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IN- SITU GEL OF CYCLOSPORINE A – LOADED SOLID LIPID NANOPARTICLES
FOR TOPICAL OCCULAR DELIVERY FOR DRY EYE DISEASE

A Thesis

Presented for the degree of

Master of Science in Pharmaceutical Sciences

With an emphasis in Pharmaceutics and Drug Delivery

The University of Mississippi

By

SAMIR SENAPATI

MAY 2019

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ABSTRACT

Cyclosporine (CSA) is a highly potent immunosuppressant drug and has been used to treat rheumatoid arthritis, psoriasis, organ rejection post-transplant, and chronic dry eye disease. The objective of this investigation was to increase the CSA residence time on the ocular surface using solid lipid nanoparticles (SLNs) and its in-situ gel formulation (CSA-SLN-IG), for treatment of dry eye disease. CSA loaded SLNs (CSA-SLN) were prepared with the homogenization method, using Precirol® ATO 5 as a solid lipid and Tween® 80 and Poloxamer 188 as surfactants. Prepared SLNs were optimized based on particle size, zeta potential (ZP), PDI, assay and stability. CSA-SLN in-situ gels (CSA-SLN-IG) were developed using gellan gum as the gelling agent and rheological properties were evaluated. DSC analysis showed that there was no interaction between lipid and drug. Optimized CSA-SLN showed the particle size, PDI, ZP and assay to be 121.20 ± 5.20 nm, 0.4 ± 0.04 , -24 ± 1 , $95 \pm 0.5\%$ respectively. CSA-SLN was stable at refrigerated and room temperature conditions over one month and no significant changes were observed before and after autoclaving up to one month. CSA-SLN-IG prepared with 0.3% gellan gum showed immediate gel formation with a gel residence time of more than 24 hours, the viscosity of 27.90 ± 2.80 cP and an assay of $85 \pm 0.5\%$. Therefore, CSA-SLN and CSA-SLN-IG could be considered as an alternative approach for dry eye disease.

LIST OF ABBREVIATIONS AND SYMBOLS

ACN Acetonitrile

CSA Cyclosporine A

IPBS Isotonic phosphate buffer saline

SLN Solid Lipid Nanoparticles

PDI Polydispersity index

ZP Zeta Potential

CSA-SLN Cyclosporine solid lipid nanoparticle

CSA-SLN-IG Cyclosporine solid lipid nanoparticle in-situ gel

STF Simulated Tear Fluid

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CHAPTER I

INTRODUCTION

Dry eye disease (DED) or kerratoconjunctivitis sicca (KCS) is a disease which is characterized by unstable tear film which is caused either by tear film deficiency (Aqueous dry eye disease) or excessive evaporation of tear film (evaporative dry eye disease). Site of action for DED is mainly extraocular organs like cornea, lacrimal glands and conjunctiva. Cyclosporine A (CSA) has been known to treat aqueous dry eye disease by acting as a T-cell inhibitor and thus decreasing interleukin-2 production which is known marker of dry DED (Ames and Galor 2015).

CSA is a cyclic undecapeptide with a very high molecular weight of 1202.61 g/mol. Its cyclic structure and lipophilic characteristic gives it a high stability (Kumar, Singhal, and Singh 2001). It is a highly hydrophobic drug with very poor water solubility of about 0.012 mg/mL at 25°C (Mondon et al. 2011). CSA is a powerful immunosuppressive agent and has been used in the treatment of dry eye disease.

Solid lipid nanoparticle (SLNs) is dispersions of solid lipid in water and allows loading of both hydrophilic and hydrophobic active ingredients. They are a colloidal carrier system for controlled drug delivery. The solid matrix protects the active ingredients against chemical

degradation and provides long term stability and flexibility in drug release profile designs. High lipid solubility of the drug means high encapsulation efficiency of the formulation (Patel, Kesharwani, and Gupta 2013). SLNs have been known to enhance the bioavailability of drug when applied topically in ophthalmology (Qaddoumi et al. 2004).

