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Exploration in the Effectiveness of Solid Lipid Nanoparticle Formulations in Enhancing Ocular Delivery of Win 55, 212 in the Management of Glaucoma

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EXPLORATION IN THE EFFECTIVENESS OF SOLID LIPID NANOPARTICLE FORMULATIONS IN ENHANCING OCULAR DELIVERY OF WIN 55, 212 IN THE MANAGEMENT OF GLAUCOMA

By
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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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Approved by

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DEDICATION

I would like to dedicate my thesis to my friends and family for supporting me through every step of my academic career and never failing to cheer me on. This thesis would not have been possible without their unrelenting encouragement.

My parents, Tom and Debra Nesbit, deserve the highest of dedications. They have loved me unconditionally and given me an ambitious spirit that has led me to where I am today. Through every adventure in my life they have been my biggest cheerleaders, showering me with grace and motivating me to be the best I can possibly be. I will never be able to thank them enough for all that they have done and continue to do for me.
ACKNOWLEDGEMENTS

Being a student of the Sally McDonnell Barksdale Honors College has been the greatest blessing of my undergraduate career at the University of Mississippi. The faculty and professors of this institution have constantly pushed me to step outside my comfort zone and to strive for greatness. With that, I would like to acknowledge the Sally McDonnell Barksdale Honors College for their support of this thesis, both financially and academically.

I would also like to acknowledge and thank Dr. Soumyajit Majumdar. He welcomed me, a student with no prior lab experience, into his laboratory through an internship position with the Center of Research Excellence in Natural Products Neuroscience. In addition, I would like to acknowledge Dr. Majumdar’s Ph.D. students, who patiently taught me the foundations on which this thesis is written.
ABSTRACT

EMILY ANN NESBIT: EXPLORATION IN THE EFFECTIVENESS OF SOLID LIPID NANOPARTICLE FORMULATIONS IN ENHANCING OCULAR DELIVERY OF WIN 55, 212 IN THE TREATMENT OF GLAUCOMA

(Under the direction of Dr. Soumyajit Majumdar)

Glaucoma is a progressive and degenerative ocular disease which, without proper treatment, can result in permanent blindness. Due to a complex organization of lipophilic and hydrophilic ocular membranes, topical delivery of therapeutic agents has also proved to be a challenging task. Cannabinoids, such as \(\Delta^9\)-Tetrahydrocannabinol (THC), have intraocular pressure (IOP) lowering and neuroprotective capabilities. Due to the unfavorable physiochemical characteristics of these molecules, delivery into the eye through a topical route is very difficult. Previous research has shown that prodrug derivatization and formulations such as solid lipid nanoparticles (SLNs) improve the physiochemical characteristics and ocular bioavailability of such molecules. WIN 55, 212 (WIN) is a synthetic cannabinoid possessing a structure similar to THC and is fifty times more potent. WIN 55, 212 (212) also possesses slightly better physiochemical characteristics than THC.

The goal of this research is to develop solid lipid nanoparticle formulations that will enhance the intraocular delivery of WIN 55, 212. The particle size, entrapment efficiency, and content of the SLN formulations will be standardized. In vitro and in vivo studies will be carried out to determine the benefit of the SLNs over conventional formulations. In vitro transcorneal permeability studies will be carried out utilizing a Franz diffusion apparatus and corneas dissected from rabbit eyes. Samples will be taken at different time intervals throughout the permeability study and analyzed using a HPLC-UV system. The in vivo IOP lowering evaluation studies will be performed on male New Zealand albino rabbits that have artificially induced glaucoma. Fifty microliters of 0.4% w/v SLN’s (Dose: 0.2 mg) will be topically applied to the cul-de-sac of each rabbit’s eye. An equivalent dose of WIN 55, 212 in Tocrisolve® will be used as the control. The study will take place until the IOP returns to 90% of the baseline levels. Following the conclusion of the study, the animals will be anesthetized and euthanized with a sufficient dose of pentobarbital injected through the marginal ear vein. Immediately, the eyes will be enucleated and the ocular tissues will be isolated, weighed, and analyzed for WIN 55, 212 content. The goal of this project is to increase the retention of WIN 55, 212 in the eye and prolong the intraocular lowering duration compared to the control formulation.
TABLE OF CONTENTS

LIST OF TABLES AND FIGURES...........................................................................................................vii

LIST OF ABBREVIATIONS...................................................................................................................viii

BACKGROUND.........................................................................................................................................1

MATERIALS AND METHODS...............................................................................................................32

RESULTS AND DISCUSSION...............................................................................................................41

CONCLUSION.......................................................................................................................................49

BIBLIOGRAPHY.................................................................................................................................51
LIST OF TABLES AND FIGURES

Figure 1  Cupping in POAG................................................................. 2
Figure 2  Anatomy of the eye............................................................ 4
Figure 3  Aqueous humor drainage in healthy and glaucomatous eyes........ 5
Figure 4  Iris hyperpigmentation due to PGA use versus normal eye........... 9
Figure 5  Structure of Δ⁹-tetrahydrocannabinol..................................... 16
Figure 6  Chemical structures of classical cannabinoids........................... 18
Figure 7  Chemical structure of WIN 55, 212......................................... 19
Figure 8  Chemical structures of THC-HS and THC-HG.......................... 22
Figure 9  Structure of a Franz diffusion apparatus................................... 27
Figure 10 Results of in vitro permeability study..................................... 32
Figure 11 Effectiveness of control formulation versus WIN-SLN.................. 37
Figure 12 Results of the histology testing............................................ 38
Table 1  Results of WIN 55, 212 SLN physiochemical testing.................... 30
Table 2  Results of in vitro drug release studies..................................... 31
Table 3  Results of basal IOP readings............................................... 33
Table 4  Results of IOP readings after installation of control formulation........ 34
Table 5  % Change in IOP from baseline with control formulation............... 34
Table 6  Results of basal IOP readings of rabbits receiving WIN-SLN........... 35
Table 7  Results of IOP readings after instillation of WIN-SLN.................... 36
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS-OCT</td>
<td>Anterior Segment Optical Coherence Tomography</td>
</tr>
<tr>
<td>CB&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Cannabinoid Receptor 1</td>
</tr>
<tr>
<td>CB&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Cannabinoid Receptor 2</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic Pulmonary Obstructive Disease</td>
</tr>
<tr>
<td>DPBS</td>
<td>Dulbecco’s Phosphate-Buffered Saline</td>
</tr>
<tr>
<td>EE</td>
<td>Entrapment Efficiency</td>
</tr>
<tr>
<td>HPβCD</td>
<td>2-Hydroxypropyl-β-Cyclodextrin</td>
</tr>
<tr>
<td>IPBS</td>
<td>Isotonic Phosphate Buffer Saline</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity Index</td>
</tr>
<tr>
<td>PGA</td>
<td>Prostaglandin Analogues</td>
</tr>
<tr>
<td>POAG</td>
<td>Primary Open-Angle Glaucoma</td>
</tr>
<tr>
<td>NRR</td>
<td>Neuroretinal Rim</td>
</tr>
<tr>
<td>RGC</td>
<td>Retinal Ganglion Cell</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
</tr>
<tr>
<td>RMβCD</td>
<td>Randomly Methylated β-Cyclodextrin</td>
</tr>
<tr>
<td>Δ&lt;sup&gt;9&lt;/sup&gt;-THC</td>
<td>Δ&lt;sup&gt;9&lt;/sup&gt;-Tetrahydrocannabinol</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>THC-HG</td>
<td>THC-Hemiglutarate Ester Prodrug</td>
</tr>
<tr>
<td>THC-HS</td>
<td>THC-Hemisuccinate Ester Prodrug</td>
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Background

I. The Pathophysiology and Epidemiology of Glaucoma

Glaucoma is an ocular disease that stands as the second leading cause of blindness in the United States and affects nearly 70 million people worldwide (Shaikh, Yu, & Coleman, 2014). Glaucoma is defined as a neuropathic ocular disease, characterized by the disruption of normal functions of the peripheral nerves within the eye (Cook & Foster, 2012). Although the pathogenesis of the disease remains poorly understood, an increase in intraocular pressure (IOP) within the eye is correlated to an increase in the death of retinal ganglion cells. This cellular process results in the most common clinical manifestation of glaucoma: vision loss (Weinreb, Aung, & Medeiros, 2014).

