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DEVELOPMENT SOLID LIPID NANOPARTICLES CONTAINING MICONAZOLE DRUG FOR THE TREATMENT OF CONJUNCTIVITIS

Dharmendra Kumar Mishra, Kalpana Badoniya, Surendra K. Jain, Krishan Kishore Badoniya*

Truba Institute of Pharmacy, Karond, Bhopal, M.P., India.

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***Corresponding Author**
Dr. Krishan Kishore Badoniya
Associate Professor
Truba Institute of Pharmacy
Bhopal, M.P.

ABSTRACT

The complexity of the eye structure and its physiology turned ocular drug administration into one of the most challenging topics in the pharmaceutical field. Conjunctivitis is one of the most common ophthalmic disorders. Topical administration of drugs leads difficulty in overcoming the eye barriers, which are both physical and chemical, reduces drug bioavailability, and the frequency of administration must be increased to reach the therapeutic effect. Lipid nanoparticles seem to be a great alternative to ocular drug delivery. In this work we developed solid lipid nanoparticles of Miconazole for the effective treatment of conjunctivitis. Drug exicipient compatibility study was done. SLNs were prepared using hot homogenization followed by probe sonication techniques. The Box-Behnken design was employed for Miconazole hydrochloride loaded nanoparticles. The particle size and entrapment efficiency showed a wide variation of 254-699 nm and 80.5 -87.8% respectively. The optimum formulation exhibited a zeta potential of 23 mV, entrapment efficiency of 84%, Miconazole release of 8% after 1 h, and time to release of 50% of the drug of 5 h. It produced a 1.5-fold and 3-fold increase in the cumulative amount permeated and cumulative percentage permeated, respectively, compared with other tested formulations.

KEYWORDS: Miconazole, Solid lipid nanoparticles, conjunctivitis, inflammation, ocular drug delivery.

1. INTRODUCTION

The eye is a distinctive anatomy and physiology in human body. Eye can be divided into anterior portion and posterior portion (Figure 1). Frontal portion of the eye contains about one-third portion and the rest of the part is hold by subsequent portion. Eye tissues like cornea, ciliary body, lens, aqueous humor, conjunctiva, iris are in the frontal portion. The posterior portion includes retinal pigment epithelium, choroid, sclera, vitreous humor, optic nerve, neural retina. The both portion of eye is mostly affect by different visual hazard diseases.

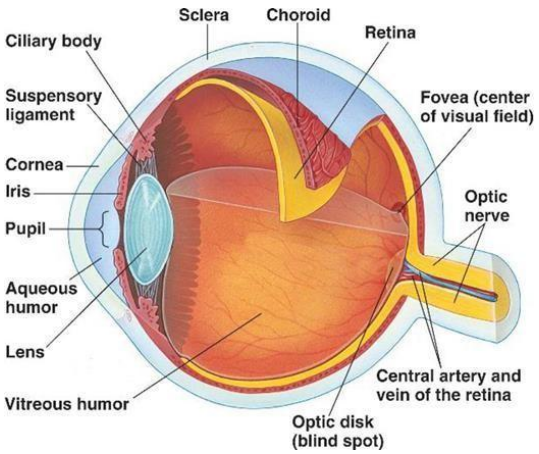

Figure 1: Anatomy of the eye.

Conjunctivitis is a common cause of eye redness and, subsequently, a common complaint in the emergency department, urgent care clinics, and primary care clinics. People of any age, demographic, or socioeconomic status can be affected. More than 80% of all acute cases are generally diagnosed by non-ophthalmologists, such as internists, primary care providers, pediatricians, and nurse practitioners.[1] In the United States, this imparts a

substantial economic burden on the healthcare system, costing about $857 million annually.[2] While conjunctivitis is typically a temporary condition that does not often lead to vision loss, ruling out other potential sight-threatening causes of red-eye during evaluation is crucial.

