This is to certify that the
thesis entitled

ENGINEERED POLYSACCHARIDE CARBOXYLATES
MATRICES FOR OCULAR DRUG DELIVERY

presented by

Laura Marie Fisher

has been accepted towards fulfillment
of the requirements for the

M. S. degree in Chemical Engineering

Major Professor's Signature

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Date

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ENGINEERED POLYSACCHARIDE CARBOXYLATE MATRICES FOR OCULAR DRUG DELIVERY

By

Laura Marie Fisher

A THESIS

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ABSTRACT

ENGINEERED POLYSACCHARIDE CARBOXYLATE MATRICES FOR OCULAR DRUG DELIVERY

By

Laura Marie Fisher

Two to three million Americans suffer from glaucoma. There exist treatments such as beta blockers, but their ability to deliver drugs to the eye is minimal (<5%). The overall goal of this project is to develop a biocompatible, biodegradable, in situ- gelling, ocular drug delivery system using proprietary cellulosic/starch carboxylate copolymers. Sustained drug delivery in topical formulations for treating glaucoma, inflammation, infection and dry eye will be targeted. There is a need for an ocular drug delivery system that has the characteristics of long retention time, ease of use, ease of manufacture and overall acceptance by the patient as evident by many review articles on the subject. This product would be formulated with current topical ophthalmic drug preparations to provide a longer retention time reducing the overall dosing frequency and increasing bioavailability of the drugs. It would use the physiological properties of the eye (pH, temperature and ionic strength) to turn a clear, topical application into a gelling matrix when placed in the eye. The kinetics of the starch and cellulose oxidation are shown to fit a third order model. Drug release studies were conducted using ofloxacin and the matrices are shown follow Higuchi's model for diffusion from a hydrogel.

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Chapter 1. Introduction

Like many current areas of scientific research, the development of drug delivery matrices lies at the intersection of two traditional disciplines. Applying traditional material science and chemical engineering knowledge to pharmaceutical knowledge has led to the development of many new drug delivery systems within the last decade. Pharmaceutical companies have found that they can reformulate current pharmaceutical compounds to have better efficacy, bioavailability and fewer side effects by improving the method of delivery. The many methods of drug delivery include transdermal, subcutaneous, intravenous, and intramuscular delivery (Saltzman). One specific current area of interest to pharmaceutical companies is ocular drug delivery because of the wide gap between the amount of drug administered and amount of drug that reaches the targeted site.

1.1 Objectives

The primary objective of the thesis is to evaluate the potential of using a carboxylated polysaccharide as an ocular drug delivery matrix that provides a controlled release gel when placed in the eye. The overall goal is increasing the bioavailability of the drug in the anterior region of the eye. It is known that carboxylated polysaccharides have the properties of being water dispersible and biocompatible. This is why materials containing carboxylated flexible chain segments were chosen to be studied. The objective the work presented here is attained by:

- Carboxylating starch and cellulose using different methods of oxidation;
- Characterizing the materials synthesized using titration, FTIR; and,

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- Conducting *in vitro* release studies of ofloxacin using the developed matrices.

1.2 Organization Of The Thesis

The thesis is divided into three main parts. The first part, Chapter 2, reviews the need of new drug delivery systems and reviews overall advances. It proceeds into detail regarding the need for new ocular delivery systems.

The second part, Chapters 3 through 8, details the experiments performed to evaluate a newengineered carboxylated polysaccharide system, in which the carboxy groups are on flexible chain segments. Flexible chain segments are important to design into the system because we want to use the carboxy groups to chelate with Ca ions and form in-situ clear gels.

The third part, Chapter 9, explores the commercial aspects of developing an improved ocular drug delivery system.

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Chapter 2. Drug Delivery Systems

Therapeutics can only be effective if they reach their intended site of action. For treatments such as topical cortisones applied to a rash on the skin, this is not difficult; but for many other treatments it can be very difficult due to such factors as absorption, distribution, binding, biotransformation and excretion. This chapter will give an overview of general drug delivery principles and techniques in the first section and then focus on issues peculiar to ocular drug delivery in the second section.

2.1 Drug Delivery Principles

Drug delivery involves getting a treatment to the site of action. This can be as simple as applying a cream transdermally, with the skin being the site of action, or as complicated as having a chemotherapy agent injected intravenously and reaching the intended cancerous lung cells. In all cases the drug must pass through membranes, and then be carried to the site of action. Cell membrane transport can be either passive or active. In passive transport systems, the approach is diffusion through a membrane due to a concentration gradient and is influenced by size and concentration; while active transport involves some type of biological assistance such as 'tricking' the body into thinking a drug is another needed structure.

Figure 1 schematically describes the relationship among the factors affecting drugs in the body. All of these factors are influenced by the characteristics of the drug including molecular size and shape, solubility at the site of absorption, degree of ionization, and relative lipid solubility of its ionized and nonionized forms which will

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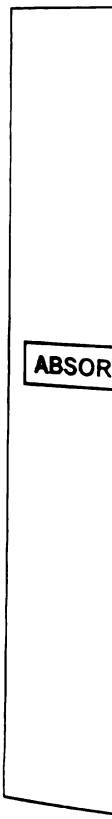

Figure 1. Drug

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affect membrane transport (Fechner, Teichmann et al. 1998). All of these factors affect the bioavailability of a drug and describe the extent to which a drug itself reaches its site of action or a biological fluid transporting the drug can reach the site of action. For example, if a drug first reaches the liver where it is mostly metabolized before it reaches the site of action, it would have very low bioavailability.

