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PREPARATION & EVALUATION OF ERYTHROMYCIN MICROEMULSION FOR OCULAR DRUG DELIVERY

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ABSTRACT

The aim of the present study is to prepare and evaluate erythromycin microemulsion for ophthalmic drug delivery. Microemulsions are in focus of recent research due to its advantages like it reduces the risk of toxicity, reduced dosing frequency, and the tendency to load both hydrophilic and lipophilic drugs.The first step in the current research was the preformulation studies of the drug. The given drug was subjected for different preformulation tests like organoleptic properties, melting point, determination of absorbance maxima (λ_{max}) by UV, preparation of standard curve in different solvents, FTIR, partition coefficient and solubility. After confirmation of the given drug, erythromycin loaded microemulsion was successfully prepared. Delivery of drugs into eyes using conventional dosage form or conventional drugs eg.solutions is a complicated and considerable challenges to the scientist for the treatment of ocular diseases. In Ocular drug delivery system the scientist faced many challenges due to unique structure, anatomy and physiology of eye. The aim of the present study was to develop Erythromycin Microemulsion for Ocular drug delivery. Micro emulsions (ME) are thermodynamically stable and clear mixtures of oil, surfactants, and water sometimes also available in combination with co surfactants. Erythromycin belongs to the class of Macrolide antibiotics & drug delivery through microemulsion via ocular enhaned bioavailability. The main objective of the present investigation was to formulate and characterise microemulsion for ocular drug delivery. 70% of the ophthalmic preparations are conventional dosage forms, extensive pre corneal loss caused by rapid drainage and high tear liquid are the main drawbacks associated with these systems, only 1 to 5% of the total drug penetrates into cornea and reaches to the intraocular tissue, to overcome these problems, microemulsion based systems are developed. The microemulsion was prepared by using different ingredients are as follows: oleic acid as an oil phase, tween 80 as surfactant, ethanol as co surfactant and 0.5 N NAOH as aqueous phase. The optimization and formulation chart was carried out using 23 factorial design. The microemulsions was prepared by using different ratios of oil and surfactant/co surfactant with water. The prepared microemulsions was evaluated for particle size, viscosity, and pH and % drug content. The particle size of prepared microemulsions was found in micro range that is **from 12.11 to 650.6 d.nm**. Drug content of all the prepared microemulsions was ranged from **79.55% to 95.56%.** In formulation FI and F5, by observing the value of n it was confirmed that the anomalous transport is dominant. Therefore it was concluded that prepared formulations was able to provide better management of eye diseases and hence improve patient compliance. After performing all evaluation parameters and observing every experimental results, formulation F1 was selected as optimized formulation. Therefore it was concluded that the optimized erythromycin microemulsion was effective in the treatment of eye diseases.

KEYWORDS: Microemulsion, Conventional dosage form, Thermodynamically, Amphiphile, patent, Drug content, Surfactant, Oleic acid, Tween 40 & 80, secretion, Conjunctivitis, Evaluation etc.

1. INTRODUCTION

Micro emulsions (ME) are thermodynamically stable and clear mixtures of oil, water and surfactants, and sometimes also available in combination with co surfactants & it is formed by surfactant, cosurfactant, oil phase, aqueous phase. In these types of preparations

there are basically 2 phases, one is aqueous phase and another one is oil phase. The aqueous phase comprises of salt and different fixings while the oil phase comprises of various hydrocarbons, oils and waxes.[1] The human eye is a Senstive and complex structure designed in a way that its physiology, anatomy, biochemistry, prephysiology parameters render or requital it almost imperviable to foreign agents, pathogens, impurities along with drugs.

The human eye is divided into two segments one is anterior segment & second one is posterior segment, in anterior segment (conjunctiva, cornea etc.) and in posterior segment (retina, vitreous humor etc.) in details it's shown in the figure1. Human eye, is the most complicated organ of human body, and also known as organ of sight and it is contained inside the cavity where it is completely protected from injuries and external damage. Eye has a spherical shape included in the orbital cavity and protected by lids, With a diameter of 24 mm and a volume of 6.5 cm3, it weigh is about 7.5g. In human eye the corneal epithelium serves or represent one of the most rate limiting barrier which inhibit or hinders permeation of macromolecules and hydrophilic drugs. In human eye another common rate limiting barrier is stroma which prevents the diffusion of highly lipophilic drugs. The diffusion of highly liophilic drugs due to high amount of hydrated collagen contents.[2]

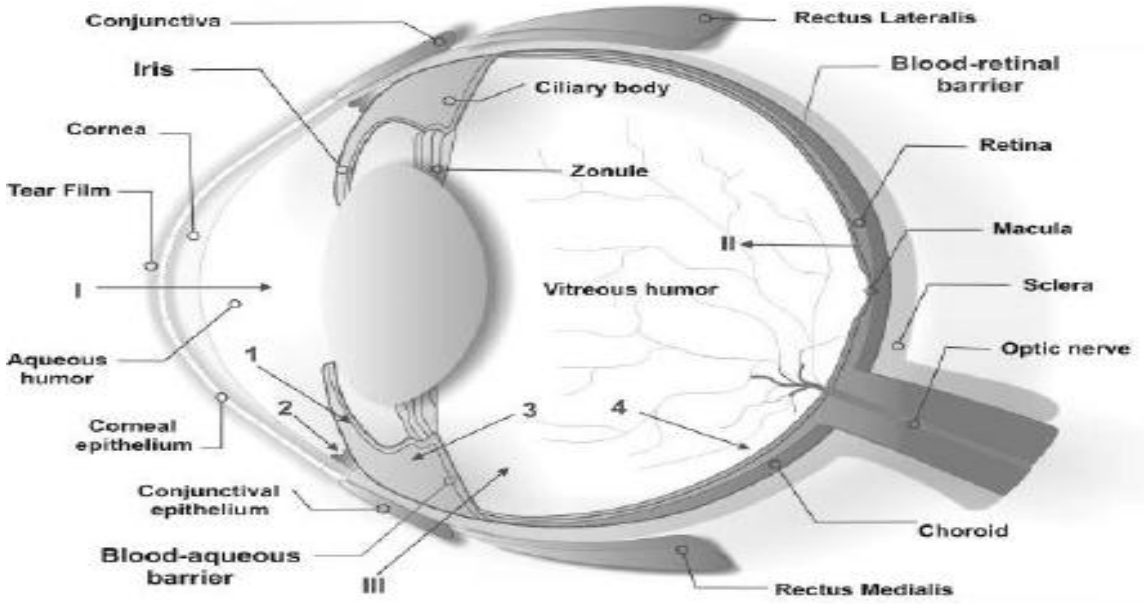

Figure1.1: Schematic illustration of ocular structures and barriers. Figure 1 is taken from Barriers et,al, 2009.[3,4]

Ocular administration of drug is primarily associated or linked with the need to treat ophthalmic Diseases & ocular dosage form prevent from infection and minimizes the chance of bacterial infection in the eye. These are specialized dosage forms designed in a manner to treat the infections or to be instilled onto the different surface of eye such as external surface of eye topical, administered inside intraocular surface or used in conjunction with an ophthalmic controlling device.[5] In human eye the topical application or administration of drugs is the most significant route for the treatment of diseases & minimizes the chances of infection in the various diseases like eye flu, dryness, redness in the eye, conjunctiva etc. Human eye have a protective criteria or mechanism such as baseline, reflex lachrymation, blinking and drainage decrease the bioavailability of drugs administered and these mechanism can also help to remove dust particle, pathogens, bacteria, including drugs from the surface of human eye.[6]

The following characteristics are required to optimize ocular drug delivery systems they are as follows[7]

- A prolonged contact time of drug with the corneal tissue.
- Good corneal penetration.
- Appropriate properties such as rheological properties and concentration of viscolyzer.
- Concentration of surfactant & co-surfactant.
- Patient awareness & know about the ocular diseases.
- Easiness of installation or administration and removal for the patient.
- Easy to administered.

The challenging task for pharmaceutical formulator or pharmaceutical researcher is to build up a formulation with enhanced visual maintenance, increased corneal absorption and lessened side effects therefore to beat and control this issue numerous plans and strategies have been produced, for example, various pharmaceutical formulation such as in situgels, nanoparticles, bio adhesive gels, liposomes, small scale emulsions.[8]

1.1 Anatomy & Physiology of Eye

Human eye, is the most sensitive & complicated organ of human body, human eye is located/ contained inside the cavity where it is protected from impurities, dust particle, injuries and external damages. Human eye is also known as organ of sight. In Ocular drug delivery system the scientist faced many challenges due to unique structure, anatomy and physiology of eye. Human eye with a diameter of 24nm & a volume of 6.5cm3, human eye weigh is about 7.5g. the human eye is composed of different layers & classified it in two segments: one is anterior and second one is posterior.

