A detailed formulation of the films containing drug-loaded SLNPs is provided in the accompanying **table 2**. To prepare the casting solutions, the required concentration of polymer is evenly dispersed in each solvent for 30 minutes using a magnetic stirrer. The SLNP dispersion, which contains the drug and 30% plasticizer, is then combined while the polymer solution is stirred continuously at 600 rpm. Afterward, the mixture is allowed to settle for 30 minutes to remove any air bubbles. The resulting liquid is transferred into a petri dish that has been lubricated with glycerine. The dish is then sealed with a reverse-leaked cover to regulate evaporation overnight. Once the evaporation process is complete, the film is carefully taken out, cut into 4x2 mm pieces, wrapped in oil paper, and kept at RT.

Evaluation of Film

Physical and Surface characteristics

Prepared compositions are visually inspected for clarity, texture and overall appearance.

pH Measurement

To promote film swelling, 0.5 ml of deionized water is introduced. After allowing the film to equilibrate for one minute, the pH is measured three times with a pH meter ensuring that the electrode makes consistent contact with the film.

Film Thickness

The film thickness is measured by a vernier calliper, taking the average of three readings following several folds to achieve uniform polymer distribution. To evaluate weight consistency, five randomly selected films from each batch are weighed three times, with the results averaged. We measure the folding durability of the film by repeatedly creasing it in the same place until it breaks. The average number of folds before failure indicates the film's overall folding endurance.

Uniformity of Drug Content

The ophthalmic film formulations (4 mm x 2 mm) will be dissolved in a buffer solution at pH 7.4 and stirred for one hour. After this, the solution is filtered, and the drug concentration is analysed using a UV spectrometer, with each sample evaluated in triplicate.

Tensile Strength

An ophthalmic film measuring 3x4 cm is placed between two supports that are 3 cm apart. The tensile strength is measured by applying weight to the film until it breaks. The total weight at the moment of rupture is recorded, and the tensile resistance is calculated using the following formula:

Table 4: Physiochemical characterization of FCZ loaded solid lipid nanoparticles

Sr. no	Batches	Particle size (nm ± SD)	Zeta potential (mV ± SD)	PDI (±SD)	Entrapment efficiency (% ± SD)
1	FUSLN-1	237 ± 2.55	−25.74 ± 0.66	0.330 ± 0.04	73.29 ± 0.12
2	FUSLN-2	250 ± 2.11††	−30.13 ± 0.58††	0.195 ± 0.05††	75.11 ± 0.05††
3	FUSLN-3	268 ± 1.53††	−28.26 ± 0.59††	0.264 ± 0.10†	79.67 ± 0.07††
4	FUSLN-4	277 ± 4.07††	−27.76 ± 0.64††	0.294 ± 0.06†	87.28 ± 0.14††
5	FUSLN-5	291 ± 4.61††	−31.89 ± 0.61††	0.235 ± 0.08†	64.93 ± 0.07††
6	FMSLN-6	311 ± 3.54	−29.49 ± 0.33	0.251 ± 0.07	62.16 ± 0.10††
7	FMSLN-7	322 ± 2.67†	−30.99 ± 0.44††	0.191 ± 0.05†	66.26 ± 0.15††
8	FMSLN-8	328 ± 4.19††	−27.86 ± 0.68††	0.340 ± 0.09†	68.16 ± 0.08††
9	FMSLN-9	338 ± 4.53††	−26.49 ± 0.43††	0.222 ± 0.05†	75.53 ± 0.08††
10	FMSLN-10	346 ± 3.54††	−31.9 ± 0.39††	0.266 ± 0.05†	63.20 ± 0.08††

