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Preparation and evaluation of pluronic lecithin organogel containing ricinoleic acid for transdermal drug delivery

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Preparation and Evaluation of Pluronic Lecithin Organogel Containing Ricinoleic acid for Transdermal Drug Delivery

By

Sindhu Prabha Bonam

Submitted to the Graduate Faculty as partial fulfillment of the requirements for

The Master of Science Degree in Pharmaceutical Sciences,

Industrial Pharmacy Option

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December 2013

An Abstract of

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Pluronic Lecithin Organogels (PLO gels) are commonly used as transdermal vehicles in compounding pharmacies to provide customized medication for pain management as well as for other therapies. The present research aims at studying the plausibility of using ricinoleic acid as an alternative to isopropyl palmitate in compounding stable PLO gels for pain and inflammation management. In the first part of the study, PLO gel containing ricinoleic acid was prepared and characterized for the transdermal delivery of ketoprofen (10%) and dexamethasone (0.5%). Blank PLO gels were prepared using ricinoleic acid as the oil phase and characterized for pH, viscosity, gelation temperature, and microscopic structure. *In-vitro* anti-inflammatory activity and cell viability tests were also performed using blank ricinoleic acid PLO and compared with the isopropyl palmitate PLO gel. The optimized PLO gel formulation was further evaluated using ketoprofen (10%) and dexamethasone (0.5%) as model drugs. The stability and *in vitro* permeability of ketoprofen and dexamethasone was evaluated and compared with the corresponding control formulation (isopropyl palmitate PLO gel). The pH and viscosity of blank PLO

gel prepared with ricinoleic acid was comparable with the isopropyl palmitate PLO gel. The anti-inflammatory effect exhibited by the blank ricinoleic acid PLO gel was significantly ($p<0.05$) higher than isopropyl palmitate PLO gel at 1 mM concentration, while both the gel formulations had no significant ($p>0.05$) cytotoxic activity. Drug loaded PLO gels behaved in a similar manner and all formulations were found to be stable at 25ºC, 35ºC, and 40ºC for up to 35 days. The penetration profile of dexamethasone was similar from both the PLO gels, while the permeability for ketoprofen from ricinoleic acid PLO gel was found to be three times higher as compared to the control formulation. The second part of the study deals with the preparation and evaluation of ricinoleic acid PLO gel acid for the transdermal eyelid delivery of tobramycin (0.3%) and dexamethasone (0.1%). PLO gel was characterized for viscosity, pH, gelation temperature, morphology, DSC and drug content. The *ex vivo* permeability of dexamethasone and bactericidal activity of tobramycin was tested and compared with the marketed Tobradex® eye ointment. The pH of the optimized ricinoleic acid PLO gel was found to be 6.54 with a gelation temperature of 31ºC. The penetration of dexamethasone from the ricinoleic acid PLO gel was found to be much higher than the marketed Tobradex® eye ointment. Ricinoleic acid PLO gel containing tobramycin showed a better anti-microbial activity and higher zones of inhibition when compared with the marketed Tobradex® eye ointment. The findings of this investigation indicate that the ricinoleic acid PLO gel has the potential for use as a transdermal eyelid delivery system. Ricinoleic acid PLO gel was found to be better than the commercial Tobradex® eye ointment with respect to the penetration of dexamethasone and anti-microbial activity of tobramycin.

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Chapter 1

Introduction

1.1. Gels

According to the USP, gel is a formulation in a water-soluble base and may be regarded as a greaseless ointment. Gels are defined as semi-solid formulations comprising of an external solvent phase, either polar or non-polar in nature, that is immobilized within the spaces of a three dimensional network [1]. This is often referred to as the gelator system. The gelator prevents the flow of solvent phase predominantly by causing a rise in the surface tension [2]. Gels may be classified, depending upon the strength of the bonds within the gelator system, as physical or chemical gels. In physical gels, the type of bonding may occur as van der Waal's forces or hydrogen bonding; however, chemical gels are held together by covalent bonds. Gels are basically classified into two types, depending upon the nature of the liquid component, as either organogels or hydrogels (Fig.1-1).

1.2. Hydrogels

Hydrogels are three-dimensional polymeric networks, that are hydrophilic in nature and capable of imbibing large quantities of water or biological fluids [3, 4]. Insoluble homopolymers or copolymers form networks, which are insoluble owing to the presence of cross-links. Physical cross-links include tie-points and junctions which contribute to the network formation and physical integrity, while chemical cross-links can be seen as entanglements or crystallites. [5-10] These hydrogels swell in the presence of an aqueous environment because of the thermodynamic compatibility they exhibit with water [3, 4, 11-13]. Hydrogels have a wide range of applications in the pharmaceutical industry [14-16]. They show a resemblance to natural living tissues in terms of their water content and soft texture [14]; while the high water content contributes to their biocompatibility. As a result, hydrogels are widely used as contact lenses, membranes for biosensors, linings for artificial hearts, materials for artificial skin, and as drug delivery devices [14-18]

1.3. Organogels

Organogels are thermodynamically stable, visco-elastic bi-phasic systems comprising of a gelator and a nonpolar phase, with or without the presence of water molecules within the network formed by the gelator system. They have a lower degree of hydration, when compared with hydrogels. They gained importance in the delivery of drugs over the past few years owing to their non-irritating nature and biocompatibility. The organogel systems exhibit morphological and rheological properties similar to solids even though

they are comprised of large amounts of liquid. The thermodynamic and kinetic stability of these systems can be attributed to the opposing forces which are operating and are associated with the organogelator's partial solubility in the continuous phase. The physicochemical properties of gel components and their resulting interactions govern the formation of the gelling matrix. Gels can be classified based on the properties of gelators, solvents and the intermolecular interactions which can occur in gels. Organogelators are mostly small molecules, while gelators in a hydrogel are polymeric in nature (Fig. 1.1). Hence, the organogelators are well known by the name Low Molecular Weight (LMW) Organogelators. Administration of drugs require variations in the formula of organogel used, depending upon the route of drug administration [1].

1.3.1. Advantages of organogels

- Ease of preparation and administration
- Less number of ingredients that minimizes the overall production cost
- Act as good permeation enhancers
- Enhanced patient compliance due to the non-toxic and non-irritating nature of gels
- Act as a carrier system for the delivery of a wide variety of hydrophilic and lipophilic drugs
- Drug release for sustained delivery can be achieved by modifying the structure of the organogelator and the type of organic phase used [19].

1.3.2. LMW Organogelators

Most organogels are comprised of solid fibers, which are produced by a fall in the temperature below the solubility limit of the gelator [20]. This is followed by a quick precipitation of gelator molecules in the organic medium to some extent, which leads to the formation of aggregates by co-operative intermolecular interactions [21]. However, the fluid fiber matrices are formed by the realignment of surfactant molecules in single or bi-layered cylindrical molecules, where polar molecules are incorporated into the surfactants present in organic solutions. The cylindrical aggregates thus formed, immobilize the solvent [21].

The solid and fluid fiber matrices vary from one another with respect to the kinetic stability of networks that form the gel system. Strong gels are formed by permanent and mostly, crystalline networks [22], while weak gels are formed from transient networks [23, 24]. Physical properties of the organogels are determined by the nature of the networks they form. Solid-matrix fibers are known for their robustness owing to their aggregation and alignment to form bundles because of their rigidity [21]. This has been demonstrated by rheological studies [3, 22]. However, this aggregation does not take place in fluid-matrix fibers. Molecular and supramolecular chirality greatly influences the formation and stability of solid fibers, while its occurance in fluid-matrix fibers is rare [20, 21].

1.3.2.1. Solid-matrix fibers

Most LMW organogelators form solid networks when combined with a specific organosolvent. Solid-matrix gels are prepared by the dissolution of a gelator in a hot solvent in a concentration ranging between 0.1-15% [25]. As the temperature falls, the affinity between the gelator and the solvent molecules decreases.

As this occurs, the gelator assembles itself into solid aggregates which are bound by intermolecular interactions. However, the system is stabilized by the remaining solvent-aggregate affinity. The formation of the aggregates occurs in two ways:

1) Unidimensional growth of fibers in high specific (length-to-width) ratios, which measures tens of nanometers and micrometers in length. Most aggregates fall under this category (e.g., L-alanine fatty acid derivatives).
2) Two dimensional growth of fibers (e.g., Hexatriacontane which forms microplatelet arrangements).

1.3.2.2. Fluid-matrix organogels

These are thermo reversible gels which may be transparent or opaque. They are also known as worm-like or polymer-like networks because of their aggregate fluidity and transient junctions. They are broadly divided into two systems: lecithin organogels and sorbitan monostearate (SMS) organogels, which have a wide range of pharmaceutical applications.

Classification of gels

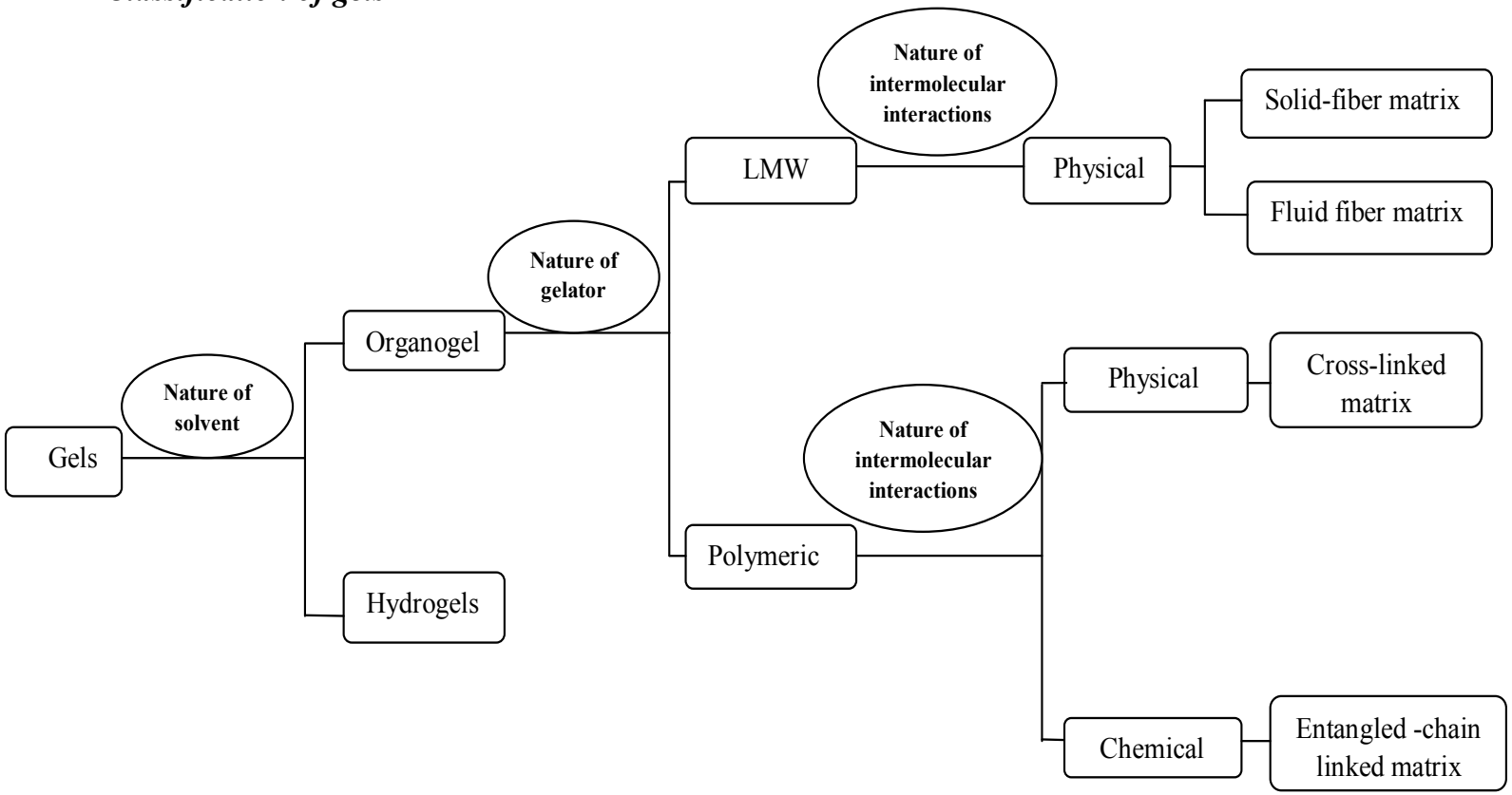

Figure 1-1: Classification of organogels. Modified from [1].

1.3.2.2.1. Lecithin organogels

These systems are gaining importance owing to their biocompatibility, amphiphilic nature which allows the permeation of lipophilic and hydrophilic drugs and their permeation enhancing properties. Lecithin has the ability to form different shapes owing to its amphiphilic nature (Fig. 1.2). Reverse micelles are formed when small amounts of organic solvent are added to lecithin. Cylindrical reverse micelles start growing, upon the addition of polar solvents until they intertwine to form a gelling network. In spite of being known as "weak" organogels, lecithin organogels have high viscosities than that of gelatin. The high viscosity of these systems was found to be a result of the growth and overlapping of the reverse tubular micelles [26-28]

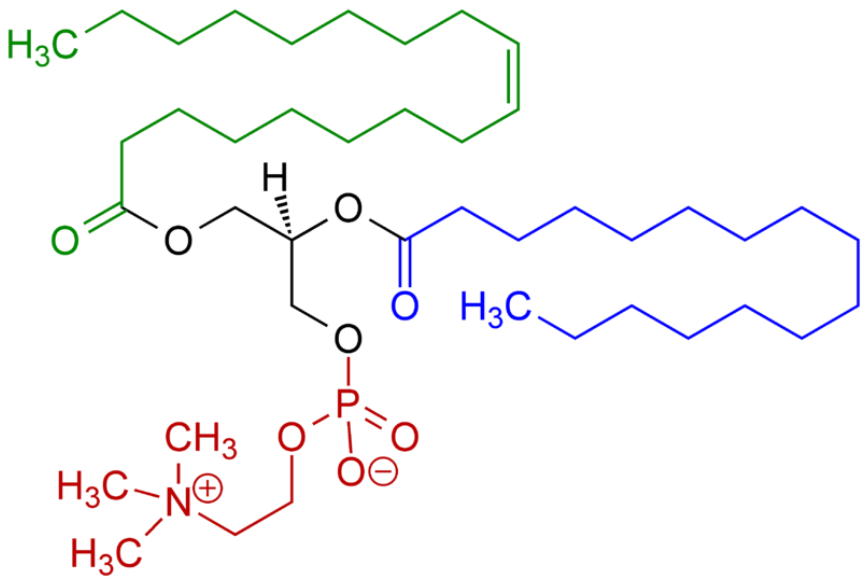

Figure 1-2: Structure of lecithin

1.3.2.2.2. Fatty acid-derived organogels

These were first reported by Murdan et al. [29-32] wherein sorbitan monostearate and sorbitan monopalmitate organogels were prepared, with and without aqueous phase. There occurs a transition from an isotropic phase comprised of reverse micelles to a phase that has the intertwined rod shaped tubules, responsible for the immobilization of the solvent [32, 33]. These systems are stabilized by the alignment of amphiphiles inside the tubules as concentric inverted bilayers. However, in the case of lecithin organogels, it is the hydrogen bonds between water and the polar head of the amphiphiles that attribute to the stability of the system [30, 32, 34].

1.3.3. Polymeric organogelators

Polymeric gels can be of different shapes including linear to hyper branched and star-shaped, with similar characteristics. Most commonly used polymeric gelators include poly (ethylene) organogels, copolymers of methacrylic acid and methyl methacrylate, etc. Poly (ethylene) organogels are generally used as ointment bases, while the later matrials are used in the preparation of organogels and sustained release formulations for rectal administration [35, 36].

