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Liquid Crystalline Nanocarriers: Treatment of Dry Eye Syndrome through Ocular Drug Delivery

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ABSTRACT

Ocular drug delivery has been a major challenge to pharmacologists and drug delivery scientists due to its unique anatomy and physiology. New and novel drug delivery systems are being developed by various researchers and developers to alter the drug release pattern, resulting in a unique plasma drug concentration versus time profile and pharmacodynamic effect. The unique structural features of the eye and the physiological ocular barriers are major challenges for effective delivery at the disease site. Recent advances in bioadhesive *in situ* gelling systems and nanotechnology-based drug delivery systems are gaining substantial attention for overcoming the drug delivery challenges. Nanocarrier-based therapeutic delivery systems have been developed to promote sustained and targeted drug delivery to both the anterior and posterior segments of the eye. However, translation of nanotechnology-based drug delivery systems from bench to bedside are associated with scale up and quality control challenges. An attempt to develop an ophthalmic drug delivery system for Cyclosporine based on glyceryl monooleate/poloxamer 407 liquid crystalline nanoparticles with reduced ocular irritancy and improved corneal penetration was prepared by hydrotropic dilution method using glyceryl monooleate, poloxamer, ethanol and water. Various nanomedicines and their findings are compiled to understand the impact of nanocarriers in the treatment of ophthalmic diseases. Moreover, this review addresses the current challenges in the translation of nanomedicine, including the large-scale production and quality control aspects of nanomedicine.

1. INTRODUCTION

Ocular drug delivery system (ODDS) is a dosage form, vehicle, or system intended for instilling, administering, or delivering drug/medicine to eye against any ailment or disorder involving or affecting vision. It ranges from simple sterile eye drop for the ocular surface to complex implants for intraocular tissue. The most common disorders that require ODDS are Glaucoma, Cataract, infection of ocular surface, dry eye syndrome etc.[1]

Ocular drug delivery has been a major challenge to pharmacologists and drug delivery scientists due to its unique anatomy and physiology. Static barriers (different layers of cornea, sclera and retina including blood aqueous and blood retinal barriers), dynamic barriers (choroidal and conjunctival blood flow, lymphatic clearance and tear dilution), and efflux pumps in conjunction pose a significant challenge for delivery of a drug alone or in a dosage form, especially to the posterior segment. Parallel, colloidal dosage form such as nanoparticles, nano micelles, liposomes and microemulsions have been widely explored to overcome various static and dynamic barriers.[2]

Various nanocarriers, including nano dispersion system, nano micelles, lipidic nanocarriers, polymeric nanoparticles, liposomes, niosomes, and dendrimers, have been investigated for improved permeation and effective targeted drug delivery to various ophthalmic sites.[3]

Most conventional oral drug products, such as capsules and tablets, are formulated to release the active drug for the treatment and cure of various ailments. In the formulation of conventional dosage forms, no effort is made to modify the drug release rate. In conventional oral products containing poorly soluble (lipophilic drug) substances, drug absorption may be gradual due to slow dissolution and delayed pharmacological action across the GI tract, resulting in a delayed onset of action. The pharmaceutical industry uses various terms to describe modified-release drug products like ocular drug delivery system, transdermal drug delivery system, bucco-adhesive drug delivery system, gastro retentive drug delivery system etc. New and novel drug delivery systems are being developed by various researchers and developers to alter the drug release pattern, resulting in a unique plasma drug concentration versus time profile and pharmacodynamic effect. Several benefits can be derived from using targeted drug delivery system. These include reduced dosing frequency to improve the patient compliance, reduced side-effect profile especially those related to rapid rise in peak serum concentration and local irritation due to slow release or targeted nature of delivery, resulting in reduction in local irritation and a steady rise in serum levels. It improves drug tolerance,

reduced peak-to-trough variations, maintaining plasma levels within therapeutic ranges and provides increased duration of drug therapeutic effect.

As per a World Health Organization (WHO) report, every five seconds someone in the world goes blind and every minute a child loses their sight.[4,54] The International Classification of Diseases (IDC-11) (2018) states that approximately 1.3 billion people live with some form of vision impairment globally.[5] These ocular diseases affect the vision and quality of life of patients. Considerable achievements have been made in the supervision of ocular diseases. In the last decade, extensive research has been done at the preclinical and clinical level for the development of therapeutics for various ocular diseases, including glaucoma, uveitis, age-related macular degeneration (AMD), cataracts, and diabetic retinopathy. Recent advances in the treatment of ophthalmic diseases at the clinical level include anti-vascular endothelial growth factor drugs, gene therapy, laser surgery on the eye, and ocular sealants. To deliver these therapeutics, various drug delivery systems, such as eye drops (solutions, suspensions, emulsions), *in situ* gels, ocular inserts, contact lenses, punctum plugs, intraocular injections, and implants, have been explored for effective ocular drug delivery.[6,7] The unique structural features of the eye and the physiological ocular barriers are major challenges for effective delivery at the disease site.[8,9] Recent advances in bio-adhesive *in situ* gelling systems and nanotechnology-based drug delivery systems are gaining substantial attention for overcoming the drug delivery challenges. Nanocarrier-based therapeutic delivery systems have been developed to promote sustained and targeted drug delivery to both the anterior and posterior segments of the eye.[10,11] However, translation of nanotechnology-based drug delivery systems from bench to bedside are associated with scale up and quality control challenges.[12]

In this article, we focus on the anatomical and physiological barriers to ocular drug delivery. Further, we discuss the limitations of conventional formulations and other routes of drug delivery. Overcoming the limitations of current therapies, advanced nanocarriers have been shown to be effective in treating ocular diseases. Various nanomedicines and their findings are compiled to understand the impact of nanocarriers in the treatment of ophthalmic diseases. Moreover, this review addresses the current challenges in the translation of nanomedicine, including the large-scale production and quality control aspects of nanomedicine.