In-situ gels are solutions under normal conditions but undergo sol-to-gel conversion only when they come in contact with certain conditions like pH, temperature or physiological fluids (Agrawal, Das, and Jain 2012). The gel formed helps in control drug release by increasing the residence time of formulation on the cornea, and thus decreasing loss caused by tear flow and blinking. The gelation mechanism depends on the type of gelling agent used.

A CSA formulation available in the market for the treatment of DED is Restasis® (Allergan) which is 0.05%w/v CSA emulsion. Recently another formulation Cequa® (Sun Pharma) has been approved by the US FDA for treatment of DED. It is a nano-micellar formulation with 0.09% w/v drug load.

Various modes of deliveries for CSA have been attempted but each delivery method has a certain drawback. Topical delivery of CSA has been seen advantageous with fewer side effects as compared to systemic delivery(Lallemand et al. 2003). CSA being a highly hydrophobic drug, it has to be dissolved or loaded in certain media for proper delivery into the cornea. As our target for DED is the cornea and not intra ocular region, the primary goal is to sustain the formulation on the cornea for longer period for which SLN would help as it would entrap more drug and at the same time help in sustaining release of drug. Further, its conversion into in-situ gel would further help in sustaining the release.

The objective of the present investigation was to develop and characterize CSA loaded SLN (CSA-SLN) and its corresponding in-situ gel (CSA-SLN-IG) formulation, which would give controlled and sustained release of CSA. Accordingly, CSA-SLN was prepared and optimized based on physico-chemical characteristics. CSA-SLN converted to CSA-SLN-IG by the addition of gellan gum as gelling agent and evaluated for rheological characteristics and stability under several conditions.

CHAPTER II

METHODOLOGY

MATERIALS

Tween® 80, Poloxamer 188 were acquired from Fischer scientific (Hampton, NH, USA). Precirol® ATO 5 (Glyceryldistearate) and Compritol 888® ATO (Glyceryldibehenate) was a generous gift sample from Gattefossé (Paramus, NJ, USA). Gellan gum was purchased from MP Biomedicals, LLC (Santa Ana, CA, USA). Cyclosporine A (CSA) was purchased from Sigma Aldrich (USA). Other chemicals and entities required for the project like HPLC grade solvents, centrifuge tubes, HPLC vials, scintillation vials were obtained from Fischer Scientific (Hampton, NH, USA).

Preparation of Cyclosporine A loaded solid lipid nanoparticle (CSA-SLN) and in-situ gels (CSA-SLN-IG):

PREPARATION OF CSA-SLN:

CSA loaded solid lipid nanoparticle (CSA-SLN) was prepared using homogenization with probe sonication method (Gokce et al. 2008) as per the composition Table 1. It mainly consists of two

phases: the lipid phase and aqueous phase. Lipid phase consists of Precirol® ATO 5 and drug (CSA) whereas the aqueous phase consists of Tween® 80, Poloxamer 188 and double distilled water (mili-Q-water). The lipid phase was melted at 65°C in a glass scintillation vial with continuous stirring at 2000 rpm. Simultaneously, in a separate vial aqueous phase was heated to the same temperature as the lipid phase. On attaining the temperature, the aqueous phase was added to the lipid phase drop by drop with constant stirring to

Table 1: Composition of Cyclosporine a loaded solid lipid nanoparticle and in-situ gel of solid lipid nanoparticles

Formulation Composition (%) w/v	CSA-SLN-C	CSA-SLN-P	CSA-SLN-IG2	CSA-SLN-IG3	CSA-SLN-IG4
Cyclosporine A	0.1	0.1	0.1	0.1	0.1
Compritol® 888 ATO	1.0	-	-	-	-
Precirol ® ATO 5	-	1.0	1.0	1.0	1.0
Tween ® 80	0.5	0.5	0.5	0.5	0.5
Poloxamer	0.5	0.5	0.5	0.5	0.5
Gellan gum	-	-	0.2	0.3	0.4
Water qs	10 ml	10 ml	10 ml	10 ml	10 ml

get a premix. This premix was homogenized at high speed with T25 digital ultra-Turrax® (IKA®, USA) for 5 min at 24000 rpm to form a hot pre-emulsion. The pre-emulsion was placed in a beaker of ice (to reduce charring caused by excess heat) and then taken for probe sonication for 10 minutes at 40% amplitude with pulse 20sec on and 10sec off. The emulsion thus obtained was allowed to cool to room temperature to form CSA-SLN.