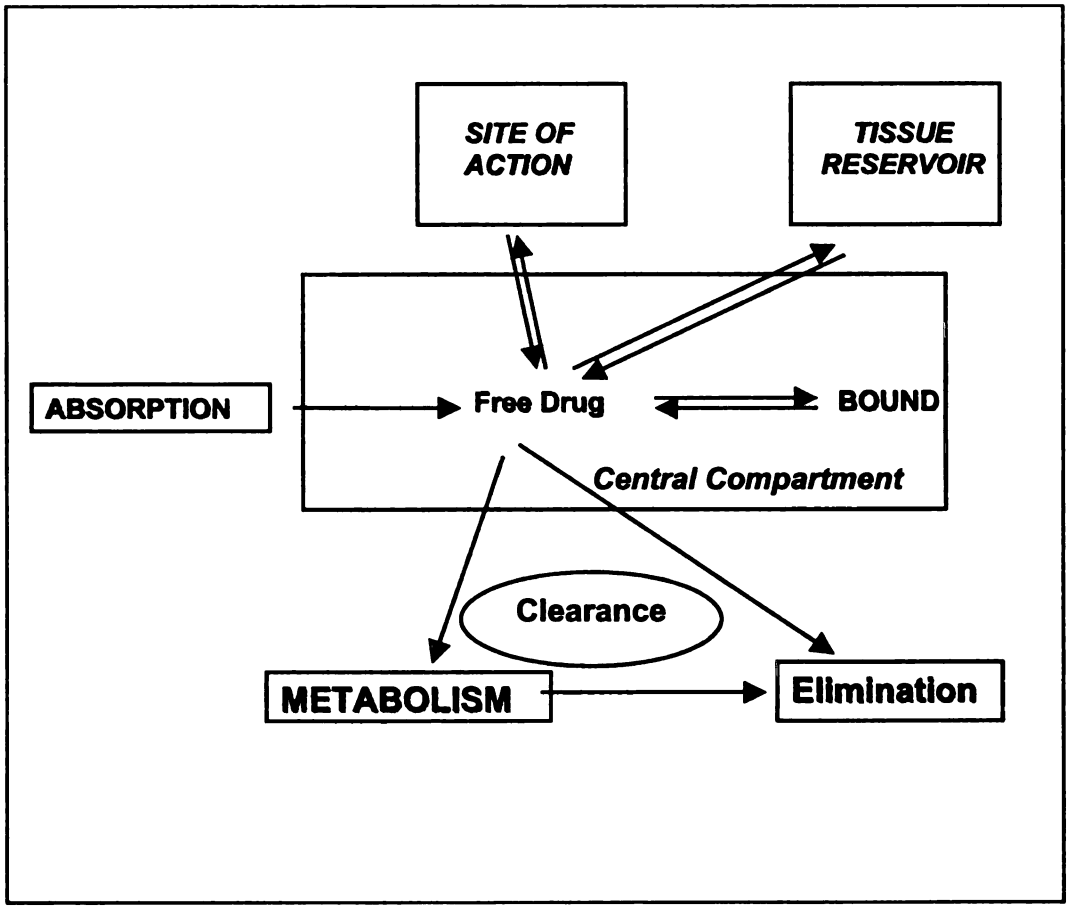

Figure 1. Drug Pathway

Solubility plays an important factor in drug administration because of the cellular transport required. A cell membrane consists of a lipid bilayer, while cellular fluid is more polar. Most drugs are weak acids or bases that are present in solution as both the nonionized and ionized species. The nonionized molecules are usually lipid soluble and

diffuse across the cell membrane, while the ionized forms are usually unable to penetrate the cell membrane because of their polarity.

If a drug has a low bioavailability, it may have to be administered in a large dose to ensure the required dose will reach the site of action. This high dose is problematic if the drug has other known side effects. Therefore, a constant steady-state dose is preferred over a large one-time (bolus) dose. This steady-state can be reached by frequent repeated administrations. Once a drug is administered, its half-life will determine how long it will remain in the body and indicate how frequently a drug must be administered to reach a steady-state concentration. Figure 2 illustrates the pharmacokinetic relationships between dosing and steady-state. The average steady-state concentration, C_{ss}, can be found using the following equation:

$$C_{ss} = F * dose / (Cl * T) \quad (1)$$

where:

F = the fractional bioavailability
T = the dose interval (time)
Cl = the clearance rate
Dose = the concentration

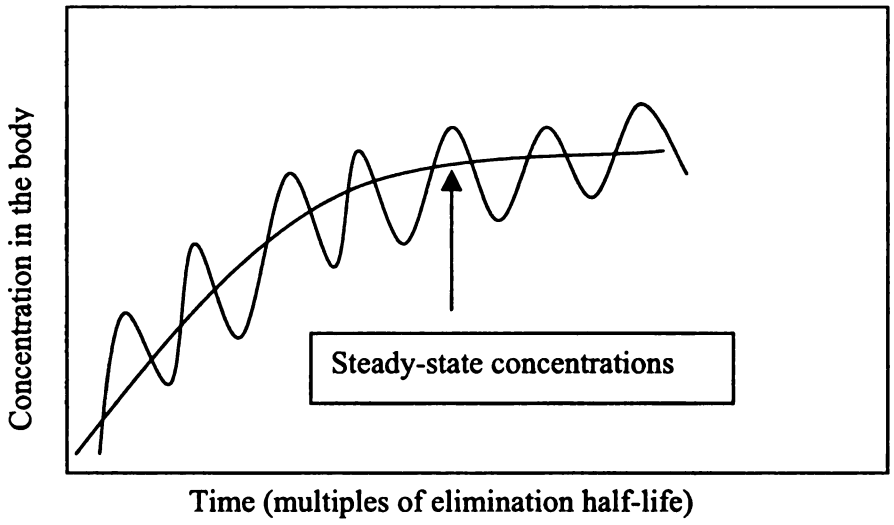

Figure 2. Generalized drug release profile

The concentration of the drug that is required at steady state is dependent on the dose-response effect of the drug which is the relationship between the amount of drug applied and the measured effect (Jose, Polse et al.). Typically, as the drug concentration is increased, the therapeutic benefit increases until a certain point called the maximum effective dose is achieved. Another important property necessary for correct dosing procedures is the time-response curve of a drug. Certain drugs may have a lag time before they become effective, while others may instantaneously act. Knowing these pharmacodynamic parameters of a drug is necessary to determine when to expect the desired effect and whether additional doses are necessary. Those properties are found experimentally in clinical trials and are not examined in this research.

2.1.1 Drug Delivery Systems

Briefly, this section will describe some current methods and materials being used to obtain a sustained delivery. Hydrogels and other biodegradable polymers will be discussed briefly. The use of biodegradable polymers for site-specific drug delivery has attracted much research.

Natural and modified natural gums (Bhardwaj, Kanwar et al.) have been widely used in pharmaceutical applications because of their wide use in the food industry and their general recognition as safe. Materials that have been widely studied include sodium alginate, carrageenans, cellulose ethers, chitosans, guar gum and modified starches. In general, these polysaccharides have significant quantities of oxidized groups in addition to their normal polyhydroxy format. Please see Figure 3 for representative structures. Simply put, they function by hydrating and forming a gel when in contact with water. The drug contained in the matrix is expected to release through the gel layer providing a constant-rate sustained release. Some polysaccharides, such as sodium alginate, form gels under more specialized conditions such as the addition of calcium ions and sensitivity to gelling at certain pH levels.