The retinal ganglion cells are the last in a chain of optic cells which begin with the photoreceptors. As pressure rises within the eye, the area where the nerve fibers of the retinal ganglion cells pass, known as the lamina cribrosa, compresses and results in thinning of the neuroretinal rim. Compression of the lamina cribrosa results in the clinical phenomenon of ‘cupping’, where the optic cup expands as a result of the decreased thickness of the neuroretinal rim (Healey & Thomas, 2010). Continual compression of the neuroretinal rim leads to retinal ganglion cell (RGC) death, which is associated with the visual impairments that glaucoma patients experience.
To understand the complexities of glaucoma and the treatment options available, a basic understanding of the anatomical structure of the eye is necessary. The eye is divided by the lens into two fluid-filled sections, the anterior segment which contains the aqueous humor and the posterior segment which contains the vitreous humor (Purves, Augustine, Fitzpatrik, Katz, LaMantia, McNamara, & Williams, 2001). The visual pathway begins with the cornea, a transparent structure that allows light to enter the eye and travel through the pupil, which is surrounded by the iris, the colored portion of the eye that expands and contracts depending on
the magnitude of light entering the eye (Peate & Jones, 2014). The final structure of the anterior segment is the lens, which is supported by the ciliary muscles and serves to refract and focus incoming light onto the retina (Peate & Jones, 2014). The aqueous humor encompasses each of these structures and is produced by the ciliary body. Inadequate drainage of the aqueous humor through the trabecular meshwork is the main culprit in the development of glaucoma (Purves, Augustine, Fitzpatrik, Katz, LaMantia, McNamara, & Williams, 2001). The current treatment options for glaucoma aim to decrease aqueous humor production or increase the drainage through the trabecular meshwork (Weinreb et al., 2014).

The space between the back of the lens and the retina, known as the posterior segment, is filled with the vitreous humor, a gelatinous substance that comprises approximately 80% of the total volume of the eye (Purves et al., 2001). The retina contains the cells involved in the visual pathway, beginning with the photoreceptors and ending with the retinal ganglion cells (RGCs). The axons of these cells synapse at the optic nerve in the lateral geniculate nucleus located in the thalamus (Healey & Thomas, 2010). The anatomy of the eye can be visualized in Figure 2.
I. 2 Different Subtypes of Glaucoma and Their Associated Risk Factors

The two broad categories of glaucoma are open-angle and angle-closure, and can manifest either randomly or be secondary conditions resulting from use of certain medications, pre-existing conditions, or trauma. Although both subtypes result in the death of retinal ganglion cells, there are distinct differences in how the aqueous humor drains through the trabecular meshwork and uveoscleral outflow pathway, which can be seen in Figure 3.
Primary open angle glaucoma (POAG) is the most prevalent form of the disease and results from increased resistance of aqueous humor outflow through the trabecular meshwork, a drainage pathway located in the anterior chamber of the eye at the junction of the cornea and...
ciliary body (Janssen et al., 2013). The pathogenesis of this phenomenon is poorly understood, and POAG is often described as being insidious due to its slow progression, with many patients only receiving a diagnosis after their vision has deteriorated quite significantly (Janssen et al., 2013). Some of the risk factors associated with the development of POAG include a family history of glaucoma, older age, belonging to the African American race, high intraocular pressure, and the use of systemic or topical corticosteroids (Weinreb et al., 2014). Research has suggested that myopia (nearsightedness), high fasting capillary blood glucose levels, and increased levels of high-density lipoprotein cholesterol may also put individuals at risk of developing POAG (Kim, Jeoung, & Park, 2014). The role of genetics has also been called into question, and although less than 10% of all glaucoma cases can be attributed to a genetic predisposition, recent studies have identified certain alleles that may increase a patient’s risk of developing the disease (Mabuchi, Sakurda, Kashiwagi, Yamagata, Ijima, & Tsukahara, 2015).

The other main categorization of the disease in angle-closure glaucoma, which is characterized by the site of aqueous humor outflow being anatomically obstructed by the iris (Weinreb et al., 2014). This subtype of glaucoma disproportionately affects people of Chinese, Mongolian, and Indian descent and the highest prevalence rates have been found in those belonging to the Eskimo and Inuit ethnicities (Tarongoy, Ho, & Walton, 2009). Angle-closure glaucoma can either be acute, which is considered a medical emergency, or chronic. The risk factors for developing chronic angle-closure glaucoma are more understood than POAG because they are primarily morphological in nature. In addition to the increased prevalence seen in Asian countries, other risk factors include a shallow anterior chamber, short axial length, thicker lens, hyperopia, and belonging to the female sex (Zhang, Wang, Aung, Jonas, & Wang, 2015).
I. 3 Diagnosing Glaucoma

Older age, a family history of glaucoma, African or Latino ethnicity, diabetes mellitus, myopia, and low blood pressure are all easily identifiable risk factors for developing POAG. However, specific diagnostic exams are required to identify the physiologic risk factors of high IOP levels, disc hemorrhage, a large cup-to-disc ratio, and a high pattern standard deviation on threshold visual field testing (McLeod et al., 2016). Proper diagnosis of PAOG begins with evaluation the patient’s self-reports of vision, with common complaints being difficulties with night driving and reading (McLeod et al. 2016). After evaluating a patient’s history visual acuity should be measured, the pupils should be dilated, and the anterior chamber of the eye should be examined using a slit-lamp (McLeod et al. 2016). Intraocular pressure in a major consideration during diagnosis, and the gold standard is by utilizing the Goldmann tonometry method. An IOP exceeding 22 mmHg is considered abnormal, but some studies have found that anywhere from 25-50% of people diagnosed with glaucoma had IOP readings less than 22 mmHg (Weinreb et al., 2014). Although the best way to visualize the effects of glaucoma is to perform visual field exams and optic nerve imaging, it is estimated that 30-50% of retinal ganglion cell death occurs before abnormalities can be detected during these tests.

In addition to assessing risk factors and elevated IOP levels, primary angle-closure glaucoma has traditionally been diagnosed using goniscopic imaging, but newly developed anterior segment optical coherence tomography imagine (AS-OCT) has proved to be more reliable (Weinreb et al., 2104). Optical coherence tomographic imaging allows for precise measurement of the angle of aqueous outflow, making it a vital diagnostic tool for PACG in ophthalmology.
II. Therapeutic Approaches in Managing Glaucoma

Following the diagnosis of glaucoma, the main therapeutic goal for the patient is to keep their IOP within the normal range of 10-21 mmHg and to prevent further damage to the optic nerve (Weinreb et al., 2014). Several large scale randomized controlled trials have shown that controlling IOP is the only effective method to control glaucoma progression (Weinreb et al., 2014). Research into the effectiveness of surgical versus pharmaceutical therapies for glaucoma have shown that no significant benefit exists for surgical interventions (Burr, Azuara-Blance, Avenell, & Tuulonen, 2012). There are a variety of different therapeutic approaches to treat glaucoma, each having their own benefits and adverse effects.