There are many factors which effects the administration of drug they are as follows[9,11]

1. Anantomical & physiological features

- Secretion of tears
- Blinking of human eye
- Reflex lachrymation
- Nasolachrimal drainage

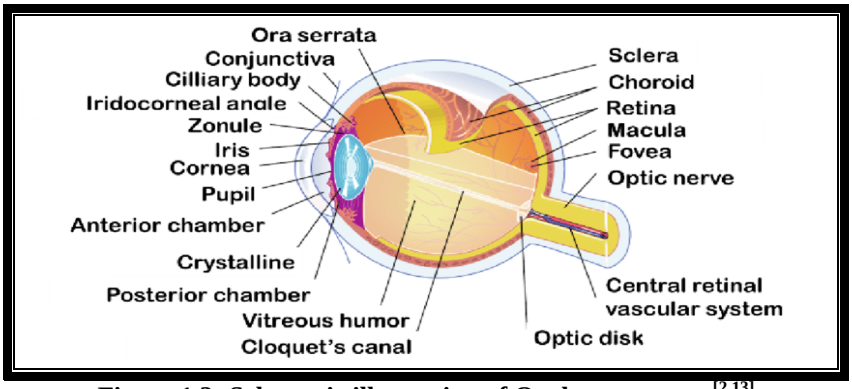

Figure 1.2: Schematic illustration of Ocular structure.[2,13]

1.1.1 Different Layers of Human Eye

Human eye is surrounded by three different layers they are as follows

- Outer layer
- Medium layer
- Inner layer

1. **The outer layer:** is composed by the cornea and the sclera. They are fibrous tissue and have a protective function for the eyeball.[14]
2. **The Middle layer:** is a vascular envelope additionally called uvea, made up of iris, the choroid and the ciliary body. The iris is a contractile, roundabout film opened at its middle. It supplies nutrients and oxygen to the iris and retinal photoreceptors.[15]
3. **The innermost layer:** tissue consist of retina and optic nerves, the neural tissue is composed of the photoreceptor (rods for the night and the peripheral vision and cones for the colour), the bipolar cells and the ganglion.[16]

1.1.2 Eye related Disease

Some eye problems are minor and go away. The disease, symptoms and treatment related to the eyes is given as well as following in table:

Table 1.1: Eyes, Disease, Symptoms and Treatments.[17]

DISEASE	SYMPTOMS	TREATMENTS
Glaucoma	Vision loss, Redness to the eye, Pain in the eyes	Eye drops, Laser surger
Low vision	Double vision, Headache, Blurred vision	Proper diet, and medicines
Eye infection	Pain/discomforting eyes, Itchy eyes, Burning in eyes, Eye won't stop tearing up	Broad-spectrum antibiotics, Macrolide antibiotics
Diabetic eye disease	Loss of vision, Discomfort in eyes	Healthy diet, Regular exercise
Conjunctivitis(pink eyes)	swollen conjunctiva, more tears than usual, itchy eyes, blurred vision	Antibiotics and Antiviral drugs

Table 1.2: Eyes Disease, marketed formulations and dosage forms.[18]

DISEASE	MARKETED FORMULATIONS	DOSAGE FORM
Glaucoma	Latanoprost (XALATAN), Travoprost (0.005%	Ophthalmic solution , Eye drops
Low vision	Dorzolamide, Brinzolamide	Ophthalmic Eye drops
e infection	Gatifloxacin(ZYMAR),Hydrocortisone	Hydrocortisone Ophthalmic eye drops, creams, lotions
Dry eye	Cyclosporine (0.05%), Lifitegrast (5%)	Ophthalmic emulsion, eye drops
Conjunctivitis (Pink Eyes)	Erythromycin, Azithromycin, Ciprofloxacin	Eye drops, ointments, suspensions

Table 1.3: Marketed Product of Ocular Drug Delivery System.[19]

Brand Name	Dosage form	Uses
Acuvail	8.5mg/ml ketorolac tromethamine solution (0.45%) in a single-use vial.	Cataract surgery
Alocril Elestat	2% is a clear, yellow, sterile solution 0.05% epinastine HCl ophthalmic solution	Allergic conjuctivis Allergic conjuctivis
Ozurdex Pred Forte	0.7 mg dexamethasone intravitreal ocular implant. 1% prednisolone acetate ophthalmic suspension, USP.	Retinal vein occlusion Bulbar conjunctiva
Zymar	0.3% gatifloxacin ophthalmic solution.	Bacterial conjunctiva
Zymaxid	0.5% gatifloxacin ophthalmic solution.	Bacterial conjunctiva
Trivaris	80mg/mltriamicinolone acetonide injectable suspension	Sympathetic ophthalmia

Various list of dosage form used in ocular drug delivery are mentioned below in table 3:

1.2 Microemulsion Science

Microemulsion can be defined in many ways, one of the best definitions of microemulsions is from Danielsson and Lindman "a microemulsion is a system of water, oil and an amphiphile which means having a tendancy of both hydrophilic and lipohilic componds or drug, which is a single optically isotropic and thermodynamically stable liquid solution". In these types of preparations there are basically 2 phases, one is aqueous phase and another one is oil phase. The aqueous phase comprises of salt and different fixings while the oil phase comprises of various hydrocarbons, oils and waxes. Microemulsion formed by using Surfactant, co surfactant, oil phase, & aqueous phase.[1]

1.2.1 Difference between emulsion & microemulsion

Microemulsion & emulsion can be defined or classified in many ways such as appearance, optical isotropy, droplet size, viscosity, surfactant concentration, size rang, miscelle diameter, microstructure, cost etc. Microemulsion have low surface pressure & little bead size in range (5-20nm), which may result about penetration and high medication retention.[20] Difference between emulsion and microemulsion are shown in table 1.4.

Property	Emulsion (Macro emulsion)	Microemulsion
Appearance	Cloudy	Transparent
Optical isotropy	Anisotropic	Isotropic
Microstructure	Static	Dynamic
Droplet size	>500 nm	20-200nm
Stability	Thermodynamically unstable	Thermodynamically stable and long shelf life
Size Range	0.5- 5 µ	<0.1 µ
Cost	Higher Cost	Lower Cost

1.2.2 Methods of preprations of microemulsion

Microemulsion prepared by following methods; these are given as follows;

1. Phase Titration Method
2. Phase Inversion Method

Phase Titration Method; The micro emulsions can be set up by the stage titration strategy (unconstrained emulsification technique which can be portrayed with the assistance of stage graphs). Construction of the phase diagram is a functional approach to study the number of events undergoing when different components are mixed. These are formed with different shapes including micelles hexagonal, cubic, and lamellar depending on the composition. As quaternary phase diagram is a time consuming study and difficult to elucidate, pseudo ternary phase diagrams are prepared to find the zones including micro emulsion zone in which each side represents a particular component. The regions are separated into o/w or w/o micro emulsion by simply taking into account weather it is oil rich or water rich.[23]

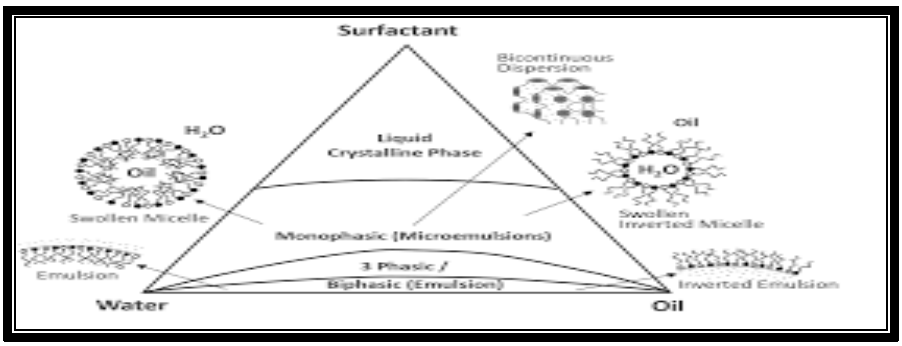

Figure 1.3: Pseudo ternary Phase Diagram of Oil, Water, and Surfactant of Microemulsion.