1.3.3.1. Mechanism of organogelation

Lecithin molecules undergo gelation on the addition of a polar solvent. Lecithin tends to self-assemble into reverse spherical micelles at a concentration of ~0.01mM in the

presence of a non-polar medium [37]. However, the addition of small and critical quantities of a polar solvent induces an aggressive uni-axial growth of the spherical micelles into tubular or cylindrical micellar aggregates (Fig. 1.3). The binding of the polar molecules in stoichiometric ratios to the hydrophobic head of lecithin molecules results in the formation of a linear framework, where two adjacent lecithin molecules are linked by a polar molecule [38, 39]. This linear framework is a result of hydrogen bonds formed by polar molecules and the phosphate group of lecithin molecules. These bonds are also responsible for the one dimensional growth of the reverse micelles of lecithin. Long tubular micelles of 2.0 - 2.5 nm radius and hundreds to thousands of nanometers will be formed on further addition of the polar solvent [40, 41]. Once these micelles attain a critical length, they start to overlap followed by the intertwining and result in the formation a three-dimensional network [23, 42-47]. This offers the system an enhanced viscosity and the visco-elastic properties, which results in the formation of a jelly- like structure. Micellar aggregates are further stabilized by the rigidity of the phospholipid in the region of the phosphate group and glycerol residue. In PLO gels, the synergistic action of phospholipids and the polymeric surfactant molecules in their respective hydrated states contribute to the gelling and structural network. The micellization of the intramolecular interactions between the gelator and organic solvent molecules. As shown in Figure 3, the lipophilic drug is solubilized in the organic phase (mixture of apolar solvent and lecithin), while the hydrophilic drug can be solubilized in the polar phase. Addition of the polar phase to the organic phase, under continuous stirring, results in the formation of an organogel. Pluronics, nonionic triblock copolymers composed of polyoxypropylene (poly(propylene oxide)) flanked by two hydrophilic chains of

polyoxyethylene (poly(ethylene oxide)), are used as cosurfactants in the preparation of a pluronic lecithin organogels, popularly known as PLO gels.

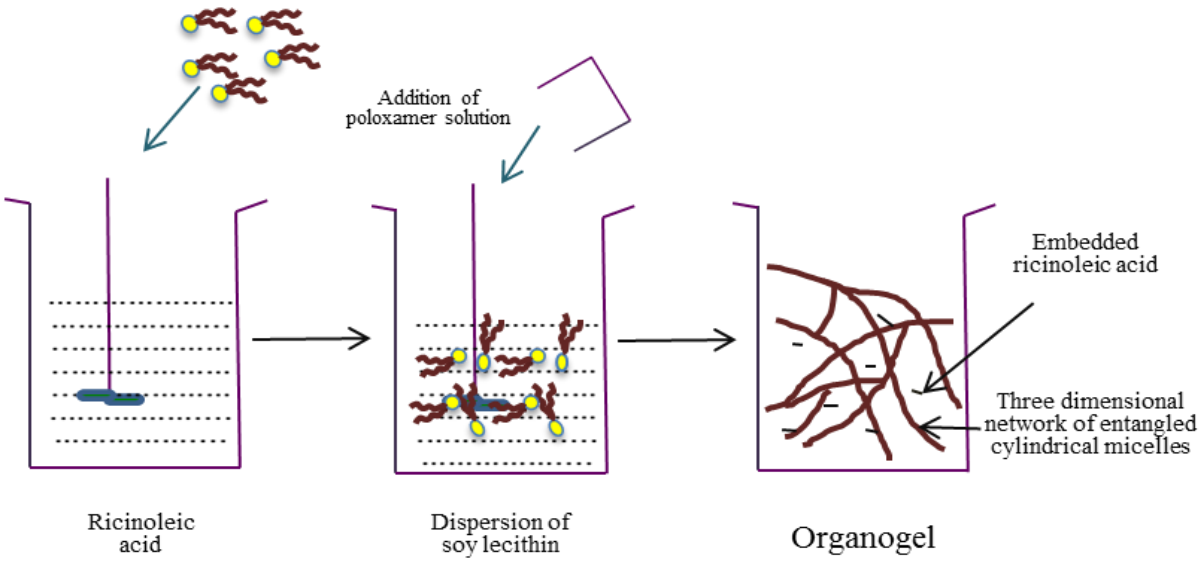

Figure 1-3: Different steps in the formation of a lecithin organogel. Modified from [48].

1.3.3.2. Poloxamer as a gelling agent

Poloxamer block copolymers were introduced in the 1950s. They were an important part in the preparation of liquids, pastes and solids. Poloxamers are polymers of ethylene oxide and propylene oxide, arranged in a triblock fashion (Fig. 1.4). The lipophilicity, hydrophilicity and size of the of the poloxamers can be modified by changing the "a" and "b" values (Table 1.1). They exhibit reversible thermodynamic properties, which plays a crucial rule in designing a formulation. Commercially, they are available under the names Pluronic®, Synpersonic® or Tetronic® [49]. They are odorless, tasteless, white waxy

granules having a free flow. They are soluble in polar and non-polar solvents. Poloxamers are preferred over other molecules owing to the stability in aqueous solutions in the presence of alkali, acid or metal ions. Different poloxamer grades are marketed, which have different physical and chemical properties.

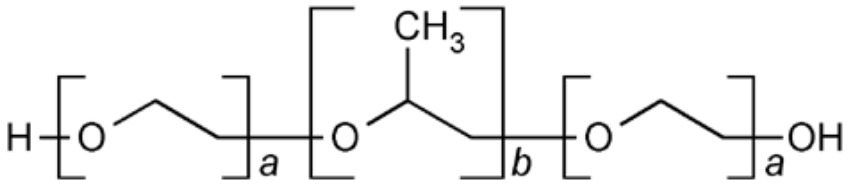

Figure 1-4: General structure of Poloxamers

Poloxamer 407 is the most commonly used pluronic in the pharmaceutical industry. It is a triblock copolymer that exhibits thermoreversible behavior in aqueous solutions; it consists of ~70% polyoxyethylene units and ~30% polyoxypropylene blocks. This

Table 1.1: Different marketed brands of poloxamers with their pluronic name [50]

Pluronic®	***Poloxamer***	***a***	***B***	***Molecular weight (gram/mole)***
L 44 NF	124	12	20	2090-2360
F 68 NF	188	80	27	7680-9510
F87 NF	237	64	37	6840-8830
F 108 NF	338	141	44	12700-17400
F127 NF	407	101	56	9840-14600

polymer is characterized by low toxicity, excellent compatibility with other chemicals, and a high solubilizing capacity for different drugs [51]. They exhibit a thermo reversible property above a certain temperature, known as the sol-gel transition temperature. They act as solids above the sol-gel transition temperature and as liquids below that same temperature. The gelation process takes place in two steps (Fig. 1.5). In the first step, co-polymers aggregate resulting in the formation spherical micelles. Hydrated swollen poly ethylene oxide (EO) chains form the outer lining of the micelles, while the dehydrated polypropylene oxide (PO) blocks occupy the inner core portion. The first step takes place as temperature increases and reaches the critical micelle temperature. A further increase in the temperature results in the alignment of micelles in a uniform fashion, which results in the formation of gels. Poloxamers varying in concentration between 20-30% is generally used in pharmaceutical formulations, which are clear liquids at 4-5°C and gels at room temperature. These solutions are generally prepared by the cold method (uses cold water), during which a layer of water molecules surrounds the poloxamer molecule. This leads to the separation of the hydrophobic portions as a result of hydrogen bonding between the water and hydrophilic chains

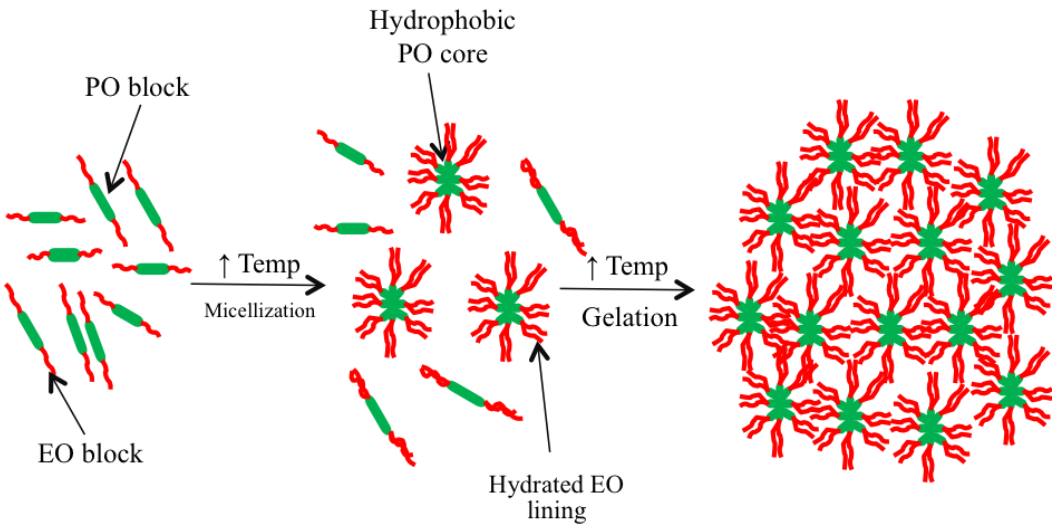

Figure 1-5: Mechanism of gelation of poloxamer in presence of water. Modified from [52].

1.4. Pluronic Lecithin Organogels (PLO gels)

PLO gels have gained importance in recent years as transdermal drug delivery systems. PLO gel was introduced in the 1990s by Jones and Kloesel [53]. It is a biphasic system consisting of an oil phase (lecithin dissolved in isopropyl myristate or isopropyl palmitate in a 1:1 ratio) and a water phase containing 20-30% Pluronic F127 [54]. It is a thermodynamically stable, visco-elastic system, which is non-irritating, odorless and biodegradable.

Pluronic F127 or poloxamer is a copolymer of polyoxyethylene and polyoxypropylene which forms a thermoreversible gel in concentrations between 15-30%w/v. Poloxomer exists in a liquid state at refrigerated conditions (4^{o}C) and forms a gel at room or body temperature. Water plays the role of a structure-forming agent and stabilizes the process of gel formation as it solubilizes the pluronic and other hydrophilic drugs. Lecithin, a lipid composed of choline and inositol, is found as a major component of cell

membranes. It is a surfactant with emulsification and lubricant properties. Isopropyl palmitate acts as an emollient, moisturizer and penetration enhancer [55]. Sorbic acid or potassium sorbate may be used as the preservative to enhance the shelf life of the gel.

PLO gels have generated significant interest in the United States as transdermal and topical drug delivery vehicles. They have been studied for the topical delivery of several other hydrophilic and hydrophobic drugs including hormones, antiemetics, opoids, antipsychotic drugs, calcium channel blockers and local anesthetics [56, 57]. The PLO gel system facilitates the delivery of hydrophilic as well as lipophilic drugs owing to the presence of both oil and aqueous phases within the gel system.

1.5. PLO gels for transdermal drug delivery

The route of drug administration wherein the active ingredients are delivered across the skin for systemic circulation is known as transdermal drug delivery. The most commonly used delivery systems for transdermal drug administration include transdermal patches, transdermal implants, transdermal gels, etc. It is important to have an insight into the skin's structure to understand the passage of drugs through the skin, which serves as a great barrier for the drug before it reaches the systemic circulation. The skin consists of three layers, namely, the epidermis, the dermis and the sub-cutaneous layer (Fig. 1.6). The epidermis is the most impermeable layer owing to its complex, multi-layered structure having a thickness of approximately 0.6 mm on the eyelids [58, 59]. The epidermis is further divided into four distinct layers, namely, stratum germinativum (inner most), stratum spinosum, stratum granulosum and stratum corneum (outer most) [58, 60]. The passage of the drug through the stratum corneum, which is composed of

flattened cornified cells, embedded in a lipoidal intercellular matrix serves as the rate limiting step in the process for transdermal drug absorption [58, 59].

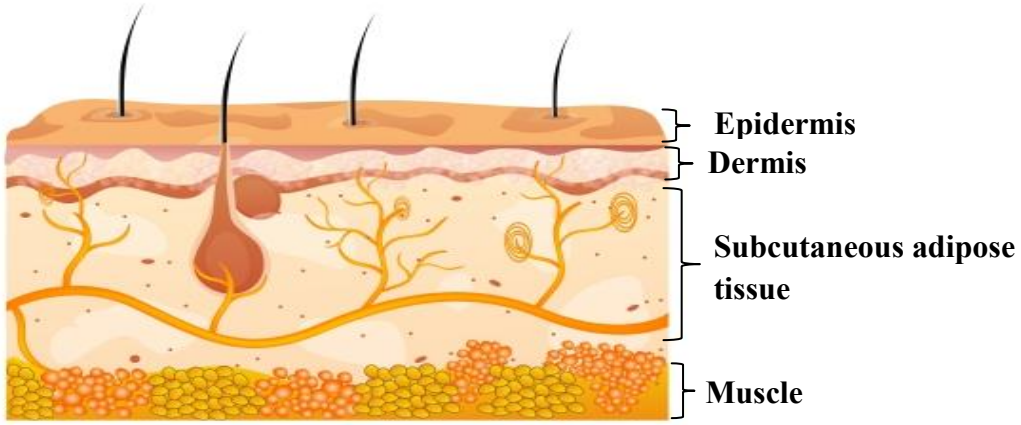

Figure 1-6: Layers of the skin.(Modified from http://www.freedigitalphotos.net/images/view_photog.php?photogid=2280)

Advantages for transdermal drug delivery

- Avoids hostile GI environment and presystemic metabolism
- Drug elimination is independent of gastric emptying
- Suitable for drugs with short biological half-life and narrow therapeutic window
- Increased therapeutic efficacy and decreased fluctuations
- Decreased dosing frequency and increased patient compliance
- Painless when compared to parenteral therapy
- Provides suitability of self-medication
- This route is most suitable for the drugs with short elimination half-life and undergo extensive first-pass metabolism

Disadvantages for transdermal delivery

- In most cases the effect is slow and sustained
- Chances of irritation to the skin from the penetration enhancers
- Only drugs small enough to penetrate the skin can be effectively delivered

Organogels serve as a matrix for the transdermal delivery of various lipophilic and hydrophilic drugs (Table 1.2). This was first studied by Willimann and Luisi [61] for scopolamine and broxaterol. Lecithin organogels containing isopropyl palmitate were found to exhibit higher drug permeation than those containing cycloctane [18]. Pluronic lecithin organogels have been proven non-irritating to the skin, odorless and allow the quick passage of drug through the skin. They are also known to be effective in moisturizing and revitalizing keratin-like tissues such as the hair, fingernails and skin. These gels consist of reversed polymer like micelles, which evolve from spherical micelles seen initially, which agglomerate to form a temporal three-dimensional network in the bulk phase and are obtained by the dissolution of trace amounts of water in a non-aqueous solution [18, 39, 43].

In vivo studies performed so far revealed the ability of PLO gels to be excellent drug carriers with penetration enhancement ability. Significant drug penetration into the systemic circulation was observed on repeated topical application of methimazole to the inner pinna of healthy cats suffering from hyperthyroidism, which was not observed after a single application [62]. This was assumed to be a consequence of the lowered resistance to the drug penetration caused by the exfoliation of stratum corneum and inflammation produced by the lecithin component in the gel.

According to Giordano and his co-workers, a topical formulation of ondansetron, a 5-HT receptor antagonist loaded gel showed a greater reduction in pain after a single application in healthy human volunteers, for whom mechanical hyperalgesia and flare were stimulated by the intradermal administration of capsaicin [63]. PLO gels were effective in producing a local action, especially in the case of nonsteroidal anti-inflammatory drugs (NSAIDS). Diclofenac loaded PLO gel was found to ameliorate pain to a greater extent along with enhancement in wrist extension strength, when used to treat osteoarthritis of the knee and lateral epicondilytis. Acetaminophen loaded PLO gel (APAP-PLO) was found beneficial for cancer patients in a hospice setting, when applied transdermally. PLO gel containing methimazole when applied to the inner pinna of cats suffering from hyperthyroidism lowered gastrointestinal adverse effects compared to orally administered methimazole. However, the transdermal application did not exhibit a higher bioavailability than the orally administered drug [64]. PLO-based gel loaded with fish oils, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) was capable of delivering the oils transdermally by a fixed repeated dosage regimen rather than by the administration of a finite dose; while retaining a part of these highly lipophilic molecules within the gel matrix. Delivery of ketoprofen through PLO gels was also observed in the same study, where the drug molecules were retained in the matrix to a lesser extent [65].

Table 1.2: Different drugs incorporated in the PLO gels [81].

Drug class	Name of the drugs/ References
Non-Steroidal anti-inflammatory drugs (NSAIDS)	Piroxicam [66], diclofenac [67], Ketoprofen [66]
Hormones	Dexamethasone[68]
Antiemetics	Promethazine [69], Ondansetron [63], scopolamine [70], metoclopramide [71]
Opiods	Methadone, morphine, buprenorphine [72]
Anesthetics	Benzocaine, Lidocaine [73]
Antipsychotic drugs	Haloperidol, prochlorperazine [74]
Calcium channel blockers	Diltiazem [67]
Miscellaneous drugs	Methimazole [62], ketamine hydrochloride [71], selegiline hydrochloride [73], fluoxetine [71], clonidine [73], carbamazepine [73], baclofen [73], insulin

Chapter 2

Significance of the Thesis Research

Though PLO gels have emerged as an effective topical vehicle base for drugs, there are several gaps in our knowledge related to the selection of the most effective ingredients for compounding PLO gels. Further investigation is needed to explain the plausibility of using ricinoleic acid as an alternative to isopropyl palmitate in compounding PLO gels for pain and inflammation management. Ricinoleic acid is an unsaturated omega-9 fatty acid that naturally occurs in a mature Castor plant. Ricinoleic acid is known for its analgesic and anti-inflammatory properties following acute or repetitive local application [75]. Topical application of ricinoleic acid has demonstrated analgesic and anti-inflammatory effects on the induced acute and subchronic inflammation in an animal model [75-77]. In a different randomized, double-blind, comparative clinical study, the effectiveness of castor oil in treating patients with knee osteoarthritis was compared to diclofenac sodium. This study concluded that diclofenac and castor oil were efficacious and well tolerated in patients with osteoarthritis, however, castor oil treated patients reported no adverse effects. The analgesic property of castor oil has been attributed to its unique chemical composition of primarily ricinoleic acid (85-95%) [78, 79]. The present research was designed to test ricinoleic acid for its ability to form a stable PLO gel.

