DEVELOPMENT AND EVALUATION OF QUERCETIN NANOPARTICLES AND HOT MELT CAST FILMS FOR RETINAL NEUROPROTECTION

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.

Oxford
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In dedication to all
my friends, family, and loved ones who
have been great support throughout
this journey
ACKNOWLEDGEMENTS

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ABSTRACT
CHAU T. TRUONG: Development and Evaluation of Quercetin Nanoparticles and Hot Melt Cast Films for Retinal Neuroprotection (Under the direction of Soumyajit Majumdar)

Purpose:
The aim of this study was to prepare optimized nanoparticles and hot melt cast films of quercetin and to investigate the release and permeability profile across corneal membranes.

Method:
Quercetin dissolved in DMSO was mixed with glycerin and added to the lipid phase of either Glycerol monostearate or Miglyol 812® combined with Compritol ATO 888®. The aqueous phase of Tween 80®, Poloxomer 188®, and water was added, and the premix was homogenized, probe sonicated, and cooled to form the nanoparticles. Quercetin and polyethylene oxide N10 were mixed, and the blend was pressed and melted to prepare the films. Physicochemical profiles for nanoparticles were analyzed, and permeability across rabbit cornea was studied for both formulations using side by side diffusion apparatuses.

Results:
The SLNs and NLCs demonstrated particle sizes of 65.4 and 46.1 nm, polydispersity indices of 0.29 and 0.18, zeta potentials of -12.3 and -16.2 mV, assay of 78.4 and 86.6, and entrapment efficiencies of 90.9 and 93.4, respectively. Transcorneal flux of quercetin nanoparticles, film and control were 0.036, 0.144, and 0.026, respectively.

Conclusion:
These results demonstrate that all formulations can be successfully employed for delivery of quercetin into the eye through the topical route of administration, with films showing significantly better transcorneal permeability.
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<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
<td></td>
</tr>
<tr>
<td>DMF</td>
<td>dimethyl formamide</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
<td></td>
</tr>
<tr>
<td>GMS</td>
<td>glycerol monostearate</td>
<td></td>
</tr>
<tr>
<td>HME</td>
<td>hot melt extrusion</td>
<td></td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
<td></td>
</tr>
<tr>
<td>IPBS</td>
<td>isotonic phosphate buffer saline</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>low density lipoprotein</td>
<td></td>
</tr>
<tr>
<td>μg</td>
<td>microgram</td>
<td></td>
</tr>
<tr>
<td>μL</td>
<td>microliter</td>
<td></td>
</tr>
<tr>
<td>MWCO</td>
<td>molecular weight cut-off</td>
<td></td>
</tr>
<tr>
<td>NLC</td>
<td>nanostructured lipid carrier</td>
<td></td>
</tr>
<tr>
<td>mV</td>
<td>millivolt</td>
<td></td>
</tr>
<tr>
<td>%w/v</td>
<td>percent weight by volume (in grams per 100 milliliters of solution)</td>
<td></td>
</tr>
<tr>
<td>%w/w</td>
<td>percent weight by weight (in grams per 100 grams of solution)</td>
<td></td>
</tr>
<tr>
<td>PDI</td>
<td>polydispersity index</td>
<td></td>
</tr>
<tr>
<td>PEO</td>
<td>polyethylene oxide</td>
<td></td>
</tr>
</tbody>
</table>
r.nm  radius in nanometers

ROS  reactive oxidative species

rpm  revolutions per minute

SLN  solid lipid nanoparticle

TGA  thermogravimetric analysis

UV   ultraviolet
1. INTRODUCTION:

With increased age comes declining health conditions and increased prevalence in diseases. Included in this broad category is the deterioration of vision due to a variety of factors contributing to a number of ophthalmic diseases. These diseases can include age-related macular degeneration, cataracts, diabetic retinopathy, and glaucoma. According to statistics by the National Eye Institute, several millions of people above the age of forty are affected by one of these conditions. From the total population of those above age forty in the 2010 U.S. Census (n = 142,648,393), 36,883,997 adults suffered from vision impairment due to all of the ophthalmic diseases listed above combined (NEI “Prevalence”, 2016). This is approximately over a quarter (25.9%) of adults over age forty who are affected with vision impairment. As age increases, the prevalence rates of all vision impairment also increase, rising significantly around ages 75-79 in all ethnicities, as shown in Figure 1.

![Image of 2010 U.S. Prevalence Rates: All Vision Impairment](https://nei.nih.gov/eyedata/vision_impaired#1)

**Figure 1.** 2010 U.S. Prevalence Rates: All Vision Impairment
Over the course of ten years, starting from the year 2000, the prevalence of vision impairment has increased by almost 130% (NEI “Vision”, 2016). These trends are expected to continue with each subsequent year so that by the year 2030, there will be almost a two-fold increase in prevalence of vision impairment compared to that in 2010, and by 2050, there will be more than a 3-fold increase in prevalence, as shown in Table 1 and Figure 2.

**Table 1.** Table Projections for Vision Impairment (2010-2030-2050)

<table>
<thead>
<tr>
<th>Year</th>
<th>All</th>
<th>White</th>
<th>Black</th>
<th>Hispanic</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>4,195,966</td>
<td>3,398,977</td>
<td>330,644</td>
<td>290,781</td>
<td>175,564</td>
</tr>
<tr>
<td>2030</td>
<td>7,169,680</td>
<td>5,277,689</td>
<td>618,110</td>
<td>840,497</td>
<td>433,383</td>
</tr>
<tr>
<td>2050</td>
<td>13,026,870</td>
<td>9,019,189</td>
<td>1,047,986</td>
<td>2,000,853</td>
<td>958,842</td>
</tr>
<tr>
<td>Total Population</td>
<td>142,648,393</td>
<td>103,846,437</td>
<td>15,190,777</td>
<td>14,901,369</td>
<td>8,709,810</td>
</tr>
</tbody>
</table>