Phase Inversion Method; Reversal of the micro emulsion happens because of the addition of excess of the dispersed phase or in response to temperature. At the season of stage reversal, extreme physical changes happen which incorporates the adjustments in the molecule estimate additionally which can additionally influence the medication discharge both in vitro and in vivo. This technique uses the changing in the unconstrained arch of the surfactant. This can be achieved by changing the temperature of the System on account of Non-Ionic surfactants, which powers the progress from an O/w micro emulsion at low temperatures to a w/o micro emulsions at higher temperatures (transitional stage reversal.) at the season of cooling, the System crosses the purpose of zero unconstrained and flow insignificant surface strain, which advances the arrangement of the finely scattered oil beads. This strategy is additionally called the Phase Inversion Temperature Method (PIT). Be that as it may, rather than the temperature different parameters to be specific the pH esteem or the convergence of the salt can even be viewed as only rather than the temperature alone. Moreover, a progress in the unconstrained range of ebb and flow can be acquired by changing the water volume division. By progressively including water into oil, at first water beads are shaped in a continuous oil stage. By just simply expanding the water volume part changes the unconstrained ebb and flow of the surfactant from at first settling a w/o micro emulsion to an o/w micro emulsion at the reversal locus. The short-tied surfactants frame adaptable monolayers at the o/w interface bringing about a bi-consistent micro emulsion at the reversal point.[24]

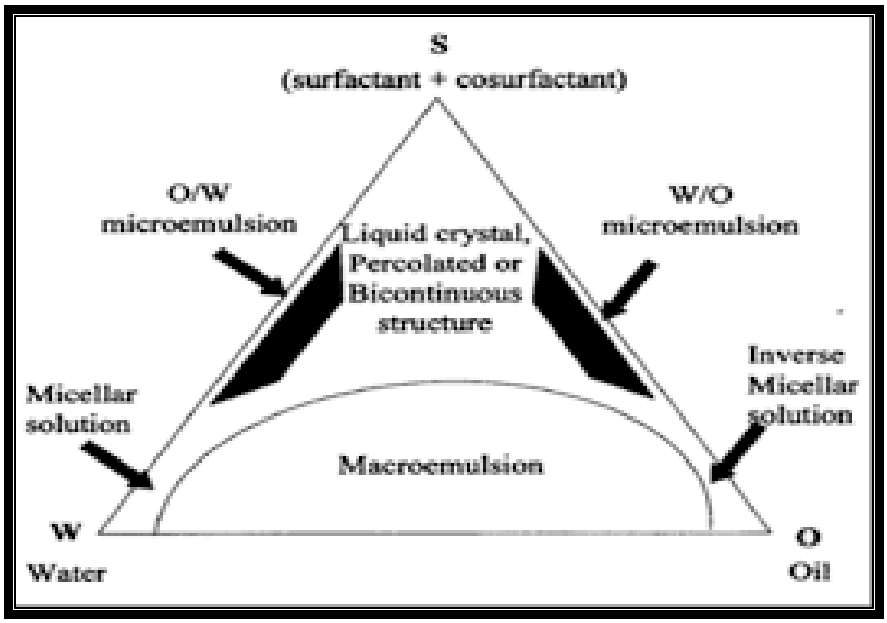

Figure 1.4: Hypothetical Phase region of Microemulsion system.

1.2.3 Chracterization of microemulsion

Characterization of microemulsion can be divide into 3 major parameters they are as follows physical evaluation, electrochemical evaluation, and microscopic evaluation. Physical nature of microemulsion can be determine by appearance, viscosity, and optical clarity etc. Conductivity measurements can be used to determine whether a microemulsion is oil-continuous or water continuous and may also be used to monitor or evaluate percolation or phase inversion phenomena. To investigate both the structural and dynamic features of microemulsion dilectric measurement have been used. Small droplet size of microemulsion give an indication that the microemulsion are formed slightly & shows optical clarity which is evaluated by microscopic methods & also with light scattering method.[25]

The basic components for the physiochemical characterization of microemulsion are as follows:

- Shape.
- Size.
- Surfactant & co surfactant ratio.
- Interfacial tension.
- Interaction and dynamics.
- The local molecular rearrangement.
- Phase behaviour and phase stability properties.
- The dimensions of the micro emulsion.
- Microstructure.
- Centrifugation; The micro emulsion systems can be centrifuged at 5000 R.P.M. for 30 minutes and then checked for phase separation.[26]

1.2.4 Advantages of microemulsion in ophthalmic drug delivery

- Ability to carry both hydrophilic & lipophilic drug.
- It is used as bioavilability enhancer for poorly water soluble drug.

- Good corneal penetration.
- High capacity of drug holding.
- Maximise ocular drug absorption through prolong contact time with corneal tissue.
- Reduces the frequency of drug adminstartion.
- It can improve the efficacy of a drug, minimizing side effects with reduced total dose.[27]

1.2.5 Components of microemulsion[28-33]

"A microemulsion is a system of water, oil and an amphiphile which means having a tendancy of both hydrophilic and lipohilic componds or drug, which is a single optically isotropic and thermodynamically stable liquid solution". Basically Microemulsion formulation involves the following components,

The components of microemulsion are given as well as following;

- Oil phase
- Aqueous phase
- Primary surfactant
- Secondary surfactant or co-surfactant

1) Oil phase

The oil being one of the most important excipients in the formulation not only because it can solubilise the required measurement of the lipophilic medication, it can expand the part of lipophilic medication transported by means of the lntestinal lymphatic system, along these lines expanding retention from the GI tract relying upon the atomic idea of the triglyceride. The oil segment impacts shape by its capacity to enter and subsequently swell the tail gather district of the surfactant monolayer. Short chain oils infiltrate the tail aggregate area to a more prominent degree than long chain alkanes, and subsequently swell the district to a more prominent degree, bringing about expanded negative flow (decreased powerful HLB).

Different oil are used in the formulation of micro-emulsion they are as follows:

- Saturated fatty acid-lauric acid, myristic acid, capric acid
- Unsaturated fatty acid-oleic acid, linoleic acid, capric acid
- Fatty acid ester-ethyl or methyl esters of lauric, linolenic acid

Saturated and unsaturated fatty acid have penetration enhancing property of their own and they have been studied since a long time.

2) Aqueous phase

The aqueous phase can have two additives and hydrophilic dynamic fixings. The most ordinarily utilized watery stage is water. It is because of the impact on the stage conduct of microemulsion which is mainly utilized for parentral organization, the fluid stage must be iso-osmotic to the blood which is highly balanced by glycerol, sorbitol and sodium chloride.

3) Primary surfactant

Surfactants are the molecules which when present in low concentration will adsorb to the face of interfaces of a system and alter the interfacial energies of the system. The interfacial vitality is the work required to make unit region of an interface. The real motivation behind surfactant is to bring down the interfacial strain to irrelevant esteem that encourages the procedure of scattering amid planning of microemulsion. It gives the microemulsion appropriate lipophilic character to outfit exact ebb and flow. This adsorption conduct can be credited to dissolvable nature and to the compound idea of surfactant that joins both polar and non-polar gartering in a solitary atom. Because of their double nature these amphiphilic "sit at interfaces so their hydrophobic moiety is repulsed from solid dissolvable associations. Surfactant screening should be possible with help of HLB (Hydrophilic lipophilic adjust) esteem. The HLB gives a numerical esteem that proposes whether o/w or w/o emulsion will shape.

The surfactants that are used to stabilize the microemulsion system can be

a) Non-ionic
b) Zwitter-ionic
a. Cationic, or Anionic surfactants

The surfactants which arecommonly preferred for the formulation of the W/O water in oil microemulsion are generally of low HLB values (3-6). On the other hand for the formation of O/W microemulsion, the surfactants with high HLB values (8-16) are favoured. The surfactants which have the HLB values higher than 20 usually needs to be added with co-surfactants which adjusts their effective HLB value within the range which is required for the formation of the microemulsion.

Table 1.5 HLB ranges and the typical applications of related surfactants.

S.NO	HLB value	Application
1.	2-3	Antifoaming agents
2.	3-6	Water in Oil Emulsion
3.	7-9	Wetting and Spreading Agents
4.	8-16	Oil in Water Emulsion
5.	13-15	Detergents
6.	15-18	Solubilizers

4) Co-Surfactant

It has been found that single-chain surfactants alone are unable to reduce the o/w oil in water interfacial tension sufficiently to enable a microemulsion to form. The nearness of co-surfactants permits the interfacial film adequate adaptability to take up various arches required to frame microemulsion over an extensive variety of structure. In the event that a solitary surfactant film is

wanted, the lipophilic chains of the surfactant ought to be adequately short, or contain fluidizing gatherings (e.g. Unsaturated Bonds). Short to medium chain length alcohols (C3 C8) are generally included as co surfactants which additionally diminish the interfacial strain and increment the ease of the interface. Average co-surfactants are short chain alcohols (Ethanol to Butanol), glycols, for example, propylene glycol, medium chain alcohols, amines or acids. The utilization of co-surfactant is to wreck fluid crystalline or gel structures that shape instead of a microemulsion stage and co-surfactant free microemulsion in most System can't be made with the exception of at high temperature.