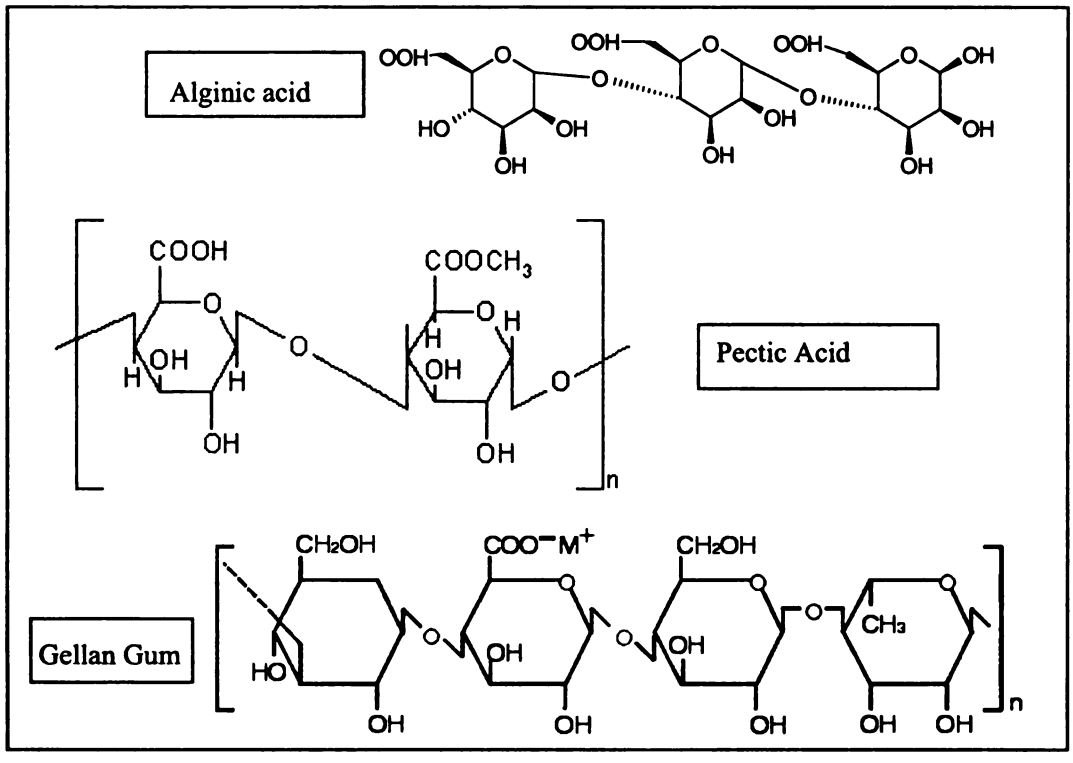

Figure 3. Structures of some natural polysaccharides

Another interesting area that affects distribution of a drug subsequent absorption is drug targeting. A drug delivery matrix can actively target a site such as "cancer"-labeled chemotherapy agents or passively target an area, such as enteric coated drugs that resist destruction in the stomach and that will release in the intestines where they can be absorbed. This is a large area of active research, but will not be reviewed further in this paper.

Hydrogels (Peppas) have been studied because of their ability to swell in water or biological fluids. Hydrogels that have been studied for drug delivery applications include ones related to poly(2-hydroxyethylmethacrylate) or HEMA, poly(ethyleneoxide), and various cellulose derivatives. Hydrogels are particularly useful in areas where there is a high moisture area such as in wounds, for vaginal treatments and drugs that act in the stomach. Peppas did an extensive review of water-swollen cellulose derived hydrogels

and their applications, specifically related to prolonged and controlled release applications. Specific to this research, Peppas notes that treated cellulose with higher carbonyl and carboxyl content are claimed to be more stable, to exhibit more regular and sustained release properties and to be effective at a much lower concentration. It is also noted that carboxyl-containing derivatives have stronger muco-adhesive capacity which is important in this research with regards to increasing retention time in the eye as the eye contains a mucin layer on the surface. Data from a controlled release study of diazepam in a 15-mg tablet show that the controlled release formulation administered once daily was as effective as five doses of a 5-mg unformulated tablet. This reduction of dosing frequency from five times per day to one time a day can increase patient compliance. This study also showed the ability of a controlled release system to lower the side effects of the drug, in this case minimizing sedation caused by the rapid raise in the plasma level.

2.1.2 Mechanisms and mathematical models for drug release from gel-forming matrices

Peppas describes the release of drugs from a gel-forming matrix as a three step process. The first is the *initial burst* when the liquid dissolves the drug present at the immediate surfaces of the matrix, creating a small "burst" effect. At this time the water or biological fluids begin to penetrate the gel at a rate that is dependent on the porosity of the matrix. The second phase is classified as the *stationary phase*, where the water continuously penetrates the matrix at a constant rate. This penetration is accompanied by an expansion of the gel layer in the direction of the external medium. This phase accounts for the majority of the drug release. It is generally accepted that the release of the drug is controlled by diffusion process, not by the rate of drug dissolution or the rate

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of penetration of the front for hydrophilic matrices. The third phase is the *exhaustion period* which begins when the penetration front has reached the center of the matrix and the drug concentration has dropped below its solubility limit in water. During this stage the release rate rapidly falls.

These controlled release systems can either be classified as systems with suspended drugs or systems with dissolved drugs. Mathematical models for both have been developed and adapted by various authors, and Higuchi's model for systems with dissolved drugs can be used for this research. This model is presented for the case that the system is homogenous, there is one plane of diffusion, there is no diffusion boundary layer present and there are sink conditions. In this situation, the initial concentration of the drug in the hydrated matrix is less than the drug solubility in it ($C_o < C_m$). Setting $C_o = M_\infty/V$, where M_∞ is the initial drug loading (the total amount of drug release at infinite time) and V, the effective volume of the hydrated matrix, the following expressions are valid for $0 \leq M_t/ M_\infty \leq 0.6$:

$$M_t = 2AC_o\left(\frac{D_m}{\pi}\right)^{1/2} \bullet t^{1/2} \qquad (2)$$

The rate of release is:

$$\frac{dM_t}{dt} = 2AC_o\left(\frac{D_m}{\pi}\right)^{1/2} \bullet t^{-1/2} \qquad (3)$$

where:
M_t = the amount of drug released at any time
M_∞ = the initial drug loading
A= the diffusional area
C_o= the initial concentration of the drug in the system
D_m = the apparent diffusion coefficient

These equations are for the planar case, but can be modified for other shapes. All of these cases will also show a $\sqrt{t}$ dependency. Peppas brings up faults with this model due to the following assumptions:

1. This model was not developed for systems undergoing dimensional change.
2. A pseudo steady-state analysis was used which ignores the external mass transfer resistance and is only valid when the solute loading is in great excess of it solubility limit.
3. The countercurrent solvent diffusion was not considered
4. Drug diffusion in the gelled matrix was assumed to be the rate limiting step.

However, the shortcoming of these assumptions are not as much a problem with the ocular system being described because the gel is already hydrated when applied, will not undergo dimensional change, and solvent diffusion can be neglected.

Matrix formulations play an important role in the drug release profiles. Release profiles can usually be modified by type and viscosity of the polymers, the polymer concentration, and the drug particle size. The type of polymer is often determined by the solubility characteristics of the drug. For example, hydrophilic matrices are generally used to prolong the release of highly water-soluble drugs. The viscosity of the polymer appears to play a role in the *initial burst* period of drug release, but have no effect in the *stationary phase* where the majority of release takes place. Polymer concentration follows the general rule that increasing the proportion of hydrophilic material decreases the rate of release. Drug particle size affects the dissolution rate of the drug with the

smaller particles having larger surface/volume ratios and, therefore, faster dissolutions rates.

2.2 Ocular Delivery Systems

The specific focus of the paper is drug delivery to the eye, which has been identified as a crucial area because of the unique physiological and anatomical properties of the eye. With current topical treatments, only 1%-5% of the drugs even reach the site of action (Mindel), which is counterintuitive considering the short distance between the site of application and the site of action. There is a need for an ocular drug delivery system that has the characteristics of long retention time, ease of use, ease of manufacture and overall acceptance by the patient as evidenced by many review articles on the subject (Urtti 1995),(Kaur and Kanwar 2002) (Jarvinen, Jarvinen et al. 1995) . Complicating these research efforts is the fact that there are unique pharmacodynamic, pharmacokinetic, and drug delivery issues for ocular drug therapy owing to the eye's distinctive anatomical and functional properties. Treatment of ocular diseases, especially diseases of the retina, is often a drug delivery problem. Improving topical applications would include not only drugs for treatment of retinal degenerative diseases and glaucoma, but also anti-bacterial agents, corneal wound repair, and intraocular treatments.

