Journal for Re Attach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 December; 6 (10s) (2): 1879-1891

Formulation and Evaluation of Econazole loaded Solid Lipid Nanoparticles based Ocular Film for Treatment of Fungal Infection

Keshav Rao Bapu Rao Kulkarni[1]*, Dr. Satish Kumar Sarankar[2]

[1*]Research Scholar, Mansarovar Global University, Faculty of Pharmacy, Sehore, M.P. 466111
[2]Professor and Principal, Faculty of Pharmacy, Mansarovar Global University, Sehore M.P. 466111

Abstract: This study evaluated the viability of using solid lipid nanoparticles for the ocular delivery of Econazole, adopting stearic acid as lipidic material, tween 80 as a stabilizer, and carbopol 934 as a controlled release agent and for increasing the precorneal residence time in the eye. The systems were prepared using two different methods, that is, the ultrasonication method and the microemulsion technique. The results showed that the larger particle size of SLNs was found with the microemulsion technique (309 ± 3.53 nm to 344 ± 3.52) compared to SLN prepared with the ultrasonication method (235 ± 3.53 nm to 289 ± 4.59 nm). All of the formulations had polydispersity index values less than 0.3, and the formulations made using these two procedures had zeta potentials ranging from -23.72 ± 0.64 mV to -29.87 ± 0.59 mV. The drug's crystallinity decreased, as shown by differential scanning calorimetry and powder X-ray diffraction. The SLN formulations made using the ultrasonication method and the in vitro release research both showed sustained release for a maximum of 12 hours. This investigation showed that, without significantly altering the amount of corneal moisture, SLN prepared using the ultrasonication method is more appropriate than that made using the microemulsion approach. The goal of the solid lipid nanoparticle-loaded ocular film of Econazole, an antifungal medication used to treat fungal keratitis, was to improve patient compliance by reducing the need for repeated dosage, lengthening the retention period, and providing sustained release. Using the retarding polymers chitosan, PVA, and ethyl cellulose, the optimal solid dispersion formulation was first created by the solvent evaporation method and then added to films made by the solvent casting method. Plasticizer PEG-400 was utilized. The films were assessed for antimicrobial activity, in-vitro and ex-vivo permeation investigations, thickness, surface pH, drug content, weight uniformity, tensile strength, and in-vitro antifungal research. The films were within an acceptable range and showed good mechanical properties with promising results. In vitro tests using the improved formulation F6 demonstrate more drug release than in vivo experiments. This results from disruption of other tissues and the cornea's variable pore size. The developed formulation proved not to irritate the eyes, according to an ocular irritation test. Therefore, it appears that the ophthalmic film formulation holds promise for the safe and efficient administration of Econazole via the ocular route in the management of fungal keratitis.

Keywords: Econazole, Fungal Infection, Solid Lipid Nanoparticles, Ocular Film, etc.

Introduction

The eye is the most easily accessible area for topical drug application. Medications are frequently administered to the eye system to produce a focused effect inside or on the surface of the eye [1]. The formulator's main task is to overcome these obstacles without endangering any tissue [2]. The cornea, which consists of endothelium, stroma, and epithelium, is the anterior layer of the eye. Nonetheless, this layer functions as a mechanical barrier that prevents medication molecules from being delivered. The epithelium and endothelium are thought to hinder the flow of hydrophilic molecules because of their high fat content. Because of its high water content, the stroma is impermeable to molecules that are lipophilic [3]. An ideal ophthalmic drug delivery must be able to release the drug in a sustained manner and remain in the front of the eye for a prolonged period of time. As a result, various attempts have been made to prolong the residence time of drug on the ocular surface and also to slow down the drug elimination [4,5]. Corneal barrier also plays a significant role in low ocular bioavailability, due to which only < 5% of the applied drugs are able to penetrate through the cornea into the intraocular tissues [6]. In order to optimize ocular drug delivery, different strategies were developed to increase the bioavailability of drugs in the front of the eye to prolong time.

To tackle the foregoing issues associated to ocular delivery, novel drug delivery systems (NDDS) consisting largely of nanoemulsions, liposomes, microemulsions, microspheres, and solid lipid nanoparticles (SLNs) have been proposed afresh for oral, topical, and parental administration of pharmaceuticals [6, 7]. Compared to other colloidal carriers including liposomes, lipid emulsions, emulsions, polymeric microparticles, and so on, SLN was a remarkable carrier system. SLNs are physiological lipid-based submicron colloidal carriers, with a size range of 50 to 1000 nm, that are dissolved in water or an aqueous surfactant solution [8]. Lipophilic, hydrophilic, and poorly water soluble medications can be added with them [9].

An imidazole chemical called Econazole nitrate (ECN) has a wide range of antifungal activity and is mostly used to treat fungal infections brought on by the fungus Candida albicans [10]. By blocking the enzyme Cytochrome P-450, Econazole nitrate prevents the synthesis of ergosterol by increasing cell permeability, resulting in the leakage of cellular content

and ultimately leading to cell death [11–13]. When applied topically, the plasma protein binding is around 98%, and the absorption is relatively low [14].

Polymer-based films, known as ophthalmic films, have the ability to remain in the external segment of the eye for extended periods of time. These consist of various polymer grades that disintegrate in the presence of physical pressures. Due to their biodegradable nature, longer half-life at the site of action, and lower cost compared to other conventional forms, they offer several advantages in terms of improved bioavailability and patient compliance [15]. Therefore, the main objective of this work was to create and assess an Econazole ophthalmic film with improved solubility, longer drug retention at the site, and enhanced bioavailability by employing solid lipid nanoparticles. PVA, chitosan, and ethyl cellulose were used as retarding polymers.

Material and Methods

Methods for preparation of Nanoparticles

Ultra sonication Method

To relax the side chains of the lipids, stearic acid will be first heated in a porcelain dish before the necessary amount of tween 80 will be added by changing the frequency to 0.5 for 30 minutesat around 45% amplitude, ultrasonication will be performed. Following sonication, the dispersionwill continuously stirred for 15 minutes while being diluted with 80 mL of distilled cold water. Drugs could successfully ultrasonically dissolved into a stable SLN solution.

Microemulsion Technique

This method involved melting stearic acid in a porcelain dish to relax the lipid side chain before adding the necessary amount of tween 80. The medication would be then introduced to the aforementioned lipid phase in dichloromethane, where it will soluble. The lipid phase will be added gradually while being constantly stirred, and the mixture will be then heated for 15 minutes at about 70 oC. Warm o/w microemulsion will be dispersed drop wise into cold water (2– 3 C) in a beaker while being continuously stirred at 2000 rpm for 4 hours to produce solid lipid nanoparticles. After stirring, SLN dispersion will immediately ultrasonically sonicated using a probe sonicator (PCI analytics, Mumbai, India). By changing the frequency to 0.5 for 30 minutesat around 45% amplitude, ultrasonication will be performed. By using the microemulsion technique, a stable SLN suspension of the medication will be produced.