1.3 Disease profile[34-36]

Bacterial conjunctivitis, also known as pink eye, is inflammation of the outermost layer of the white part of the eye and the inner surface of the eyelid It makes the eye appear pink or reddish Pain, burning, scratchiness. or itchiness may occur The affected eye may have increased tears in the morning, Swelling of the white part of the eye may also occur. Itching is more commonside effect or reaction in cases due to allergies. Conjunctivitis can affect one or both eyes. Various risk factors for bacterial keratitis are those that cause dismission of the integrity of the corneal epithelium. The most common risk factor for bacterial keratitis is contact lens wear Contact lens wear has been associated with 19%-42% of cases of culture proven corneal infections. Overnight wear and inadequate or inproper lens disinfection have been associated with increased risk of infection. Other prodoposing factions include trauma (muhading fireign bodies and chemical and thermal injuries), contaminated ocular solutions, changes in the corneal surface (from dry eye, eyelid misdirection, and exposure), shered sedar defense mechanisms (from topical and systemic immune suppressioni, kose sutures with adjacent infections (hlepharis and viral keratitis), and corneal edema. According to a research it was found that In younger patients, trauma and contact lens wear are the most common predisposing factors while in comparison to older patients, chronic corneal disease such as dry eyes, surgical trauma, and bullous keratopathy are also important causes

1.3.1 Types of Bacterial Conjunctivitis

- Keratais bacteria come in two varieties. The outer layers of the cornea are affected by superficial keratitis, which normally cures without leaving a scar.
- Deep keratitis affects the cornea's deeper layers and might leave a scar after it heals. This can have a long-term effect on vision

1.3.2 Causes of bacterial conjunctivitis

The following are the two types of bacteria that most usually cause bacterial keratitis:
Staphylococcus aureus and Pseudomonas aeruginosa
Others are

- Recent injury to the eye
- Recent refractive corneal surgery
- Immune system deficiency
- Abnormal eyelids

1.3.3 Symptoms of bacterial conjunctivitis: pain in the eye (often sudden)

- Unusual eye redness
- Reduced vision
- Increased light sensitivity
- Excessive tearing
- Discharge from your eye

1.3.4 Advantages of Microemulsion in ophthalmic drug delivery

- High drug holding capacity, and good corneal penetration.
- It is used as bioavailability enhancers for poorly water soluble drug.
- Maximise ocular drug absorption through prolong contact time with corneal tissue.
- It reduces the frequency of administration.
- Minimal pre corneal drug loss.
- Doesn't cause blurred vision and non-greasy

1.3.5 Challenges in microemulsion based Ocular Drug delivery

- The concentration of surfactants and co-surfactants used must be kept low for toxicological reasons.
- Micro emulsion also suffers from limitations of phase separation.
- Sometimes causes blurring of vision.
- Dosage form cannot be terminate easily during emergency.
- Occasional drug loss during sleep or while rubbing eyes.

1.3.5 Treatments

Antibiotic	Class	Marketed available formulations
Azithromycin	Macrolide	Azasite 1
Besifloxacin	Fluroquinolone	Besivance0.6%
Chloramphenicol	Chloramphenicol	Optrex infected eyes 0.5%
Ciprofloxacin	Fluroquinolone	Ciloxan0.3%
Gatifloxacin	Fluroquinolone	Zymar 0.3%
Gentamycin	Aminoglycoside	Genric0.3% drops

4. MATERIALS AND METHODS

4.1 Materials

Table 4.1.1 list of material used.

S. NO.	NAME OF MATERIAL	SOURCE/ SUPPLIER
1	Erythromycin	Medmenor (P) Ltd Bhagwanpur
2	Oleic acid	Central drug house (P) Ltd.
3	Tween 80	Central drug house (P) Ltd.
4	Ethanol	Central drug house (P) Ltd.
5	Span 80	Central drug house (P) Ltd.
6	Distilled water	Central drug house (P) Ltd.
7	Castor oil	Central drug house (P) Ltd.

Table 4.1.2: List of equipment and instruments used.

S. NO.	EQUIPMENTS	SPECIFICATIONS
1	Digital weighing balance	Sansui, Japan
2	Magnetic stirrer	Remi Elecktrotechnik
3	P^H meter	Elico, Japan
4	Bath sonicator	Sonar India
5	UV spectrophotometer	Carywin UV Agilent technologies
6	FTIR	Perkin Elmer Spectrum Two
7	Digital melting point apparatus	Rexnord model REC-28025A2
8	Viscometer	Brokfield, India

4.2 Drug profile

Erythromycin[67-69]

Erythromycin is a broad-spectrum, macrolide antibiotics with antibacterial spectrum similar to or slightly wider than that of penicillin, and is often used for people who have an allergy to penicillin. For respiratory tract infection, it has better coverage of atypical organism, including mycoplasma. Adminstration of erythromycin is used to treat trachoma.

4.2.1 Chemical structure

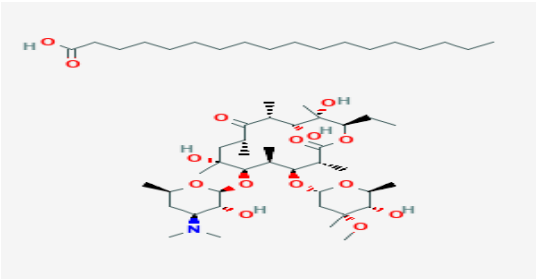

Fig. 4.1: Chemical structure of erythromycin drug.

4.2.2 Chemical and physical properties of drug.

Name of drug	Erythromycin
Chemical name	4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-7,12,13-trihydroxy-4-[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,7
Molecular formula	$C_{37}H_{67}NO_{13}$
Molecular Weight	733.937g/mol
Melting point	135-140°C
Solubility	Very soluble in acetone, ethanol, and methanol and freely soluble in chloroform
Appearance	Fine white powder
Log P (Octanol/ water) and protein bindin	3.06 and 90%

4.2.3 Pharmacokinetics

Absorption: Orally administered erythromycin base and its salts are easily absorbed from substances with microbial activity. Topical application of ophthalmic ointment to the eyes can cause absorption into the cornea and aqueous humor.

Distribution: It is widely distributed in most body tissues and fluids, except cerebrospinal fluid, and only a small amount is distributed in cerebrospinal fluid. The drug crosses the barrier and approximately 80% of the alkali and 96% of the erythromycin estolate protein bound.

Metabolism: Most of erythromycin is metabolized by demethylation in the liver by the hepatic enzyme CYP3A4.

Elimination: elimination half-life ranges between 1.5 and 2.0 hours and is between 5and 6 hours in patients with end-stage renal disease. Its main elimination route is the bile with little reneal excretion, 2%-15% unchanged drug.

Pharmacokinetic properties	
Bioavailbility	30-65%
Elimination half life	1.5 hours
Metabolism	Liver
Dose	0.5%w/v

4.2.4 Pharmacodynamics

Erythromycin is a broad-spectrum, macrolide antibiotic with antibacterial activity. Erythromycin diffuses through the bacterial cell membrane and reversibly binds to the 50S subunit of the bacterial ribosome. This prevents bacterial protein synthesis Erythromycin may be bacteriostatic or bactericidal in action, depending on the concentration of the drug at the site of infection and the susceptibility of the organism involved.

4.3 Methods

Preformulation studies related to drug

Preformulation studies of pure drug are the first step in the rational development of dosage form of a drug substance. The object of pre formulation studies are to develop a portfolio of information about the drug substance, so that this information useful to develop formulation. Preformulation can be defined as investigation of physical and chemical properties of drug substances alone and when combined with excipients.

4.3.1 Organoleptic properties

Preformulation studies are defined as an investigation of physical and chemical properties of drug. The organoleptic properties of the drug were observed by physical examination.[70]

4.3.2 Melting Point

The melting point of the sample was calculated using the capillary melting method. Initially, take a single-sided fused capillary and fill it with the sample. Then place the capillary and thermometer in the sample tank of the melting point apparatus. Record the temperature range in which the sample begins to melt and completely melt.[71]

4.3.3 Identification of drug by FTIR

Perform Fourier Transform Infrared (FTIR) studies to verify any physical or chemical interactions between the drug and the excipients used in the formulation. Comparing the spectra obtained from functional groups, FTIR studies of pure erythromycin and optimized formulations were carried out. It was performed or implemented by Diamond ATR (model 630). After cleaning the glass and collecting the infrared background, simply pour the liquid onto the glass. All glass or crystal must be covered for qualitative and quantitative analysis. The glass is recessed into the metal plate to retain the sample. Horizontal ATR devices are used to quantify transmission cell preferences because they are easier to clean and maintain. Once the liquid sample has been placed on the crystal area, the pressure arm should be positioned over the crystal/sample area. When using Spectrum 100 series universal ATR accessories, the pressure arm is locked in a precise position on the diamond crystal. Apply force to the sample to push it towards the diamond surface.[72,74]

4.3.4 Determination of absorbance maxima (max) by UV

To determine the maximum absorbance of the drug, 25 mg of the drug was dissolved in 25 ml of phosphate buffer and the same as methanol, and a stock solution was prepared to obtain a solution concentration of 1 mg/ml. A 10 µg/ml dilution was prepared from the stock solution and analyzed by UV at Amax 285 nm between 200,400 nm to obtain the maximum wavelength of the drug.[75]

4.3.5 Solubility analysis

The solubility of the drug was determined in different solvent systems. A sufficient amount of drug was added to 5 ml of each solvent in the volume flask and stirred. The samples were maintained at room temperature: 24 hours. After 24 hours, the sample were filtered and diluted and examined for the absence or presence of drug particles. Determination of qualitative solubility by UV spectrophotometer at 285 nm.[76]

Table 4.3.5: Standard table of solubility as per IP.