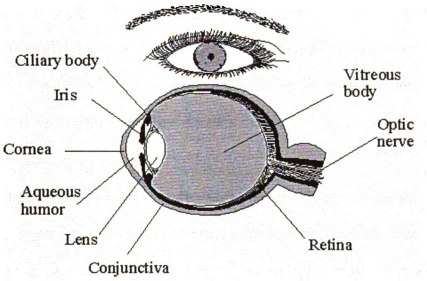

Figure 4. Structure of the eye

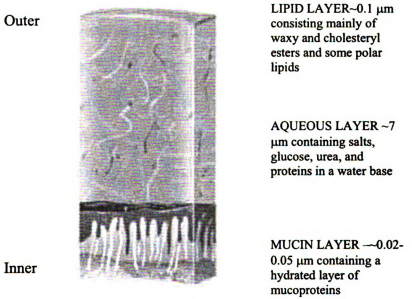

Figure 5. Tear fluid composition

2.2.1 Bioavailability

To understand the unique drug delivery issues associated with the eye, the structure of the eye is the first consideration. Reasons for low bioavailability include the blinking reflex,

tear turnover, and low corneal permeability (Burrows, Tsibouklish et al.). Figure 3 shows a schematic cross section of the eye. Treatments for glaucoma, infections and allergies target the anterior section of the eye, while treatments for retinal diseases must reach the posterior section of the eye.

Anatomy of the eye

The unique anatomy of the eye must be understood before attempts at drug delivery can be made. The three areas of interest in the eye with respect to topical application of drugs are the cornea, the conjunctiva and the nasolachrymal drainage system (Burrows, Tsibouklish et al.). The cornea is considered to be the main pathway for the permeation of drugs into the eye. It is approximately 0.5 mm thick in the central region and up to 0.7 mm thick at the periphery. It is an optically transparent tissue that conveys images to the back of the eye and covers about one-sixth of the total surface of the eyeball. In terms of drug delivery, the cornea can be considered to comprise three distinct layers, which accounts for the poor permeability characteristics. First, the outer epithelium is lipophilic in nature consisting of 5-6 layers of cells and tight junctions, thereby making it the most significant barrier to drug delivery. The second area is the stroma, which accounts for approximately 90% of the corneal thickness and which is a relatively open hydrophilic region. The final important region is the inner endothelium, which consists of a single layer of flattened cells and which is in direct contact with the anterior chamber. Because of both hydrophilic and hydrophobic regions, the cornea provides an effective barrier to drug transport.

The second major region of the eye with importance to drug delivery is the conjunctiva - a thin, vascularized mucous membrane that lines the inner surface of the

eyelids and the outer region of the cornea. It is involved in the formation and the maintenance of the tear film that coats the outer region of the cornea. The precorneal region of the human eye has a surface area about 17 times greater than (Jarvinen, Jarvinen et al.) the cornea and provides an alternative absorption path for drugs applied topically. Intercellular spaces of the conjuctival epithelium are wider than those in the corneal epithelium, making permeabilities of hydrophilic drugs typically an order of magnitude greater than corneal permeabilities. This order of magnitude difference does not occur for moderately lipophilic, small molecules. Also considered part of the precorneal region is the tear film which covers the cornea (Figure 5). This region is of importance because the applied drops will mix with tear film and must be compatible therewith to reach the cornea. The tear film is approximately 9-10 μm thick and consists of a superficial oily layer, an aqueous layer with proteins, electrolytes and other small molecules, and a mucin layer.

The nasolachrymal drainage system accounts for most of the drug loss. It consists of the secretory, distributive and excretory systems. The excretory part of the nasolachrymal drainage system includes the lachrymal puncta, the superior, inferior and common canaliculi, the lachrymal sac and the nasolachrymal duct. It is thought that tears are largely absorbed by the mucous membranes of the ducts and that only a small amount reaches the nasal passages (Davies 2000). This leads to another route of systemic absorption of the applied drugs. The cul-de-sac of the eye normally holds 7-9 μl of tears and can contain up to 20-30 μl if care is taken not to blink. The normal tear flow rate is 1 μl per minute and the pH is maintained at 6.5-7.6. The high turnover of tear fluid

(approximate 15% per minute) and the limited capacity of the sacs lead to a high clearance rate of drugs once applied.

Eye diseases

Glaucoma, eye allergies and irritation, and eye infections are generally treated topically. Topical application of drugs is preferred because (Davies): 1)drug effects are localized and less drugs enter the systemic circulation., 2) it facilitates drug absorption into the eye that is otherwise hard to target, 3) it avoids hepatic first-pass metabolism and 4) it is a relatively convenient, simple and painless method of administration. Glaucoma is a group of disorders characterized by progressive damage to the eye at least partly due to intraocular pressure damaging the optic nerve. It is the second most common cause of blindness in the USA, with roughly 2 million Americans affected . Because glaucoma comes in many forms, there is not a universal treatment for it. In general, glaucoma is caused by disruption of the aqueous outflow of the eye through the anterior chamber angle by either a physical obstruction or by other factors, such as hypertension or diabetes. Treatments can be categorized by either increasing outflow or by decreasing aqueous production. Table 1 shows a list of drugs that are commonly used to treat glaucoma and their mechanism of action.

Table 1. Glaucoma treatments and their method of action

Type	Drug Examples	Mechanism of Action
Miotics, direct and indirect acting	Pilocarpine Carbachol Physostigmine Neostigmine	Causes miosis, increase aqueous outflow, cause accomodation
Carbonic anhydrase inhibitors	Acetazolamide Dorzolamide	Decrease aqueous production
Non-selective adrenergic agonists	Epinephrine Dipivefrin	Cause mydriasis, increase outflow and decrease fluid production
α_2-selective adrenergic agonists	Apracionidine Brimonidine	Decrease aqueous production, increase uveoscleral aqueous outflow
β-blockers	Timolol Betaxolol Levobunolol	Decrease aqueous production, does not affect pupil size
Prostaglandin analogs	Latanoprost	Increase uveoscleral outflow rather than altering conventional aqueous outflow
Osmotic diuretics	Glycerin Mannitol(IV)	Hypertonic plasma draws fluid from eye

Other common diseases of the anterior section of the eye include (Burrows, Tsibouklish et al.):

Conjunctivitis - an inflammation of the conjunctiva that may be caused by bacterial and viral infection, pollen and other allergens, smoke and pollutants.

Dry eye syndrome - the inadequate wetting of the ocular surface.

Keratitis - an inflammation of the cornea, caused by bacterial, viral or fungal infection.

Iritis (anterior uveitis) - commonly having acute onset with the patient suffering pain and inflammation of the eye. Other rare conditions include the ophthalmic complications of rosacea, blepharitis (inflammation of the lid margins) and chalazia (Meibomian cysts of the eyelid).

Table 2 lists some of the common treatments for these conditions.

Table 2. Common ocular treatments

Classification	*Examples*	*Typical Indications*
Antibacterials	Chloramphenicol, gentamicin, fusidc acid	Conjunctivitis, keratitis, blepharitis
Anitvirals	Aciclovir, idoxuridine	Viral infections such as dendritic corneal ulcers, keratitis
Corticosteroids	Betamethasone, prednisolone, hydrocortisone	Uvetis, scleritis
Local anaesthetics	Amethocaine, lignocaine	Anesthesia during treatments
Anit-inflammatory agents	Cromoglycate, nedocromil, antihistamines	Inflammation and allergic conjunctivitis

Retinal disorders encompass a group of diseases that are not currently treated topically but potentially could be if the drug delivery vehicle was improved. Age-related macular degeneration is the leading cause of visual loss in the elderly.