**Figure 2.** Chart Projections for Vision Impairment (2010-2030-2050)

One cause of vision impairment is due to uncontrolled exposure to reactive oxidative species, or ROS. These are species that are produced naturally in the body as a byproduct of adenosine triphosphate, or ATP, production, the energy source that is used for every day function and processes (Prunty, 2015). They are free radicals that contain oxygen and are the most common type produced in tissues (NCI, 2014). They can affect cells by damaging important cellular components, such as DNA, proteins, and membranes. The mitochondria, an efficient organelle found abundantly in the body’s cells and tissues, produce the largest quantities of ROS as it is the largest contributor to ATP synthesis (90% of the body’s energy) [UMDF, 2017]. The body has natural antioxidant mechanisms to protect against ROS, but when ROS are overproduced, these mechanisms are overwhelmed (Prunty, 2015). This leads to oxidative stress cascades on cells and tissues, which causes them to become damaged and eventually die off. In the eye, ROS damage the cells of the retina, which plays an important role in converting visual images into electrical impulses for the brain to interpret. As the photoreceptor cells of the retina die off, vision worsens as the eye has less capability to convert those images to impulses. The longer the period of exposure to ROS, the more damage is done to the retina. Prolonged damage to the retina exacerbates retinal degradation and degeneration, further impairing vision and may even lead to permanent blindness. This is why as a person ages, their vision becomes progressively worse.

A protective measure against ROS is the use of antioxidant treatments. Antioxidants can be used to slow the rate of retinal degradation by counteracting damage from the reactive oxidative species. The body produces some antioxidants endogenously, but they can also be obtained outside of the body. They can be found in a variety of
antioxidant-rich foods, such as fruits, leafy green vegetables, and grains, and can also be obtained through dietary supplements. These include carotenoids like beta-carotene, lycopene, and vitamins A, C, and E (NCI, 2014). There are a variety of substances that act as antioxidants, like the ones mentioned before, in addition to glutathione, coenzyme Q10, lipoic acid, flavonoids, phenols, polyphenols, phytoestrogens, etc., all having different properties and roles against ROS (Harvard, 2017). In the case of preventing retinal degradation to improve outcomes in terms of vision integrity and function, antioxidants can be employed to protect against the damage from reactive oxidative species. However, because of physiological barriers, antioxidants cannot simply be taken orally and expected to be able to reach its target, i.e. the retina, to enact its protection.

This is due to the way that the eye is structured. The retina is located in the posterior chamber of the eye, which is generally challenging to access (Kaufman, 2011). There is a blood-retinal barrier, which is formed by retinal capillaries and tight junctions of retinal vascular endothelium that limit drug access from the general circulation. If taken orally, antioxidants would face a number of problems in trying to reach the retina. Firstly, it would undergo first-pass metabolism to which oral drugs are susceptible. This limits the amount of drug available to be absorbed and to be effective in the body. Secondly, dietary antioxidants, such as flavonoids, tend to be poorly soluble. Low solubility negatively affects dissolution rates, which limits how much of the drug can be absorbed into the circulatory system. This, in turn, limits the bioavailability of the drug for use in the body. Lastly, even if the drug were able to be absorbed into the circulatory system, not only would it be severely limited in terms of bioavailability, but also it would not be able to effectively reach its target due to the blood-retina barrier and lack of
circulation in the back of the eye. Thus, taking antioxidants as an oral dosage form would not be a viable option when targeting the retina.

Drug administration directly to the eye would be a more effective route compared to oral dosing. There are several ocular dosage forms and drug delivery systems, though these too are not without their challenges (see Figure 3 and Table 2). Some routes of administration include topical and injectable. Drugs administered through the topical route are most commonly in the form of eye drops, are noninvasive, and have high patient compliance. These are generally better for delivery to the anterior part of the eye. This is because there are several barriers that prevent absorption towards the back of the eye. Firstly, blinking and the production of tears can quickly wash away the drug and clear it from the eye. In fact, the majority of topically administered dosages are washed away within 15-30 seconds after instillation (Gaudana, 2010). Thus, very little of the drug is in contact with the eye long enough to be absorbed, accounting for less than 5% of the applied dose. Secondly, even if the drug were absorbed from the surface of the eye, it would have to pass through all layers of cornea, including the epithelium, stroma, and endothelium, to reach the aqueous humor, the fluid that fills the anterior part of the eye. Each layer has alternating hydrophilicity and lipophilicity that the drug must be compatible with in order to pass through the layers. The epithelium is lipophilic and so resists the permeation of hydrophilic drugs. The stroma is hydrophilic and resists the permeation of lipophilic drugs. The endothelium is similar to the epithelium in that it is lipophilic and resists hydrophilic drugs (Kaufman, 2011). In order to permeate through the corneal layers, the drug must be amphipathic, meaning it must possess both lipophilic and hydrophilic characteristics to pass through the respective layers. A way to bypass this
requirement would be to deliver the drug via injection, specifically intravitreal injections. Injections bypass the mechanical barriers, i.e. the different layers of the eye, to deliver the drug directly to the posterior segment of the eye into the vitreous humor (Gaudana, 2010). It is a more effective route compared to topical administration in terms of drug delivery to the back of the eye, but it is not very patient compliant as it is very invasive. Additionally, because drug distribution in the vitreous humor is not uniform due to lack of circulation, only small molecules can rapidly distribute through the vitreous humor as distribution of larger molecules is limited.

**Figure 3.** Routes of Administration for Ocular Drug Delivery
Table 2. Benefits and Challenges of Ocular Routes of Administration