1.1 OCULAR DELIVERY

1.1.1 Eye

The human eye has a spherical shape with a diameter of 23-24 mm.[54] Eye is a unique organ of our body from anatomical and physiological point of view.

There are many eye diseases that can affect physiology of eye and vision as well. Topical application of drugs to the eye is the most popular and well-accepted route of administration for the treatment of various eye disorders.

The bioavailability of ophthalmic drugs is, however, very poor due to efficient protecting mechanisms of the eye. Blinking, baseline and reflex lachrymation, and drainage remove rapidly foreign substances, including drugs, from the surface of the eye.[55] Therefore, many ophthalmic drug delivery systems are accessible. These are classified as conventional and non-conventional (newer) drug delivery systems.

Most commonly accessible ophthalmic preparations are eye drops and ointments about 70% of the eye dosage formulations in market. But these preparations when instilled into the culde-sac are rapidly drained away from the ocular cavity due to tear flow and lachrymal nasal drainage. Only a small amount is available for its therapeutic effect resulting in frequent dosing. So, to overcome these problems newer pharmaceutical ophthalmic formulation such as in-situ gel, nanoparticles, liposome, nanosuspension, microemulsion, into phoresis and ocular inserts have been developed in last three decades, increased the bioavailability of the drug in a sustained and controlled manner.[14,15]

1.1.2 Dry Eye

Dry eye disease (DED) is common; its prevalence around the world varies from 5% to 345. It is a common chronic multifactorial condition of ocular surface characterized by failure to produce high quality or sufficient amount of tears to moisturize the eyes. [16-18]

Dry eye is currently one of the most common ocular surface disease. It can lead to ocular discomfort and even cause visual impairment, which greatly affects the work and quality of life of patients.[19]

Dry eye is a disease which starts with unpleasant levels of symptoms of dryness of eyes and an uncomfortable feeling, greatly preventing people from performing daily activities when

the disease worsens.[20,21] It can lead to ocular discomfort and even cause visual impairment. It is a disorder of tear film which occur due to tear deficiency or excessive tear evaporation, it causes damage to the interpalpebral ocular surface and is associated with a variety of symptoms reflecting ocular discomfort.[22]

1.1.3 Dry eye syndrome

Dry eye syndrome (DES, Keratoconjunctivitis sicca) is an extremely common and often unrecognized pathological entity.[23,57] It is an ordinary disorder of the tear film caused by decreased tear production on corneal and conjunctival surface or increased evaporation.[4] Tears are needed to lubricate the eyes and to wash away sand particles and foreign objects. There should be a healthy tear film on the eye which is necessary for good vision. Dry eyes mainly develop when eye is not capable to maintain a healthy coating of tears.[24]

According to "National Health Service" (NHS), U.K, approximately 17% to 30% of people have Dry Eye at same time in their life.

DES may be divided into 2 main types as follows:

- DES associated with Sjogren Syndrome (SS)
- DES unassociated with SS (non-SS KCS)[25]

1.1.4 Mechanism and route of transport of drug molecules

Drug absorption is determined by drug's physicochemical properties, formulation, and route of administration.[26] There are two general processes of absorption of drug across barrier membranes. The first process is known as "transcellular transport process" and the second process is "paracellular transport process". In transcellular process, drug molecules have to pass through the barrier cells to reach the circulation and in paracellular process, drug molecules travel across an epithelium by passing through the intercellular space between cells to reach the circulation. Transcellular transport process is a two-step process, which starts with the drug uptake into cells, and ends with drug efflux out of cells.[27,28] Both pathways are important in transport of most of molecules. Generally lipophilic molecules follow transcellular route and hydrophilic molecules (lacks transcellular route) follows paracellular route.

The mechanism of transcellular pathway includes passive diffusion, facilitated diffusion, endocytosis, exocytosis and active transport. Passive diffusion is also known as simple diffusion, which does not require cellular energy but requires chemical gradient for transport of molecules.

Facilitated diffusion is just similar to passive diffusion (does not require cellular energy but requires chemical gradient) but it requires a carrier for the transport of molecules. The mechanism of paracellular pathway includes diffusion of molecules between adjacent cells restricted by presence of a series of junctional strands known as tight junction, gap junction and desmosomes.[27,28]