Lyophilization

% w/v mannitol will be added as a cryoprotectant to the aqueous dispersions mentioned above, and after that, lyophilization will done for 24 hours to ensure physical stability and redispesibility. Prior to keeping the vials in the adapter, prefreezing will be carried out by freezing the mixture at 74 oC and 0.02 mm Hg pressure. In order to obtain free-flowing powder of drug-loaded SLN, the adapter will be subsequently fitted into a freeze-dryer (Lyophilizer FD-5-3, Allied Frost, New Delhi, India).

Methods to be used for preparation of Films

Spin coating

Among solution processable thin film techniques, spin coating is the preferred methodfor small-scale laboratory studies, yielding thin, uniform films on flat substrates.

Solvent casting Method

As casting solvents, acetic acid, distilled water, chloroform, and distilled water were employed with the retarding polymers chitosan, ethyl cellulose, and PVA, respectively. Plasticizer PEG
400 will be utilized the casting solutions were made by uniformly dispersing the specified concentration of polymers in the appropriate solvents for 30 min. using a magnetic stirrer

Formulations

Table 1: Composition of Econazole loaded solid lipid nanoparticles (EUSLN and EMSLN-Econazole solid-lipid nanoparticles)

Batches	Drug (% w/v)	Stearic acid (% w/v)	Tween 80 (ml)	Carbopol 934 (mg)	Dichloromethane(ml)	Distilledwater
EUSLN-1	0.02	0.3	2	25	2	q. s
EUSLN-2	0.02	0.3	2	50	2	q. s
EUSLN-3	0.02	0.3	2	75	2	q.s
EUSLN-4	0.02	0.3	2	100	2	q.s
EUSLN-5	0.02	0.3	2	125	2	q.s
EMSLN-6	0.02	0.3	2	150	2	q.s
EMSLN-7	0.02	0.3	2	175	2	q.s
EMSLN-8	0.02	0.3	2	200	2	q.s
EMSLN-9	0.02	0.3	2	225	2	q.s
EMSLN-10	0.02	0.3	2	250	2	q.s

Physicochemical Characterization of drug loaded SLN

Particle Size, Polydispersity Index, and Zeta Potential Measurements

Using a Zetasizer Nano ZS-90, photon correlation spectroscopy (PCS) will be used to measure the SLN's Polydispersity index (PDI) and particle size (Malvern Instruments Ltd., Worcestershire, UK). All SLN formulation samples were diluted with double-distilled water before being analysed. To verify the electrophoretic mobility of the particles, the zeta potential measurements were carried out using a laser-doppler-anemometer in conjunction with a Zetasizer Nano ZS-90 (Malvern Instruments Ltd., Worcestershire, UK). All analyses were performed three times.

Determination of Entrapped drug

It will be computed using the following equation and represents the proportion of the actual mass of drug that will be trapped in the polymeric carrier as compared to the original amount of medication loaded:

$$\text{Entrapment efficiency\%} = \frac{\text{Actual loading}}{\text{Theoretical loading} \times 100}$$

Theoretical drug loading will be calculated from the amount of drug taken relative to the amount of total drug and excipients used in the preparation of nanoparticles as follows:

$$\text{Theoretical loading (\%)} = \frac{\text{Total drug}}{\text{Total drug} + \text{Total excipients}}$$

For the actual drug loading, the SLN dispersion will be centrifuged at 13000 rpm for 20 minutes using 25 mg of the lyophilized SLN powder in methanol: distilled water (1:4). Using a UV- visible spectrophotometer to measure absorbance at a specified wavelength, the clear supernatant's free drug content will be determined (Systronics, Mumbai). The total amount of drug present in the SLN will be calculated by sonicating of the lyophilized SLN powder into dichloromethane, filtering through a micro syringe filter (0.2 m), and then analyzing the filtrate for drug using a UV visible spectrophotometer to measure absorbance at a particular wavelength. Actual loading will be calculated using the formula below:

$$\text{Actual loading (\%)} = \frac{\text{Total drug} - \text{Free drug}}{\text{Mg of lyophilized powder} \times 100}$$

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Utilizing the potassium bromide (KBr) disc approach, FTIR spectra of produced SLN were captured by a Bruker spectrophotometer (Bruker IFS 66/S, Germany) (5 mg samples for 100 mg dry KBr). The lyophilized formulations were produced as KBr discs and examined between 400 and 400 cm1 in wavelength.

Electron Microscope Examination
Using a transmission electron microscope, the drug-loaded SLN's morphology will be observed (TEM). 2% (w/v) phosphotungstic acid will be used to stain the nanoparticle samples. The copper grids with films were put with the nanoparticle suspension for TEM observation (Hitachi H7500, Tokyo, Japan). Software for imaging viewers and digital micrographs were used to take the picture.

Powder X-Ray Diffraction (PXRD) Analysis
PXRD will be used to look at the SLN formulation's crystalline state. The samples' X-ray powder diffraction patterns were captured using the PRS measurement software on the Ni-filtered, CuK radiation generated at 45 kV and a current intensity of 40 mA in the XPERT-PRO multifunctional X-ray diffractometer (PAN analytical, Netherlands). The instrument's diffraction angle range will be operated over a range of two angles, from 5 to 40.

Differential Scanning Calorimetry (DSC) Analysis
A DSC TA-60 (Shimadzu, Tokyo, Japan) 208 calorimeter will be used to obtain the thermo grams of the various materials. At a scanning rate of 10 C/min, samples were heated in crimped aluminium pans from 40 C to 200 C. In every instance, analyses were performed using an inert nitrogen purge (35 mL/min) and a reference pan of empty alumina.

In- Vitro Drug Release from drug loaded-SLN
The modified USP dissolution apparatus 1 (37 0.5 C), which contains a two-sided open glass cylinder, will be used for the 12-hour in vitro drug release tests. Dialysis membrane served as the diffusion barrier, while the release barrier will be the molecular weight cut-off 12000–14000 A (Himedia, Mumbai). The glass cylinder's end will be modified to fit a pre-soaked dialysis membrane. Each time, 5 mL of a solid lipid nanosuspension were carefully added to the glass cylinder from the open side, which will be then fastened to the stirrer. The stirrer will be submerged in synthetic tear fluid (pH 7.4) that will be dissolving in medium that will be kept at 37 oC ± 0.5 oC and 100 rpm. At set intervals, aliquots of the samples were taken out, and the volume will be replaced. Utilizing a UV-visible spectrophotometer to measure absorbance at a specified wavelength, the removed samples were examined for drug content (Systronics, Mumbai, India). For the duration of the release time, sink conditions were maintained. A graph plotting the percent drug release versus time will be used to graphically examine the data that were acquired in triplicate.