<table>
<thead>
<tr>
<th>Route</th>
<th>Benefits</th>
<th>Challenges</th>
<th>Application in the treatment of diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical</td>
<td>High patient compliance, self-administrable and noninvasive</td>
<td>Higher tear dilution and turnover rate, cornea acts as barrier, efflux pumps, BA &lt;5%</td>
<td>Keratitis, uveitis, conjunctivitis, scleritis, epideratitis, blepharitis</td>
</tr>
<tr>
<td>Oral/Systemic</td>
<td>Patient compliant and noninvasive route of administration</td>
<td>BAB, BRB, high dosing causes toxicity, BA &lt;2%</td>
<td>Scleritis, episcleritis, CMV retinitis, PU</td>
</tr>
<tr>
<td>Intravitreal</td>
<td>Direct delivery to vitreous and retina, sustains drug levels, evades BRB</td>
<td>Retinal detachment, hemorrhage, cataract, endophthalmitis, patient in compliance</td>
<td>AMD, PU, BRVO, CRVO, DME, CME, UME, CMV retinitis</td>
</tr>
<tr>
<td>Intracameral</td>
<td>Provides higher drug levels in the anterior chamber, eliminates usage of</td>
<td>TASS, TECDS</td>
<td>Anesthesia, prevention of endophthalmitis, inflammation and pupil dilation</td>
</tr>
<tr>
<td></td>
<td>topical drops, reduces corneal and systemic side effects seen with</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>topical steroid therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subconjunctival</td>
<td>Delivery to anterior and posterior segment, site for depot formulations</td>
<td>Conjunctival and choroidal circulation</td>
<td>Glaucoma, CMV retinitis, AMD, PU</td>
</tr>
<tr>
<td>Subtenon</td>
<td>High vitreal drug levels, relatively noninvasive, fewer complications</td>
<td>RPE, chemosis, subconjunctival hemorrhage</td>
<td>DME, AMD, RVO, uveitis</td>
</tr>
<tr>
<td></td>
<td>unlike intravitreal delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrobulbar</td>
<td>Administer high local doses of anesthetics, more effective than peribulbar,</td>
<td>Retrobulbar hemorrhage, globe perforation, respiratory arrest</td>
<td>Anesthesia</td>
</tr>
<tr>
<td></td>
<td>minimal influence on IOP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior</td>
<td>Safe for delivery of depot formulations, sustain drug levels up to 6</td>
<td>Requires surgery and RPE acts as barrier</td>
<td>AMD</td>
</tr>
<tr>
<td>Juxtascleral</td>
<td>months to the macula, avoids risk of endophthalmitis and intracocular</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


BA = bioavailability, BAB = blood–aqueous barrier, BRB = blood–retinal barrier, AMD = age-related macular degeneration, DME = diabetic macular edema, BRVO = branched retinal vein occlusion, CRVO = central retinal vein occlusion, RVO = retinal vein occlusion, CME = cystoid macular edema, UME = uveitic macular edema, CMV = cytomegalovirus, IOP = intraocular pressure, TASS = toxic anterior segment syndrome, TECDS = toxic endothelial cell destruction syndrome, RPE = retinal pigmented epithelium, PU = posterior uveitis
Many studies over the past few years have demonstrated the effectiveness of nanotechnology in ocular drug delivery. In one study, the administration of very small sized molecules (20 nm) of gold resulted in the particles readily passing through the blood-retinal barrier, which, as stated before, is a major barrier in getting the drug to the retina, and distributing in all the retinal layers without cytotoxicity (Gaudana, 2010). Increased penetrance and better distribution of small molecules, specifically nanoparticles, in the posterior segment of the eye would increase bioavailability and consequently pharmacological activity, which in turn can improve health outcomes. In this research, two nanoparticle formulations, solid lipid nanoparticles and nanostructured lipid carriers, were being investigated for efficacy in delivering quercetin, an antioxidant flavonoid, into the eye for retinal neuroprotection, compared against hot melt cast films. Many studies have been performed with quercetin nanoparticles targeted at various areas of the body and through different dosage routes, but to our knowledge, there are no known studies aimed for delivery into the eye. The advantages of using a topical administration route are high patient compliance and ease of modification to improve drug solubility, and these paired with the advantages of nanoparticle formulations makes them promising candidates for ocular drug delivery.

1.1 Solid Lipid Nanoparticles:

Solid lipid nanoparticles, or SLNs, are dispersions that contain particles that have an average size of 500 nanometers or smaller and that contain low microparticle content (Mehnert, 2012). These nanoparticle dispersions are commonly and effectively produced via a high pressure homogenization process. As the name suggests, solid lipid
nanoparticle formulations use solid lipids, such as trilaurin, tripalmitin, glyceryl monostearate, glyceryl behenate, and stearic acid, for the lipid phase instead of lipid oils, primarily as a means to control drug release and to increase stability in vivo. Drugs encapsulated in a solid lipid have considerably lower mobility, and thus slower drug release from the lipids, compared to drugs encapsulated in liquid oil. The solid lipids also remain in a solid form at body temperature, which contributes to higher stability of the delivery system in vivo (Beloqui, 2016). In addition to controlled drug release, SLNs have many other advantages as a drug delivery system. These advantages include increased drug stability, high drug payload, ability to incorporate lipophilic and hydrophilic drugs, no biotoxicity or use of organic solvents, and ease in large scale production and sterilization (Mehnert, 2012).

The general formulation of SLNs includes a lipid phase containing the solid lipid and drug, an aqueous phase, and emulsifiers to stabilize the lipid dispersion and to prevent particle agglomeration. Formulations can also include cyclodextrins, which are cyclic oligosaccharides with hydrophobic inner cores and hydrophilic outer surfaces. Many studies have been performed to demonstrate that the addition of cyclodextrins is beneficial in the formulation process (Adelli “Effect”, 2015; Srirangam, 2012). The use of cyclodextrins in the SLN formulations not only enhances the solubility of lipophilic drugs by forming soluble complexes with the drugs (i.e., encapsulating the drug in the core), but it also improves drug permeability across biological membranes by increasing the availability of drug molecules at the surface of the membranes (Adelli “Effect”, 2015). These advantages, in turn, increase the bioavailability and consequently, the action of the drug at the target site.
There are many different methods and techniques that can be used to produce SLN formulations (Mehnert, 2012). Some examples include high shear homogenization and ultrasound, high pressure homogenization, solvent emulsification and evaporation, and microemulsion dilutions. Under the high pressure homogenization method, there are two different techniques: hot homogenization and cold homogenization. A schematic of these two techniques is shown below in Figure 4. In this research study, the hot high pressure homogenization technique was used, which produces a nanoemulsion that sports the advantages of highly impeded lipid crystallization and prolonged storage as a supercooled melt.

**Figure 4. Hot vs. Cold Homogenization Techniques**

In characterizing SLN formulations, the goal parameters are small and uniform particle sizes (less than 500 nm), low polydispersity index, and high magnitude of zeta potential. These specific parameters would ensure the most stable and homogeneously distributed formulation. Studies suggest that smaller (nanoparticle) sizes have slightly faster release rates when compared to larger (microparticle) sizes (Dan, 2016). Polydispersity index measures the degree of non-uniformity, or rather, the deviation from uniformity in a dispersion. A low value for PDI would indicate a more evenly distributed formulation with uniform particle sizes. Zeta potential measures the magnitude of electric repulsion between particles. A high magnitude for zeta potential would mean that there is strong electric repulsion between the particles, which would decrease the likelihood of particle aggregation in the dispersion, and thus, would increase the stability of the formulation.