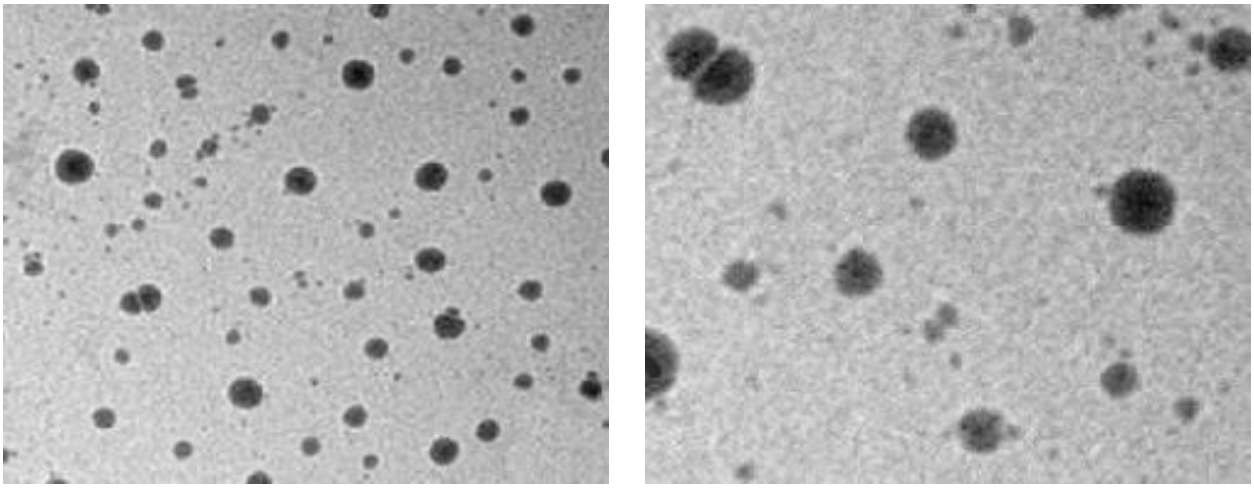

Fig. 7: TEM images of FUSLN-4 (left) and TEM images of FMSLN-9 (right)

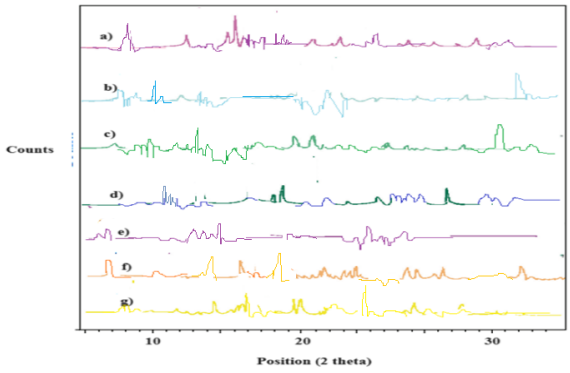

Figure 8: PXRD spectra of (a) Fluconazole, (b) stearic acid, (c) Fluconazole, stearic acid, and Carbopol physical mixture, (d) Carbopol 934, (e) Mannitol, (f) FUSLN-4, and (g) FMSLN-9

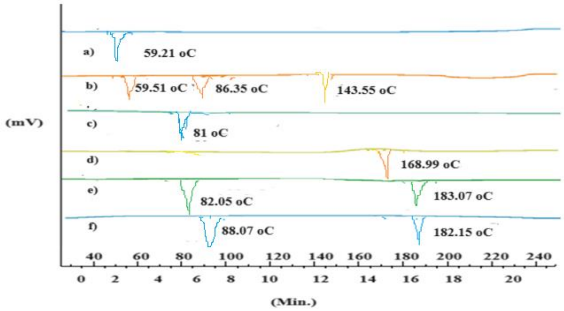

Fig. 9: DSC of (a) stearic acid, (b) Fluconazole, stearic acid, and Carbopol physical mixture, (c) Carbopol 934, (d) mannitol, (e) FUSLN-4, and (f) FMSLN-9.

Tensile resistance (g/ (cm²) = (failure load (G) x 100) / (cross-sectional area (cm²)).

Humidity Absorption Rate

This test evaluates the ability of the ophthalmic film to withstand high humidity. The film's weight is recorded over a three-day period in an environment of 79.5% humidity using a desiccant. After three days, the film is reweighed to assess moisture absorption.