Formulation of drug loaded SLN Incorporated Ophthalmic Films
The solvent casting approach will be used to create six SLN-loaded ophthalmic films. As casting solvents, acetic acid + distilled water, chloroform, and distilled water were employed with the retarding polymers chitosan, ethyl cellulose, and PVA, respectively. Plasticizer PEG 400 will be utilised. The Table provides a full breakdown of the film compositions incorporating drug-loaded SLNPs. The casting solutions were made by uniformly dispersing the specified concentration of polymers in the appropriate solvents for 30 min. using a magnetic stirrer. SLNPs dispersions containing medication and 30 % plasticizer will be added while the polymeric solution will be constantly stirred (at a speed of 600 rpm). To get rid of the bubbles, it will be then placed aside for a half-hour. The liquid will be added to a glycerin-filled pre-lubrication petridish. For controlled evaporation, the petridish will be sealed in an upside-down funnel and left out overnight. After being carefully removed, the films were cut into 4x2mm pieces, wrapped in butter paper, and kept at room temperature for storage.

Table: 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)	0.2	0.3	-	-	-	-
Ethyl cellulose (gm)	-	-	0.25	0.3	-	-
Chitosan (gm)	-	-	-	-	0.2	0.25
Chloroform (ml)	-	-	10	10	-	-
Distilled water (ml)	15	15	-	-	-	-
Distilled water + acetic acid (ml)	-	-	-	-	15 + 0.5	15 + 0.5
PEG 400 % W/V	30 %	30 %	30 %	30 %	30 %	30 %

Evaluation of Film

Physical Appearance and Surface Texture

The prepared formulations were inspected visually for their appearance, texture, and clarity.

Surface pH

The film will be kept in contact with 0.5ml of neutral water to swell it. After one minute of equilibration, the electrode will be brought into contact with the ocular film, and the pH will be measured in triplicate using a pH meter (Elite Scientific Corporation, Haryana, India).

Thickness of Film

A Vernier calliper will be used to gauge the film's thickness. The measured value will be divided by the quantity of folds after the film will be folded a specific number of times. This illustrates both the homogeneous distribution of the polymer and the uniform thickness of the film. Three measurements were made for each measurement.

Uniformity of Weight

A digital balance will be used to determine the weight of five films that were randomly selected from each batch of the formulation. Three copies of the average weight were recorded.

Folding Endurance

The film will be folded over and over in the same place until it finally snapped. The film's breakage threshold will be determined by how many folds were needed. The average of three analyses will be taken into account.

Drug Content Uniformity

All 4 mm x 2 mm ophthalmic film formulations were dissolved in phosphate buffer solution with a pH of 7.4, agitated for an hour on a magnetic stirrer, filtered, and the filtrate will be examined for drug content using a UV spectrophotometer. A triple research will be conducted for each formulation.

Tensile Strength

The 3x4 cm ophthalmic film will be secured by two clamps that were 3cm apart from one another. By adding weights to the pan until the film broke, the tensile strength of the film will be ascertained. The total weight will be noted, and a triple tensile strength calculation will be performed. Using, adhering to the formula.

Tensile strength g/(cm)2 = (Load at failure (g)X100) / (Cross sectional area ((cm)2))

Percent Moisture Absorption

It is one method of evaluating the ophthalmic film's physical stability in high-humidity circumstances. The prepared ophthalmic films were weighed and kept in the desiccator for three days. Aluminum chloride solution is used as a desiccant (79.5 percent humidity). All ocular films were weighed, and the method will be used to calculate the percentages of moisture absorption in triplicate.

Percent moisture absorption= (Final weight-Initial weight)/ (Final weight) X 100

Percent Moisture Loss

In the dissector, anhydrous calcium chloride is present. Three days were spent weighing and storing the films. After the films were reweighed, the moisture loss will be calculated using the following equation.

Percent moisture loss= (Initial weight-final weight)/ Initial weight ×100

In-vitro Diffusion Study

To ascertain the in-vitro drug release profile of ophthalmic films, the Franz diffusion cell will be utilized. The 8 mm films were in the donor compartment, and the simulated tear solution will be in the receptor compartment. With the rpm set to 50 and the temperature set to 37.5°C, the dialysis membrane (molecular weight cut off of 12,000–14,000 Dalton) will be fixed as a semipermeable membrane between the donor and receptor compartments. Samples were taken out up to 8 hours apart at predetermined intervals. Samples were examined at a given wavelength while the sink state will be preserved.

In-vitro Trans corneal Permeation Studies

The goat eye will be given via a nearby slurry. The core layer will be removed, cleaned, and then stored at 4°C until it will be needed. To get rid of the protein, the cornea will be rinsed in cold normal saline. The outer epithelium of the cornea faced the donor compartment when it will be positioned halfway between the donor and receptor compartments. The receptor compartment will be filled with simulated tear fluid for 8 hours, which will be continuously stirred using a Teflon-coated magnetic bead at 50 rpm while held at 37°C. A 4x2mm ophthalmic film will be used to cover the cornea

in the donor compartment, and a glass cover slip will be used to seal it. To keep the sink state, fluid will be taken out of the receptor compartment at predetermined intervals and refilled with new simulated tear fluid. Samples that had been withdrawn were examined for drug content at a specific wavelength.

Ocular Toxicity Study

To evaluate ocular toxicity in compliance with OECD recommendations, the Draize test will be modified. The management of experimental animals (albino rabbits of either sex) received ethical approval from the Institutional Animal Ethical Committee (IAEC) the animals were split into three groups, each of which contained six rabbits designated as the test, control, or placebo groups. They were provided with regular food and water during the trial while being kept in an animal enclosure at room temperature. The control group did not receive anything, while the placebo group will be given a fake movie. The best formulation will be given to the test group. Every day for seven days, an ocular film will be applied to the rabbits in the test group's cul-de- sac, and corneal irritancy tests were performed at set intervals of 24 hours, 48 hours, and 72 hours. All animals were examined for redness, swelling, discharge, hemorrhaging, cloudiness, ulceration, and blindness after one week.

Antimicrobial Activity

In vitro evaluation of the antibacterial activity will be carried out using a Candida albican media with Sabouraud dextrose agar. The samples underwent inoculation using the streak method and were incubated at 354°C for 24 hours. After being sterilized in the autoclave, Aproximate of Sabouraud dextrose agar will be added to the pre-sterilized petridish and allowed to solidify at room temperature. A 9cm diameter petridish will be used to hold 1ml of the sterile saline water- based fungal suspension, which will be then incubated at a temperature of 354°C. Cork bores were used to create wells (8mm). A 42mm (30mg) optimized ocular film, For 24 hours, all petridish were incubated at 354°C. After 24 hours, the zone of inhibition will be assessed.

Kinetic Modelling of the Drug Release

In order to determine the release constant and regression coefficients (R2), the total amount of medication that pierced each square centimetre of the ocular film will be fitted into the zero order, first order, Higuchi kinetic model, and Korsmeyer peppas models using PCP DISSO V3 software.

