Lipid & surfactant based systems for improved delivery of poorly soluble APIs

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LIPID & SURFACTANT BASED SYSTEMS FOR IMPROVED DELIVERY OF POORLY
SOLUBLE APIs

A Dissertation Submitted
In The Partial Fulfillment of Requirements For
The Doctor of Philosophy Degree in Pharmaceutical Sciences
With an emphasis in Pharmaceutics & Drug Delivery

by

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ABSTRACT

A large number of pharmaceutical compounds belong to class II and IV of biopharmaceutical classification of drug (BCS). Class II compounds are limited by their poor aqueous solubility, while class IV compounds suffer from poor solubility and permeability. This translates to poor absorption and low plasma levels post oral administration. Lipids comprise of fatty acids and their derivatives and are considered a biocompatible option for drug delivery. In the studies discussed in following chapters, lipid based drug delivery systems (LBDDS) have been utilized in improving the handling, ease of formulation, solubility and bioavailability of three compounds. In the first study, studies have been performed to formulate $\Delta^9$-THC into a sustained release tablet, using lipid matrices, for the treatment of chemotherapy induced nausea and vomiting (CINV). In the second study, LBDDS such as solid lipid nanoparticles were prepared for non-invasively enhancing the ocular penetration of $\Delta^8$-THC for the treatment of Glaucoma. In the third study, the handling and oral bioavailability of dihydroartemisinin dimer oxime was studied using various lipid based systems for potential use in the treatment of malaria.

The lipid based tablet formulation of $\Delta^9$-THC was successful in achieving a 24 hour release profile and can be potentially be used as a one dose per day medication for CINV. The topical ocular penetration of $\Delta^8$-THC significantly when formulated as solid lipid nanoparticles and the drug was able to reach the posterior ocular segments, with levels being maintained at the end of three hours. The dimer oxime was able to achieve an 8 hour plasma profile through various lipid based systems and these levels were above the IC$_{50}$ values of the malarial parasite.
The tested prodrug showed a lot of promise and further optimization of these formulations would help in developing a new line of malarial therapy.
DEDICATION

I would like dedicate this thesis to my parents Ananth Babu Punyamurthula and Vydehi Prasanna Punyamurthula for their constant encouragement, unyielding support and love throughout the course of my life.
ACKNOWLEDGEMENTS

Foremost, I would like to thank my adviser Dr. Soumyajit Majumdar for his support, patience and guidance throughout my graduate studies. He has been a great teacher and a constant source of innovation and inspiration. I am grateful to my dissertation committee members Dr. Michael A. Repka, Dr. S. Narasimha Murthy and Dr. John O’Haver for their guidance and time. Finally, along with my parents, I would like to thank Harika Tadepally, Dr. Yoganand Kanduri, Sandeep Tadepally, Rajesh Satyavolu & Padmaja Ayyagari without whose support this would not have been possible.
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CHAPTER 1

INTRODUCTION
A large number of pharmaceutical compounds, established and newer ones with promising therapeutic potential, belong to class II and IV of biopharmaceutical classification of drug (BCS). Class II compounds are limited by their poor aqueous solubility, while class IV compounds suffer from poor solubility and permeability. This mostly translates to poor absorption and low plasma levels post oral administration. To overcome these issues, over the years, various strategies have been investigated, e.g. using salt forms, co-crystals, complexation, micellar solutions, co-solvents, lipid systems. Even though all of these approaches have shown some degree of success in improving the delivery of poorly soluble compounds, surfactant & lipid based systems have probably demonstrated the highest versatility in terms of improving delivery of a large number of compounds.

Lipids comprise of fatty acids and their derivatives and are considered a biocompatible option for drug delivery[1]. The formulations vary from simple oil based solutions to the more complex self-emulsifying drug delivery systems (SEDDS). Depending on the formulation characteristics and behavior in vivo, lipid based drug delivery systems (LBDDS) LBDDS are classified into 4 types as shown in Table 1-1.

In the studies discussed in following chapters, these lipid based carriers have been utilized in improving the handling, ease of formulation, solubility and bioavailability of three compounds, namely, Δ⁹-Tetrahydrocannabinol (Δ⁹-THC), Δ⁸-Tetrahydrocannabinol (Δ⁸-THC) & a prodrug of dihydroartemisinin dimer (dimer oxime).
Table 1-1: Classification of Lipid Based Drug Delivery Systems

<table>
<thead>
<tr>
<th>Formulation type</th>
<th>Material</th>
<th>Characteristics</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Oils without surfactants (e.g., tri-, di-, and monoglycerides)</td>
<td>Nondispersing - requires digestion</td>
<td>Generally recognized as safe (GRAS) status; simple; and excellent capsule compatibility</td>
<td>Formulation has poor solvent capacity unless drug is highly lipophilic</td>
</tr>
<tr>
<td>Type II</td>
<td>Oils with water insoluble surfactants</td>
<td>SEDDS formed without water-soluble components</td>
<td>Unlikely to lose solvent capacity on dispersion</td>
<td>Turbid o/w dispersion (particle size 0.25–2µm)</td>
</tr>
<tr>
<td>Type III</td>
<td>Oils with surfactants, and cosolvents (both water-insoluble and water-soluble excipients)</td>
<td>SEDDS/SLNs formed with water-soluble components</td>
<td>Clear or almost clear dispersion, drug absorption with digestion</td>
<td>Possible loss of solvent capacity on dispersion, less easily digested</td>
</tr>
<tr>
<td>Type IV</td>
<td>Water-soluble surfactants and cosolvents</td>
<td>Formulation disperses typically to form a micellar solution</td>
<td>Formulation has good solvent capacity for many drugs</td>
<td>Likely loss of solvent capacity on dispersion may not be digestible</td>
</tr>
</tbody>
</table>
i. In the first study, an effort will be made to formulate $\Delta^9$-THC into a sustained release tablet using lipid matrices. Tetrahydrocannabinol, the active constituent of Cannabis sativa is used in the treatment of chemotherapy induced nausea and vomiting (CINV). This compound binds to the cannabinoid receptors, CB1 in the central (brain) and CB2 in the peripheral nervous systems (spleen), thereby controlling vomiting and is also beneficial in the treatment of weight loss.[2] They are very beneficial in the treatment of CINV and added benefits include analgesia, anti-tumor effects, mood elevation and cure for insomnia in cancer patients.[3, 4] Commercially available pharmaceuticals containing cannabinoids include Nabilone (Cesamet®) and Dronabinol (Marinol®). Nabilone is given twice a day up to 48 hours post chemotherapy[5] and Dronabinol, every 2-4 hours for a total of 4-6 doses a day,[6, 7] a common reason for patient non-compliance. Also, use of Dronabinol is contraindicated in case of patients with hypersensitivity reaction to sesame oil[6, 7]. The aim of the present study is to formulate an oral sustained release tablet formulation of $\Delta^9$-THC, which can be administered once a day and provides a sustained effect over a longer period of time. Also, by eliminating the sesame oil in the formulation one can enhance the acceptance among a majority of patients.

ii. In the second study, LBDDS will be applied for enhancing the ocular penetration of $\Delta^8$-THC for the treatment of Glaucoma. $\Delta^8$-Tetrahydrocannabinol ($\Delta^8$-THC) is the primary active constituent of Cannabis sativa and an isomer of $\Delta^9$-THC. This compound has shown potential in treatment of glaucoma through its intra-ocular pressure (IOP) lowering and neuroprotective effects, through its agonistic action on the CB1 and CB2 receptors. It
presents many challenges, however, in formulation and delivery owing to its high lipophilicity, poor aqueous solubility and resinous nature. The fact that the eye is an organ with complex physiological barriers further complicates the problem. Using solid lipid nanoparticles as carriers, studies will be undertaken to evaluate, and possibly enhance, penetration of Δ⁸-THC to the posterior ocular segment following topical application.

iii. In the third study, various surfactant & LBDDS will be utilized for improving the oral bioavailability of a novel dihydroartemisinin dimer. Dihydroartemisinin (DHA) is the active metabolite of Artemisinin, which in turn is the active constituent of Artemisia annua L and is used in the treatment of malaria. This compound was proven to be highly effective on the malarial parasite and has a short fever clearance time and low toxicity. Inherently, Artemisinin or DHA are both BCS class IV compounds, which means low solubility and permeability. For this study, a novel DHA dimer with an oxime group was synthesized and this compound’s physicochemical characteristics will be delineated, following which, the compound will be incorporated into various surfactant & lipid based systems. These formulations will be studied with respect to their performance, both in vitro and in vivo.
CHAPTER 2

AIMS OF THE STUDY
**Overall Objective:**

The goal of the studies is to improve the handling and bioavailability of these three compounds. Surfactant & lipid based systems will be utilized for this purpose and various formulation approaches such as solid lipid nanoparticles (SLNs), nanolipid carriers (NLCs) and sustained release systems will be explored. This is based on the hypothesis that orally, the surfactants will help in enhancing the solubility of the compounds in solution and LBDDS will help bypass the dissolution step by presenting the drug in a pre-dissolved form and avoidance of re-precipitation from this pre-dissolved state, emulsify the drug in the intestinal milieu and finally, enhance the lymphatic uptake processes. From an ophthalmic delivery point of view, the lipid based nanoparticles will help increase the residence time on the ocular surface because of entrapment in the mucosal layer and active epithelial uptake and size.

**Specific Aims:**

1. To screen various lipids and fillers and finally suggest an optimal composition for a sustained release tablet of $\Delta^9$-THC.

2. To screen $\Delta^8$-THC for its physicochemical characteristics, formulate various LBDDS and evaluate their *in vitro* release, entrapment efficiencies and transcorneal permeability.
3. To evaluate the \textit{in vivo} bioavailability of these formulations in rabbits & compare it with conventional solution formulations.

4. To screen the DHA dimer oxime for its solubility, stability and other physicochemical characteristics.

5. To formulate various DHA incorporated surfactant, LBDDS and compare their \textit{in vitro} permeability across intestinal segments.

6. To compare the oral bioavailability of these formulations and suggest a platform for enhancing the oral bioavailability of DHA dimer oxime.
CHAPTER 3
CONTROLLED RELEASE TABLET FORMULATION CONTAINING
NATURAL Δ⁹ - TETRAHYDROCANNABINOL
3.1. Introduction

Chemotherapy Induced Nausea and Vomiting (CINV) is the most common and feared post chemotherapy side-effect [2, 8-13]. Around 30 to 90% of patients undergoing chemotherapy experience this, thereby reducing the quality of life (QOL) [2]. Tetrahydrocannabinol (THC), a component of cannabis is used against CINV. THC binds to the cannabinoid receptors, thereby controlling vomiting and is also beneficial in the treatment of weight loss [2, 14-20]. Commercially available pharmaceutical cannabinoids include Nabilone (Cesamet®) and Dronabinol (Marinol®)[5-7]. Synthetic THC used in Dronabinol as well as the natural THC obtained from the plant, is a resinous sticky oil that hardens on refrigeration. THC undergoes degradation through several mechanisms [21, 22] and handling of the resinous form is very difficult from a formulation point of view. Dronabinol is thus formulated as a sesame oil solution of THC and is supplied in a soft-gelatin capsule. Nabilone, on the other hand, is a synthetic THC derivative that is crystalline in nature and is thus filled into a hard gelatin capsule.

The goal of this research project was to develop an oral tablet formulation using naturally occurring THC. To overcome the challenges in handling the oily resinous characteristics of THC, a lipid based formulation approach was selected to develop controlled release THC tablets [23, 24].
In vivo, it has been shown that THC undergoes high first pass metabolism, transforming it into its 11-hydroxy metabolite, which results in very low oral bioavailability [22, 25]. Therefore, the lipid based systems could promote lymphatic uptake of THC and thus decrease first-pass metabolism, resulting in greater plasma concentrations at lower doses[24]. Also, to our knowledge this is the first attempt at producing a sustained release solid dosage form for THC. The instability of THC in solid state has been a major obstacle previously[26], which may also be mitigated through the use of a lipid matrix.

3.2. Materials and methods

Precirol® ATO 5 (Glycerol Distearate) and Compritol® 888 ATO (Glycerol Dibehenate) were obtained as gift samples from Gatefosse (St.Priest, France), Avicel® 102 (Micro-crystalline Cellulose) was obtained from FMC Biopolymer (Philadelphia, PA), Emcompress® (Dicalcium Phosphate Anhydrous, DCPA), from JRS Pharma (Rosenberg, Germany), Ludipress® (composed of Lactose monohydrate, Povidone K30 (Kollidon® 30) and Crospovidone (Kollidon® CL) from BASF Fine chemicals (Switzerland), Pluronic® F68 from Sigma Aldrich (St.Louis, Missouri), Aerosil® R972 from Evonik Industries (Germany), Magnesium Stearate (Mg. Stearate) from Spectrum Chemicals (Gardena, CA). All solvents used for analysis were of analytical grade.
Methods

Three types of THC containing tablets were prepared. The variations involved the use of the lipid component either in the lipid-THC matrix, lipid in external phase (added during the blending phase) or incorporating lipid in the matrix as well as in the blend stage. The various formulations evaluated are shown in Table 2.1.