II. 1 Prostaglandin Analogues – Pharmaceutical Therapies for Glaucoma

A study published in 1996 provided evidence that latanoprost, a newly approved prostaglandin analogue, showed significant reduction in IOP when compared to timolol, a β-adrenergic blocker (Camras, 1996). This study, along with evidence provided by additional research, quickly made prostaglandin analogues (PGAs) the first-line therapeutics for the treatment of glaucoma. Currently there are five FDA-approved PGAs on the market: latanoprost, travoprost, tafluprost, unoprostone, and bimatoprost (Weinreb et al., 2014). PGAs exert their mechanism of action by increasing the outflow of aqueous humor through the uveoscleral pathway and have been proven to lower IOP levels by up to 50% (Drug Therapy of Glaucoma, 2015). If target IOP levels are not being attained with a PGA alone, it is possible to add other medications to a patient’s therapeutic regimen. As with any pharmaceutical therapy, adverse effects have been reported, but in comparison to other glaucoma treatments PGAs have less serious systemic side effects. Some of the common local adverse effects include conjunctival hyperemia, lengthening and darkening of the eyelashes, brown discoloration of the iris, uveitis,
and macular edema (Weinreb et al., 2014). Interestingly, one of these effects, lengthening of the eyelashes, gave rise to the first FDA-approved drug for inadequate eyelashes, Latisse. Studies into iris pigmentation changes caused by PGAs have shown that up to 70% of individuals may experience this adverse effect, although many changes were subtle (Figure 4). PGAs have few, if any, systemic side effects, although some studies suggest that their use may lead to the development of headaches (Weinreb et al., 2014).

Iris hyperpigmentation due to PGA use versus normal eye (Teus, Arranz-Marquez, & Lucea-Suescun, 2002)

**Figure 4**
II. 2 β-Adrenergic Blockers – Pharmaceutical Therapies for Glaucoma

Prior to the introduction of prostaglandin analogues, β-Adrenergic blockers, commonly referred to as beta blockers, were the first-line therapeutics for the treatment of glaucoma (Glaucoma, 2015). Currently there are five FDA-approved beta blockers for the management of glaucoma: timolol, levobunolol, carteolol, metipranolol, and betaxolol (Weinreb et al., 2014). Beta blockers exert their therapeutic effect by decreasing the amount of aqueous humor produced by the ciliary body in the eye (Weinreb et al., 2014). Beta blockers typically decrease IOP levels by 25%, only slightly less than PGAs, but carry with them more serious systemic side effects and their use is contraindicated in some patients (Glaucoma, 2015). PGAs exert their action for a full 24 hours, while beta blockers typically only last 12 hours. In addition, beta blockers must be administered by the patient in the morning, a factor thought to decrease patient compliance (Glaucoma, 2015).

The local adverse effects of beta blockers are minor and include irritation and dry eyes. Beta blockers are contraindicated in patients with obstructive respiratory illnesses, and patients with even mild cases of COPD can experience serious exacerbations after using these therapeutics (Healey & Thomas, 2010). Although beta blockers no longer serve as the first-line treatment for glaucoma, their use in combination with other classes of glaucoma drugs, most commonly PGAs, have been shown to further reduce IOP levels (Healey & Thomas, 2010). Currently there are three combinations of PGAs and beta blockers available to treat glaucoma: bimatoprost/timolol, latanoprost/timolol, and travoprost/timolol (Healey & Thomas, 2010).

II. 3 α-Adrenergic Agonists – Pharmaceutical Therapies for Glaucoma

There are currently two FDA-approved α-adrenergic agonists on the market, brimonidine and apraclonidine (Weinreb et al., 2014). These therapeutics, commonly referred to as alpha
agonists, exert their action both by reducing aqueous humor production and increasing aqueous humor outflow (Weinreb et al., 2014). Alpha agonists prove effective at lowering IOP levels, but patient adherence is a concern because the medication must be applied topically three times daily to achieve maximum results (Healey & Thomas, 2010). Alpha agonists are contraindicated in patients taking monoamine oxidase inhibitors and may produce unpleasant side effects including drowsiness (Healey & Thomas, 2010). Alpha agonists serve as a good alternative for patients experiencing bothersome or allergic reactions from other classes of glaucoma drugs, and they can be used in combination with other therapeutics to further lower IOP (Weinreb et al., 2014). There are currently two FDA-approved combination drugs containing alpha agonists: Combigan (brimonidine/timolol) and Simbrinza (brimonidine/brinzolamide).

**II. 4 Carbonic Anhydrase Inhibitors – Pharmaceutical Therapies for Glaucoma**

Carbonic anhydrase inhibitors (CAIs) are unique because they can be delivered both topically and orally, making them a good option for patients not responding well to topically applied eye drops (Healey & Thomas, 2010). There are two topical CAIs, dorzolamide and brinzolamide, and an oral CAI, acetazolamide. CAIs, like beta blockers, exert their effects by decreasing the amount of aqueous humor produced (Weinreb et al., 2014). Topical administration of CAIs results in minimal adverse effects, but oral delivery can result in paresthenia, nausea, diarrhea, loss of appetite, and renal stones (Weinreb et al., 2014). This class of drugs are most often utilized in combination formulations. The two combination drugs that include CAIs are Cosopt (dorzolamide/timolol) and Simbrinza (brinzolamide/brimonidine). Simbrinza does not contain a beta blocker, making it an ideal addition for a patient with an obstructive respiratory disease that does not respond well to a PGA alone (Glaucoma, 2015).
II. 5 Cholinergic Agonists – Pharmaceutical Therapies for Glaucoma

Although cholinergic agonists are the least prescribed class of glaucoma medications prescribed today, they became the first viable treatment option in 1875 with the discovery of pilocarpine (Weinreb et al., 2014). The two cholinergic agonists approved for the management of glaucoma, pilocarpine and carbachol, work by increasing aqueous humor outflow by contracting the ciliary muscles, resulting in decreased resistance in the trabecular meshwork drainage pathway (Weinreb et al., 2014).

II. 6 Laser Trabeculoplasty – Surgical Approaches to Glaucoma Management

The laser trabeculoplasty procedure was first performed in 1974 by Worthen and Whickham, and became a common surgical option for POAG after the publication of Wise and Witter’s pilot study in 1979 (Sayyad & Helal, 2009). This procedure has an excellent safety record and is indicated for patients who cannot tolerate topically applied medications or patients who cannot undergo more invasive forms of glaucoma surgery, like a trabeculotomy (Dietlein, Hermann, & Jordan, 2009). This procedure utilizes the energy of a laser to lower the resistance in the trabecular meshwork and induce an increase in aqueous humor outflow (Healey & Thomas, 2010). Research has shown that this procedure results in a 20% reduction in IOP levels (Dietlein et al., 2009).

