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R E S E A R C H A R T I C L E

Formulation and Evaluation of Chitosan Gels Enriched with Tropicamide Loaded Solid Lipid Nanoparticles for Ocular Drug Delivery

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A B S T R A C T

The aim of present work Formulation & Evaluation of chitosan gels enriched with tropicamide loaded solid lipid nanoparticles for ocular drug delivery. The preparation of tropicamide loaded solid lipid nanoparticles by cold homogenization method. The interaction between drug, lipids & polymer by performing with FTIR there is no incompatibility. The morphological particles were carried out by TEM in solid lipid nanoparticle formulation. The average particle size was ranges from 212.7 ± 10.27 nm to 530.3±6.14nm. The zeta potential ranges from -0.26 ± 1.2 mV to 6.16 ± 0.9 mV. The entrapment efficiency of solid lipid nanoparticles contains free tropicamide were ranges from 78.16 % to 94.37 %. The polydispersity index of Tropicamide loaded solid lipid nanoparticles was ranges from 0.612 ± 0.12 to 0.916 ±0.24. The drug content of Tropicamide loaded solid lipid nanoparticles was ranges from 0.121mg/ml to 0.916 mg/ml. The prepared chitosan gels enriched in solid lipid nanoparticles, the pH of the formulations was in the range of 6.4 to 6.8. The gelling strength was ranges from 65 ± 1 sec to 124 ± 2 sec. The Bio adhesive force was ranges from 10.15 ±1.20 dynes/cm^2 to 18.19 ± 1.31 dynes/cm^2. The viscosity studies were ranges 412 ± 1.25 cps to 623 ± 1.48 cps.. Among the sixteen formulation *In vitro* diffusion studies of TSLNGF10 shows the 98%, it follows the zero order kinetics. The ocular irritancy studies among all the formulation it indicates the TSLNGF10 was shows the non-irritant with excellent ocular tolerance, does not find out redness, there is no swelling towards watering of the eye, it may shows the excellent mydriatic activity.

Keywords: Solid Lipid Nanoparticles, Gels, Chitosan, in vitro diffusion studies, Ocular drug delivery.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Solid lipid Nanoparticles are introduced as a carrier system for poorly water soluble drug and cosmetic active drug. Colloidal particles ranging in size between 10 and 1000 nm are known as nanoparticles [1]. The release of drug continuously at a controlled rate for a period of several days. In 1990s the Solid lipid nanoparticles was introduced for considering as being promising drug delivery system. The Gels forming system are those when exposed to physiological condition will shift into a gel phase. In the early 1980s this new concept was suggested for the first time [2]. Gel formation occurs through the cross linking of polymer chains that can be achieved by covalent bond or non covalent bond formation. The aim of present work Formulation & Evaluation of chitosan gels enriched with Tropicamide loaded solid lipid nanoparticles for ocular drug delivery. The SLN which dissolved rapidly in chitosan gels introduced in the Ocular drug delivery system of mydriatic activity. Muscarinic antagonists having the tropicamide mainly used as mydriatic & cycloplegia. The cholinergic stimulation of pupil dilation, ciliary muscle paralysis and block the sphincter muscle in the iris, receptors in the muscles of the eye. Since then they have attracted scientists as a widely used, stable, non-toxic, and reliable particulate drug delivery vehicle [3,4]. SLNs can be an effective ocular drug delivery system by enhancing corneal absorption, improving ocular bioavailability, prolonging the ocular retention time, and providing a sustained drug release profile. SLNs control and stop the degradation process of sensitive lipophilic materials and drugs due to the fact that the mobility of their active agents is hindered in solid state when compared to liquid state. SLNs lipophilic properties should effectively cross the epithelium. Moreover, the epithelium is slightly negatively charged, hence cationic SLNs can increase the corneal residence time of the drug and increase its absorption levels

2. Materials and Methods

The pure drug of tropicamide was obtained as a gift sample Ashford Laboratories Ltd, China Macau, lipid Dynasan 118 was procure from S.D. Fine Chemical Ltd, Mumbai, Glycerine trimyristate, polycaprolactone procure from sigma Aldrich, Phosphatidylcholine procure from Drugs India. Polysorbate 80 acquires from Micro fine chemicals in Erode, India and Polymer like Poloxamer 118 acquire from Merck Life Science Private Limited Mumbai. All other reagents used were of analytical grade.

Methodology:

Preparation of Tropicamide loaded solid lipid nanoparticles: The Accurate weight of Dynasan 118, Glycerin trimyristate and phosphatidylcholine were dissolved in 10 ml of chloroform and methanol (1:1). Tropicamide, soyabean lecithin & polycaprolactone was kept in a mixture [5]. Organic solvents were evaporated and a physical conjugate of embedded with drug & lipid layer was performed by rota evaporator. In separate polysorbate 80 & poloxamer was heated to a same temperature as the molten lipid phase. Hot aqueous phase was transfer in to the molten lipid phase and in homogenization to maintain at 8000 rpm, temperature at 5^oC for 1-5 minutes. The nanoparticles were freeze dried at -80^o C for 4 h followed by lyophilisation for 24 hours using mannitol (1%, w/v) as cryoprotectant, Finally you can obtained nanoparticles. Formulation design shown in table 01 & 02.