S. NO	Descriptive term	Parts of solvent required for 1part of solute
1	Very soluble	<1
2	Freely soluble	From 1-10
3	Soluble	From 10-30
4	Sparingly soluble	From 30-100

5	Slightly soluble	From 100-1000
6	Very slightly soluble	From 100-10,000
7	Practically insoluble, Soluble	10,000 or more

4.3.6 Partition coefficient determination

Calculate the partition coefficient of the drug in n-octanol and phosphate buffer by the shake flask method. Add 25ml of phosphate buffer and 25ml of n-octanol into the separatory funnel and shake for half an hour. 25 mg of the drug was added to the mixture, the solution was stirred for a further 2 hours, and then the phase separation was maintained. After phase separation, the aqueous phase is collected and fully diluted, and the amount of drug is determined by measuring the absorbance.[77]

Partition coefficient, P = [Organic] /[Aqueous] Where []-Concentration Log P log 10 (partition coefficient)

4.3.7 Preparation of standard curve in different solvents

50 mg drug was accurately weighed and transferred to 50 ml of volumetric flask and dissolved in 50ml of solvent. From the stock solution dilutions having concentration Jug/ml 6pg/ml 9µg/ml, 12µg/ml, 15µg/ml (Beer's range 0-15µg/ml) was prepared These dilutions were observed in UV Spectrophotometer and absorbance was measured at 285mm.[78]

4.3.8 Drug- Excipient compatibility study

Fourier Transform Infrared spectra of drug with polymer was taken by using Diamond ATR (model no 630) technique using Albunt FTIR Spectrophotometer in the wavelength region of 100 to 4000cm The procedure consist of dispersing ATR spectrophotometer Force is applied to the sample, pushing it into the diamond surface. After the spectrum has been collected which should typically take no more than 32 seconds, the user must return to the preview mode.[79]

4.9 Preparation of microemulsion

In this method erythromycin microemulsion was prepared by mixing the adequate quantity of drug in oleic acid le. (oil phase) for about 20 minutes at 200 rpm in magnetic stirrer. Then the mixture of surfactant and co-surfactant (Tween 80 was taken as surfactant and ethanol was taken as a co-surfactant which provides clarity to the microemulsion) was added in the oil phase through pippete and allow it to rotate at 300 rpm, finally an appropriate amount of aqueous phase (Water) was added to the mixture drop by drop to get a clear and transparent microemulsion.[80]

Table 4.9: Formulation table of microemulsion.

Formulation code	Ratio	S/co Ratio(1:1)	Oil (ml)	Water (ml)	Total (ml)
F1	9:1	4.5	0.5	5ml	10
F2	8:2	4	1	5ml	10ml
F3	7:3	3.5	1.5	5ml	10ml
F4	6:4	3	2	5ml	10ml
F5	5:5	2.5	2.5	5ml	10ml

4.10: Evaluation of prepared erythromycin

4.10.1 Determination of pH

The pH of the prepared microemulsion was determined using calibrated digital pH meter. In this method 1gm of microemulsion was added and dissolved in 100ml of distilled water and kept for 2 hours. Then, the pH of the following medium was measured using digital pH meter.[81]

4.10.2 Viscosity

Viscosity of the prepared microemulsion was evaluated by using Brookfield viscometer. The prepared microemulsion was subjected to a specific spindle at different rpm. The data was collected during the procedure and a graph was prepared to determine the viscosity.[82]

4.10.3 Drug content

For determination of drug content, microemulsion containing 100mg drug was dissolved in 100ml of 0. IN HCL taken in volumetric flask Then the solvent was filtered, 1 ml was taken in 50ml volumetric flask and diluted up to the mark with 0. IN HCL and then subjected to UV spectrophotometer at 285mm.[83]

4.10.4 Particle size distribution, and PDI study

Particle size of the micro formulation was determined by using Differential light scattering with a zetasizer. The micro particles were dispersed into water and diluted accordingly. This solution was then analysed using zetasizer for particle size. List of polydispersity index for different type of dispersion were like.

Polydispersity index	Types of dispersion
0-0.05	Monodisperse standard
0.05-0.08	Nearly monodisperse
0.08-0.07	Mid-range polydisperse
>0.7	Very polydisperse

5. RESULTS AND DISCUSSION

In the present study, several different formulations were prepared by varying the ratios of oil and surfactant/co-

surfactant Before developing formulations an extemive Preformulation studies were abo conducted.

Preformulation studies

5.1 Organoleptic Properties

The study of organoleptic properties was conducted through visual observation using senses. From the above data it was observed that the organoleptic properties of test drug matches with the given reported description Experimental result reported on the **(Table no 5.1)**

5.2 Melting Point

The melting point study was conducted using digital melting point apparatus. The experimental result (**Table no 5.2)** reveals that the observed average melting point value was 138.3°C for test drug which matches with the standard given value. This can be used as preliminary identification tool for drug.

5.3 Identification of drug by FTIR

The IR spectrum of the test drug was obtained by using FTIR instrument. The obtained FTIR spectrum of drug **(Fig 5.1)** shows the detection of different functional groups **(Table no 5.3)** which was compared with the standard spectra **(Fig 5.2)** no significant difference was observed hence, it was confirmed that the obtained sample is Erythromycin.

5.4 Determination of absorbance maxima (λ) by UV

The absorbance maxima of test drug was determined using UV spectrophotometer The absorbance maxima of the test drug in methanol was observed at 285am which perfectly matched the standied value given in I.P 2014.

5.5 Solubility analysis

The solubility of the test drug was performed by prescribed method. The prepared solution subjected to UV for determination of absorbance at specific wavelength (285mm) As per the standand table given in I.P, regarding solubility, the observed data was compared and reported **(Table 5.4)** In respect of all solvents obtained test sample produce solubility data in specified range which proves the purity of the substance.

5.6 Partition coefficient determination

Partition coefficient of the drug was calculated by shaking flask method using n-octanol and phosphate buffer pH 74, as oil and aqueous phase respectively Concentration of drug in oil and aqueous phase was determined and reported in **(Table no 5.5)** the obtained result proves the lipophilicity characteristics of erythromycin, hence makes it a good candidate for corneal permeation.

5.7 Preparation of standard curve in different solvent

Standard curve of test drug (Erythromycm) was prepared in different solvents such as methanol and phosphate buffer pH 7.4 **(Table no 5.6)** The r^2 value was found to be 0999 and 0.9994 respectively. The observed r^2 value of sample suggested that the linearity in equation So, this equation can be used for further calculation purpose **(Fig 5.3, 5.4).**

5.8 Drug- Excipient compatibility study

Drug-excipient Compatibility study was performed by using FTIR instrument. After comparing the peaks of the pure drug with drug and excipients, **(Table no 5.7)** no significant difference was found. Thus, confirming the compatibility of drug with excipients **(Fig 5.5. 5.6).**

Evaluation of erythromycin microemulsion

5.9 Determination of pH

The pH of all formulations were found to be in the range of 6.9±0.04 to 7.4±0.12 as shown in **(Table no 5.9)** and **(Fig 5.16)** Which perfectly suited with ophthalmic pH environmental requirements Thus may not produce any irritation after application.

5.10 Viscosity

The viscosity of prepared microemulsion was evaluated using Brookfield viscometer **(Table no 5.10)**, the graph was plotted between rpm and viscosity **(Fig 5.17)** and shear stress **(Fig 5.18).** The viscosity of all formulations ranges from 119±0.79 to 153±40.83cp. The graph plotted confirm the pseudo plastic nature of flow of prepared formulation.

5.11 Drug content

The drug content of the prepared formulations was reported in **(Table no 5.11)**, the drug content estimation was done by measuring absorbance using UV spectrophotometer. Drug content of all formulations was found to be between 79.55±0.33 to 95.56±0.23 **(Fig 5.19)**. Experimental results suggested that amount of surfactant and co-surfactant present in formulations affect loading of drug in microemulsion. As the amount of surfactant/co-surfactant increases in the formulation the loading of drug also increases significantly.

5.12 Particle size determination

The particle size of prepared formulations were evaluated using zetasizer. The particle size of the prepared formulations lied in a range 12.11±0.15 to 743.4± 0.29nn **(Table no 5.12)**. Formulation FI was found to have the smallest particle size 12.11 with 100% intensity **(Fig5.20 to 5.24)** From the above data it was concluded that with increase in surfactant and co-surfactant ratio with external phase preparation contributed to smaller particle size. Which leads to promote corneal –permation.

Table 5.1:Organoleptic Properties.

S.no	Organoleptic properties	Result
1	Description	Yellowish white

		powder
2	Taste	Biter
3	Odour	Odourless

4	Colour	Off white

Table 5.2: Melting point determination of drug.