Production parameters like different rpm for ultra-Turrax®, and different ratios of lipid and surfactants in the formulation were also tested. Other parameters like mixing time and probe sonication time were selected based on literature(Gokce et al. 2008).

PREPARATION OF CSA-SLN-IG:

CSA –SLN loaded in situ gels were prepared in the same way as CSA-SLN. The only additional step was the addition of a gelling agent (Gellan gum) to the aqueous phase after homogenization. The composition of CSA-SLN-IG3 is given in table 1

HPLC CHROMATOGRAPHIC CONDITIONS:

A previously used high pressure liquid chromatography (HPLC) method was used for analysis of CSA-SLN and CSA-SLN-IG(Xu et al. 2013). HPLC system comprised of Waters® e2695 equipped with column oven, in-line degasser, auto sampler and PDI detector. The column used was C₈Luna® 5 μ (250 mm x 4.6 mm) and was protected by guard column. The mobile phase comprised of Acetonitrile (ACN): water: phosphoric acid (H₃PO₄) in ratio 75:25:0.1v/v/v respectively. The column temperature was set at 60°C using a column oven and the elution flow rate was 1ml/min. The Detection wavelength was set at 210 nm and auto sampler was maintained at 10°C. Injection volume for each sample was 10 μ L.

CHARACTERIZATION OF SLN:

Measurement of Particle size, Zeta potential (ZP) and PDI:

Zeta potential is the overall charge of the nanoformulation and its magnitude (positive or negative) indicates the physical stability of the colloidal system as higher the magnitude, more the repulsion and less is the aggregation (Riddick 1968).

The hydrodynamic radius, PDI and zeta potential(ZP) of the CSA-SLN formulation was determined by photon correlation spectroscopy at 25°C with 173°backscatter detection using a Zetasizer Nano ZS Zen3600 (Malvern Instruments, Inc., MA, USA). Clear disposable folded capillary cells (for particle size and PDI measurement) and special Zeta cells (for zeta potential measurement) were used to place the sample in Zetasizer. Particle size analysis data was evaluated based on the volume distribution for which about 10 µL of the sample was diluted up to 1000µL (100 times dilution) using double distilled (mili-Q) 0.2µm filtered water.

DRUG CONTENT OF CSA-SLN AND CSA-SLN-IG

Assay of CSA-SLN and CSA-SLN-IG was done by using lipid precipitation method. About 100 µL of CSA-SLN and CSA-SLN-IG formulation was extracted using 900 µL of ACN (as CSA is soluble in ACN) and sonicated for 15 minutes (for lipid precipitation). It was then centrifuged for 15 minutes at 13,000 rpm. The supernatant thus obtained was then analyzed for CSA content using the HPLC method mentioned above.

DRUG-EXCIPIENT COMPATIBILITY STUDY - DIFFERENTIAL SCANNING

CALORIMETRY (DSC)

DSC analysis was used to check the crystallinity of the drug as well as the interaction of the drug with other lipid components. DSC of pure CSA, Precirol®ATO5 and physical mixture of Precirol®ATO5 and CSA (CSA: Precirol: 1:1) were obtained by DSC 250 (TA Instruments, USA). Approximately 5 mg of solid samples were hermetically sealed in the T0 aluminum pans and scanned from 0 to 225 °C with a heating rate of 10°C/min with constant nitrogen purging.

Empty pan was used as a reference. The thermograms thus obtained were overlaid and compared for results.

MEASUREMENT OF pH:

The pH of CSA-SLN and CSA-SLN-IG formulations was measured using pH meter (MettlerToledo, USA) after the preparation. The pH meter was calibrated every time using standard pH buffers 4.01, 7.00 and 10.01.

RHEOLOGICAL AND IN VITRO GELLING CHARACTERISTICS OF CSA-SLN-IG:

CSA-SLN-IG was evaluated for viscosity, gelling time and gel residence time.