II. 7 Trabeculotomy – Surgical Approaches to Glaucoma Management

A trabeculotomy is the most commonly performed incisional surgical procedure for the treatment of glaucoma, and is indicated in patients whose glaucoma has advanced despite the use of several topical medications or for patients who are non-compliant to medication regimens (Dietlein et al., 2009). A large scale randomized controlled trial showed that IOP levels were
significantly lower in surgically treated patients when compared to those using topically administered glaucoma medications (Musch, Gillespie, Palmberg, Spaeth, Niziol, & Lichter, 2014). The downside of a trabeculotomy is the possibility of excessive scarring, which results in failure of the procedure, but antifibrotic agents including mitomycin-C and 5-fluorouracil are frequently applied to the Tenon’s capsule during the procedure to combat this (Healey & Thomas, 2010).

II. 8 Other Surgical Options

Silicon tube implants are an alternative to a trabeculectomy and allow the aqueous humor to drain into an external reservoir. These drainage devices are not as effective at lowering IOP, but carry fewer risks than incisional procedures (Weinreb et al., 2014). Congenital glaucoma, characterized by an abnormal iridocorneal angle, is most effectively treated by a combination of a trabeculotomy and a goniotomy, where the trabecular meshwork is cut from both the inside and outside of the eye (Healey & Thomas, 2010). The oldest surgical method for glaucoma treatment, the iridectomy, is indicated for patients who develop narrow-angle glaucoma as a result of a pupillary block (Dietlein et al., 2009).

III. The Challenges of Managing Glaucoma

Proper management of glaucoma proves difficult for a variety of reasons, ranging from issues with patient adherence to the difficulties of designing an effective ocular drug delivery system. Compliance rates vary widely depending on the study, but the disease’s slow progression and the tedious medication regimen required to adequately manage the symptoms have convinced practitioners that there is a need for improvement (Hoevenaars, Schouten, van den Borne, Beckers, & Webers, 2008).
The human eye has several physiologic protective barriers, and has always posed a challenge to pharmaceutical scientists designing ocular drug delivery systems. Topical administration is the most common delivery route of glaucoma medications, but pre-corneal elimination and high tear fluid turnover reduce the bioavailability, and it is estimated that only 1-5% of the active drug reach the target site of action (Achouri, Alhanout, Piccerelle, & Andrieu, 2013). The low bioavailability of topical glaucoma drugs has led researchers to explore other ocular drug delivery systems, such as the solid lipid nanoparticle formulation designed in our project.

The low efficacy of topical glaucoma medications means that patients must frequently administer their eye drops to achieve desirable results, a factor that contributes to low adherence rates (Guadana, Jwala, Boddu, & Mitra, 2008). Studies have attempted to identify factors influencing drug persistency, which is defined as continuous therapy without more than a 90-day gap in treatment (Owen, Carey, de Wilde, Whincup, Wormald, & Cook, 2009). One study found no significant variability when analyzing gender and social factors, but did find that approximately one-third of all patients would fail to properly follow their medication regimens within the first year of treatment (Owen et al., 2009). Another study followed 10,260 subjects over a 12-month period and found that more than half stopped and restarted their medications, and only 10% followed their prescribed medication regimens continuously for the entire time period (Enhancing Adherence and Persistency in Glaucoma, 2008).

Additional studies have identified strong correlations between adherence rates and psychological factors, including motivation for treatment, intention to take medication, and the number of doses required in a given day (Cook, Schmiege, Mansberger, Kammer, Fitzgerald, & Kahook, 2015). Research into the psychometric properties determining medication adherence
found that white race, older age, and married marital status contributed to higher rates of persistency (Barker, Cook, Schmiege, Kahook, Kammer, & Mansberger, 2015).

Low bioavailability and adherence rates are not the only issues in glaucoma management. All current therapeutic options, whether pharmaceutical or surgical, exert their effect by lowering the intraocular pressure of the eye. However, vision impairments associated with glaucoma occur as a result of cellular and molecular changes that are detrimental to the retinal ganglion cells, nerve fiber layer, superior colliculi, lateral geniculate nuclei, and the visual cortex (Liu & Pang, 2013). These damages place glaucoma into the category of neurodegenerative disorders, and the need for a drug offering neuroprotection cannot be ignored.

In response to the need for a neuroprotective treatment option, memantine, currently FDA-approved for the treatment of Alzheimer’s Disease, entered clinical trials as a potential therapy providing neuroprotection in glaucoma patients. Unfortunately, the drug failed in phase III trials, deterring optimism of finding a viable neuroprotective therapy for glaucoma (Baltmr, Duggan, Nizari, Salt, & Cordeiro, 2010). However, recent advances in nanotechnology have provided researchers with an innovative way to design a drug delivery system capable of providing neuroprotection. Nanotechnology provides a way to deliver drugs to the anterior segment of the eye, where the detrimental neurologic damage takes place (Cholkar, Patel, Vadlapudi, & Mitra, 2013). Research has indicated that nanotechnology can provide a delivery system capable of prompting retinal ganglion cell regeneration, thus providing neurological protection (Zarbin, Montemagno, Leary, & Ritch, 2013).
IV. Cannabinoids and Their Potential in Glaucoma Management

In 1971, a study conducted by Hepler and Frank reported a 25-30% reduction in IOP levels after smoking marijuana, sparking interest in utilizing cannabinoids in the treatment of glaucoma (Hepler & Frank, 1971). Documentation of the medicinal capabilities of marijuana date back to 2737 BC, when the Chinese emperor Shen Nung wrote about its many medicinal purposes in a traditional Chinese medicine book (Tomida, Pertwee, & Azuara-Blanco, 2004). Marijuana was believed to treat a variety of ailments, including stomach pain, psychosis, chronic cough, and epilepsy, and was frequently prescribed by physicians until its removal from the United States pharmacopeia in 1942 (Tomida et al., 2004). Medicinal marijuana is currently legal in 23 states, carrying indications for treating multiple sclerosis symptoms, neuropathic pain, Tourette syndrome, insomnia, chemotherapy-induced nausea and vomiting, and cachexia associated with HIV infection (Metts, Wright, Sundaram, & Hashemi, 2016).

The cannabis plant itself contains more than 480 chemical constituents, but Δ⁹-tetrahydrocannabinol has been identified as the active ingredient. The structure of Δ⁹-THC, seen in Figure 5, was first determined in the 1960s by Gaoni and Mechoulam.

![Structure of Δ⁹-tetrahydrocannabinol](Tomida et al., 2004)

**Figure 5**
Marijuana, along with other cannabinoids, have been found to exert their effects through action at cannabinoid receptors, which include two subtypes: CB\textsubscript{1} and CB\textsubscript{2} (Pertwee, 2006). CB\textsubscript{1} receptors are predominantly found at the nerve terminals of central and peripheral cells, although some are expressed in non-neuronal cells in the immune, reproductive, and endocrine systems. CB\textsubscript{2} receptors are expressed primarily by immune cells (Tomida et al., 2004). In 1996, CB\textsubscript{1} receptors were discovered in the eye, and further research found them to be localized in the anterior chamber and on the retina (Straiker, Maguire, Mackie, Lindsey, 1999). These discoveries helped to explain the findings of Hepler and Frank’s study in 1971, and provided researchers with hope that these receptors could be selectively targeted as a treatment option for glaucoma (Straiker et al., 1999).

IV. 1 The Classification of Cannabinoids

Although $\Delta^9$-tetrahydrocannabinol is the most studied cannabinoid, numerous other cannabinoids, both synthetic and naturally occurring, exist and have been used in exploring the ability of cannabinoids to treat glaucoma (Jarvinen, Pate, & Laine, 2002). Cannabinoids are generally classified into four different categories based on their chemical structures.

