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Curcumin loaded in-situ gel for ocular complication due to diabetes mellitus: Dry eye syndrome

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Abstract
Diabetes mellitus is a very common turmoil in all over the world. Often minimally referred to as diabetes, is a cluster of metabolic diseases (Also known as metabolic syndrome and a slow poison) in which a person has high blood sugar. It happens either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced causes established symptoms like polyuria (frequent urination), polydipsia (augmented thirst) and polyphagia (increased food shortage). Diabetes is reported as the one of the most common cause of death in the world.
Diabetes mellitus is a chronic syndrome which cannot be cured. Management concentrates on keeping blood sugar levels as secure to normal ("euglycemia") as possible, without causing hypoglycemia. This can usually be proficient with diet, exercise, and use of appropriate medications (insulin in the case of type 1 diabetes, oral medications, as well as possibly insulin, in type 2 diabetes). In the present study to find out new dimensions for the treatment of diabetes by understanding the versatile role of calcium in diabetes associated cellular events.

Keywords: Curcumin, Situ gel, topical drug delivery

Introduction

Materials and Methods

Material

Tween® 80, Poloxamer 188 were acquired from Fischer scientific (Dr.reddy lab Hyderabad,). Precirol® ATO 5 (Glyceryldistearate) and Compritol 888® ATO (Glyceryldibehenate) was a generous gift sample from Gattefossé (Thermo scientific Banglore). Gellan gum was purchased from MP Biomedicals, LLC (Biocon Cennai). Cyclosporine A (CSA) was purchased from IMTECH chandigarh). Other chemicals and entities required for the project like HPLC grade solvents, centrifuge tubes, HPLC vials, scintillation vials were obtained from Fischer Scientific (Delhi, India).

Methods

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

A (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 3. It mainly consists 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 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).

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

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 3.

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 C_8Luna® 5µ (250 mm x 4.6 mm) and was protected by guard column. The mobile phase comprised of Acetonitrile (ACN): water: phosphoric acid (H_3PO_4) 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 10µL.

Results and Discussion

An mounting number of examinations are carried out using live-cell imaging techniques to afford critical insight into the essential nature of cellular and tissue meaning, especially due to the rapid advances that are at this time being witnessed in progress of the fluorescent protein and synthetic fluorophores. Because of these advances, live-cell imaging has become a vital analytical tool for the cell biologist, as well as a routine methodology that is practiced in the wide ranging area of neurobiology, developmental biology, pharmacology, and numerous other associated biomedical investigate disciplines. Among the most significant technical challenges for performing triumphant live-cell imaging experiments is to maintain the cells in a vigorous state and functioning normally on the microscope point while being illuminated in the existence of synthetic fluorophores and/or luminous proteins. From the above discussions it is clear that in almost all cases, live-cell microscopy represents a compromise between achieving the best possible image quality and preserving the health of the cells. Present instrumentation enables the imaging of source of revenue specimens at high signal-to-noise ratios using extremely low levels in incident illumination and now stands as a powerful technique for the analysis of molecular dynamics within cells. Sustained advances in imaging techniques and fluorescent probe propose enhance the power of this approach and ensure its future as an important tool in modern biology. Technological and conceptual advances in instrumentation are also likely to push three- dimensional and chronological resolution to new limits, as well as perfecting the modes of fluorescence microscopy currently in use. The results in detail are as follows.

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 And Precirol® ATO5, the later one gave smaller PS and PDI (Table 5) 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, 24,000rpm for 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 7). 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 and were selected for CSA-SLN development. Table 2 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 4: 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 5: 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 6: 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 ºC, 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® ATO5in 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.

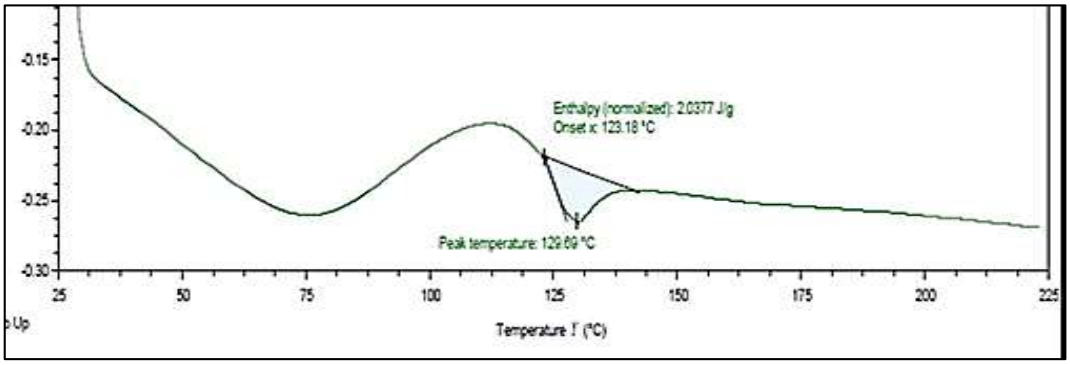

Fig 1: DSC Thermogram for pure Cyclosporine A

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.

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