Preparation of lipid-drug matrix

The lipid-drug matrices were prepared by solid dispersion technique. THC lipid dispersions, 25% w/w, were made by heating the mixture to 70°C followed by molding of the dispersion into slugs using 1 mL tuberculin syringes. The matrix was cryo-milled using a Fitz Mill L1A (The Fitzpatrick Company, Elmhurst, Illinois) and sieved through ASTM sieve # 70.

Preparation of tablets

Granules were compressed using a Manual Tablet Compaction Machine, MTCM-I (Globe Pharma Inc., New Brunswick, NJ) with 8mm flat faced punches at compression forces ranging from 7.6kN to 9.8kN. THC-lipid matrices were blended with the other excipients and directly compressed. In some formulations additional lipid was included during the blending stage also. Alternatively, THC (in hexane) was coated on the filler (DPCA/ MCC/ Lactose), dried at 25°C, blended with the other excipients including the lipids under evaluation. The blend was directly compressed into tablets.
Evaluation of tablets

Physical characteristics

The physical properties of the tablets such as appearance, texture, hardness, weight variation, friability were determined in accordance to standard protocols. Hardness was determined using VK-200 tablet hardness tester (Varian Inc, NC).

Assay and Content uniformity of tablets

About 5 tablets for assay and 3 tablets separately for content uniformity were powdered in a mortar and pestle; about 2 mg of the powder was accurately weighed and extracted in 1mL of methanol, followed by centrifugation (13000 rpm, 20 min). The supernatant was collected and diluted suitably with mobile phase and was analyzed in triplicate by HPLC using the analytical method described in the section below.

Analytical method

A Waters HPLC system (Mildford, Massachusetts) consisting of Waters 600 pump controller, refrigerated Waters 717 plus autosampler, Waters 2487 UV detector, and Agilent (Santa Clara, California) 3395 integrator was used in the present investigation. Initial stock solution (1mg/mL) of THC was prepared in ethanol and stored at -20° C till used. Standards were
prepared by pipetting out a known amount of stock solution and evaporating it under a stream of nitrogen gas. Suitable standards were prepared by reconstitution in the mobile phase. A Luna PFP (2), 4.6 × 250mm column, Phenomenex (Torrance, CA) was used for the separation and analytical purposes. Mobile phase had a mixture of methanol – water [containing 0.84% (v/v) glacial acetic acid] in 85:15 ratios. The detector was set at an analytical wavelength of 226nm, while the injection volume was 20uL. The standards were prepared in the concentration range of 1-100 µg/mL. The standard calibration curve was derived and the parameters such as regression coefficient ($r^2$), slope and Y- intercept were noted to establish the linearity of the method. Response after repeated injections from the same sample was recorded to check for precision and dissolution medium with the placebo formulations were injected to ensure there was no interference. The method was also validated with respect to limit of detection (3 times signal-to-noise ratio) and limit of quantification (10 times signal-to-noise ratio).

**In vitro drug release studies**

The *in vitro* drug release (dissolution) studies were performed using USP type I (basket) and type II (paddle) apparatus at 100 rpm and 50 rpm, respectively, and THC release from the tablets was evaluated. The dissolution medium consisted of 900 mL of water containing sodium lauryl sulfate (SLS) (0.5 %w/v), while the temperature was maintained at 37°C±0.5°C. At predetermined time intervals, samples (1 mL) were withdrawn and replaced with an equal volume of dissolution media. The samples were then analyzed by HPLC.
Stability studies

Stability studies were performed as per the ICH guidelines. The tablets were packed into small aluminum pouches which were then sealed under nitrogen. The samples were loaded into stability chambers maintained at 25±2°C/60±5 % RH and 40±2°C/75±5% RH. At predetermined time points, samples were withdrawn for evaluation.

3.3. Results and Discussion

Formulations

A total of 14 formulations with varying excipient proportions were prepared. Qualitative and quantitative composition of all formulations are listed in Table 3-1.
**Table 3-1:** Quantitative composition of the various formulations tested. Values represent mg/tablet.

<table>
<thead>
<tr>
<th>Code</th>
<th>THC</th>
<th>Precirol in Matrix</th>
<th>Compritol in Matrix</th>
<th>Avicel</th>
<th>Ludipress</th>
<th>Pluronic</th>
<th>Precirol in Blend</th>
<th>Compritol in Blend</th>
<th>Aerosil</th>
<th>Mg. Stearate</th>
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<tr>
<td>F-1</td>
<td>10</td>
<td>30</td>
<td>159.7</td>
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<tr>
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<td>0.1</td>
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</tr>
<tr>
<td>F-3</td>
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<td>30</td>
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<td></td>
<td></td>
<td>0.2</td>
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<tr>
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<td>F-5</td>
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<td>159.7</td>
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<tr>
<td>F-13</td>
<td>10</td>
<td></td>
<td>149.7</td>
<td>20</td>
<td>0.2</td>
<td>0.1</td>
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<tr>
<td>F-14</td>
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<td></td>
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<td>20</td>
<td>0.2</td>
<td>0.1</td>
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</tbody>
</table>

Precirol in matrix: Precirol incorporated along with THC into the drug-lipid matrix.
Precirol in blend: Precirol added directly in the blend, after THC was coated onto DCPA.
Compritol in matrix: Compritol incorporated along with THC into the drug-lipid matrix.
Compritol in Blend: Compritol added directly in the blend, after THC coated onto DCPA.
Mg. Stearate: Magnesium Stearate

**Analytical method**

The analytical method showed linearity within the range 0.5 – 100 µg/mL, with an $r^2$ value of 0.99. Limit of detection and limit of quantification were found to be 10 ng/mL and 30 ng/mL, respectively.
respectively, and the retention time for THC was about 11.2 min. The method was observed to be specific and precise.

**Evaluation of the tablets**

**Assay, Content uniformity and Physical characteristics**

THC assay in all formulation batches, was between 95 to 102%. Content in each tablet was between 94 to 101%. The target tablet weight and hardness were 200 mg and 5.4 kp, respectively. Weight variation (200±2 mg), friability and thickness were all observed to be within limits. Tablet hardness was found to range between 5.3 - 5.6kp (n=3) over the entire range of formulations.

**In vitro drug release studies**

Release of THC from the formulations was seen to be influenced by the type of filler (DCPA/MCC/Lactose) used in the formulation, as shown in Figure 3-1. In the case of MCC (Avicel) as the filler (F-2), the drug release was more uneven with about 70 % THC being released within 1h, followed by minimal release at further time points. Additionally, ‘tablet splitting’ was observed in the dissolution media in these formulations (F-2). Although hardness was kept similar in all three formulations, this phenomenon was seen only in formulation F-2.
Figure 3-1: Percentage THC released from the tablets as a function of type of the filler used in the composition. F-1 (DCPA); F-2 (Avicel); F-3 (Lactose). Each data point represents mean ± SD (n=3). Dissolution conditions: Basket apparatus operated at 100rpm, 37°C in 0.5% SLS medium.

Tablets prepared using lactose as the filler (F-3), showed good physical characteristics, but a release of 55% in 1h and 78% in 6 h was observed. On the other hand, tablets with DCPA as the filler (F-1) released about 34% in 1h and about 74% by the end of 6 h. Moreover, the THC release profile from formulation F-1 was much smoother and more uniform compared to formulations F-2 and F-3. On the basis of these results, DCPA was selected as the filler for further studies.
The dissolution conditions recommended by FDA for the marketed THC formulations, Nabilone and Dronabinol, are shown in Table 3-2 [27].

**Table 3-2:** FDA recommended dissolution conditions for the marketed THC formulations.

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Dosage Form</th>
<th>USP Apparatus</th>
<th>Speed (RPMs)</th>
<th>Medium</th>
<th>Volume (mL)</th>
<th>Recommended Sampling Times (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dronabinol</td>
<td>Capsule</td>
<td>II (Paddle)</td>
<td>100 and 150</td>
<td>10% Labrasol in Water; (In addition, the USP capsule rupture test should also be conducted)</td>
<td>500</td>
<td>5, 10, 15, 30, 45, 60, and until at least 80% of the labeled content is released</td>
</tr>
<tr>
<td>Nabilone</td>
<td>Capsule</td>
<td>II (Paddle)</td>
<td>50</td>
<td>0.1% Tween 80 solution</td>
<td>1000</td>
<td>15, 30, 45 and 60</td>
</tr>
</tbody>
</table>

The currently marketed dosage forms are immediate release capsules. Thus, there was a need to develop a dissolution method to study THC release from the proposed controlled release formulations. Since THC release is known to be pH independent and the dissolution medium was able to maintain sink conditions, apparatus type (basket or paddle) and rpm (50 or 100) were the only parameters that were varied.

As shown in Figure 3-2, THC release from F-1 was 41% and 52% at the end of 6 hours when a paddle was employed at 50 and 100 rpm, respectively. When an USP Type I Apparatus (basket) was used THC release was 48% and 76% in 6 hours at 50 and 100 rpm, respectively. The
higher drug release in the basket apparatus, could be due to the greater erosion effect on the bottom layers of the tablets, due to abrasion.

**Figure 3-2:** In vitro release profile of THC controlled release tablets (F-1) as a function of dissolution apparatus type and paddle/basket speed (rpm). Each data point represents mean ± SD (n=3). Dissolution conditions: Paddle, basket apparatus operated at 50rpm, 100rpm at 37°C in 0.5% SLS medium.

In order to optimize the release characteristics of THC from the formulations, it was decided that the more stressful dissolution conditions (producing faster release rates) would be used since these formulations would be more rugged in nature. Considering this, the basket
apparatus at 100 rpm was selected for studying the release characteristics of the formulations. Release profile with the paddle method at 100 rpm, as per the FDA approved method, will be determined on the formulation showing a sustained 24h release profile under the stress conditions.

As discussed earlier, F-1 released 76% of the THC in the formulation at the end of 6 hours (Fig. 3-2), when a basket at 100 rpm was employed. In order to slow down the release, formulation F-4 was prepared using 20 mg of additional Precirol® in the blend phase (on top of the 30 mg in the lipid matrix). The tablet weight was kept at 200 mg by adjusting the amount of DCPA. Another formulation, F-5, was prepared with the total amount of lipid added externally in the blend phase. In this case, THC, dissolved in hexane, was coated onto DCPA and dried in an oven at 25°C for 30 minutes, before blending it with the other excipients. Formulation F-4, showed 51% THC release at the end of 6 hours with insignificant release at the later time points (Fig. 3-3). Formulation F-5 exhibited a much faster release profile with a t₉₀ (time taken to release 90 % of drug) of less than 6 hours. From these results, it can be concluded that Precirol®, used internally in the drug-lipid matrix or externally in the blend phase significantly diminished drug release, but the inclusion of the lipid in the internal phase had a greater sustained release effect. On the other hand, when Compritol® was used in the lipid matrix (F-8), the release was found to be very erratic. From the data (not shown), it was evident that the drug was being released in uneven bursts.
Figure 3-3: In vitro release profile of THC as a function of lipid distribution between the matrix and/or blend phases of the formulation (basket, 100rpm). F-4: Precirol® distributed between matrix and blend; F-5: Precirol® in blend only. Each data point represents mean ± SD (n=3). Dissolution conditions: Basket apparatus operated at 100rpm, 37°C in 0.5% SLS medium.

In view of the relative rapid and uniform release profile of THC from formulation F-5, the matrix phase was not studied any further and the lipid content in the external phase was increased to 60 mg per tablet in formulation F-6. As can be seen from Figure 3-4, 83% THC release was observed in 10 hours.
Figure 3-4: In vitro release profile of THC from F-6 (Precirol® in the blend), F-7 (F-6 with Pluronic® F68), F-9 (Compritol® in the blend) and F-10 (F9 with Pluronic® F68) tablets. Each data point represents mean ± S.D. (n=3). Dissolution conditions: Basket apparatus operated at 100rpm, 37°C in 0.5% SLS medium.

In vivo, one of the major factors that could significantly affect the release profile of the drug from a lipid based tablet is lipolysis. The lipases present in the GIT digest the lipids rapidly, thereby causing rapid drug release. An approach to counter the effect of these enzymes is to use long chain lipids [28] and stabilizers [29]. Stabilizers act by inhibiting the degradation of drugs through lipolysis. Pluronic® F68, a triblock polymer, acts by blocking the activity of co-lipase enzyme. For lipolysis, the particle surface needs to have a lipase anchored to it with co-lipase playing an important role in facilitating the activity of lipase on the lipid surface. One of the
predominant mechanism of co-lipase is to anchor the lipase to the particle surface and other is to prevent the inactivation of lipase by the action by bile salts [29]. A stabilizer reduces the adsorption of lipases on the particle surface through a mechanism called the “windscreen wiper effect” [29] wherein the lipases are prevented from anchorage and thus blocking the initial necessary step for degradation of lipid [30]. Several studies have shown that use of Pluronic® F68, as a stabilizer has been successful in preventing lipolysis in vitro [31]. Also, the property of Pluronic® F68’s increased efficiency with increasing protein molecular weight, further facilitates the anti lipolytic activity, considering the high molecular weight of the lipases, which is approximately 50,000 kDa [30].