VISCOSITY:

The viscosity measurement was carried out using a Brookfield cone and plate viscometer (LV-DV-II+ Pro Viscometer, Middleboro, USA). The distance between the cone spindle and sampler cup was maintained to the minimum and the temperature of the system was set to $34 \pm 0.2^\circ\text{C}$. About 500 μL of the sample was placed in a cup and CPE 52 spindle was used at different shear stress to measure the viscosity. Two types of viscosity were calculated: a) viscosity for in-situ gel formulation alone and, b) viscosity of in-situ gel formulation with simulated tear fluid (STF) in the ratio of 50:7 respectively (as it mimics viscosity of formulation after application into eyes). Composition of STF : 0.678% of NaCl (sodium chloride), 0.0084% of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (calcium chloride), 0.138% of KCl (potassium chloride), and 0.218 % of NaHCO_3 (sodium bicarbonate) in deionized water with pH of 7.0 ± 0.2 was maintained (Tatke et al. 2018). Rheocalc® software was used to register the viscosities.

GEL FORMATION TIME:

Gel formation time is the time taken by CSA-SLN-IG to form gel when it comes in contact with STF without any agitation. STF was prepared as mentioned earlier. Gel Formation time of formulation with different concentration of gellan gum was determined by taking 50 μ l of CSA-SLN-IG in 2 ml of STF (in glass vial) maintained at 34 °C temperature. Time taken for the formation of gel without any agitation was noted as gel formation time(Tatke et al. 2018).

GEL RESIDENCE TIME:

Gel residence time is the time the formed gel remains intact in STF with continuous shaking. Fifty microliters of in situ gel formulation were added to 2 mL of STF in a glass vial and were then kept in a shaking water bath, maintained at a temperature of 34°C. Reciprocation rate of the shaker was kept at 100 times per minute. Time for gel breaking was noted(Tatke et al. 2018).

STABILITY STUDIES:

Stability studies of optimized CSA-SLN and CSA-SLN-IG were performed at 4°C, 25°C and 40°C temperature conditions. At specific time intervals, the formulations were evaluated for particle size, ZP, PDI and Assay according to the procedure mentioned earlier.

STERILIZATION:

It is necessary that all ophthalmic formulations be sterile, making it a challenge for formulations. The two available methods are either sterile filtration or autoclave sterilization. Autoclave sterilization was done on both CSA-SLN and CSA-SLN-IG formulations to check if the formulations were stable during the autoclave process. About 5 mL of both CSA-SLN and CSA-SLN-IG formulation was taken in an autoclavable glass vial and placed in the autoclave (3850EL, Tuttnauer) at 121°C for 15 minutes at 15 psi pressure. Post-autoclave the formulations were checked for physical characteristics and compared to the pre-autoclave values.

CHAPTER III

RESULTS AND DISCUSSIONS

Formulation development and optimization of CSA-SLNs:

The CSA-SLN composition was selected as per the reported literature and slightly modified(Gokce et al. 2008).From the results, it has been observed that Tween 80® helps in reduction of aggregation as compared Poloxamer 188® alone(Gokce et al. 2008) and hence, Tween® 80 was added to the development of SLN. Tween® 80 and Poloxamer 188are non-ionic surfactants and are known to be less toxic surfactant as compared to cationic surfactants (Lallemand et al. 2012). Also, Tween® 80 is known to be well-tolerated emulsifier. The concentration of surfactants used in the CSA-SLN formulation development was within the FDA Inactive Ingredient Database (IIG) system.

Lower particle size gives more surface area and better interaction with body fluids because of which lower particle size and lower PDI are preferred. Thus, these parameters were considered of utmost importance while fixing the processing parameters and other composition concentrations. Numerous works of literatures are available on the CSA which helped us select Compritol® 888 ATO and Precirol® ATO 5 as the desired lipids of interest. Out of Compritol ®

and Precirol® ATO5, the later one gave smaller PS and PDI (Table 2) and thus Precirol® ATO5 was selected as the lipid of interest.