The conjunctiva is the transparent, lubricating mucous membrane covering the eye's outer surface and comprises 2 parts: the bulbar conjunctiva that covers the globe and the tarsal conjunctiva that lines the eyelid's inner surface.[3]

Conjunctivitis refers to the inflammation of the conjunctival tissue, engorgement of the blood vessels, pain, and ocular discharge, and is classified as acute or chronic and infectious or noninfectious. Acute conjunctivitis refers to symptom duration of 3 to 4 weeks from presentation, usually only lasting 1 to 2 weeks, whereas chronic is defined as lasting more than 4 weeks. Solid Lipid Nanoparticle (SLN) has distinctive properties like forming micron size particle, higher drug loading capacity, high surface area and thus improves performance of pharmaceuticals. SLN formulation used in drug delivery through parenteral, ocular, oral, rectal, pulmonary topical route.[4] In this research work we developed Miconazole loaded solid lipid nanoparticles for the treatment of conjunctivitis.

2. MATERIAL AND METHODS

2.1. Materials

Miconzole was obtained from ThermoFisher Scientific. Glycerin and Tween® 80 were obtained from Spectrum Pharmaceuticals. GeleolTM and all other chemicals were obtained from Fisher Scientific Hampton. High-performance liquid chromatography (HPLC) grade solvents were used for analysis.

2.2. Analytical Method

Miconazole content in all samples was analyzed using a reversed-phase HPLC-UV system consisting UV/Vis detector. The mobile phase was made up of 18 mM phosphate buffer containing 0.1% *v*/*v* triethylamine (pH adjusted with phosphoric acid) and methanol in a 60:40 *v*/*v* ratio. The mobile phase was pumped isocratically at 1.2 mL/min through a Waters Symmetry C_{18} column set at 25 °C, with a detection wavelength (λ_{max}) set at 294 nm. The samples were analyzed using a Waters chromatography data system coupled with Empower software.[5] The analytical method was linear over a miconazole concentration range of 1–100 μg/mL.

2.3. Lipid screening studies

Various liquid and solid lipids were evaluated for miconaole solubility. Nine different liquid lipids (castor oil, sesame oil, mineral oil, oleic acid, isopropyl myristate, Labrafac® Lipophile WL 1349, olive oil, and Miglyol® 829) and nine solid lipids (Softisan 154, Precirol® ATO 5, Compritol® 888 AO, Geleol™, Dynasan™ 114, Dynasan™116, Gelucire™ 44/14, Gelucire™ 43/01, and Gelucire™ 50/13) were evaluated in the solubility screening studies. An 10 mg of miconazole was added to each liquid or solid lipid of 200 mg in separate glass vials of 3 mL. This mixture was stirred at 2000 rpm under ambient temperature (80 ± 2 °C) for 10 min.[6] After 10 min, stirring was discontinued and the mixtures were removed from the heat. Upon cooling to room temperature, the vials were visually examined for deposits of Miconazole. The lipids which visually showed no signs of precipitation/deposits were selected for further saturation solubility studies.

2.4. Preparation method of miconazole HCl loaded SLN

Miconaole-SLNs are formulated using the hot homogenization–probe sonication method. The lipid phase in SLNs comprised of miconazole in a solid lipid. The aqueous phase contained Tween® 80 (surfactant), glycerin (tonicity adjustment), and TPGS (permeation enhancer) in Milli-Q water based on previous studies.[7] Both the phases were allowed to reach ambient temperature in a hot water bath (80 ± 2 °C). In a dropwise fashion, the aqueous phase was added to the molten lipid phase under constant stirring (2000 rpm), forming an emulsion. This emulsion was subjected to homogenization at 14,000 rpm at 70 ± 2 °C for 5 min using a T25 digital Ultra-Turrax homogenizer. Upon cooling to RT, the emulsion was then subjected to probe sonicated (Sonics Vibra-Cell™, Newtown, CT, USA) at 40% amplitude for 10 min using a 3 mm stepped microtip (10 s pulse on; 10 s pulse off), 500 watts power supply, and 115 volts. Placebo formulations (absence of drug) were prepared and visually examined for excipient–excipient compatibility before preparing drug-loaded SLNs.