Based on this, formulation F-7 was prepared with Pluronic® F68, added as a stabilizer, to study the effect of its inclusion on the release of THC from F-6. It was seen that inclusion of Pluronic® F68 did not significantly affect THC release.

Also, Compritol®, a C-22 chained ester of behenic acid, is impervious to emulsification, a step that is pre-requisite for lipolysis [28, 32, 33], and formulations based on Compritol® would likely show minimum change in release profiles as a result of enzymatic degradation of the lipids. Thus, formulations F-9 & F-10 were prepared substituting Compritol® in place of Precirol® in F-6 and F-7. Compritol® was added directly to the blend. These formulations, F-9 and F-10, showed a very slow release rate of 5% and 4%, respectively, at the end of 10 hours (Fig. 3-4).
The combined effect of Compritol® and Precirol® was then investigated (F-11, F-12, F-13 and F-14). Formulations F-12 & F-14 contained Pluronic® F68 in the composition. Figure 3-5 shows that 100% drug release was observed from all formulations. F-13 and F-14 had better release profiles compared to F-11 and F-12.

**Figure 3-5:** In vitro THC release from tablets prepared using a combination of lipids added to the blend phase. F-12 and F-14 contained Pluronic® F68. Each data point represents mean ± S.D. (n=3). Dissolution conditions: Basket apparatus operated at 100rpm, 37°C in 0.5% SLS medium.
**Effect of THC dose on the release profile**

To simulate the effect of dose on percentage release, the release studies were also carried out using two tablets of formulation F-14 (containing 10 mg THC each) per jar. Though this does not mimic adding a single 20 mg THC tablet, the results would provide additional information with respect to the THC release profiles at higher THC doses (dose weight ratio formulations) and the performance of the dissolution medium (0.5% SLS in water). THC release profiles from the lower dose, 10 mg (single tablet), and higher dose, 20 mg (double tablet), formulations was observed to be similar. Similarity ($f_2$) factors were calculated and the $f_2$ value was greater than 50, suggesting that the drug release profile remained significantly unchanged.

The dose effect dissolution study was done using the paddle apparatus, as suggested by the FDA for testing Dronabinol. About 100% THC release was seen at 22 hours at a paddle speed of 100 rpm from both the single and double doses (Fig. 3-6). These results suggest that doubling the dose to 20 mg in a dose-weight ratio based formulation would not affect the release profile.
**Figure 3-6:** In vitro THC release profiles from 10 mg and 20 mg tablets. Each data point represents mean ± S.D. (n=6). Dissolution conditions: Paddle apparatus operated at 100rpm, 37°C in 0.5% SLS medium.

**Drug release model**

The release profile obtained from formulation (F-14) was fitted into various models (Zero order, First order, Higuchi diffusion kinetics, Korsemeyer Peppas, Hixon Crowel model). It was seen that the zero order release kinetic model ($r^2$ adjusted = 0.99) was the best-fit for the THC release profiles observed.
**Stability studies**

The stability study results demonstrated that formulation F-14 was physically and chemically stable at 25±2°C/60±5 %RH for 3 months. About 7% drug loss however, was observed at 40±2°C/75±5 %RH in the same time period. The thermolabile characteristics of THC and susceptibility to oxidation is probably responsible for this. The stability can be improved with the addition of antioxidants and an intermediate stress condition, 30±2°C/65±5 %RH, should probably be more applicable for accelerated testing.

**3.4. Conclusions**

This study demonstrates that THC can be successfully formulated into a controlled release tablet. With an optimal lipid combination and proportion, it is possible to tailor the drug release to meet the desired plasma concentration time profile. Also, dispersion of THC in melted lipids followed by cryo-milling or the coating of THC (in hexane) on DCPA followed by drying before blending and compression proved to be good methods for overcoming the handling issues. The use of Pluronic® F68 as a stabilizer against lipolysis did not alter the release profile *in vitro*. Further studies are being planned to establish the stabilizing activity of Pluronic® F68 and subsequent oral bioavailability, *in vivo*. 
CHAPTER 4

OCULAR DISPOSITION OF $\Delta^8$-TETRAHYDROCANNABINOL FROM VARIOUS TOPICAL FORMULATIONS
4.1. Introduction:

Glaucoma is a primary cause for irreversible loss of vision. The onset of this condition is multifactorial which involves an increase in the intra-ocular pressure (IOP), loss of retinal ganglion cells (RGC) due to apoptosis, vascular insufficiency etc.[34, 35]. Studies have shown that this condition is widely prevalent with around 2 million people diagnosed in the United States, and accounts for 17.8% of the medical costs towards major eye diseases. These numbers are expected to further increase with more than 3 million of the populace being afflicted by this disease by the year 2020 [34, 36]. On a global scale, this number is expected to reach a staggering 79.3 million by 2020, with more than 11 million falling into a state of “complete loss of vision” [37].

Elevation of the IOP, an important factor in glaucoma, is as a result of altered aqueous humor flow and drainage dynamics which is caused by a change in the trabecular meshwork structure. Death of RGC has also been shown to have an effect along with elevated IOP on the ultimate loss of vision in glaucoma patients [38, 39]. It has also been shown that a substantial number of patients have been diagnosed with glaucoma with no elevated IOP [40]. This shows that elevated IOP is a determining but not the only factor causing this condition. More recent studies focus on the loss of RGC due to apoptosis, a mechanism of programmed cell death, which is believed to be the main reason for neuronal damage, ultimately leading to RGC death [41, 42].
Δ⁹-Tetrahydrocannabinol (Δ⁹-THC) & Δ⁸-Tetrahydrocannabinol (Δ⁸-THC) are the primary active constituents of Cannabis sativa. Δ⁹-THC has shown potential in the treatment of glaucoma through its IOP lowering and neuroprotective effects [43-45]. The mechanism of action is not completely understood, though it has been said to have an agonistic action on the CB1 and CB2 receptors [46, 47]. These receptors are expressed on the iris-ciliary, retina choroid and the trabecular meshwork [48]. THC, through these receptors, causes relaxation of the trabecular meshwork which results in increased aqueous humor drainage and subsequent IOP reduction [44]. Neuroprotective action of Δ⁹-THC was also recently studied by El-Remessy et al. in NMDA induced retinal toxicity [44], making Δ⁹-THC a promising candidate in glaucoma therapy. Previous reports from our group have demonstrated the physicochemical characteristics and permeability and in vivo disposition of Δ⁹-THC and it’s relatively water soluble prodrugs in the eye. The effects of ion pairing and micellar solutions on the disposition of Δ⁹-THC in the eye were studied in these reports [49, 50].

While all the above literature focuses completely on Δ⁹-THC, little has been said and reported about the potential of its isomer, Δ⁸-THC. This compound exhibits a stereochemistry similar to Δ⁹-THC and is also chemically more stable than the latter [51]. In addition, the efficacy of Δ⁸-THC in reducing IOP has been demonstrated previously in rabbits [51, 52].

Although Δ⁸-THC is chemically more stable than Δ⁹-THC, delivery of this compound to the deeper ocular tissues is challenging. Like Δ⁹-THC, this compound is also highly lipophilic
and poorly soluble and resinous in nature. Table 4-1 compares the properties of these two isomers.

**Table 4-1: Physicochemical Properties of Δ⁹ & Δ⁸-Tetrahydrocannabinol**

<table>
<thead>
<tr>
<th>Property</th>
<th>Δ⁸-Tetrahydrocannabinol</th>
<th>Δ⁹-Tetrahydrocannabinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight</td>
<td>314.4</td>
<td>314.4</td>
</tr>
<tr>
<td>Log P</td>
<td>7.53 ± 0.36</td>
<td>7.68 ± 0.35</td>
</tr>
<tr>
<td>mLog P</td>
<td>3.96</td>
<td>3.96</td>
</tr>
<tr>
<td>Log D</td>
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<td>7.07</td>
</tr>
<tr>
<td>pKa</td>
<td>9.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Polar surface area</td>
<td>29.46</td>
<td>29.46</td>
</tr>
<tr>
<td>Solubility (µg/mL)</td>
<td>0.26 ± 0.03</td>
<td>1 - 2</td>
</tr>
</tbody>
</table>


Solid lipid nanoparticles (SLNs) have been studied over the years as a platform for enhancing topical administration [53, 54]. Ease of fabrication, stability, targeted delivery, non-toxicity, small size, prolonged release are some of the advantages of this delivery system. Additionally, the small size of the SLNs can enhance ocular delivery by increasing the corneal residence time and penetration [55-57]. Ibrahim et al reported increased bioavailability of gatifloxacin using mucoadhesive nanoparticles synthesized using Eudargit RS 100 and hyaluronic acid [58]. Cavalli et al. reported that ocular bioavailability of tobramycin increased 4-fold on incorporation of the drug into an SLN formulation. The authors attributed the increased
bioavailability to the trapping of the nanoparticles in the epithelial mucus layer, thus, resulting in prolonged release of the drug into the aqueous humor for up to 6 hours [56]. Although the utility of SLNs in ocular therapy in conditions pertaining to the anterior chamber has been investigated and reported, the same cannot be said for delivery to the posterior segment.

Another ocular delivery platform that has promise is drug loaded films. These systems, have the advantage of providing increased contact time with the ocular surface and prolonged release, which reduces the dosing frequency. Depending on the mechanism of drug release post application, the films are classified as either soluble or insoluble. Soluble films are generally made of soluble or erodible polymers and therefore circumvent the need of removal from the eye. The polymer can be either natural or synthetic and release from these kinds of delivery systems is mainly by diffusion as the polymer undergoes gradual gelling followed by dissolution in the tear fluid. Hermans et al reported the improved bioavailability of cyclosporin A using chitosan films [59], while Attia et al used erodible gelatin films for improving the bioavailability of dexamethasone [60].

In this study we evaluate the efficacy of topically administered SLNs in delivering Δ8-THC to the posterior ocular tissues. Further, we also study the utility of a melt-cast film formulation. This film formulation technique, unlike previous reports, avoids the use of solvents, thereby eliminating the risks posed by residual solvents to the ocular tissues.
A comparative evaluation of the SLNs, films, nanoemulsion and a cyclodextrin solution in terms of their ability to deliver the drug to various ocular tissues, has been undertaken both at _in vitro_ and _in vivo_ levels.

**4.2. Materials & Methods:**

**Materials**

Compritol® ATO 888 and Precirol® ATO 5 were gift samples from Gattefosse, France. Poloxamer® 188 was obtained from BASF, Chattanooga, TN. Lipoid® E 80 (Lipoid, Ludwigshafen, Germany) was a gift sample. Propofol, randomly methylated beta cyclodextrin, hydroxypropyl methyl cellulose (4000 cps), Polyethylene Oxide N10 were purchased from Sigma (St. Louis, MO). All other chemicals were purchased from Fisher Scientific (St. Louis, MO). Solvents used for analysis were of HPLC grade.

**Animal Tissues**

Whole eye globes of New Zealand Albino rabbits were purchased from Pel Freez Biologicals (Rogers, AK). Eyes were shipped overnight in Hanks Balanced Salt Solution (HBSS) over wet ice. Corneas were isolated and used immediately on receipt.
Animals

Male New Zealand White Albino Rabbits were procured from Harlan Labs (Indianapolis, IN). Animal experiments conformed to the tenets of the Association for Research in Vision and Ophthalmology statement on the Use of Animals in Ophthalmic and Vision Research and followed the University of Mississippi Institutional Animal Care and Use committee approved protocols.

Formulations:

Solid Lipid Nanoparticles Containing Δ⁸-THC:

SLNs were prepared as per previously established protocols [61], using a high speed & high pressure homogenization method. Δ⁸-THC was accurately weighed and melted along with Compritol® 888 ATO or Precirol® ATO 5 (Gattefosse, France) to obtain a clear lipid phase. An aqueous phase containing 0.25% Poloxamer® 188, 0.75% Tween® 80 and 2.25% Glycerin (w/v) in distilled water, was heated and added to the melted lipid phase under stirring. A coarse emulsion from this pre-mix was formed using an Ultra-Turrax®, followed by high pressure homogenization. The temperature during this entire process was maintained at 70°C. The hot emulsion was slowly cooled to room temperature to form Δ⁸-THC SLNs.