Processing parameter like Ultra-Turrax® speed was fixed at 16,000 and 24,000 rpm for 5 minutes for pre-emulsion preparation of the CSA-SLN. From the results, pre-emulsion which were obtained at 24, 000 rpm showed better size reduction as compared to 16,000 rpm (Table 3). Therefore, 24,000rpmfor 5 minutes was selected for preparation of the CSA-SLN. Other processing parameters like probe sonication time and mixing time were optimized with slight modifications from the earlier report.

The effect of lipid and surfactant concentrations on particle size and PDI were studied. From the results, as the concentration of lipid increases and surfactant decreases, increase in particle size and PDI were observed (Table 4). SLN prepared with 1%w/v of Precirol® ATO5, 0.5%w/v of Tween® 80, and0.5% w/v of Poloxamer 188 resulted in lower PS and PDI andwere selected for CSA-SLN development.

Table 1 shows the formulation composition for SLN and in-situ gel. Drug load of 0.1% was fixed as it is the maximum amount of drug load which shows a direct effect on dry eyes reduction,increasing the drug load more than 0.1%w/v does not increase the effect on treating DED.

Table 2: Effect of different lipids (Compritol® 888 ATO and Precirol ® ATO 5) on Physical characteristics of Cyclosporine A solid lipid nanoparticles (mean ± SD, n=3)

Formulation	Size (nm)	PDI	Zeta (mV)	Assay (%)
CSA-SLN-C	631± 112.2	0.5± 0.08	-21± 0.5	90.1 ± 1
CSA-SLN-P	121.20 ± 5.2	0.4 ± 0.04	-24 ± 1	95 ±0.5

Table 3: Effect of Ultra-Turrax® rpm on particle size and PDI (mean ± SD, n=3)

Ultra-Turrax® RPM	Particle size(nm) ± S.D	PDI ± S.D
16000	140.80 ± 5.3	0.56 ± 0.06
24000	121.20 ± 5.2	0.4 ± 0.04

Table 4: Effect of lipid and surfactants concentration on physical characteristics of CSA-SLN (mean ± SD, n=3)

Concentration (%)				Physical characters	
Precirol® ATO5	Poloxamer 188	Tween® 80	CSA	Particle size (nm)	PDI
4.5	0.25	1.5	-	210 ± 4.8	0.5 ± 0.03
1	0.5	0.5	-	119 ± 3.9	0.48 ± 0.02
0.4	0.4	0.2	-	130 ± 4.6	0.60 ± 0.05
1	0.5	0.5	0.1	121.2 ± 5.2	0.4 ± 0.04

Compatibility Studies - DSC

DSC studies were carried out to determine the purity, crystalline behavior and interaction of the drug with physical mixture. DSC thermograms of CSA, Precirol® ATO5 and physical mixture of CSA with Precirol® ATO5 are showed in Figure 1, 2 and 3 respectively. From the results, DSC thermogram of CSA showed an endothermic peak at 130.34°C, and confirming its crystalline nature. A sharp melting point endothermic peak was observed for Precirol® ATO5 at 56.6°C in individual as well as in the physical mixture, whereas a shift in melting point of CSA seen in thermograms of CSA with lipid physical mixture. The shift of CSA in the physical mixture and the short DSC peak for crystalline CSA is in accordance with the literature (Guada et al. 2015). The decrease in intensity of Precirol® ATO5 peak in the physical mixture is likely due

to the decrease in the quantity of Precirol® ATO5 in the physical mixture(1:1:Precirol® ATO5: CSA). Thus no interaction was found between the CSA and Precirol® ATO5 and thus they were compatible for SLN development.