Reasons for not reaching

While there are many topical applications, all of them in common have very low bioavailability. The most common method of ocular drug delivery is the instillation of 30-50 μl drops into the lower cul-de-sac. The concentration of the drug in the precorneal area provides the driving force for its transport across the cornea via passive diffusion.

Therefore, efficient ocular drug absorption requires good corneal penetration as well as prolonged contact time with the corneal tissue (Burrows, Tsibouklish et al.). These are hindered by the rapid solution drainage, systematic absorption through the conjunctiva, and the limited surface area of the corneal barrier. Figure 6 shows schematically the competing factors related to topical ocular drug administration.

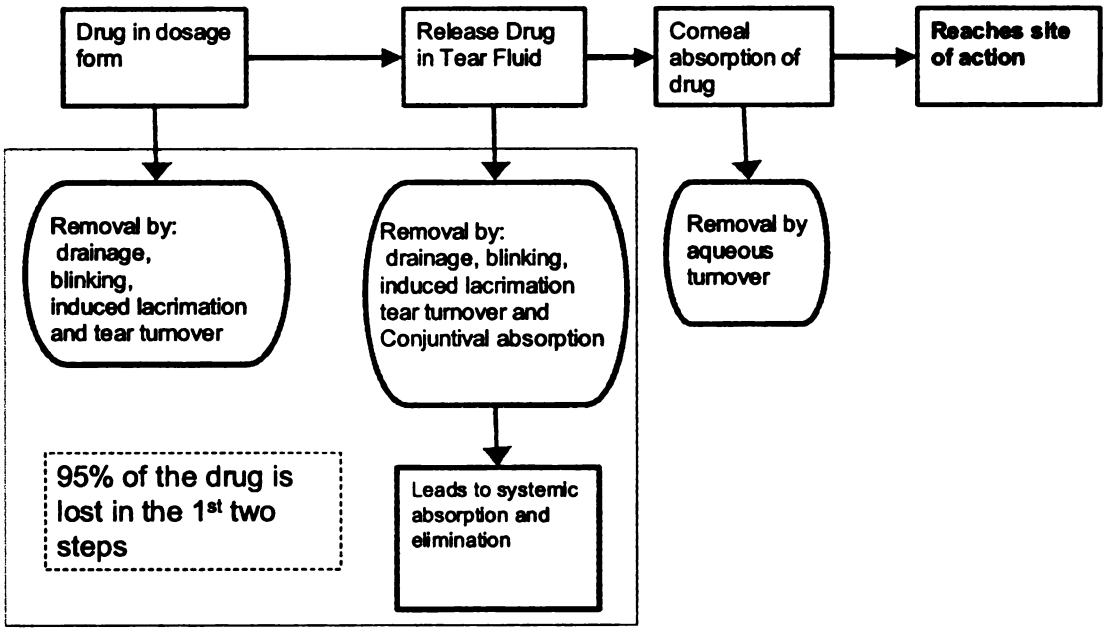

Figure 6. Drug pathway in the eye

Drug release in vivo and precorneal kinetics

An ocular drug must reach its action site in adequate concentration to be efficacious.

The steady-state concentration of a drug in the precorneal tear fluid is a function of drug release rate in *vivo,* rate of drug clearance via tear turnover, drug clearance from the lacrimal fluid to the cornea, drug clearance from the lacrimal fluid to the conjunctiva, drug permeability in the conjunctiva, conjunctival surface area, corneal permeability of the drug and the corneal surface area.

If the dosage form flows partly or completely from the eye after administrations, as viscous vehicles, gelling systems, nanoparticulates and liposomes do, it is very important that the drug release rate and the rate of dosage form drainage from the eye match each other. If the release rate from the matrix is too slow and not compensating for the drainage of the free drug, the overall bioavailability could be reduced. The mechanism for the increased rate of drug release in lacrimal fluid is not known exactly, so it is important to collect both *in vivo* and *in vitro* data to correctly design a system.

As can be seen by Figure 6, there are many paths leading to systemic absorption. The main routes of absorption are the nose and conjunctiva (Urtti). It has been shown that by using a viscous carboxymethyl cellulose vehicle without release rate control, the systemic peak concentrations of timolol were decreased in rabbits 2-3 times, probably due to the longer precorneal retention and the slower vehicle spread to the nasal mucosa. This is a very important finding for some ocular drugs such as beta blockers which can lead to heart attacks in patients with heart conditions and even for other glaucoma drugs which may have side effects such as drowsiness

2.2.2 Materials

Natural Polymers

Natural polymers and gums have been used in pharmaceutical formulations of sustained-release carriers, and modified celluloses; carboxy methylcellulose (CMC) and MMC are found in a large number of ocular formulations as viscosity enhancers. Because of the wide acceptance of these modified natural polymers, pharmaceutical companies are interested in the use of modified natural polymers for their ocular drug

delivery systems. Natural polymers with gelling properties that have been successfully used in ocular topical formulations include gellan gum and carrageenans. Ocular topical formulations with gelling properties afford increased ocular bioavailability of certain drugs. Figure 7 below is work done by Vrbanac that shows the increased bioavailability of timolol, a glaucomic agent, in rabbits with a formulation including Gelrite.