2.5. Measurement of Particle Size (PS), Polydispersity Index (PDI), and Zeta Potential (ZP)

SLN formulations are analyzed for average PS (d.nm), PDI, and ZP (mV) using Nano ZS Zen3600 Zetasizer in disposable, clear, micro cuvettes. The SLN formulation was diluted (100 times) with water prior to initiating the measurements (n = 3) at 25 °C. After being evaluated for PS and PDI, the ZP was evaluated by placing these diluted samples in Zetasizer (DTS1070) cells for measurement (n = 3) at 25 °C.

2.6. Assay of Miconazole content

miconazole content in the miconazole-SLN formulation was determined by extraction of the drug in methanol. Briefly, the nanodispersion was diluted 100-fold with methanol in a volumetric flask. The mixtures were sonicated for 10 min and then centrifuged at 13,000 rpm at 25°C for 20 min. The supernatant was analyzed for Miconazole content using the previously mentioned HPLC method.

2.7. Entrapment Efficiency (EE)

The EE (%) of Miconazole in the nanodispersions was calculated based on the amount of unentrapped drug in

the aqueous phase of the prepared formulations. A total of 300 µL of GTX nano formulation was transferred into Amicon® filter devices (pore size 100 kDa) and centrifuged at 13,000 rpm, following which the filtrate was collected and diluted with methanol (10 times), and the amount of miconazole content was quantified using the HPLC. The percentage of miconazole entrapped in the lipid phase was calculated using the formula

$$\%EE = \left[\frac{\text{Amount of GTX quantified in assay} - \text{Amount of free GTX}}{\text{The amount of GTX quantified in assay}}\right] \times 100$$

2.8. Measurement of pH and Viscosity

The pH was measured (n = 3) using a Mettler Toledo pH meter equipped with an Inlab® Micro Pro-ISM probe. Prior to the measurement of the samples, the pH meter was calibrated using standardized buffers of known pH.

The viscosity of the lead GTX-NLC was measured using an Ares G2 strain-controlled rotational rheometer at 25° C. The rheometer was equipped with a stainless steel cone-plate geometry of 40 mm diameter and a 2° cone angle, having a 0.047 mm truncation gap. The sample was loaded on the platform until it was sandwiched entirely between the geometry and the platform. A flow ramp over a shear rate range of 0.1–100 s^{-1} was used to measure the viscosity of the formulation.

2.9. Antimicrobial Efficacy

The antimicrobial activity of the lead Miconazole-SLN formulation was evaluated against *Pseudomonas aeruginosa*. An adapted version of the Clinical and Laboratory Standards Institute (CLSI) method was used to perform susceptibility testing. The lead miconazole-NLC formulation and its corresponding placebo were diluted 1:2 fold using cation-adjusted Mueller–Hinton medium at pH 7.0. Diluted samples (10 µL) of the lead formulation were transferred to 96 well assay plates (n = 3). Inocula was prepared as per CLSI protocol by correcting the OD_{630} of microbe suspensions in incubation broth. Alamar Blue™ (5% *w*/*v*) was added to both organisms' plates. GTX-C was included as a positive control in the microbial assays. The optical density was determined at 35°C before and after incubation for 24 h, using the Bio-Tek plate reader. Minimum Inhibitory Concentrations (MICs), described as the lowest miconazole concentration that allows no visual microbial growth, were calculated for the samples.

2.10. Stability Studies

The lead Miconazole-SLN was investigated at three different storage conditions (4 ± 2.0 (RF), 25 ± 2.0 (RT), and 40 ± 2 °C (accelerated)). Samples kept at 25 and 40°C in incubator. These samples were assessed at predetermined time points for any possible changes in PS, PDI, ZP, pH, miconazole content.

3. RESULTS AND DISCUSSION

3.1. Screening of Lipids

The determination of the solubility of the drug in the lipid is critical in the selection of the liquid and solid lipid combinations. Drug entrapment and loading efficiency are directly impacted by the solubility of the miconazole in the lipid (s).[8,9] From the lipid screening studies, three solid lipids and two liquid lipid were identified as lead candidates for the lipid nano formulation development (**Table 1**).

Table 1: Lipid screening studies for miconazole-loaded lipid nano formulations.