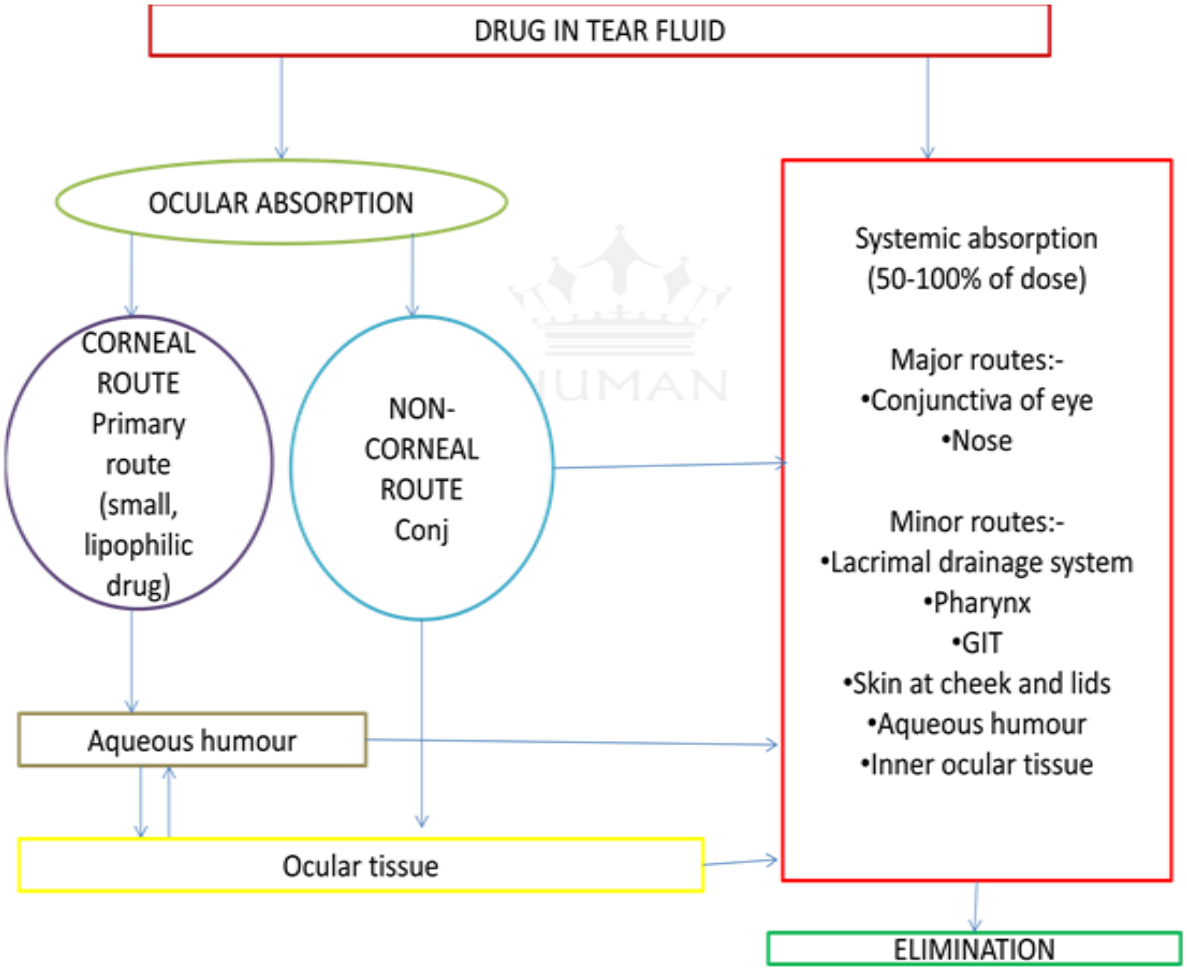


Fig. 1.1: Flow chart of mechanism and routes of penetration of topically administered drug [29]

Table 1.1: Approaches to ocular drug delivery [29]

Dosage form	Advantages	Disadvantages
Solutions	□ Convenience	□ Rapid precorneal elimination □□Loss of drug by drainage □□Nosustained action
Suspensions	□Patient compliance □ Best for drugs with slow dissolution	□ Drug properties decide Performance □ Loss of both solution and suspended solid
Emulsions	□ Prolonged release of drug from Vehicle □Enhanced pulsed entry	□ Patient non compliance □ Blurred vision □Possible oil entrapment
Ointment	□Flexibility in drug choice	□ Sticking of eyelids
	□ Improved drug stability	□Blurred vision
	□Inhibition of dilution by tears	□ Poor patient compliance
	□ Increased tissue contraction time	□ No true sustained effect
	□ Resistance to nasolacrimal drainage	□ Drug choice limited by partition Coefficient
Gels	□ Comfortable	□No rate control on diffusion
	□ Less blurred vision than ointment	□Matted eyelids after use
Erodible Inserts	□ Need only be introduced into eye and not removed	□ Movement of system around eye can cause abrasion
	□ Sophisticated and effective delivery System	□ Requires patient insertion
		□ Patient discomfort
	□ Flexibility in drug type and dissolution rate	□ Occasional product
Non-Erodible Inserts	□ Flexibility for type of drug selected	□Inadvertent loss of system from eye
	□□Controlled rate of release	
	□□ Prolonged delivery	□Patient discomfort
	□□Sustained release	□Irritation to eye
		□Patient placement
		□Patient removal
		□ Tissue fibrosis

1.1.5 NANOCARRIER

Nanocarriers offers several advantages in treating DED by better drug solubility, improved precorneal retention, enhanced permeability, site-specific, high drug payload, tear film stability, osmoregulation and target- oriented delivery of encapsulated bioactive.[1]

Nanocarriers are colloidal drug carrier systems having submicron particle size typically <500 nm.[30,52] The overall goal of utilizing nanocarriers in drug delivery is to treat a disease effectively with minimum side effects.[31]

Nanocarriers are nanoparticles which can be used to deliver biological active molecules at the desired rate for desired time the drug concentration level in therapeutic window. Nanoparticles system accelerate the drug penetration, increases corneal uptake, and avoid systemic absorption. It is able to deliver more intact drug at site of action as compared to free drug.[58-60]

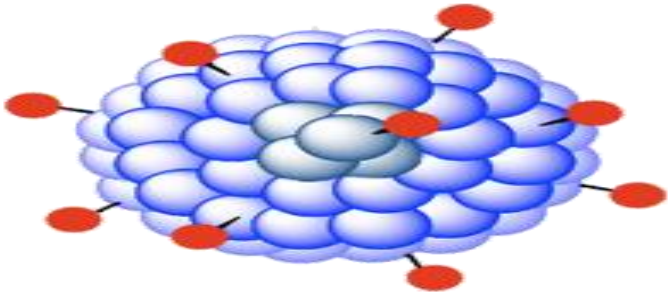


Fig. 1.2: Structure of nanocarriers

Solid-Lipid nanoparticles (SLN):

SLNs are discovered by "Gasco and Muller in 1991. SLNs provides a highly lipophilic lipid matrix for drugs to be dispersed or dissolved.[34] These are made from solid lipids i.e. lipids solid at body temperature as well as room temperature. By definition, the lipids can be highly purified triglycerides (Tri-stearin), partial glycerides, fatty acids (Stearic acid, Palmitic acid), waxes, steroids (Cholesterol) and complex glycerides mixture. Colloidal particles ranging in size between 10 & 1000 mm are known as nanoparticles. SLNs are generally spherical in shape.[32,33,56]