S.no	Observed melting point of test sample	Average	Standard melting point range
1	138.2-139.3°C	138.3°C	135-140°C
2	138.5-139.4°C		
3	138.3-139.7°C		

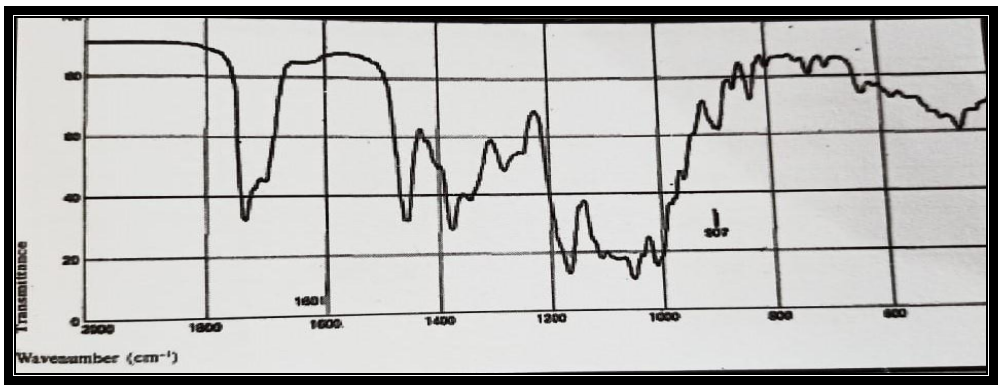

Fig.5.1: IR Spectra of eryrthromycin (I.P2014).

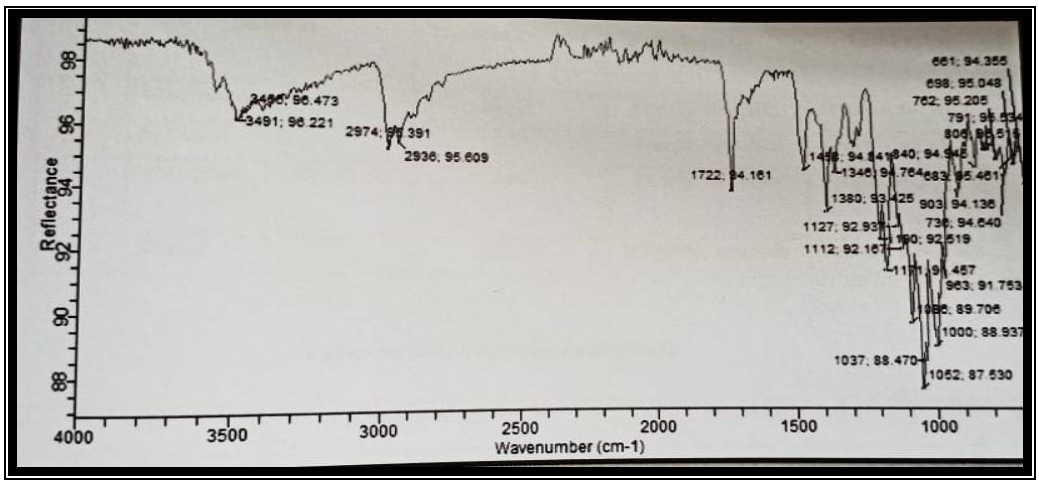

Fig. 5.2: IR Spectra Test (erythromycin).

Table 5.3: Functional group detection of erythromycin.

Material	Standard	Test	Peaks of functional group assignment
Erythromycin	1870-1540	1722	C=O
	1440-1395	1380	C-OH
	1375-1450	1348	CH_3
	2800-3000	2930	Alkane, streching
	2815-2832	2974	O-CH_3 Bending

Table 5.4: Solubilites study of drug in different solvents.

S.no	Solvent	Concentration	Solubility	Indication
1	Methanol	5.53	Freely soluble	From 1 to 10
2	Ethanol	5.11	Freely soluble	From 1 to 10
3	Phosphate buffer(7.4)	5.67	Freely soluble	From 10-10
4	Water	120.22	Slightly soluble	From100-1000

Table 5.5: Partition Coefficient.

S.no	Observed value	Standard log P
1	2.702	3.06

Table 5.6: Standard curve data of erythromycin in methanol.

S.no	Concentration (ug/ml)	Absorbance in methanol	Bsorbance in phosphate buffer (pH7.4)
1	0	0	0
2	3	0.1011	0.33
3	6	0.1569	0.62
4	9	0.2179	0.92
5	12	0.2841	1.24
6	15	0.3576	1.51

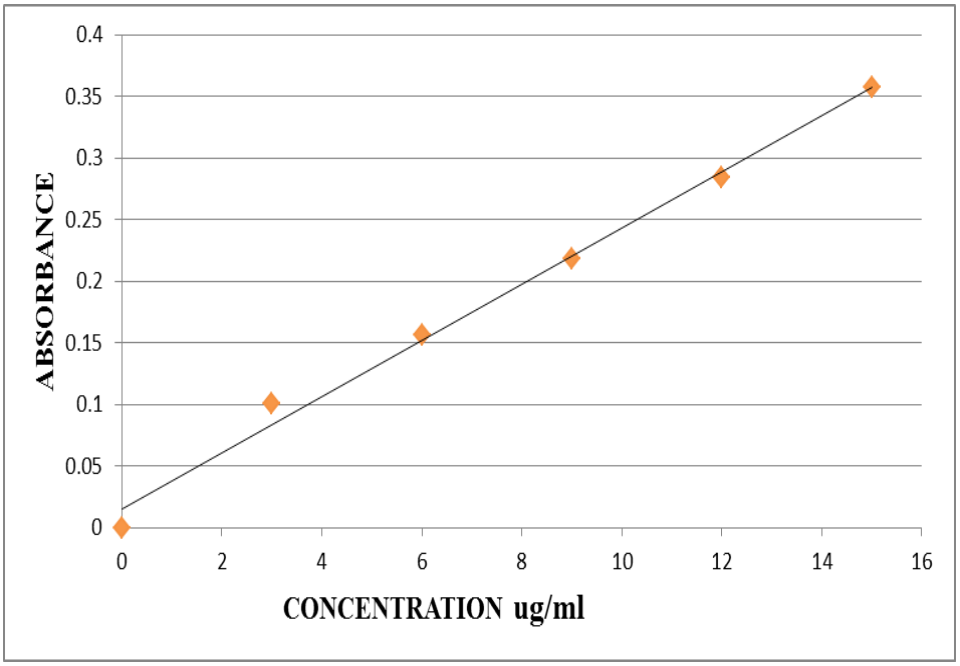

Fig. 5.3: Standard curve of erythromycin in methanol.

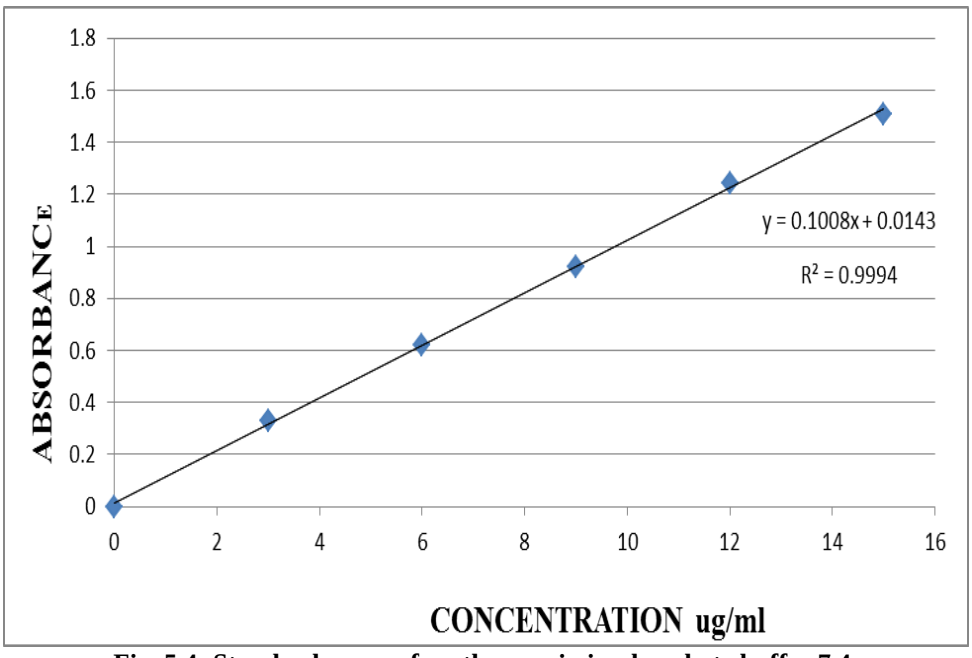

Fig. 5.4: Standard curve of erythromycin in phosphate buffer 7.4.