Preparation of solid lipid nanoparticles enriched in gels: The optimized Tropicamide loaded SLN assimilate in prepared gels using chitosan (1% w/v) and glycerol (5% v/v) as hydrating agent [6]. For the preparation of chitosan gels, was kept in a distilled water containing glycerol (5%). Finally add preservatives to store for long time. The gels are composed of chitosan were arranged up to the pH 6.5 to neutralize the chitosan. The gel formulations were designated as TSLNGF1 to TSLNGF16.

Compatibility studies of drug, the lipid and polymers: The FTIR spectrum of pure form of tropicamide, physical mixtures are carried out with FT-IR.

Morphological studies: The following methods are used to determine over all shape and morphology of solid lipid nanoparticles performed with Transmission electron microscopy [7].

Evaluation parameters for Tropicamide Loaded Solid Lipid Nanoparticles:

Particle size analysis: The SLN was inflexible by using Malvern particle size analyzer [8]. The Polydispersity index (Đ) is a measurement of distribution of molecular mass in a given polymer. PDI of a polymer is calculated as follows

PDI= 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^oc by keeping the electric field strength around 23.2 V/cm [9].

Entrapment efficiency:

Tropicamide encapsulated in the nanoparticles, the prepared SLN dissolved in distilled water was kept in centrifuged up to 14,000 rpm for 40 mints at 10^oC. The sample was observed in UV Visible spectrophotometer at lambda max 254 nm.

Drug content:

Accurately weight 10 mg of 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 whattman filter paper [10]. The volumetric flask make up to the mark by methanol to give concentration of 1000µg/ml for tropicamide. The volumetric flask to give concentration of 10µg/ml and then absorbance was measured at 254 nm.

Evaluation of solid lipid nanoparticles Loaded Gels: The SLNs were encapsulating into the 1% Chitosan gels. The Formulated SLN enriched in Chitosan gel characterization as listed below.

Physical Evaluations:

Visual appearance & pH: The pH of tropicamide loaded SLN incorporated gels formulations were measured in pH paper [11].

Gelling strength:

In 100 ml measuring cylinder containing 50 gm of 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 [12].

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 inverted position while 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 [13].

Viscosity: The prepared Gels enriched in SLN were carried out by using Brookfield viscometer [14].

Spread ability coefficient:

The gels was 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 [15]. The time in which the upper glass slide moves over to the lower plate was taken as measure of spread ability (S).

$$S = ML/T$$

Where

M = weight tide to upper slide (g)

L = length moved on the glass slide (cm)

T = time taken (sec)

***In vitro* anti microbial activity:**

There should be intimate control between the test organism and substance to be evaluated. Microorganism should be provided with the required conditions for growth. The paper disc diffusion method is one of the methods that may be used for determining the relative effectiveness of the anti-microbial activity. The anti-microbial activity of the prepared gels was screened against the following bacteria [16]. The gram positive organisms like *Staphylococcus aureus.* All the Petri dishes were incubated for 24 hr at the required temperatures, i.e., 37°C for bacteria. After incubation the diameters of the circular inhibition zones were measured and from these values Minimum Inhibitory Concentration and biological activities were calculated.

***In vitro* Franz's diffusion:** *In-vitro* franz's diffusion drug release can be performed in a Franz diffusion cell. The formulation was placed in an acceptor compartment and is quantified using a suitable method of determination Such as UV Visible spectroscopy tropicamide ƛ max 254nm. The sink condition is usually maintained by replacing the volume of aliquots taken by similar volumes of the buffer to resemble constant clearance of drugs from their physiological site of action the drug release [17].

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.

Zero order kinetics:

It describes the system in which the drug release rate is independent of its concentration [18].

$$Q_{ts} = Q_0 + K_0 t$$

Where, Qt = amount of drug dissolved in time "t"

Q0 = initial amount of drug in the solution

K_0 = Zero order release constant

First order kinetics:

It describes the drug release from the systems in which the release rate is concentration dependent [19].

$$\text{Log}Q_t = \text{Log}\, Q_0 + K_1 t/2.303$$

Where, Q_t = amount of drug release in time "t"

Q_0 = initial amount of drug in the solution

K_1 = first order release constant

Higuchi model:

It describes the fraction of drug release from a matrix is proportional to square root of time [20].

$$Mt/M\alpha = K_H t^{1/2}$$

Where, Mt & Mα = cumulative amounts of drug release at time "t" and infinite time

K_H = Higuchi dissolution constant reflection formulation characteristics.