SLNs are new generation of submicron sized lipid emulsion where the liquid lipid (oil) has been substituted by a solid lipid. SLNs are prepared by using general ingredients including solid lipid, emulsifier & water. Lipid contains triglycerides, partial glycerides, fatty acids,

steroids, waxes. Combination of emulsifier might prevent because it can cause particle agglomeration. Emulsifier includes soybean, egg lecithin, poloxamer etc.[32]

SLNs can be prepared by various methods like Homogenization, Ultrasonication, Solvent emulsification, Micro emulsion, using Supercritical fluid and by Spray drying method.[34]

SLNs are quite similar to nanoemulsions except that different kinds of lipids are used in both formulations. Lipid that are solid at room temperature are used in SLNs instead of liquid lipid (oils) used in nanoemulsions.[35] SLNs are submicron colloidal carriers ranging from 50-1000 nm, which are composed of a physiological lipid dispersed in water or in aqueous surfactant solution.[36]

2. METHODS

2.1 Preformulation study

2.1.1 Organoleptic properties: The colour, odour and taste of cyclosporine were assessed as per the USP 2009.[37]

2.1.2 Melting point determination: Melting point of cyclosporine was determined by using scientific melting point apparatus by using a capillary tube sealed from one side. Then the temperature at which drug melts is compared with the melting point given in literature.[38,39]

2.1.3 Microscopy: The particle size and shape of cyclosporine was observed by using 10X and 40X magnification lens of digital research microscope (Motic BA310).[40]

2.1.4 Partition co-efficient: The partition-coefficient of cyclosporine was determined by "shake-flask" method in octanol and water. 10 mg drug was weighed and placed in solvent system containing 10 mL octanol and 10 mL distilled water and shaken for 24 h at 37°C to achieve pre-saturation of both phases. Mixture was kept for 10 min. Layers of octanol and water was separated with aid of separating funnel using Whatman's filter paper grade number 41. The aliquots were analysed spectrophotometrically at λ_{max}205 nm for Cyclosporine.[41] The partition coefficient was calculated by following formula:

Partition coefficient, PC = Ct-Ca/Ca

Where,

Ct = Concentration of the total drug taken.

Ca = Concentration of the drug in octanol phase.

2.1.5 Solubility studies: Solubility studies were performed by dissolving excess amount of drug in water by taking it in vials and stirred by wrist hand shaker for 24 h at 37ºC. The samples were filtered by Whatman filter paper. The solubility of drug was analysed spectrophotometrically (UV-Agilent Technologies Cary 60) at 205 nm**.**

Solubility of cyclosporine was determined in different solvents like methanol, ethanol, ethyl acetate, chloroform, and distilled water. The initial solubility of Cyclosporine was determined by weighing out 10 mg Cyclosporine. To this add excess amount of solvent of interest. If compound doesn't dissolve, further excess amount of solvent was added. Successive amount of solvent was then added until the compounds were dissolved. This method gives an approximate value of solubility.[42]

2.1.6 Infra-red analysis of drug: Cyclosporine was characterised by FTIR (Fourier Transform Infrared Spectroscopy) at ARBRO analytical laboratory, New Delhi, India. KBr pellets of drug were prepared and analysed by FTIR. The obtained spectrum of drug was compared with reference spectrum of Cyclosporine.[37]

2.1.7 UV Visible spectrophotometric analysis

UV scan in solvent system (Acetonitrile: Methanol: Water)

The diluted drug solution (10 µg/mL) was prepared in solvent system (Acetonitrile:Methanol: Water) and λ_{max} was observed. Before this, blank solution was scanned between 200nm-400nm using Agilent UV 60, single beam spectrophotometer. The wavelength at which maximum absorbance observed highest peak was considered as λ_{max}.

Calibration curve in solvent system (Acetonitrile: Methanol: Water)

Solvent was prepared by dissolving acetonitrile, methanol and water in (65 : 20 : 15) (V/V/V) ratio. The calibration curve of cyclosporine was prepared by dissolving drug in solvent system (Acetonitrile: Methanol: Water) (V/V/V). Further dilutions were done to get concentrations of 1.0 µg/ml, 2.0 µg/ml, 4.0 µg/ml, 6.0 µg/ml, 8.0 µg/ml, 10 µg/ml respectively.[43]

2.2 FORMULATION AND DEVELOPMENT

LCN of Cyclosporine were prepared by hydrotropic dilution method by using GMO, Poloxamer 407, ethanol and water as excipients. Box-Behnken design was employed to observe the effects of 3 variables on formulation at 3 levels (high, intermediate, low) by formulating 17 experimental trials.[44,45]

Formulation variables were determined by preliminary trials. Cyclosporine possesses highest solubility in ethanol as compared to GMO and poloxamer. GMO was kept constant. Ethanol along with Cyclosporine considered as independent variables (X1, X2, & X3) to observe the effect over dependent variables (particle size, entrapment efficiency).

Table 2.1: Formulation variables LCNs of Cyclosporine

Sr. No.	Code	Independent variables
1.	X_1	Poloxamer
2.	X_2	Cyclosporine
3.	X_3	Ethanol

Table 2.2: Response variables LCNs of Cyclosporine

Sr. No.	Code	Dependent variables / Responses
1.	Y_1	Particle size
2.	Y_2	Entrapment efficiency

Table 2.3: Actual and coded values of independent factors

Level	Code	Poloxamer (mg) (X_1)	Cyclosporine (mg) (X_2)	Ethanol (mL) (X_3)
Low	-1	25	1.25	0.3
High	+1	50	5	0.5

Table 2.4: List of formulation designed in coded values according to response surface design (BBD)

Run	Independent variable Coded		
	X_1	X_2	X_3
1.	-1	-1	0
2.	-1	0	+1
3.	0	0	0
4.	+1	-1	0
5.	+1	0	-1
6.	0	-1	-1
7.	0	0	0
8.	+1	+1	0
9.	-1	0	-1
10.	0	0	0
11.	+1	0	+1
12.	0	0	0
13.	0	+1	+1
14.	0	0	0
15.	0	-1	+1
16.	0	+1	-1
17.	-1	+1	0

