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Research Article

FORMULATION AND EVALUATION OF TROPICAMIDE *IN-SITU* GELS LOADED SOLID LIPID NANOPARTICLES FOR OCULAR DRUG DELIVERY

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ABSTRACT

The aim of present work Formulation and Evaluation of Tropicamide *In-situ* Gels loaded Solid Lipid Nanoparticles for Ocular Drug Delivery. The surface morphological of SLN was carried out by TEM. The Tropicamide loaded solid lipid nanoparticles was measured the average particle size was ranges from $182.1+3.12$nm to 390.1 ± 2.10 nm. The zeta potential ranges from -0.17 ± 1.4 mV to -3.80 ± 1.5 mV. The entrapment efficiency 66.2 % to 89.2 %. Drug content was ranges from 0.112mg/ml to 0.502 mg/ml. The percentage yield ranges from was ranges from 0.112mg/ml to 0.502 mg/ml. The polydispersity index ranged from 1.011 ± 0.15 to 1.327 ± 0.13. These SLN enriched in Chitosan gels the pH of the formulations range from 6.8 to 6.9. The gelling strength ranged from 129 sec to 152 sec. The bioadhesive force was ranges from 10.21 ± 1.15 dynes/cm^2 to 15.23 ± 1.22 dynes/cm^2. The viscosity was ranges from 2212 ± 1.14 cps to 2420 ± 1.19 cps. The spreadability coefficient was ranges from 11.2 ± 1.10 gms/sec to 13.3 ± 1.21 gms/sec. The *in-vitro* diffusion release studies carried out at 12 hrs TSLNGF19 shows the 79.2 ± 0.32. The ex vivo permeation studies for optimized formulation the increased drug permeation and corneal accumulation. In vitro corneal permeation profile of tropicamide loaded SLN from the chitosan gels and commercial eye drop solution (Tropicacyl) across the isolated porcine cornea. The ocular tolerance studies performed with HETCAM assay, corneal hydration study, histopathological studies. The stability studies of chitosan gels for long-term stability as per ICH guidelines (25°C $\pm 2^{\circ}$C / 60% RH $\pm$ 5% RH) &accelerated stability as per ICH guidelines (40°C $\pm 2^{\circ}$C / 75% RH $\pm$ 5% RH) there is no changes in gelling strength, bioadhesive force, viscosity, spreadability coefficient in optimized formulation.

Keywords: Chitosan, Corneal hydration studies, ex vivo permeation, in *vitro* diffusion studies, Solid Lipid Nanoparticles

INTRODUCTION

Ocular drug delivery systems using a nanotechnology have the potential to enhance the corneal residence time of the drug to reduce the faster clearance of drug from the eyes [1]. So many attempts were performed in the history to fix this problem in ocular drug delivery. The solid lipid nanoparticles are the most promising method for ocular drug delivery [2]. Nanotechnology has properties like smaller size range, enhanced surface area and easily suspending in liquids, various optical and magnetic properties are offered by nanotechnology than conventional drug delivery systems. The residence time of drugs in the precorneal area can be enhanced by use of mucoadhesive polymers [3]. Among them, chitosan shows several important biological properties, like biodegradability, low toxicity, biocompatibility and mucoadhesiveness. Chitosan is a deacetylated form of chitin, after cellulose it is the second most abundant polymer in nature [6]. Chitin is hydrophobic but in contrast, chitosan is soluble in acidic solutions, the ionic interaction between positively charged amino group of

chitosan and negative charged sialic acid residue in mucus lead to mucoadhesion mechanism [5]. These unique properties makes chitosan a versatile biopolymer and useful for development of ocular drug delivery systems. Tropicamide (BCS Class-II drug) is an antimuscarinic agent used to produces mydriasis [4]. It is frequently used during eye examinations for eye surgery, funduscopic examination, cycloplegic retinoscopy, cycloplegia. It blocks receptor in the muscles of the eye and control the size of pupil and lens shape. Tropicamide produces mydriasis due to blockage of receptors [4]. It has the fastest (25-45 min.) and briefest (4-6 hours) action. It is used as a short-acting mydriatic in fundoscopy. The positive or negative charge of the hydrophilic polymer was complexes with a multivalent cationic or anionic to form viscous gel particles having size range of nanometer [7]. The goal of present work SLNs can be an effective ocular drug delivery system by enhance the residence time of drug in eye by mucoadhesion which can leads to enhance the bioavailability and reducing dosing frequency [9].

MATERIALS & METHODS

The tropicamide was purchased from Optica Pharmaceuticals Pvt. Ltd (Yamunanagar, India). lipid Glyceryl trimyristate was procured from S.D. Fine Chemical Ltd, Mumbai, Dioleyl trimethyl ammonium propane was procured from Avanti Polar Lipids, Alabaster, Alabama. Polycaprolactone, Glyceryl

monostearate, chitosan was procured from sigma Aldrich Chemical Pvt Limited. Polysorbate 80 acquires from Microfine chemicals in Erode, India. All other reagents used were of analytical grade.

METHODOLOGY

Preparation of solid lipid nanoparticles:

In this technique, the drug (tropicamide) containing lipids in different ratios melt in cooled, solid lipid group these lipid nanoparticles are dispersed in cold surfactant (polysorbate 80) solution yielding a pre-suspension. Homogenizations was carried out at 8000 rpm reduce into small particles temperature was maintained at -5°C - 10°C with the help of a Remi homogenizer for 5 mints [8]. Then this presuspension is homogenized at below room temperature the gravitational force is strong enough to break the lipid into microparticles directly to SLN. The tropicamide loaded SLN were Lyophilised using a programmable freeze dryer (FDU-1100; Eyela, Japan). Before freezing, cryoprotectants were added to the SLN dispersion. Maltose and sucrose were screened at the levels of 5% and 10% (w/v) for their cryoprotectant ability, while lactose was screened at the levels of 2.5% and 5% (w/v). The freeze drying was conducted as follows: primary drying of the dispersion was at -35°C for 1 h. The shelf temperature was then raised to -25°C for 12 h. secondary drying was performed at 15°C for 4 h. Formulation design is shown in table 1.

Table 1: Formulation Design of solid lipid nanoparticles

Formulation code	Tropicamide drug (mg)	Lipids (gms)			
		Glyceryl trimyristate	Dioleyl trimethyl ammonium propane	Polycarpol acetone	Glyceryl monostearate
TSLNF1	1	1	-	-	-
TSLNF2	1	1.5	-	-	-
TSLNF3	1	2	-	-	-
TSLNF4	1	2.5	-	-	-
TSLNF5	1	-	1	-	-
TSLNF6	1	-	1.5	-	-
TSLNF7	1	-	2	-	-
TSLNF8	1	-	2.5	-	-
TSLNF9	1	-	-	1	-
TSLNF10	1	-	-	1.5	-
TSLNF11	1	-	-	2	-
TSLNF12	1	-	-	2.5	-
TSLNF13	1	-	-	-	1
TSLNF14	1	-	-	-	1.5
TSLNF15	1	-	-	-	2
TSLNF16	1	-	-	-	2.5
TSLNF17	1	1	0.2	-	-
TSLNF18	1	1	0.4	-	-
TSLNF19	1	1	0.6	-	-
TSLNF20	1	1	-	0.2	-
TSLNF21	1	1	-	0.4	-
TSLNF22	1	1	-	0.6	-
TSLNF23	1	1	-	-	0.2
TSLNF24	1	1	-	-	0.4
TSLNF25	1	1	-	-	0.6

Note: 5% of polysorbate 80 was used as a surfactant in all above formulations, 1:1 ratio of chloroform and menthol used as organic solvents in all the above formulations.