Liquid Oil	Solubility	Solid Lipids	Solubility
Castor oil	(−)	Precirol® ATO 5	(−)
Sesame oil	(−)	Compritol® 888	(+)
Oleic acid	(−)	Dynasan™ 114	(+)
Mineral oil	(−)	Geleol™	(+)
Capryol® 90	(−)	Gelucire™ 43/01	(−)
Labrafac® Lipophile WL 1349	(+)	Dynasan™ 116	(−)
Isopropyl Myristate, NF	(−)	Gelucire™ 50/13	(−)
Olive oil	(+)	Gelucire™ 44/1	(−)
Miglyol® 829	(−)	Softisan 154	(−)

(+): miconaole dissolves in the lipid and does not precipitate after cooling to RT. (−): miconazole does not dissolve in the lipid, or dissolves in the lipid but precipitates on cooling.

3.2. Preparation of GTX-Loaded SLNs and NLCs

The physicochemical characteristics and visual observations of different miconazole-loaded SLNs is presented below in **Table 3**.

Table 3: Physicochemical characteristics and visual observations miconazole loaded lipid nano formulations (mean ± SD, n = 3).

Formulation *		Precirol® ATO 5 (% *w/v*)	Oleic Acid (% *w/v*)	Physicochemical Characteristics						Visual Observations
				PS (nm)	PDI	ZP (mV)	pH	Assay (%)	EE (%)	
NLCs	F1	3.0	2.0	266.8 ± 6.4	0.42 ± 0.05	−32.2 ± 0.5	6.37 ± 0.03	99.2 ± 2.6	87.3 ± 2.0	Milky-white dispersion
	F2	2.5	2.5	216.2 ± 6.8	0.32 ± 0.03	−30.6 ± 1.2	6.37 ± 0.03	98.6 ± 2.1	91.63 ± 1.5	Milky-white dispersion
	F3	3.0	1.0	229.5 ± 3.2	0.16 ± 0.06	−26.3 ± 0.9	5.62 ± 0.03	100.5 ± 2.4	73.9 ± 3.2	Milky-white dispersion
	F4	2.0	2.0	209.2 ± 3.2	0.14 ± 0.03	−31.2 ± 1.3	6.24 ± 0.02	102.3 ± 1.1	81.2 ± 1.8	Milky-white dispersion
SLNs	F9	5.0	-	200 ± 11.0	0.20 ± 0.03	−21.3 ± 0.4	7.17 ± 0.03	101.0 ± 2.5	80.29 ± 2.3	Drug expulsion at day 15 with 20.3 ± 2.1% EE
	F10	4.0	-	182.7 ± 4.3	0.15 ± 0.02	−18.2 ± 0.6	7.12 ± 0.02	97.9 ± 3.1	71.7 ± 2.8	Drug expulsion at day 15 with 12.2 ± 3.6% EE

* All formulations contained miconazole (0.5% *w/v*). Note: aqueous phase contains Tween® 80 (3% *w/v*), Glycerin (2.25% *w/v*), TPGS (0.002% *w/v*), and Milli-Q water (q.s. to 10 mL) for all nanocarriers.

3.3. Stability Studies

The lead NLC colloidal dispersion (F2) was analyzed at scheduled time intervals for any changes in PS, PDI, ZP, pH, assay, and EE (%) on storage (**Figure 4**).