Table 2.5: Composition of cyclosporine loaded liquid crystalline nanoparticles as per Box-Behnken design

Formulation code	Poloxamer (mg)	Cyclosporine (mg)	Ethanol (mL)	Glyceryl Monooleate (mg)	Water (qs to mL)
F1	25	1.25	0.4	250	Qs
F2	25	3.125	0.5	250	Qs
F3	37.5	3.125	0.4	250	Qs
F4	50	1.25	0.4	250	Qs
F5	50	3.125	0.3	250	Qs
F6	37.5	1.25	0.3	250	Qs
F7	37.5	3.125	0.4	250	Qs
F8	50	5	0.4	250	Qs
F9	25	3.125	0.3	250	Qs
F10	37.5	3.125	0.4	250	Qs
F11	50	3.125	0.5	250	Qs
F12	37.5	3.125	0.4	250	Qs
F13	37.5	5	0.5	250	Qs
F14	37.5	3.125	0.4	250	Qs
F15	37.5	1.25	0.5	250	Qs
F16	37.5	5	0.3	250	Qs
F17	25	5	0.4	250	Qs

(Every formulation contains total 5ml of liquid dispersion)

Optimization and formulation of LCN

For the development of formulation, Box-Behnken design (BBD) was employed to study the effect of independent variables over the dependent variables. Independent variables were Poloxamer (X_1), Cyclosporine (X_2) and Ethanol (X_3). Particle size (Y_1) and entrapment efficiency of Cyclosporine (Y_2) were considered as dependent variables. Effects of independent variables were studied and statistical model incorporating interactive and polynomial equations were utilized to evaluate the stated responses.

$$Y_1 = b_0 + b_1 X_1 + X_2 + {}_{b1}X_1X_2 + b_1 X_2 + b_2X_2$$

$$Y_2 = b_0 + b_1 X_1 + X_2 + {}_{b1}X_1X_2 + b_1 X_2 + b_2X_2$$

where,

Y= Dependent variable

b_0= arithmetic mean response

X= independent variable

X_1 and X_2 are the polynomial terms which were included to investigate the non-linearity. From the Box-Behnken outcomes (responses) it had been found that all the dependent variables are strictly dependent over the selected independent variables, as they showed a wide variation among the 17 batches (F1-F17). The polynomial equation would be used to draw a conclusion after considering the magnitude of coefficients and the sign carries i.e. positive or negative. The high value of correlation coefficient for the dependent variables showed a good fit. These equations may be used to estimate the response because small errors of variance were observed in the replicates.

The results of ANOVA of each response were carried out and the F statistics was applied to check whether the non-significant terms can be eliminated or not for the model.

Stability study

Physical stability of LCN of Cyclosporine (optimized formulation) was evaluated at 2-8°C and 25ºC/60% RH maintained by stability chamber for three months.[46]

Three important measuring parameters were:

1. Organoleptic evaluation:

Observed responses of F_5 formulation was compared with predicted responses obtained by DoE. The closeness between the responses was basis for the optimization of final formulation. Organoleptic evaluation included phase separation, creaming and discoloration. In phase separation, a 5 mL quantity of LCN of optimized formulation was stored for 3 months at ambient temperature 25°C/60% RH in stability chamber and visually observed for phase separation and drug precipitation. Optimized formulation was visually examined on weekly basis for 3 months.[47]

Creaming involves the separation of dispersed phase form the liquid crystalline dispersion on storage under normal condition at room temperature. The formed dispersion was visually assessed for creaming during the storage period at 25°C/60% RH on weekly basis up to three months.[48]

Discoloration (change in colour) was assessed by visual inspection. Optimised formulation was observed for change in colour after 1 week of preparation and continued on weekly basis for next three months.[48]

2. Particle size

The mean particle size and polydispersity index were determined using laser diffraction on a Malvern Zetasizer (Ver. 6.0.1r) at 37°C. Samples were diluted with deionised water prior to measurement by setting the dispersant viscosity to 0.8872 cP at 37°C.[49,50]

3. Entrapment efficiency

Entrapment efficiency of cyclosporine was determined by using a NANOSEP (MWCO: 2-3 KD, Pall USA, India) device. The aqueous phase was analysed for cyclosporine at 205 nm by UV spectroscopy.

The entrapment efficiency (E.E %) was determined by using following equation.[49,50]

$$\text{E.E (\%)} = \frac{\text{Total mass of Cyclosporine} - \text{Mass of Cyclosporine in phase}}{\text{Total mass of Cyclosporine}} \times 100$$

Formulation of cyclosporine loaded nanocarriers by dilution method

Nanocarriers were prepared by dilution method by using poloxamer 407, glyceryl monooleate, cyclosporine and ethanol.