Stability Studies

Drugs' or dosage forms' recommended storage conditions and shelf lives are revealed through stability testing. In a stability chamber, the stability investigation of the improved formulation F6 will be conducted over a 90-day period under normal conditions of 25°C and 60 RH% and accelerated conditions of 40°C and 75 RH%. Films were placed in an aluminum pouch after being covered in butter paper, then foil. After 15, 30, 60, and 90 days of storage, they were tested for drug content, tensile strength, and in-vitro diffusion investigations.

Result and Discussion

Solubility

Econazole Organic solvents including ethanol, DMSO, and dimethyl formamide (DMF) are soluble in Econazole (nitrate). Econazole (nitrate) dissolves in ethanol with a solubility of about 0.1 mg/ml and in DMSO and DMF at a solubility of around 25 mg/ml. In aqueous buffers, Econazole (nitrate) is only weakly soluble.

Particle Size, Polydispersity Index, and Zeta Potential Analysis

Particle Size and Polydispersity Index Analysis

The following table shows the particle size data of freshly prepared lyophilized Econazole loaded formulations, which are formulated using two different processes. The average particle size for all SLN formulations was less than 400 nm, which is ideal for ocular administration because particles less than 10 μm are tolerated by the human eye. The EUSLN-1 to EUSLN-5 formulations range in particle size from 235 ± 3.53 nm to 289 ± 4.59 nm using the ultrasonication method, whereas the EMSLN-6 to EMSLN-10 formulations range in particle size from 309 ± 3.53 nm to 344 ± 3.52 nm using the microemulsion technique. Particle size increases with increasing polymer concentration were primarily caused by the dispersed phase's increased viscosity, which led to the creation of larger nanodroplets. When compared to the mean particle size of formulations (EUSLN-1 to EUSLN-5) prepared using the ultrasonication method, the particle size of SLNs prepared using the microemulsion technique (EMSLN-6 to EMSLN-10) was considerably $p < 0.01$. This was clarified by the fact that, during the preparation process, the ultrasonication approach typically causes the disintegration of particles into smaller droplets.

Polydispersity Index

A measure of the distribution of particle sizes is the polydispersity index (PDI). When it comes to submicron particles, a score between 0.16 and 0.4 denotes size homogeneity; a number higher than 0.4 denotes heterogeneity. As shown in the following table, the polydispersity index of all SLNs varied from 0.189 ± 0.014 to 0.338 ± 0.016, demonstrating a limited size distribution that demonstrates the higher stability of solid lipid nanoparticles. The polydispersity index was significantly $p < 0.05$.

Zeta Potential Analysis

Zeta potential is a crucial surface characterization method that aids in figuring out the surface charge and potential stability of a system of nanoparticulates. For colloidal dispersion stability, an absolute big negative or positive zeta potential value is typically needed because electrostatic repulsion between particles carrying the same charge prevents aggregation. Due to the inclusion of stearic acid (0.3 % w/v), all formulations showed negative zeta potential values, which were significantly $p < 0.01$. The values range from -23.72 ± 0.64 mV to -29.87 ± 0.59 mV, as shown in Table, with the latter being closer to -31 mV, guaranteeing physical stability.

Entrapment Efficiency (EE %)

From 62.92 ± 2.05 % to 85.26 ± 1.12 % (EUSLNs) prepared by ultrasonication method and 60.14 ± 1.98 % to 73.51 ± 2.06 % (EMSLNs) prepared by microemulsion technique, respectively, the corresponding percent entrapment efficiency of SLN was found to be satisfactory high, as shown in Table. According to the data, the drug entrapment effectiveness rose significantly (P < 0.01) as the polymer concentration increased because of its higher viscosity. Since entrapment efficiency is the ratio of actual drug loading to theoretical drug loading, a further increase in polymer concentration (EUSLN-5 and EMSLN-10 having higher polymer concentrations than the others) resulted in a decrease in entrapment efficiency, which is essentially caused by a decrease in drug loading. Less particle size as compared to the microemulsion approach accounts for the higher entrapment efficiency of SLNs manufactured using the ultrasonication method.

Table: Physiochemical characterization of ECZ loaded solid lipid nanoparticles

Sr. no	Batches	Particle size (nm ± SD)	Zeta potential (mV ± SD)	PDI (±SD)	Entrapment efficiency (% ± SD)
1	EUSLN-1	235 ± 1.53	−23.72 ± 0.64	0.328 ± 0.02	71.27 ± 0.10
2	EUSLN-2	248 ± 2.09††	−28.11 ± 0.56††	0.193 ± 0.03††	73.09 ± 0.03††
3	EUSLN-3	266 ± 1.51††	−26.24 ± 0.57††	0.262 ± 0.08†	77.65 ± 0.05††
4	EUSLN-4	275 ± 4.05††	−25.74 ± 0.62††	0.292 ± 0.04†	85.26 ± 0.12††
5	EUSLN-5	289 ± 4.59††	−29.87 ± 0.59††	0.233 ± 0.06†	62.92 ± 0.05††
6	EMSLN-6	309 ± 3.52	−27.47 ± 0.31	0.249 ± 0.05	60.14 ± 0.08††
7	EMSLN-7	320 ± 2.65†	−28.97 ± 0.42††	0.189 ± 0.03†	64.24 ± 0.13††
8	EMSLN-8	326 ± 4.17††	−25.84 ± 0.66††	0.338 ± 0.07†	66.14 ± 0.06††
9	EMSLN-9	336 ± 4.51††	−24.47 ± 0.41††	0.220 ± 0.03†	73.51 ± 0.06††
10	EMSLN-10	344 ± 3.52††	−29.7 ± 0.37††	0.264 ± 0.03†	61.18 ± 0.06††

Electron Microscope Examination

Transmission electron microscopy was used to examine the morphology of the ECZ-SLN formulations (TEM). TEM pictures of SLN formulations made using the two techniques are displayed in (Figures (a) and (b)). This demonstrated that every created formulation had a smooth surface, spherical shape, and potential for nanoparticle stability. Given that isometric particles with obtuse angles and edges have been shown to cause less discomfort than particles with sharp angles and edges, it is possible that these nanoparticles have no effect on the ocular surface. The basic structure of the Econazole SLN generated by both approaches showed a few small changes. This may be supported by the observation that lipid properties such as solubility and film-forming ability mostly determine the structure of solid lipid nanoparticles.