Moisture absorption = (Final Wt - Initial Wt) / Final Wt x 100

Percentage of Moisture Loss

The film sample is placed in a anhydrous CaCl2 desiccator for a drying period of 72 hours. After this time, the sample is reweighed, and the amount of moisture loss is calculated using a straightforward formula.

% Moisture Loss = (Initial Wt. -Final Wt.) / Initial Wt. x 100

In-vitro Diffusion Study

To evaluate the efficacy of our ophthalmic films in delivering medication, we will employ a Franz diffusion cell setup. The films, each measuring 8mm in diameter, will be placed in one chamber (the donor) that is separated from another chamber containing simulated tear fluid (the receptor) by a membrane with a Mol. W.t 13,000–54,000 Dalton. This entire assembly will be steady temperature of 37.5°C and stirred at a rate of 50 rpm. Throughout 8-hour duration, samples will be collected at regular intervals to ensure that sink conditions are upheld, and the drug content will be analysed using a UV spectrophotometer.

Kinetic Modelling

The drug release from each square centimeter of the ocular film using PCP DISSO V3 software. This involves fitting the release data to several kinetic models—zero-order, first-order, Higuchi, and Korsmeyer Peppas—to determine the goodness of fit (R^2) and associated release constants.

Stability Studies

We will perform a 90-day stability study to determine the ideal storage conditions and the shelf life of our new F6 formulation. Both regular conditions (25°C, 60% RH) and accelerated conditions (40°C, 75% RH) will be applied to the samples while they are in a stability chamber. After being carefully wrapped with butter paper and foil, each film sample will be put into an aluminum pouch. At 15, 30, 60, and 90-day intervals, we will assess the films for drug content, tensile strength, and in-vitro diffusion.

Results and Discussion

Preformulation Study

λ max Determination: One milligram of the medication was precisely measured and put into a 100 mL volumetric flask. After dissolving the medication in methanol, the flask

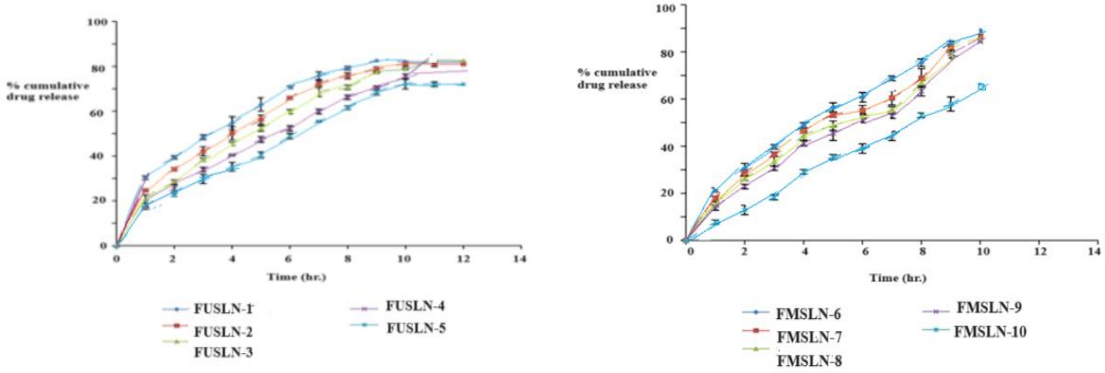

Fig.10: (a) Using a dialysis membrane, the in vitro release profile of fluconazole from solid lipid nanoparticle (SLN) formulations made by the ultrasonication approach was assessed. (b) A dialysis membrane was used to assess the in vitro release profile of fluconazole from solid

