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DEVELOPMENT OF NOVEL CYCLOSPROINE OPHTHALMIC EMULSION FORMULATIONS FOR TREATING DRY EYE DISORDER

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1. INTRODUCTION

Dry eye is a multifactorial disorder of the ocular surface involving the tear film and the reflex control of tear homeostasis. There are two major forms of dry eye: lacrimal-deficient or aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE). In the United States and worldwide, dry eye has been estimated to affect 5–30% of the population. Patients with dry eye complain of a variety of symptoms including poor visual quality, pain (burning, aching) and tearing. Symptoms associated with dry eye are a leading cause of visits to eye clinics and its treatment has significant cost implications. Dry eye adversely impacts quality of life as its symptoms interfere with activities of daily living such as driving, reading and watching television. Studies using the Impact of Dry Eye on Everyday Life questionnaire have confirmed that dry eye negatively affects physical and mental functioning.[1]

Despite its high frequency and morbidity, there is no gold standard for dry eye diagnoses. As such, most clinicians rely on a combination of symptoms and signs to detect and monitor the disorder. Several questionnaires are available to document dry eye symptom severity, the most popular being the ocular surface disease index (OSDI). Common tear film and ocular surface assessments in dry eye include tear breakup time (TBUT) [an assessment of tear film stability, lower scores are indicative of tear instability], corneal staining [an assessment of corneal epithelial cell disruption, higher scores indicative of more disruption], basal or reflex tear secretion test (Schirmer's strips) [an assessment of tear secretion, lower scores are indicative of less secretion], and morphologic and qualitative characterization of the eyelid margin and meibomian glands. Newer tests that can provide subclinical information on the ocular surface environment have more recently become available including measurement of tear osmolarity and of tear MMP-9 as an index of ocular surface inflammation. Unfortunately, many groups have demonstrated poor correlation between dry eye symptoms and signs, a fact that makes diagnosing, treating and researching dry eye challenging. Even when separately measuring the two major subtypes of dry eye – ADDE and EDE, neither were significantly correlated with the presence of symptoms. It can also be difficult to appraise certain tests like osmolarity. There is currently no commercially available way to measure osmolarity in the central cornea which is believed to greatly exceed the osmolarity levels found in the inferior tear meniscus and be responsible for the discomfort symptoms. Likewise, there are likely unmeasured factors in dry eye, such as ocular sensory apparatus function, that may become sensitized in patients with ocular surface inflammation and high osmolarity.[2]

It is well recognized that inflammation plays an important role in dry eye. Early studies demonstrated that patients with dry eye had increased CD4+ T cells and HLA-DR expression in their conjunctivae and higher levels of inflammatory mediator expression like ICAM-1. A classic paper that established this concept was published by Niederkorn et al. in 2006. In this paper, mice were first subjected to a low humidity environment and were given scopolamine which causes decreased aqueous tear production. These experimental conditions led to the development of T-cell-mediated inflammation on the ocular surface with clinical manifestations that resembled dry eye in humans (i.e. corneal staining). The authors were then able to induce a similar disease picture in nude mice by adoptively transferring CD4(+) T cells from the affected animals. Since then, many experimental models have expanded on this concept and have found that other parts of the immune system, like antigen presenting cells and immunoglobulins, are also important in the development of experimental dry eye. In mice, several therapies that interfere with the

inflammatory cascade, like IL-17, IL-1 and chemokine receptor 2 inhibition, were found to improve experimental dry eye. Inflammation is also a component of dry eye in humans. As above, T cells have been described in the conjunctivae of patients with dry eye and elevated levels of various inflammatory cytokines have been found in their tears. Specifically, tear levels of IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17, TNF-α and IL-6 have all been found to be elevated in dry eye compared with control subjects. It is not clear, however, what percentage of patients with dry eye symptoms has underlying ocular surface inflammation and to what degree this inflammation drives symptoms.[3]