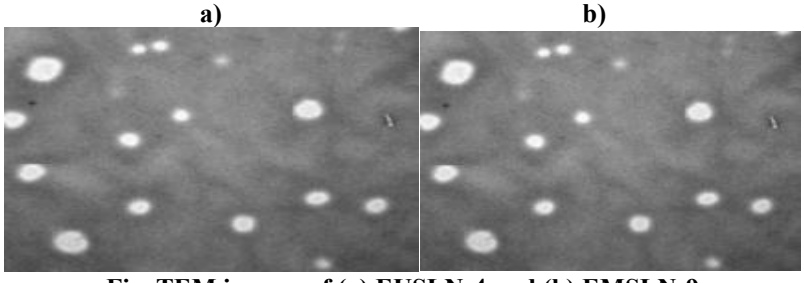

Fig. TEM images of (a) EUSLN-4 and (b) EMSLN-9

Powder X-Ray Diffraction (PXRD) Analysis

The evaluation of the crystalline nature of lipid matrices and ECZ-SLN produced using both techniques was done using X-ray diffraction, as demonstrated in Figure, which also shows the X-ray diffractograms of drugs, lipids, polymers, physical mixtures, mannitol, and lyophilized SLNs. The unique peaks of Econazole were seen at 13.14∘, 14.46∘, 15.21∘, 20.31∘, 22.15∘, 28.16∘, and 35.16∘ 2θ. Econazole diffractograms showed the presence of strong peaks, which suggested that the compound was crystalline. Since no distinct peaks are seen in the Carbopol 934 XRD patterns, it is evident that the polymer is entirely amorphous. The physical combination of stearic acid, carbopol 934, and Econazole produced a form that was comparatively less crystalline and showed peaks at 6.3∘, 15.53∘, and 21.29∘ 2θ. The polymorphic form of mannitol, which exists in α, β, and δ forms, is represented by the multiple different peaks it displayed at 11.56∘, 16.36∘, 19.79∘, 20.90∘, 24.40∘, 25.23∘, 30.44∘, 34.30∘, 35.99∘, and 40.54∘ 2θ.The peaks that the freeze-dried SLNs with ECZ showed at 9.95∘, 10.77∘, 16.99∘, 20.71∘, 21.43∘, 21.55∘, 22.45∘, and 25.61∘ 2θ appear to be caused by stearic acid and mannitol. Peak intensity was becoming more intense, which could indicate that the SLN product is crystalline in form.

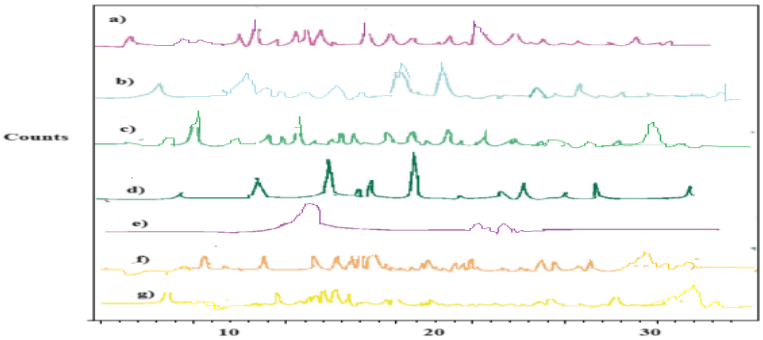

Fig. PXRD spectra of (a) Econazole, (b) stearic acid, (c) Econazole, stearic acid, and Carbopol physical mixture, (d) Carbopol 934, (e) mannitol, (f) EUSLN-4, and (g) EMSLN-9.

Differential Scanning Colorimetry (DSC) Analysis

The method used to study a substance's melting and recrystallization behavior is called DSC. The samples' DSC thermograms are shown in Figure. Stearic acid's heat of fusion is 158.20 J/g, and its melting endotherm is sharply defined at 57.19∘ C on its thermal curve. With no melting point depression, the physical mixture of carbopol 934, stearic acid, and Econazole displayed typical peaks at 141.53∘ C, 57.49∘ C, and 84.33∘ C, respectively. Carbopol 934's DSC scan revealed a large endothermic peak at 79°C. Mannitol has an endothermic peak at 166.97∘ C and a heat of fusion of 292.32 J/g, which define its thermal behavior. The lyophilized SLNs' DSC curve revealed a sharp endotherm at 165.65∘ C, which appears to represent the depressed endothermic peak of the β polymorph of mannitol, followed by a small endotherm at 82.03∘ C, which corresponds to the melting point of Carbopol 934. Thermograms did not reveal a medication melting peak. This implies that the medication is fully encapsulated in the lipid matrix and that ECZ was present in an amorphous state.

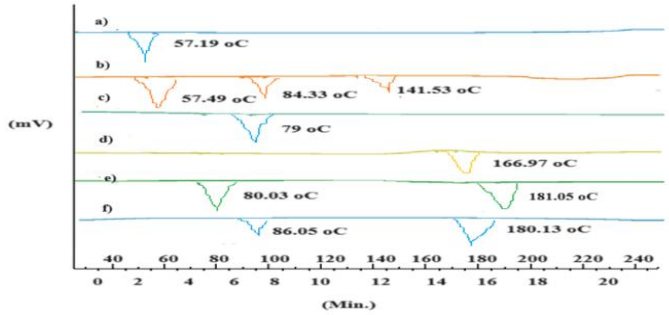

Fig. DSC of (a) stearic acid, (b) Econazole, stearic acid, and Carbopol physical mixture, (c) Carbopol 934, (d) mannitol, (e) EUSLN-4, and (f) EMSLN-9

In-Vitro Drug Release from ECZ-SLN

Figures (a) and (b) show the in vitro release of ECZ from solid lipid nanoparticles made using both techniques. By employing simulated tear fluid (pH 7.4) as the release medium, the dialysis membrane was used to measure the drug release from SLN. Solid lipid nanoparticles prepared by microemulsion technique (EMSLN-9) demonstrated 14.87 ± 0.10 % and 85.28 ± 1.02 % drug release in 1 hr and 10 hr, respectively, while solid nanoparticles made with the ultrasonication method (EUSLN-4) showed 18.20 ± 0.10 % drug release in 1 hr and 87.77 ± 0.79 % drug release in 12

hr. Regarding the drug release aspect, there was a statistically significant difference ($p < 0.01$) between the two techniques. The % drug release vs. time graph (Figure (a)) showed that the ultrasonication approach produced a sustained medication release for up to 12 hours. The findings indicated that drug entrapment in solid lipid nanoparticles inhibits drug release, i.e., in the case of the ultrasonication approach, an initial burst release followed by a delayed release phase. This could be explained by the fact that the ultrasonication process produced smaller particle sizes, which in turn led to greater surface area and a longer diffusional channel. Furthermore, the entrapment efficiency was lower in the case of the microemulsion approach, which ultimately affected the drug release.
The polymer concentration makes the drug release more sustained; however, because of insufficient drug loading, the drug release is reduced in EEUSLN-5 and EMSLN-10. Solid lipid nanoparticles created using the microemulsion approach showed controlled drug release for up to 10 hours, whereas SLNs prepared using the ultrasonication method (EUSLN-4) were able to regulate the drug release for up to 12 hours. Because it prolongs the precorneal residence duration and offers continuous drug release, the ultrasonication method proved to be superior to the microemulsion technique.