SLNs with randomly-methylated beta cyclodextrins (RMβCD) in the lipid phase were prepared by dissolving Δ⁸-THC & RMβCD in acetonitrile and keeping in a water bath for 24h at 25°C for complex formation. At the end of 24h, the organic solvent was evaporated under
nitrogen and molten lipid was added to this $\Delta^8$-THC-RMβCD complex and the above procedure was repeated for SLN production. Alternately, $\Delta^8$-THC-RMβCD SLN formulation was prepared without the $\Delta^8$-THC-RMβCD complexation step. Instead, RMβCD was dissolved in the aqueous phase along with Poloxamer 188®, Glycerin, Tween® 80 and the procedure described above was followed for SLN production.

**Nanoemulsion Containing $\Delta^8$-THC:**

The nanoemulsion containing $\Delta^8$-THC was prepared according to previously published protocols [52, 62]. Briefly, $\Delta^8$-THC (0.1% w/v), $\alpha$-tocopherol (0.02 %w/v) and oleic acid (6 %w/v) were added to super refined soybean oil (14 %w/v) to prepare the oil phase. Poloxamer® 188 (2% w/v) and glycerin (2.25% w/v) were added to deionized water to prepare the aqueous phase. Lipoid E 80® (1 % w/v) was dispersed in the aqueous phase. Both phases were heated to 70°C. The aqueous phase was first added to the oil phase under constant mixing using Ultra-Turrax® to form a coarse emulsion. It was then passed through a high pressure homogenizer (Avestin C5 Emulsiflex®) and later allowed to cool down to room temperature. The pH of the final emulsion was adjusted to pH 7.4 using 1N sodium hydroxide and filtered through a 0.45 μM membrane filter.

**Film Formulation Containing $\Delta^8$-THC:**

Hot melt cast method was utilized to prepare the polymeric film. Polyethylene oxide (PEO N10: MW 100000 Daltons) was used as the matrix forming material. $\Delta^8$-THC (20% w/w) was dissolved in acetonitrile and dispersed in PEO N10 with adequate mixing. The mixture was
placed in a vacuum chamber to evaporate the organic solvent. A 13 mm die was placed over a brass plate and the brass plate was heated to 70 °C using a hot plate. The drug-polymer mixture was placed in the center of the die, compressed and further heated for 2-3 min. Following cooling, 4 mm x 2 mm film segments were cut from the film.

**Solution Formulations containing Δ8-THC:**

Solutions were prepared by adding excess of Δ8-THC to 2.5% and 10% aqueous RMβCD solutions in isotonic phosphate buffered solution (IPBS) and allowing the solutions to equilibrate for 24h at 25°C in a reciprocating water bath. At the end of 24h, solutions were centrifuged and the supernatant was analyzed for Δ8-THC content. These solution formulations served as controls for all the experiments.

The formulation compositions have been presented in Table 4-2
Table 4-2: Composition (%w/v) of the various Δ⁸-Tetrahydrocannabinol Formulations:

<table>
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<th>Formulation Code</th>
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<th>C 2</th>
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<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5*</th>
<th>F6</th>
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</thead>
<tbody>
<tr>
<td><strong>Formulation Type</strong></td>
<td>Solution</td>
<td>Solid Lipid Nanoparticles (SLNs)</td>
<td>Film</td>
<td>Nanoemulsion</td>
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<td>Δ⁸-THC</td>
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<td></td>
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<td>1</td>
</tr>
</tbody>
</table>

*: F-5 values expressed in % w/w terms.

Determination of Particle Size and Polydispersity Index:

Particle size and Polydispersity Index (PDI) of the SLNs and nanoemulsion formulations were measured using a dynamic light scattering instrument, Zetasizer Nano ZS (Malvern Instruments Inc., Westbrough, MA), at 25 °C. A high concentration zeta cell was used to measure both mean particle size (z averaged) and PDI.
Assay Procedure for the Formulations:

$\Delta^8$-THC content in the SLNs and nanoemulsions was assayed according to the following procedure. An accurately measured amount of the formulation, was extracted in 1 mL of ethanol and the suspension was centrifuged for 10 minutes. The supernatant was diluted in mobile phase and was analyzed for $\Delta^8$-THC content.

$\Delta^8$-THC content in the film was determined by placing the film in 10 mL of acetonitrile and sonicating for 15 min. The film dissolves completely in acetonitrile. This mixture was centrifuged at 13000 rpm for 10 min and supernatant was collected. The supernatant was then analyzed by HPLC. To determine the content uniformity of the film three separate sections from the same film were analyzed following the same procedure as described above.

Entrapment Efficiency for Solid Lipid Nanoparticles:

Entrapment efficiency was determined using AMICON® Ultra centrifugal filters with a 10000 KDa membrane. A measured amount of formulation was taken and placed in the centrifugal filter and the sample was spun at 13000 rpm for 30 min, following which the filtrate was collected and analyzed for free $\Delta^8$-THC content. Percentage $\Delta^8$-THC entrapped was calculated using the formula.

$$\%\text{ Entrapped} = \frac{\text{Total Amount of Drug} - \text{Free Drug}}{\text{Total Amount of Drug}} \times 100$$
**In vitro Release from Solid Lipid Nanoparticles:**

Slide-A-Lyzer® mini dialysis cassettes (0.5mL; 10k membranes) were used for studying release. The receiver medium contained a solution of IPBS with 2.5% RMβCD. A volume of 500 µL of the formulation was placed in the cassette. Samples, 1 mL aliquots, were drawn at regular intervals from the receiver (18mL) which was then immediately replaced with an equal volume of fresh receiver solution. The samples were analyzed and the percentage drug released was determined. Experiments were carried out in triplicates.

**In vitro Transcorneal Transport Studies:**

Corneas were excised from whole rabbit eye globes (Pel-Freez Biologicals; Rogers, AK). Briefly an incision was made about 2mm from the corneal-scleral junction and the cornea was excised by cutting radially along the sclera. The excised corneas were immediately mounted on side by side permeation cells (Permgear Inc, Hellertown, PA) (Figure 4-1). A circulating water bath was used to maintain the temperature at 34°C during the transport studies. Receiver solution for all permeability studies consisted of 2.5% RMβCD solution in IPBS with pH adjusted to 7.4. The volume of the receiver solution was 3.2 mL, 0.2 mL more than the donor to maintain the natural curvature of the corneas, and the solution in the chamber was stirred continuously using magnetic stirrers. SLNs and nanoemulsion formulations were diluted in a 2:1 ratio with IPBS to yield donor solution for these studies. The initial donor concentrations were 1.33mg in the case of F-0 and F-6, 10mg in the case of F-1, F-2, F-3, and F-4. Aliquots, 600µL, were withdrawn from the receiver chamber every thirty minutes for three hours and immediately replaced with an
equal volume of the receiver solution. Samples were analyzed following the method described in the analytical methods section. All experiments were carried out in triplicate.

**Figure 4-1:** Side-by-side diffusion apparatus setup for studying *in vitro* trans-corneal transport of Δ⁸-Tetrahydrocannabinol from solid lipid nanoparticles, nanoemulsion and solution formulations.
In vitro transcorneal flux of Δ⁸-THC from the matrix film was evaluated by sandwiching the film (4 mm x 2 mm) in between a Spectra/Por® membrane (MWCO: 10,000 2 Daltons) and isolated rabbit cornea (Pel-Freez Biologicals; Rogers, AK). Corneas were excised from whole eye globes, with approximately 1 mm scleral portions remaining for ease of mounting. The membrane-film-cornea sandwich was then placed in between the side-by-side diffusion cells with the chamber towards the Spectra/Por® membrane representing the perioccular surface and the chamber towards the cornea representing the aqueous humor (Figure 4-2). The side-by-side diffusion cells were maintained at 34 °C using a circulating water bath. 2.5% RMβCD in IPBS (pH 7.4) was used as the receiver medium on both sides. The initial donor concentration was 1.6 mg and aliquots, 600μL, were drawn every thirty minutes for three hours and replaced with an equal volume of receiver solution. Samples were analyzed following the method described in the analytical methods section. The experiment was carried out in triplicate.
Figure 4-2: Side-by-side diffusion apparatus setup for studying *in vitro* trans-corneal transport of Δ^8^-Tetrahydrocannabinol from film formulation

**In vivo Ocular Bioavailability Studies:**

Ocular bioavailability of Δ^8^-THC was evaluated from the solution, SLN and film formulations, in male New Zealand albino rabbits weighing 2-2.5 Kg. Rabbits were anesthetized at the start of the experiment using a combination of ketamine (35 mg/kg) and xylazine (3.5 mg/kg) injected intramuscularly and were maintained under anesthesia throughout the experiment. Fifty microliters (375 µg dose) of the SLNs formulation or a 4x2x2 mm (1.6 mg
dose) melt-cast film was instilled/placed topically into the conjunctival cul-de-sac of the rabbits. At the end of one hour after topical application, the rabbits were euthanized with an overdose of pentobarbital injected through the marginal ear vein under deep anesthesia. The eye was washed with ice cold IPBS and immediately enucleated and washed again. The ocular tissues were separated, weighed and stored at -80°C until further analysis. All experiments were carried out in triplicate.

**Sample Preparation for Analysis:**

**Standard Solutions:**

Stock solutions of Δ⁸-THC were prepared in acetonitrile. Known quantities of Δ⁸-THC from these stock solutions were spiked in blank ocular tissues and allowed to stand for 10 minutes before protein precipitation using ice cold acetonitrile (1:1 ratio for aqueous, vitreous humor and 1 mL for all the other tissues). The samples were then centrifuged for 15 minutes at 4°C and 13000 rpm and the supernatant was collected. Standard curves were prepared in aqueous humor (10 ng – 200 ng), vitreous humor (20 ng-200 ng), cornea (20ng-200ng), iris ciliary body (10ng-200ng), retina choroid (10-200 ng) and sclera (20-200 ng). Propofol was used as the internal standard during the analysis.
Sample Preparation:

Hundred microliters of aqueous humor and 500 microliters of vitreous humor was collected from each test eye into individual centrifugal tubes. All other tissues, retina-choroid, iris-ciliary, cornea & sclera, from each test eye were cut into very small pieces and placed into individual vials. Protein precipitation was carried out similar to the standard solution preparation and analyzed using the method described below.

Analytical Methods:

Bio-analytical Method for in vivo Samples:

A previously published bio-analytical method [50] using fluorescence detection was modified and used for analyzing THC content in the ocular tissues. HPLC system comprised of a Waters 600 pump, Waters 717 plus refrigerated autosampler and Waters 2475 fluorescence detector, set at an excitation wavelength of 220 nm and Δ⁸-THC was detected at emission wavelength of 305 nm. EUFS was set at 150 and gain was set at 30. Phenomenex PFP (2) (5µM, 4.6 x 250 mM) column was used. The mobile phase consisted of 30% water containing 0.5% o-phosphoric acid and 70% acetonitrile, with a flow of 1mL/min. Injection volume was 50 µL. Retention time for propofol and THC were 7.0 min and 11.9 min, respectively.

The standard calibration curve was derived and the parameters such as regression coefficient ($r^2$), slope and Y- intercept were noted to establish the linearity of the method. The
analytical method was also validated with respect to precision, accuracy, recovery and specificity. Limit of detection for $\Delta^8$-THC in various ocular tissues was, aqueous humor (5 ng), vitreous humor (10 ng), cornea (10 ng), iris ciliary body (5 ng), retina choroid (5 ng) and sclera (10 ng) in the fluorescence method.

**HPLC-UV Method for in vitro Samples:**

A Previously published Waters HPLC-UV system with a Phenomenex PFP(2) (5 µM, 4.6 x 250 mM) column was used for analysis [63]. The mobile phase consisted of 18% water, 0.75% acetic acid, 30% Acetonitrile and 52% methanol at a flow rate of 1.2 mL/min. The UV detector wavelength was set at 226 nm. Injection volume was 25 µl and retention time for THC was 13.1 min.

The standard calibration curve was derived and the parameters such as regression coefficient ($r^2$), slope and Y- intercept were noted to establish the linearity of the method. The analytical method was also validated with respect to precision, accuracy, recovery and specificity. Limit of detection and limit of quantification were found to be 10 ng/mL and 30 ng/mL, respectively, and the retention time for THC was about 13.1 min. The method was observed to be specific, precise and reproducible.
4.3. Results:

Particle Size and Polydispersity Index of the Formulations:

The particle size and PDI of the SLN and nanoemulsion formulations are as described in Table 4-3.

Assay of the Various Formulations:

$\Delta^8$-THC content in all the formulations ranged between 95 to 104%.

Entrapment Efficiency (EE) of the Solid Lipid Nanoparticles:

The degree of entrapment varied in each formulation with highest EE shown by F-2, (92.5%), followed by F-1 (89.6%), F-4 (85.7%) and F-3 (82.4%).

In vitro Release from the Solid Lipid Nanoparticles:

$\Delta^8$-THC release was studied from F-1 and F-2 formulations in vitro. Similar release profiles were observed from both formulations till the end of 12 hours ($f_2 > 50$), as shown in Figure 4-3.
**Figure 4-3:** Release profiles of Δ⁸-Tetrahydrocannabinol from solid lipid nanoparticles (F1 and F2) using Slide-A-Lyzer® mini dialysis cassettes. Data represents mean±SD (n=3).