Given the role of inflammation in dry eye, it makes sense that anti-inflammatory agents have been evaluated in its treatment. Cyclosporine A (CsA) emulsion 0.05% (Restasis®, Allergan, CA, USA) has been the only product to receive US FDA approval for the treatment of dry eye. CsA is an immunosuppressant medication that was originally used to prevent rejection after organ transplantation. It affects immune function by interfering with the activity and growth of T-cells. In the normal situation, T-cell receptor activation leads to the influx of calcium (Ca^{2+}) into the cytoplasm. Intracellular calcium binds the cytosolic protein calmodulin, which in turn binds and activates calcineurin. This calmodulin/calcineurin complex then dephosphorylates the transcription factor nuclear factor of activated T cells (NFATc), which translocates into the nucleus and increases the activity of genes coding for IL-2 and other inflammatory cytokines. CsA exerts its action after it enters the cytoplasm of T cells and binds to cyclophilin. The CsA/cyclophilin complex affects T-cell activity by blocking the action of calcinuerin and preventing NFATc dephosphorylation. The subsequent reduction in IL-2 levels also reduces the function of effector T cells. CsA can also affect mitochondrial activity in some cells. In human conjunctival epithelial cells, the inflammatory mediators TNF-α and IFN-γ induce mitochondrial permeability transition pore (MPTP) opening, upregulate Fas/FasL and caspase, and increase cell apoptosis. CsA prevents epithelial cell death by blocking MPTP opening, Fas/FasL and caspase activation. Interestingly, a similar effect of CsA on blocking MPTP opening was not seen in activated T cells.[4]

2. Novelty, Rationale, and Innovations

1. Development of Ophthalmic Emulsion of Cyclosproine for better treatment option for dry eye syndrome.
2. Use of novel excipients for development formulation which will have better pharmaceutical as well as pharmacokinetic excellence.
3. Comprehensive characterisation of formulation for better understanding of product and its overall stability.

3. MATERIALS AND METHODS

3.1. Pre-formulation studies

Cyclosporine A will be comprehensively studied for its physical properties, melting point, solubility studies, partition coefficient, and drug interactions (FT-IR studies) as per the methods/protocols provided by Kim et al., 2015.[5]

3.1.1. Physical properties

The physical properties of Cyclosporine A will be determined in terms of color, odor, and taste.

3.1.2. Melting point

The melting point of the Cyclosporine A will be determined by taking a small amount of drug in a capillary tube closed at one end and will be placed in Thiele's melting point apparatus and the temperature at which the drug melts will be noted. Averages of triplicate readings will be noted.

3.1.3. Solubility studies

The solubility of Cyclosporine A will be determined in distilled water, different buffers, viz., pH 4.0, pH 7.4, and pH 8.0 and in methanol. Triplicate readings will be taken and the average will be calculated.

3.1.4. Partition coefficient

The partition coefficient of the drugs will be determined by taking equal volumes of n-octanol and aqueous phases in a separating funnel. A drug solution will be prepared and 1 ml of the solution will be added to octanol: stimulated tear solution (pH 7.4) (50:50 v/v) will be taken in a separating funnel and shaken for 10 minutes and allowed to stand for 1 hr and is continued for 24 hr. Then aqueous phase and octanol phase will be separated, centrifuged for 10 min at 2000 rpm. The aqueous phase and octanol phase will be assayed before and after partitioning using UV-Vis Spectrophotometer at their respective λ max to get the partition coefficient.

3.1.5. Preparation of calibration curve

UV-Vis spectrophotometry will be used to identify Cyclosporine A. Stimulated tear solution from 200 nm to 400 nm qualitatively exhibits the same absorbance characteristics at identical wavelengths as does a similarly prepared and concomitantly measured solution of a Cyclosporine A standard. Quantitatively, the equimolar sample and standard solutions will exhibit absorbances at 295 nm (λmax).

3.1.6. Fourier transform-infrared (FT-IR) spectroscopy

All of the excipients will be analyzed with Fourier transform-infrared (FT-IR) spectroscopy. The pellets will be scanned in an inert atmosphere over a wave number range of 4000-400 cm^{-1} over 128 scans at a resolution of 4 cm-1 and an interval of 1 cm-1. Each spectrum will be background subtracted.

3.2. Formulation development

Cyclosporine A was added to oil (arachis oil) phase for 24 hr to complete biopolymer hydration and saturation. Then aqueous (distilled water) phase was added to the above solution and homogenization was carried out in presence of emulsifiers Tween 20 and Span 80 under ultrasound for 2 min using nominal power of 160 W. After homogenization, emulsions were placed in an ice bath until they reach room temperature. The pH was adjusted further with triethanolamine.[6]

Table 1: Formulation chart.