Korsemayer-Peppas model (Power law): The powerful law describes that the fractional amount of drug release is exponentially related to the release time and adequately describes the release of drug from slabs, cylinders and spheres [21].

$$Mt/M\alpha = Kt^n$$

$$\text{Log}\,[Mt/M\alpha] = \text{Log}\, K + n \log t$$

Where, Mt & Mα = cumulative amounts of rug release at time "t" and infinite time.

K = constant incorporating structural and geometrical characteristics of CR device.

n = diffusion release exponent indicative of the mechanism of drug release for drug dissolution.

Accelerated stability studies:

The sixteen formulations were filled in each glass vials, closed with rubber closures and sealed with an aluminium caps [22]. The vials contain optimized formulation were kept in stability chamber, maintained at 40 ± 2°C and 75 ± 5 % RH. Samples were withdrawn monthly and estimated the viscosity, Gelling strength and pH. The vials contain optimized formulation were kept in stability chamber, maintained at 40 ± 2°C and 75 ± 5 % RH for 2nd month, 4th Months, 6th months. Samples were withdrawn monthly and estimated the viscosity, Gelling strength and pH, bio adhesive force & spreadability coefficient.

Ocular irritation studies: Ocular irritation study was performed for selected formulations by using male albino rabbits, each weighing about 2 to 3 kg. 1 gm in each formulation was instilled in to cul-de-sac twice a day for a period of 14 days [23]. The rabbits were monitored

periodically for redness, swelling, watering of the eye and mydriatic activity.

3. Results and 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 04 & interpretation was display in table 01.

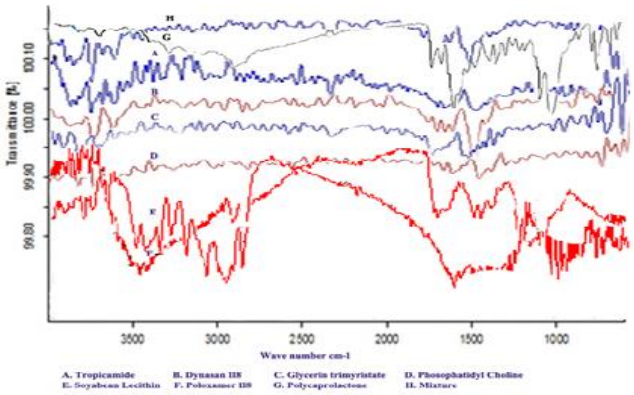

Figure 1: FT-IR spectrum of Drug, Lipids, Excipients & Mixture of compounds

Morphological characters of Tropicamide Loaded SLN:

Transmission electron microscopy:

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 02.

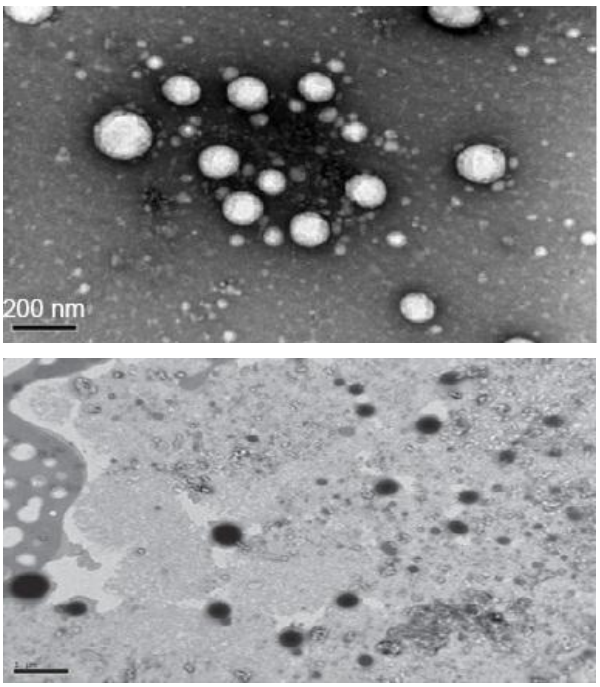

Figure 02: Tropicamide loaded Solid lipid nanoparticles best formulation TSLNF10

Characterization of tropicamide loaded solid lipid nanoparticles

Particle size analysis:

The solid lipid nanoparticles were to settle by PCS using Zetasizer Nano-Series. The average particle size was ranges from 212.7 ± 10.27 nm to 530.3±6.14nm. All these observations values are display in Table 06.

Zeta potential:

The solid lipid nanoparticles were estimated by Malvern zetasizer. The zeta potential ranges from -0.26 ± 1.2 mV to 6.16 ± 0.9 mV. All these observations values are display in Table 06.

The entrapment efficiency: The solid lipid nanoparticles contain free tropicamide were ranges from 78.16 % to 94.37 %. All these observations values are display in Table 06.

Polydispersity Index:

Tropicamide loaded solid lipid nanoparticles was ranges from 0.612 ± 0.12 to 0.916 ±0.24. All these observations values are display in Table 06.

Drug content:

Tropicamide loaded solid lipid nanoparticles was ranges from 0.121mg/ml to 0.916 mg/ml. All these observations values are display in Table 06.