Preparation of solid lipid nanoparticles enriched in gels:

The optimized Tropicamide loaded SLN assimilate in prepared gels using chitosan (1%, 1.5%, & 2% w/v), Carboxymethylcellulose Sodium (0.5%) used as lubricating agent and glycerol (1% v/v) as hydrating agent taken in a beaker with continues stirring finally add preservatives (Benzalkonium chloride 0.02%) to store for a long time [11], finally adjust with pH 6.5.

Compatibility studies of the drug, the lipid, and polymers:

The FTIR spectrum of the pure form of tropicamide, physical mixtures are carried out with FTIR [12].

Morphological studies:

The following methods are used to determine overall shape and morphology of solid lipid nanoparticles performed with Transmission electron microscopy [13].

Evaluation parameters for Tropicamide Loaded Solid Lipid Nanoparticles:

Particle size analysis:

The SLN was inflexible by using Malvern particle size analyzer. The Polydispersity index ($Đ$) is a measurement of the distribution of molecular mass in a given polymer [14]. PDI of a polymer is calculated as follows

$$PDI = \frac{Mw}{Mn}$$

Where,

Mw is the weight average molecular weight and

Mn is the number average molecular weight.

Zeta potential:

The SLN enriched gels was estimated by zeta analyzer. The SLN dispersions and tropicamide SLN were diluted up to (1:100) and it was measured at 25°C by keeping the electric field strength around 23.2 V/cm.

Entrapment efficiency:

The prepared SLN dissolved in distilled water was kept in centrifuged up to 14,000 rpm for 40 mins at 10°C. The sample was observed in UV Visible spectrophotometer at λ max 254 nm.

$$\text{Entrapment Efficiency} = \frac{\text{Total amount of drug} - \text{Free drug}}{\text{Total amount of drug}} \times 100$$

Drug content:

Accurately weight 10 mg of the formulation was taken and mixed with small quantity of methanol. Then the formulation is warmed on the water bath so that the drug was easily dissolved in the formulation. Then the solution was placed in whatman filter paper [15]. The volumetric flask makes up to the mark by methanol to give a concentration of 1000 µg/ml for tropicamide. The volumetric flask to give a concentration of 10µg/ml and then absorbance was measured at 254 nm.

Percentage Yield:

It was calculated to determine whether the drug into the polymer was efficient. The raw material, active ingredients, when the manufacturing process of nanoparticles [16]. The produced results were determined by weighing the nanoparticles and determined the percentage result of added materials weight that was the weight of drug and polymer which were added.

$$\text{Percentage Yield} = \frac{\text{Total solid lipid nanoparticles weight}}{\text{Total solid weight}} \times 100$$

Evaluation of solid lipid nanoparticles Loaded Gels:

The SLNs were encapsulating with different concentration 1%, 1.5% & 2% Chitosan gels. The Formulated SLN enriched in Chitosan gel characterization as listed below.

Gelling strength:

In 100 ml measuring cylinder containing 50 gm of a gel at thermostat at 37°C, it allows to penetrate into the Chitosan gel. At physiological temperature while applying pressure on the device sink at 5cm down, to measure the time in seconds [17].

Bioadhesive force:

All the optimized batches as follows, the chicken cheek portion of a mucosa was kept in a glass vial. The vial connected with mucosa in an inverted position while the first vial was placed on a height adjustable pan. Then the second vial was placed on mucosal surfaces of both vials. Then weight was kept rising in the pan until vials get detached. The minimum weight required to detach two vials [18].

Viscosity:

The prepared Gels enriched in SLN were carried out by using Brookfield viscometer.

Spreadability coefficient:

The gels were placed in between the two glass slides and compressed to uniform thickness then kept weight up to 1000g for 5 min. weight (50 g) was placed on the pan. The time in which the upper glass slide moves over to the lower plate was taken as a measure of spreadability (S).

S= ML/T

Where

M = weight tide to upper slide (g)

L = length moved on the glass slide (cm)

T = time taken (sec)

Visual appearance & pH:

The pH of tropicamide loaded SLN incorporated gels formulations were measured in pH paper.

In vitro Franz's diffusion:

In vitro release studies were performed using standard Franz diffusion cells (FDC-6, LOGAN Instrument Corp., Somerset, NJ, USA). The diffusion area was 0.75 cm2 and receptor volume was 5.0 mL. Receptor chambers were filled with 5 ml of PBS (pH 7.4; osmolality 297 mosm/kg) and constantly stirred by small magnetic bars. The receptor fluid was stirred with a magnetic rotor at a speed of 600 rpm and the

temperature was maintained at 35 ±0.5°C in order to mimic the ocular surface temperature [19]. Donor and receptor chambers were separated by means of activated dialysis membrane bag (molecular weight cut off 12,000 Da). One millilitres of each formulation was loaded into the donor compartment before occluding the chamber with Para-film. Samples were withdrawn at regular intervals (1, 3, 5, 8, and 12h filtered through a 0.45-µm membrane filter and analyzed for drug content by UV-Visible spectrophotometer method measured at λmax 254nm.

Mathematical modeling for drug release profile:

The cumulative amount of tropicamide released from the chitosan gels at different time intervals were fitted with Zero order, First order, Higuchi model, and Korsemayer-peppas model.

Ex vivo permeation studies:

It was performed by Franz diffusion cell on the excised goat eye collected within 30 min after sacrifice collect the freshly excised cornea was tied to one side of the open tube (donor compartment) in such a way that its epithelial surface faced the donor compartment while the endothelial part was exposed to Phosphate buffer pH 7.4 (receptor compartment). Then you can place Tropicamide loaded solid lipid nanoparticles chitosan gels in donor compartment. The Phosphate buffer was stirred at 50 rpm and maintained the temperature at 37± 0.5 °C. Samples (0.5 mL) were withdrawn from the receptor cell after 12hrs analyzed by HPLC. The Permeation flux was calculated as the ratio of drug permeation rate of the corneal tissue and the cross-sectional area of the tissue [20]. The amount of drug retained within the corneal tissue was determined using HPLC after rinsing (with PBS) and homogenizing the tissue.

Animal Ethical Approval:

All animal experiments were carried out after approval of the protocol by the Institutional Animal Ethical Care committee (IAEC), Sri venkateswara college of pharmacy, RVS Nagar, Tirupathi road, Chittoor, Andhrapradesh (vide letter no. SVCP/IAEC/0172017-18) and conducted according to the Indian National Science Academy (INSA) guidelines for the use and care of experimental animals.