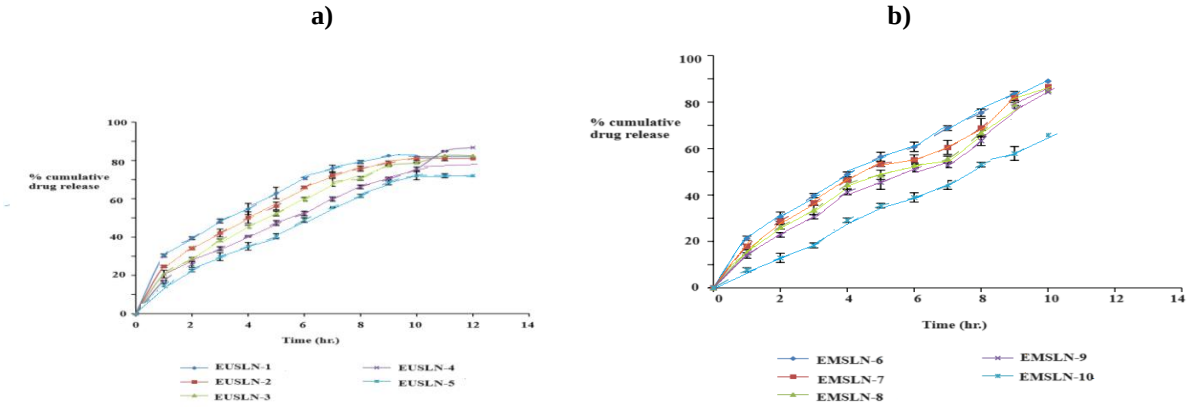

Fig. (a) In vitro release profile of Econazole from SLN formulations prepared by ultrasonication method through dialysis membrane. (b) In vitro release profile of Econazole from SLN formulations prepared by microemulsion technique through dialysis membrane.

Evaluation of Econazole Ophthalmic Films

Physical Appearance, Surface Texture and pH of Ophthalmic Films

The films featured a clean surface free of flaws and cracks, and all of the created formulations (EF1–EF6) had an attractive and transparent look. The pH of the film's surface determines how acidic and alkaline the film is. The ocular fluid has a pH between 6.9 and 7.6. The different alkalinity of the film may irritate or damage the eyes. The EF1-EF6 formulation's surface pH fell within the acceptable range of 6.9-7.3 pH, suggesting that patients find these films to be extremely acceptable and less likely to cause eye irritation. Every produced film had a surface pH of 7.4 that was consistent with the pH of the simulated tear fluid (Table).

Table: Evaluation of Econazole SLN Incorporated Ophthalmic Films

Formulation Code	Surface Texture	Surface pH	Thickness(mm)	Weight (mg	Folding Endurance
EF1	Smooth	7.2 ± 0.242	0.025 ± 0.39	127 ± 1.253	261 ± 0.452
EF2	Smooth	6.9 ± 0.153	0.071 ± 0.76	129 ± 2.517	454 ± 1.733
EF3	Smooth	7.4 ± 0.116	0.064 ± 0.11	127 ± 2.082	351 ± 0.578
EF4	Smooth	7.1 ± 0.193	0.044 ± 0.26	128 ± 1.245	346 ± 1.255
EF5	Smooth	7.3 ± 0.211	0.071 ± 0.76	130 ± 2.026	281 ± 1.357
EF6	Smooth	7.3 ± 0.121	0.036 ± 0.43	128 ± 1.528	312 ± 2.646

Thickness, Weight Variation and Folding Endurance

The formulas EF1-EF6 have thicknesses between 0.0246 ± 0.39 mm and 0.07032 ± 0.76 mm. These results showed that the medication and polymer were dispersed evenly. The permissible limit was reached by the average weight of all formulations (EF1-EF6), which ranged from 127 ± 1.253 mg to 130 ± 2.03 mg. The film's mechanical strength is reflected in the folding endurance, which for the (EF1-EF6) formulations fell between 261-454 (Table).

Drug Content and Tensile Strength

Ensuring that the drug is delivered uniformly throughout the picture is the aim of determining the drug content. The medication concentration of formulations EF1–EF6 ranged from 96.31% to 99.29%. The tensile strength of the formulations EF1-EF6 fell within an acceptable range, measuring between 30 ± 0.813 and 46 ± 0.8780 (Table). The films of all six formulations (EF1 to EF6) had good mechanical strength, flexibility, and elasticity at the specific polymer concentration. It was also noted that the folding endurance and tensile strength steadily rose as the plasticizer concentration increased.

Table: Evaluation of SLN Incorporated Ophthalmic Films

Formulation code	Tensile strength (g/cm2) ± SD	Drug content (%) ± SD	Moisture Absorption (%) ± SD	Moisture Loss (%) ± SD
EF1	36.0 ± 0.77	97.83 ± 0.29	3.76 ± 0.35	3.83 ± 0.43
EF2	46.0 ± 0.88	98.28 ± 0.42	3.93 ± 0.45	3.71 ± 0.40
EF3	43.6 ± 0.63	96.31 ± 0.39	4.27 ± 0.39	3.12 ± 0.06
EF4	33.0 ± 0.82	99.29 ± 0.48	4.87 ± 0.25	3.68 ± 0.25
EF5	31.5 ± 0.13	96.75 ± 0.42	4.97 ± 0.43	3.56 ± 0.28
EF6	32.3 ± 1.23	97.21 ± 0.47	4.59 ± 0.40	2.39 ± 0.77

Moisture Absorption and Moisture Loss

All formulations' moisture absorption and moisture loss were found to be within an acceptable range, with values ranging from 3.754 ± 0.350 to 4.98 ± 0.424 and from 3.389 ± 0.770 to 4.823 ± 0.423, respectively, as shown in the table.

In-vitro Permeation Studies

The results of an in-vitro permeation research for EF1, EF2, EF3, EF4, EF5, and EF6 revealed the release of, in that order, 80.49 percent, 91.64%, 86.18%, 86.73%, 83.82%, and 81.22%. EF1, EF3, and EF5 were rejected since their in-vitro investigation demonstrated a faster and more complete release in 4–5 hours. The prolonged release of the medication was effectively aided by the formulation's usage of retarding polymers (PVA, ethyl cellulose, and chitosan). The in-vitro permeability of EF2, EF4, and EF6 for eight hours was determined to be in the range of 91.64%, 86.15%, and 81.22%, respectively. EF6 was found to be the best formulation out of the three based on the overall assessment criteria. Higher quantities of chitosan (EF5 and EF6) and PVA (EF1 and EF2) may have reduced drug release because of their propensity to create a denser and more intensive molecular network. Because it is less expensive, more adaptable, more biocompatible than other polymers, ethyl cellulose (EC) is the most commonly used polymer in the creation of continuous drug delivery systems. The in vitro drug release reduced when the EC concentration ratio increased from 0.25 gm to 0.30 gm (EF3 and EF4); see Figure. This could be because the drug's longer diffusion pathway in the greater ratio caused the release to be delayed.