Optimized PLO gel of ricinoleic acid was investigated for its potential use in transdermal drug delivery.

1. In the first part of the study, we examined the *in vitro* transcutaneous permeability and stability of two model drugs, ketoprofen and dexamethasone incorporated in PLO gel containing ricinoleic acid, for transdermal delivery.
2. The second part includes the development and characterization of a PLO gel containing ricinoleic acid for the transdermal eyelid delivery of dexamethasone and tobramycin.

Chapter 3

Chemicals used *(partial listing)*

Ketoprofen

Ketoprofen, (RS)2-(3-benzoylphenyl)-propionic acid, is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and anti-pyretic activities (Fig. 3.1). A 10% ketoprofen gel is most commonly prescribed as a topical NSAID for severe back and shoulder pains [80]. The anti-inflammatory activity is believed to be exerted by the inhibition cylooxygenase-2 (COX-2), an enzyme which is involved in prostaglandin synthesis via the arachidonic acid pathway [81], while the antipyretic activity is attributed to its action on the hypothalamus.

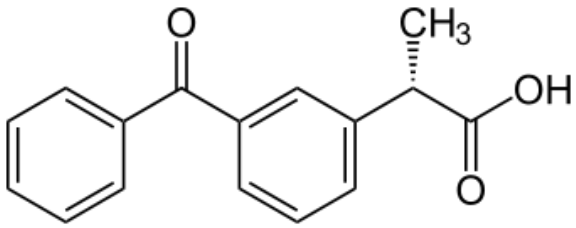

Figure 3.1: Structure of ketoprofen

Dexamethasone

Dexamethasone is a glucorticoid with anti-inflammatory and immunosuppressant activities (Fig.3.2). Dexamethasone acts by inhibiting the phospholipase A2 activity needed for arachidonic acid release and thus preventing the release of prostaglandins, thromboxanes and leukotrienes [82]. PLO gel containing 0.5% dexamethasone is commonly used for veterinary purpose.

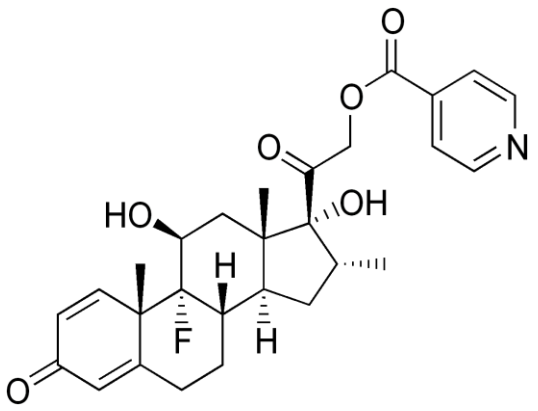

Figure 3.2: Structure of dexamethasone

Tobramycin

Tobramycin is an aminoglycoside antibiotic (Fig. 3.3) derived from the microorganism Streptomyces tenebrarius. It is used to treat various types of bacterial infections, particularly Gram-negative infections. It is known to be effective against the *Pseudomonas* species. Tobramycin works by binding to a site on the bacterial 30S and 50S ribosome, thereby preventing formation of the 70S complex. This results in the failure of the mRNA to be translated into protein, thereby causing the cell death. It is

preferred over gentamicin for *Pseudomonas aeruginosa* pneumonia due to better lung penetration.

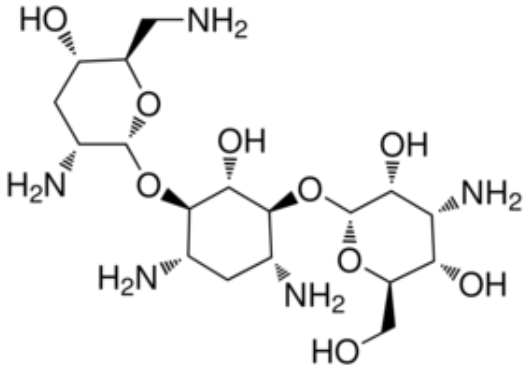

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Figure 3.3: Structure of tobramycin

Ricinoleic acid

Ricinoleic acid is an unsaturated omega-9 fatty acid (Fig. 3.4) that naturally occurs in a mature Castor plant. Ricinoleic acid is known for its analgesic and anti-inflammatory properties following acute or repetitive local application [75]. It acts by specifically activating the EP3 prostanoid receptor for prostaglandin E2 [83].

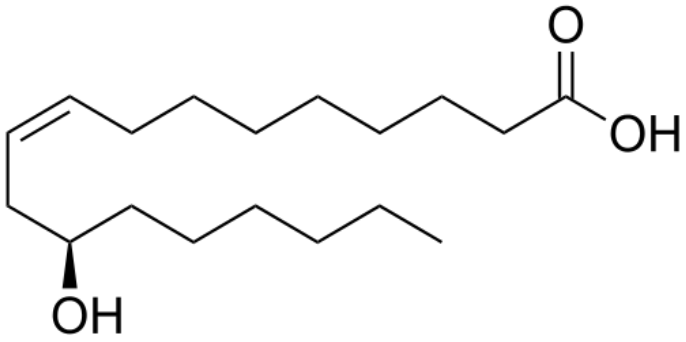

Figure 3.4: Structure of ricinoleic acid

Chapter 4

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Chapter 5

Preparation and *In vitro* Evaluation of a Pluronic Lecithin Organogel Containing Ricinoleic acid for Transdermal Delivery of Ketoprofen and Dexamethasone

5.1. Abstract

The present study deals with the preparation and *in vitro* evaluation of a pluronic lecithin organogel (PLO gel) containing ricinoleic acid for transdermal delivery. Blank PLO gels were prepared using ricinoleic acid as the oil phase and characterized for pH, viscosity, gelation temperature, and microscopic structure. *In-vitro* anti-inflammatory activity and cell viability tests were also performed using blank ricinoleic acid PLO and compared with the isopropyl palmitate PLO gel. The optimized PLO gel formulation was further evaluated using ketoprofen (10%) and dexamethasone (0.5 %) as model drugs. The stability and *in vitro* permeability of ketoprofen and dexamethasone was evaluated and compared with the corresponding control formulation (PLO gel made with isopropyl palmitate as the oil phase). The pH and viscosity of blank PLO gel prepared with ricinoleic acid was comparable with the isopropyl palmitate PLO gel. The thixotropic property of ricinoleic acid PLO gel was found to be better than the control. The anti-inflammatory effect exhibited by ricinoleic acid PLO gel was significantly ($p < 0.05$) higher than isopropyl palmitate at 1 mM concentration, while both the gel formulations had no significant ($p > 0.05$) cytotoxic activity.

Drug loaded PLO gels behaved in a similar manner and all formulations were found to be stable at 25ºC, 35ºC, and 40ºC for up to 35 days. The penetration profile of dexamethasone was similar from both the PLO gels, while the permeability for ketoprofen from PLO gel containing ricinoleic acid was found to be three times higher as compared to the control formulation.

5.2. Introduction

Most commonly prescribed pain relief medications result in unwanted side effects that include stomach irritation, when taken orally [1]. Currently, there is a growing trend among those affected patients for alternative topical medications which can eliminate these annoying side effects. Pluronic Lecithin Organogels (PLOs) are commonly used as transdermal vehicles in compounding pharmacies to provide customized medication for pain management. PLO gel was introduced in 1990s by Jones and Kloesel [2]. Since then, PLO gels have been increasingly used in delivering a variety of drugs in humans and animals. PLO gel is a two-phase system consisting of an oil phase (lecithin dissolved in isopropyl myristate or isopropyl palmitate in a 1:1 ratio) and a water phase containing 20-30% Pluronic F127 [3]. Pluronic F127 or poloxamer is a copolymer of polyoxyethylene and polyoxypropylene, which forms a thermoreversible gel in concentrations between 15-30% w/v. Poloxomer exists in a liquid state at refrigerated conditions (4^{o}C) and forms a gel at room or body temperature. Lecithin, a lipid composed of choline and inositol, is found as a major component of cell membranes. Lecithin is a surfactant with emulsification and lubricant properties. Isopropyl palmitate acts as

an emollient, moisturizer and penetration enhancer with limited benefits in pain management [4, 5]. PLO gels have generated significant interest in the United States as transdermal and topical drug delivery vehicles. They have been studied for the topical delivery of several other hydrophilic and hydrophobic drugs including hormones, antiemetics, opioids, antipsychotic drugs, calcium channel blockers and local anesthetics [6, 7].

Though PLO gels have emerged as an effective topical vehicle base for drugs, there are several gaps in our knowledge related to the selection of the most effective ingredients for compounding PLO gels. We are thus investigating the plausibility of using ricinoleic acid as an alternative to isopropyl palmitate in compounding PLO gels for pain and inflammation management. Ricinoleic acid is an unsaturated omega-9 fatty acid that naturally occurs in a mature Castor plant. Ricinoleic acid is known for its analgesic and anti-inflammatory properties following acute or repetitive local application [8]. Topical application of ricinoleic acid has demonstrated analgesic and anti-inflammatory effects on the induced acute and subchronic inflammation in an animal model [8-10]. In a different randomized, double-blind, comparative clinical study, the effectiveness of castor oil in treating patients with knee osteoarthritis was compared to diclofenac sodium. This study concluded that diclofenac and castor oil were efficacious and well tolerated in patients with osteoarthritis, however, castor oil treated patients reported no adverse effects. The analgesic property of castor oil has been attributed to its unique chemical composition of primarily ricinoleic acid (85-95%) [11, 12].

This study was designed to test ricinoleic acid for its ability to form a stable PLO gel and we further examined the *in vitro* transcutaneous permeability and stability of two model

drugs, ketoprofen and dexamethasone. Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and anti-pyretic activity. A 10% ketoprofen gel is most commonly prescribed as a topical NSAID for severe back and shoulder pains [13]. The anti-inflammatory activity is believed to be exerted by the inhibition of cyclooxygenase-2 (COX-2), an enzyme which is involved in prostaglandin synthesis via the arachidonic acid pathway [14], while the antipyretic activity may be attributed to its action on the hypothalamus. Dexamethasone is a glucorticoid with anti-inflammatory and immunosuppressant activity. Dexamethasone acts by inhibiting the phospholipase A2 activity needed for arachidonic acid release and thus preventing the release of prostaglandins, thromboxanes and leukotrienes [15]. PLO gel containing 0.5% dexamethasone is commonly used for veterinary purposes. The objectives of the present study were to (a) compare the physicochemical properties of blank PLO gel containing ricinoleic acid with the conventional PLO gel (PLO gel containing isopropyl palmitate) and (b) assess and compare the stability and *in vitro* percutaneous absorption of ketoprofen or dexamethasone from the two base formulations.

5.3.Materials and Methods

Materials

Lecithin (Lot RWUG0 IT), ricinoleic acid (Lot 2DYB0), and Phosphate buffer saline (Lot AXK46323) were obtained from Fisher Scientific (Pittsburgh, PA). Ketoprofen (Lot C142119), dexamethasone (Lot C137572), poloxamer 407 (Lot 2433346) were procured from PCCA (Houston, TX). High Performance Liquid Chromatography (HPLC) solvents including acetonitrile (Lot 121151), methanol (Lot 113904) and DMSO (Lot 121001)

were supplied by Fisher Scientific (Pittsburgh, PA). Griess reagent (Lot A0322539) was purchased from AcrosOrganic, Growth medium for the Human RA synovial fibroblasts, RPMI 1640 (Lot 10040539) was purchased from CORNING cellgro. MTT reagent (Lot MKBD4946V) was purchased from Sigma. Distilled deionized water was used for the preparation of PLO gels.

Preparation and characterization of the blank PLO gels

The oil phase was prepared by mixing lecithin and ricinoleic acid in 1:1 ratio or 50:50 (w/w) mixture of lecithin in ricinoleic acid. The mixture was set to stand overnight to allow the complete dissolution of lecithin in the ricinoleic acid. Poloxamer solution (20% w/v) was prepared using the cold method. The poloxamer solution was stored under refrigerated conditions at 4°C overnight, in order to enhance the dissolution of the polymer. PLO gel was prepared by mixing 1 part of oil phase (mixture of lecithin and ricinoleic acid) with 4 parts of aqueous phase (20% w/v poloxamer 407 solution) using a vortex mixer (VORTEX – T, Genie® 2). Conventional PLO gel was also prepared in a similar manner using isopropyl palmitate and used as the control. The blank PLO gels were characterized for pH, viscosity, gelation temperature and morphology.

Determination of pH

For the determination of pH, 1 g of PLO gel was dispersed in 25 mL of distilled deionized water and the pH was determined using an Accumet® excel XL 25 pH meter (Fisher Scientific, Pittsburgh, PA). The pH meter was calibrated with standard buffer solutions of pH 4, 7, 10 before each use.

Determination of viscosity

A Brookfield HBDV-III+ Ultra Cone/Plate Rheometer (Brookfield Engineering Laboratories, Middleboro, MA) was used with CPA-52X Cone Spindle to determine the viscosities. Tests were performed at 25ºC and the temperature was controlled by using a Brookfield Programmable Bath, type TC-550MX-115 (Brookfield Engineering Laboratories, Middleboro, MA). The viscosities of blank PLO gels prepared with ricinoleic acid and isopropyl palmitate were measured at varying shear rates (10, 40, 70, 100 sec^{-1}).

Determination of the gelation temperature

A 25 mL glass vial containing 10 gm of PLO gel formulation was placed in a water bath at 4°C. A magnetic bar placed at the bottom of vial and a digital thermo sensor (Fisher Scientific, Pittsburgh, PA) was dipped into the formulation to monitor the temperature. The formulation was heated gradually at a rate of 1°C/min with continuous stirring at 60 rpm. The temperature at which the magnetic stirrer stops rotating is known to be the gelation temperature of the organogel [16].

Transmission Electron Microscopy (TEM)

TEM (HITACHI HD-2300 A, Ultra-thin Film Evaluation System, Hitachi High Technologies America, Pleasanton, CA) was employed in studying the morphology of the PLO gel. Sample preparation was done by placing a small drop of ricinoleic acid PLO gel on a copper grid. The sample was then stained using 2% phosphotungstic acid

solution (Fisher Scientific, Pittsburgh, PA). The copper grid was allowed to dry overnight undisturbed. The samples were then observed using the TEM.

Anti-inflammatory assay

The anti-inflammatory activity of blank ricinoleic acid PLO gel was compared with the isopropyl palmitate PLO gel. Blank PLO gels were used for the study in order to show the inherent anti-inflammatory activity of ricinoleic in the PLO gel. Human Rheumatoid Arthritis synovial fibroblast cell lines were used for this study. The cell lines were isolated by digesting the synovial membrane of Rheumatoid Arthritis (RA) patients, purchased from Cooperative Tissue Network by our collaborator [19]. The cells were maintained in a DMEM supplemented with 10% fetal bovine serum (FBS), 100 µg/ml streptomycin and 10 U/mL penicillin at 37^{o}C and 5% CO_2. The medium was changed every alternate day until the cells reached a confluency of 80-90%. The cells were then harvested and the final cell concentration was adjusted to $2*10^6$ cells/mL. Fifty microliters of cell suspension was seeded in a 96-well plate ($4*10^5$ cells/well) and incubated for 2 hours at 37^{o}C and 5% CO_2 for attachment. The cells were then stimulated with 150 µg/mL (50 µL) interferon gamma-γ (IFG- γ) and 50 µL each of control and formulation at different concentrations of 1, 0.1, 0.01 and 0.001 mM, making the final volume to 100 µL/well. DMSO was used at a concentration of 0.1% to enhance the solubility of PLO gels. Cells were then incubated at 37^{o}C and 5% CO_2 for 24 hours. The cells were then subjected to the Griess assay for the nitrite determination and cell viability test for the determination of viable cells using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent.