1.2 Nanostructured Lipid Carriers:

Nanostructured lipid carriers, or NLCs, constitute another drug delivery system with controlled drug release from nanoparticles. Not only have NLCs been shown to improve drug permeation and aqueous solubility of drugs, but also these formulations have demonstrated to increase drug retention and more importantly, to enhance the antioxidant and anti-inflammatory effects of quercetin (Beloqui, 2016). The differentiating characteristic of NLCs from SLNs is the incorporation of a liquid lipid, such as caprylic or capric triglycerides, lauroyl polyoxyglycerides, monoacylglycerols, and soy lecithin, in the solid lipid matrix, which results in an unstructured solid lipid matrix. The use of a liquid lipid in the lipid matrix increases the amount of openings and
gaps in the solid matrix, allowing for more of the drug to be encapsulated into the nanoparticles as well as increasing the drug release rate by increasing the surface permeability of the nanoparticles. Comparatively, drug release through the solid lipid is slower and more limited than the drug release through the liquid lipid due to the lower solubility of the drug in the solid lipid (Dan, 2016). The incorporation of the liquid lipid improves drug solubility in the nanoparticles and increases the rate and amount of drug release while still controlling how much is released into the body at a time. The lipid blends can be mixed in any ratio from 70:30 up to 99.9:0.1, solid lipid to liquid lipid (Beloqui, 2016). Otherwise, the general formulation is the same as that of SLNs. The NLC formulation consists of a lipid phase which contains the drug, an aqueous phase, and surfactants accounting for about 1.5-5% w/v of the formulation.

NLCs can be made using a variety of methods, such as micro-emulsification and solvent displacement, but similar to SLNs, the most preferred method is high pressure homogenization, specifically hot homogenization, as it does not require the use of solvents during the preparation process and can be easily implemented in large scale production. Due to the presence of water in these formulations, physical instability caused by microbial growth is a major concern. To circumvent this problem and to preserve ideal nanoparticle characteristics, the formulation can either be lyophilized to remove water content and form a solid formulation, or a preservative can be added to the formulation to inhibit microbial growth while maintaining a liquid formulation. Criteria for a lyophilized formulation include short reconstitution time, easy resuspension in water, no changes to particle size distribution of nanoparticles, and preserved drug activity. To prevent aggregation of the lyophilized particles, a cryoprotectant is required.
One such cryoprotectant shown in studies to effectively prevent particle aggregation while preserving the stability of the nanoparticle shell structure is trehalose. However, caution should be taken when using a lyophilization method as the use of cyroprotectants often implies alteration of initial nanoparticle surface properties. Other preservation methods may be more favored and advised in this case, such as the use of a preservative while maintaining the water content of the formulation. This method ensures that there are minimal changes to the nanoparticle surface properties (e.g., zeta potential). The ideal preservative should be highly hydrophilic, non-ionic, and have little affinity to the particle surface.

The goal parameters of NLCs are also the same as those for SLNs. The desired formulation should have small (less than 500 nanometers) and uniform particle sizes, low polydispersity index, and high magnitude of zeta potential to ensure the greatest stability and homogeneous distribution.

1.3 Hot Melt Cast Films:

Hot melt cast films are yet another drug delivery system that is used for improving bioavailability of poorly soluble drugs. They are solid molecular dispersions of a drug or active pharmaceutical ingredient in a polymeric matrix that provide controlled and sustained drug release while eliminating the use of solvents in the preparation process (Repka, 2007). These formulations have the advantages of being quicker and more efficient to produce as well as increased efficiency of drug delivery.
The general formulation for hot melt films includes a blend of the active ingredient or drug, a thermoplastic polymeric carrier, and other processing aids like plasticizers or antioxidants. This blend is then heated and softened before being pressed in a die to form the film. This method is known as hot melt extrusion, or HME, when an extruder is used, and it can be used to form a variety of other dosage forms as well, such as granules, pellets, tablets, capsules, and implants. There are specific criteria for materials used in the HME process, a major one being thermal stability as the materials must be able to withstand the high temperatures at which the process is performed without degrading (Crowley, 2007). They must also be able to easily deform while in the extruder and solidify upon exiting the extruder to form a solid dosage form.

In regards to the active pharmaceutical ingredients, the drug contained in the dosage form may be dispersed in the polymeric matrix as undissolved particles, a solid solution, or some combination of both. Because of this, the dosage form may be a solid dispersion system, in which the drug is undissolved and dispersed in the carrier matrix, or a solid solution system, in which the drug is dissolved in the carrier matrix. Solid dispersions have the advantages of being more stable and more easily produced compared to solid solutions. However, solid solutions have the advantage of exhibiting potentially higher bioavailability of poorly soluble drugs over solid dispersions. The properties of the drug can also affect the functionality of other materials used in the formulation. The drug can negatively affect the formulation by decreasing the viscosity or inhibiting the hardening of the matrix, which results in a dosage form that will be poorly handled and unusable. On the other hand, the drug could positively affect the formulation by lowering the glass transition temperature of the polymeric carrier, which
can improve processing conditions. Thus, the drug should be compatible with the other ingredients used in the formulation in order to yield a good dosage form.

A second major component of the formulation is the carrier, which is generally a polymer, a low melting point wax, or a mixture of low melting point substances in which the drug or active pharmaceutical ingredient is embedded. The compatibility of the drug and carrier compounds should be taken into account as there is the possibility of the formation of a eutectic mixture when mixing a low melting drug with a low melting carrier, which would result in a dosage form that would not be able to solidify. The physical and chemical properties of the carriers can also highly affect the drug release from the dosage form. Drug release mechanisms differ depending on the type of carrier used. Water insoluble carriers exhibit a diffusion controlled drug release rate while water soluble carriers exhibit a diffusion and erosion drug release mechanism. Ionic or pH dependent carriers can be used to achieve zero-order or site-specific drug release. Functional excipients can be added to the carrier to modulate the rate of drug release as well, by altering either the porosity, tortuosity, viscosity, or rate of disintegration of the polymeric matrix and the resulting dosage form.