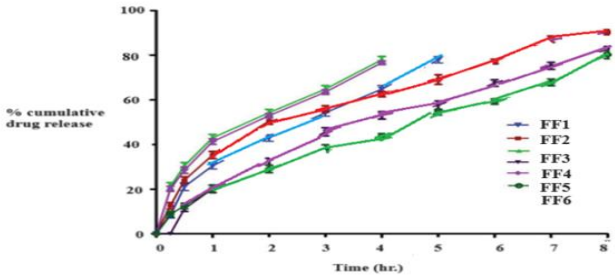

Fig. 11: In-vitro release profile of all Fluconazole ophthalmic films

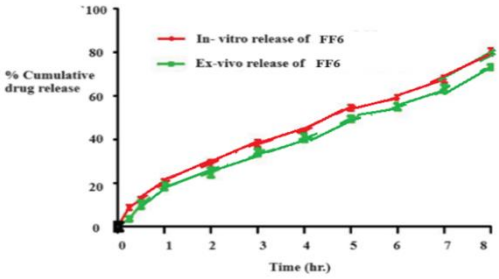

Fig. 12: Comparative in-vitro and ex vivo drug release profile of optimized formulation

was filled to the 100 mL mark. The absorbance of this solution was then measured using a UV spectrophotometer over the wavelength range of 200–400 nm. UV Spectra of Fluconazole shown in **fig. 1.**

The highest absorbance was observed at 265 nanometres, which was selected as the maximum wavelength (λmax) for additional analysis.

Calibration Curve

A standard solution of fluconazole was created strength of 1 mg/100 mL in methanol. The UV analysis indicated a peak absorbance at 265 nm for a 5 µg/mL solution. Using this UV method, a linear relationship between concentration and absorbance was determined within the range of 5 to 25 µg/mL. Calibration curve of Fluconazole in Methanol shown in **fig. 2 and table 3.**

Solubility study

Figure 3 shows the solubility of Fluconazole in aqueous solutions and buffer systems.

Analysis of drug and polymer interactions

Fourier Transform Infrared Spectroscopy Analysis

An examination of fluconazole FTIR spectrum **(figure 4)** validated the existence of specific peaks that align with its anticipated functional groups. This spectral information reinforces the assertion that the fluconazole sample is of high purity.The identity of Fluconazole was verified using FT-IR spectroscopy. The analysis showed that all the distinctive peaks corresponding to its functional groups were found within the anticipated range.

FTIR spectral analysis of the polymer validated the presence of all anticipated chitosan functional group peaks within the standard range, thereby confirming its identity shown in **fig.5.**

Differential Scanning Calorimetry (DSC)

We examined the thermal characteristics of fluconazole using differential scanning calorimetry (DSC). The obtained DSC curve **(figure 6)** shows an endothermic peak at around 140°C, signifying the melting point of the compound.

Two separate approaches were used to produce fluconazole-loaded solid lipid nanoparticles (SLNs) in order to examine the effects of various preparation processes on the physicochemical characteristics of drug-loaded SLNs. In order to reduce the size and polydispersity of the nanoparticles by disaggregating clusters, the ultra-sonication approach needs enough energy input to break down the droplets into the nanometer range. This method offers several advantages, including ease of use, no special environmental conditions necessary, and a faster manufacturing process. In contrast, the microemulsion technique involves introducing the microemulsion into water, causing the lipid phase to precipitate and resulting in smaller particles. This approach requires low mechanical energy input and is relatively easy to manage.

Particle Size, Polydispersity Index, and Zeta Potential Analysis

The results regarding the particle sizes of the formulations containing newly prepared freeze-dried fluconazole using the two methods are summarized in the table below. All SLN formulations demonstrated an average particle size<400 nm, which is ideal for ocular delivery, as particles