In vitro corneal permeation study:

The *In vitro* Corneal permeation studies of tropicamide-loaded solid lipid nanoparticles enriched in chitosan gels were comparatively evaluated with the commercially available eye drops (Tropicacyl 1%, (w/v)) using isolated porcine eyes cornea as model Fresh whole eyeballs were obtained from the local butcher shop immediately after slaughtering and transported to the laboratory in cold normal saline within an hour. The Cornea was carefully excised along with 2-4 mm of scleral tissue and finally cleaned and washed till free from proteins with cold normal saline. The isolated cornea was mounted by clamping between the donor and receptor compartments of modified Franz diffusion cell, with endothelial side facing the receptor and epithelial side facing the donor. The receptor compartment

contained 11.5 ml of freshly prepared phosphate buffered saline (PBS) pH 7.4 maintained at 35 ±0.5°C under magnetic stirring. Area available for corneal permeation was 0.785 cm². The TSLNGF19 (1 ml) was placed in the donor compartment over the cornea. An aliquot of 1 ml of the sample was withdrawn from receptor compartment at fixed time intervals and analyzed for the contents of tropicamide. The study was conducted using paired corneas, i.e. one cornea of the animal was used for the permeation study of Chitosan gels and the contralateral cornea was used for conventional eye drops of aqueous drug solution [21].

Determination of tropicamide flux and permeability:

The cumulative amount of tropicamide permeating across the porcine cornea was plotted against time and slope of the linear portion of the graph was calculated. The steady state flux (J, mg/h/cm2) and apparent permeability coefficient (K, cm/h) were calculated as follows.

$$Js = \frac{dQ}{A \times dt}$$

Where dQ/dt is the linear portion of the slope (mg/h), A is the corneal surface area (in this study, 0.785 cm2), and C is the initial drug concentration (mg/ml).

Ocular tolerance studies:

i) Hen's Egg Test Chorio Allantoic Membrane (HETCAM) assay:

HETCAM assay was developed for toxicity and irritation studies as an alternative method to replace the Draize test. In this study, fertilized chicken eggs (pathogen-free) were incubated at 37 ± 0.5 ºC, 58 ± 2% relative humidity in a standard cell culture incubator up to 10 days. At the beginning of the test procedure, the outside of the eggs was disinfected and a hole was drilled through the pointed pole of the shell, 3ml of albumin was removed to allow the CAM to develop in an accessible way to treatment [22]. The hole was sealed with molten candle wax. On the 10 th day, the eggs were opened at their blunt ends and the gel substances (0.3 mL) were applied to the CAM surface before reactions were observed within a specific time limit: 0.5 min (Ist phase), 2 min (II nd phase) and 5 min (III rd phase)

ii) Corneal hydration study:

The corneal hydration study is one of the important parameters to assess any damage to corneal tissue (epithelium and/or endothelium). At the end of the ex vivo study, each cornea was weighed, soaked in methanol, and dried overnight at 90 °C, and reweighed [22]. The percent of corneal hydration was calculated from the difference in weight.

iii) Histopathology:

The evaluation of cornea provides useful descriptive information of corneal damage as well as an assessment of the extent of the corneal injury. The isolated corneas were exposed to test material followed by fixing in an appropriate fixative (e.g., 10% neutral buffered formalin). The corneal tissues were then dehydrated

using alcohol followed by embedding in low melting point paraffin. The corneal sections of thickness 5μ were cut, placed on clean glass slides and then depart finished [22]. Later, the sections were stained with hematoxylin-eosin and examined under an optical microscope for the presences of any changes/damage in the corneal tissues.

Stability studies:

Stability study was performed as per International Conference on Harmonisation guidelines (ICH) Q1A (R2) to assess the prepared hydrophilic drug loaded solid lipid nanoparticles enriched in chitosan gels for its stability and potential to withstand atmospheric/environmental changes [23]. Prepared Tropicamide loaded solid lipid nanoparticles enriched in chitosan gels was subjected to stability were packed in a clear glass vial and stored at a long term condition (25°C ± 2°C / 60% RH ± 5% RH) for 12 months and accelerated stability condition (40°C ± 2°C / 75% RH ± 5% RH for 6 months in stability chamber. The stored samples were analyzed mean gelling strength, Bioadhesive force, viscosity, spreadability coefficient, pH.

RESULTS & DISCUSSION

Compatibility studies:

Physical mixture of samples was characterized by FTIR spectral analysis for any physical as well as chemical drug characteristics. There was no interference in the functional groups as the principal peaks. The FTIR spectrum is display in Figure 02 & interpretation was display in table 2.
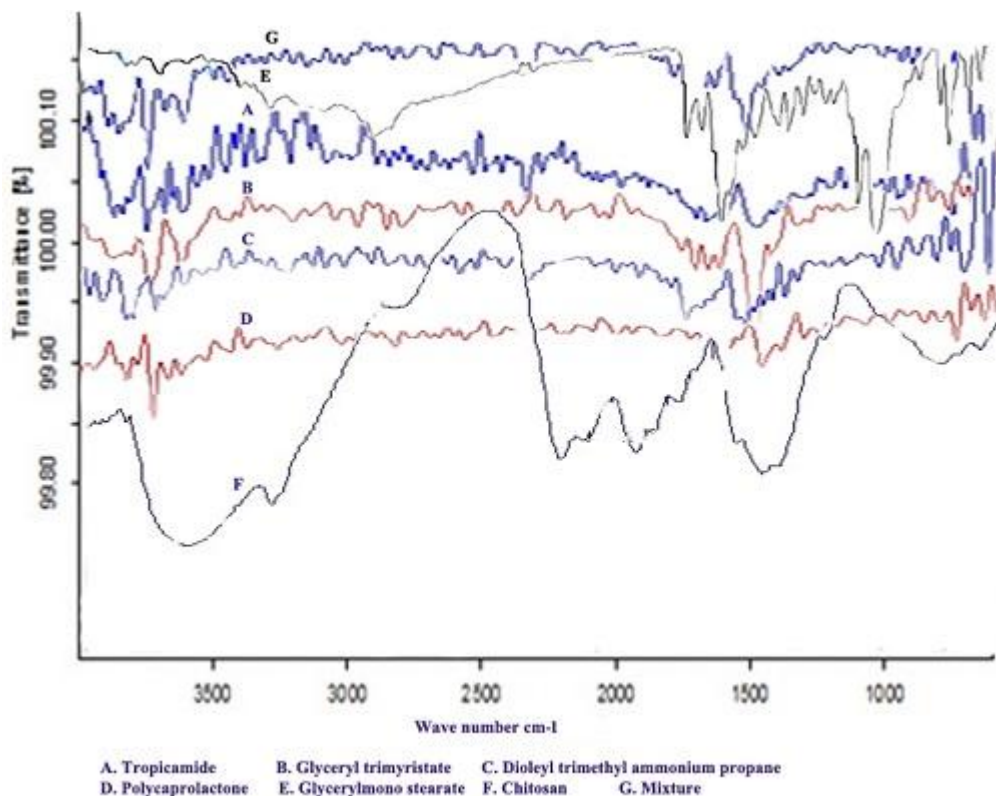

A. Tropicamide   B. Glyceryl trimyristate   C. Dioleyl trimethyl ammonium propane
D. Polycaprolactone   E. Glycerylmono stearate   F. Chitosan   G. Mixture

Figure 1: FTIR spectrum of Drug, Lipids, Excipients & Mixture of compounds

Table 2: FTIR spectrum of the observed and characteristic peak of Drug, Lipids, Excipients & Mixture of compounds