Plasticizers are the third important component of the formulation. Plasticizers are low molecular weight compounds that can have two roles in the formulation process: increase polymeric flexibility and decrease processing temperatures. They can be used to soften the polymers to make the resulting dosage form more flexible. They can also improve processing conditions by decreasing the glass transition temperature and melt viscosity of a polymer by increasing the free volume between polymer chains while decreasing the ease of their movement with respect to each other,
which allows the HME process to be conducted at lower temperatures and with less energy. This improves the drug and carrier stability by reducing their degradation. Plasticizers used in the formulation should have good efficiency, stability, polymer-plasticizer compatibility, and permanence, as these can affect the physical and mechanical properties as well as the drug release rate of the dosage form.

Lastly, there are other processing aids, like antioxidants, acid receptors, and light absorbers, which can be used to improve the stability of the other components and the overall formulation. Antioxidants, which can be either preventive or chain-breaking, are used to protect the compounds against free radicals and oxidative degradation. Preventive antioxidants prevent the initiation of free radical chain reactions while chain breaking antioxidants inhibit free radical chain reactions. Preventive antioxidants include reducing agents, which preferentially undergo oxidation and thus protect the other compounds from oxidative damage, and chelating agents, which form stable complexes with the metal ions to prevent them from catalyzing the formation of free radicals and thus decrease the number of free radicals produced. Chain breaking antioxidants include hindered phenols and aromatic amines, which have very weak O-H and N-H bonds, respectively, that will undergo a higher rate of oxidation and thus reduce oxidation of the other formulation components. Other processing aids can be used to improve processing conditions, such as glycerclyl monostearate, which can act as a thermal lubricant, and Vitamin E TPGS, which can enhance drug absorption.

The main goal parameters for hot melt cast films are homogenous distribution of the drug in the polymeric matrix, high stability of the drug, polymer, and any additives, and good rate and quantity of drug release.
1.4 Quercetin:

Quercetin, which has the chemical name of 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4\(H\)-1-benzopyran-4-one, is a bioflavonoid aglycone, specifically of the flavonol subclass, meaning that it has a 3-hydroxyflavone backbone and lacks attached sugars, as shown in Figure 5 (Kelly, 2011).

![Quercetin Structure](Source: Quercetin [product insert]. Ann Arbor, MI: Cayman Chemical Company; 2016.)

It is a crystalline solid that has a bright citron yellow color. It is lipophilic and hydrophobic in nature so its solubility in water is poor to nonexistent (poor in hot water and entirely insoluble in cold water). A way to improve its solubility in water is to convert it to a glycoside. This can be done by replacing one of the hydroxyl groups, commonly the one at position 3, with a glycosyl group, which can be any sugar such as glucose, rhamnose, or rutinose (see Figure 6). A glycoside group at position 3 is known as isoquercitin. The addition of a glycosyl group changes the chemical properties of the drug, including solubility, absorption, and in vivo effects. Specifically, the addition of the glycosyl group increases the water solubility of quercetin.
Otherwise, if quercetin were to be solubilized in an aqueous buffer, it would first need to be dissolved in DMSO and then diluted with the aqueous buffer. Quercetin is rather well soluble in organic solvents, such as ethanol, acetic acid, dimethyl formamide, and dimethyl sulfoxide. In ethanol, quercetin has a solubility of 2 mg/mL whereas in DMF and DMSO, it has a solubility of 30 mg/mL (up to 100 mM) [Cayman, 2016; Abcam, 2017].

Quercetin is found in a variety of foods, such as onions, shallots, apples, berries, grapes, and tea leaves, as well as in some medicinal botanicals, such as *Ginkgo biloba*, *Hypericum perforatum* (St. John’s wort), and *Sambucus canadensis* (elder) [Kelly, 2011]. It is commercially available as dietary supplements and as additives to foods and beverages. It has many potential uses, as demonstrated in several test tube studies, though more research is required in humans to prove efficacy. Quercetin has been demonstrated to prevent immune cells from releasing histamine, which is responsible for allergic reactions, as well as to prevent free radicals from activating transcription factors that generate pro-inflammatory cytokines, making it a good
candidate for reducing symptoms of allergies and inflammatory conditions (Erhlich, 2015; Bentz, 2009). In respect to heart disease and hypertension, various studies, including test tube, animal, and population-based studies, suggest that the flavonoids found in red wine like quercetin may reduce blood pressure and the risk of atherosclerosis. It can also help in hypercholesterolemia by reducing LDL levels. It does so by exhibiting a potent inhibitory effect on lipid absorption in the gastrointestinal tract, and it also helps eliminate lipids from the body by enhancing lipid metabolism in the liver, which ultimately protects the body against damage by LDL cholesterol (Flavonoids, 2017). Quercetin has also been shown to exhibit antitumor characteristics in terms of inhibiting the growth of cancer cells and tumors. In one study, it was suggested to be more efficacious in tumor growth inhibition than resveratrol (Erhlich, 2015). These are only a few of the numerous clinical indications in which quercetin may have beneficial effects.

In respect to this research, the most important characteristic of quercetin, however, is its antioxidant properties. Quercetin is a strong antioxidant that can bind transition metal ions, scavenge free radicals, and increase glutathione levels (Bentz, 2009). Specifically it can inhibit lipid peroxidation, which is the process by which unsaturated fatty acids are converted to free radicals by hydrogen extraction. When the free radicals are oxidized by molecular oxygen, lipid peroxy radicals are created, which then extract hydrogen molecules from other unsaturated fatty acids and produce more free radicals in an amplifying cascade process. Trace amounts of transition metal ions can catalyze this process. The overproduction of free radicals over a prolonged period of time can lead to extensive damage to various body tissues, including those of the heart, brain,
eye, and associated structures. Quercetin not only hinders the production of free radicals significantly by mopping up any transition metal ions, but also it greatly reduces the number of free radicals already made in the body by scavenging and neutralizing them. Additionally, it can increase glutathione levels in the brain to protect neurons from oxidative damage by competitively converting hydrogen peroxide to oxygen and water, instead of allowing superoxide dismutase to convert it to a superoxide radical. This allows it to reduce oxidative stress in the body, protecting it from a myriad of ailments, such as cardiovascular disorders, neurodegenerative diseases, atherosclerosis, chronic inflammation, and retinal degeneration.