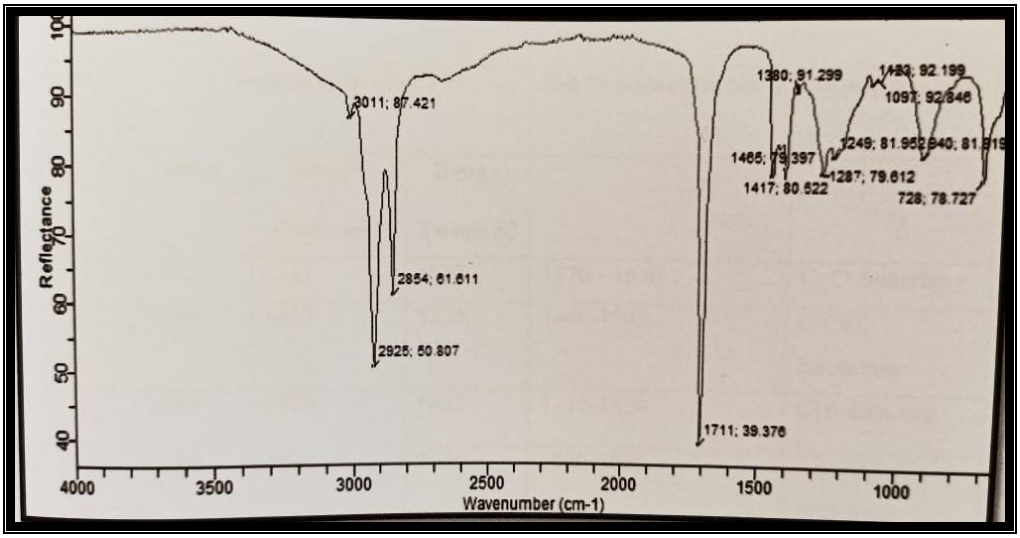

Fig. 5.5: IR of erythromycin & oleic acid.

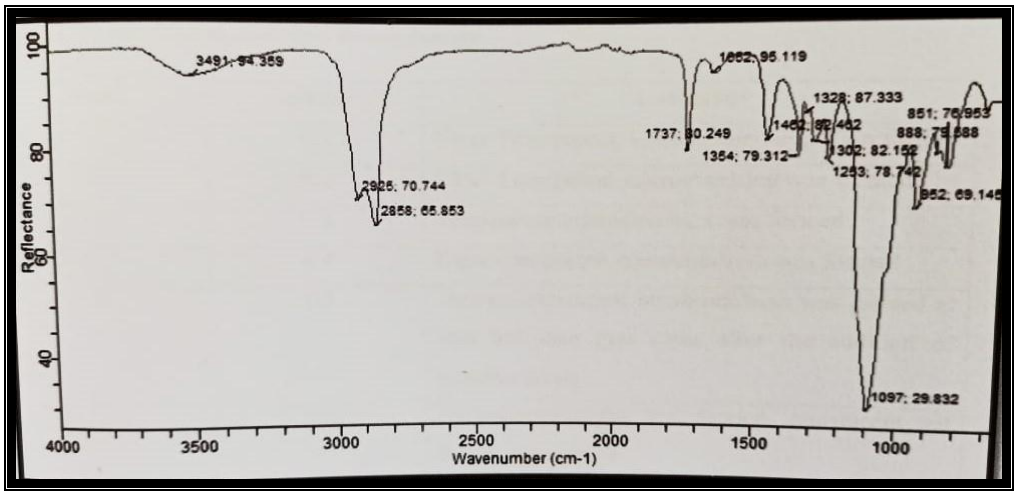

Fig. 5.6: IR of erythromycin and tween 80.

Table 5.7: Comparison of peak of functional group of pure drug and excipient mixture.

S. no.	Wavelength (cm^{-1})			Standard wavelength (cm^{-1})	Interpetation
	Drug	Drug + Oleic acid	Drug + Tween 80		
1	1722	1711	1652	1870-1540	C=O Streching
2	1380	1417	1354	1440-1395	C-OH, Streching
3	1348	1380	1452	1375-1450	CH_3 Bending
4	2930	3011	2925	2800-3000	Alkane, stretching
5	2974	2854	2858	2815-2832	O-CH_3 Bending

Table 5.8: After conducting all Preformulation studies related to drug , several trials were conducted for formulation and development.

Trial	Ratio	Comment
1	9:1	Clear transparent, viscous microemulsion formed
2	8:2	Clear transparent microemulsion was formed
3	7:3	Transparent microemulsion was formed
4	6:4	Clear transparent microemulsion was formed
5	5:5	Viscous translucent microemulsion was formed at first but then gets clear after the addition of aqueous phase
6	4:6	No microemulsion was formed, translucent gel like in appearance
7	3:7	White, opaque Emulsion was formed
8	2:8	Microemulsion was not formed
9	1:9	Microemulsion was not formed