Classical cannabinoids retain the natural cannabinoid ring structures with their oxygen atoms, as seen in Figure 6. Naturally occurring classical cannabinoids include $\Delta^9$-THC, cannabidiol, and cannabinol. Synthetic classical cannabinoids include synhexyl and nabilone. As a result of Hepler and Frank’s study, which utilized $\Delta^9$-THC, many studies of cannabinoids in humans and animals have been carried out using classical cannabinoids (Jarvinen et al., 2002).
The second category of cannabinoids, the non-classical cannabinoids, are bicylic analogs of $\Delta^9$-THC that lack a pyran “B” ring. One member of this group, CP-55, 940, is commonly used in cannabinoid receptor-binding studies (Jarvinen et al., 2002). In regards to glaucoma, CP-55, 940 was found to lower IOP in both normotensive rabbits and rabbits with elevated IOP levels (Sugrue et al., 1996).

Aminoalkylindoles form the third category of cannabinoids. The prototype molecule of this category is WIN 55, 212, which has been utilized widely in studies demonstrating the IOP lowering effects and is the molecule we used in our project. Although it targets the same receptors, WIN 55, 212 bears little resemblance to the chemical structure of classical cannabinoids, as seen in Figure 7.
The final category, the endocannabinoids, are molecules produced within the body that naturally activate cannabinoid receptors. Numerous endocannabinoids have been identified, including arachidonylethanolamid, bimatoprost, and HU-211 (Jarvinen et al., 2002).

IV. 2 The Neuroprotective Capabilities of Cannabinoids

The ability of cannabinoids to lower IOP triggered initial research into their use as glaucoma treatments, and recent discovery that cannabinoids may offer neuroprotection has fueled researchers to conduct further studies (Yazulla, 2008). Research has found that cannabinoids may offer protection against traumatic, ischemic, inflammatory, and neurotoxic damage in the central nervous system (Yazulla, 2008). The vision loss that occurs in glaucoma results from ischemia of the retinal ganglion cells. Ischemia triggers excessive aspartate and glutamate release, which subsequently activates NMDA receptors, the first step in the apoptotic pathway that leads to cell death (Yazulla, 2008). A study done by Shen and Thayer showed that administration of a synthetic cannabinoid in rats inhibited hippocampal neurons from releasing glutamate (Shen & Thayer, 1999). Research conducted on the retinas of cattle concluded that cannabinoids exerted inhibitory action on both potassium and ischemia induced release of
aspartate through the action of CB₁ receptors (Opere, Zheng, Zhao, Lee, Kulkarni, & Ohia, 2006). These findings have lead researchers to believe that in addition to lowering IOP, cannabinoids also offer something that no other glaucoma drug can: neuroprotection.

V. Designing a Viable Ocular Drug Delivery System for Cannabinoids

Following the publication of Hepler and Frank’s study discussing the effectiveness of marijuana cigarettes in lowering IOP, several additional studies were conducted in the 1970s and 1980s utilizing different delivery methods. By the end of the 1980s, publications had shown the ability of Δ⁹-THC to lower IOP when delivered intravenously, orally, or through inhalation (Porcella, Maxia, Gessa, & Pani, 2001). The systemic effects resulting from these delivery methods discouraged many from thinking that Δ⁹-THC could serve as a viable glaucoma treatment option, but the discovery of localized cannabinoid receptors in the eye left researchers hopeful for a targeted drug delivery system (Straiker et al., 1999).

Following the discovery of cannabinoid receptors in the eye, researchers became interested in the local effects of CB₁ agonists. Researchers found that activating CB₁ receptors in the ciliary muscle induced its contraction, subsequently widening the intercellular spaces of the trabecular meshwork and increasing the outflow of aqueous humor, a process that results in lower IOP levels (Llobet, Gasull, & Gual, 2003). One of the first published studies on the effectiveness of topical administration utilized 0.05% Δ⁹-THC in mineral oil and reported a 4.8 mm drop in IOP without a subsequent drop in systemic blood pressure, indicating that Δ⁹-THC was acting on local receptors in the eye (Merritt, Olsen, Armstrong, & McKinnon, 1981). Δ⁹-THC is a highly lipophilic molecule, which hinders its permeability across the cornea when delivered topically, explaining why early studies of topical administration did not show significant drops in IOP (Hingorani, Gul, ElSohly, Repka, & Majumdar, 2011). Significant increases in the concentration of Δ⁹-THC in
topical formulations successfully reduced IOP, but resulted in systemic effects, highlighting the need for a formulation that would successfully deliver the molecule through the cornea without dramatically increasing the concentration (Hingorani et al., 2011).

Successfully formulating topical $\Delta^9$-THC drug delivery systems is challenging for a variety of reasons, most notably because of its poor solubility in aqueous media and its susceptibility to oxidation, hydrolysis, thermal, and photolytic degradation. To enhance the delivery of $\Delta^9$-THC, researchers have focused on formulating complex prodrugs. One method is to modify the solution that the active drug ingredient is in. Cyclodextrins are molecules with hydrophilic surfaces and hydrophobic cores, and are widely used in formulations of poorly soluble drugs to enhance their solubility, bioavailability, and stability profiles (Gaurav, Tiwari, & Rai, 2010). 2-Hydroxypropyl-β-cyclodextrin (HPβCD) is one of the most commonly used cyclodextrins, and has been shown to be safe and well tolerated when topically administered to the eye (Loftsson & Stefansson, 1997). $\Delta^9$-THC in 2.5% HPβCD solutions with Dulbecco’s phosphate-buffered saline (DPBS) had a permeability 300 times that of $\Delta^9$-THC in light mineral oil-based formulations, which had been the most common drug delivery vehicle used in early studies (Hingorani et al., 2011). In the same study, prodrugs of $\Delta^9$-THC were synthesized to form hemisuccinate (THC-HS) and hemiglutarate (THC-HG) esters, seen in Figure 8. Researchers found that the ester prodrugs, in combination with a buffered solution and prodrug-ion-pair complexes formed using L-arginine or tromethamine, resulted in significant improvements of permeability (Hingorani et al., 2011). Although in vitro results from this study showed that the use of an ion-pair complex of THC-HG could be an effective strategy for topical delivery of THC, our research focused on other prodrug formulations to further enhance the ocular delivery and bioavailability.
Solid lipid nanoparticles (SLNs) were first patented by Muller and Lucks in 1996, and are a type of nanotechnology delivery system that is capable of accommodating drugs with poor physiochemical properties by increasing their solubility (Seyfoddin, Shaw, & Al-Kassas, 2010). It has been documented that SLNs are capable of enhancing the corneal permeability of drugs, leading to an increase in the drug’s uptake by the ocular tissues and bioavailability (Seyfoddin & Al-Kassas, 2013). The aim of this study was to create an SLN formulation that would enhance the corneal permeability and bioavailability of the synthetic cannabinoid WIN 55, 212. First, a formulation with favorable physiochemical characteristics was made, then the formulation was subjected to both in vitro and in vivo studies to measure the viability of the drug delivery system as a treatment option for the management of glaucoma.
Materials and Methods

I. Materials

In the formulation of the SLN, the aqueous phase contained Poloxamer 188, Tween 80, and glycerin were used. The lipid phase contained WIN 55, 212 and Compritol® 888 ATO (glyceryl behenate). The phases were combined to form a premix and then subjected to emulsification using the T 25 digital Ultra-Turrax (IKA® Works, Inc.). The control formulation was prepared using WIN 55, 212 and Tocrisolve™. After the formulation had been made, its particle size and poly dispersity index were determined by photon correlation spectroscopy using Zetasizer Nano ZS Zen3600 (Malvern Instruments, Inc.). The entrapment efficiency and assay were determined after dilution with 190-proof alcohol using an HPLC system.