FTIR Spectrum	IR absorption bands (cm-1)		Bond	Functional group
	Observed peak	Characteristic peak		
Tropicamide	3678.49	3000-3700	O-H stretch	Alkenes,aromatic
	3562.90	3000-3700	O-H stretch	Alkenes, aromatic
	3220.11	3000-3700	O-H stretch	Alkenes, aromatic
	3090.71	3000-3700	O-H stretch	Alkenes, aromatic
Glycerin trimyristate	3623.09	3000-3700	O-H stretch	Alkenes, aromatic
	3410.81	3000-3700	O-H stretch	Alkenes, aromatic
	3215.31	3100-3330	N-H stretch	Aromatic ring
Dioleyl trimethyl ammonium propane	3647.52	3000-3700	O-H stretch	Alkenes, aromatic
	3473.00	3000-3700	O-H stretch	Alkenes, aromatic
	3170.27	3000-3700	O-H stretch	Alkenes, aromatic
	3035.53	3000-3700	O-H stretch	Alkenes, aromatic
	2967.04	2500-3000	C-H stretch	Alkenes, aromatic
Polycaprolactone	3647.88	3000-3700	O-H stretch	Alkenes, aromatic
	3557.92	3000-3700	O-H stretch	Alkenes, aromatic
	3298.14	3000-3700	O-H stretch	Alkenes, aromatic
Glyceryl monostearate	2928.92	2960-2850	C-H stretch	Alkanes
	3679.72	3700-3500	O-H stretch	Free OH alcohols
	1091.08	1150-1070	C-O stretch	Ethers
	1297.40	1300-800	C-C stretch	Alkenes
Chitosan	3359	3000-3700	O-H stretch	Free OH alcohols
	2872	2500-3000	C-H stretch	Alkenes, aromatic
	1085	1150-1070	C-O stretch	Ethers
	1025	1150-1070	C-O stretch	Ethers
Mixture	3682.31	3000-3700	O-H stretch	Alkenes, aromatic
	3154.37	3000-3700	O-H stretch	Alkenes, aromatic
	2718.03	2500-3000	C-H stretch	Alkenes, aromatic
	2355.95	2100-2660	C=C stretch	Alkynes

Morphological characters of Tropicamide Loaded SLN:

The TEM evaluates particle morphology by examining the electrons that are transmitted through the specimen.

An image is produced by interpreting the interaction of the electrons passed through the specimen, which is visualized by an imaging device or detected by a special sensor as shown in figure 2.
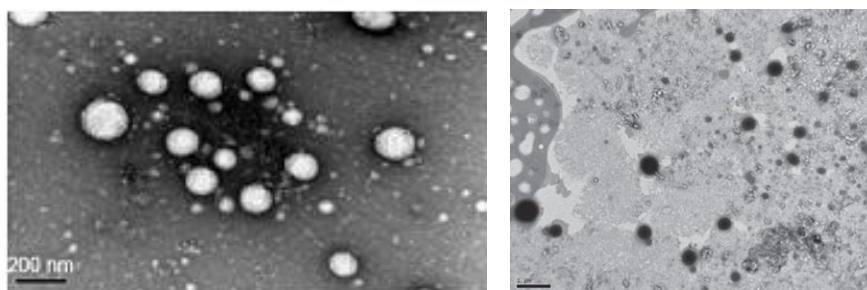
Figure 2: Tropicamide loaded Solid lipid nanoparticles best formulation TSLNF10

Characterization of tropicamide loaded solid lipid nanoparticles

Particle size analysis:

It was analyzed by using Zetasizer Nano-Series. The average particle size ranged from 182.1+3.12nm to 390.1±2.10 nm. All these observations values are displayed in Table 3.

Zeta potential:

The solid lipid nanoparticles were estimated by Malvern zetasizer. The zeta potential ranges from -0.17±1.4 mV to -3.80±1.5 mV. All these observations values are displayed in Table 3.

The entrapment efficiency:

The solid lipid nanoparticles contain free tropicamide ranges from 66.2 % to 89.2 %. All these observations values are displayed in Table 3.

Drug content:

Tropicamide loaded solid lipid nanoparticles was ranges from 0.112mg/ml to 0.502 mg/ml. All these observations values are displayed in Table 3.

Percentage yield: Tropicamide loaded solid lipid nanoparticles ranges from 0.8% to 3.8 %. All these observations values are displayed in Table 3.

Polydispersity Index:

Tropicamide loaded solid lipid nanoparticles was ranges from 1.011 ± 0.15 to 1.327 ± 0.13. All these observations values are displayed in Table 3.

Table 3: Evaluation parameters of solid lipid nanoparticles

Formulation code	Particle size* (nm)	Zeta potential* (mv)	Entrapment efficiency (%)	Drug content (mg/ml)	% yield	Poly dispersity index*
TSLNF1	182.1+3.12	-0.17±1.4	66.2	0.112	1.8	1.011±0.15
TSLNF2	191.2±3.19	-0.27±1.6	68.4	0.131	2.4	1.121±0.17
TSLNF3	201.5±2.18	-0.31±1.8	70.2	0.142	2.9	1.215±0.19
TSLNF4	213.6±2.10	-0.39±1.9	72.6	0.212	3.4	1.245±0.23
TSLNF5	190.1±3.23	-1.21±1.2	67.8	0.121	1.9	1.025±0.16
TSLNF6	210.2±3.40	-1.25±1.3	69.3	0.133	2.3	1.128±0.18
TSLNF7	227.5±2.17	-1.40±1.5	72.5	0.144	2.8	1.229±0.20
TSLNF8	235.1±2.13	-2.12±1.7	74.6	0.216	3.4	1.250±0.22
TSLNF9	192.3±3.81	-0.18±1.6	68.9	0.124	1.9	1.027±0.17
TSLNF10	212.3±3.91	-0.30±1.9	70.5	0.132	2.3	1.130±0.20
TSLNF11	240.1±2.12	-0.50±2.0	72.4	0.146	2.9	1.205±0.23
TSLNF12	310.2±2.09	-1.80±2.2	75.6	0.218	3.4	1.315±0.15
TSLNF13	194.2±3.61	-0.22±2.0	69.8	0.131	1.9	1.131±0.18
TSLNF14	240.5±2.15	-0.59±2.3	71.4	0.134	2.4	1.141±0.20
TSLNF15	267.3±2.16	-0.90±2.7	74.3	0.149	2.8	1.230±0.22
TSLNF16	340.1±3.12	-1.18±2.9	77.6	0.223	3.4	1.245±0.23
TSLNF17	211.1±3.12	-2.18±2.1	79.2	0.316	2.0	1.219±0.19
TSLNF18	250.6±2.19	-2.60±1.8	82.4	0.391	2.2	1.215±0.24
TSLNF19	301.5±2.15	-3.80±1.5	85.6	0.412	2.5	1.326±0.29
TSLNF20	212.2±2.15	-2.85±1.6	81.3	0.418	2.1	1.220±0.20
TSLNF21	251.3±3.21	-2.95±1.8	84.5	0.487	2.3	1.241±0.15
TSLNF22	351.3±1.67	-3.12±1.5	87.3	0.512	2.4	1.327±0.13
TSLNF23	210.1±3.18	-2.90±1.6	83.4	0.412	2.1	1.225±0.22
TSLNF24	261.4±2.15	-3.181.9	86.3	0.451	2.2	1.245±0.13
TSLNF25	390.1±2.10	-3.20±2.0	89.2	0.502	2.5	1.326±0.19

*Values are mean of triplicate ± SD

Evaluation of solid lipid nanoparticles loaded Chitosan Gels:

The tropicamide SLNs were incorporated in different concentration 1%, 1.5%, 2 % of Chitosan gels. From the above 25 formulation selected formulations chosen the Chitosan gel TSLNGF3, 4, 7, 8, 11, 12, 15, 16, 17, 18, 19, 21, 22, 24, & 25 characterization as displayed below.