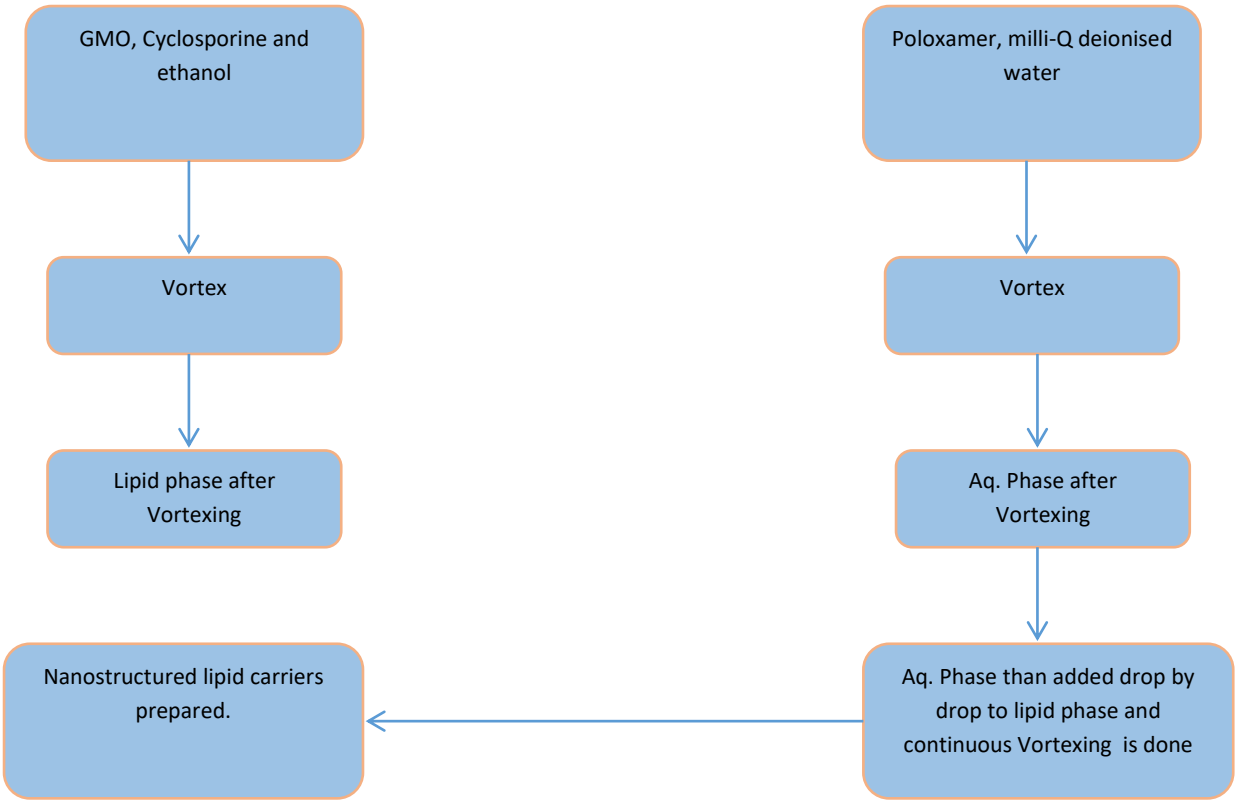


Fig. 1.3: Schematic presentation of formation of nanostructured lipid carriers of cyclosporine.

➢ ***In Vitro* Release Studies**

In vitro release study of cyclosporine was performed by using modified Franz diffusion cell. The dialysis membrane having pore size 2.4 nm and a molecular weight cut off 12000-14000 (DM-150, HiMedia, India) was utilized. The dialysis membrane retained the nanoparticles and released the free drug as barrier to diffusion. The donor compartment was filled with 2ml of cyclosporine containing nanoparticle suspension. The dialysis membrane was pre-treated with Sodium bicarbonate and EDTA solution and kept in diluted EDTA solution prior to use and the membrane was washed with distilled water prior to use and mounted in diffusion cell upward to donor chamber. Balanced salt solution (BSS) containing 10% ethanol (4 mL), maintained at 37°C was used as receptor medium and stirred continuously. Then 2 mL samples were withdrawn at 0.5, 1, 2, 3, 4, 5, 6 h after beginning the experiment and replaced by equal volume of media. Then % drug release was evaluated by U.V Spectroscopy at 205 nm.

$$\% \text{ Cyclosporine released} = \frac{\text{Amount of Cyclosporine in releasing medium}}{\text{Total amount of Cyclosporine}} \times 100$$

3. RESULT AND DISCUSSION

3.1 Organoleptic Evaluation

The colour and odour of cyclosporine was observed and recorded. Cyclosporine was found to be white-off white crystalline powder having pungent odour.

3.2 Melting point

Melting point of cyclosporine was determined by capillary rise method and melting point of pure drug was found 148°C which is in conformity with the reported range of literature values and indicated that procured drug was Cyclosporine as given in Table 3.1[51]

Table 3.1: Melting point of cyclosporine

Parameter	Drug	Observed value	Reference value
Melting point	Cyclosporine	148°C	148-151°C

3.3 Solubility studies

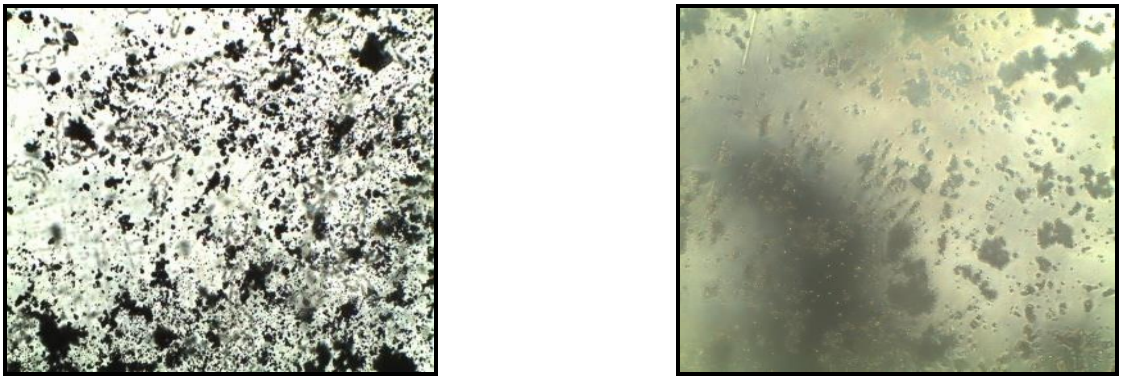
Solubility of Cyclosporine, in distilled water was 9.41×10^{-3} ± 0.21 mg/mL and in isotonic buffer (pH 7 .4) was found to be 8.67×10^{-3} ± 0.29 mg/mL indicating that solubility of cyclosporine is pH independent. Cyclosporine was found to be soluble in methanol, acetone, acetonitrile, ethanol, ether, and chloroform and methylene chloride as shown in Table 3.2.