In terms of safety and toxicity, quercetin has been demonstrated to be well tolerated in human studies. Doses as high as 1,000 mg/day were administered for several months, and no adverse effects on liver and kidney functions, hematology, or serum electrolytes were produced as a result (Kelly, 2011). However, one potentially significant concern with respect to toxicity is the concomitant use of digoxin with high doses of quercetin, which has been shown to have a lethal effect in one pig study. More research is needed to determine safe dosage levels of quercetin when used concomitantly, but for the purpose of our research, the dose of quercetin used was deemed to be safe for human use.
2. MATERIALS AND METHODS:

2.1 Materials:

Quercetin was purchased from Tocris Bioscience. Glycerol monostearate and Compritol ATO 888® were graciously donated by Gatrefossé. Miglyol 812® was purchased from Condea. Tween 80 and methyl-beta-cyclodextrin were purchased from Acros Organic. Poloxamer 188 was purchased from Spectrum. Glycerin was purchased from PCCA. Acetonitrile and dimethyl sulfoxide were purchased from Fisher Chemical. Whole rabbit eye globes were purchased from Pel-Freez Biologics. The rabbit eye globes were dissected in lab to collect the corneas for use in the permeability studies.

2.2 Methods:

Solid Lipid Nanoparticles:

Quercetin solid lipid nanoparticles, or SLNs, were prepared by probe sonication method. The lipid phase was prepared with a solid lipid (0.7% w/v Glycerol monostearate) in combination with 1.3% w/v Compritol ATO 888® and heated on a hot plate at 80°C. Quercetin was dissolved in 100 µL of dimethyl sulfoxide and added to 2.25% w/v glycerin. This mixture was added to the melted lipid phase. The aqueous phase was prepared using 0.75% w/v Tween 80®, Poloxamer 188®, and filtered water and heated on the hot plate at 80°C. The aqueous phase was then added to the lipid phase
while stirring at 600 rpm for 2 minutes. The final concentration of quercetin was 0.1% w/v. The premix was homogenized with an Ultra-Turrax® at 16,000 rpm for 3 minutes to form a coarse emulsion. This coarse emulsion was then subjected to probe sonication at a 15-second pulse rate for 3 minutes. The final emulsion was allowed to cool to form the nanoparticles.

**Nanostructured Lipid Carriers:**

Quercetin nanostructured lipid carriers, or NLCs, were also prepared by probe sonication method. The method is almost exactly the same as that for the SLN formulation, except that the lipid phase was prepared with a liquid lipid (0.7% w/v Miglyol 812®) in combination with 1.3% w/v Compritol ATO 888®, which was then heated on a hot plate at 80°C. Quercetin was dissolved in 100 µL of dimethyl sulfoxide and added to 2.25% w/v glycerin. This mixture was then added to the melted lipid phase. The aqueous phase was prepared using 0.75% w/v Tween 80®, Poloxomer 188®, and filtered water and heated on the hot plate at 80°C. The aqueous phase was added to the lipid phase while stirring at 600 rpm for 2 minutes. The final concentration of quercetin was 0.1% w/v. The premix was homogenized with an Ultra-Turrax® at 16,000 rpm for 3 minutes to form a coarse emulsion, which was then subjected to probe sonication at a 15-second pulse rate for 3 minutes. The final emulsion was allowed to cool to form the nanoparticles.

**Hot Melt Cast Films:**

Quercetin films were prepared by melt-cast method. Polyethylene oxide N10 was used as the matrix forming polymer. Quercetin and PEO N10 were mixed via
geometric dilution to prepare the physical mixture. The drug load in the film was 10% w/w. A 10 mm die was placed over a brass plate and heated on a hot plate at 75°C for at least one minute. The physical mixture was poured into the center of the die and compressed for a few seconds to form a flat matrix surface. The film was heated on the hot plate for an additional minute so that the mixture was completely melted and then removed to cool. When completely cooled, the film was cut to collect samples that weighed approximately 8 mg each.

Characterization of Nanoparticles:

Mobile phase of acetonitrile:water (ACN:H₂O, 40:60) was prepared. Quercetin was dissolved in dimethyl sulfoxide (DMSO) to make a 1 mg/mL stock solution for the standards. Varying volumes of the stock solution were diluted with the mobile phase to make standards of the following concentrations: 1 μg, 2 μg, 5 μg, 10 μg, and 20 μg. The standards were analyzed using UV analysis at a wavelength of 369 nm to determine the light absorbance trend, or calibration curve, of quercetin to which the nanoparticle emulsions will be compared.

The SLN and NLC formulations were diluted 500 times with purified water and analyzed using a zetasizer (Malvern Instruments, Ltd.) to determine the size, polydispersity index, and zeta potential of the particles. The goal was to have small particle sizes, low polydispersity index, and high magnitude of zeta potential for the most stable and homogeneous formulation.

Entrapment efficiency, which is a measure of how much drug is entrapped in the nanoparticles, was performed by centrifuging 500 μL of each formulation with a
filter for 15 minutes. 100 μL of the centrifuged filtrate was drawn, diluted with 900 μL of the mobile phase, and then vortexed to ensure thorough and homogeneous mixing.

Assay was performed to determine quercetin content in the nanoparticles. For each formulation, 100 μL of formulation were added to 900 μL of a mixture of DMSO and methanol (50:50) and sonicated for 10 minutes. After sonication, the mixture was centrifuged for 15 minutes. From this, 100 μL of the supernatant was drawn and diluted with 900 μL of the mobile phase before being vortexed to ensure thorough and homogeneous mixing.

All samples collected from entrapment efficiency and assays were analyzed in triplicates using HPLC-UV method with a Kinetex® 5 μm EVO C18 100 Å LC column (250 x 4.6 mm).

**Characterization of Films:**

Mobile phase of acetonitrile:water (ACN:H₂O, 40:60) was prepared. Quercetin was dissolved in dimethyl sulfoxide (DMSO) to make a 1 mg/mL stock solution for the standards. Varying volumes of the stock solution were diluted with acetonitrile to make standards of the following concentrations: 1 μg, 2 μg, 5 μg, 10 μg, and 20 μg. The standards were analyzed using UV analysis at a wavelength of 369 nm to determine the calibration curve of quercetin to which the film samples will be compared.