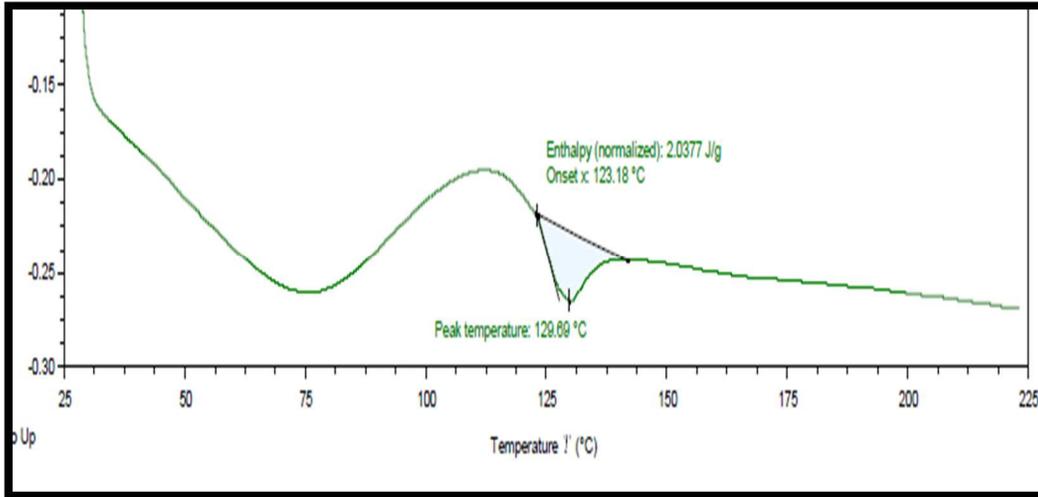


Figure 1: DSC Thermogram for pure Cyclosporine A

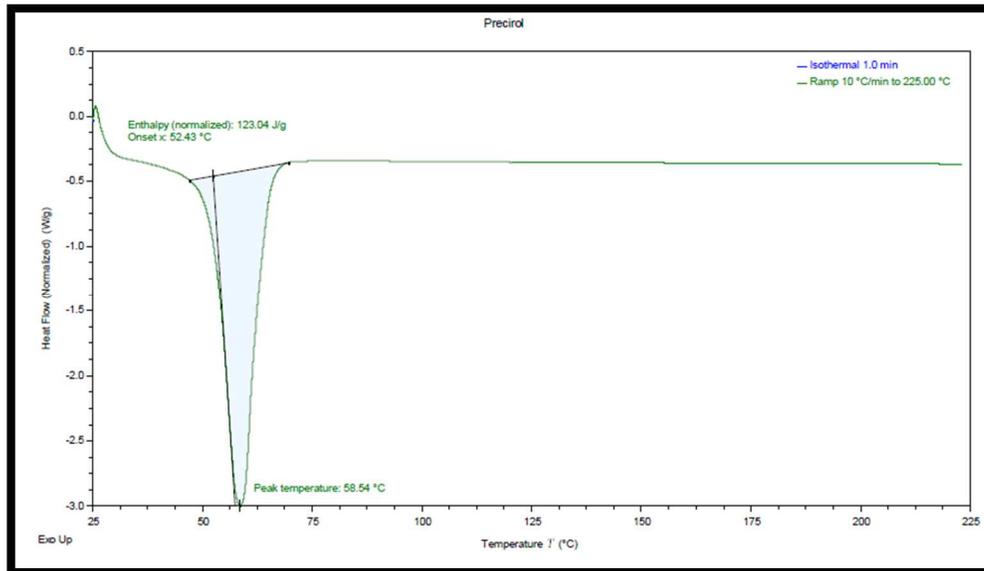


Figure 2: DSC Thermogram for pure Precirol®

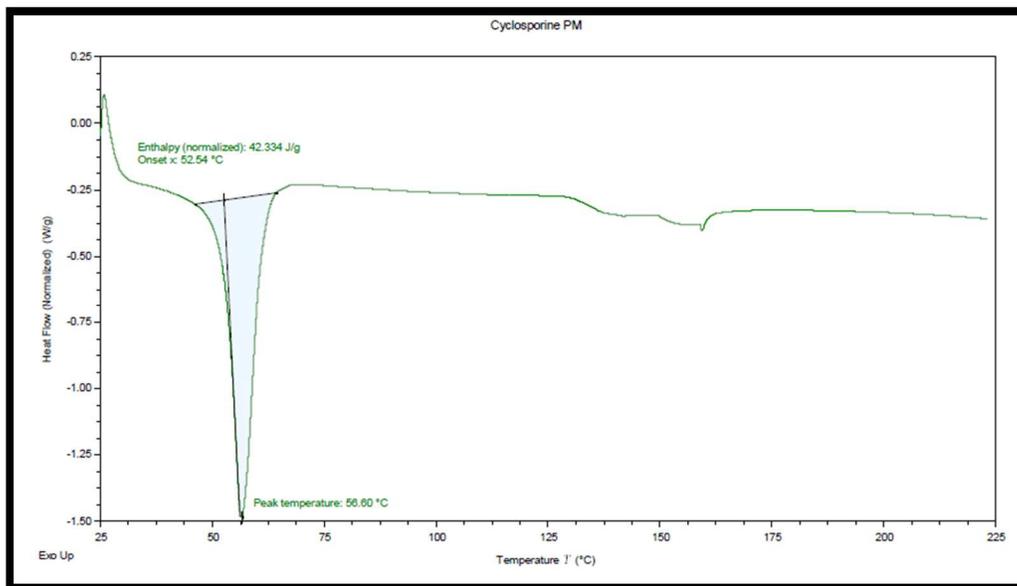


Figure 3: DSC thermogram Cyclosporine A and Precirol® ATO5 physical mixture

Stability Studies of CSA-SLN:

The physical stability of CSA-SLN-P was checked for 30 days at refrigerator (4°C), room (25°C) and accelerated (40°C) temperature conditions. The results are presented in Table 5. The samples were checked for physical appearance, physical and chemical parameters like particle size, ZP, PDI and assay, respectively at predetermined time intervals. The formulation was found stable as there was no significant difference found in the results as well as there were no physical separation or breaking observed during storage at refrigerator and room temperatures upto 30

days. In the case of formulation stored at 40°C, precipitate formation (might be CSA) was observed after 15 days.