Ingredients	F1	F2	F3
Cyclosporine A (in mg)	20	20	20
Arachis Oil (in mL)	10	20	30
Triethanolamine (in mL)	0.5	0.5	0.5
Tween 20 (in mL)	0.5	0.75	1.0
Span 80 (in mL)	0.5	0.75	1.0
Distilled water (in mL)	30	20	10

3.3. Evaluation parameter

The optimized formulation will be comprehensively evaluated for pH measurement, electrical conductivity, zeta potential, globule size measurement, viscosity, optical microscopy, and creaming index as per the methods/protocols given by Campelo et al., 2017.[7]

3.3.1. Physical appearance

The physical properties of the emulsion formulation will be determined in terms of color, odor, and taste.

3.3.2. pH measurement

Emulsion pH measurement was carried out in triplicate using a PHS-3E pH meter. The equipment was calibrated with buffer solutions and pH values were measured by inserting the electrode directly into the sample at 25°C.

3.3.3. Electrical conductivity

The emulsion electrical conductivity measurement will be determined in triplicate, using a benchtop conductivity meter. The conductivity values will be measured by inserting the electrode directly into the sample at 25°C.

3.3.4. Zeta potential

The surface charge density (Zeta potential) will be determined by Electrophoretic Light Scattering using ZetaSizer Nano-ZS. The emulsions will be diluted in Milli-Q water, according to the equipment optimal detection range. The measurements will be performed in duplicate at 25°C.

3.3.5. Droplet size measurement

The Z-Average diameter will be determined by dynamic light scattering technology using Zetasizer Nano ZS. The emulsions will be diluted in Milli-Q water to 2.0% (v/v), according to the equipment optimal detection range. The measurements will be performed in duplicate at 25°C.

3.3.6. Viscosity

Emulsion rheological behavior and viscosity will be evaluated using an oscillatory rheometer coupled to a temperature controller. The emulsions will be evaluated immediately after preparation and after 4 hr. Analyses will be performed in triplicate at 25°C. The rheological behavior will be analyzed by the deformation rate (0-300 s^{-1}) increase, decrease and increase. After removal of time dependence (thixotropic), the third curve will be obtained. The model adequacy will be evaluated by the coefficient of determination (R^2), the residual mean square and the significance of the parameters ($p < 0.05$). Apparent viscosity of emulsions will be studied at shear rate. The parameter will be evaluated at this shear rate since it is the typical rate for food processes, such as the flow through tubes in industry and stirring and mastication processes.

3.3.7. Optical microscopy

Optical microscopy will be performed to assess the emulsion destabilization processes on a microstructural level. The analysis will be performed after the emulsion preparation and after 4 hr of storage, in an optic microscope. For this, an aliquot of emulsion will be placed on the slide, covered with a cover slip and will be examined with a 100x objective lens.

3.3.8. Creaming index

The creaming index will be determined by adding 5 mL of emulsion in plastic pots, immediately after preparation. After 4 hr, the volume of the formed cream will be measured and creaming percentage will be determined accordingly.

3.4. Statistical analysis

All the data will be represented in Mean ± SD. Data will be analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests using Sigmastat® software. The group means will be considered significantly significant when p-value is < 0.05.

4. RESULTS AND DISCUSSION

4.1. Preformulation

4.1.1. Physical appearance study

The Cyclosporine A was white in color, odorless, with characteristic bitter taste.

4.1.2. Melting point study

The melting point of Cyclosporine A was found to be 149-151°C.

4.1.3. Solubility study

The Cyclosporine A was found to be most soluble in acetone, indicating non-polar nature (Table 2). The same phenomenon was proved from solubility in polar solvents where the solubility was found to be quite limited.

Table 2: Solubility profile of Cyclosporine A.

S. No.	SOLVENT	SOLUBILITY PROFILE	INTERPRETATION
1.	Ethanol	+++	Soluble
2.	Methanol	+++	Soluble
3.	Ethyl acetate	+++	Soluble
4.	Distilled water	++	Low solubility
5.	Petroleum ether	+++	Soluble
6.	Acetone	++++	Highly soluble

4.1.4. Determination of absorption maxima

The λmax of Cyclosporine A was found to be 234 nm (Figure 1).