To test the \textit{in vitro} transcorneal permeability, a Franz diffusion apparatus (PermeGear, Inc., Hellertown, PA) was used with Spectra/Por® membranes (molecular weight: 10,000 Daltons). The results of the \textit{in vitro} studies were analyzed using the HPLC-UV method utilizing a Phenomenex® Luna® C18(2) HPLC column and acetonitrile and trifluoroacetic acid for the mobile phase. For the \textit{in vivo} studies, glaucoma was induced in male albino New Zealand rabbits by injecting alpha-chymotrypsin intravitreally. After administering the SLNs, IOP levels were measured using a TONO-PEN® vet tonometer. The rabbits were subsequently euthanized with a dose of phenobarbital, and their eyes were enucleated and sent to Excalibur Pathology Inc. for corneal histology testing to be done.
II. 1 Experimental Methods - Designing a Solid Lipid Nanoparticle Formulation of WIN 55, 212 with Appropriate Physiochemical Properties

Formulating WIN-SLN$s$ utilizes the hot homogenization method and begins by preparing the aqueous phase. The aqueous phase contains two surfactants: 0.25% w/v Poloxamer 188 and 0.75% w/v Tween 80. In addition, the aqueous phase contains 2.25% w/v Glycerin. To prepare the lipid phase, 4% w/v of the lipid excipient Compritol 888 ATO was melted followed by the addition of 0.4% w/v of WIN 55, 212. During the preparation of the lipid phase, the aqueous phase was heated and added to the melted lipid phase in a beaker being magnetically stirred at a speed of 600 rpm. The two phases were stirred for a total of two minutes to form the premix. The premix was then emulsified using the T 25 digital Ultra-Turrax (IKA® Works, Inc.) dispersion homogenizer. The premix was subjected to emulsification for 5 minutes at a speed of 16,000 rpm, forming the heated pre-emulsion. The pre-emulsion was then subjected to high-pressure homogenization using the EmulsiFlex-C5 (Avestin) homogenization using a pulse of 15 seconds and amplitude of 80% for a total of 5 minutes. The temperature throughout the entire homogenization process was held constant at $80^\circ C \pm 2^\circ C$. After the 5-minute homogenization period was complete, the emulsion was allowed to slowly cool to room temperature to form the WIN-SLN formulation. After the preparation of the SLN complete, several physiochemical characteristics were assessed to ensure the efficacy of the formulation. The characteristics measured were particle size, polydispersity index, zeta potential, entrapment efficiency, and assay.

To assess the average particle size of the formulation, photon correlation spectroscopy technology was employed by using the Zetasizer Nano ZS Zen3600 (Malvern Instruments, Inc.). Backscatter detection at $25^\circ C$ and $173^\circ C$ was utilized in disposable folded capillary clear cells and the measurements were obtained using a He-Ne laser of 633 nm. This same technology was also
employed to determine the polydispersity index (PDI) of the SLN. The PDI is indicative of the degree of “non-uniformity” of a sample. To assure stability, uniformity of SLNs is desired. Zeta-potential, a measurement of the magnitude of the electrostatic repulsion or attraction between particles, was assessed using the same instrument and was carried out at a temperature of 25°C in folded capillary cells. Zeta-potential measurements are frequently taken when assessing the stability of nanoparticles with any measurement between -10 and +10 mV indicating neutrality and any measurement greater than +30 mV or less than -30 mV indicating strong cationic or anionic properties. Cellular membranes are typically anionic, and a strong cationic zeta potential may pose a threat to cell membrane integrity, making this property an important consideration in formulations.

Entrapment efficiency of the SLN formulation was the next parameter analyzed. The entrapment efficiency is the percentage of drug encapsulated within the solid lipid nanoparticle core. The percentage of free drug in solution should be kept to a minimum to assure that the formulation is stable and that bioavailability will be high. To measure the entrapment efficiency (EE) of the SLN, the lipid was precipitated using 190-proof alcohol, and the drug content in the supernatant after 20 minutes of centrifugation at 13,000 rpm was measured using the HPLC-UV method. To measure the concentration of free drug in the aqueous phase of the WIN-SLN formulation, an ultrafiltration technique was employed using a 100-kDa centrifugal filter device composed of a regenerated cellulose membrane (Amicon Ultra). An aliquot containing 500 mL of the undiluted WIN-SLN was added to the sample reservoir and centrifuged for 10 minutes at 5,000 rpm. The filtrate obtained was further diluted with 190-proof alcohol, and then analyzed for drug content using the HPLC-UV method. The entrapment efficiency was then estimated based on an equation that considers the total drug content and the amount of free drug in the aqueous phase.
II. 2 Testing the Solid Lipid Nanoparticle Formulation in vitro by Conducting Permeability and Release Studies

After formulating a WIN-SLN with desirable physiochemical characteristics, the drug’s efficacy was determined by conducting in vitro permeability and drug release studies. Before conducting these studies, a control formulation was prepared. To prepare the control, an accurately weighed amount of WIN 55, 212 was combined with Tocrisolve™ to give a 0.4% w/v WIN 55, 212 control formulation. The combined mixture of the drug and Tocrisolve™ emulsion was subjected to sonication for 10 minutes, and the drug content was evaluated using the HPLC-UV method. When analyzing the control formulation, a Phenomenex® Luna® C18(2) column was used. The mobile phase contained a 50:50 solution of acetonitrile/water + 0.5% trifluoroacetic acid. The flow rate was 1 mL/min, the injection volume was 25 μL, and the UV wavelength was set at 315 nm.

After successfully preparing the Tocrisolve™ control formulation, the in vitro release and permeability studies were conducted using a Franz diffusion apparatus (PermeGear, Inc., Hellertown, PA). The Franz diffusion apparatus is comprised of an entry for the donor compound, a receptor chamber containing a stir bar, and a sampling port as seen in Figure 9.
The two *in vitro* studies differed only in which membrane was used – the drug release study used the synthetic Spectra/Por® membrane and the permeability studies used corneas excised from whole New Zealand rabbit eye globes. Both *in vitro* studies began by adding 100 μL of the 0.4% w/v WIN-SLN to the donor portal of one apparatus and 100 μL of the 0.4% w/v Tocrisolve™ control formulation was added to the entry portal of the other apparatus. The receiving chambers of both contained 5 mL of 5% w/v of Randomly Methylated β-Cyclodextrin (RMβCD) in isotonic phosphate buffer saline (IPBS) having a pH of 7.4. Both studies took place over a duration of 3 hours, and samples were collected at 30 minute intervals. After each sample of 0.6 mL had been taken from the sampling port, 0.6 mL of the IPBS buffer were subsequently added to keep the total volume in the receiving chamber constant. After both studies were complete, the collected samples were then analyzed using the HPLC-UV method.
Analysis of the samples was done under the same conditions as the control formulation using a Phenomenex® Luna® C18(2) column and a mobile phase of 50:50 acetonitrile/water + 0.5% trifluoroacetic acid with a flow rate of 1 mL/min and an injection volume of 25 μL. As with the analyzation of the control formulation, the UV wavelength was set at 315 nm.