Table 3.2: Solubility of Cyclosporine in different solvents

Solvent	Observed value (mg/mL)	Reference value (mg/mL)
Methanol	8	10
Ethanol	48	50
Chloroform	5	6
Methylene chloride	9	10
Distilled water	8.89×10^{-3}	$9.41\times10^{-3}\pm$ 0.21
IPB pH 7.4	7.99×10^{-3}	8.67×10^{-3} ± 0.29
DMSO	47	50 ±

3.4 Microscopic Analysis:

Particle size and shape of cyclosporine was observed by microscopic examination by research microscope at magnification of 10 X and 40 X. Particles of cyclosporine drug were found to be irregular in shape which confirmed the crystalline structure of the drug. Maximum particles of cyclosporine were found to be in the size range of micrometre.



(a) **(b)**

Fig. 3.1: Microscopic images of drug and excipients. (a) Pure cyclosporine at 10 X, (b) Pure cyclosporine at 40 X

3.5 Partition coefficient

Partition coefficient of cyclosporine was determined by shake flask method, it was found that the value of partition coefficient of cyclosporine was compiled with the reference values represented in Table 3.3. Obtained result indicated that this drug is highly lipophilic in nature.

Table 3.3: Partition coefficient

Parameter	**Drug**	**Observed value**	**Reference value**
Log $P_{o/w}$	Cyclosporine	3.71	3.64-4.12

3.6 Determination of absorption maxima (λ_{max}) of Cyclosporine

3.6.1 Determination of absorption maxima (λ_{max}) of cyclosporine in solvent system (Acetonitrile: Water: Methanol)

Absorption maxima (λ_{max}) of Cyclosporine in mobile phase was found at 205 nm and obtained UV spectra of scanned sample of pure drug was shown in Fig. 3.2.

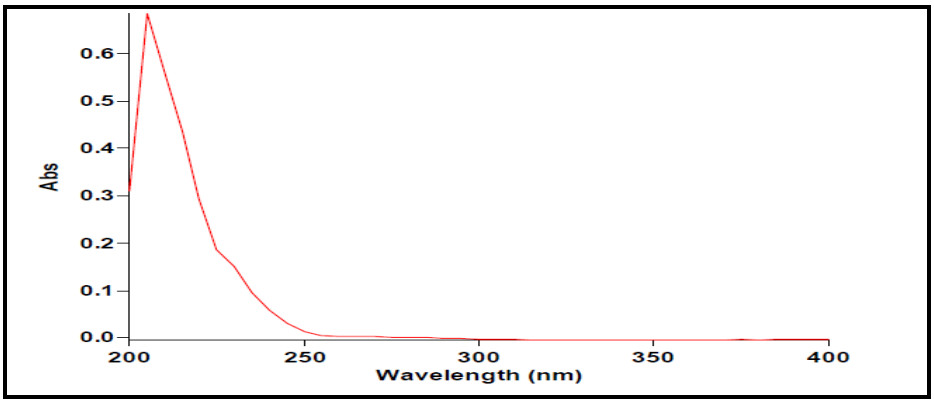


Fig. 3.2: U.V. spectra of drug in solvent system

Table 3.4: Calibration curve of cyclosporine in solvent system

Concentration (µg/mL)	Absorbance
1.0	0.0287
2.0	0.1256
4.0	0.2433
6.0	0.3871
8.0	0.5091
10.0	0.6071

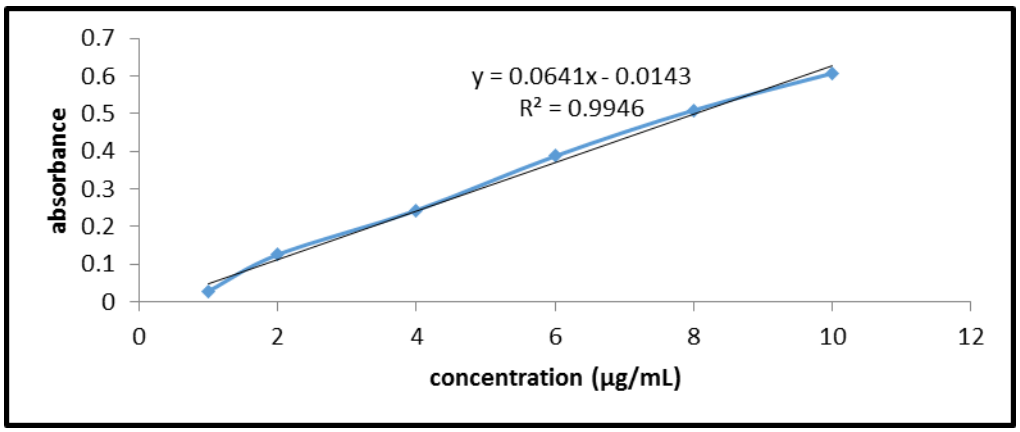


Fig. 3.3: Calibration curve of cyclosporine in solvent system

3.6.2 Determination of absorption maxima (λ_{max}) of cyclosporine in phosphate buffer pH 7.4

Table 3.5: Preparation of calibration curve of cyclosporine in phosphate buffer (pH 7.4):

Concentration (µg/mL)	Absorbance
1.0	0.0527
2.0	0.1425
4.0	0.2654
6.0	0.3916
8.0	0.4829
10.0	0.5901