Nitrite determination

Equal volumes of the Griess reagent and the culture supernatant were mixed and incubated for 10 minutes. The wells were observed for the development of color at 550 nm using a Bio Tek Synergy HT microplate reader (MTX Lab Systems, Inc. McLean, VA) The amount of nitrite in the culture supernatant was determined by plotting a standard curve of sodium nitrite in deionized water.

Cell viability

MTT reagent (5mg/mL) was added to the wells and incubated at 37ºC for 2 hours. The yellow medium was aspirated and 100 µL of 100 % DMSO was added to each well to allow the dissolution of the formazam salts formed. The absorbance was measured using a microplate reader at 570 nm.

Preparation of drug loaded PLO gels

The oil phase was prepared by mixing lecithin and ricinoleic acid in 1:1 ratio. The mixture was allowed to stand overnight to allow the complete dissolution of lecithin in the ricinoleic acid. Poloxamer solution (20% w/v) was prepared using the cold method. The poloxamer solution was stored under refrigerated conditions at 4°C overnight, in order to enhance the dissolution of the polymer. Ketoprofen (10% w/v) or dexamethasone (0.5% w/v) was dissolved in the mixture of lecithin and ricinoleic acid. Butylated hydroxytoluene (BHT) was used as an antioxidant at a concentration of 0.1% w/w. BHT was also dissolved in the oil phase containing ketoprofen or dexamethasone. PLO gel was prepared by mixing oil phase (mixture of lecithin and ricinoleic acid) and aqueous phase (20% w/v poloxamer 407 solution) using a vortex mixer (VORTEX – T, Genie® 2).

Conventional PLO gels were also prepared in a similar manner using isopropyl palmitate as the oil phase and used as control formulations in this study. Drug loaded PLO gels were evaluated for pH, stability and *in vitro* permeability. Ricinoleic acid PLO gels of dexamethasone and ketoprofen were considered as test formulations and corresponding isopropyl palmitate PLO gels were considered as controls.

Stability study

PLO gel formulations of dexamethasone and ketoprofen were stored at 25ºC, 35ºC and 40ºC in glass vials. At regular time intervals of 0, 7, 14, 21, 28, and 35 days, PLO gels were tested for drug content. For drug content analysis, 0.25 gm dexamethasone loaded PLO gel and 0.05 gm of ketoprofen loaded PLO gel was dissolved in 5 mL of methanol. Further dilutions were made with distilled water and analyzed using high-performance liquid chromatography (HPLC) technique.

Chromatographic conditions

Samples were then analyzed using a high-performance liquid chromatography system (HPLC) (Waters Alliance e2695 separation module, Milford, MA), equipped with a 2998 PDA detector. Ketoprofen was analyzed using a reverse-phase C18 column (5 µm, 100A, Microsrob, Woburn, MA, USA) with a mobile phase composed of acetonitrile: 0.1% trifluoroaceticacid in water (50:50) pumped at a flow rate of 1 mL/min. Dexamethasone was analyzed by an isocratic method with a mobile phase containing water and acetonitrile (50:50) pumped at a flow rate of 1 mL/min. The retention times for ketoprofen (λ_{max}= 254 nm) and dexamethasone (λ_{max}= 242nm) were found to be 6.7

minutes and 2.9 minutes, respectively. The drug content was determined quantitatively by plotting a calibration curve in each case.

Calibration curve

A stock solution 1 mg/mL of ketoprofen and dexamethasone was used in preparing the calibration curve standards. Various calibration concentration standards of ketoprofen and dexamethasone were prepared ranging from 1-30 μg/mL, in their respective mobile phases. For the calibration curve, each standard concentration was analyzed in triplicate and the average peak area was plotted against concentration.

In vitro percutaneous absorption

Bovine ear and porcine stomach tissue were procured from a nearby slaughterhouse (Kastel's Slaughter House & Processing Center, Riga, MI) and cleaned. Permeability experiments were initiated within 2-3 hours after the sacrifice. The permeability of dexamethasone and ketoprofen from PLO gels were studied across the bovine ear skin and porcine stomach tissue, respectively. Tissues were cleaned using Dulbecco's phosphate buffered saline (pH-7.4) and the hair was shaved using a razor. The thick dorsal skin of the ears was separated from the underlying cartilage using a scalpel. Approximately, 2 cm^2 specimens were cut and mounted on Franz-type cells (PermeGear Inc., Hellertown, PA) for carrying out the permeability study. The outer surface was placed towards the donor chamber on which the drug depot (PLO gel of dexamethasone or ketoprofen prepared using ricinoleic acid) was placed. PLO gels of dexamethasone or ketoprofen made of isopropyl palmitate were used as controls. The receptor chamber was

filled with phosphate buffered saline containing sodium azide. An aliquot (300 μL) was withdrawn at regular time intervals for up to 24-48 hours and replaced with equal amounts of fresh buffer. All the experiments were carried out under sink conditions. Samples were analyzed by HPLC as previously described. Permeability studies were carried out in triplicate.

Permeability (P_{app}) of the dexamethasone and ketoprofen from PLO gel formulations was calculated using Eq. 1.

$$\text{Permeability } (P_{app}) = \text{Flux}/C_d \qquad \text{Eq. 1}$$

Flux (J) is calculated by dividing the slope obtained by plotting the cumulative amount of drug permeated (M) through the skin vs time (t) with the cross-sectional area of the membrane (A) exposed to the drug. C_d is the initial drug concentration in the donor chamber [17].

5.4.Results

Characterization of the blank PLO gels

The blank PLO gel was prepared successfully using ricinoleic acid as the oil phase and its physicochemical properties were compared with isopropyl palmitate PLO gel. Both the gel formulations resulted in a smooth feeling when rubbed onto the skin surface and both appeared to be similar in consistency. The pH of both ricinoleic acid and isopropyl palmitate PLO gels was found to be in the range of 6.0-6.5. The viscosity of the PLO gels was measured at varying shear stresses. PLO gel made of ricinoleic acid exhibited a similar viscosity as that of the control formulation (PLO gels made with isopropyl

palmitate. Figure 5-1 shows the apparent viscosity vs. RPM (shear stress) for the formulations.

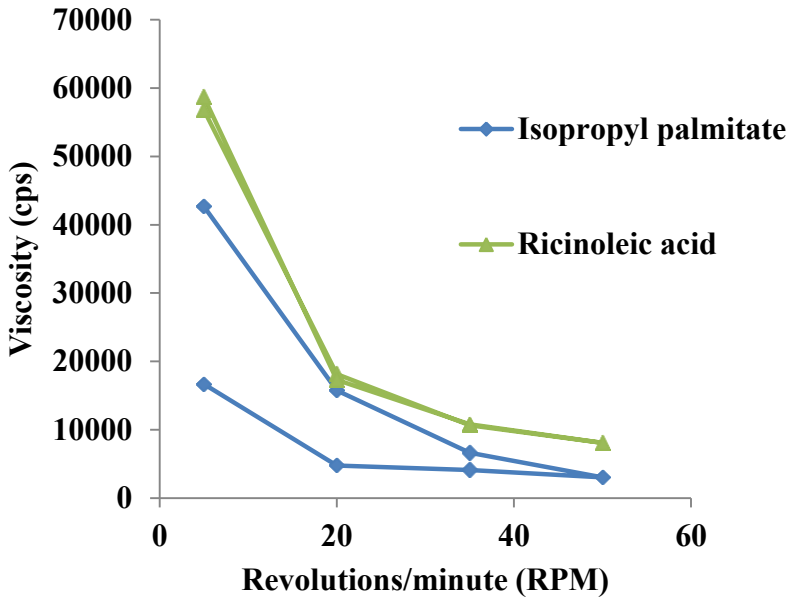

Figure 5-1: Viscosity data of PLO gels containing isopropyl palmitate and ricinoleic acid

The viscosities for the PLO gels containing isopropyl palmitate and ricinoleic acid at 5 rpm were found to be 42704.29 cps and 56883.22 cps, respectively. Both formulations exhibited a non-Newtonian behavior with pseudoplastic flow and the shear thinning behavior, indicated by the downward sloping curves. Also, isopropyl palmitate PLO gel showed a significant loss of viscosity at the end of the test, while ricinoleic acid PLO gel exhibited only a minor viscosity loss. The gelation temperature for the organogel containing ricinoleic acid was found to be 22ºC, at which the liquid form converted into a semi-solid gel. The TEM image of a PLO gel containing ricinoleic exhibited a vesicular

framework formed due to the presence of lecithin in the organogel (Fig. 5-2). These results clearly indicate the ability of ricinoleic acid to form PLO gels with comparable physicochemical properties.

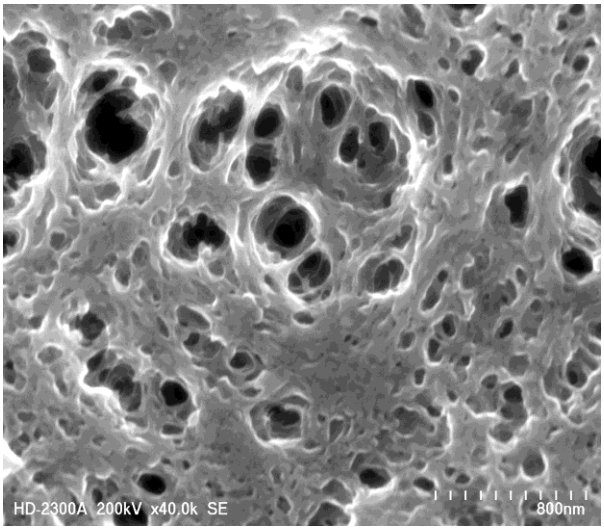

Figure 5-2: Transmission Electron Microscopy image of ricinoleic acid PLO gel

In the anti-inflammatory assay, the amount of nitrite produced by fibroblast cells was determined using the Griess reagent. The amount of nitrite produced by the cells treated with ricinoleic acid PLO gel was lower than that produced by the isopropyl palmitate treated cells (Fig. 5.3). The anti-inflammatory effect exhibited by ricinoleic acid PLO gel was significantly ($p < 0.05$) higher than isopropyl palmitate at 1 mM concentration. Results also indicated that both the ricinoleic acid and isopropyl palmitate PLO gels were not cytotoxic in concentrations ranging between 0.001-1 mM in fibroblasts (Fig. 5-4).

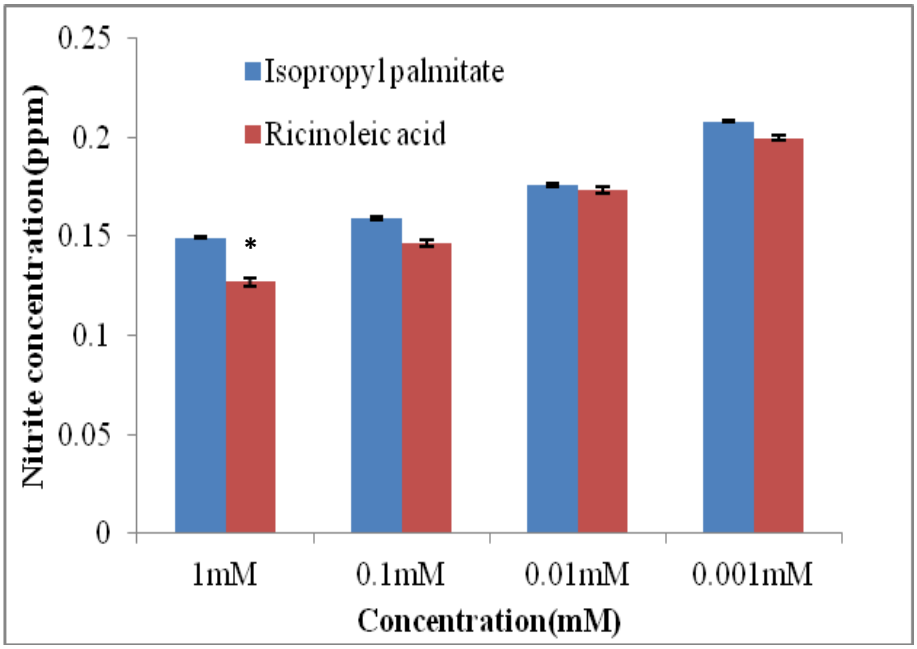

Figure 5-3: Griess assay showing the anti-inflammatory activity exhibited by the blank isopropyl palmiate and ricinoleic acid PLO gels, (n=8), * represents $p<0.05$, Error bars represent the standard error of mean (S.E.M)

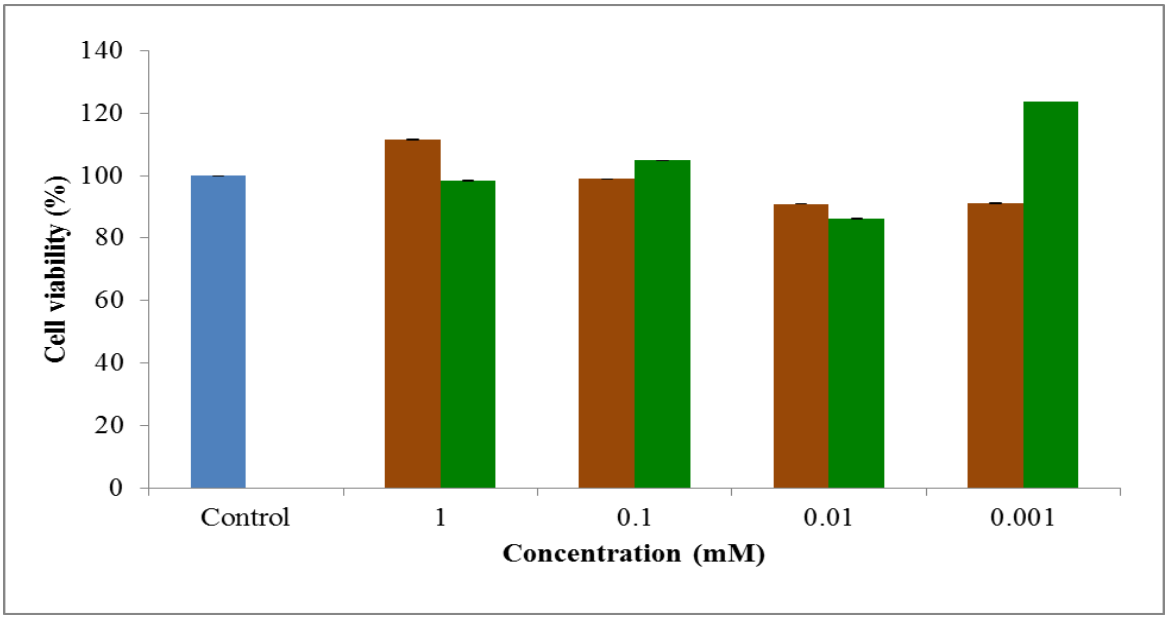

Figure 5-4: Cell viability test performed after treating fibroblasts with different concentrations of blank isopropyl palmitate and ricinoleic acid PLO gels, (n=8). Blue bar for untreated cells, brown bars for cells treated with isopropyl palmitate PLO gel, green bars for cells treated with the ricinoleic acid PLO gel. Error bars represent the standard error of mean

Characterization of the drug loaded PLO gels

PLO gel prepared using ricinoleic acid as the oil phase was able to dissolve ketoprofen (10%) and dexamethasone (0.5%) similarly as the control formulations. The retention time for ketoprofen (λ_{max}= 254 nm) was found to be 6.7 minutes. Concentrations ranging between 1-30 µg/mL were used for plotting the calibration curve. A straight line (y = 34522x - 3041) was obtained with a correlation coefficient (r^2) value of 0.9998 (Fig. 5.5). The retention time for dexamethasone (λ_{max}= 242 nm) was found to be 2.9 minutes. Concentrations ranging between 1-30 µg/mL were used for plotting the calibration curve. A straight line (y = 43098x - 5679.5) was obtained with a correlation coefficient (r^2) value of 0.9989 (Fig. 5-5).