Evaluation of Chitosan Gels enriched in Tropicamide loaded solid lipid nanoparticles:

The tropicamide SLNs were incorporated into the Chitosan gels at the concentration of 1%. The Evaluated Solid lipid nanoparticles enriched in Chitosan gel characterization as displayed below.

Physical Evaluations:

Visual appearance and pH:

The pH for all formulations gels enriched in Tropicamide loaded were found to be transparent white colour and semi solid consistency. The pH of the formulations was in the range of 6.4 to 6.8.

Gelling strength:

The gelling strength of gels enriched in Tropicamide loaded solid lipid nanoparticles loaded gels for all formulations was ranges from 65 ± 1 sec to 124 ± 2 sec. All these observations values are display in Table 07.

Bioadhesive force:

The Bio adhesive force of all formulations was ranges from 10.15 ±1.20 dynes/cm^2 to 18.19 ± 1.31 dynes/cm^2. All these observations are display in Table 07.

Viscosity:

The rheological studies of all formulations were ranges from 412 ± 1.25 cps to 623 ± 1.48 cps. All the values are display in Table 07.

Spreadability coefficient: The spreadability coefficient of all the formulation was ranges from 08gms/sec to 18gms/sec. All these observations are listed in Table 07.

***In vitro* anti microbial activity:** This was determined by the agar diffusion test employing "cup plate technique". Sterile solutions of Tropicamide (standard solution) and the developed formulations were diluted at different concentration (test solutions) these solutions were poured in to cups bored into sterile nutrient agar previously seeded with test organisms (*Staphylococcus aureus*). After allowing diffusion of the solutions for 2 hours, the Agar

plates were incubated at 37°C for 24hrs. The gram-positive organisms like *Staphylococcus aureus.* The zone of inhibition of *S.aureus* at 100 µg/ml of all formulations ranges from 17mm to 25mm. The 200 µg/ml of all formulations ranges from 21mm to 29mm. The pure form of tropicamide 100 µg/ml was 23mm & 200 µg/ml ranges from 27mm. The control in both 100 µg/ml & 200µg/ml containing 21mm & 26mm.

***In-vitro* diffusion release studies:**

The all Formulations were subjected to *in-vitro* drug release studies. The release studies were performed in a phosphate buffer of pH 7.4, suspending the formulation with 15mg equivalent of the drug. For all formulations respectively, in a span of 6hrs of study. The results revealed that, about 92%, 84%, 90%, 91%, 87%, 81%, 85%, 91%, 93 %, 98%, 89%, 87%, 97%, 92%, 95% and 96% of drug release Values are display in table 9 & 10. The optimized formulations were subjected in the data analysis. The average of 16 optimized formulations was subjected to data analysis and it was found to be first order drug release. The values are display in Table 9, 10 & Figures 3.

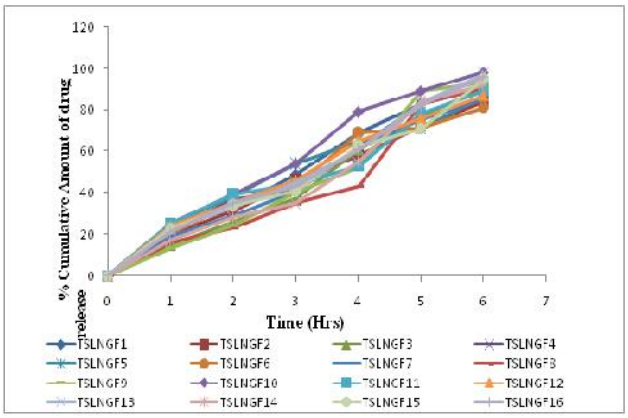

Figure 3: *In-Vitro* Franz's diffusion studies of Chitosan Gels enriched in Tropicamide loaded Solid lipid Nanoparticles TSLNGF1 to TSLNGF16

Accelerated stability studies

In accelerated stability testing chamber maintained at 40 ± 2°C and 75 ± 5 % RH for two months, four months and six months, the optimized formulation TSLNGF10 during at the end of the stability study, the selected formulations did not undergo any chemical Changes/interaction and remained stable during the study period and showed almost similar in viscosity, gelling strength, pH, Bioadhesive force & spreadability coefficient as shown in Table 14 & 15.

Ocular irritancy studies:

The observations of ocular irritancy studies for all the formulation was shows the non-irritant with excellent ocular tolerance, does not find out redness, there is no swelling towards watering of the eye, TSLNGF10 it may shows the excellent mydriatic activity.