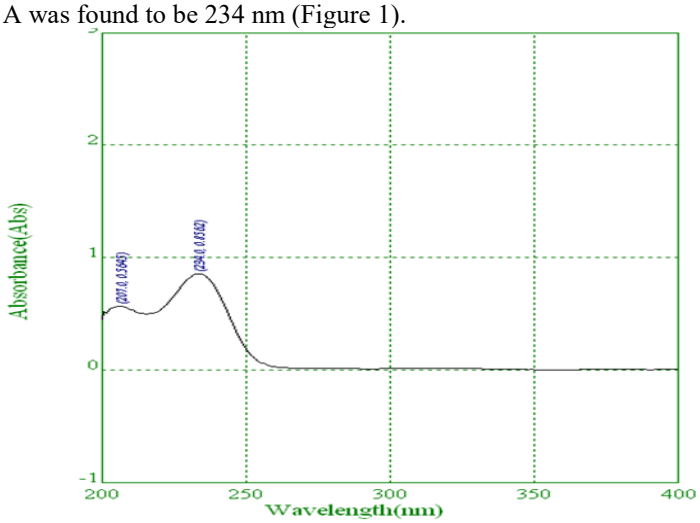

Figure 1: UV-Vis spectra of Cyclosporine A.

4.1.5. Partition coefficient study

The partition coefficient of Cyclosporine A was found to be 1.07, indicating lipophilic behavior.

4.1.6. FT-IR spectroscopy

The FT-IR spectra of Cyclosporine A highlighted the presence of various functional groups as observed from the wavenumbers indicating carbonyl (-C=O), nitro (-NO2), aromatic (-C6H5), hydroxyl (-OH), and amine (-NH2) groups (Figure 5.2).

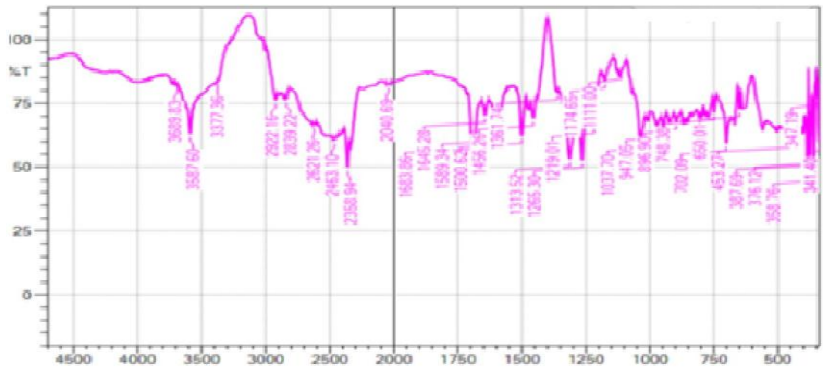

Figure 2: FT-IR spectra of Cyclosporine A.

4.2. Characterization of formulations

4.2.1. Physical appearance

The formulations appeared transparent, no odor, and completely free from taste.

4.2.2. pH measurement

The pH of the formulations was found to be in the range of 6.5-6.8 which lies in close proximity with eye environment (nearly neutral).

4.2.3. Electrical conductivity

The electrical conductivity of the formulations was found to be sufficiently high in the range of 7.63 S/m to 9.42 S/m which indicates formation of O/W formulation.

4.2.4. Zeta potential

The zeta potential of the formulations was found to be in the range of -29.7 mV to -33.2 mV which indicated that the formulations were stable in continuous phase.

4.2.5. Droplet size measurement

The droplets in each of the formulations appeared round, non-congealed, and equally distributed with size range of 49.8 nm to 57.4 nm. Figure 3 represented emulsion droplet of optimized formulation F3.

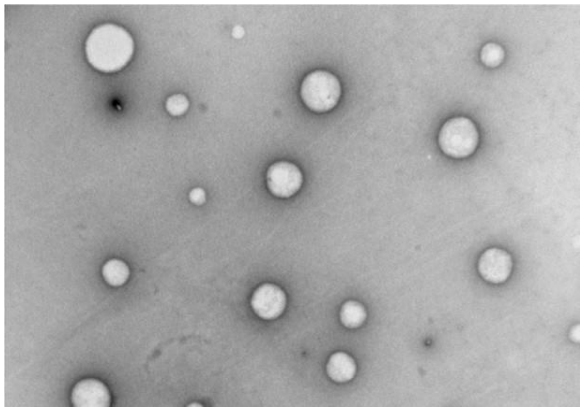

Figure 3: Emulsion droplet of optimized formulation.