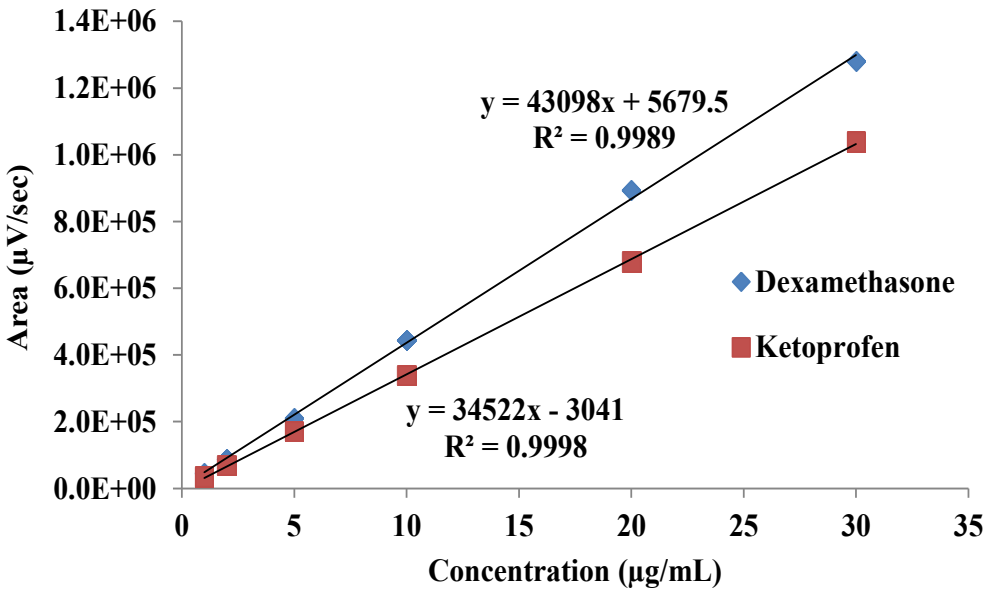

Figure 5-5: Calibration curves for ketoprofen and dexamethasone

The pH of dexamethasone loaded PLO gel prepared using isopropyl palmitate and ricinoleic acid were found to be 6.12 and 6.15, respectively. However, there was a slight drop in pH when ketoprofen was incorporated and the pH was found to be 4.59 and 4.37 for PLO gels prepared with isopropyl palmitate and ricinoleic acid, respectively. The samples were stored at 25ºC, 35ºC and 40ºC and analyzed for drug content at regular intervals of 0, 7, 14, 21, 28, and 35 days. All the formulations were found to be stable under all three storage conditions. The concentration of dexamethasone and ketoprofen remained between 95-105% for up to 35 days at 25ºC, 35ºC and 40ºC (Table I).

Table 5.1: Percent drug content of gel formulations after 35 days, (n=3).
S.E.M: Standard Error of Mean

Formulation	**Temperature (^{o}C)**		
	25ºC (mean±S.E.M)	**35ºC** (mean± S.E.M)	**40ºC** (mean± S.E.M)
Dexamethasone (IPP)	101.46±0.85	97.08±1.77	98.14±0.20
Dexamethasone (RA)	100.14±0.64	102.13±1.65	101.03±0.34
Ketoprofen (IPP)	100.90±1.62	100.42±0.80	100.27±0.65
Ketoprofen (RA)	102.86±0.11	102.05±4.25	99.16±0.43

The *ex-vivo* permeability study for dexamethasone was carried out across the excised bovine ear skin. The penetration profile of dexamethasone from PLO gel containing ricinoleic acid was found to be similar to the control formulation (Fig. 5.6).

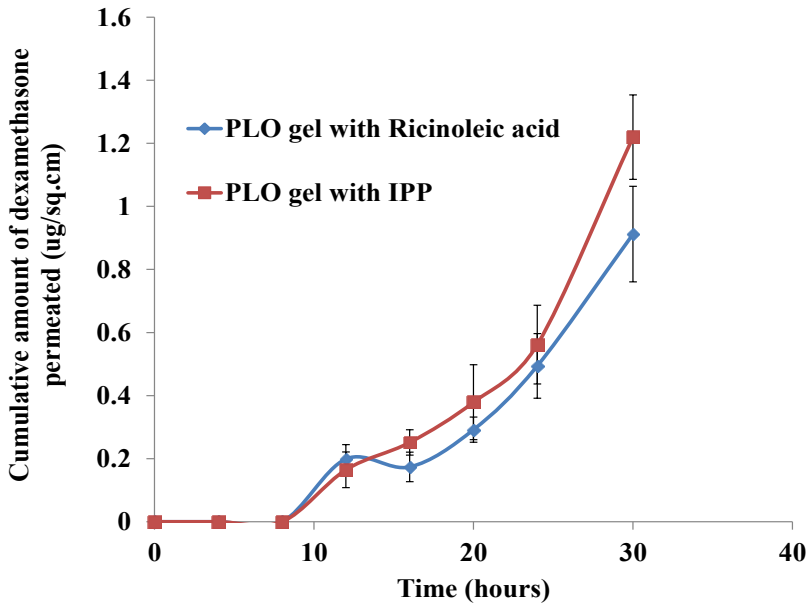

Figure 5-6: *Ex vivo* permeation of dexamethasone across the excised bovine ear skin, (n=4). Error bars represent the standard error of mean.

Permeability values ($PX10^5$) for dexamethasone from PLO gel containing ricinoleic acid and the control were found to be 1.54±0.35 and 2.04±0.40 cm/hour, respectively. The *ex-vivo* permeability study for ketoprofen was carried out across the excised porcine abdominal skin. The permeability for ketoprofen from PLO gel containing ricinoleic acid was found to be three times higher as compared to the control formulation (Fig. 5-7). Permeability values ($PX10^4$) for ketoprofen from PLO gel containing ricinoleic acid and the control were found to be 3.3±0.4 and 1.1±0.6 cm/hour, respectively.

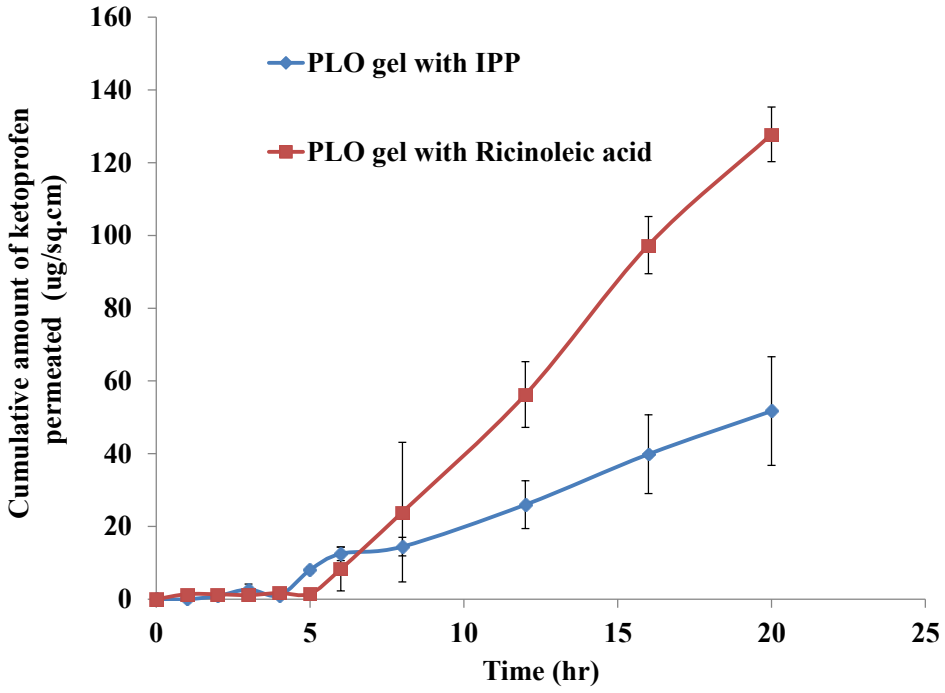

Figure 5-7: *In* vitro permeation study of ketoprofen across the excised porcine abdominal skin, (n=4). Error bars represent the standard error of mean.

5.5. Discussion

PLO gels are widely used in compounding pharmacies to customize medications in order to meet specific patient needs and minimize systemic adverse effects associated with the oral delivery of NSAIDs [3]. Poloxamer gels have potential advantages over traditional bases in terms of drug release and absorption properties. PLO gels can be topically applied to the affected areas in patients who cannot tolerate oral drugs or who have trouble swallowing medications. In this study, we have shown the ability of ricinoleic acid to form a stable gel with 20% poloxamer solution. Ricinoleic acid is an unsaturated

omega-9 fatty acid that naturally occurs in mature Castor plant beans. More than 90% of the fatty acid content present in castor oil is from ricinoleic acid [4, 18]. Ricinoleic acid has been studied extensively for its analgesic and anti-inflammatory properties. Unlike the conventional PLO gels prepared using isopropyl palmitate or isopropyl myristate in pain management, ricinoleic acid is a better alternative due to its inherent analgesic and anti-inflammatory properties. More precisely, PLO gels of ricinoleic acid can work synergistically with analgesics and anti-inflammatory drugs in reducing pain and inflammation. In this study, our objective was to compound a PLO gel containing ricinoleic acid and study the influence of ricinoleic acid on the physicochemical properties of poloxamer gels. Dexamethasone (0.5%) and ketoprofen (10%) were used as model drugs for stability and *in vitro* permeation studies.

PLO gel containing ricinoleic acid was found to exhibit similar physicochemical properties as the control formulation. Both the formulations had a similar physical appearance and they exhibited pseudoplastic behavior. Flow property is an important parameter for pharmaceutical ointments or gels. For example, most gels and ointments are intended to be highly viscous and thick at rest in order to prevent them from flowing away from the intend area of use. However, they must become thin when pressure is applied in order to facilitate spreadability when applied onto the skin. The viscosity of the gel containing ricinoleic acid was found to be similar to that of the control which had isopropyl palmitate as the oil phase. Both the gel formulations exhibit shear thinning behavior, indicated by the downward sloping curves. However, a significant loss of viscosity was observed with the isopropyl palmitate PLO gel, while ricinoleic acid PLO

gel retained its initial viscosity. This clearly indicates the better thixotropic properties of ricinoleic acid PLO gel compared to the control. The TEM study revealed the presence of a vesicular framework in the PLO gel, which is due to the presence of lecithin. This indicates the effectiveness of the preparation method and also explains the phenomenon by which the gel interacts with the skin, thereby causing the penetration of drug molecules through the skin.

Griess test was performed to identify the presence of nitrite compounds. Nitric oxide is produced as a result of inflammation. Nitrite is a stable, non-volatile and an oxidized form of nitric oxide. Griess assay is based on the diazotization reaction between the nitrite and sulphanilamide present in the Griess reagent. The nitrites react with sulphanilic acid to form a diazonium salt which reacts with the azo dye agent (N-alpha-naphthyl-ethylenediamine) to develop a pink color. A pink color develops when an azo dye (included in the Griess reagent) is added to the diazonium salt formed, by which the nitrite present in a sample is estimated. As determined from the amount of nitrite produced by the fibroblasts, the extent of anti-inflammatory effect exhibited by the ricinoleic acid PLO gel was higher than the isopropyl palmitate PLO gel. The anti-inflammatory effect increased with increase in the ricinoleic acid concentration and the anti-inflammatory effect exhibited by ricinoleic acid PLO gel was significantly ($p < 0.05$) higher than isopropyl palmitate at 1 mM concentration. The MTT assay is a colorimetric test for determining the cell viability by measuring the cellular metabolic activity through NAD(P)H-dependent cellular oxidoreductase enzymes. It is a measure of the activity of cellular enzymes that reduce the tetrazolium dye, MTT, to its insoluble formazan, giving

a purple color. The MTT assay performed showed that these PLO gels had no cytotoxic effect on the fibroblasts.

The pH of drug loaded ricinoleic acid PLO gels was close to the control formulations. The addition of ketoprofen reduced the pH of the PLO gels from 6.2 to 4.5 in both test and control formulations. This might be due to the inherent acid nature of ketoprofen which is chemically a propionic acid derivative. Ricinoleic acid did not alter the stability of dexamethasone and ketoprofen. All formulations were found to be stable with respect to drug content at 25ºC, 35ºC, and 40ºC. An *in vitro* percutaneous study was performed to compare the penetration of drugs across various animal tissues for both the test and control formulations. The penetration profile of dexamethasone from ricinoleic acid PLO gel was found to be similar to the control formulation (isopropyl palmitate PLO gel). However, a significantly higher permeability of ketoprofen was observed from the ricinoleic acid PLO gel. This might be attributed to the binding of isopropyl palmitate to ketoprofen in the control formulation thus resulting in decreased penetration. The penetration profile for dexamethasone and ketoprofen from the test formulations were found to be either similar or higher than the control formulations.

5.6.Conclusion

In this study, we examined the plausibility of preparing PLO gels with an oil phase consisting of lecithin dissolved in ricinoleic acid in a 1:1. The results observed in this study clearly indicate the formation of stable PLO gel with ricinoleic acid and the thixotropic property and anti-inflammatory activity of ricinoleic acid PLO gel was found

to be better than the isopropyl palmitate PLO gel. Although the permeability study indicated either similar or higher penetration of dexamethasone and ketoprofen from PLO gels, further studies comparing their anti-inflammatory properties should be performed in animal models.

5.7. References

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Chapter 6

Development and Characterization of a Ricinoleic acid Poloxamer Gel System for the Transdermal Eyelid Delivery

6.1. Abstract

The present study deals with the preparation and evaluation of a pluronic lecithin organogel (PLO gel) containing ricinoleic acid for the transdermal eyelid delivery of dexamethasone and tobramycin. PLO gel containing tobramycin and dexamethasone was prepared and compared with a conventional PLO gel (PLO gel prepared using light mineral oil as the oil phase) with respect to physical appearance and viscosity. The optimized ricinoleic acid PLO gel was further characterized for pH, gelation temperature, morphology and drug content. The *ex vivo* permeability of dexamethasone and bactericidal activity of tobramycin was tested and compared with the marketed Tobradex® eye ointment. Five different PLO gel formulations containing tobramycin (0.3%) and dexamethasone (0.1%) were prepared using ricinoleic acid as the oil phase. No apparent change in the physical appearance and consistency was observed when ricinoleic acid was used as the oil phase. The pH of the optimized ricinoleic acid PLO gel (formulation F2) was found to be 6.54 with a gelation temperature of 31ºC. The drug content of tobramycin and dexamethasone were found to be 102.8 % and 100.14%, respectively. The penetration profile of dexamethasone from formulation F2 was found to

be much higher than the marketed Tobradex® eye ointment. F2 showed a better anti-microbial activity and higher zones of inhibition when compared with the marketed Tobradex® eye ointment. The findings of this investigation indicate that the ricinoleic acid PLO gel has the potential for use as a transdermal eyelid delivery system.

6.2. Introduction

Young children and adults with external ocular infections present unique challenges in the treatment of these infections. Early recognition and treatment of ocular infections is crucial in a child in order to prevent devastating visual consequences such as corneal scarring [1, 2]. Most ocular infections are chronic in nature and characterized by inflammation of the cornea, conjunctiva and eyelids. The management of ocular infection can be challenging, and a stepwise approach to treatment begins with eyelid hygiene followed by topical or systemic antibiotics with or without topical steroids [3]. Antibiotics and steroid eye drops or ointments are typically applied into the conjunctival sac three to six times daily for up to two weeks and then less frequently (once a night for eight weeks) for several weeks after the inflammation has settled [4]. A therapeutic concentration of drugs in the infected tissues should be attained for effective treatment.

Administration of drugs to the eye by means of droptainer bottle or ointment tube is challenging in young children and adults due to the lack of physical acuity and inability to aim adequately [5]. Most patients are unwilling to submit to instillation of eye drops and ointments, and this often results in a little of the active agent getting to its site of action. A variation in the administration technique can have a significant impact on the

ocular bioavailability of the drug [6]. In addition to the difficulties in administration, conventional formulations have their own setbacks. Even when used correctly, the ocular bioavailability of drugs is very low (<5%) due to blinking, draining, non-productive absorption, tear production, transient residence time, and impermeable corneal epithelium [7]. Drug absorption from eye drop suspensions is highly unpredictable, and identical formulations with the same concentrations of active and inactive ingredients tend to exhibit differences due to varying physicochemical properties [8]. The erratic absorption of drugs from a suspension dosage form is attributed to clearance of a large percentage of drug particles from the precorneal region before dissolution and absorption can occur. Moreover, the intrinsic dissolution rate of the drug varies due to constant inflow and outflow of lachrymal fluids [9-11]. Ointments are known to provide enhanced ocular contact time, however, experimental data indicates that steroid ointments do not penetrate into the eye like eye drops because of the binding of the drug to the ointment base [12]. Despite intense research efforts, no alternative delivery system has become widely used. Initial attempts to alter the method by placing the eye drops on the inner canthus with the subject lying supine and eyes tightly closed failed to overcome the difficulties of administering these drugs to children [13-15].

Though ophthalmic inserts provide sustained delivery, they are difficult to insert, and there are several hurdles to commercialization [16]. Even biodegradable nanocarrier systems suffer from several disadvantages, including lack of particle size uniformity, poor formulation stability, burst release of drugs, and large-scale manufacturing difficulties [17, 18]. Transdermal patches applied onto the eyelid with a pressure-

sensitive adhesive layer have shown some success in animals; nonetheless, these have yet to demonstrate a clinical benefit [19]. An ideal vehicle should provide prolonged residence time at the site of application and improve tissue penetration. Pluronic lecithin organogel (PLO gel) based transdermal eyelid delivery system might hold a significant promise in treating anterior segment ocular infections, and improves the drug administration in young children and adults. PLO gel can be applied topically to the skin surface of the eyelid resulting in percutaneous absorption of drugs into anterior segment tissues (cornea and conjunctiva) without causing any side effects as observed with conventional dosage forms (Fig. 6.1).