Visual appearance and pH:

All the formulations were found to be transparent white color and in semi-solid consistency. The pH of the formulations ranges from 6.8 to 6.9.

Gelling strength:

The gelling strength of solid lipid nanoparticles loaded gels range from 129 sec to 152 sec. All these observations values are displayed in Table 4.

Bioadhesive force:

The Bioadhesive force of all formulations ranged from 10.21 ±1.15 dynes/cm^2 to 15.23 ± 1.22 dynes/cm^2. All these observations are displayed in Table 4.

Viscosity:

The rheological studies of all formulations were ranges from 2212 ± 1.14 cps to 2420± 1.19 cps. All the values are displayed in Table 4.

Spreadability coefficient:

all the formulation ranged from 11.2 ± 1.10 gms/sec to 13.3 ± 1.21 gms/sec. All these observations are listed in Table 4.

Table 4: Evaluation parameters of Solid lipid nanoparticles loaded chitosan gels

Formulation code	Gelling properties				
	Gelling strength (sec)	Bioadhesive force* (dynes/cm^2)	Viscosity* (cp)	Spreadability Coefficient* (gm/sec)	pH
TSLNGF3	129	10.21±1.15	2212±1.14	11.2±1.10	6.9
TSLNGF4	139	11.21±1.17	2321±1.10	11.5±1.13	6.9
TSLNGF7	141	12.49±1.20	2352±1.18	12.0±1.16	6.9
TSLNGF8	131	13.19±1.24	2215±1.16	11.5±1.12	6.9
TSLNGF11	142	9.21±1.03	2325±1.10	11.9±1.15	6.8
TSLNGF12	144	10.18±1.21	2349±1.20	12.1±1.18	6.8
TSLNGF15	133	10.29±1.42	2216±1.21	10.5±1.15	6.9
TSLNGF16	145	11.16±1.53	2330±1.24	12.2±1.14	6.8
TSLNGF17	146	13.41±1.18	2418±1.20	12.5±1.20	6.8
TSLNGF18	135	14.01±1.20	2420±1.19	12.4±1.19	6.9
TSLNGF19	148	15.23±1.22	2519±1.15	13.3±1.21	6.9
TSLNGF21	150	11.21±1.42	2418±1.10	12.9±1.23	6.8
TSLNGF22	139	12.18±1.51	2412±1.16	12.6±1.22	6.9
TSLNGF24	150	11.89±1.35	2398±1.24	13.0±1.25	6.9
TSLNGF25	152	12.15±1.21	2410±1.20	13.2±1.26	6.9

*Values are mean of triplicate ± SD

In-vitro diffusion release studies:

The drug release studies were performed in a phosphate buffer of pH 7.4 suspending the formulation with the 5mg equivalent of the drug. For all formulations respectively, in a span of 12hrs of study. The optimized formulations were subjected in the data analysis. The all formulations were subjected to data analysis and it was found to be first order drug release. The values are displayed in Table 5 & 6 Figures 3.

Table 5: in Vitro Franz's diffusion studies of Chitosan Gels enriched in Tropicamide loaded Solid Lipid Nanoparticles

Time (hrs)	Cumulative Percent drug release after each time interval*								
	TSLNGF3	TSLNGF4	TSLNGF7	TSLNGF8	TSLNGF11	TSLNGF12	TSLNGF15	TSLNGF16	TSLNGF17
1	5.1 ± 0.61	6.5±0.92	8.3 ± 0.82	9.3 ± 0.51	8.3 ± 0.12	9.4 ± 0.22	10.2 ± 0.27	09.2 ± 0.15	06.3 ± 0.17
2	11.3±1.11	14.2± 0.86	16.1 ± 0.62	13.2 ± 1.46	12.1 ± 0.41	13.3 ± 0.15	15.1 ± 1.62	13.5 ± 0.24	13.3 ± 0.42
3	12.4 ±1.52	19.2±0.63	23.3 ± 0.82	19.4 ± 0.22	19.3 ± 0.53	25.3 ± 0.23	21.5 ± 0.36	24.2 ± 0.26	19.2 ± 1.63
4	19.3 ±2.21	29.2±1.2	29.1 ± 1.32	24.5 ± 0.62	25.4 ± 0.15	34.2 ± 0.43	33.2 ± 0.48	31.2 ± 0.53	23.8 ± 0.54
5	24.5±1.41	34.7±1.6	36.2 ± 1.33	29.3 ± 0.31	33.1 ± 0.53	39.1 ± 0.41	39.5 ± 0.52	39.2 ± 0.53	29.4 ± 0.53
6	34.7±1.31	40.2±1.54	41.2 ± 1.63	35.1 ± 0.52	39.7 ± 0.62	42.2 ± 0.22	42.5 ± 0.62	43.4 ± 1.63	38.2 ± 0.63
7	41.8±1.61	47.4±1.32	48.5 ± 1.57	39.1 ± 1.23	43.1 ± 0.25	49.2 ± 0.42	48.3 ± 0.32	52.4 ± 1.32	42.4 ± 0.34
8	49.7± 3.21	52.5±1.34	53.8 ± 1.63	46.5 ± 1.41	49.3 ± 0.28	53.2 ± 0.48	52.2 ± 1.46	59.4 ± 1.42	53.3 ± 1.32
9	53.6 ±2.42	58.6± 2.13	61.7 ± 1.73	52.4 ± 1.52	54.3 ± 0.53	61.3 ± 1.86	58.4 ± 1.82	65.3 ± 0.34	60.4 ± 1.34
10	59.4 ±2.61	64.8± 1.54	62.8 ± 1.53	59.2 ± 1.23	60.5 ± 0.52	68.3 ± 1.56	67.6 ± 1.73	63.3 ± 0.43	68.3 ±1.53
11	63.7±1.31	67.1± 1.63	68.4 ± 2.54	62.2 ± 0.12	65.2 ± 0.15	70.3 ± 1.35	71.3 ± 1.47	71.3 ± 1.24	73.5 ± 2.42
12	67.9 ±2.40	68.5 ± 1.55	70.4 ± 1.11	65.3 ± 2.01	69.3 ± 1.51	72.64 ± 1.78	75.27 ± 2.51	76.51 ± 1.21	78.31 ±1.21