**In vitro Transcorneal Transport:**

Flux values across corneas were calculated for all the formulations (Table 4-3 & Figure 4-4). The control (C-1) and film (F-5) formulations depicted higher flux in comparison to nanoemulsion (F-6) and SLN (F-0) formulations. Increasing the drug load from 0.1% to 0.75% in F-2, F-3 & F-4 resulted in several folds increase in the flux.
Figure 4-4: Permeability and flux of Δ⁸-Tetrahydrocannabinol across isolated rabbit corneas from various formulations. Data represents mean±SD (n=3).

Table 4-3: Particle size characterization & transcorneal Δ⁸-Tetrahydrocannabinol flux from various formulations. Transcorneal flux determined using side-by-side diffusion apparatus at 34°C. Data represents mean±SD (n=3)
<table>
<thead>
<tr>
<th>Code</th>
<th>Formulation</th>
<th>Particle Size (nm)</th>
<th>Polydispersity Index</th>
<th>Flux Across Isolated Corneas (µg/min/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>THC in 2.5% RMßCD</td>
<td>-</td>
<td>-</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>F-0</td>
<td>SLNs with Compritol - 0.1% Load</td>
<td>495</td>
<td>0.39</td>
<td>0.014 ± 0.005</td>
</tr>
<tr>
<td>F-2</td>
<td>SLNs with Compritol - 0.75% Load</td>
<td>390</td>
<td>0.32</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>F-3</td>
<td>SLNs with Compritol &amp; RMßCD in Aqueous Phase - 0.75% Load</td>
<td>395</td>
<td>0.34</td>
<td>0.21 ± 0.009</td>
</tr>
<tr>
<td>F-4</td>
<td>SLNs with Compritol &amp; RMßCD Complexed with THC - 0.75% Load</td>
<td>410</td>
<td>0.34</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>F-5</td>
<td>Film</td>
<td>-</td>
<td>-</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>F-6</td>
<td>Nanoemulsion</td>
<td>237</td>
<td>0.28</td>
<td>0.02 ± 0.001</td>
</tr>
</tbody>
</table>

**In vivo Ocular Bioavailability:**

The Δ⁸-THC levels in various ocular tissues, from the above formulations, one hour post topical administration, are illustrated in Table 4-4 and Figure 4-5. Δ⁸-THC formulated in RMßCD solutions (2.5% and 10%), which served as controls, did not show any detectable levels in the aqueous humor, vitreous humor, iris ciliary and retina-choroid. Cornea and sclera on the other hand showed significant Δ⁸-THC accumulation levels.
With the SLN and film formulations, $\Delta^8$-THC was observed in all the ocular tissues except the vitreous humor. The SLNs were able to deliver higher amounts to the tissues compared to the film.

**Figure 4-5:** Obtained levels of $\Delta^8$-THC in ocular tissues from various formulations, 1h post topical administration. Dose: 375µg in 50µL for SLNs, 1.6mg in 8mg for film, 15µg in 50µL for 2.5% CD solution & 70µg in 50µL for 10% CD solution. Data represents mean±SD (n=3).
Table 4-4: Ocular disposition of Δ⁸-Tetrahydrocannabinol one hour post topical administration of the selected formulations. Values expressed in µg/g of tissue. Data represents mean±SD (n=3)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>SLN (Precirol)</th>
<th>SLN (Compitrol)</th>
<th>SLN with CD in Aq.Phase</th>
<th>SLN with THC-CD Complex</th>
<th>2.5% CD Solution</th>
<th>10% CD Solution</th>
<th>Film</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formulation Code</strong></td>
<td><strong>F-1</strong></td>
<td><strong>F-2</strong></td>
<td><strong>F-3</strong></td>
<td><strong>F-4</strong></td>
<td><strong>Control 1</strong></td>
<td><strong>Control 2</strong></td>
<td><strong>F-5</strong></td>
</tr>
<tr>
<td><strong>Drug Load (%w/v)</strong></td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.003</td>
<td>0.014</td>
<td>20% w/w</td>
</tr>
<tr>
<td><strong>Dose</strong></td>
<td>375ug</td>
<td>375ug</td>
<td>375ug</td>
<td>375ug</td>
<td>15ug</td>
<td>70ug</td>
<td>1.6mg</td>
</tr>
<tr>
<td><strong>Volume Instilled / film weight</strong></td>
<td>50µl</td>
<td>50µl</td>
<td>50µl</td>
<td>50µl</td>
<td>50µl</td>
<td>50µl</td>
<td>8mg</td>
</tr>
<tr>
<td><strong>Aqueous Humor</strong></td>
<td>4.0±1.5</td>
<td>4.6±1.3</td>
<td>5.3± 0.3</td>
<td>4.9±2.1</td>
<td>N.D</td>
<td>N.D</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td><strong>Cornea</strong></td>
<td>9.2±0.5</td>
<td>8.4±1.9</td>
<td>10.7±1.7</td>
<td>10.1±0.8</td>
<td>1.2±0.1</td>
<td>7.9±0.5</td>
<td>22.4± 1.2</td>
</tr>
<tr>
<td><strong>Iris-Ciliary</strong></td>
<td>0.33±0.2</td>
<td>0.38±0.1</td>
<td>0.66±0.1</td>
<td>0.76± 0.2</td>
<td>N.D</td>
<td>N.D</td>
<td>0.26 ± 0.1</td>
</tr>
<tr>
<td><strong>Vitreous Humor</strong></td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
</tr>
<tr>
<td><strong>Retina-Choroid</strong></td>
<td>0.44±0.1</td>
<td>0.42±0.3</td>
<td>0.3±0.1</td>
<td>0.4± 0.2</td>
<td>N.D</td>
<td>N.D</td>
<td>0.1± 0.02</td>
</tr>
<tr>
<td><strong>Sclera</strong></td>
<td>1.008±0.4</td>
<td>0.8±0.2</td>
<td>1.2±0.6</td>
<td>1.2 ± 0.1</td>
<td>0.4±0.1</td>
<td>0.8±0.2</td>
<td>2.3 ± 0.5</td>
</tr>
</tbody>
</table>
4.4. Discussion:

Evidence of reduction in IOP through marijuana smoking, a phenomenon that has been studied and reported as early as in the 70s [64], was not concrete enough to underline the utility of cannabinoids in glaucoma therapy. Variation in the therapeutic outcome of cannabinoid therapy was the main reason for this. Previous reports have shown the ability of the relatively hydrophilic prodrug of Δ⁹-THC to reach into the iris-ciliary bodies, though no drug was seen to reach the retina choroid [49, 50]. The micellar solutions of the parent Δ⁹-THC on the other hand could not penetrate the surface tissues such as the cornea and sclera, highlighting the innate inability of the highly lipophilic molecule to permeate across the surface layers. Thus, either hydrophilic prodrug derivatization or design of formulation approaches which enhance ocular penetration will be needed for lipophilic therapeutic agents intended for topical ophthalmic application.

Δ⁸-THC, being a highly lipophilic compound, like Δ⁹-THC, poses significant challenges in its delivery across the eye into the deeper ocular tissues. Therefore, in this study we have utilized SLNs and films for improving the ocular penetration of Δ⁸-THC through the topical route. Initial in vitro transcorneal transport studies of the formulations was a comparison between C-1 (2.5% RMβCD solution), F-0 (SLNs with Compritol®), F-5 (Film) & F-6 (Nanoemulsion). The control formulation in 2.5% RMβCD solution showed the highest flux in this study, followed by the film. Nanoemulsion and SLNs on the other hand were not as good comparatively. The lower ability of the lipid based system, in particular the SLNs, to deliver more drug across the cornea could be
because of the preference of $\Delta^8$-THC for the lipids, lack of lipases in the experimental set-up, decreased or absence of phagocytic uptake by the epithelial cells, low drug content in the SLNs (low $\Delta^8$-THC to lipid ratio). Moreover, the side-bi-side diffusion apparatus may not be a good system to study transcorneal flux from particulate systems because contact profile in vivo in the conjunctival sac would be different from the side-bi-side apparatus. A vertical diffusion apparatus may be more appropriate. Formulations F-2 (SLNs with Compritol®), F-3 (SLNs with RMβCD in the aqueous phase) and F-4 (SLNs with RMβCD complexed with the drug) had a drug load of 0.75%, as opposed to 0.1% in F-0. Significant improvement in flux, 12-16 fold increase, was observed. The nanoemulsion was not a part of this study due to poor physical stability of the formulation at room temperature.

In order to test the effect of lipid on SLN release characteristics, two formulations were studied - one with Precirol ® as the lipid (F-1) and the other with Compritol ® (F-2). The formulations were similar in all other aspects. In vitro drug release profiles of both these formulations were not very different, with the drug being released at almost similar rates ($f_2 > 50$) from both formulations. Both formulations showed good characteristics in terms of their size, PDI and entrapment efficiencies (Table 3.3).

In vivo, with the cyclodextrin solutions (C-1 & C-2), at the end of 1h post administration $\Delta^8$-THC content was undetectable in all the tissues tested, with the exception of the cornea and sclera. Cyclodextrins have been documented to enhance solubility and ocular bioavailability of various drugs such as diclofenac, dexamethasone, and hydrocortisone [65-67]. Although RMβCD
enhanced the aqueous solubility of Δ⁸-THC and the solution showed comparatively higher transcorneal flux *in vitro*, an increased penetration was not seen *in vivo*. This can be attributed to the lipophilic Δ⁸-THC getting entrapped in the corneal and scleral membranes and not penetrating into the deeper tissues. The 10% solution only resulted in a higher amount of Δ⁸-THC accumulating in the cornea and sclera in comparison to the 2.5% solution, showing the inability of the solution formulation in delivering Δ⁸-THC to deeper tissues. Also, the comparatively lower instilled doses due to limited solubility, lower viscosity in comparison to the SLNs, leading to pre-corneal loss, could be some other factors.

Ocular inserts were reported as early as 1978 when Bloomfield et al suggested the use of collagen shields for delivery of gentamycin [68]. Since then, a number of reports using these invasive techniques have appeared for various other drugs such as diclofenac sodium, cyclosporin A, dexamethasone, pilocarpine etc [69-73]. While ocular inserts were effective in improving the bioavailability of the tested drugs, the fact they are required to be either surgically implanted or removed after a certain period of time makes them unattractive. In contrast, a topical film which gels on application, thereby providing a prolonged release platform and then ultimately dissolving in the tear fluid appears to be a more convenient system. Moreover, the melt cast method eliminates any concerns regarding the toxic effects of residual solvents associated with solvent cast films preparation technique and was found to be successful in producing films that had good physical characteristics. The process was fast and reproducible and the films produced were able to deliver the drug to the deeper ocular tissues. The formulation gelled in the tear fluid within 30-40 seconds on application and stayed in contact with the corneal surface. No irritation of ocular
The ocular tissue concentrations obtained suggest involvement of the conjunctival-scleral diffusional pathway. Nonetheless, the observed $\Delta^8$-THC levels from the film were much lower compared to that observed with the SLNs. This can be attributed to the fact that even though the film was able to achieve a longer residence time and sustained release, it had no effect on the inherent transmembrane permeability characteristics of $\Delta^8$-THC. As a result, $\Delta^8$-THC efficiently partitioned from the formulation into the corneal and scleral membranes but could not diffuse any further.

The SLNs, in contrast, were able to deliver a higher amount of $\Delta^8$-THC to all ocular tissues, except vitreous humor. Formulations F-1 & F-2, made with Precirol® and Compritol®, respectively, were very similar to each other. The observed $\Delta^8$-THC levels in the ocular tissues, particularly the retina-choroid, may be due to an uptake of these nanoparticles by the conjunctival and corneal membranes, as suggested in earlier studies [56]. The advantages of SLNs and their utility in delivering a host of drugs such as ibuprofen, flurbiprofen, cyclosporine A, rapamycin, gatifloxacin, indomethacin etc. has been tested and published before [55, 57, 58, 61, 74-81]. While most of the observations in these earlier reports have been made at in vitro and ex vivo studies, only a handful of the studies reported improved in vivo bioavailability and that too only to the anterior chamber delivery.
The efficacy of SLNs in posterior segment delivery, post topical administration, to our knowledge, has not been reported elsewhere and the results from this study are very encouraging. At almost a fifth of the dose, compared to the matrix film formulation, the SLNs were able to generate ocular concentrations significantly greater than that obtained with the film formulation in the deeper ocular tissues such as the aqueous humor, retina-choroid and iris-ciliary bodies. Incorporation of cyclodextrins into the SLN formulations (F-3 & F-4) did not improve ocular penetration of Δ⁸-THC.