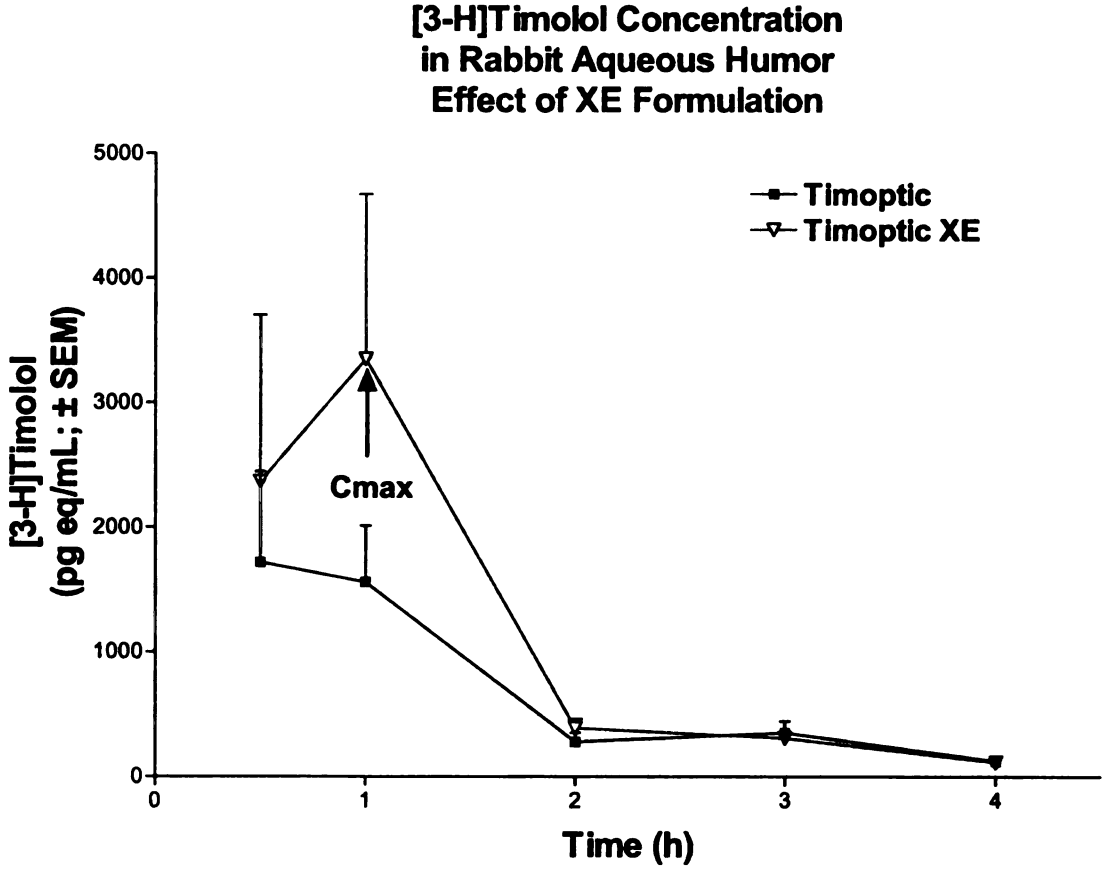

Figure 7. Timolol concentration in rabbit aqueous humor

A review of literature and patents shows that much of the focus in this area is centered around natural polymers. Gelrite®, a registered trademark of Monsanto, is used by Merck in a preparation of timolol, Timoptic XE. This is the only known *in situ*

gelling drug delivery system currently on the market. According to the package insert it requires half of the doses as the standard Timoptic. It is a low-acetyl gellan gum which would have a structure similar to Figure 3 and can ionically crosslink in the presence of a divalent cation such as calcium. Rozier et al has shown that the in *in vivo* testing, Gelrite behaved similar to HEC (hydroxyethylcellulose), a known viscosity enhancer. It significantly reduced intraocular pressure over the HEC, which was determined to be caused by an increased residence time at the surface of the eye.

Another natural gel-forming polysaccharide is alginate. Cohen et al. describe an alginate system that gels in the presence of calcium ions in the eye. Alginate is a mixture of guluronic and mannuronic acids as seen in Figure 3. They suggest using a mixture with the guluronic acid concentrations higher than 65% to form a suitable gel. When testing pilocarpine, a common glaucoma treatment, the alginate formulated system demonstrated a correlation between the gelation capability of the alginate formulation, the speed at which it occurs and the sustained release properties. It was also claimed that there was excellent ocular tolerance in the test rabbits; even though redness of the conjunctivae was reported for 1-2 hours after instillation of the drops.

A final natural polysaccharide that can form gels in situ is pectin (see Figure 3). A patent filed by Ni and Yates claims that pectin isolated from Aloe Vera, which contains a higher galacturonic acid ratio will form a gel when subjected to mono- or divalent ions at a low pectin of concentration of 0.25% w/v. It will also form a gel in the presence of small organic compounds, proteins, nucleic acid, and live cells.

Gelfoam ® is a structured matrix of gelatin has been studied for the release of pilocarpine. The matrix is a structured water-insoluble sponge prepared from purified

po

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pork skin gelatin that will biodegrade. Because this simple matrix released most of the drug within 15 minutes, retardants had to be added. This matrix embedded in cetyl ester wax demonstrated zero-order release kinetics while the matrix impregnated with polyethylene glycol 400 monostearate exhibited close to first-order kinetics. The results show that gelatin itself does not provide for good sustained release. The following table summarizes work in ocular drug delivery systems.

Table 3. Ocular drug delivery systems

Matrix Material	Method of action	Author
Natural Polymers		
Alginate	Ionic concentration	Cohen (Cohen 1998)
Gellan Gum	Ionic concentration	Rozier(Rozier, Mazuel et al. 1989)
Pectin	Ionic concentration	Ni(Ni and Yates 2002)
Gelatin	Not *in situ* gelation	Nadkarni(Nadkarni and Yalkowsky 1993)
Cyclodextrins	Not *in situ* gelation	
Synthetic Polymers		
Poloxamer	Temperature change	Lin(Lin and Sung 2003)
Pluronic	Temperature change	Lin (Lin and Sung 2003)
Carbopol	pH change	Lin (Lin and Sung 2003)
Cellulose acetophthalate	pH change	Gurney (Gurney 1986)

Synthetic polymers

Many synthetic polymers have been tested for sustained release in the eye. While they have the advantage of being engineered to specific applications, their breakdown products are not always known potentially leading to extended FDA testing. A patent by Hong-Ru Lin explains a formulation approach of combining Carbopol and Pluronic. Carbopol is a high molecular weight carboxy vinyl polymer and Pluronic is a class of

block copolymers containing polyoxyethylene and polyoxypropylene. This formulation claims to be free-flowing at non-physiological conditions (pH 4.0 and 25° C), but forming a gel at physiological conditions (pH 7.4 and 37° C). A disadvantage to this system is the high amount of Pluronic (14%) required for optimal gel formation. Again there are many disadvantages of synthetic polymers including high polymer concentration, irritancy and potentially harmful breakdown products.

Dicarboxy polysaccharides were chosen for this work because of their water dispersibility, their potential muco-adhesive capacity and because their breakdown products are known and safe. Experimental data by Singh et al. has shown that similar dicarboxy cellulose matrices are biocompatible and can be broken down in the body by metabolism into 2-carbon and 4- carbon intermediaries.

Chapter 3. Engineering Carboxy Containing Flexible Chain Segment Polysaccharides

3.1 Synthesis

The objective of the work is to find a flexible, water dispersible, biocompatible material that would have the ability to encapsulate a drug in the physiological conditions of the eye. The natural polysaccharides listed in the previous chapter exhibit some of these characteristics such has biocompatibility and water dispersibility but they were not optimized for the conditions of the eye. The carboxyl content of these polysaccharides were improvements could be made. It was determined that engineering carboxy groups onto a natural polysaccharide backbone would be the best method to achieve this. Starch and cellulose were chosen as the polysaccharide backbone because of their abundance and their current acceptance in other pharmaceutical applications. The oxidation of starch and cellulose is not a new science, but the application of these oxidized polysaccharides to ocular drug delivery systems is novel. Different methods of oxidation are known including the use of sodium periodate, hypochlorite, or ozone. All of these methods can be used separately or combined until the desired material properties are achieved. Oxidation by sodium periodate was studied most extensively in this research because it has the best method to control the position and extent of oxidation.

Cellulose and starch both consist of repeating glucose units with only the glycosidic bond differing as seen in Figure 8. The oxidation methods could be applied to either structure theoretically, though there would be differences in the kinetics because of

the structure of the materials. Starch is composed of amylose that forms a helical structure. When the material is hydrated the helices open and water can penetrate the material easily. Cellulose on the other hand forms a tight crystalline structure that is not as easily hydrated.

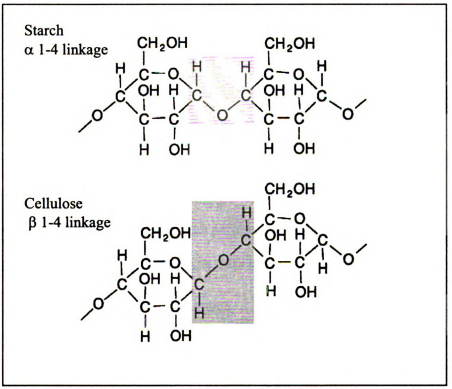


Figure 8. Structure of starch and cellulose

In the periodate method the starch/cellulose ring is opened between the C-2 and C-3 using $NaIO_4$ in the first step (Floor, Kieboom et al.) which forms an aldehyde structure. Secondly, that dialdehyde is oxidized using any oxidizing agent (i.e. NaOCl) and carboxyl groups will be formed at the C-2 and C-3 (see Figure 9).