The in vitro permeability studies were then carried out to further assess the bioavailability of the drug. Whole rabbit eye globes were obtained and the corneas were enucleated to be used as the membrane in the Franz diffusion apparatus. As in the drug release study, 100 μL of both formulations was added to the entry portal and the receiving chambers contained 5 mL of RMBβCD in IPBS having a pH of 7.4. This study also had a duration of 3 hours, with samples being collected every 30 minutes. The samples for this study were assessed on three parameters: rate, flux, and permeability.

III. 3 Testing the Pharmacodynamic Response in vivo

After formulating a WIN 55, 212 SLN with appropriate physiochemical characteristics and carrying out the initial in vitro studies, the in vivo studies were initiated. The animal model chosen for the in vivo studies were male albino New Zealand rabbits, primarily because of the size of their eye globes and docile nature. Male New Zealand albino rabbits (2-2.5 kg) procured from Harlan Laboratories® (Indianapolis, IN) were used in all the studies. All 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.
The in vivo experiments were carried out in two separate phases. The first phase analyzed the effect of the Tocrisolve™ control formulation on IOP levels of two of the rabbits involved in the study. Prior to testing the formulation, glaucoma was induced in the right eye of each rabbit by administering an intravitreal injection of 50 μL of 20 mg/mL of α-chymotrypsin. The enzyme α-chymotrypsin results in increased IOP levels, the phenomenon that ultimately leads to the pathophysiology of glaucoma. After the intravitreal injections were administered, the study population was closely monitored for any sign of infection or inflammation the IOP levels were allowed to stabilize over a two-week time period.

After the two-week stabilization period, the basal IOP readings of each rabbit were taken prior to the administration of the control formulation using the TONO-PEN®. After the basal IOP values were established, the WIN 55, 212 Tocrisolve™ formulation was topically administered to the right eye of both rabbits involved in the study. A total of 50 μL of 0.4% w/v control formulation was administered to each eye. Each dose contained 0.2 mg of WIN 55, 212. The study took place over a 120-minute duration, and IOP readings were taken at 30-minute time intervals to determine when the maximum IOP reductions took place.

After the completion of the first phase of the in vivo study, the remaining three rabbits of the study population were given instillations of the WIN-SLN formulations. Glaucoma was induced in the same fashion as in the first phase, by administering an intravitreal injection of 50 μL of 20 mg/mL of α-chymotrypsin two the right eye, followed by a two-week time period for stabilization of IOP levels. After the IOP levels had stabilized, basal IOP levels were recorded before administering the SLN formulations.

After the baseline IOP readings were recorded, 50 μL of the 0.4% w/v WIN-SLN formulation was topically administered to the right eyes of the three rabbits involved in the
study. Each instillation contained a 0.2 mg dose of WIN 55, 212. The duration of the study was dependent on the IOP readings taken at each 30-minute time interval, and the study was terminated when the IOP levels of the glaucomatous eyes had returned to 90% of the baseline IOP reading. The study had a duration of 180 minutes and IOP readings were recorded every 30 minutes.
Results and Discussion:

Prior to initiation of the *in vitro* and *in vivo* testing, a solid lipid nanoparticle formulation was prepared with favorable physiochemical properties. Several solid lipid nanoparticle formulations were prepared, but the 0.4% w/v formulation had characteristics that most aligned with the goals of the remaining *in vitro* and *in vivo* testing of the study. The results of the physiochemical property testing are shown below in Table 1. A favorable particle size for SLN formulations ranges from 200-500 nm. A particle size below 200 nm carries with it the risk of being washed away in the blood, and a particle size exceeding 500 nm will not successfully permeate the ocular tissues. Poly dispersity index is used as a measure of the uniformity of the particles in the formulation. A range of 0.2-0.4 is acceptable for SLN formulations. Zeta potential represents a way to estimate the formulation’s stability, and any value exceeding 20 mV is considered stable. Although an entrapment efficiency exceeding 80% is desired, our formulation’s entrapment efficiency was deemed acceptable because it had optimal physiochemical characteristics otherwise.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>0.4% w/v SLN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size (nm)</td>
<td>372 ± 21 nm</td>
</tr>
<tr>
<td>Poly dispersity index (PDI)</td>
<td>0.24 ± 0.04</td>
</tr>
<tr>
<td>Zeta potential (mV)</td>
<td>21.0 mV</td>
</tr>
<tr>
<td>Entrapment efficiency</td>
<td>72.6%</td>
</tr>
<tr>
<td>Assay</td>
<td>100-106%</td>
</tr>
</tbody>
</table>

Results of WIN 55, 212 SLN physiochemical testing

Table 1
After the SLN formulation’s physiochemical characteristics had been analyzed and deemed favorable, the *in vitro* drug release and permeability studies were carried out. The release study was conducted over three hours, and the samples in the donor chamber of the diffusion apparatus were analyzed to determine the percentage of drug crossing the membrane. The results, seen in Table 2, show that the SLN allowed a higher concentration of WIN 55, 212 to cross the membrane when compared to the control.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% of Total WIN 55, 212 Crossing the Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4% w/v Tocrisolve™ Control Formulation</td>
<td>21.3 ± 1.7%</td>
</tr>
<tr>
<td>0.4% w/v WIN-SLN Formulation</td>
<td>29.1 ± 3.1%</td>
</tr>
</tbody>
</table>

Results of *in vitro* drug release studies

Table 2

The *in vitro* permeability studies were then completed to further estimate the WIN SLNs bioavailability *in vivo*. Samples were analyzed using the HPLC-UV method on three different parameters: rate (μg/min), flux (μg/min/cm²), and permeability x 10⁶ (cm/sec). The rate simply quantifies the amount of WIN 55, 212, in μg, that was able to pass through the rabbit cornea membrane each minute over the duration of the study. The rate of the Tocrisolve™ control formulation was found to be 0.063 μg/min, while the WIN-SLN had a membrane crossing rate of 0.13 μg/min. The next parameter analyzed was flux. The WIN-SLN was analyzed to have a flux of 0.2 μg/min/cm², twice that of the 0.1 flux value of the Tocrisolve™ control formulation. The final parameter analyzed was the permeability. The WIN-SLN had a permeability of 0.84 x 10⁶.
cm/sec, while the Tocrisolve™ emulsion had a permeability of $0.41 \times 10^6$ cm/sec. The results, seen in Figure 10, show the SLN as being twice as permeable when compared to the control formulation based on rate, flux, and permeability.

![Transcorneal permeability of WIN 55 with different formulations](image)

**Results of in vitro permeability study**

**Figure 10**

The 0.4% w/v WIN SLNs continued to show favorable experimental results, leading to the initiation of the *in vivo* testing of the formulation in an animal model. The *in vivo* testing was the final component to this study. After the rabbits were induced with glaucoma and their IOP levels stabilized, basal IOP levels were recorded using the TONO-PEN® prior to the administration of the control formulation. Each rabbit eye was subjected to a total of three readings, and the average of the three was recorded, as seen in Table 3.
After the basal IOP readings were recorded, both animals received instillations of the control formulation in the right eye. A total of 50 μL of 0.4% w/v control formulation was administered, with each dose containing 0.2 mg of WIN 55, 212. The study took place over a 120-minute duration, and IOP readings were taken at 30-minute time intervals. A total of three readings were taken of the right eye at each interval, and the average of the three was recorded, shown in Table 4.
<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Average IOP Reading (mmHg) of Rabbit Number: 87</th>
<th>Average IOP Reading (mmHg) of Rabbit Number: 91</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 minutes</td>
<td>12.7</td>
<td>13.7</td>
</tr>
<tr>
<td>60 minutes</td>
<td>15.0</td>
<td>17.7</td>
</tr>
<tr>
<td>90 minutes</td>
<td>17.7</td>
<td>20.0</td>
</tr>
<tr>
<td>120 minutes</td>
<td>22.3</td>
<td>24.0</td>
</tr>
</tbody>
</table>

Results of IOP readings after installation of control formulation

Table 4

After the study was complete, the IOP levels were recorded as a function of the percent of baseline IOP. This allows the determination of when the maximum IOP reduction was for each animal. Both animals saw a maximum IOP reduction at the 30-minute time interval. Rabbit 87 experienced a 37.6% maximum IOP reduction rabbit 91 experienced a 29.2% maximum IOP reduction, shown in Table 5.