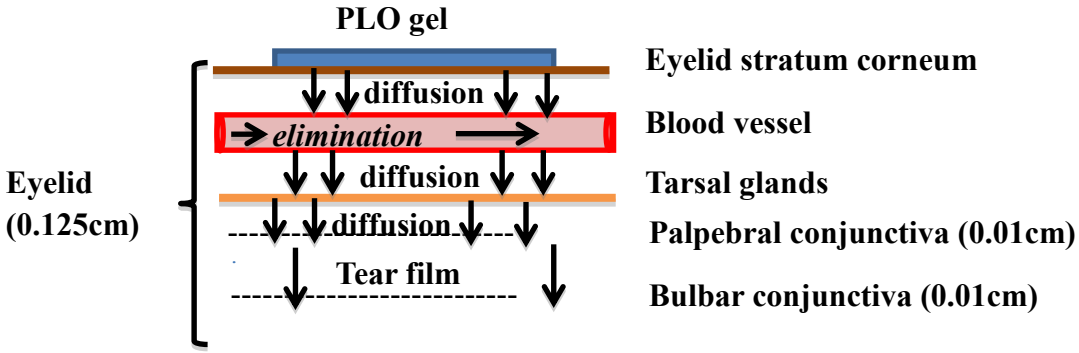


Figure 6-1: Schematic of ocular drug absorption from PLO gel

PLO gels are commonly used as transdermal vehicles in compounding pharmacies to provide customized medication for the management of pain and inflammation. Conventional PLO gel is a two-phase system consisting of an oil phase (lecithin dissolved in isopropyl myristate or isopropyl palmitate or light mineral oil in a 1:1 ratio) and a water phase containing 20-30% Pluronic F127 [20]. PLO gels have been successfully used in the topical delivery of several other hydrophilic and hydrophobic

drugs including hormones, antiemetics, opoids, antipsychotic drugs, calcium channel blockers and local anesthetics [21, 22]. The oil phase used in the preparation of PLO gels only act as an emollient, moisturizer and penetration enhancer with limited therapeutic benefits [23]. We hypothesize that PLO gels made of oils such as ricinoleic acid which is well known for its analgesic, anti-inflammatory and antibacterial properties can work synergistically with drugs to provide better therapy. Ricinoleic acid is an unsaturated omega-9 fatty acid that naturally occurs in a mature castor plant. Ricinoleic acid is known for its analgesic, anti-inflammatory and antibacterial properties following acute or repetitive local application [24-26].

The objective of the present study was to develop and characterize a PLO gel containing ricinoleic acid as the oil phase for the transdermal eyelid delivery. Dexamethasone and tobramycin were chosen as model drugs. Currently, tobramycin and dexamethasone combination is marketed in the form of eye drop suspension (Tobradex® and Tobradex ST®) and eye ointment (Tobradex®) by Alcon Laboratories. It is widely prescribed for the treatment of bacterial eye infections. Ricinoleic acid PLO gel containing tobramycin and dexamethasone was prepared and compared with a conventional PLO gel (PLO gel prepared using light mineral oil as the oil phase) with respect to physical appearance and viscosity. The effect of varying lecithin and poloxamer concentrations on the viscosity of PLO gel was also studied. The optimized ricinoleic acid PLO gel was further characterized for pH, gelation temperature, morphology and drug content. The *ex vivo* permeability of dexamethasone and bactericidal activity of tobramycin from the optimized PLO gel formulation was compared with Tobradex® eye ointment.

6.3. Materials and Methods

Materials

Lecithin (Lot RWUG0 IT), ricinoleic acid (Lot 2DYB0), mineral oil (Lot 110685), sodium chloride (Lot 123819) were obtained from Fisher Scientific (Pittsburgh, PA). Dexamethasone (Lot C137572), tobramycin (Lot C144169), and poloxamer 407 (Lot 2433346) were procured from PCCA (Houston, TX). Butylated hydroxytoluene (Lot U41659D01) was obtained from the Amend Drug & Chemical, Irvington, CA. Distilled deionsed water was for the preparation of PLO gels. High Performance Liquid Chromatography (HPLC) solvents including acetonitrile (Lot 121151) and methanol (Lot 113904) were purchased from Fisher Scientific (Pittsburgh, PA). Mueller- Hinton agar (Lot 1333385) was obtained from Oxoid Ltd (Basingstoke, Hampshire, England), 6mm blank discs (Lot 3031129) and gentamicin 10 µg standard discs (Lot 2163220) were purchased from Becton, Dickinson and Company (Sparks, MD) .

Preparation of the PLO gel

The oil phase was prepared by mixing lecithin and ricinoleic acid in 1:1 ratio or 50:50 (w/w) mixture of lecithin in ricinoleic acid. The mixture was allowed to stand overnight to allow the complete dissolution of lecithin in the ricinoleic acid. Dexamethasone (0.1% w/v) was dissolved in the mixture of lecithin and ricinoleic acid. Butylated hydroxytoluene (BHT) was used as an antioxidant at a concentration of 0.1% w/w. BHT was also dissolved in the oil phase containing dexamethasone. Poloxamer solution (20% w/v) was prepared using the cold method. The poloxamer solution was stored under refrigerated conditions at 4°C overnight in order to enhance the dissolution of the

polymer. Tobramycin (0.3%) was dissolved in the aqueous phase. PLO gel was prepared by mixing 1 part of oil phase (mixture of lecithin and ricinoleic acid) with 4 parts of aqueous phase (20% w/v poloxamer 407 solution) using a vortex mixer (VORTEX – T, Genie® 2). The conventional PLO gel was prepared in a similar manner using light mineral oil and used as the control. PLO gels with lower lecithin and poloxamer concentrations were prepared by mixing oil phase containing lecithin and ricinoleic acid in 1:5 ratio with 17.5%, 15%, 12.5% and 10% poloxamer solutions. The exact concentrations of each ingredient are shown in Table I.

Determination of viscosity

A Brookfield HBDV-III+ Ultra Cone/Plate Rheometer (Brookfield Engineering Laboratories, Middleboro, MA) was used with CPA-52X Cone Spindle to determine the viscosities. Tests were performed at 25ºC and the temperature was controlled by using a Brookfield Programmable Bath, type TC-550MX-115 (Brookfield Engineering Laboratories, Middleboro, MA). The viscosities of PLO gels prepared with ricinoleic acid (F1, F2 and F3) were measured at varying shear rates (10, 40, 70, 100 sec^{-1}) and compared with the control PLO gel (F6).

Determination of the gelation temperature

A 25 mL glass vial containing 10 gm of formulation F2 was placed in a water bath at 4°C. A magnetic bar placed at the bottom of vial and a digital thermo sensor (Fisher Scientific, Pittsburgh, PA) was dipped into the formulation to monitor the temperature. The formulation was heated gradually at a rate of 1°C/min with continuous stirring at 60

rpm. The temperature at which the magnetic stirrer stops rotating is known to be the gelation temperature of the organogel [27].

Determination of pH

For pH determination, 1 g of formulation F2 was dispersed in 25 mL of distilled deionsed water and the pH was determined using the Accumet® excel XL 25 pH Meter pH meter. The pH meter was calibrated with standard buffer solutions of pH 4, 7, 10 before each use.

Transmission Electron Microscopy (TEM)

TEM (HITACHI HD- 2300 A, Ultra-thin Film Evaluation System) was employed in studying the morphology of PLO gel. Blank and drug loaded PLO gels (formulation F2) were placed on a copper grid. The samples were then stained using 2% phosphotungstic acid solution. The copper grid was allowed to dry overnight undisturbed. The samples were then observed using the TEM.

Estimation of dexamethasone and tobramycin

The drug content of dexamethasone was estimated by dispersing 0.5 gm of F2 in 5 mL of methanol. Samples were analyzed using a high-performance liquid chromatography system (HPLC) (Waters Alliance e2695 separation module, Milford, MA), equipped with a 2998 PDA detector. Dexamethasone was analyzed by an isocratic method with a mobile phase containing water and acetonitrile (50:50) pumped at a flow rate of 1 mL/min. The retention time of dexamethasone (λ_{max}= 242 nm) was found to be 2.9 minutes. Different calibration standards of dexamethasone ranging in between 1-50 µg/mL were prepared in

the mobile phase. For calibration curve, each standard was analyzed in triplicate and the average peak area was plotted against concentration. The drug content was determined quantitatively by plotting a calibration curve. The estimation of tobramycin was measured using an UV-Vis spectrometer (Agilent UV spectrophotometer 8453) [28]. Accurately measured tobramycin standards ranging from 25-150 µg/mL were mixed with 1mL of 2% sodium bicarbonate solution and mixed vigorously. Then, 2 mL of freshly prepared 95% ethanolic solution of 2,4-dinitro fluoro benzene was added to the above mixture and vortexed for 2 minutes. This solution was kept aside for 20 minutes, after which 0.5mL of 0.1N HCl solution was added and tapped so as to remove any bubbles of carbon dioxide. The solution was filtered and the filtrate was analyzed using the UV-Visible spectrophotometer at 415 nm for plotting the calibration curve. The tobramycin content in the PLO gel was estimated by treating 0.283 gm of the gel in a similar manner and the drug content was determined from the tobramycin calibration curve.

Ex vivo permeation of dexamethasone

Bovine eyelids were procured from a nearby slaughterhouse (Kastel's Slaughter House & Processing Center, Riga, MI) and cleaned. Permeability experiments were initiated within 2-3 hours after the sacrifice. Bovine eyelids were using Dulbecco's phosphate buffered saline (pH-7.4) and the hair was shaved using a razor. Approximately, 2 cm^2 specimens were cut and mounted on Franz-type cells (PermeGear Inc., Hellertown, PA) for carrying out the permeability studies. The outer surface was placed towards the donor chamber on which the drug depot (formulation F2 or Tobradex® eye ointment) was placed. The receptor chamber was filled with phosphate buffered saline containing 0.025% w/v

sodium azide and 0.02% (w/v) tween 80 to maintain sink condition. An aliquot (300 µL) was withdrawn at regular time intervals and replaced with equal amounts of fresh buffer. All the experiments were carried out under sink conditions. Samples were analyzed for dexamethasone by HPLC as previously described. Permeability studies were carried out in triplicate.

Permeability (P_{app}) of dexamethasone from PLO gel formulation was calculated using Eq. 1.

Permeability (P_{app}) = Flux/C_d Eq. 1

Flux (J) is calculated by dividing the slope obtained by plotting the cumulative amount of drug permeated (M) through the skin vs. time (t) with cross-sectional area of the membrane (A) exposed to the drug. C_d is the initial drug concentration in the donor chamber [29].

Anti-microbial disk diffusion susceptibility test

Microorganisms were grown on Mueller-Hinton agar in the presence of filter paper disks impregnated with antibiotic. The presence or absence of growth around the disks or cylinders indicates the ability of the anti-microbial agent to inhibit that organism.

Preparation of Mueller-Hinton (MHA – GMB) plates

Initially, Mueller-Hinton agar medium was prepared according to the manufacturer's instructions and autoclaved for 15 minutes at 20 psi, followed by the cooling of the agar medium to 40-45^{o}C in a water bath. The pH of the agar medium adjusted before autoclaving. The cooled agar was then poured into flat bottomed petri dishes so as to

obtain a uniform depth of 4mm under aseptic conditions. The petri dishes were allowed to cool to room temperature and maintained in the refrigerator (2-8^{o}C) until used.

Preparation of inoculum

Pseudomonas aeruginosa (ATCC 27853) was used for performing the anti-bacterial activity of the PLO gel formulation containing tobramycin, as tobramycin has the most effective anti-bacterial activity against Pseudomonas species. The organisms were sub-cultured the previous day so as to ensure that the organisms tested are in their log phase of growth so as to make sure that the results produced are valid. Four or five isolated colonies of the organism to be tested were touched using a sterile cotton swab. The organisms were suspended in 5ml of saline medium and vortexed well so as to obtain a uniform suspension. The turbidity of the suspension was adjusted to a 0.5 McFarland standard by adding more organisms if the suspension was too light or diluting with sterile saline if the suspension was too turbid. The suspension was prepared just before inoculating the organisms on the agar plate.

Inoculation of the MH plate

A sterile cotton swab was introduced into the inoculum tube and rotated against the walls of the tube by applying firm pressure, ensuring the removal of excessive fluid so that the swab is not dripping wet. The dried surface of the MH agar plate was inoculated by streaking the swab six times over the entire agar surface by rotating the plate approximately 60 degrees each time assuring an even distribution of the inoculum all over the plate. The plate was rimmed with the swab to remove any excess liquid present

and the swab was disposed appropriately. The lid of the jar was slightly opened and the plates were allowed to sit at room temperature for at least 3 to 5 minutes so as to ensure the drying of the plates

Preparation and placement of the impregnated disks or cylinders

Formulation F2 containing tobramycin equivalent to 15, 20 and 40 µg was weighed carefully onto sterile disks and refrigerated for 15 minutes. PLO gel liquefies at 4-8^{o}C and allows uniform absorption of gel into the disk. Similarly, the Tobradex® ointment containing tobramycin equivalent to 15, 20 and 40 µg was weighed carefully onto sterile disks and heated slightly to melt the ointment base. This allowed uniform absorption of ointment into the disk. Gentamicin 10 µg standard discs were used to ensure that the agar medium was good enough to support the growth of micro-organism beyond the zone of inhibition. Sterile disks containing different formulations were placed uniformly, equidistant from one another using a sterile forceps. All the disks were pressed gently onto the agar surface with the forceps so as to ensure complete contact with the agar surface and avoid irregular zone shapes. Care was taken such that no disks were placed close to the edge of the plate as the zones will not be fully round and would be difficult to measure.

Incubation of plates and measurement of the zone sizes

The inoculated petri dishes were incubated at 37^{o}C for a period of 24 hours, after which the zones of inhibition were measured. The zone sizes were measured to the nearest millimeter using a ruler, which includes the diameter of the zone of inhibition. All the

measurements were done with an unaided eye while viewing the back of the petri dish against a black, non-reflecting surface illuminated with reflected light.

Anti-microbial activity of formulations F2 and F6 without drugs

The anti-microbial activity of blank ricinoleic acid PLO gel was compared with the light mineral oil PLO gel. Formulation F2 without any drugs was used for the study in order to show the inherent anti- microbial activity of ricinoleic in the PLO gel. Cylinder plate assay was performed to observe the anti-microbial activity exhibited by ricinoleic acid. In this study, *Escherichia coli* ATCC 25922 was used as a model organism. *E.coli* was cultured similar to *P.aeruginosa* as described earlier. Cylinders were bored using a sterile borer in a perti-dish inoculated with *E.coli.* Ten milligrams of blank ricinoleic acid or mineral oil PLO gel was added to the cylindrical wells. Cylinder plate method was employed in order to facilitate the penetration of the gel into the agar medium. The inoculated petri dish was incubated at 37^{o}C for a period of 24 hours, after which the zones of inhibition were measured. The zone sizes were measured to the nearest millimeter using a ruler, which includes the diameter of the zone of inhibition. All the measurements were done with the unaided eye while viewing the back of the petri dish against a black, non-reflecting surface illuminated with reflected light.

6.4. Results

Five different PLO gel formulations containing tobramycin (0.3%) and dexamethasone (0.1%) were prepared using ricinoleic as the oil phase (Table 6-1). F1 and F6 containing

similar concentrations of lecithin and poloxamer but with a different oil phase appeared to be similar in consistency. No apparent change in the physical appearance was observed when ricinoleic acid was used as the oil phase. Lowering the concentrations of lecithin and poloxamer in F2 and F3 resulted in a slight change in the overall consistency. No gelation occurred when the poloxamer concentration was less than 12% w/w. So, formulations F4 and F5 were discontinued from further characterization studies.

Table 6.1: PLO gels prepared with varying concentrations of lecithin and poloxamer

Formulation	Lecithin (%w/v)	Ricinoleic acid (%w/v)	Light mineral oil (%w/v)	Poloxamer (%w/v)
F1	10	10	-	16
F2	1	5	-	14
F3	1	5	-	12
F4	2	10	-	10
F5	2	10	-	8
F6	10	-	10	16

The viscosities of ricinoleic acid PLO gels (F1, F2 and F3) were compared with light mineral oil PLO gel (F6) at varying shear stresses. Formulation F1 exhibited a slightly higher viscosity than the corresponding control formulation (F6). Figure 6.2a shows the apparent viscosity vs. RPM (shear stress) of the formulations. The viscosities of F1, F2, F3 and F6 were found to be at 5 rpm were found to be 56883.22 cps, 30956.64 cps, 15875.20 cps and 46673.09 cps, respectively.