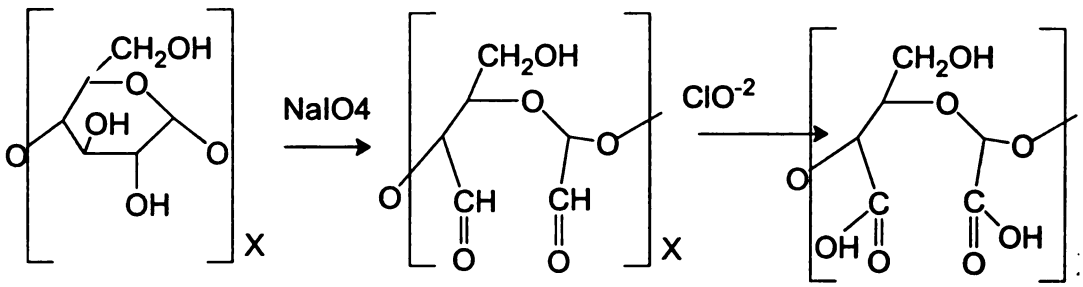


Figure 9. Oxidation of starch reaction scheme

In this method, by controlling the amount of ring opening, the total amount of carboxylation can be controlled. Floor (Floor, Kieboom et al.) described a process where the second step oxidation uses hydrogen peroxide as an inexpensive HOCl scavenger that will reduce the HOCl. The reaction is as follows:

$$R\text{-}CHO + ClO^{-2} \rightarrow R\text{-}COO^{-} + HOCl$$

$$HOCL + H_2O_2 \rightarrow HCl + H_2O + O_2$$

This was important improvement over previous methods which used ClO^{-2} as a scavenger. Besides being less toxic and less expensive, Floor reports that this method gives higher yields of the dicarboxy polysaccharide with superior calcium sequestering properties as compared to the reactions using chlorite as the scavenger.

By controlling the amount, nature, and conditions of oxidation or hydrolysis, the percent carboxyl groups incorporated, the position of attachment, and the molecular weight can be controlled. By effectively controlling the first periodate oxidation step,

copolymers can be formed that contain both the structure of the glucose ring and the flexibility of the open ring structure with –COOH groups on them (structure IV).

Structure IV

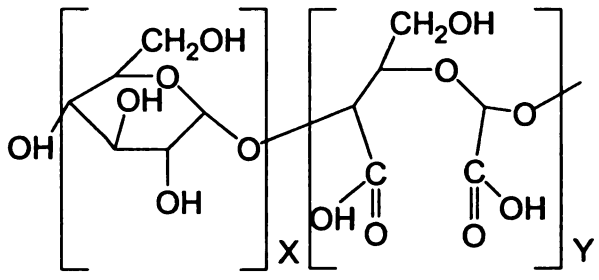


Figure 10. Copolymer starch structure

A completely flexible copolymer structure can be engineered by partial oxidation of the –CHO groups to –COOH and reducing the remaining aldehyde groups to –OH using sodium borohydride. Polycaprolactone is a biodegradable polymer that can be grafted on the backbones allowing for a method of increasing the hydrophobicity of the drug delivery system as required by certain hydrophobic drugs. The controlled grafting of polycaprolactone onto polysaccharide backbones has been patented by Narayan (Narayan)

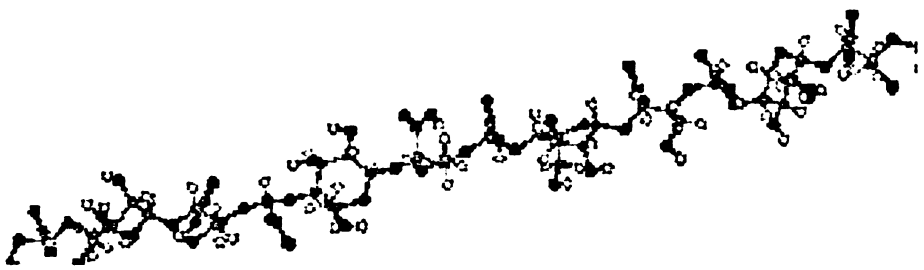


Figure 11. 3D Structure of 50% dicarboxy starch

Also, the dicarboxy polysaccharides are stable at the alkaline pH of the washing process but are degraded under acidic wastewater (pH 4-5) conditions due to their polyacetal structure. The resulting mono- and oligomeric fragments are readily biodegradable but will not form the structure needed for this application. Floor(Floor, Kieboom et al.) shows that at a pH = 3 the dicarboxy starch can degrade up to 80% in 24 hrs, while at a pH=7 it will only degrade 20% over a 24hr period. This is important to note since ophthalmic solutions are usually formulated around pH=7.4. Also this brings to light how easily hydrolyzed this material is. Erythronic and glyoxylic acids are the principal acidic hydrolysis fragments with minor amounts of glycolic, oxalic and formic acids. This indicates the C2-C3 dicarboxy polysaccharide structure stays intact. (Floor, Kieboom et al.)

These carboxylated cellulosic derivatives can then form gels with the addition of divalent cations, such as Ca^{+2}, which is naturally found in the eye. The rate of gelation, the gel strength and the release profile are controlled by percent carboxyl group engineered onto the polymer chain, its position on the polymer chain, and the molecular weight of the polymer chain. Floor (Floor, Kieboom et al.) has also shown that the calcium complexing properties does not differ with respect to the type of glycosidic bond

(i.e the β-1-4 linkages of cellulose compared to the α 1-4 linkages of starches) . It is also important to note that the calcium complexing ability is strongly dependent on the molecular weight in the region M_W 10^4 to 10^5 and at least a M_W of 10^5 is required for superior calcium complexation.

The dialdhyde reaction may lead into other formations of including a hydrated aldehyde, an hemiacetal, or a hemiadol (Fan, Lewis et al. 2001) . The structures of these are below in Figure 12.