4.5. Conclusion:

The SLN formulations hold great potential in the delivery of lipophilic compounds, such as Δ⁸-THC, to the deeper ocular tissues. Other formulations such as solution or film delivery systems depend on the physico-chemical characteristics of the agent to diffuse across the tissues. The SLNs on the other hand probably penetrate the tissues on their own to a certain extent and deliver the drug into the deeper ocular tissues.
CHAPTER 5
PHYSICOCHEMICAL CHARACTERIZATION AND ORAL BIOAVAILABILITY EVALUATION OF A NOVEL DIHYDROARTEMISININ DIMER PRODRUG: THE DIMER OXIME
5.1. Introduction:

Malaria is a very serious mosquito borne infectious disease that affects millions of people worldwide. According to the CDC, around 3.4 billion people worldwide are at risk of malarial transmission [82]. The WHO, in 2013, reported around 190 million cases with more than half a million of them resulting in deaths [83]. Though this disease was eliminated in the US by the early 1950s, it is still a problem in other parts of the world, especially in the sub-Saharan parts of Africa where more than 80% of the global malarial deaths occur. Even in the US, about 2000 cases are diagnosed every year [84, 85] and majority of these cases are immigrants or tourists coming in or returning from endemic countries [84, 86].

Pathologically, malaria is caused by different species of Plasmodium parasites, such as falciparum, vivax, ovale, malariae, knowlesi etc. The successive infection of two hosts by these parasites results in the disease’s transmission. One of the hosts is the female anopheles mosquito while the other is a human. Of all the above mentioned species, falciparum is more popular in the malaria caused in human beings [87]. Inside the human body, the parasite undergoes its “asexual cycle” where it attacks and then multiplies in the liver cells and then moves onto the red blood cells. Upon division inside the RBCs, the parasites burst the cell open and start invading neighboring RBC cells for further multiplication. The stage inside the RBC is called the “merozoite” stage of the parasite’s life cycle and this is what causes the symptoms of malaria. From here the merzoites are ingested by the anopheles mosquito when it takes a bite on the
human host and then, after approximately 10-18 days, the parasites’ cycle (sexual) inside the mosquito is completed at the end of which they start residing in its salivary glands. When this mosquito feeds next time on a human, the parasite is transferred into a new host and once again starts its cycle by attacking the liver cells [88].

During the merozoite phase, various symptoms such as fever, chills, sweats, malaise etc are seen in patients, while the more severe form of malaria would cause seizures, impairment of consciousness, acute kidney failure, respiratory distress, hypoglycemia [89] etc. Numerous drugs such as pyrimethamine, mefloquine, primaquine, chloroquine, progaunil, atovaquone are available to counter this disease [90, 91], but due to the parasite’s multidrug resistance [92], the disease keeps recurring.

Artemisinin, the active constituent of Artemisia annua L. was first identified during the Vietnam War. Advantages of artemisinin over the other compounds include rapid activity, low toxicity, short fever clearance time, and wide spectrum of activity across various stages of the parasite’s cycle in the body, higher efficacy [93]. Disadvantages of this compound includes short half-lives which results in fever recurrence when administered by itself. Artemisinin monotherapy was banned by WHO in 2006 and currently, combination therapies exist [94]. Also, a number of side effects from this compound exist, due to higher and frequent dosing [95-97].

Inside the body, post dosing, artemisinin breaks down into its derivatives such as artemether, arteether, artesunate, artenimol also known as dihydroartemisinin (DHA). Of these, DHA is the most important metabolite for exerting the anti-malarial activity [98, 99]. Its short
half-life is advantageous as it theoretically reduces the risk of the parasites developing resistance, while the disadvantage would be fever recurrence, as mentioned above, due to short half-life.

The mechanism of action of artemisinins is different from the other classes of antimalarial compounds. The peroxide ring within the trioxane system is crucial for its activity [100]. Some of the artemisinins have a carbon in the place of the peroxidic oxygen and these are devoid of any activity [101]. Compounds like DHA on the other hand have the oxygen intact and execute anti-malarial action. It has been suggested that the reactive peroxides act inside the parasitized RBCs, cause oxidative stress and cause haemolysis [102].

From a formulation standpoint, the limitations with artemisinin and its endoperoxide group of compounds has always been their poor solubility and bioavailability [103, 104]. Therefore, for this study, we have synthesized a DHA dimer and attached a nitrogen group into the linker with the hope of overcoming these obstacles (Patent number EP1753419A1).

The novel DHA dimer prodrug, dimer oxime (DO), will be utilized in this project, and studies will be carried out to elucidate its physicochemical characteristics such as solubility in buffer and surfactant solutions, stability, metabolic profile & thermal profile. Based on the results from these preformulation studies, DO will be incorporated into various formulation platforms and they will be used to study the in vitro intestinal permeation characteristics of the compound. Finally, the oral bioavailability of the DO through all these formulations will be evaluated.
Using surfactant systems for enhancing solubility and permeability has been extensively documented[30]. Edwards et al reported improved solubilization of aromatic hydrocarbons using nonionic surfactants [105], Chiou et al reported and discussed the usefulness of these systems in their study where the solubility and dissolution of chloramphenicol and prednisolone was enhanced [106]. Along with these, there have also been plenty of reports with surfactants as additives in particulate systems and dispersions for similar purposes [107-109]. Similarly, lipid based systems have also been reported extensively for their versatility as carriers for a host of compounds and improving their bioavailability. The ability of lipids to promote absorption of active moieties was proven as early as 1987 [110]. The mechanism of bioavailability enhancement by the lipid systems is mainly owed to their adhesiveness to physiological membranes and absorption enhancing effect [111]. Also, lipids with a fatty acid chain length of C-14 to C-18 are reported to enhance and promote lymphatic uptake [112-114] and this aspect would be very useful as it helps avoid first pass metabolism [112]. Yang et al studied the bioavailability enhancement of camptothecin using SLNs prepared using stearic acid and reported significant increase of total camptothecin delivered to brain after oral administration [115], while Chen et al reported improved bioavailability (4%-13%) of lovastatin using precirol and squalene based NLCs [116]. Nepal et al evaluated the efficacy of Witepsol based SNEDDS in the oral delivery of coenzyme Q$_{10}$ and reported 4-5 fold increase in absorption[117]. Studies and reports similar to these made by various other research groups also validate the efficacy of SLNs, NLCs, SNEDDS in improving the oral bioavailability of various classes of compounds [118-129].
Therefore, all these platforms will be utilized to evaluate the oral bioavailability of the DO. The formulations will be compared against each other, \textit{in vivo}, through plasma profile elucidation, post oral administration. Also, the compound’s intravenous (IV) and intraperitoneal (IP) profiles will be studied in order to gauge its clearance from the body.

A basic understanding regarding the properties and behavior of DO, both \textit{in vitro} and \textit{in vivo}, would aid in potentially developing a new line compounds for efficient therapy against malaria.

\textbf{5.2. Materials & Methods:}

\textbf{Materials:}

Compritol® ATO 888, precirol® ATO 5, capryol® 90, transcutol® P, labrafil M® 1944, gelucire® 50/13, labrasol®, labrafac lipophile®, lauroglycol® were gift samples from Gattefosse, France. Poloxamer® 407, kolliphor® EL were obtained from BASF, Chattanoga, TN. Randomly methylated beta cyclodextrin, polyethylene Glycol 400, oleylamine, chitosan chloride, tween® 80, propylene glycol were purchased from Sigma (St. Louis, MO). Avicel® RC591 was a gift from FMC biopolymer (Philadelphia, PA). All other chemicals were purchased from Fisher Scientific (St. Louis, MO). Solvents used for analysis were of HPLC grade.
Animal Tissues:

Whole small intestines of male Sprague Dawley rats were purchased from Charles River (Wilmington, MA). Intestines, perfused with saline were shipped fresh overnight over cool packs. Segments were isolated and used immediately on receipt.

Animals:

Male Sprague-Dawley rats (200-250g) were procured from Harlan Labs (Indianapolis, IN). Animal experiments conformed to the tenets of the Association for Research in Vision and Ophthalmology statement on the Use of Animals in Ophthalmic and Vision Research and followed the University of Mississippi Institutional Animal Care and Use committee approved protocols.

Differential Scanning Calorimetry:

DSC thermograms for pure dimer was collected using a Diamond Differential Scanning Calorimeter (Perkin-Elmer® Life and Analytical Sciences). The samples were weighed and sealed in aluminum pans and were heated from 0°C to 270°C at a heating rate of 10°C/min under nitrogen purge (20 mL/min).

Hot Stage Microscopy:

Hot stage microscopy under polarized light was conducted on a hot-stage (FTIR 600, Linkam Technologies & Agilent Technologies Cary 620 IR microscope) to observe morphological changes in the API as a function temperature.
**Solubility Studies:**

**pH Dependent Solubility:**

Solubility of dimer oxime in various buffers, 1.2, 4.5, 6.8, 7.4, 8.0, was evaluated by adding 1mg of the same into respective 1mL buffer which was equilibrated for 24h on a reciprocating water bath at 25°C. At the end of shaking, the vials were centrifuged and the supernatant was analyzed for the solubilized drug content. Experiment was carried out in triplicate.

**Solubility in Surfactants & Cyclodextrins:**

Solubility of dimer oxime in 5% surfactant solutions of poloxamer 188, poloxamer 407, tween 80, cremophor EL & sodium lauryl sulfate was evaluated by adding 1mg of the same into 1mL of respective surfactant solutions. The vials were then allowed to equilibrate for 24h on a reciprocating water bath at 25°C. At the end of shaking, the vials were centrifuged and the supernatant was analyzed for the solubilized drug content. Experiment was carried out in triplicate.

Cyclodextrin (CD) solubility was evaluated in 5% and 10% solutions of hydroxypropyl β cyclodextrin (HPβCD) and randomly methylated β cyclodextrin (RMβCD). 1mg of the dimer oxime was added to 1mL of respective CD solutions and was allowed to equilibrate for 24h on a reciprocating water bath at 25°C. At the end of shaking, the vials were centrifuged and the
supernatant was analyzed for the solubilized drug content. Experiment was carried out in triplicate.

**Stability Studies:**

Stability of dimer oxime was evaluated in mixtures of water-acetonitrile, acetonitrile-simulated intestinal fluid (SIF) and acetonitrile-simulated gastric fluid (SGF). Briefly, known amount of drug was spiked in 50:50 and 60:40 mixtures of the above combinations and sampling was done at 0, 6, 12, 24 hours and analyzed for amount of drug remaining. Experiment was carried out in triplicate.

**Metabolic Stability Studies:**

Metabolic stability of dimer oxime was evaluated in whole rat liver homogenates. Briefly, the whole liver was cut into small pieces and then homogenized in 10 mL DPBS using a tissue-mizer. To one mL of this homogenate, 5 mL of Bradford reagent was added and was allowed to stand for 5 minutes before being analyzed for protein content in a UV/VIS spectrophotometer (standard curve prepared using a protein standard set). Once the concentration was determined, the study was performed at an initial drug concentration of 25µg/mL and a liver protein concentration of 1 mg/mL. NADPH was supplied through a regenerating system consisting of NADP+, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, magnesium chloride.
The mixture was incubated at 37\(^\circ\)C and at each time point, 1 mL was sampled and reaction was terminated using ice cold acetonitrile, followed by storing at -20\(^\circ\)C for 15 minutes. At the end of which, it was centrifuged at 4\(^\circ\)C & 13,000rpm for 30 minutes. The supernatant was then analyzed for remaining drug content. Verapamil hydrochloride was used as the positive control in this study for validating the viability of the liver homogenates and the NADPH regenerating system.

**Formulation Development:**

**Solution Containing Dimer Oxime:**

Solution formulation containing the dimer oxime was prepared by weighing an accurate amount of dimer oxime, dissolving it in ethanol. In a separate vial, cremophor EL was dissolved in water and stirred until the solution was clear. Then, the aqueous phase was added to organic phase and kept overnight for stirring till a clear solution was obtained.

A second solution formulation was prepared to achieve a higher drug loading. Briefly, the dimer oxime was first dissolved in ethanol. Once a clear solution was obtained, tween 80 was added to this mixture followed by equal amounts of PEG 400 and water. The mixture was kept stirring until a clear solution was obtained.

**Nano-lipid Carriers Containing Dimer Oxime:**

Nano-lipid carriers (NLCs) were prepared using a high speed homogenization followed by ultra-sonication method. Dimer was accurately weighed and melted along with Precirol\textsuperscript{®}
ATO 5 & Capryol® 90 (Gattefosse, France) to obtain a clear lipid phase. An aqueous phase containing Gelucire® 50/13 in distilled water, was heated and added to the melted lipid phase under stirring. A coarse emulsion from this pre-mix was formed using an Ultra-Turrax® operated at 16000 rpm for 5 minutes, followed by sonication for 6 minutes. This resulted in a fine emulsion which was slowly cooled to room temperature to form dimer oxime NLCs.

A second NLC formulation was prepared with Gelucire® 50/13 in lipid phase where it was melted along with the dimer and other components of the lipid phase and the aqueous phase consisted of only distilled water.