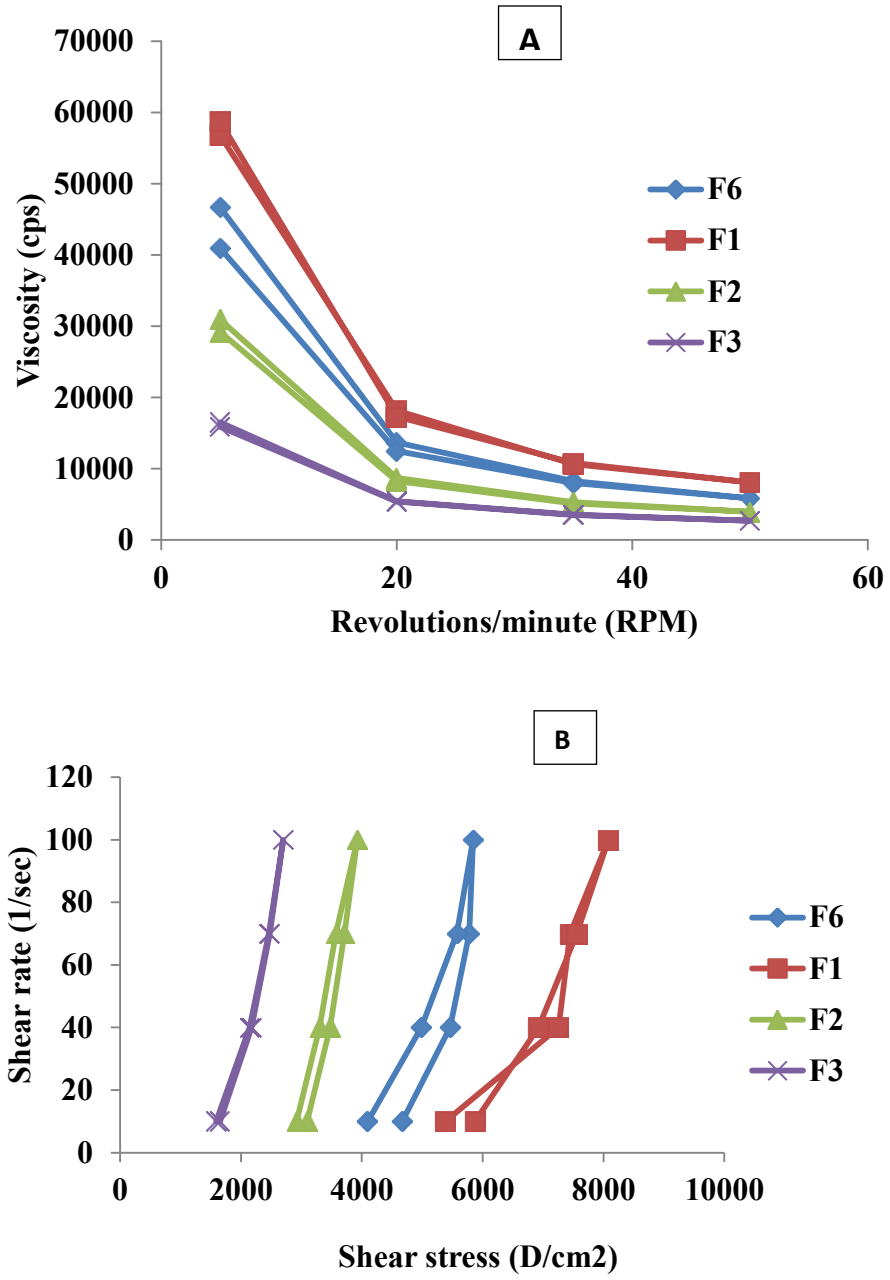
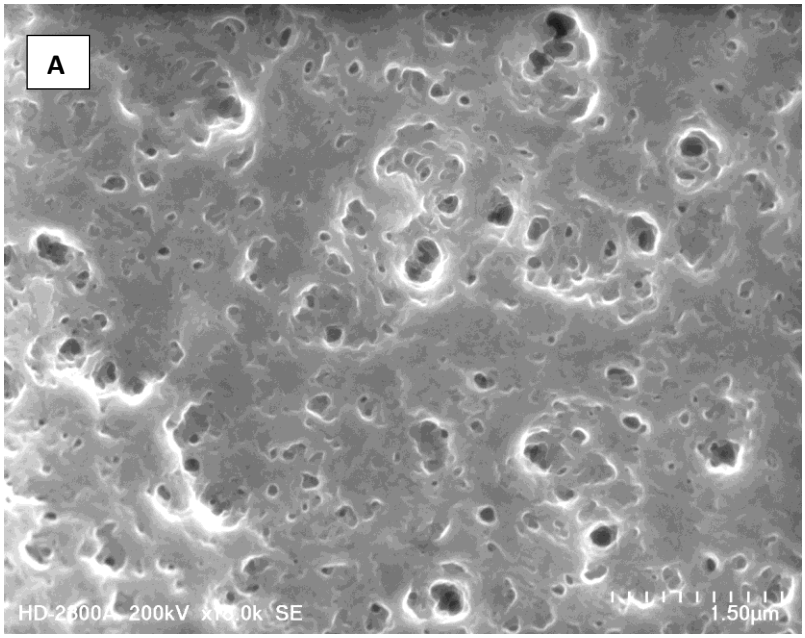


Figure 6-2: Viscosity data of PLO gels containing mineral oil and ricinoleic acid. (A) apparent viscosity vs. RPM (shear stress) of the formulations, (B) thixotropic behavior of the formulations.

With same lecithin and poloxamer concentrations, the viscosity of PLO gel was slightly higher in the presence of ricinoleic acid as the oil phase when compared to light mineral oil. All the formulations exhibited a non-Newtonian behavior with pseudoplastic flow and the shear thinning behavior, indicated by the downward sloping curves. No significant loss of viscosity was observed in the tested formulations at the end of the test (Fig. 6.2a).

Considering the sensitivity of eyelids, formulation F2 containing lesser concentrations of lecithin and poloxamer (1% w/v lecithin and 14% w/v poloxamer) was considered for later studies. F2 was characterized for pH, gelation temperature, morphology and drug content. The pH of the blank and drug loaded PLO gels was found to be 5.72 and 6.54, respectively. The gelation temperature of the organogel containing ricinoleic acid was found to be around 31ºC, at which the liquid form of polymer solution turned into a semi-solid gel. The TEM images of F2 (blank and drug loaded PLO gel) containing ricinoleic showed a vesicular framework formed due to the presence of lecithin in organogel (Fig. 6-3). The drug content of dexamethasone was estimated by the HPLC technique. The retention time of dexamethasone (λ_{max}= 242 nm) was found to be 2.9 minutes (Fig. 6-4a). Concentrations ranging between 1-50 mcg/mL were used for plotting the calibration curve. A straight line (y = 43098x - 5679.5) was obtained with a correlation coefficient (r^2) value of 0.9989 (Fig. 6-4b). Tobramycin was analyzed using a UV spectrophotometer. Tobramycin concentrations ranging between 25-150 µg/mL were used for plotting the calibration curve. A straight line (y = 0.0051x - 0.0181) was obtained with a correlation coefficient (r^2) value of 0.9995 (Fig. 6-5). The drug content of tobramycin and dexamethasone were found to be 102.8 % and 100.14%, respectively.



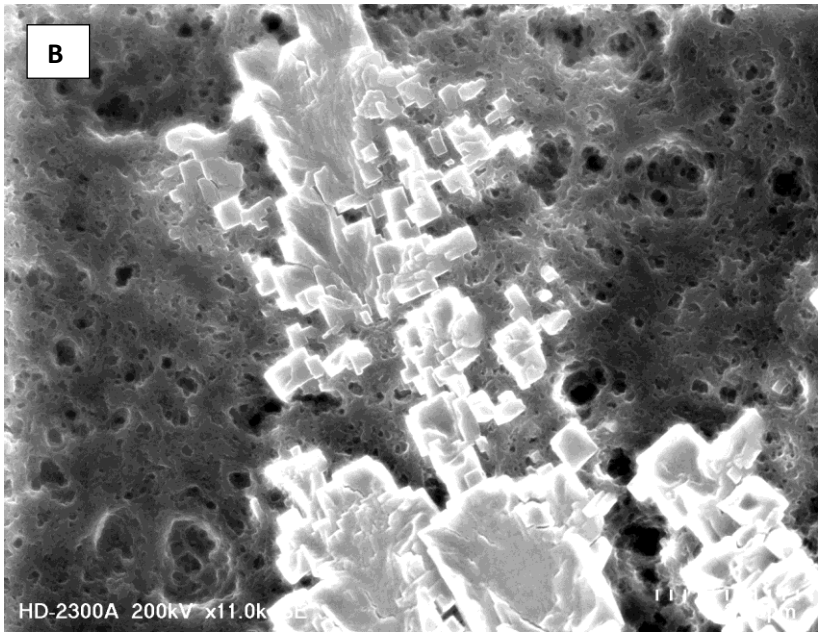


Figure 6-3: Transmission electron microscopy image of ricinoleic acid PLO gel (formulation F2), (a) blank and (b) drug loaded

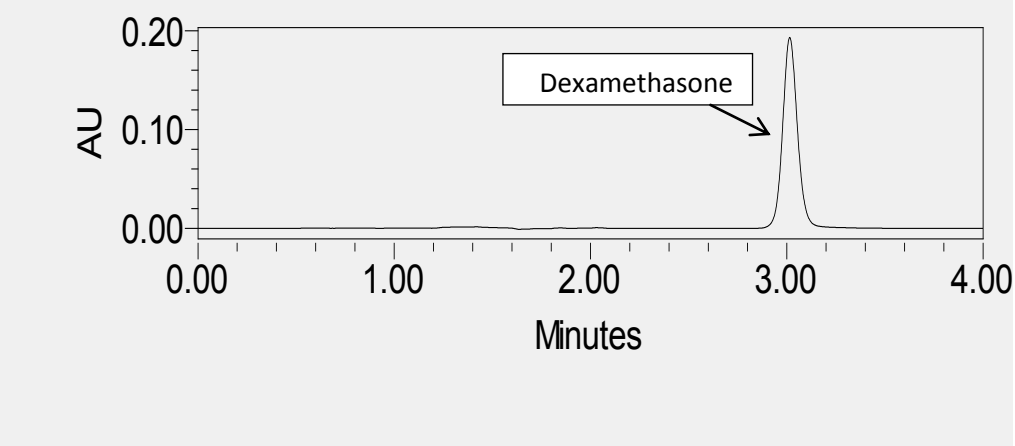


Figure 6-4a: Sample HPLC chromatogram for dexamethasone

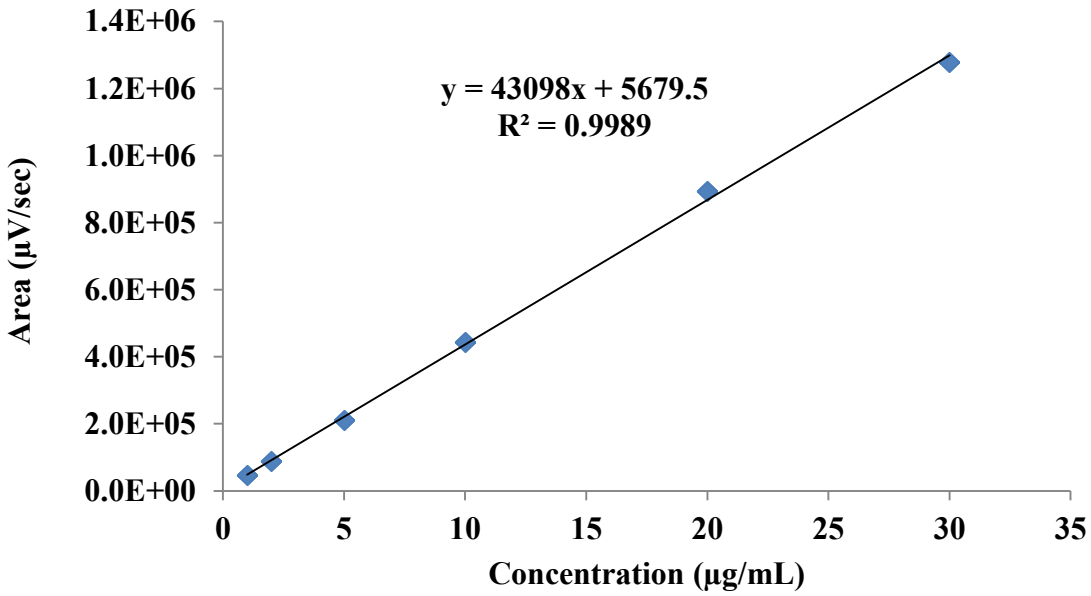


Figure 6-4b: Calibration curve for dexamethasone

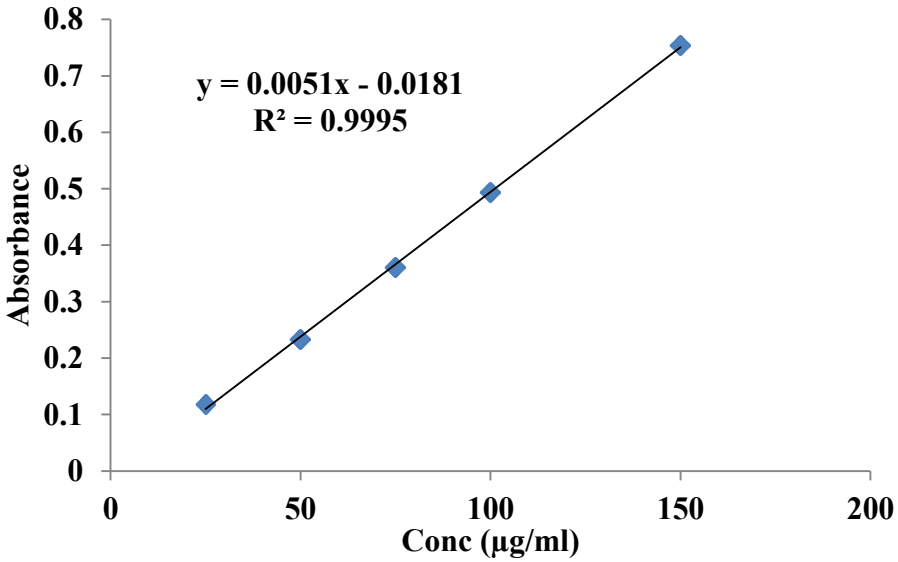


Figure 6-5: Calibration curve for tobramycin

An *ex-vivo* permeability study of dexamethasone was carried out across the excised bovine eyelids using a Franz-type vertical diffusion cell. The penetration profile of dexamethasone from F2 was found to be much higher than the marketed Tobradex® eye ointment. Permeability values ($PX10^5$) of dexamethasone from F2 and Tobradex® eye ointment (control) were found to be 19.04 and 1.15 cm/hour, respectively (Fig. 6-6). A significant difference in the permeability of dexamethasone was evident. To our surprise, no dexamethasone concentrations were observed in the receptor chamber from Tobradex® formulation until 16 hours after administration. In the case of PLO gel containing ricinoleic acid (formulation F2), permeation of dexamethasone was observed after one hour in a continuous manner.