Table 5: Stability studies of cyclosporine A solid lipid nanoparticles with Precirol® ATO5 at refrigerator (4°C), room (25°C) and accelerated (40°C) temperature conditions (mean ± SD. n=3)

CSA-SLN (P)	4°C			
	Particle size (nm)	PDI	Zeta Potential (mV)	Assay (%)
Day 1	121.2 ± 5.2	0.4 ± 0.04	-24 ± 1.0	95 ± 0.5
Day 15	119.0 ± 2.2	0.43 ± 0.02	-24 ± 0.5	94 ± 0.1
Day 30	123.1 ± 3.4	0.35 ± 0.01	-23.62 ± 0.2	90 ± 0.3
25°C				
Day 1	118.2 ± 8.0	0.55 ± 0.05	-24 ± 0.0	94 ± 0.5
Day 15	120 ± 10	0.31 ± 0.06	-24.9 ± 1.5	92 ± 0.4
Day 30	125.7 ± 5	0.41 ± 0.13	-24.8 ± 1.2	91.5 ± 1.0
40°C				
Day 1	135.6 ± 12.1	0.42 ± 0.09	-29.9 ± 1.0	90 ± 0.2
Day 15	139 ± 1.1	0.41 ± 0.02	-26.4 ± 1.0	41 ± 0.07
Day 30	130 ± 1.5	0.36 ± 0.06	-22.3 ± 0.0	38.32 ± 0.1

pH of CSA-SLN

The pH of the CSA-SLN-P formulation was checked every time during stability studies, in order to observe if there was any difference in pH. The pH of the CSA-SLN-P comes in the range of 6-6.5, which is nearly equal to human tears (6.5-7.5) (Abelson, Udell, and Weston 1981). The pH of all formulations was within this range.