Samples of approximately 8 mg were cut from quercetin films and added to 2 mL of acetonitrile. This mixture was then sonicated for 5 minutes until the film was completely dissolved. After sonication, the stock was diluted by a factor of 20. All
samples were collected and analyzed in triplicates using UV analysis at a wavelength of 369 nm.

**Permeability Studies:**

Release and permeability of quercetin from the nanoparticles were studied using a vertical dialysis cassette with a 10,000 Dalton MWCO membrane. Isotonic phosphate buffer saline, or IPBS, was made with 5% methyl-beta-cyclodextrin. IPBS served as the receiver medium. 18 mL of IPBS was filled into the vial, and 1 mL of formulation was filled into the cassette. Measurements were taken at 15, 30, 60, 90, 120, 150, and 180 minute time points, at which time 1 mL of the receiver medium was drawn and 1 mL of IPBS was added into the vial. All samples collected were analyzed in triplicates under HPLC-UV method.

Permeability of quercetin from NLCs and films was studied using fresh rabbit cornea in a side-by-side diffusion apparatus, maintained at 34°C using a circulating water bath. IPBS with 5% methyl-beta-cyclodextrin was made. Spectra/Por® membrane (10,000 Daltons MWCO) were cut and soaked in IPBS for 30 minutes. Side-by-side diffusion cells were set up so that the fresh rabbit cornea and Spectra/Por® membrane were sandwiched between the cells. For the film studies, the films were cut to approximately 45 mg and wetted with IPBS. They were then sandwiched between the rabbit cornea and Spectra/Por® membrane, in the following order: donor cell→Spectra/Por® membrane→quercetin film→cornea→recipient cell (see Figure 7).
Both the donor and recipient cells for the film studies were filled with 3.2 mL of IPBS. For the NLC formulations and the control, which was 3 mg of pure quercetin mixed with IPBS, 3.2 mL of each formulation were added into their respective donor cells, and 3.2 mL of IPBS were added to the recipient cells. Measurements were taken at 30, 60, 90, 120, 150, and 180 minute time points. After flushing a few times to mix the receiver medium, 0.6 mL samples were drawn from the recipient cell, except for the films, for which samples were collected from both donor and recipient cells, and 0.6 mL of IPBS was added to the cells to replace the volume removed. The samples were analyzed in triplicates using UV analysis at a wavelength of 369 nm.

**Figure 7.** Side-by-Side Diffusion Apparatus Setup
3. RESULTS:

3.1 Characterizations of Nanoparticles and Films:

The solid lipid nanoparticles demonstrated an average particle size of 65.4 nm, polydispersity index of 0.29, and zeta potential of -12.3 mV. Assay and entrapment efficiency results were 78.4 ± 1.6% and 90.9 ± 0.3%, respectively. Drug release across the Spectra/Por® membrane was 33.3 ± 1.5%. These parameters are summarized below in Table 3. Particle size distribution is shown in Figure 8.

Table 3. Physicochemical Characterization of SLN Formulations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Solid Lipid Nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle Size (nm)</td>
<td>65.4</td>
</tr>
<tr>
<td>Polydispersity Index</td>
<td>0.29</td>
</tr>
<tr>
<td>Zeta Potential (mV)</td>
<td>-12.3</td>
</tr>
<tr>
<td>Assay</td>
<td>78.4 ± 1.6</td>
</tr>
<tr>
<td>Entrapment Efficiency</td>
<td>90.9 ± 0.3</td>
</tr>
<tr>
<td>% Release across Spectra/Por® Membrane</td>
<td>33.3 ± 1.5</td>
</tr>
</tbody>
</table>

Figure 8. SLN Particle Size Distribution by Intensity
The nanostructured lipid carriers demonstrated an average particle size of 46.1 r.nm, polydispersity index of 0.18, and zeta potential of -16.2 mV. Assay and entrapment efficiency results were 86.6 ± 0.2% and 93.4 ± 0.1%, respectively. Drug release across the Spectra/Por® membrane was 47.1 ± 7.9%. These parameters are summarized below in Table 4, and particle size distribution is shown below in Figure 9. The comparison of the particle size distributions for the SLN and NLC formulations is shown below in Figure 10.

**Table 4. Physicochemical Characterization of NLC Formulations**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Nanostructured Lipid Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle Size (r.nm)</td>
<td>46.1</td>
</tr>
<tr>
<td>Polydispersity Index</td>
<td>0.18</td>
</tr>
<tr>
<td>Zeta Potential (mV)</td>
<td>-16.2</td>
</tr>
<tr>
<td>Assay</td>
<td>86.6 ± 0.2</td>
</tr>
<tr>
<td>Entrapment Efficiency</td>
<td>93.4 ± 0.1</td>
</tr>
<tr>
<td>% Release across Spectra/Por® Membrane</td>
<td>47.1 ± 7.9</td>
</tr>
</tbody>
</table>

**Figure 9.** NLC Particle Size Distribution by Intensity
Hot melt cast films demonstrated assay results of 76.3 ± 4.1%. Particle size, polydispersity index, zeta potential, and entrapment efficiency are parameters designed more for nanoparticle systems and were not evaluated with the hot melt cast films. Drug release across Spectra/Por® membranes was also not evaluated with the films.

3.2 Corneal Permeability Studies:

All samples were analyzed via UV analysis at a wavelength of 369 nm, at which the standard calibration curve had an R² value of 0.9989. TGA data indicated that physical mixtures were stable under the utilized processing temperature. Permeability across the rabbit cornea for quercetin films, NLCs, and control are shown in Figures 11, 12, and 13, respectively.
**Figure 11.** Permeability of Quercetin Films

**Figure 12.** Permeability of Quercetin NLCs
The results from the permeability studies, in terms of rate, flux, and permeability, are summarized in Table 5 and Figure 14. Transcorneal flux, which is the amount of drug that crosses the cornea per minute per area squared (in this case, the area of the cornea is 0.636 cm), of quercetin control, NLCs, and films were $0.026 \pm 0.002$, $0.036 \pm 0.006$, and $0.144 \pm 0.009$, respectively. Permeability was calculated as flux normalized by assay.