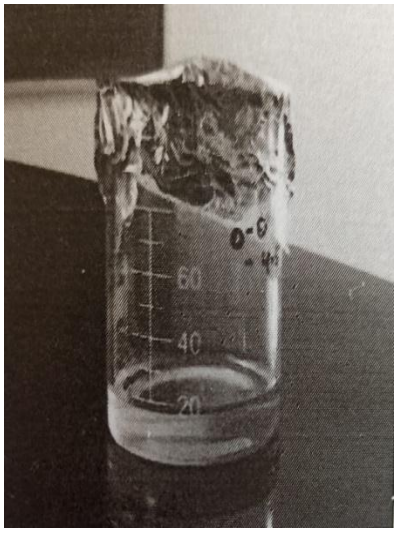

Fig. 5.7: Trial 1

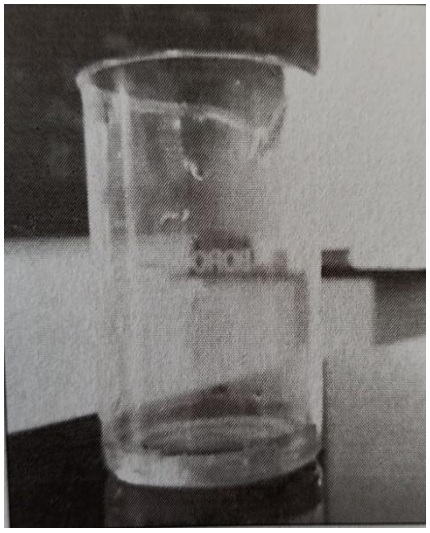

Fig. 5.8: Trial 2

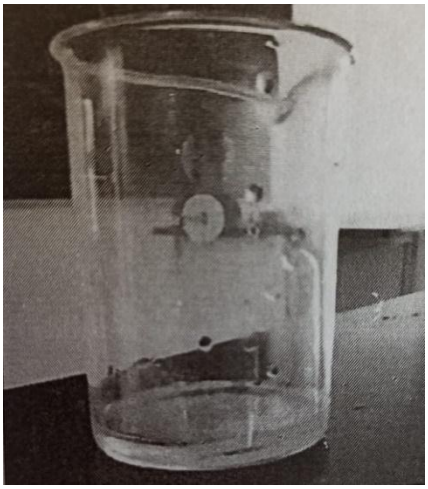

Fig. 5.9 Trial 3

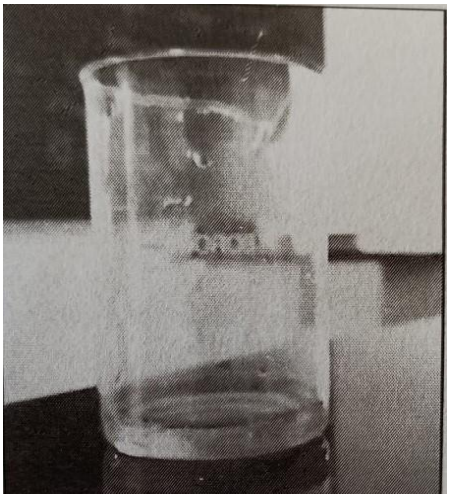

Fig. 5.10 Trial 4

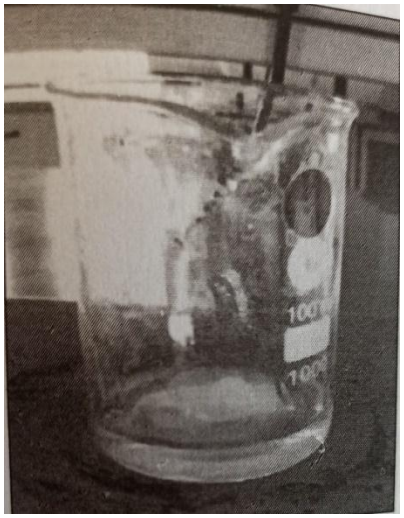

Fig. 5.11: Trial 5

Fig. 5.12: Trial 6

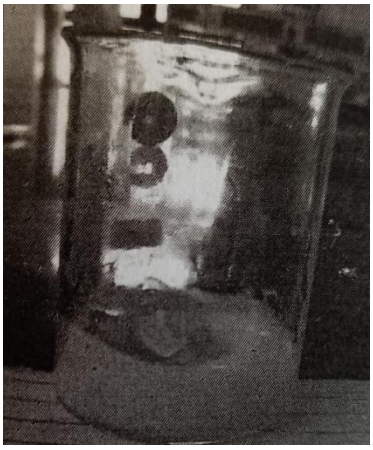

Fig. 5.13: Trial 7

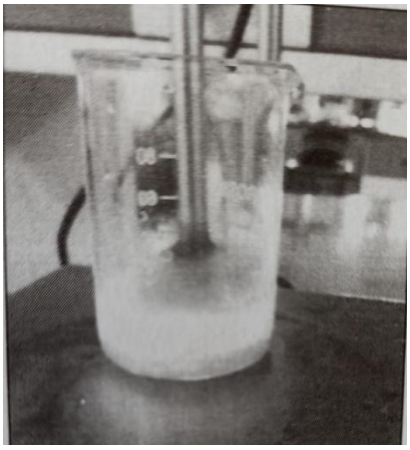

Fig. 5.14: Trial 8

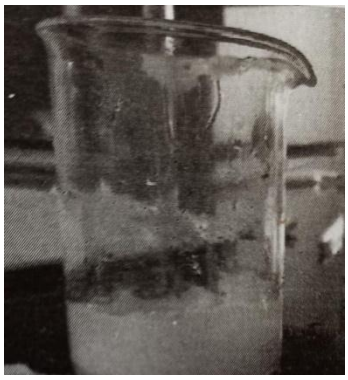

Fig. 5.15: Trial 9

Table 5.9: pH of prepared of formulations.

S.no	Formulation code	pH
1	F1	7.4±0.12
2	F2	7.0±0.09
3	F3	7.2±0.03
4	F4	7.3±0.15
5	F5	6.9±0.04

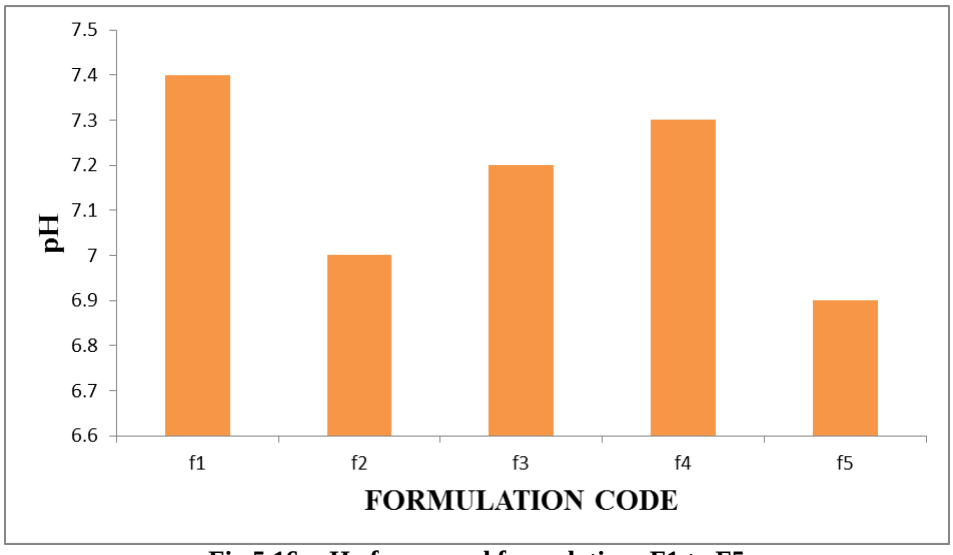

Fig 5.16: pH of prepared formulations F1 to F5

Table 5.10: Viscosity of prepared formulations.

S.no	Formulation code	Viscosity (Cp)
1	F1	119.23±0.83
2	F2	123.10±0.92
3	F3	137.45±1.19
4	F4	145.66±1.26
5	F5	1.53.85±0.79

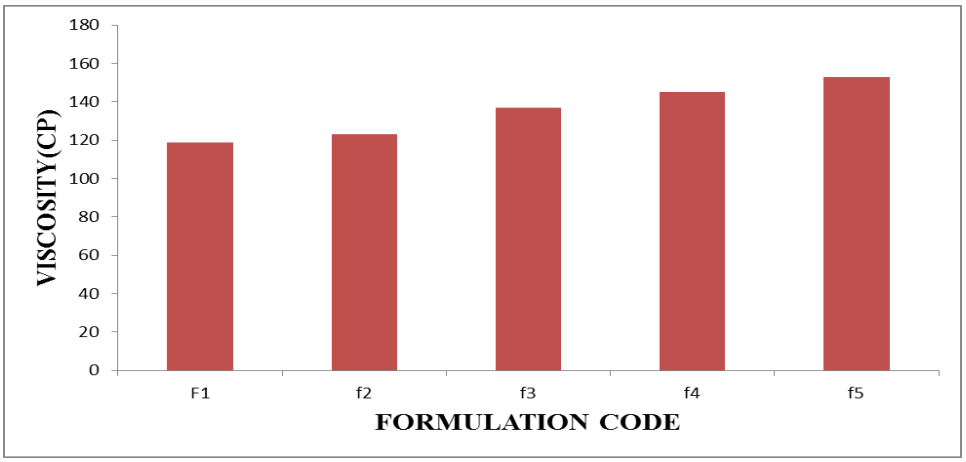

Fig 5.17: viscosity of prepared formulations F1 toF5

Table 5.11: Drug content of prepared formulations.

S.no	Formulation code	Drug content (Mean ±std)
1	F1	95.56±0.22
2	F2	91.89±0.88
3	F3	89.76±0.78
4	F4	85.91±0.66
5	F5	79.55±0.22

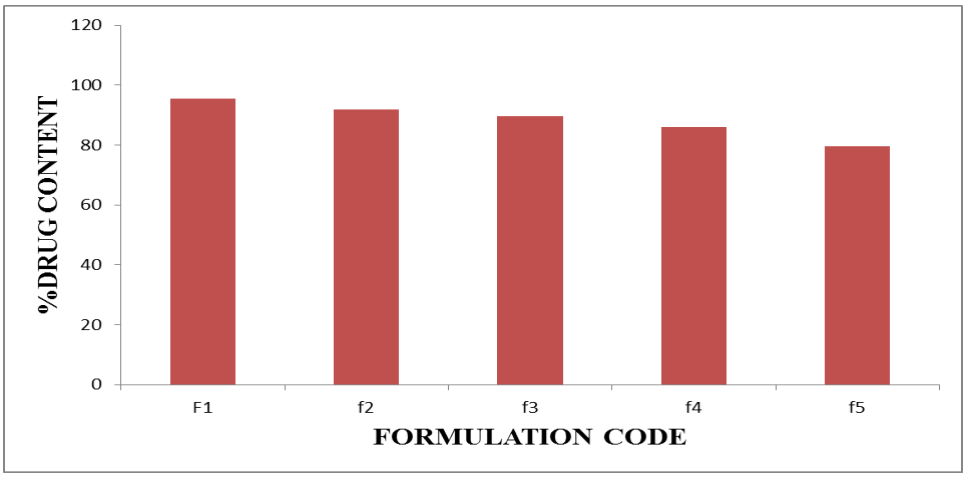

Fig. 5.18: % Drug content of formulation F1to F5.

CONCLUSION

The aim of the present work was used to prepare, evaluate and optimize microemulsion of erythromycin for ophthalmic drug delivery. This study clearly demonstrated that erythromycin can be successfully delivered through cornea by preparing the microemulsion. Through this method it is possible for the drug to act directly on the site of administration and reduce the drug dosage, and also increases the efficiency By formulating we can achieve higher bioavailability, allowing low dose, modified release pattern, and reducing dosing frequency etc. The main advantages of microemulsion are that it can be used to carry both the lipophilic drugs as well as the hydrophilic drugs, the absorption rate is tremendously increased in the case of microemulsion, they are thermodynamically more stable than conventional dosage forms, The microemulsion

systems are very much advantageous because of its many applications in the colloidal drug delivery systems for the purpose of controlled release of the drugs and for drug targeting Drug delivery to the eye is the most challenging part because of several problems like lachrymal drainage and corneal barrier In the present research work drug loaded microemulsion was prepared by taking different ratios of oil, surfactant and co-surfactant and evaluated for various parameters like viscosity, particle size, in-vitro drug permeation and its kinetic data. On the basis of all parameters formulation FI was selected to optimise for the formulation of ophthalmic microemulsion with pH 7.4+0.12, viscosity 119.23+0.83cp, drug content 95.56±0.23%, and particle size 12.1 nm. This optimised formulation is efficient to deliver drug into cornea with increased corneal absorption and lessened side effects.

Future Prospects

The ME systems for ocular delivery have been reported to possess excellent physicochemical properties and stability. Apart from this, they are easy to fabricate and characterize. MEs are expected to deliver any drug to both the anterior and posterior segments of the eye, at the right time in a safe and reproducible manner at required level. MEs is the effective treatment of ocular diseases.

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