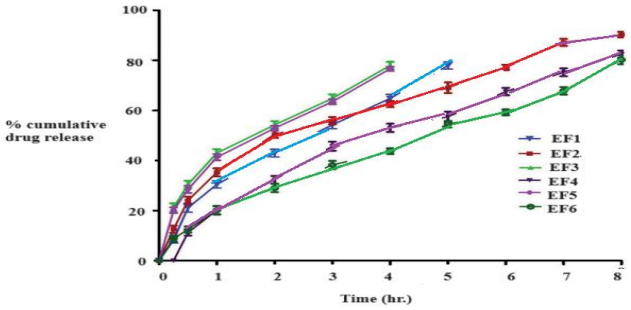

Fig. In-vitro release profile of all Econazole ophthalmic films

Ex-vivo Permeation Studies

A goat's cornea was used for an ex-vivo permeation assay, and after eight hours, the percentage cumulative drug penetration of the optimized formulation EF6 was discovered to be 77.31%. EF6 was able to permeate both in vitro (81.22%) and ex vivo (77.31%). When compared to ex-vivo trials, the in-vitro tests showed a higher drug penetration rate. This is because certain other fatty tissues obstruct the passage of medications through the cornea, and the actual cornea is a semipermeable membrane with variable pore size. Additionally, the dialysis membrane functions as a mechanical barrier, and the cornea is composed of the lipophilic epithelium, the hydrophilic stroma, and the less lipophilic endothelium. These components work together to form a lipophilic–hydrophilic barrier that prevents corneal penetration. Figure shows the comparative in vitro-ex vivo release of the optimized formulation as well as the in vitro release profile of all formulations (EF1-EF6).

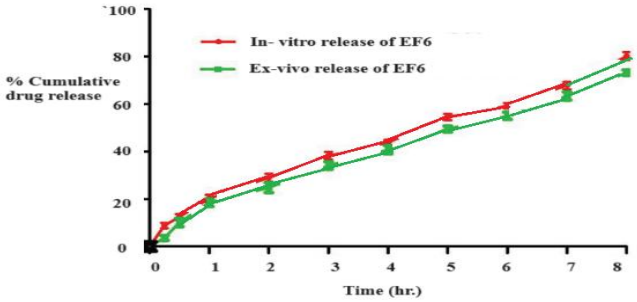

Fig. Comparative in-vitro and ex vivo drug release profile of optimized formulation EF-6

Antimicrobial Activity
Using Cup-let Method

As seen in Figure, the zone of inhibition for the improved EF-6 film was 10.6 mm, 11 mm for the commercial formulation, and 4 mm for the placebo after 24 hours. The optimized formulation produced satisfactory results. A placebo report states that chitosan is an antibacterial agent in and of itself. Following an incubation period of 10 to 15 days at 35°C±4°C, the ocular film was carefully released. The results showed that there was no microbial growth in the area that was suppressed. The zone of inhibition was sustained for 24 hours and the same zone size was maintained for up to 15 days, according to in vitro antimicrobial studies. The final eye irritation score was calculated by dividing the total irritation score—which was obtained by adding the individual rabbits' eye irritation scores—by the total number of rabbits utilized in the ocular irritancy test. The computed eye irritation score for EF6 was 0.48, indicating good ocular tolerance, compared to 0.24 for the control group. Every rabbit was under constant observation for a full day. When comparing the optimized formulation EF6 and control eye to the placebo, there was no irritation, inflammation, or redness. The produced compound proved to be non-irritating when administered topically, according to the ocular irritation test (Figure).

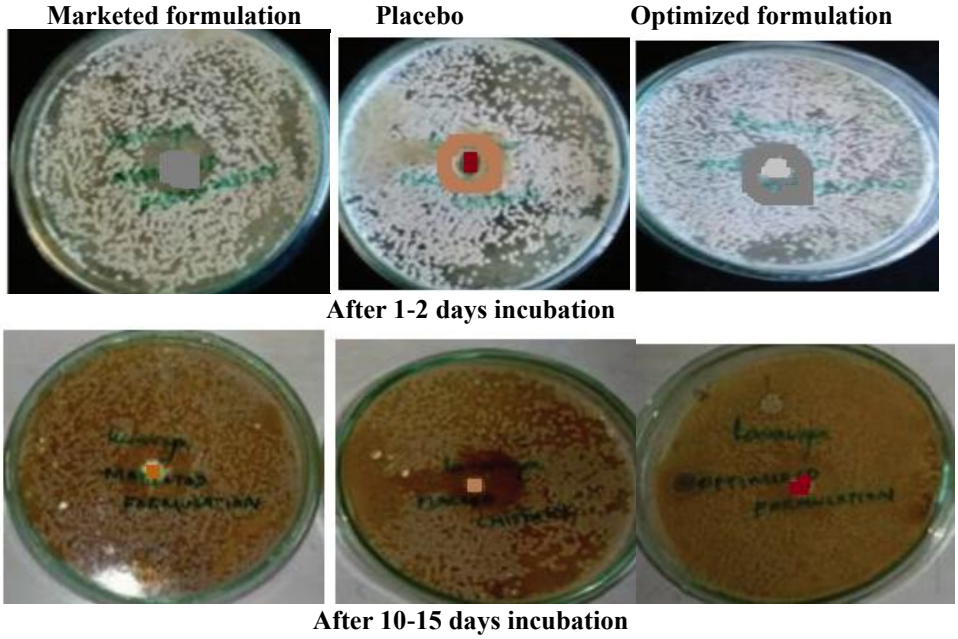

Fig. In-vitro antifungal activity (zone of inhibition) of optimized formulation EF-6 against strain Candida albicans

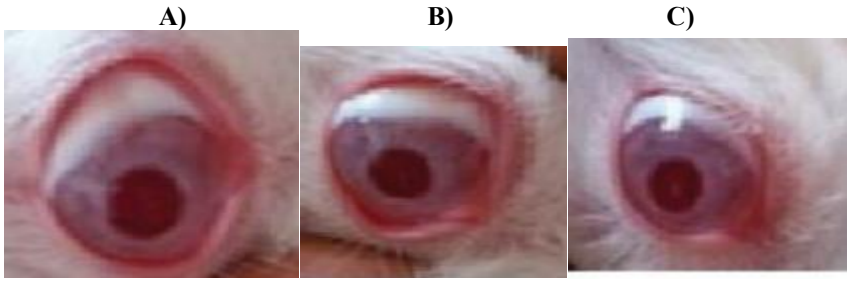

Fig. Results of ocular irritation study A-Placebo film administered rabbit eye, B -Control group rabbit eye and C-Optimized film EF-6 administered rabbit eye

Kinetic Modelling of the Drug Release

The release data was fitted into a number of kinetic models in order to determine the release constant and regression coefficients (R2). Among the models examined, the drug release profile for all formulations, EF1 to EF6, showed Higuchi kinetics. The release profiles were caused by the diffusion process. Diffusion exponent (n) values of less than 0.5 were observed in all formulations (EF1-EF6), suggesting a Fickian mechanism of drug release.