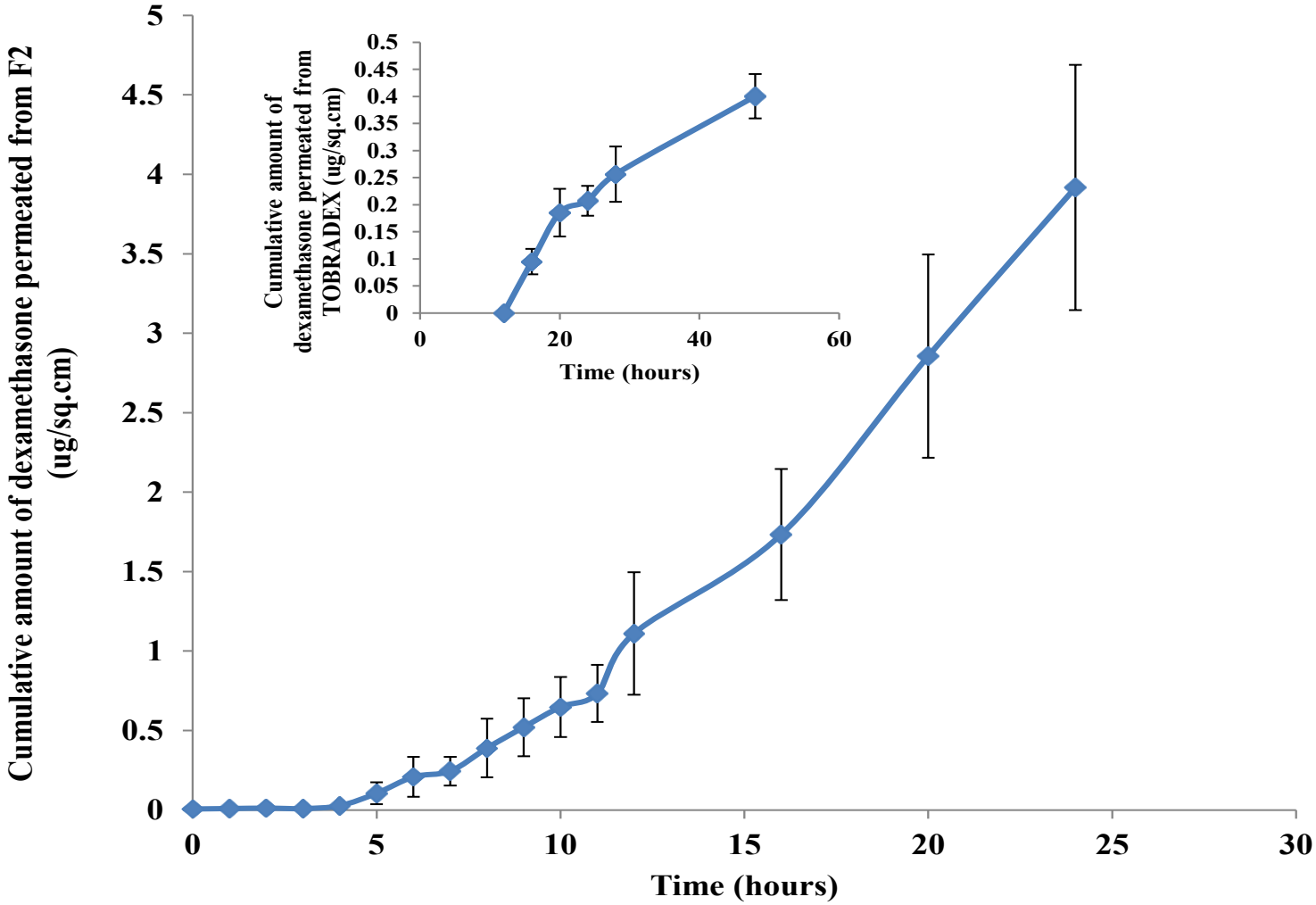


Figure 6-6: *In* vitro permeation of dexamethasone across bovine eyelid from formulation F2 and Tobradex® eye ointment

The results of the anti-microbial disk diffusion susceptibility study indicated that the formulation F2 (ricinoleic acid PLO gel with tobramycin) had a better anti-microbial activity (higher zone of inhibition) compared with the marketed Tobradex® eye ointment. The average zones of inhibition of F2 and Tobradex® ointment were found to be 17.75 ± 0.5 mm (n=4) and 12.875 ± 1.55 mm (n=4), respectively (Table 6-2). Similar results were obtained when the experiment was repeated with higher tobramycin concentrations of 20 and 40 µg (Table 6-3). Surprisingly, no significant increase in the zones of inhibition was observed with increasing tobramycin concentrations.

Table 6.2: Anti-bacterial activity of different formulations containing 15µg of tobramycin and standard gentamicin disk against Pseudomonas aeruginosa (n=4)

Formulation	**Zones of inhibition (mm) ± S.D**
Formulation F2	17.75 ± 0.5
Tobradex ointment	12.875 ± 1.55
Standard gentamicin disk 10µg	18 ± 0

Table 6.3: Anti-bacterial activity of different formulations containing different amounts of tobramycin and standard gentamicin disk against Pseudomonas aeruginosa (n = 2) with their respective zones of inhibition (mm).

Amount of the drug	**Formulation F2**	**Tobradex ointment**	**Gentamicin standard 10ug**
15 µg	18± 0	16± 0	18± 0
20 µg	19 ± 0	16± 0	18± 0
40 µg	20 ± 0	14.5± 3.54	18± 0

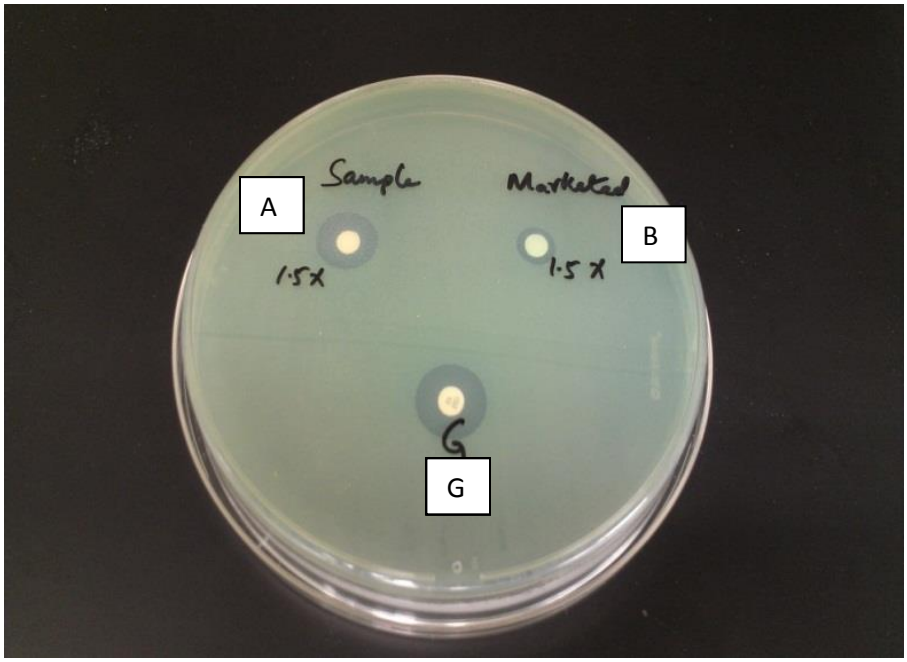


Figure 6-7a: Zones of inhibition produced by discs loaded with (A) PLO gel containing 15 µg of tobramycin, (B) Tobradex® ointment containing 15 µg of tobramycin, (G) gentamcin 10 µg standard disc

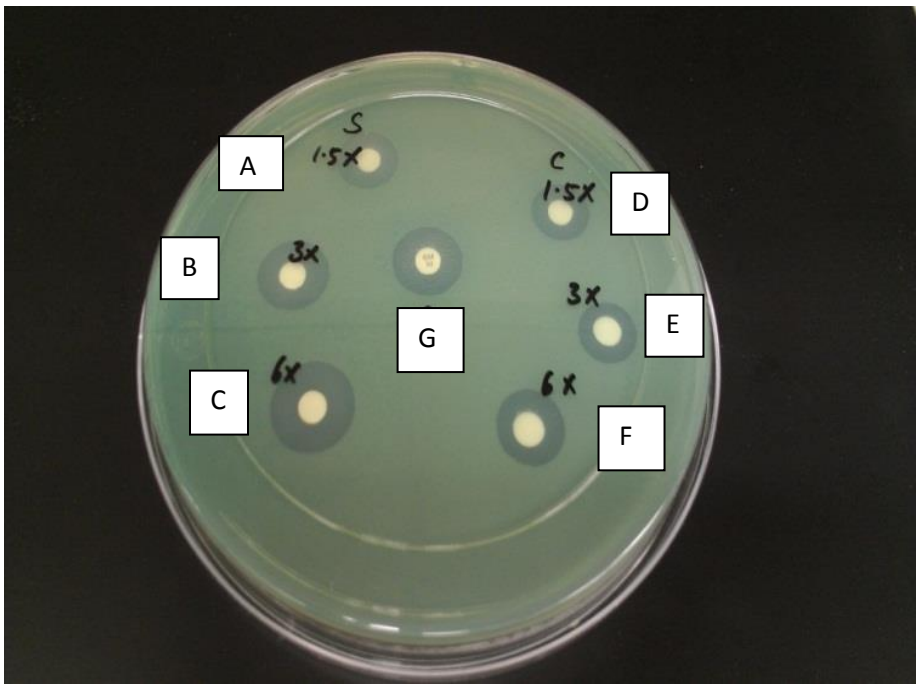


Figure 6-7b: Zones of inhibition of PLO gel with varying amounts of tobramycin (A =15 µg; B = 20 µg; C = 40 µg) and Tobradex® ointment with varying amounts of tobramycin (D = 15 µg; E = 20µg; F =40 µg), gentamicin 10 µg standard disc (G)

Also, the inherent anti-microbial activity of blank ricinoleic acid PLO gel was studied using *E.coli* as a model micro-organism. In this study, the PLO gel containing mineral oil exhibited no zone of inhibition, while ricinoleic acid PLO gel exhibited an average zone of inhibition of 10.83 $\pm$ 1.16 mm (n = 6).

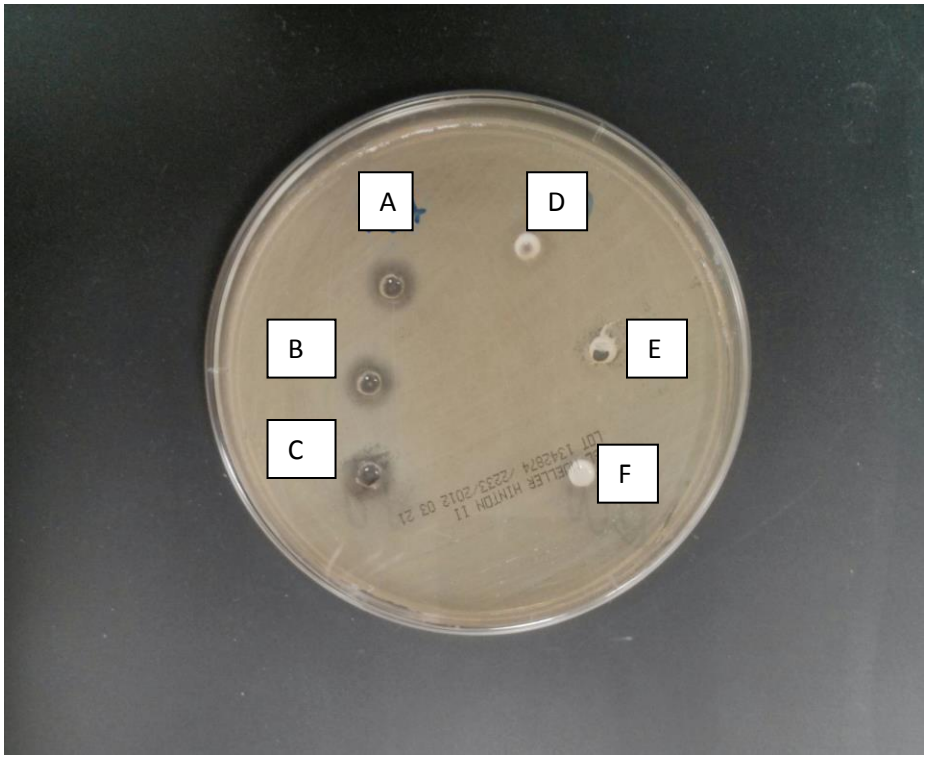


Figure 6-8: Zones of inhibition produced by blank formulations F2 (A, B and C) and F6 (D, E, and F)

6.5. Discussion

Ophthalmic infections in young children usually affect both eyes and pose unique challenges in diagnosis and treatment. Some common pediatric eye infections include conjunctivitis (inflammation of the conjunctiva), blepharitis (inflammation of the eyelids), keratitis (inflammation of the cornea), keratoconjunctivitis (inflammation of the cornea and conjunctiva), chalazion (cyst on the eyelid) and keratoconus **(**a progressive

thinning of the cornea). Inflammation is generally caused by a bacterial or viral infection or an allergic reaction. Ocular infections require prompt diagnosis and treatment in order to decrease the risk of serious visual complications. Most bacterial infections are commonly treated with compresses, anti-bacterial and steroidal drugs administered in the form of either eye drops or ointments. Administering eye drops or ointment in the eyes can be a real challenge, especially in young children who are reluctant to tolerate conventional instillation. Children intuitively resist putting any medication in the eye and the necessity to administer the formulation three to six times a day adds to the problem.

This study aimed at the development of a novel PLO gel formulation that can be applied to the outer surface of eyelid skin to ensure drug penetration into various ocular tissues (eyelids, cornea and conjunctiva) in a sustained manner. Application of drug to the eyelid skin in the form of a gel provides a constant concentration in the tear film for a longer duration. Transdermal ophthalmic PLO gel of tobramycin and dexamethasone was formulated successfully using ricinoleic acid as the oil phase. This study clearly indicates the ability of ricinoleic acid to form PLO gels with comparable physicochemical properties. The prepared formulations were gels at room temperature and liquefied when stored at 4ºC, which is due to the presence of poloxamer. Viscosity studies revealed the pseudoplastic behavior of PLO gels. The viscosity of PLO gels decreased with decreasing poloxamer concentrations. This indicates the influence of poloxamer concentration on the rheological characteristics of ricinoleic acid PLO gel. Formulations F4 and F5 resulted in a suspension instead of homogenous gel when poloxamer concentration was less than 12%. Formulations F1, F2, F3 and F6 exhibited some thixotropy and the concentration of poloxamer had an influence on this parameter. The shape of rheograms remained

constant but a shift in rheograms was observed in the yield values and the size of hysteresis loop increased with poloxamer concentration. The increase in the yield value indicates the strengthening of the network structure or the vesicular framework of the PLO gels. The yield value in the case of topical gel formulations should be sufficiently low so as to facilitate the spreadability when applied onto the skin and permit its removal from the container.

Formulation F2 was considered suitable as it showed required gelation properties with lower lecithin and poloxamer concentrations. So, further characterization studies were carried out for the formulation F2. TEM study revealed the presence of a vesicular framework in the organogel, which is due to the presence of lecithin. It explains the phenomenon by which the gel interacts with the skin, thereby causing the penetration of the drug molecule through the skin. Product performance tests serve as a useful tool in the product development process. We studied the *ex vivo* permeability of dexamethasone and bactericidal activity of tobramycin was tested for F2 and compared with Tobradex® eye ointment. Franz diffusion cells are widely used for testing the permeation of drugs from a gel formulation. The formulation F2 showed significantly higher penetration of dexamethasone across bovine eye lids compared with Tobradex® eye ointment. No measurable drug release observed up to 16 hours from Tobradex®, while the formulation F2 exhibited a continuous release and permeation of dexamethasone after one hour. This study clearly indicated the lack of release and penetration of dexamethasone from Tobradex® ointment which might be due to the binding of the drug to the ointment base. Moreover, PLO gels have the ability to pass through the lipid barrier in the keratin layer of the skin and thereby enhancing the drug absorption [30].

Kirby Bauer disk susceptibility test was performed to study the anti-microbial activity of tobramycin and ricinoleic acid against *P.aeruginosa* and *E.coli* respectively. Anti-microbial disk susceptibility test is preferred for studying the *in vitro* anti-microbial activity of an antibiotic, especially in a semi-solid formulation, as the formulation intended for the study remains intact throughout the study. Kirby-Bauer disk diffusion susceptibility aims at determining the sensitivity or resistance of a pathogenic microorganism to various antimicrobial compounds (commonly known as the antibiotics), which help in treating the infection against a particular microorganism [31]. The pathogenic microorganisms are known to grow well on Mueller-Hinton agar medium in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks or the cylinders reflects the ability of that anti-microbial agent to inhibit that organism. The anti-microbial disk susceptibility test performed in this study revealed the fact that the PLO gel containing tobramycin (formulation F2) was more effective against the micro-organism *P.aeruginosa* than the Tobradex® ointment. However, no significant increase in the zones of inhibition was observed with increasing tobramycin concentrations. This could be attributed to the retention of tobramycin inside the gel/ointment structure and only the drug adsorbed onto the gel/ointment surface is resulting in the zone of inhibition. Moreover, the inherent anti-microbial activity of ricinoleic acid used in the formulation of the PLO gel was confirmed by the cylinder plate assay performed on *E.coli.* The results clearly indicate that the ricinoleic acid PLO gel has the potential for use as a transdermal eyelid delivery system for both hydrophilic and hydrophobic drug molecules. PLO gels are cost-effective and easy to manufacture on a large scale. This new delivery system would improve

compliance and enhance the ease of administration of ophthalmic drugs. This system can also be implemented for treating other anterior segment diseases that require a very high frequency of eye drop instillation.

6.6. Conclusion

The findings of this investigation conclusively indicate the formation of a stable PLO gel with ricinoleic acid. Ricinoleic acid PLO gel was found to be better than the Tobradex® eye ointment with respect to the *ex vivo* permeation of dexamethasone and anti-bacterial property of tobramycin. However, *in vivo* pharmacokinetic studies should be carried out to determine the anterior segment concentrations and the irritation potential of the ricinoleic acid PLO gel.

6.7. References

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