Table 5: Evaluation of Fluconazole SLN Incorporated Ophthalmic Films

Formulation Code	Surface Texture	pH of Surface	Thickness(mm)	Weight (mg)	Folding Endurance
FF1	Smooth	7.4 ± 0.244	0.027 ± 0.41	129 ± 2.255	263 ± 1.454
FF2	Smooth	7.1 ± 0.155	0.073 ± 0.78	131 ± 1.519	456 ± 0.735
FF3	Smooth	7.6 ± 0.118	0.066 ± 0.13	129 ± 1.084	353 ± 1.580
FF4	Smooth	7.3 ± 0.195	0.046 ± 0.28	130 ± 2.247	348 ± 0.258
FF5	Smooth	7.5 ± 0.213	0.073 ± 0.78	132 ± 1.028	283 ± 1.359
FF6	Smooth	7.5 ± 0.123	0.038 ± 0.45	130 ± 1.530	314 ± 2.650

smaller than 10 μm, can be accommodated by the human eye. The particle sizes of the formulations produced via the ultra-sonication method (FUSLN-1 to FUSLN-5) ranged from 237 ± 3.55 nm to 291 ± 4.61 nm, whereas those made using the microemulsion method (FMSLN-6 to FMSLN-10) varied from 311 ± 3.55 nm to 346 ± 3.54 nm. Higher polymer concentrations resulted in larger nano droplets due to the increased viscosity of the dispersed phase. Because the particles were broken up into smaller droplets during the preparation process, the ultra-sonication approach (FUSLN-1 to FUSLN-5) produced noticeably lower particle sizes than the microemulsion method (FMSLN-6 to FMSLN-10).

Polydispersity Index

The distribution of particle sizes was measured by the polydispersity index (PDI), which varied between 0.191 ± 0.016 and 0.340 ± 0.018. PDIs for submicron particles usually range from 0.18 to 0.6, with values above 0.6 suggesting significant size fluctuation. A restricted size distribution is suggested by a PDI of less than 0.6, which indicates the solid lipid nanoparticles' high stability. There was statistical significance in these findings. **Table 4** shows Physiochemical Evaluation of Solid Lipid Nanoparticles Loaded with Fluconazole.

Electron Microscope Analysis

Transmission electron microscopy (TEM) was used to evaluate the FCZ-SLN formulations morphologically. **Figures 7 (a) and (b)** show the TEM pictures of the SLN compositions produced by the two techniques. The results showed that all formulations had spherical forms and smooth surfaces, suggesting that the nanoparticles might be stable. Previous studies have shown that particles with rounded corners and edges tend to be less irritating compared to those with sharp edges, suggesting that these nanoparticles are less likely to cause discomfort on the ocular surface. There were slight differences in the fundamental structures of the fluconazole SLNs created by the two methods, with these variations primarily attributed to the lipid properties, such as solubility and membrane-forming abilities, which affect the structure of solid lipid nanoparticles.

Powder X-Ray Diffraction (PXRD) Analysis

The fluconazole, lipid, polymer, physical mixture, mannitol, and lyophilized solid lipid nanoparticles (SLN) XRD patterns are shown in the **figure 8**, providing insight into the crystallinity of the lipid matrix and the FCZ-SLN generated using both methods. Fluconazole's crystalline nature was confirmed by the identification of distinct peaks at angles of 15.16°, 16.48°, 17.23°, 22.33°, 24.17°, 30.18°, and 37.18° 2θ. Fluconazole's X-ray diffraction pattern showed notable peaks that confirm its crystalline structure. Carbopol 934's XRD pattern, on the other hand, showed no discernible peaks, suggesting that the polymer is entirely amorphous. With peaks at 7.5°, 17.55°, and 23.31° 2θ, the physical mixture of carbopol 934, stearic acid, and fluconazole showed a less crystalline profile. Mannitol showed several distinct peaks associated with its β polymorphic form at 13.58°, 18.38°, 21.81°, 22.92°, 26.42°, 27.25°, 32.46°, 36.32°, 37.96°, and 42.56° 2θ. The lyophilized SLN containing FCZ displayed peaks at 11.97°, 12.79°, 18.97°, 22.73°, 23.45°, 23.57°, 24.47°, and 27.63° 2θ, likely influenced by the presence of stearic acid and mannitol. The heightened peak intensities indicate that the SLN product may exhibit crystalline characteristics.