*Values are mean of triplicate ± SD

Table 6: *In Vitro* **Franz's diffusion studies of Chitosan Gels enriched inTropicamide loaded Solid lipid Nanoparticles**

| Time (hrs)	Cumulative Percent drug release after each time interval*					
	TSLNGF18	**TSLNGF19**	**TSLNGF21**	**TSLNGF22**	**TSLNGF24**	**TSLNGF25**
1	07.5 ± 0.27	05.2 ± 0.76	08.4 ± 0.26	08.7 ± 1.08	03.6 ± 1.27	08.3 ± 0.36
2	15.6 ± 0.35	19.6 ± 0.45	19.4 ± 0.33	15.5 ± 0.56	13.7 ± 0.14	16.6 ± 0.56
3	20.6 ± 0.43	24.3 ± 1.53	24.6 ± 0.53	21.6 ± 0.27	19.7 ± 0.25	23.7 ± 0.65
4	28.2 ± 0.53	35.6 ± 1.73	31.5 ± 0.43	28.5 ± 0.53	28.7 ± 0.59	29.7 ± 0.32
5	32.4 ± 0.53	40.4 ± 1.23	40.7 ± 0.63	37.5 ± 0.45	33.8 ± 0.37	34.7 ± 1.72
6	36.2 ± 0.46	49.3 ± 0.93	48.6 ± 0.23	41.6 ± 0.35	39.6 ± 0.64	39.7 ± 1.63
7	42.5 ± 1.32	54.2 ± 1.23	53.5 ± 1.34	49.5 ± 0.45	48.3 ± 1.64	42.7 ± 1.87
8	48.3 ± 1.54	59.5 ± 1.35	59.6 ± 1.53	52.6 ± 0.53	51.6 ± 2.74	49.6 ± 0.63
9	52.3± 0.34	63.7 ± 1.45	61.7 ± 0.53	60.3 ± 0.72	59.6 ± 1.74	54.7 ± 1.63
10	62.3 ± 1.34	71.3 ± 0.36	68.4 ± 0.47	67.8 ± 1.52	63.7 ± 0.64	63.8 ± 1.25
11	72.4 ± 2.45	75.4 ± 2.65	72.5 ± 0.53	72.7 ± 0.63	69.4 ± 1.63	72.7 ± 0.36
12	78.3 ± 1.22	79.2 ± 0..32	75.4 ± 2.14	76.2 ± 1.21	74.1 ± 2.65	77.1 ± 0.42

*Values are mean of triplicate ± SD
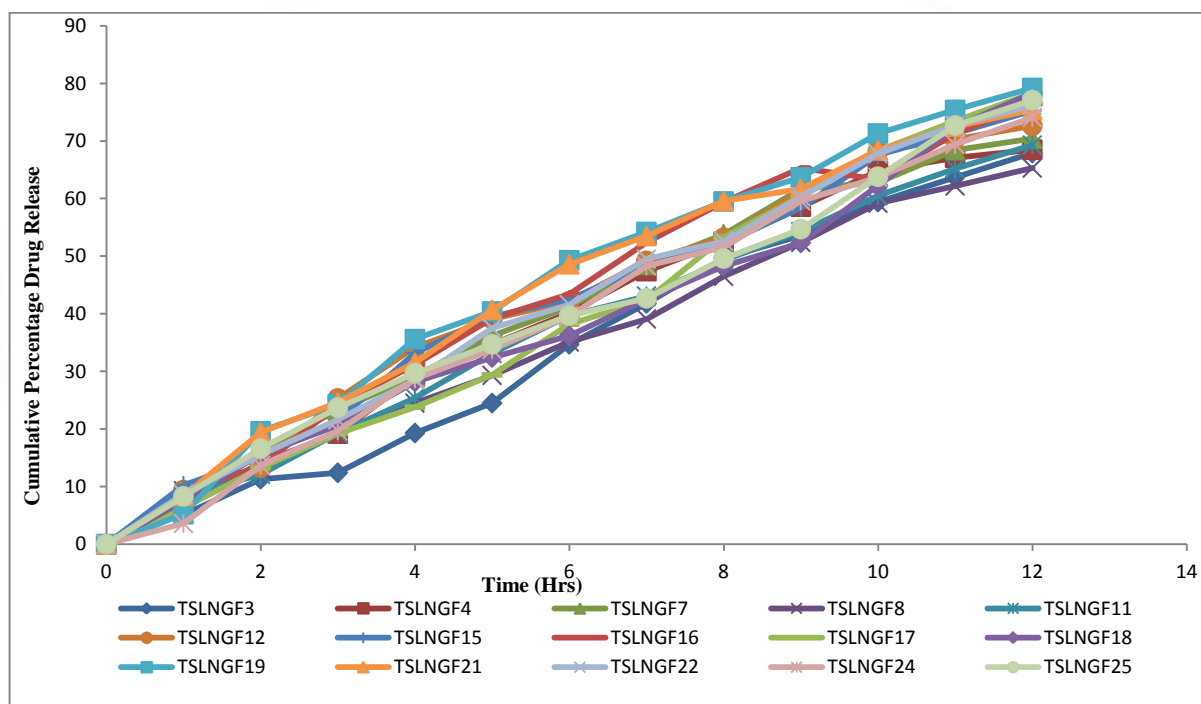
Figure 3: *In Vitro* **Franz's diffusion studies of Chitosan Gels enriched inTropicamide loaded Solid lipid Nanoparticles**

Table 7: Drug Release Kinetics of Chitosan Gels loaded in Solid lipid Nanoparticles Formulations

Order of Process		Formulation code							
		TSLNGF3	**TSLNGF4**	**TSLNGF7**	**TSLNGF8**	**TSLNGF11**	**TSLNGF12**	**TSLNGF15**	**TSLNGF16**
Zero order	R^2	0.961	0.967	0.963	0.968	0.921	0.967	0.924	0.932
First order	R^2	0.974	0.979	0.975	0.973	0.965	0.961	0.967	0.964
Higuchi	R^2	0.984	0.981	0.984	0.987	0.986	0.983	0.982	0.984
Hixon	R^2	0.853	0.880	0.980	0.927	0.879	0.922	0.845	0.861
Korsmeyer	R^2	0.853	0.952	0.840	0.918	0.873	0.835	0.912	0.961
	n	0.758	0.755	0.835	0.758	0.792	0.737	0.732	0.774
Mechanism		Non-Fickian	Non-Fickian	Non-Fickian	Non-Fickian	Non-Fickian	Non-Fickian	Non-Fickian	Non-Fickian

Table 8: Drug Release Kinetics of Chitosan Gels loaded in Solid lipid Nanoparticles Formulations

Order of Process		Formulation code						
		TSLNGF17	TSLNGF18	TSLNGF19	TSLNGF21	TSLNGF22	TSLNGF24	TSLNGF25
Zero order	R^2	0.934	0.964	**0.978**	0.989	0.980	0.962	0.961
First order	R^2	0.970	0.974	**0.971**	0.978	0.977	0.978	0.976
Higuchi	R^2	0.986	0.984	**0.987**	0.983	0.980	0.982	0.987
Hixon	R^2	0.830	0.874	**0.917**	0.841	0.835	0.814	0.696
Korsmeyer	R^2	0.892	0.871	**0.990**	0.876	0.892	0.815	0.889
	n	0.857	0.860	**0.937**	0.851	0.838	0.825	0.862
Mechanism		Non-Fickian	Non-Fickian	**Fickian**	Non-Fickian	Non-Fickian	Non-Fickian	Non-Fickian

Ex vivo permeation study:

The solid lipid nanoparticles enriched in chitosan gels exhibited about 3-fold higher permeation of TSLNGF19 through the excised cornea in comparison to the drug suspension after 12 h. The corneal flux of tropicacyl was found significantly higher for TSLNGF19 in contrast with the drug suspension figure 4. A statistically significant drug accumulation in corneal tissue was found for TSLNGF19 when compared with tropicacyl. These results support the enhanced release of drug from the chitosan gels. The increased drug permeation and corneal accumulation could be further justified by the permeation enhancing properties of oleic acid used as a carrier for solid lipid nanoparticles.
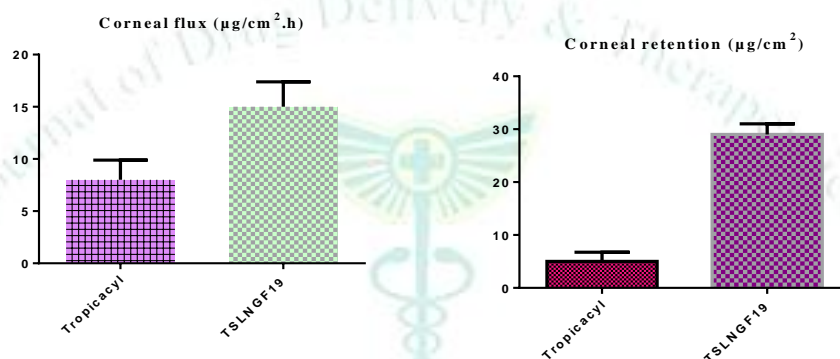

Figure 4: Ex-vivo corneal permeation of TSLNGF19 formulations (Mean±SD, n=3) statistically significant as analyzed by Dunnett comparison test)

In vitro corneal permeation study:

It presents the results of in vitro corneal permeation profile of tropicamide loaded SLN from the chitosan gels and commercial eye drop solution (Tropicacyl) across the isolated porcine cornea. It can be seen that permeation characteristics of tropicamide-loaded chitosan gels are comparable to that of conventional commercial preparation (Tropicacyl). Furthermore, no remarkable difference in values of flux (Jss) and apparent permeability (K) was seen as listed in Table 9. The preservative was added in the chitosan gels it provided % permeation of tropicamide comparable to the commercial formulation. This could be attributed to the nanometric size of the solid lipid nanoparticles enriched in gels. Earlier it was reported that nanoparticles provide higher corneal permeation due to endocytic uptake. In addition, lipid dioleyltrimethyl ammonium propane has also been reported to provide corneal penetration enhancing effect.

Table 9: Steady state flux (Jss) and permeability (Kp) of tropicamide through the porcine cornea and corneal hydration level.

Formulation	Flux Jss ($\mu g/h/cm^2$)	Apparent permeability coefficient Kp (x 10^3 cm/h)
Tropicamide loaded solid lipid nanoparticles	67.32	6.86
Tropicacyl	72.23	7.15

HETCAM assay:

The HETCAM assay has been considered as one of the most sensitive and robust screening tests for the prediction of eye irritation properties, especially for identifying slightly irritating chemicals. Based on the scoring methodology, the irritancy score (IS) of control (0.9% NaCl) and TSLNGF19 as observed as 0 (lack of coagulation, hemorrhage and lysis during the given time limit) that suggested the non-irritating nature of the formulations. Unlike, the IS of Irritant (0.1N NaOH) was reported 9 as coagulation and hemorrhage occurred within the Period of 0.5 min while lysis was observed during the 2nd min. (Table 10). Figure 5 exhibited the effect of test formulations on HETCAM after the specific time limit i.e. 0.5, 2 and 5 min.
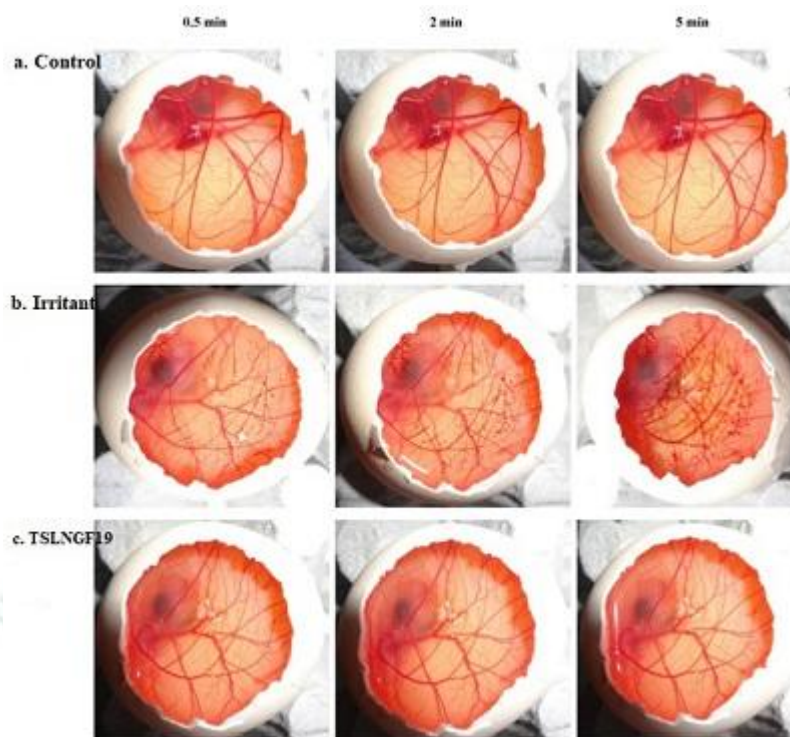

Figure 5: HET-CAM assay after treatment with (a) Control, (b) Irritant and (c) TSLNGF19

Table 10: Scoring for irritation testing (HET-CAM assay)

Test Formulation	Effect	Score			Net score	Inference
		0.5 min	2 min	5 min		
Control (0.9% Nacl)	Lysis	--	--	--	0	Non - Irritant
	Haemorrhage	--	--	--		
	Coagulation	--	--	--		
Irritant (0.1N NaOH)	Lysis		3		0	Severe irrtant
	Haemorrhage	7	--	--		
	Coagulation	9	--	--		
TSLNGF19	Lysis	--	--	--	0	Non - Irritant
	Haemorrhage	--	--	--		
	Coagulation	--	--	--		

Corneal hydration study:

The percent corneal hydration for the TSLNGF19 treated cornea was found as $77.21 \pm 2.50\%$, which denotes good corneal integrity (Figure 6). For a healthy cornea, the hydration level should be in the range between 76 and 80%. The irritant (0.1N NaOH) exhibited a statistically significant (P = 0.05) value of corneal hydration i.e. $85.15 \pm 2.15\%$ when compared with the control and TSLNGF19. The corneal injury, if any, caused by the solid lipid nanoparticles was under safe level and could be reversible. Earlier studies proposed that the enhancement in hydration level (>83%) may cause irreversible damage to corneal tissues. In most species, the water content of the normal cornea ranges from 3.2 to 4 g/g dry weight, corresponding to a 76-80% hydration level. An increase in hydration levels up to 83-92% indicates damage of the epithelium and/or of endothelium that allows extra water to enter the stroma leading to corneal edema.
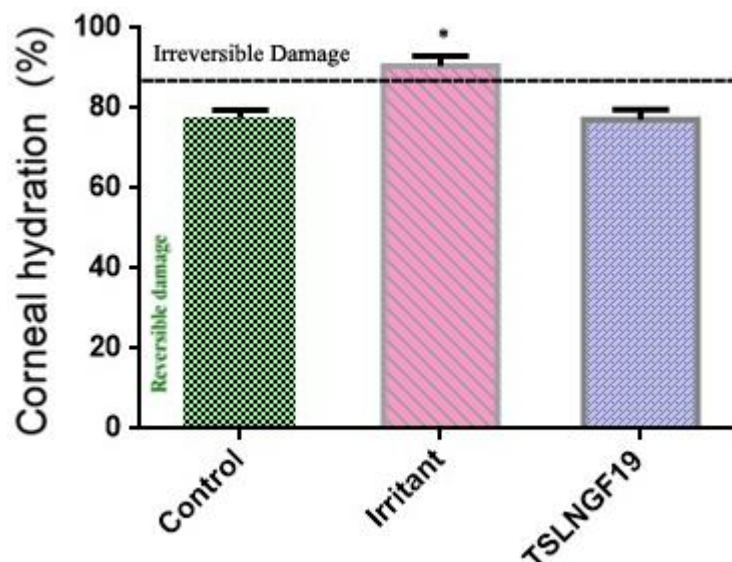
Figure 6: Corneal hydration study for TSLNGF19 (Mean ± SD, n=3) (*P = 0.05; statistically significant as analyzed by Dunnett multiple comparison test)

Histopathology:

The histopathological images of control and TSLNGF19 treated cornea exhibited intact epithelial layer without any stromal swelling. While the epithelial layer was found to be severely damaged at various regions in the irritant (0.1N NaOH) treated cornea (Figure 7). In addition, highly swelled stroma (corneal edema) was observed as result of excessive and uncontrolled fluid entry into the stroma through the highly injured epithelia.
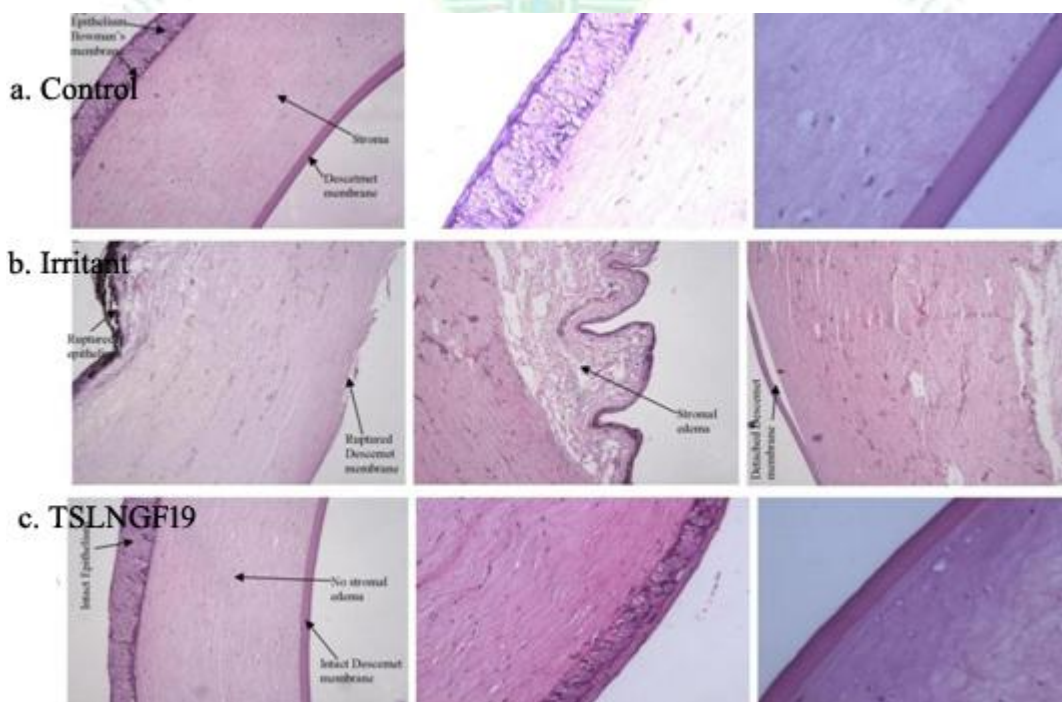

Figure 7: Images of histopathology of the isolated cornea after treatment with (a) Control (b) Irritant and (c) TSLNGF19

Stability studies:

The prepared tropicamide loaded solid lipid nanoparticles enriched in chitosan gels were subjected to stability study. At the regular intervals, the stored samples were evaluated for mean gelling strength, Bioadhesive force, viscosity, spreadability coefficient, pH. The results were summarized in table 11 to 12.

Table 11: Mean particle size, zeta potential and drug content estimation of prepared Tropicamide loaded solid lipid nanoparticles enriched in chitosan gels subjected to long-term stability as per ICH guidelines (25°C ± 2°C / 60% RH ± 5% RH)

Test duration (Months)	Physical change	Gelling strength (sec)	Bioadhesive force* (dynes/cm^2)	Viscosity* (cp)	Spreadiability Coefficient* (gm/sec)
0	No change	148	15.23±1.22	2519±1.15	13.3±1.21
3	No change	148	15.33±1.36	2519±1.17	13.1±1.25
6	No change	148	15.57±1.41	2520±1.16	13.6±1.24
9	No change	149	15.55±1.52	2520±1.24	13.7±1.23
12	No change	149	15.81±1.27	2521±1.27	13.8±1.25

The values are expressed as mean ± SD; n=3;

Table 12: Mean particle size, zeta potential and drug content estimation of prepared Tropicamide loaded solid lipid nanoparticles enriched in chitosan gels subjected to accelerated stability as per ICH guidelines (40°C ± 2°C / 75% RH ± 5% RH)

Test duration (Months)	Physical change	Gelling strength (sec)	Bioadhesive force* (dynes/cm^2)	Viscosity* (cp)	SpreadiabilityCoefficient* (gm/sec)
0	No change	148	15.23±1.22	2519±1.15	13.3±1.21
3	No change	148	15.34±1.40	2513±1.20	13.7±1.26
6	No change	149	16.06 ± 0.12	2622±0.12	14.1±0.24

The values are expressed as mean ± SD; n=3;

CONCLUSION

The present study concludes that Tropicamide can be entrapped in the form of solid lipid nanoparticles using chitosan polymer to achieve sustained release action. The mucoadhesive property of chitosan and sustain release action of solid lipid nanoparticles may contribute to enhance corneal residence time of Tropicamide by formulating its eye drop. The SLN enriched in chitosan gels pH, spreadability coefficient, bioadhesive force & viscosity. The invitro diffusion release studies carried out at 12 hrs TSLNGF19 shows the 79.2 ± 0.32. The ex vivo permeation studies for optimized formulation the increased drug permeation and corneal accumulation. In vitro corneal permeation profile of tropicamide loaded SLN from the chitosan gels and commercial eye drop solution (Tropicacyl) across the isolated porcine cornea. It can be seen that permeation characteristics of tropicamide-loaded chitosan gels are comparable to that of conventional commercial preparation (Tropicacyl). The ocular tolerance studies performed with HETCAM assay, corneal hydration study, histopathological studies. The stability studies of chitosan gels for long-term stability & accelerated stability there is no changes in optimized formulation.

Acknowledgment

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