4. Conclusion

Finally I concluded that chitosan gels enriches in solid lipid particles the morphological characters of Solid lipid nanoparticles were performed by Transmission electron Microscopy. The particle size of solid lipid nanoparticles of TSLNF10 was found to be 212.7±1.27 nm. The zeta potential of solid lipid nanoparticles of TSLNF10 was found to be -0.261±1.2 mV, The drug content of solid lipid nanoparticles of TSLNF10 was found to be 0.916 mg/ml, The polydispersity index of solid lipid nanoparticles of TSLNF10 was found to be 0.590 ± 0.49, The Entrapment efficiency of solid lipid nanoparticles of TSLNF10 was found to be 96.18%, Solid lipid nanoparticles enriched in Chitosan gel, pH TSLNGF10was found to be 6.7, The gelling strength of TSLNGF10 was found to be 105±2 sec, The bioadhesive force of TSLNGF10 was found to be 14.89±1.34 dynes/cm^2, The viscosity of formulation TSLNGF10 612±1.48 cp. The spreadability coefficient of TSLNGF10 was found to be 13gms/sec. The *in vitro* anti microbial activity of zone of inhibition performed with *S.aureus* 100 µg/ml 23mm & 200 µg/ml 27mm. *In Vitro* Franz's diffusion studies of Chitosan Gels enriched in Tropicamide loaded Solid lipid Nanoparticles of TSLNGF10 was found to be 98%. It follows zero order kinetics. The accelerated stability studies performed with all parameters do not change the values. The Ocular irritation studies of there are no redness, swellings, watering of the eye & it shows the mydriatic activity.

5. Acknowledgement

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Table 01: Formulation Design of Tropicamide loaded solid lipid nanoparticles TSLNF1 to TSLNF8

Ingredients	Formulation Codes							
	TSL NF1	TSL NF2	TSL NF3	TSL NF4	TSL NF5	TSL NF6	TSL NF7	TSLN F8
Tropicamide (mg)	15	15	15	15	15	15	15	15
Dynosan 118 (gms)	1	1	1	1	1.5	1.5	1.5	1.5
Glyceryl trimyristate (gms)	0.5	0.5	0.5	0.5	1	1	1	1
Phosphatidylcholine (gms)	-	-	-	-	-	-	-	-
Polycaprolactone (gms)	0.5	1	1.5	2	2.5	0.5	1	1.5
Soyabean lecithin (gms)	1	1.5	2	2.5	1	1.5	2	2.5
Polysorbate 80 (gms)	3	3	3	3	3	3	3	3

Poloxamer 188 (gms)	1	1	1	1	1	1	1	1

Table 02: Formulation Design of Tropicamide loaded solid lipid nanoparticles TSLNF09 to TSLNF16

Ingredients	**Formulation Codes**							
	TSLNF9	**TSLNF10**	**TSLNF11**	**TSLNF12**	**TSLNF13**	**TSLNF14**	**TSLNF15**	**TSLNF16**
Tropicamide (mg)	15	15	15	15	15	15	15	15
Dynosan 118 (gms)	2	2	2	2	2.5	2.5	2.5	2.5
Glyceryl trimyristate (gms)	1.5	1.5	1.5	1.5	0.5	2	2	2
Phosphatidyl choline (gms)	-	-	1	1.5	2	2.5	3	1
Polycaprolactone (gms)	2	2.5	-	-	-	-	-	-
Soyabean lecithin (gms)	1	1.5	2	2.5	1	1.5	2	2.5
Polysorbate 80 (gms)	3	3	3	3	3	3	3	3
Poloxamer 188 (gms)	1	1	1	1	1	1	1	1

Table 03: FTIR spectrum of 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
Dynosan 118	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
Glycerin trimyristate	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
Phosophatidyl Choline	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
Soyabean Lecithin	3618.64	3000-3700	O-H stretch	Alkenes, aromatic
	3583.92	3000-3700	O-H stretch	Alkenes, aromatic
	3217.42	3010-3300	N-H stretch	Aromatic ring
	2893.37	2850-2960	C-H stretch	Alkanes
	2225.96	2100-2660	C=C stretch	Alkynes
Poloxamer 188	3414.17	3010-3300	N-H stretch	Aromatic ring
	3128.69	3010-3300	N-H stretch	Aromatic ring
Polycaprolactone	2960-2850	2928.92	C-H stretch	Alkanes
	3700-3500	3679.72	O-H stretch	Free OH alcohols
	1150-1070	1091.08	C-O stretch	Ethers
	1300-800	1297.40	C-C stretch	Alkenes
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

Table 04: Evaluation parameters of tropicamide loaded solid lipid nano particles TSLNGF1 to TSLNGF16