Differential Scanning Calorimetry (DSC) Analysis

The thermal properties of the samples during melting and recrystallization were analyzed using the Differential Scanning Calorimetry (DSC) technique, with the resulting thermograms illustrated in the **figure 9.** Stearic acid displays a notable melting point of 59.21 °C and a fusion enthalpy of 160.22 J/g. The physical blend of carbopol 934, stearic acid, and fluorinazole does not show a reduction in melting point, instead revealing distinct peaks at 143.55 °C, 59.51 °C, and 86.35 °C, respectively. The DSC analysis for carbopol 934 revealed a significant endothermic peak at 81 °C. Mannitol exhibited an endothermic peak at 168.99 °C, with a heat of fusion measuring 294.34 J/g. The DSC curve for lyophilized solid lipid nanoparticles (SLNs) showed a clear endothermic peak at 167.67 °C, likely related to the subtle endothermic peak of the mannitol β-polymorph,

Table 6: Evaluation of SLN Incorporated Ophthalmic Films

Formulation code	Tensile strength (g/cm2) ± SD	Drug content (%) ± SD	Moisture Absorption (%) ± SD	Moisture Loss (%) ± SD
FF1	38.0 ± 1.79	99.85 ± 0.31	4.78 ± 0.37	5.85 ± 0.45
FF2	48.0 ± 01.90	96.30 ± 0.44	4.95 ± 0.47	5.73 ± 0.42
FF3	45.8 ± 0.65	98.33 ± 0.41	5.29 ± 0.41	5.14 ± 0.08
FF4	35.0 ± 0.84	98.31 ± 0.50	5.89 ± 0.27	5.70 ± 0.27
FF5	33.7 ± 0.15	98.77 ± 0.44	5.99 ± 0.45	5.58 ± 0.30
FF6	34.5 ± 1.25	99.23 ± 0.49	5.61 ± 0.42	4.41 ± 0.79

followed by a smaller peak at 83.05 °C, corresponding to the melting point of carbopol 934. The thermograms did not show a distinct melting peak for fluconazole, suggesting that FCZ exists in an amorphous state.

In Vitro Drug Release Study

Figure 10 (a) and (b) show the in vitro release of FCZ from solid lipid nanoparticles (SLN) produced using both methods. A dialysis membrane was used to measure the drug release from the SLN, and simulated tear fluid with a pH of 7.4 was used for the release evaluation.

The solid lipid nanoparticles produced through the ultrasonication method (FUSLN-4) showed a greater drug release of 20.22 ± 0.12% after one hour and 89.79 ± 0.81% after twelve hours. In contrast, the solid lipid nanoparticles developed using the microemulsion method (FMSLN-9) exhibited a drug release of 16.89 ± 0.12% after one hour and 87.30 ± 1.04% after ten hours. The drug release profiles for the two methods were notably different.

lipid nanoparticle (SLN) formulations made utilizing the microemulsion process.

Evaluation of Fluconazole Ophthalmic Films

Physical Appearance, Surface Texture, and pH of Ophthalmic Films

All of the prepared batches (FF1–FF6) displayed smooth, defect-free surfaces, with each film having a clear and attractive appearance. The surface pH of the film is crucial for determining its acidity or alkalinity, which is vital for ensuring eye safety. The typical pH range for eye fluid is between 7.1 and 7.8, and deviations from this range could cause discomfort or harm to the eyes. Films with a surface pH between 7.1 and 7.5 are more likely to be well-tolerated by patients, as they decrease the chances of eye irritation and enhance patient acceptance. This was demonstrated by all the formulations (FF1–FF6), which each had a surface pH of 7.4, aligning well with the properties of simulated tear fluid, as shown in the **table 5.**

Thickness, Weight Variation, and Folding Endurance

The formulations FF1 through FF6 ranged from 0.0248 ± 0.41 mm to 0.07034 ± 0.78 mm, indicating a uniform distribution of both the medication and polymer. The average weight of these formulations varied between 129 ± 1.255 mg and 132 ± 2.05 mg, which is within acceptable parameters. The flexural strength of the formulations (FF1 to FF6) ranged from 263 to 456, highlighting the mechanical integrity of the films.