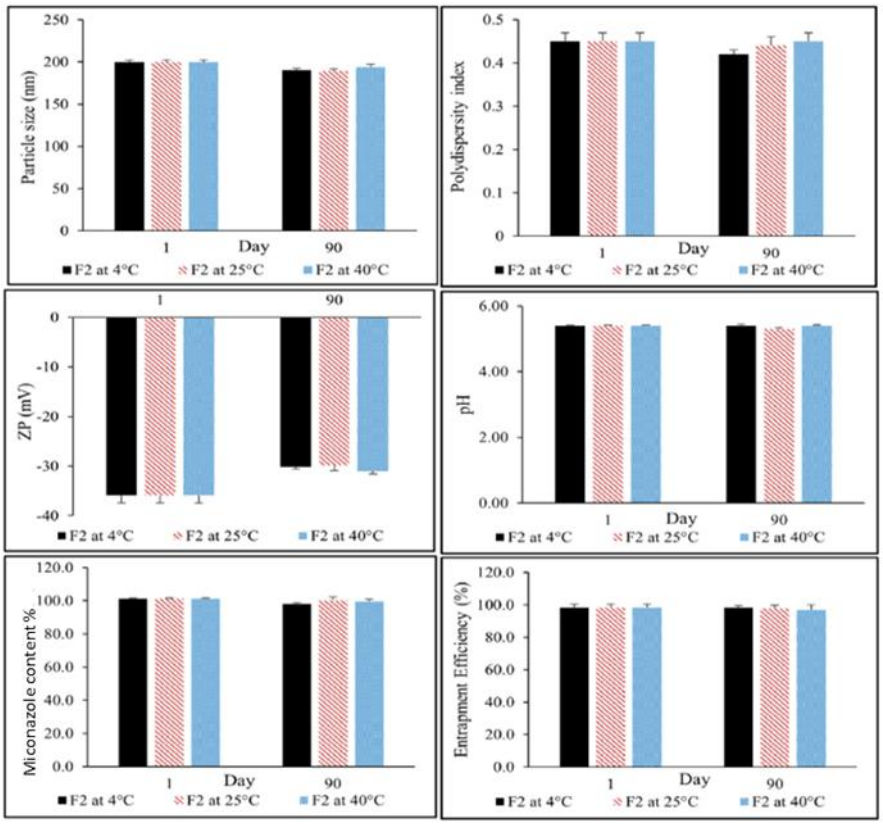

Figure 4: Particle size, polydispersity index, zeta potential (ZP), pH, drug content, and entrapment efficiency of miconazole-SLN (formulation F2) over three months of storage at 4, 25, and 40 °C (mean ± SD, n = 3).

3.4. Antibacterial Activity Testing

Antibacterial studies were conducted to determine the antimicrobial efficiency of the lead formulation against miconazole-C. F2 was chosen as the lead formulation over F6, as it had a desired release profile while maintaining similar physiochemical characteristics. The antimicrobial efficiency was evaluated against the two commonly isolated leading pathogens in ocular infections, MRSA and *P. aeruginosa.* Post-operative and nosocomial ocular infections due to MRSA have also been observed, with *P. aeruginosa* being the most frequent isolate among Gram-negatives.[10] In-house antibacterial studies showed a MIC of 6.25 µg/mL against MRSA and *P. aeruginosa* for both the lead

formulation as well as miconazole-C (**Table 6**). Moreover, this experimental MIC was achieved at all release time points samples during the study (0.5 to 24 h). The antibacterial activity is due to the miconazole present in the formulations, as the placebos did not show any such activity.

Table 6: MIC_{90} values for the control, F2, and placebo formulations against methicillin-resistant *Staphylococcus aureus.*

Formulation	MIC_{90} (µg/mL)
	Methicillin-Resistant *Staphylococcus aureus*
Miconazole-C	6.25
F2	6.25
Placebo F2	Not achieved

F2—miconazole loaded nanostructured lipid carrier formulation.

4. CONCLUSIONS

Miconazole-SLN formulations were successfully prepared and characterized. The lead SLN (F2) was physiochemically stable for over three months at the three tested storage conditions. The formulation showed an extended Miconazole release profile, over a 12 h period, and demonstrated similar antibacterial activity as the Miconazole eyedrops. Moreover, the MIC_{90} against the tested microorganism was attained with the very first time-point tested. Ex vivo transcorneal permeation studies showed significant improvement in Miconazole permeation from the Miconazole-SLN formulation, compared to the commercial ophthalmic solution eyedrops. Overall, the Miconazole-SLN formulation developed in the current investigation could reduce the frequency of dosing, by improving treatment outcomes, thereby increasing patient compliance compared to commercial miconazole eyedrops. Future in vivo research could reveal the difference in ocular surface residence time and biodistribution after topical administration. In conclusion, the SLN formulations could provide an efficient miconazole delivery platform for the management of conjunctivitis as well as various other ocular bacterial infections.

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