Formulation code	Average Particle size (nm)	Zeta Potential (mV)	Drug Content (mg/ml)	Poly Disparity Index	Entrapment efficiency (%)
TSLNF1	421.1±4.12	-316±1.4	0.121	0.841 ± 0.16	84.12
TSLNF2	530.3±6.14	-1.17±1.8	0.219	0.612 ± 0.12	82.67
TSLNF3	311.3±1.81	-4.21±1.3	0.381	0.576 ± 0.69	83.53
TSLNF4	216.5±9.51	-5.13±1.9	0.512	0.719 ± 0.43	89.44
TSLNF5	316.2±5.19	-3.29±1.6	0.716	0.813 ± 0.59	90.16
TSLNF6	420.6±5.21	-2.16±1.5	0.629	0.630 ± 0.29	94.37
TSLNF7	412.5±3.18	-2.89±0.9	0.489	0.789 ±0.50	85.13
TSLNF8	317.6±7.12	-7.6±1.6	0.812	0.529 ± 0.61	79.28
TSLNF9	214.9±2.17	-6.16±2.1	0.324	0.631 ±0.31	78.16
TSLNF10	**212.7±1.27**	**-0.261±1.2**	**0.916**	**0.590 ± 0.49**	**96.18**
TSLNF11	481.1 ±5.21	-3.61 ± 1.2	0.446	0.916 ±0.24	89.19
TSLNF12	521.5 ±6.31	-1.76 ± 1.9	0.517	0.816 ± 0.41	83.12
TSLNF13	316.5 ±2.89	-2.16 ± 1.6	0.627	0.613 ± 0.13	85.86
TSLNF14	489.6 ±4.16	-0.14 ± 2.6	0.357	0.718 ± 0.42	86.12
TSLNF15	319.7 ±4.12	-4.16 ± 2.1	0.565	0.826 ± 0.36	88.13
TSLNF16	478.6 ±3.27	-5.16 ± 1.6	0.612	0.976 ± 1.16	87.37

Table 05: Evaluation parameters of Chitosan gels enriched in Tropicamide loaded solid lipid nanoparticles

Formulation Code	Viscosity (cp)	Gelling Strength (sec)	Bio Adhesive force (dynes/cm^2)	Spreadability co-efficient (gms/sec)	pH
TSLNGF1	418± 1.08	65±1	10.15±1.20	08	6.5
TSLNGF2	428±1.31	69±4	12.16±1.01	11	6.6
TSLNGF3	435 ±1.54	70±3	13.15±1.15	12	6.7
TSLNGF4	500±1.23	82±5	15.10±1.03	13	6.4
TSLNGF5	462±1.82	68±2	11.16±1.12	09	6.6
TSLNGF6	481±1.73	73±5	13.17±1.24	12	6.5
TSLNGF7	495±1.52	79±3	14.16±1.41	14	6.4
TSLNGF8	512±1.61	86±3	16.18±1.12	15	6.5
TSLNGF9	523±1.42	98±2	12.18±1.12	10	6.6
TSLNGF10	**612±1.48**	**105±2**	**14.89±1.34**	**13**	**6.7**
TSLNGF11	418±1.16	124±2	15.12±1.42	15	6.6
TSLNGF12	485±1.23	112±3	16.89±1.61	16	6.5
TSLNGF13	412±1.25	101±3	13.89±1.21	11	6.7
TSLNGF14	487±1.81	121±4	16.21±1.81	14	6.8
TSLNGF15	523±1.54	97±2	17.18±1.41	16	6.5
TSLNGF16	623±1.48	131±3	18.19±1.31	18	6.6

Table 06: *In-Vitro* Franz's diffusion studies of Chitosan Gels enriched in Tropicamide loaded Solid lipid Nanoparticles TSLNGF1 to TSLNGF8

Time (hrs)	TSLN GF1	TSLN GF2	TSLN GF3	TSLN GF4	TSLN GF5	TSLN GF6	TSLN GF7	TSLN GF8
1	24±0.6	20±0.9	15±0.8	22±0.5	25±0.1	20±0.2	19±0.2	16±0.1
2	32±1.2	31±0.8	26±0.6	37±0.7	39±0.2	35±0.1	29±0.6	23±0.4
3	49±0.9	46±0.6	37±0.9	41±0.2	54±0.5	43±0.2	41±0.3	35±0.6
4	69±1.1	58±1.2	61±1.3	62±0.6	65±0.1	69±0.4	62±0.4	43±0.5
5	83±0.3	72±1.6	77±1.3	83±0.3	71±0.5	71±0.4	75±0.5	83±0.5
6	92±0.6	84±1.5	90±1.6	91±0.5	87±0.6	81±0.2	85±0.6	91±0.6

Table 7: *In-Vitro* Franz's diffusion studies of Chitosan Gels enriched in Tropicamide loaded Solid lipid Nanoparticles TSLNGF9 to TSLNGF16

Time (hrs)	TSLN GF9	TSLN GF10	TSLNG F11	TSLN GF12	TSLNG F13	TSLN GF14	TSLNG F15	TSLNGF16
1	13±0.2	25±0.7	25±0.6	24±1.0	23±1.2	17±0.3	23±0.2	21±0.4
2	24±0.3	39±0.4	39±0.3	35±0.5	33±0.4	28±0.5	34±0.8	35±0.5
3	40±0.4	54±1.5	44±0.5	46±0.2	45±0.5	35±0.6	41±0.4	43±0.9