Drug Content and Tensile Strength

Evaluating the formulation composition is crucial to confirm uniform distribution throughout the film. The drug content in formulations FF1 to FF6 was recorded at between 98.33% and 98.31%. These formulations exhibited tensile strength within an acceptable range, measuring from 32 ± 0.815 to 48 ± 0.8782 **(table 6)**. At the designated polymer concentration, all films from FF1 to FF6 demonstrated exceptional mechanical strength, flexibility, and elasticity. Furthermore, it was shown that both folding endurance and tensile strength were continuously improved by increasing the plasticizer's concentration.

Moisture Absorption and Moisture Loss

The figures presented in the table illustrated that the values for moisture absorption and retention across all compositions are within the acceptable ranges, which are 2.755 ± 0.351 to 5.99 ± 0.425 for absorption and 2.390 ± 0.771 to 3.824 ± 0.424 for retention.

In-vitro Permeation Studies

An in vitro permeation study illustrated drug release percentages of 82. 51%, 93.66%, 88.15%, 85.75%, 85.64% and 83.24% for formulations FF1, FF2, FF3, FF4, FF5 and FF6 respectively. FF1, FF3 and FF5 were removed from the selection as they displayed quicker and more complete drug release within 4-5 hours. Conversely, formulations FF2, FF4, and FF6 demonstrated sustained drug release over 8 h with release rates of 93. 66%, 88.17%, and 83. 24%, respectively. This sustained release arises from the effective incorporation of retarding polymers (PVA, ethyl cellulose, and chitosan) in the formulations. Through thorough assessment, FF6 was identified as the optimal formulation. Elevated concentrations of PVA and chitosan (FF1 and FF2) appeared to create a denser and more robust molecular network, which seemed to hinder drug release (FF5 and FF6). Ethyl cellulose (EC), a commonly utilized polymer for continuous drug delivery systems, has been proven to be biocompatible, cost-effective, and versatile. A notable reduction in drug release was noticed when the CE concentration increased from 0. 25 to 0.30 g (FF3 and FF4), potentially due to a prolonged diffusion path that delayed the release. In-vitro release profile of all Fluconazole ophthalmic films are shown in **fig. 11.**

Ex-vivo Permeation Studies

In vitro drug permeation tests conducted with goat corneas indicated that the enhanced FF6 formulation achieved a cumulative drug permeability of 79.33% after 8 hours. Of this total, 3.24% of the FF6 was absorbed in vitro, while 72.33% was absorbed ex vivo. The results from in vitro studies suggested that drug penetration was more significant than what was seen in ex vivo studies. The variations in absorption rates are explained by the fact that the natural cornea functions as a semipermeable membrane with different pore diameters and that the fatty tissues around it prevent medications from passing through the cornea. The cornea is made up of a hydrophilic stroma, a less lipophilic endothelium, and a lipophilic epithelium. The dialysis membrane acts as a physical barrier to prevent liquids from passing through the cornea. A comparison of the release profiles for the optimized formulations in both in vitro and ex vivo conditions is presented in the **figure 12**, along with the in vitro release profiles for all formulations (FF1–FF6).

Kinetic Modelling of Drug Release

The data were analysed using various kinetic models, calculating regression coefficients (R^2) and release constants. The findings showed (FF1-FF6) that all formulations adhered to Higuchi kinetics, indicating that the release mechanism primarily relied on diffusion. Each formulation displayed a Fickian diffusion mechanism, characterized by a diffusion exponent (n) <0.5.

Short-term Stability Evaluations

The improved formulation's 90-day stability evaluation revealed no appreciable changes in its drug content, tensile strength, or in vitro diffusion properties. These results demonstrated the formulation's appropriateness for the

long-term preservation of ocular films by confirming its stability under both ambient temperature and accelerated stability settings.

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