4.2.6. Viscosity

The viscosity of the formulations was found to be in the range of 2800 cps to 3150 cps (Table 3) which indicated that the formulation is stable enough in keeping the droplet floated in the continuous phase.

4.2.7. Creaming index

All formulations were stable and free from any deformities like creaming, froathing, etc. The formulations demonstrated creaming index of <5% which indicated that formulations were stable as well as within the pharmacopoeial limit.

Table 3: Pharmaceutical characteristics of Cyclosporine A ophthalmic formulations.

Characteristics	F1	F2	F3
Physical appearance	Round	Round	Round
pH	6.6 ± 0.16	6.5 ± 0.47	6.8 ± 0.91
Electrical conductivity (S/m)	9.42 ± 1.34	8.11 ± 1.66	7.63 ± 1.82
Zeta potential (mV)	-31.7 ± 0.46	-29.7 ± 0.27	-33.2 ± 0.33
Droplet size (nm)	57.4 ± 4.77	53.1 ± 5.28	49.8 ± 6.13
Viscosity (cps)	2950 ± 100	2800 ± 50	3150 ± 150
Creaming index (%)	3.92 ± 0.86	4.28 ± 0.54	2.77 ± 0.99

5. CONCLUSION

In the coming years, topical drug delivery is expected to be extensively used for better patient compliance and tolerance. Since ophthalmic emulsion is helpful in enhancing spreadability, adhesion, viscosity and extrusion, this novel drug delivery system garners more and more attention. In addition, ophthalmic emulsion is also favorable to become a solution for loading hydrophobic drugs in emulsion system for the long-term stability. Moreover, to the best of our knowledge, no investigation on Cyclosporine A ophthalmic emulsion formulations has been reported before. Based on the above results, which showed that the prepared Cyclosporine A ophthalmic emulsion formulations exhibited acceptable physical properties and drug release, remaining unchanged upon storage for short period, we can arrive at the conclusion that Cyclosporine A ophthalmic emulsion formulations is able to extend the retention time on the ocular surface as well as improve the ocular bioavailability without any lesions. Therefore, the ophthalmic emulsion is proved to be an ideal choice for topical drug delivery in ophthalmic therapy.

6. REFERENCES

1. Akpek EK, Amescua G, Farid M, Garcia-Ferrer FJ, Lin A, Rhee MK, Varu DM, Musch DC, Dunn SP, Mah FS. Dry eye syndrome preferred practice pattern®. Ophthalmology, 2019; 126(1): P286-334.
2. Brunton LL, Chabner B, Knollmann BC, editors. Goodman & Gilman's the pharmacological basis of therapeutics. McGraw-Hill Education, 2018.
3. Flower RJ, Henderson G, Loke YK, MacEwan D, Rang HP. Rang & Dale's Pharmacology. Elsevier Health Sciences, 2018.
4. Ames P, Galor A. Cyclosporine ophthalmic emulsions for the treatment of dry eye: a review of the clinical evidence. Clinical Investigation, 2015; 5(3): 267-85.
5. Kim SJ, Lee SE, Ng CL, Lee JK, Kim TH, Cho CW, Park JS. Preformulation and in vitro physicochemical characterization of fenofibrate-loaded emulsion. Journal of Pharmaceutical Investigation, 2015; 45(7): 669-74.
6. Khan BA, Akhtar N, Khan HM, Waseem K, Mahmood T, Rasul A, Iqbal M, Khan H. Basics of pharmaceutical emulsions: A review. African Journal of Pharmacy and Pharmacology, 2011; 5(25): 2715-25.
7. Campelo PH, Junqueira LA, Resende JV, Zacarias RD, Fernandes RV, Botrel DA, Borges SV. Stability of lime essential oil emulsion prepared using biopolymers and ultrasound treatment. International Journal of Food Properties, 2017; 20(S): S564-79.