4	52±0.5	79±1.7	53±0.4	65±0.3	61±0.9	55±0.7	63±0.2	61±0.6
5	89±0.5	89±1.2	78±0.3	76±0.4	83±0.3	84±0.5	71±0.3	82±0.5
6	93±0.4	98±0.9	89±0.2	87±0.3	97±0.4	92±0.5	95±0.6	96±0.2

Table 8: Drug Release Kinetics of Chitosan Gels loaded in Solid lipid Nanoparticles (TSLNGF1 to TSLNGF8) Formulations

Order of Process		**Formulation code**							
		TSLNGF1	**TSLNGF2**	**TSLNGF3**	**TSLNGF4**	**TSLNGF5**	**TSLNGF6**	**TSLNGF7**	**TSLNGF8**
Zero order	R^2	0.987	0.996	0.989	0.981	0.968	0.971	0.007	0.030
	Slope	7.928	7.035	7.928	7.785	6.928	6.964	1.214	2.25
First order	R^2	0.974	0.979	0.97	0.963	0.925	0.701	0.077	0.005
	Slope	0.044	0.035	0.041	0.042	0.033	0.215	0.076	0.008
Higuchi	R^2	0.981	0.991	0.954	0.967	0.826	0.981	0.349	0.032
	Slope	29.32	26.04	28.90	28.68	28.91	25.16	21.42	18.35
Hixon	R^2	0.853	0.980	0.980	0.927	0.879	0.922	0.445	0.461
	Slope	2.213	0.776	1.993	1.940	1.849	1.887	1.492	1.497
Korsmeyer	R^2	0.953	0.982	0.840	0.918	0.873	0.822	0.815	0.961
	Slope	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 9: Drug Release Kinetics of Chitosan Gels loaded in Solid lipid Nanoparticles (TSLNGF9 to TSLNGF16) Formulations

Order of Process		**Formulation code**							
		TSLNGF9	**TSLNGF 10**	**TSLNGF11**	**TSLNGF12**	**TSLNGF13**	**TSLNGF14**	**TSLNGF15**	**TSLNGF16**
Zero order	R^2	0.134	**0.978**	0.964	0.989	0.990	0.972	0.981	0.991
	Slope	3.892	**8.464**	7.107	7.357	8.142	8.035	7.571	8.123
First order	R^2	0.020	**0.966**	0.942	0.969	0.972	0.947	0.96	0.973
	Slope	0.010	**0.047**	0.037	0.038	0.0043	0.044	0.034	0.042
Higuchi	R^2	0.136	**0.987**	0.964	0.989	0.990	0.972	0.981	0.991
	Slope	14.55	**31.54**	7.107	7.357	8.142	8.032	7.571	8
Hixon	R^2	0.530	**0.917**	0.674	0.041	0.735	0.814	0.696	0.740
	Slope	1.566	**1.994**	0.211	36.16	0.226	0.237	0.212	0.226
Korsmeyer	R^2	0.730	**0.817**	0.687	0.787	0.892	0.715	0.689	0.703
	n	0.757	**0.737**	0.810	0.851	0.758	0.725	0.862	0.978
Mechanism		Non-Fickian	**Non-Fickian**	Non-Fickian	Non-Fickian	Non-Fickian	Non-Fickian	Non-Fickian	Zero order

Table 10: Accelerated stability studies chitosan gels enriches in Tropicamide loaded solid lipid particles Viscosity, Gelling strength, & pH of TSLNGF10

Formulation Code	**Viscosity (cp)**			**Gelling strength(sec)**			**pH**		
	Two months	**four months**	**six months**	**Two months**	**four months**	**six months**	**Two months**	**four months**	**six months**
TSLNGF10	612 ± 1.48	613 ± 1.49	613 ± 1.48	105 ± 2	104 ± 2	105 ± 2	6.7	6.8	6.8

Table 11: Accelerated stability studies chitosan gels enriches in solid lipid particles Bioadhesive force & Spreadability coefficient of TSLNGF10

Formulation Code	**Bio Adhesive force (dynes/cm^2)**			**Spreadability co-efficient (gms/sec)**		
	Two months	**four months**	**six months**	**Two months**	**four months**	**six months**
TSLNGF10	14.89±1.34	14.80±1.34	14.70±1.34	13	12.9	12.8

6. References

[1] Rainer H. Muller, Jana Pardeike, Aiman Hommoss, International Journal of Pharmaceutics 2009; 366, 170–84.

[2] Michael D. Triplett, Enabling Solid Lipid Nanoparticle Drug Delivery Technology by Investigating Improved Production Techniques, 2004.

[3] Triplett II M.D and Rathman J.F., Journal of Nanoparticle Research, 2009, 11:3, 601-614.

[4] Bhaskar K. Anbu J Ravichandiran V. Venkateswarlu V. and Rao Y.M, Lipids in health and disease, 2009, 6.1, 434 - 444.

[5] W. Mehnert, K. Mader, Solid lipid nanoparticles, production, characterization and applications, Adv. Drug Deliv. Rev. 47 2001, 165-196.