Formulation development and Optimization of CSA-SLN in-situ gels (CSA-SLN-IG):

The biggest advantage of an in-situ gel as compared to normal gel is that it is patient compliance, as it is easy to apply and forms gel immediately on contact with tear fluid. Conversion of CSA-SLN to in-situ gel would further enhance the retention of the formulation on the corneal surface. The critical quality attributes (CQA) for an in-situ gel is its rheological property and viscosity. CSA-SLN-IG was formulated using different percentage of gellan gum to get optimum CQA. Ideal viscosity for an ophthalmic solution lies between 25-50 cP (Uddin et al. 2017). Table 6 presents various gellan gum concentration along with their viscosities and rheological properties. From the table it is evident that the formulation with 0.3%w/v gellan gum (CSA-SLN-IG3) has a viscosity of about $27.90 \text{ cP} \pm 2.8$ (without STF) and $1400 \pm 6.9 \text{ cP}$ (with STF) which shows that it forms strong gel after coming in contact with STF as compared to SLN with 0.2%w/v gellan gum. Even though CSA-SLN-IG4 has high viscosity with STF as compared to CSA-SLN-IG3, but it had lower assay and a viscosity (without STF) above the permitted range of 25-50cP (Tatke et al. 2018) because of which CSA-SLN-IG4 was not selected. CSA-SLN-IG-3 (0.3%w/v gellan gum) also showed immediate gelling time and gel residence time greater than 24 h, thus CSA-SLN-IG3 was selected as an optimized formulation. The reason behind gel formation is that gellan gum is an anionic polysaccharide and forms clear gel when comes in contact with ions present in the tear film.

Thus, in accordance with results and inference discussed above, formulation CSA-SLN-IG3 (0.3% gellan gum) was selected as an optimized formulation.

Table 6: Rheological evaluation and physical characterization of in-situ gel of cyclosporine A solid lipid nanoparticle in-situ gel (mean \pm SD, n=3)

Formulation	Gellan Gum (%w/v)	In-vitro gelling time	Gel Residence time (h)	Viscosity (cP) Without STF	Viscosity (cP) With STF	Assay (%)
CSA-SLN-IG2	0.2	Immediate	>24	23.00 \pm 1.5	306.90 \pm 4.5	78 \pm 0.1
CSA-SLN-IG3	0.3	Immediate	>24	27.90 \pm 2.8	1400 \pm 6.9	85 \pm 0.5
CSA-SLN-IG4	0.4	Immediate	>24	120 \pm 5.6	2278 \pm 20	50 \pm 0.5

Stability studies for CSA-SLN-IG:

CSA-SLN-IG3 was found stable for one month at refrigerated and room temperature conditions and no significant changes were observed in the assay, viscosity, gelling time, in-vitro gel residence time and pH. There was no physical separation also observed.

Sterilization of CSA-SLN

Post- production stability of CSA-SLN-P and CSA-SLN-IG3 were tested by subjecting to autoclave. The formulations were found to be stable until 30 days (last point tested) indicating the potential for sterilization using autoclave method. The sterilized formulations were evaluated for parameters like assay, entrapment efficiency, particle size, PDI, ZP and compared with the values before autoclaving. The results of pre and post-autoclave of CSA-SLN-P are shown in Table 7. Sterilization increased the particle size and decreased the PDI. Similarly, CSA-SLN-IG was also stable during the autoclave processing.

Table 7: Effect of pre and post-autoclaving of CSA-SLN-P (mean± SD, n=3)

	Before Autoclave			
	Particle size (nm)	PDI	Zeta Potential (mV)	Assay (%)
	Day 1	125.8 ± 3.6	0.55±0.05	-24 ±0.0
Day 15	119 ± 1.2	0.31 ± 0.06	-24.9 ± 1.5	92 ± 0.4
Day 30	125 ± 3	0.41 ±0.13	-24.8 ± 1.2	91.5 ± 1
	After autoclave			
Day 1	165.1 ± 8.0	0.20 ±0.01	-25 ± 0.6	94 ± 0.02
Day 15	160 ± 3.5	0.18± 0.04	-23.3 ±1	91.5 ± 0.11
Day 30	158 ± 4.0	0.25 ±0 .06	-24.2 ± 0.7	90.8 ± 0.42

CONCLUSION

In this study, CSA loaded solid lipid nanoparticles and corresponding in-situ gel was successfully formulated using Precirol® ATO5 as the lipid, Tween®80 and Poloxamer 188 as surfactants and gellan gum as a gelling agent. The formulations were autoclavable and were stable for one month at different storage conditions. Further studies evaluating the in-vivo activity of the formulations are needed.

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