<table>
<thead>
<tr>
<th>Rabbit Number</th>
<th>% Change from Baseline</th>
<th>30 mins</th>
<th>60 mins</th>
<th>90 mins</th>
<th>120 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>87 Right</td>
<td>100.0</td>
<td>62.4</td>
<td>73.9</td>
<td>87.0</td>
<td>110.0</td>
</tr>
<tr>
<td>87 Left</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>91 Right</td>
<td>100.0</td>
<td>70.8</td>
<td>91.5</td>
<td>103.6</td>
<td>124.4</td>
</tr>
<tr>
<td>91 Left</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

% Change in IOP from baseline with control formulation

Table 5

44
After the control formulations had been tested and the results were quantified, the three remaining rabbits in the study population were given instillations of the WIN SLN formulations. As with the control formulations, the basal IOP readings were taken after a two-week stabilization period. The results can be seen in Table 6.

<table>
<thead>
<tr>
<th>Rabbit No</th>
<th>Basal IOP Reading</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td>Right (G)</td>
<td>27</td>
<td>29</td>
<td>30</td>
<td>28.7</td>
</tr>
<tr>
<td></td>
<td>Left (N)</td>
<td>16</td>
<td>14</td>
<td>17</td>
<td>15.7</td>
</tr>
<tr>
<td>88</td>
<td>Right (G)</td>
<td>26</td>
<td>26</td>
<td>27</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td>Left (N)</td>
<td>16</td>
<td>15</td>
<td>17</td>
<td>16.0</td>
</tr>
<tr>
<td>89</td>
<td>Right (G)</td>
<td>35</td>
<td>36</td>
<td>33</td>
<td>34.7</td>
</tr>
<tr>
<td></td>
<td>Left (N)</td>
<td>19</td>
<td>20</td>
<td>19</td>
<td>19.3</td>
</tr>
</tbody>
</table>

Results of basal IOP readings of rabbits receiving WIN-SLN

Table 6

After the baseline IOP levels were recorded, each rabbit received 50 μL of the 0.4% w/v WIN-SLN formulation containing a total of 0.2 mg of WIN 55, 212. The study was terminated when the IOP levels of the glaucomatous eyes had reached 90% of the baseline IOP levels, which was 180 minutes. The IOP readings were taken every 30 minutes. The results can be seen in Table 7.
<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Average IOP Reading (mmHg) of Rabbit Number 85</th>
<th>Average IOP Reading (mmHg) of Rabbit Number 88</th>
<th>Average IOP Reading (mmHg) of Rabbit Number 89</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 minutes</td>
<td>22.0</td>
<td>21.7</td>
<td>26.3</td>
</tr>
<tr>
<td>60 minutes</td>
<td>21.0</td>
<td>16.0</td>
<td>26.0</td>
</tr>
<tr>
<td>90 minutes</td>
<td>21.0</td>
<td>17.3</td>
<td>22.7</td>
</tr>
<tr>
<td>120 minutes</td>
<td>20.3</td>
<td>18.7</td>
<td>29.3</td>
</tr>
<tr>
<td>150 minutes</td>
<td>27.7</td>
<td>24.3</td>
<td>29.7</td>
</tr>
<tr>
<td>180 minutes</td>
<td>29.0</td>
<td>26.7</td>
<td>33.0</td>
</tr>
</tbody>
</table>

Results of IOP readings after instillation of WIN-SLN

Table 7

The results of the WIN-SLN and control formulation studies were then compared to determine the effectiveness of the solid lipid nanoparticle. The duration of effect of the 0.4% w/v WIN-SLN formulation was found to be four times that of the control formulation, which can be seen in Figure 11.
Immediately following the conclusion of the WIN 55, 212 SLN study, the rabbits were euthanized by a lethal dose of pentobarbital, given through the marginal ear vein. After being euthanized, the right eye of each rabbit was enucleated and washed. The ocular tissues were then separated, weighed, and preserved at -80°C until they were shipped to Excalibur Pathology to assess them for any damages or changes when compared to a control. The corneal cross-sections were stained with hematoxylin-eosin. The top cross-sections were the controls that were exposed to phosphate-buffered saline. The bottom cross-sections were the corneas exposed to the SLN formulation. The results show that corneas exposed to the SLN did not have any significant changes when compared to the control. Figure 12 shows the results of the histology testing, with
(1) representing the control corneas that were exposed to a phosphate-buffered saline and (2) showing the corneas exposed to the SLN formulation.

Results of the histology testing

Figure 12
Conclusion

When used correctly, current marketed glaucoma treatments have proven effective in lowering intraocular pressure levels. However, the search continues for a drug that could successfully provide an increased duration of action and protection of the optic nerve. Topically administered eye drops for glaucoma are not able to permeate the posterior segment where the optic nerves are found, and are confined to the anterior segment where they exert their action by modifying aqueous humor production and outflow. Drug delivery systems utilizing nanotechnology have been speculated as a viable option to provide what other glaucoma drugs cannot, the ability to reach the posterior segment and to sustain a drug’s length of action. Solid lipid nanoparticles serve as a vehicle for drugs with poor physiochemical properties, and we explored the effectiveness of these formulation systems in our research.

After the preparation of the solid lipid nanoparticle is complete, we had to confirm that the formulation possessed favorable physiochemical characteristics that would allow it to be successful in subsequent in vitro and in vivo studies. Our WIN 55, 212 SLN showed favorable physiochemical characteristics, prompting us to move forward with the in vitro studies. When compared to the control formulation, the WIN 55, 212 SLN showed a two-fold increase in rate, flux, and transpermeability. These results confirm the solid lipid nanoparticle delivery systems ability to enhance the permeability of the drug, and is indicative that higher bioavailability will be found during in vivo studies.

When tested in vivo, the WIN 55, 212 SLN formulation sustained a drop in intraocular pressure that was four times as long as the control formulation. These results confirm the solid lipid nanoparticle’s ability to extend the duration of action of WIN 55, 212. The results of this project provide evidence that solid lipid nanoparticles are a drug delivery system that can provide
increased bioavailability and can lengthen the duration of action. This delivery system was able to effectively deliver the cannabinoid WIN 55, 212 into the ocular tissues, and it can be extrapolated that other topically administered ocular drugs may benefit from this delivery system or other forms of nanotechnology.

Overall, the WIN 55, 212 formulation exhibited desirable physiochemical characteristics and was successful in the *in vitro* and *in vivo* studies. Although the SLN formulation extended WIN 55 212’s ability to lower intraocular pressure, it did not outlast Pilocarpine eye drops, one of the many glaucoma treatments available on the market. Further studies are required to optimize the SLN formulation to increase its efficacy when compared to current FDA marketed glaucoma drugs.
BIBLIOGRAPHY