Short-term Stability Studies

After 90 days of storage, there was no discernible change in the drug content, tensile strength, or in-vitro diffusion studies, indicating that the optimized formulations were stable both at room temperature and under accelerated stability conditions. This further supports the films' potential for extended storage.

Conclusion

The current study ultimately came to the conclusion that lipophilic drugs, such as Econazole, can also be successfully incorporated into solid lipid (stearic acid), with the use of tween 80 as a stabilizer. Additionally, the study was able to investigate the potential of both methods of preparation of Econazole loaded solid lipid nanoparticles, namely, ultrasonication method and microemulsion technique. Carbopol 934 was used as a controlled release agent during the preparation of SLN; increasing the concentration of the polymer results in more sustained drug release of SLN due to the formation of strong matrices because of its highly cross-linked structure. In terms of particle size study, the SLNs were obtained below 400 nm for all formulations with good PDI and negative zeta potential with optimal physiochemical parameters.

Precorneal residence period is extended in part by the small particle size and negative zeta potential. PXRD and DSC measurements showed that the drug's crystallinity had decreased in the nanoparticles. Drug release from SLNs in vitro and ex vivo corneal penetration were shown to be promising without having a discernible impact on the degree of corneal moisture. It was discovered that the nanoparticle had a biphasic release pattern, best fitting Higuchi-square root release kinetics with an initial burst release followed by a continuous release. In comparison to the microemulsion approach, the results showed that SLN (EUSLN-4) generated by ultrasonication method could sustain the drug release for up to 12 hours. At room temperature, the optimized formulation (EUSLN-4) would have a shelf life of more than two years. The resulting solid lipid nanoparticles, however, appear to hold promise in addressing the issue raised by the ineffective ocular delivery.

With the aim of avoiding drug loss, prolonging drug retention, avoiding repeated dosing, and improving drug release, diffusion, and patient compliance, this study assessed the suitability and feasibility of administering Econazole as an ophthalmic film via the ocular route. Using polymers (PVA, ethyl cellulose, and chitosan) and the solvent casting process, ophthalmic films were effectively created. It was found that the tensile strength and folding endurance significantly increased as the plasticizer concentration rose. All of the formulas' films—F1 through F6—had high folding endurance, no weight variation, and good mechanical strength. In vitro tests using the improved formulation F6 demonstrate more drug release than in vivo experiments. This results from disruption of other tissues and the cornea's variable pore size. The developed formulation proved not to irritate the eyes, according to an ocular irritation test. In order to treat fungal keratitis, the developed ophthalmic film appears to be a viable formulation for the safe and efficient delivery of Econazole through the ocular channel.

References

1. R. Malviya, A. Kumar, and P. K. Sharma. “Recent trends in ocular drug delivery: a short review”. Europian Journal of Applied Science. 2011; 3(3): 86–92.
2. P. Tangri and S. Khurana. “Basics of ocular drug delivery systems”. International Journal of Pharmaceutical Biomedical Research. 2011; 2(4): 1541–1552.

3. D. Achouri, K. Alhanout, P. Piccerelle, and V. Andrieu. “Recent advances in ocular drug delivery”. Drug Development and Industrial Pharmacy. 2013; 39(11): 1599–1617.
4. D. M. Maurice and S. Mishima. “Ocular pharmacokinetics”. In Handbook of Experimental Pharmacology, M. L. Sears, Ed., Springer, Berlin, Germany, 1984.
5. S. Ding. “Recent developments in ophthalmic drug delivery”. Pharmaceutical Science and Technology Today. 1998; 1(8): 328–335.
6. M. Kumar and G. T. Kulkarni, “Recent advances in ophthalmic drug delivery system,” International Journal of Pharmacy and Pharmaceutics Sciences. 2012; 4(1): 387–394.
7. H. A. Patel, J. K. Patel, N. K. Patel, and R. R. Patel. “Ophthalmic drug delivery system-a review”. Der Pharmacia Lettre. 2010; 2(4): 100–115.
8. M. Mudgil and P. K. Pawar. “Preparation and in Vitro/Ex Vivo evaluation of moxifloxacin-loaded PLGA nanosuspensions for ophthalmic application”. Scientia Pharmaceutica. 2013; 81(2): 591–606.
9. M. A. Kalam, Y. Sultana, A. Ali, M. Aqil, A. K. Mishra, and K. Chuttani. “Preparation, characterization, and evaluation of gatifloxacin loaded solid lipid nanoparticles as colloidal ocular drug delivery system”. Journal of Drug Targeting. 2010; 18(3):191– 204.
10. Mendes AI., Silva AC., Catita JA., Cerqueira F., Gabriel C., Lopes CM. Miconazole-loaded nanostructured lipid carriers (NLC) for local delivery to the oral mucosa: improving antifungal activity. Colloids Surf B Biointerfaces. 2013; 111: 755-763.
11. Piemi MPY., Korner D., Benita S., Marty JP. Positively and negatively charged submicron emulsions for enhanced topical delivery of antifungal drugs. J. Control. Release. 1999; 58(2): 177–187.
12. Passerini N., Gavini E., Albertini B., Rassu G., Sabatinoet MD., Sanna V., Giunchedi P., Rodriguez L. Evaluation of solid lipid microparticles produced by spray congealing for topical application of Econazole nitrate. J. Pharm. Pharmacol. 2009; 61(5): 559–567.
13. Albertini B., Passerini N., Sabatinoet MD., Vitali B., Brigidi P., Rodriguez L. Polymer-lipid based mucoadhesive microspheres prepared by spray congealing for the vaginal delivery of Econazole nitrate. Eur. J. Pharm. Sci. 2009; 36(4-5): 591-601.
14. Vera-Cabrera L., Campos-Rivera MP., Escalante Fuentes WG., Pucci MJ., Ocampo-Candiani J., Welsh O. In-vitro activity of ACH-702, a new isothiazoloquinolone, against no cardiabrasiliensis compared with Econazole and the carbapenemsimipenem and meropenem alone or in Combination with clavulanic acid.Antimicrob. Agents Chemother. 2010; 54: 2191-2193.
15. Gurtler F., Kaltsatos V., Boisrame B., Gurny R. Long-acting soluble bioadhesiveophthalmic drug insert (BODI) containing gentamicin for veterinary use: optimization and clinical investigation. J Control Release. 1995; 33(2):231-36.