[6] R.H. Muller, M. Radtke, S.A. Wissing, Solid lipid nanoparticles and nanostructured lipid carriers in cosmetic and dermatological preparations,Adv. Drug Deliv. Rev. 2008 (1) 88-96.

[7] R.H. Muller, K. Mader, S. Gohla, Solid lipid nanoparticles for controlled drug delivery a review of the state of the art, Eur. J. Pharm. Biopharm. 50, 2000, 161– 177.

[8] Jitendra V. Shinde, Kailas K. Mali, Remeth J. Dias, Vijay D. Havaldar, Niranjan S.Mahajan , In situ Mucoadhesive Nasal Gels of Metoclopramide Hydrochloride : Preformulation chandra mohan, jagan mohan kandukuri, venkatesham allenki., preparation and Evaluation of In-Situ-Gels For Ocular Drug Delivery, Journal Of Pharmacy Research 2009, 2(6),1089-1094

[9] Wei Yang, Jay I. Peters, Robert O. Williams Iii, Nhaled Nanoparticles A Current Review, International Journal of Pharmaceutics 356, 2008, 239–247

[10] Teo Johnson, Jaya Raja Kumar, Hiew Mei Yi,Yeap Su Yong,Kasturi. A, Factorial Design Of New Surfactant Hibiscus Rosasinensis leaf For Nanoparticles: Glimepiride Vs Hibiscus, Rapports De Pharmacie 2015,1(2):73-80

[11] Prasanth V, Della Grace Thomas Parambi, Shashi Ranjan, Formulation And Evaluation Of In Situ Ocular Gel Of Levofloxacin, Journal Of Drug Delivery & Therapeutics. 2017, (5):68-73

[12] Laith Hamza Samein, Preparation And Evaluation Of Nystatin Loaded-Solid-Lipid Nanoparticles For Topical Delivery, International Journal of Pharmacy And Pharmaceutical Sciences, 2014, 6(2): 592-597.

[13] Ankit chokriwal, Madan mohan sharma, Abhijeet singh, Biological synthesis of nanoparticles using bacteria and their applications, Am. J. Pharm Tech Res, 4(6), 2014, 38 -61.

[14] Laith hamza samein, Preparation and evaluation of nystatin loaded-solid-lipid nanoparticles for topical delivery, International Journal of pharmacy and pharmaceutical sciences, 2014, vol 6 suppl 2, 592-597.

[15] Jayachandra Reddy Peddappi Reddigari, Yerikala Ramesh et al.; Chandrasekhar B.Kothapalli, Formulation and Evaluation of in situ gels enriched with tropicamide loaded solid lipid nanoparticles, *International Journal of Research in Pharmaceutical sciences*, 2018; 9(1), 216-225.

[16] Zih rou huang, Shu chiou hua, Yueh lung yang, Jia you fang, Development and evaluation of lipid nanoparticles for camptothecin delivery: a comparison of solid lipid nanoparticles, nanostructured lipid carriers, and lipid emulsion, Acta Pharmacol Sin. 2008, 29 (9): 1094–1102.

[17] Ketkee deshmukh, Purnima amin, Formulation and evaluation of solid lipid nanoparticle based 0.1% soy isoflavone dermal gels, J. Pharm. Biosci. 1, 2013, 7-18.

[18] Umaretiya ghanshyam M, Patel priyal R, Patel Jayvadan K, Formulation and characterization of solid lipid nanoparticles dry powder inhaler containing triamcinolone acetonide , IJRPC 2011, 1(3) ,662-673.

[19] Sonali D labhade, Dipti G Phadtare, Amrutaner, Dimple pandya, R B Saudagar, Formulation and Evaluation of carvedilol loaded solid lipid nanoparticles, international journal of scientific progress and research, 2015,13 (02): 74-78.

[20] Madhushri M, R. S. Thakur, Kiran K Jadhay, Ronak N. Patel, Formulation And Evaluation Of Solid Lipid Nanoparticles Containing Clotrimazole, Am. J. Pharmtech Res. 2012, 2(3), 537-550.

[21] Rahul Nair, K.Vishnu Priya, K.S.Arun Kumar, Sevukarajan M, Formulation And Evaluation Of Solid Lipid Nanoparticles Of Water Soluble Drug: Isoniazid, J. Pharm. Sci. & Res, 2011, 3(5),1256-1264.

[22] R. Cavalli, O. Caputo, M.E. Carlotti, M. Trotta, C. Scarnecchia, M.R. Gasco, Sterilization And Freeze-Drying of Drug-Free And Drug-Loaded Solid Lipid Nanoparticles, Int. J. Pharm. 148 1997, 47-54.

[23] T. Joseph Sunder Raj , Ch. Bharathia, M. Saravana Kumar, Joseph Prabahar, P. Naveen Kumar, Hemant Kumar Sharma, Kalpesh Parikh. Identification, Isolation and Characterization of Process-Related Impurities in Rizatriptan Benzoate. Journal of Pharmaceutical and Biomedical Analysisx 2009; 49, 156-162.