**Figure 13.** Permeability of Quercetin Control

<table>
<thead>
<tr>
<th></th>
<th>Rate (µg/min)</th>
<th>Flux (µg/min/cm²)</th>
<th>Permeability X 10⁶ (cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$0.0165 \pm 0.002$</td>
<td>$0.0260 \pm 0.002$</td>
<td>$0.135 \pm 0.012$</td>
</tr>
<tr>
<td>NLCs</td>
<td>$0.0227 \pm 0.004$</td>
<td>$0.0357 \pm 0.006$</td>
<td>$0.186 \pm 0.030$</td>
</tr>
<tr>
<td>Films</td>
<td>$0.1435 \pm 0.006$</td>
<td>$0.1435 \pm 0.009$</td>
<td>$0.747 \pm 0.047$</td>
</tr>
</tbody>
</table>
Figure 14. Transcorneal Permeability of Quercetin in Different Formulations
4. DISCUSSION:

The goal parameters for the nanoparticle formulations were to have small particle sizes, low polydispersity index, and high magnitude of zeta potential. Both the solid lipid nanoparticles and the nanostructured lipid carriers successfully met and fit well within these goal parameters. Particle sizes for both formulations were well below 500 nm. The peak of the particle size distribution for the NLC formulation was higher and narrower compared to the peak for the SLN formulation, as the intensity was noticeably greater and the particle sizes were less variable. Polydispersity indices for both were close to zero (0), which is the most ideal polydispersity index in order to have a completely uniform formulation. Zeta potentials for both were relatively high in magnitude as well (between -10 and -20 mV). Both entrapment efficiencies were at least 90% or higher. Drug releases across the Spectra/Por® membrane for both formulations, on the other hand, were relatively low (around 30-50%). When comparing assays for all three formulations (SLNs, NLCs, and films), film assay results were the lowest (by 2%), and NLCs were the highest (by about 8%), with SLNs in between the two.

In all regards, the NLC formulation had better physicochemical characteristics compared to the SLN formulation. This is due to the nature of the lipid matrix shells of the nanoparticles. Because solid lipid nanoparticle formulations use only solid lipids, the matrix shells are completely solid and continuous, which has limited permeability. Not only does this affect how much drug can be encapsulated in the
nanoparticles, but it also affects how the drug diffuses out of the nanoparticles. Specifically, this structure results in a relatively low drug load, limited drug release, and drug expulsion during storage. With nanostructured lipid carriers, on the other hand, the incorporation of a liquid lipid to the solid lipid matrix results in a disrupted and highly permeable nanoparticle shell. This structure allows for a higher drug load, greater drug release, and long term drug stability. Thus, from these differences (summarized in Figure 15), we would expect to see that the NLC formulation would show better characteristics than the SLN formulation, which it did.

Figure 15. Advantages of NLC Structure over SLN Structure
To compare how well quercetin permeates from the hot melt cast film across corneas as opposed to permeation from the nanoparticles, we used a control of pure quercetin in addition to the NLC formulation, which was chosen because it demonstrated better parameters and physicochemical characteristics compared to the SLNs. The three main parameters tested in the permeability studies were rate, flux, and permeability. Rate was determined by how much drug, in micrograms (μg), crossed the cornea from the donor to the receiver cells, per minute, with concentrations extrapolated from the calibration curve based on the absorbance of the samples. The rate of drug release permeation for the control was the slowest, and the rate for the films was the highest by a significant margin (7-14x higher), with the rate of the NLCs in between the two. Flux was calculated as the amount of drug in micrograms that crosses the cornea per minute per area squared (area = 0.636 cm). Again, the flux for the control was the lowest, and the flux for the films was the highest by a good margin (4-5x higher). Flux for NLCs was in between the other two flux values. Lastly, permeability was calculated as flux normalized by assay and reported as centimeters per second. These results showed the same trends as with the other two parameters. The permeability of the control across the cornea was the lowest, the permeability of the drug from the NLCs was second highest, and the permeability of the drug from the films was the highest, once again by a good margin (4-6x higher).

In total, both formulations (NLCs and films) were more successful compared to the control in drug permeability across the cornea. However, the hot melt cast film formulation showed significantly better parameters in all respects compared to both the control and the NLC formulation.
5. CONCLUSION:

Vision problems have become an issue that progressively affects more and more people each year. With age and prolonged exposure to excessive amounts of reactive oxidative species comes increased potential for retinal degeneration, and consequently, vision impairment and/or loss. To protect against this, antioxidants can be used to neutralize the reactive oxidative species and inhibit excess production by not only scavenging ROS, but also by mopping up the metal ions that catalyze their production, thereby limiting the amount of ROS in the body. One such antioxidant is quercetin, a bioflavonoid that is found commonly in many foods and that exhibits strong antioxidant properties, among many other potential clinically beneficial uses.

Ocular drug delivery to the back of the eye is difficult, and many barriers must be overcome for successful drug delivery. Specialized drug delivery systems must be employed in such cases. Among these are nanoparticle emulsions, such as solid lipid nanoparticles and nanostructured lipid carriers, as well as hot melt cast films. These delivery systems have been demonstrated in many studies to be successful in delivering drugs to the eye, and they have the added benefit of high patient compliance, compared to other systems, such as intravitreal injections, which are successful but have less patient compliance.

Solid lipid nanoparticles, nanostructured lipid carriers, and hot melt cast films were employed in this study in hopes to be able to successfully deliver quercetin to
the back of the eye where it can act on the retina and protect it from degeneration by damage from reactive oxidative species. Upon optimization and characterization, quercetin nanoparticles were successfully prepared with a good particle size distribution and other physicochemical parameters. Further, in-vitro release and permeability values demonstrate that nanoparticles can be successfully employed for delivery of quercetin into the eye through the topical route of administration. The hot melt cast films were also demonstrated to have good release and permeability profiles. In fact, there was a significant enhancement of transcorneal permeability of quercetin films compared to the control and nanoparticles. These results demonstrate that hot melt cast films can also be successfully employed for delivery of quercetin into the eye through the topical route of administration and may be a better delivery system for quercetin administration compared to the nanoparticles.

Future direction for this research includes in-vivo testing of these drug delivery systems. Specifically, drug delivery in live animals can be performed to test permeability and absorption across corneas under physiologic conditions. A long term study can also be performed to test the extent of retinal neuroprotection that quercetin may have in protecting against damage from reactive oxidative species and consequent vision impairment.
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