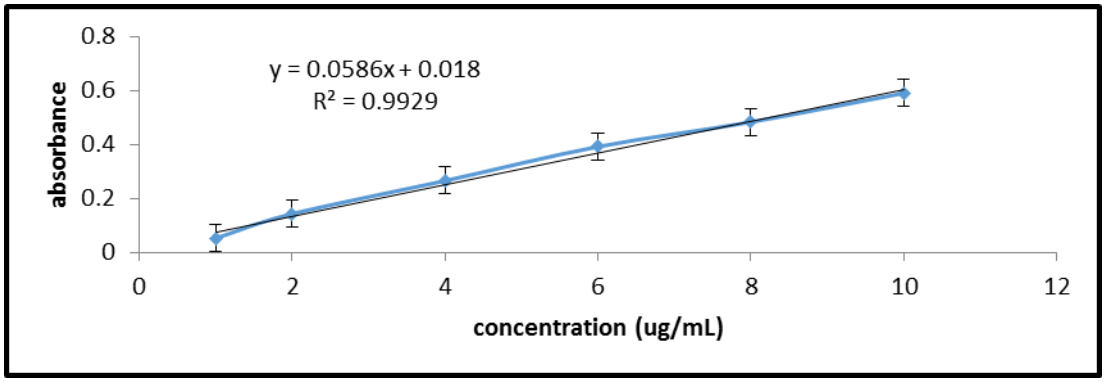


Fig. 3.4: Calibration curve of cyclosporine in 7.4 pH phosphate buffer

3.7 FTIR spectroscopy

Fig. 3.5 and Fig. 3.6 representing the reference spectrum and sample spectrum of cyclosporine respectively. FTIR analysis, suggested that the peaks and bonds of test sample of drug was similar to the reference spectra given in USP 2009. The peaks obtained in FTIR spectrum of test sample were examined and found in accordance with functional groups present in reference spectra of cyclosporine. From this study it was confirmed that procured drug sample of cyclosporine was pure.

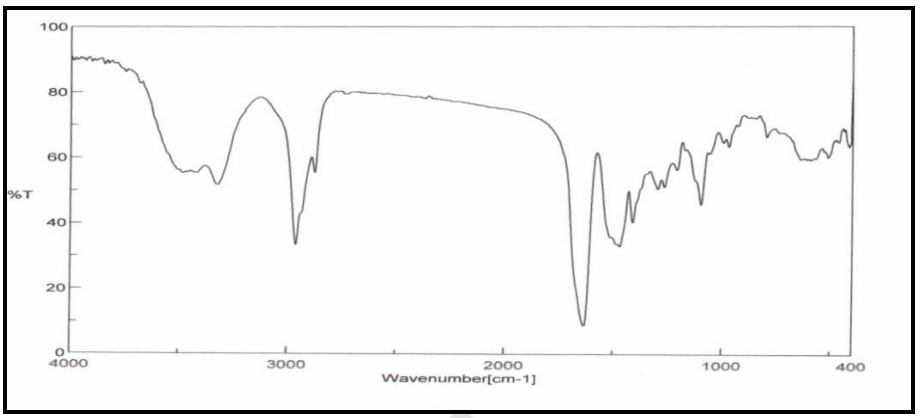


Fig. 3.5: Reference FTIR spectrum of Cyclosporine

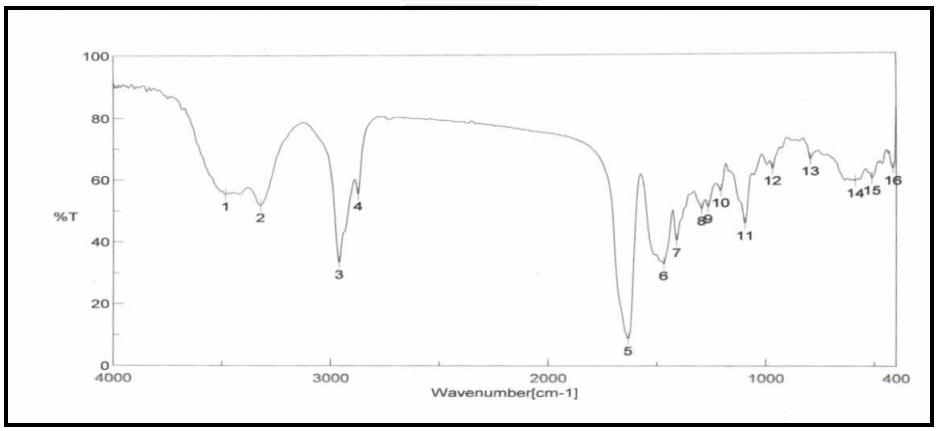


Fig. 3.6: FTIR spectrum of test sample of Cyclosporine

Table 3.6: IR spectral analysis of Cyclosporine

Std. Wave number (cm^{-1})	Test wave number (cm^{-1})	Interpretation
3180-3500	3483.78	N-H (Amide)
3300	3322.75	Aliphatic O-H
2996	2961.16	C-H
1640	1632.45	C=O (Amide)
1425	1469.49	C-N (Amide)
1050	1096.33	Aliphatic C-O

In conclusion it was stated that an attempt to develop an ophthalmic drug delivery system for Cyclosporine based on glyceryl monooleate/poloxamer 407 liquid crystalline nanoparticles with reduced ocular irritancy and improved corneal penetration was prepared by hydrotropic dilution method using glyceryl monooleate, poloxamer, ethanol and water. The optimization of liquid crystalline nanoparticles was achieved by response surface methodology (BBD).

- Pure sample of cyclosporine was supplied and used throughout the experiments.
- Cyclosporine was practically insoluble in water but soluble in ethanol, methanol, ether, and chloroform and methylene chloride.
- The FTIR spectra of drug was compared with reference spectra and was found to be identical.
- Partition coefficient for cyclosporine was found 3.71.
- λ max of cyclosporine was 205nm, determined by UV visible spectroscopy.
- Out of 17 trials, trial F8 was suggested as an optimized formulation. The selection was made on the basis of particle size and entrapment efficiency of cyclosporine.
- Optimized formulation (OF8) derived by BBD contained particle size 185.19 nm with a PDI value of 0.16 indicating uniform dispersion.
- Entrapment efficiency of cyclosporine was found 91.69 %.
- Drug release study of cyclosporine revealed that the drug was released in a remarkably controlled manner up to 6 h.

➢ Stability study revealed that the OF8 (optimized formulation) stable over a period of three months.

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Conflict of Interests

Authors declare that there is no conflict of interest.

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