**Self-Nanoemulsifying Drug Delivery Systems Containing Dimer Oxime:**

Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) were prepared by the following method. An accurately weighed amount of dimer oxime was dissolved in Labrafac Lipophile and stirred until a clear solution was obtained. In a separate vial, accurately measured amounts of Cremophor EL, Labrasol, Lauroglycol, Labrafil 1944 were dissolved in to Transcutol P and stirred until a aqueous phase was obtained. Now, the aqueous phase was added to the lipid phase under constant stirring. The formulation at the end was visually inspected for clarity. For evaluating the emulsion forming capability, a measured amount was added to 20mL water and visual inspection was made followed by particle size measurement.

Alternately a surface charge modified formulation was prepared using the same method with Oleylamine as charge inducer added to the lipid phase.
Solid Lipid Nanoparticles Containing Dimer Oxime:

Solid lipid nanoparticles (SLNs) were prepared as per previously established protocols, using a high speed & high pressure homogenization method [129]. Dimer oxime was accurately weighed and melted along with Compritol® 888 ATO or Labrafil® 1944M (Gattefosse, France) to obtain a clear lipid phase. An aqueous phase containing Poloxamer® 407, propylene glycol and tween 80 in distilled water, was heated and added to the melted lipid phase under stirring. A coarse emulsion from this pre-mix was formed using an Ultra-Turrax® operated at 16000 rpm for 10 minutes, followed by high pressure homogenization at 15000 psi for 5 minutes. The temperature during this entire process was maintained at 60°C. The hot emulsion was slowly cooled to room temperature to form dimer oxime SLNs.

A second SLN formulation was prepared, where the surface the particles were modified by adding chitosan chloride to the aqueous phase along with distilled water and other surfactants. A procedure similar to the above was followed to fabricate these nanoparticles.

Suspension Containing Dimer Oxime:

Suspension formulation was prepared with half of the dimer oxime dispersed in a Cremophor® EL, Avicel® RC 591 & water vehicle and other half dissolved in ethanol and added to the suspension. Briefly, an accurately amount of cremophor and avicel were dissolved in water. Half of the dimer was dispersed in this using an Ultra-Turrax and the other half of dimer was dissolved in ethanol and added to the suspension. Composition of all the above formulations is detailed in table 5-1.
### Table 5-1: Composition of the Various Dimer Oxime Formulations

<table>
<thead>
<tr>
<th>Formulation Code Type</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
<th>F11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Units</strong></td>
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<td>% w/v</td>
<td>% w/w</td>
<td>% w/w</td>
<td>% w/v</td>
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<td>% w/v</td>
<td>% w/v</td>
<td>% w/v</td>
</tr>
<tr>
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<td>50</td>
<td>50</td>
<td>50</td>
<td>4</td>
<td>50</td>
<td>50</td>
<td>3</td>
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<tr>
<td><strong>Dosing Volume (ml)</strong></td>
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<td>12.5</td>
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<td><strong>Propylene Glycol</strong></td>
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<tr>
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<td>80</td>
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</table>

*NLC: Nano lipid carriers; SNEDDS: Self Nano-emulsifying drug delivery systems; SLN: Solid lipid nanoparticles; SLNC: Solid lipid nanoparticles with Chitosan; IV: Intravenous; IP: Intraperitoneal*
**Determination of Particle Size and Polydispersity Index:**

Particle size and Polydispersity Index (PDI) of the SLNs, NLCs and SNEDDS formulations were measured using a dynamic light scattering instrument, Zetasizer Nano ZS (Malvern Instruments Inc., Westbrough, MA), at 25 °C. A high concentration zeta cell was used to measure both mean particle size (z averaged) and PDI.

**Assay Procedure for the Formulations:**

Dimer oxime content in the SLNs, NLCs, SNEDDS and suspension was assayed according to the following procedure. An accurately measured amount of the formulation was extracted in 1 mL of ethanol and was centrifuged for 10 minutes. The supernatant was diluted in mobile phase and was analyzed for dimer oxime content.

**In vitro Intestinal Permeability Studies:**

*In vitro* permeability of the dimer oxime across small intestinal segments of rats was carried out using the everted gut sac technique [130] (Fig. 5-1). Briefly, duodenum, jejunum and ileum segments were measured and cut from the whole small intestines. The segments were flushed with Tween 80 solution to remove fat bodies. One end of each segment was sutured and formulation (1mL) was filled, following which the other end was also tied up. These sacs were suspended in vials containing 9mL of 10% RMβCD solutions and were placed in a water bath set at 37 degrees Celsius. Samples (0.5mL) were withdrawn every 30 minutes for 3 hours and
analyzed. The initial donor concentration was 2mg/mL per sac. For the purpose of this study, three formulations, a solution, nano lipid carriers and self-emulsifying systems, were used and the permeability of dimer oxime from these formulations was tested. The SNEDDS were diluted with IPBS to achieve the predetermined donor concentration.

**Figure 5-1**: Scheme for *In Vitro* Permeability using Everted Gut Sac Technique

**In vivo Bioavailability Studies:**

Oral bioavailability of dimer oxime through the above formulations was tested in male Sprague-Dawley rats. Studies were carried out as per the protocol approved by the Institutional animal care and use committee (IACUC), University of Mississippi. Rats were fasted overnight, but were allowed water ad libitum and were divided into groups of four. A single dose of the
formulations was administered orally by gavage (50mg/kg dose) or intravenously (4 mg/kg dose) through the catheter or intra-peritoneally (3mg/kg dose). Samples (200µL) were drawn through the jugular vein at pre-determined time points. Heparinized saline (200 IU/ml) was injected through the jugular vein following sampling to maintain patency and a constant blood volume. The samples were centrifuged at 9000 rpm at 4°C for 3 minutes and the separated plasma stored at -80°C, was then analyzed for drug content. At the end of an experiment animals were euthanized by an overdose of pentobarbital injected into the jugular vein.

**Analytical Method:**

**HPLC-ELSD Method for in vitro Samples:**

Analysis of all the in vitro samples was carried out using a HPLC coupled with an evaporative light scattering detector (ELSD). The system comprised of a Waters 2695 autosampler and Waters 2424 evaporative light scattering detector (ELSD). Phenomenex Synergi™ 4 µm Max-RP 80 Å, LC Column 100 x 4.6 mm column was used. The Drift tube in the detector was set at 50°C and nebulizer was set in the heating mode with 0% power level. The mobile phase consisted of 20% water containing and 80% acetonitrile, with a flow of 1mL/min. Injection volume was 20 µL.

The standard calibration curve was derived and the parameters such as regression coefficient ($r^2$), slope and Y- intercept were noted to establish the linearity of the method. The analytical method was also validated with respect to precision, accuracy, recovery and specificity. Limit of detection and limit of quantification were found to be 500ng/mL and 1mg/mL,
respectively, and the retention time for THC was about 13.1 min. The method was observed to be specific, precise and reproducible.

5.3. Results:

Differential Scanning Calorimetry:

Pure dimer oxime was tested and the results showed that the drug recrystallizes at 116°C and degrades at 160°C. The recrystallization was validated through hot stage microscopy. The resulting thermogram is shown in figure 5-2.
Figure 5-2: Thermal Profile of DHA Dimer Oxime using a Differential Scanning Calorimeter.

**Hot Stage Microscopy:**

Images from the hot stage microscopy depicted the melting, recrystallization and degradation characteristics of the compound. It was seen that at room temperature, the compound
is a mirror like crystal. Upon heating, the compound started melting at 90°C and as the temperature was increased gradually at a rate of 2°C per minute, recrystallization was observed at 116°C. Upon holding at this temperature for 10 minutes, crystal growth was observed. Upon further increase, these crystals also started melting above 120°C and a color change (degradation) was observed above 150°C in accordance with the results observed on DSC.

**Solubility Studies:**

**pH Dependent Solubility:**

The solubility of dimer oxime across various pH was seen to be less than 1mg/ml as shown in table 5-2.

**Table 5-2: Solubility of Dimer Oxime in Various Buffers. Data represents mean±SD (n=3).**

<table>
<thead>
<tr>
<th>pH</th>
<th>Solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>4.5</td>
<td>&lt; 1</td>
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<tr>
<td>6.8</td>
<td>&lt; 1</td>
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<tr>
<td>7.4</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>8.0</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>
Solubility in Surfactants & Cyclodextrins:

The solubility of dimer oxime in all the surfactants tested, was seen to be less than 1mg/mL, whereas, solubility in 5% and 10% HPβCD solutions was seen to be 6µg/mL and 14µg/mL respectively, while it was 116µg/mL and 353µg/mL in 5% and 10% RMβCD solutions respectively.

The above results have been tabulated in tables 5-3 & 5-4.

**Table 5-3:** Solubility of Dimer Oxime in Surfactant Solutions. Data represents mean±SD (n=3).

<table>
<thead>
<tr>
<th>Surfactant (5% Solutions)</th>
<th>Solubility (mg/mL)</th>
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<tbody>
<tr>
<td>Poloxamer 188</td>
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</tr>
<tr>
<td>Poloxamer 407</td>
<td>&lt; 1</td>
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<tr>
<td>Tween 20</td>
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</tr>
<tr>
<td>Tween 80</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Cremophor EL</td>
<td>&lt; 1</td>
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</table>

**Table 5-4:** Solubility of Dimer Oxime in Cyclodextrin Solutions. Data represents mean±SD (n=3).

<table>
<thead>
<tr>
<th>Cyclodextrin</th>
<th>Solubility in 5% Solutions (µg/mL)</th>
<th>Solubility in 10% Solutions (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxpropyl Methyl β Cyclodextrin</td>
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<td>14</td>
</tr>
<tr>
<td>Randomly Methylated β Cyclodextrin</td>
<td>116</td>
<td>353</td>
</tr>
</tbody>
</table>
**Stability Studies:**

The stability of dimer oxime in mixtures of water-acetonitrile, SGF-water and SIF-water is shown in table 5-5, in terms of half-lives. It was seen that as the stability is directly proportional to the acetonitrile content in all 3 mixtures.

**Table 5-5:** Stability of Dimer Oxime in Various Mixtures. Data represents mean±SD (n=3).

<table>
<thead>
<tr>
<th>Mixture</th>
<th>50:50 Mixture (Half life in hours)</th>
<th>60:40 Mixture (Half life in hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water: Acetonitrile</td>
<td>277</td>
<td>40</td>
</tr>
<tr>
<td>Acetonitrile: SIF</td>
<td>17.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Acetonitrile: SGF</td>
<td>71</td>
<td>49.5</td>
</tr>
</tbody>
</table>

**Metabolic Stability:**

The metabolic stability of dimer oxime is shown in figure 5-3. It was observed that the compound is highly metabolized in the whole liver homogenate. The degradation profile of verapamil hydrochloride confirms the viability of the tissue and the method.
**Figure 5-3:** Metabolic Stability of Dimer Oxime & Verapamil Hydrochloride in Rat Liver Homogenates. Data represents mean±SD (n=3).

**Assay of the Formulations:**

The dimer oxime content in all the formulations was seen to be between 92 and 106%.

**Particle Size and Polydispersity Index (PDI):**

The particle size and PDI characteristics of the formulation have been listed in table 5-6.
**Table 5-6:** Particle size and Polydispersity Index (PDI) of Various Formulations. Data represents mean±SD (n=3).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Particle Size (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-2</td>
<td>395</td>
<td>0.22</td>
</tr>
<tr>
<td>F-3</td>
<td>129</td>
<td>0.13</td>
</tr>
<tr>
<td>F-4</td>
<td>194</td>
<td>0.17</td>
</tr>
<tr>
<td>F-5</td>
<td>246</td>
<td>0.34</td>
</tr>
<tr>
<td>F-7</td>
<td>350</td>
<td>0.29</td>
</tr>
<tr>
<td>F-11</td>
<td>374</td>
<td>0.27</td>
</tr>
</tbody>
</table>

*In vitro* Intestinal Permeability Studies:

The intestinal permeation of dimer oxime across duodenum, jejunum and ileum from the solution, NLC and SNEDDS formulation is shown in figure 5-4. It was seen that the permeability did not depend on the intestinal segment, as there was no significant difference in permeability values between segment types from each formulation.
**Figure 5-4:** Permeability of Dimer Oxime across Rat Intestinal Segments. Data represents mean±SD (n=3).

*In vivo Bioavailability Studies:*

The compound reached maximum level of 1665 ng/mL in 30 minutes and then the levels started dropping rapidly with some amount of drug still remained in the system even at the end of 8 hours, while a peak plasma concentration of 100ng/mL was seen at the end of 90 minutes with levels seen till the end of 8 hours post IP administration.

The plasma profiles post oral administration showed that the solution formulation showed the highest $C_{max}$ (345.3ng/mL) followed by the SLNs (266.4ng/mL). The NLCs (242.5ng/mL) and suspension (160ng/mL) were the next best followed by the SNEDDS formulations.
(44.6ng/mL & 77.8ng/mL for uncharged & charged respectively). No improvement in absorption was seen between both the solutions, when half of the water content was replaced with PEG 400 (245.7ng/mL). Presence of chitosan did not show any increase in blood levels in the second SLN formulation (135.6ng/mL) and the addition of surfactant in the aqueous phase instead of lipid phase in NLCs also yielded lower $C_{\text{max}}$ and absorption values (191ng/mL).

Nonetheless, all the formulations were also able to depict an 8 hour profile, with highest systemic bioavailability values shown by solution, followed by the NLCs. The plasma profiles from all the formulations post IV, IP and oral administration have been shown in figures 5-5, 5-6 & 5-7 and the corresponding pharmacokinetic parameters have been depicted in table 5-7.

![Pharmacokinetic Disposition of Dimer Oxime in Sprague-Dawley Rats, Post Intravenous Administration. Mean±S.D (n=3).](image)

**Figure 5-5:** Pharmacokinetic Disposition of Dimer Oxime in Sprague-Dawley Rats, Post Intravenous Administration. Mean±S.D (n=3).
**Figure 5-6:** Pharmacokinetic Disposition of Dimer Oxime in Sprague-Dawley Rats, Post Intraperitoneal Administration. Mean±S.D (n=3).
**Figure 5-7:** Pharmacokinetic Disposition of Dimer Oxime in Sprague-Dawley Rats, Post Oral Administration. Mean±S.D (n=3).
Table 5-7: Pharmacokinetic Disposition of Dimer Oxime from Various Formulations in Sprague-Dawley Rats. Data represents mean±SD (n=3).

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Type</th>
<th>Dose (µg)</th>
<th>C&lt;sub&gt;Max&lt;/sub&gt; (ng/mL)</th>
<th>T&lt;sub&gt;Max&lt;/sub&gt; (min)</th>
<th>AUC (µg.min/mL)</th>
<th>Absolute Bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-1</td>
<td>Solution</td>
<td>12500</td>
<td>345.3</td>
<td>40</td>
<td>69.37</td>
<td>6.4</td>
</tr>
<tr>
<td>F-2</td>
<td>NLC</td>
<td>12500</td>
<td>242.5</td>
<td>40</td>
<td>38.18</td>
<td>4.4</td>
</tr>
<tr>
<td>F-3</td>
<td>SEDDS</td>
<td>12500</td>
<td>169.5</td>
<td>20</td>
<td>11.32</td>
<td>1.3</td>
</tr>
<tr>
<td>F-4</td>
<td>Charged SEDDS</td>
<td>12500</td>
<td>82.9</td>
<td>20</td>
<td>17.15</td>
<td>2.0</td>
</tr>
<tr>
<td>F-5</td>
<td>SLN</td>
<td>12500</td>
<td>266.4</td>
<td>90</td>
<td>36.25</td>
<td>4.2</td>
</tr>
<tr>
<td>F-6</td>
<td>I.V</td>
<td>1000</td>
<td>1665</td>
<td>20</td>
<td>69.50</td>
<td>100</td>
</tr>
<tr>
<td>F-7</td>
<td>SLN with Chitosan</td>
<td>12500</td>
<td>135.6</td>
<td>60</td>
<td>14.70</td>
<td>1.7</td>
</tr>
<tr>
<td>F-8</td>
<td>Suspension</td>
<td>12500</td>
<td>160.4</td>
<td>90</td>
<td>20.00</td>
<td>2.3</td>
</tr>
<tr>
<td>F-9</td>
<td>I.P</td>
<td>750</td>
<td>100.5</td>
<td>90</td>
<td>26.14</td>
<td>50.2</td>
</tr>
<tr>
<td>F-10</td>
<td>Solution</td>
<td>12500</td>
<td>245.75</td>
<td>45</td>
<td>30.62</td>
<td>3.5</td>
</tr>
<tr>
<td>F-11</td>
<td>NLC</td>
<td>12500</td>
<td>191</td>
<td>45</td>
<td>32.79</td>
<td>3.8</td>
</tr>
</tbody>
</table>

*NLC: Nano lipid carriers; SEDDS: Self emulsifying drug delivery systems; SLN: Solid lipid nanoparticles; IV: Intravenous; IP: Intraperitoneal

5.4. Discussion:

Though there have been many modes of therapy for treatment of malaria, none of them was proven to be effective for a long time mainly due to the resistance developed by the parasites [131-133]. It has been shown that the parasites’ ability to develop this resistance depends on the
class and type of the anti-malarial [133, 134]. Also, some parasites have a higher ability to
develop resistance in comparison to others [135]. Resistance to atovoquone was developed in a
little over a decade, while it took parasites almost only half the time in developing resistance
towards mefloquine [136, 137]. Therefore combination therapies were developed and an
artemisinin derivative was more often than not was the partnering compound with another
compound [138, 139]. The main active metabolite of artemisinin is DHA, which has proven
activity against the malarial parasite [99] due to its peroxide bridge containing ring system [100].
But, like other endoperoxide group of drugs, this compound also has very poor physicochemical
characteristics such as very poor solubility, stability, bioavailability [103, 104]. Haynes et al
depicted the thermal instability of this compound, which makes any attempt towards developing
a formulation difficult [140]. Therefore, for this project, we have synthesized a novel dimer of
the DHA with a nitrogen group in the linker functionality. The dimer oxime was expected to be
more soluble, bioavailable and show improved activity. It may be possible to develop
formulations which can act at lower doses and thereby avoid the side effects seen due to higher
doses of DHA [141-144]. In the studies described and carried out in this part of the project, we
have attempted to get a basic understanding of the compound’s physicochemical characteristics
and evaluate its bioavailability through various formulations.

The initial set of studies carried out, were to check the solubility of the DO in buffer, 5%
surfactant and 5%, 10% cyclodextrin solutions. It was observed that the solubility was below the
detecting limit and less than 1mg/mL in all the buffered and 5% surfactant solutions. On the
other hand, the DO showed very little solubility in 5% and 10% HPβCD, while the highest was
seen in 10% RMβCD solution. Stability of the compound in mixtures of water-acetonitrile, water-SGF and water-SIF showed that the half-life dropped with decreasing organic content and increasing aqueous content. Also, metabolic stability in whole liver homogenates showed rapid degradation of the pure DO. Going by the solubility and stability profiles, surfactant, co-solvent based and lipid based systems were deemed to be the suitable platforms for formulation development.

The profile on DSC showed degradation at 150°C, while there was small thermal event observed at 116°C. Further investigation of this under a hot stage microscope with a 2°C step increase temperature profile showed the compound, molten, started recrystallizing at 116°C. While there was no change in the retention time for the recrystallized DO on HPLC, the activity of the recrystallized compound might still be different. That investigation was not a part of this part of the project, but the thermal profile provided valuable information regarding the processing conditions that must be maintained during formulation development to avoid any possible polymorphic changes in the compound.

Based on the above studies, we have decided to focus mainly on lipid based systems for evaluating the compound’s plasma profile. Initial *in vitro* evaluation of the compound’s permeation characteristics was performed using three formulations, namely a surfactant-ethanol based solution, NLCs and SNEDDS. This study was mainly performed to look at the effect on intestinal segments on the DO’s permeability. Everted duodenum, jejunum and ileum sacs were used and the results showed that, there was no significant difference in permeability between
segments within each formulation. Higher permeability was seen from the solution, followed by NLCs and SNEDDS across all segments.

Having seen that the permeability was not segment dependent, we have proceeded to evaluate the DO’s bioavailability in vivo. It was seen that the drug levels peaked very early, as expected, post IV dose, and the quick drop in levels after this showed the rapid elimination profile of the compound. Similar was the case post IP dose, though some levels were still being detected at the end of 8 hours in both cases.

Following this, the oral bioavailability was evaluated from a wide range of formulations. It was surprising to see that none of the lipid based formulations were able to surpass the solution’s plasma levels. The SLNs were the best formulation after the solution followed by the NLCs. A possibility that lymphatic uptake of the nanoparticles can result in higher plasma levels through avoiding of first-pass effect [112, 113] was not seen and adding chitosan to the SLN formulation and adding surfactant to the aqueous phase of the NLCs also did not yield better results. Also, the SNEDDS were highly unsuccessful and surface modification by imparting a positive charge also did not provide any improvement and similar results were seen when a suspension was utilized to control the release. Seeing that the solution was the best formulation of all, we have attempted to sustain the levels achieved for a longer time by replacing half of the water content in the formulation with the more viscous PEG 400 and also, this allowed for a higher loading capacity (15mg/mL in this formulation as opposed to 5mg/mL in the initial
solution formulation). It was seen that this formulation also failed to achieve any levels higher than the initial solution formulation.

Going by previous reports on enhanced drug delivery using lipid based systems [118, 119, 121-125], the results seen in these studies were in contrast. One of the possibilities could be the lipid getting digested and the drug, once out of the lipid core, is not able to form micelles [112] and/or partition effectively and get into solution. This is not surprising going by the aqueous stability and solubility observed in vitro, in the pre-formulation studies. The solution on the other hand, was able to deliver the compound immediately into the blood upon administration and so the higher plasma peak level was observed. A second reason could be the immediate partitioning of drug in to the RBCs post absorption. It has been reported previously that Artemisinin and its derivative compounds partition into the RBCs quickly [145, 146], and it is logical to make this assumption as the site of action for this compound is inside the RBCs, where the parasite merozoites reside and multiply. Seeing that we have only analyzed the plasma fraction in all the experiments, analyzing the RBC fraction also might give a more definite idea about the compound’s disposition post absorption.

It has to be noted here that while the plasma levels were not expectedly high, all the formulations showed levels till the end of 6-8 hours and these levels are higher than the IC$_{50}$ values of DHA which are 1.79ng/mL in D-6 type $P. falciparum$ parasite and 1.83ng/mL in W-2 type [147]. Also, these levels were achieved with a smaller dose (50mg/kg) in contrast to the much higher dose of DHA employed previously in other reported studies [141, 142, 144].
At this point, we surely know that the DO is absorbed into the blood at lower doses and levels above the IC$_{50}$ are being observed even at the end of 6-8 hours. Going by the plasma profiles from all the above formulations, a dosing frequency of twice a day may be suggested. This can be further improved through optimization of the solution by testing other and better suited viscous additives. Also, an RBC partitioning study can help in complete elucidation of the compound’s disposition and may help take the next step towards formulation optimization.

5.5. Conclusion:

It has been understood from all the above studies that the DHA dimer oxime is poorly soluble and highly metabolized by the hepatic enzymes. The lipid based formulations prepared to circumvent these issues were less successful than a surfactant-ethanol based solution. It remains to be seen whether this is due to the poor partitioning of the compound into the solution form the lipid cores or higher partitioning into the site of action, the RBCs, from the same. A deeper understanding of these characteristics maybe helpful in developing a formulation which performs better and helps in developing a long acting anti-malarial drug with lower dose and subsequently, lesser side effects.
CHAPTER 6

SUMMARY OF ALL THE STUDIES
All the approaches used have shown considerable promise and success in achieving the desired goals in all the studies.

i. The controlled release formulation of Δ⁹-THC can be a potential one dose per day formulation used for treating CINV. This formulation eliminates frequent dosing and the hypersensitivity inducing sesame oil. Δ⁹-THC, as mentioned is a resinous compound that is difficult to handle and the processing method described helps in overcoming this aspect. Also, the composition of this tablet may help circumvent lipolysis and maintain the release profile of the dosage form.

ii. The SLNs instilled topically showed good penetration ability and were able to deliver Δ⁸-THC to the posterior ocular segment. *In vitro*, they exhibited good characteristics in terms of particle size, PDI, assay, entrapment efficiency and permeability across corneas. *In vivo*, they showed the highest efficiency in delivering the intact Δ⁸-THC when compared to the other formulations tested, in spite of carrying a lower dose of Δ⁸-THC. This topical formulation not only is a promising method for delivery to the anterior chamber, but also has the potential to delivery compounds non-invasively to the back of the eye and therefore help in treating afflictions pertaining to the posterior ocular segment.

iii. The surfactant solution and lipid based systems had good *in vitro* intestinal permeability. The formulations had good characteristics in terms of particle size & PDI. The *in vivo* plasma profiles showed that the solution was the best formulation in terms of levels achieve. Nonetheless, all the formulations showed levels till the end of 8 hours and these levels were above the IC₅₀ values for the malarial parasite. The tested novel prodrug
showed promise, and further investigation into its disposition would help in developing an effective line of therapy for malaria.
CHAPTER 7

BIBLIOGRAPHY
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46. He, F. and Z.H. Song, Molecular and cellular changes induced by the activation of CB2 cannabinoid receptors in trabecular meshwork cells. Mol Vis, 2007. **13**: p. 1348-56.


<table>
<thead>
<tr>
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<tr>
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</tr>
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