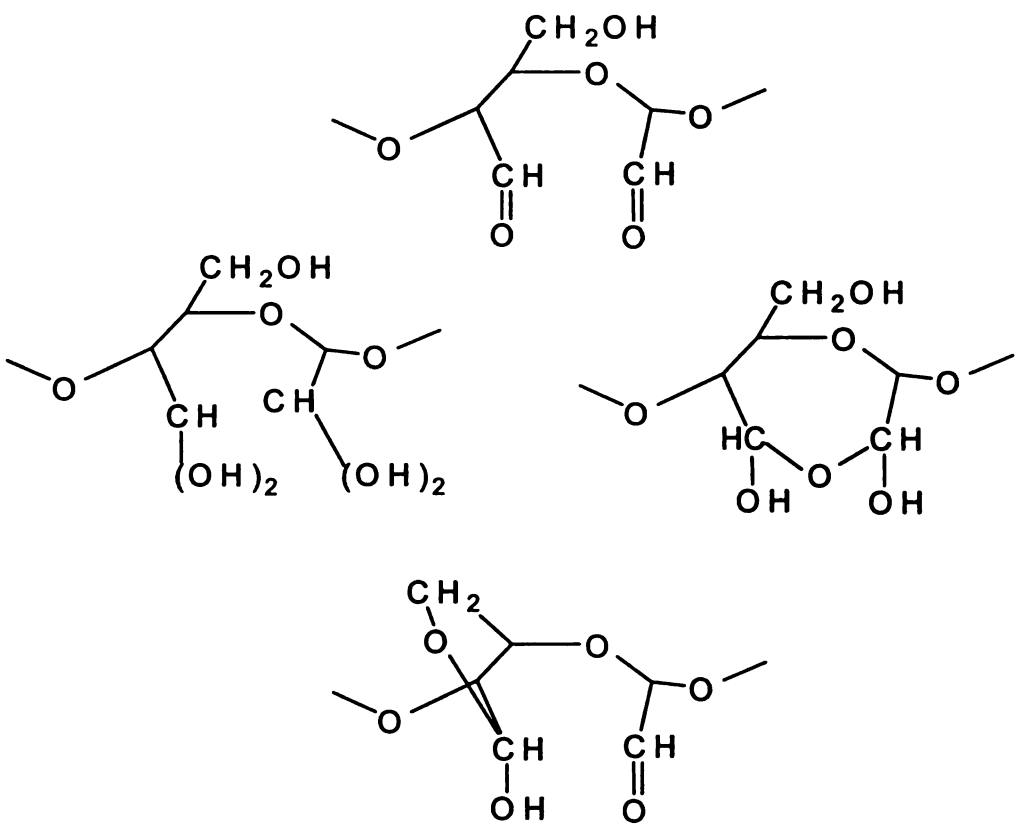


Figure 12. Possible structures of periodate-oxidized starch. 1) free aldehyde 2) hydrated aldehyde 3) hemialdol 4) hemiacetal

3.2 Kinetics of the periodate reaction

The periodate reaction is light sensitive and, therefore, care was taken to exclude light. While, some authors (Besemer, deNooy et al.; deNooy, Besemer et al.; Kim, Kuga et al.) suggest running the reaction at room temperature or colder, Narayan (Engineering, 1984) et al reported the reaction could be run at slightly elevated temperatures with little interference from side reactions. For the following work, the periodate reaction was run at 40° C. Concentrations were used that were similar to earlier work by others. A full description of the reaction conditions is listed in Chapter 5.

It was first proposed (Narayan (Engineering), 1984) that the periodate oxidation of cellulose follow the rate law:

$$r = -\frac{d[P]}{dt} = \frac{K_1[P][C]}{K^{-1} + [P]} \qquad (4)$$

This rate law was explained by being consistent with a mechanism involving the formation of an intermediate cellulose-periodate complex, most likely a cellulose-periodate cyclic diester which would then slowly decompose to the final products

Later an improved explanation of the starch oxidation by periodate was proposed. It has been suggested that the kinetecs follow a 2nd order dependence at t=0, then change to another model at approximately t=10 minutes (Veelaert, Dewit et al. 1994). This work

was conducted using granular potato starch and HPLC for analysis an improvement method over previous papers which used titration to analyze the dialdehyde formed.

Veelaert proposes that after 5 to 10 minutes the reaction deviates from second order kinetics because of the polymeric structure of the material and the possibility of hemi-acetal or acetal formation. The following two rate laws are defined for free and inhibited anhydroglucose units (an acetal neighbor):

$$\frac{d[X]}{dt} = k_1 \mu^2 [S_o]([P_o]-[X]) \tag{5}$$

$$\frac{d[X]}{dt} = k_2 \mu(1-\mu)[S_o]([P_o]-[X]) \tag{6}$$

where [X]=the erythritol concentration at any time

$[S_o]$ = the initial starch concentration expressed as total initial anhydroglucose units

$[P_o]$ = initial periodate concentration

μ = 1-degree of oxidation (1-X/g)

These two equations are combined and from experimental data they observed that k_2 was much smaller than k_1. The previous formulas then can be simplified into:

$$\frac{d[X]}{dt} = \frac{k_1}{[S_o]}([S_o]-[X])^2([P_o]-[X]) \tag{7}$$

Chapter 4. Analytical Methods

4.1 FTIR

A Perkins Elmer System 2000 FTIR was used to characterize samples. The samples were pressed in KBR pellets and run for 16 scans. The wavelength range was 4000 cm^{-1} to 400 cm^{-1}

4.2 Titration

Sodium hydroxide was used to titrate against the COOH groups. The sodium hydroxide was standardized against potassium acid pthalate to obtain its normality. It was titrated to an endpoint indicated by phenolphthalein. A concentration of approximately 1-5wt % was used. Because of the viscous nature of the material, the indicator did not react very quickly and a false endpoint would show up. The protocol used was the indicator staying pink (acid) for 15 minutes without lightening to be considered the endpoint.

4.3 In vitro UV-Visible Spectroscopy

The drug release profiles were conducted using two different set-ups as more equipment became available.

Stir plate method

In the first set up a 15 ml polystyrene centrifuge tube was modified by cutting the tip off and placing a dialysis membrane (Sigma) with a molecular cut-off of 12,000 over the open end. The membrane was secured by wrapping Teflon taping tightly around the tube. The diffusion surface with this setup was 15 mm and 5 ml of the formulated drug was placed in the tube. Twenty-five milliliters of release medium were placed in a

polyethylene cup which was modified by cutting a hole the diameter of the tube in the top along with another hole for sampling and temperature measurements. A 1" stir bar was placed in this cup and the cup was placed in a water bath kept constant at 37° C on a stir plate. One-milliliter samples were taken at time varying intervals and 1 ml of fresh release medium of was added to keep the volume constant at 25 ml. The composition of the release medium, simulated tear solution was as follows:

Simulated Tear Solution I

Sodium Chloride	0.67 g
Sodium bicarbonate	0.2 g
Calcium chloride dihyrdate	0.008 g
Water	to 100 g

This method led to variability because of the variations in the rpm of the stir bar between stir plates. Below is a schematic of the system (Figure 13).

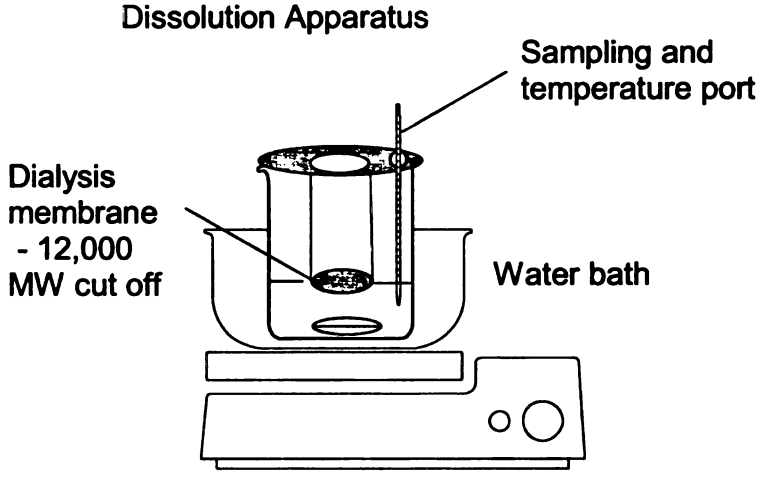


Figure 13. Stir plate dissolution system

USP dissolution method

The second method for obtaining release profiles was using a Hanson EZ-lift dissolution system which had 6 separate chambers that were all kept in the same constant

temperature bath which was regulated by a feedback loop. Each chamber had a rotating paddle attached to the same drive motor. One-liter beakers were used to hold the release medium and they were filled with a specified amount ranging from 400 ml-650 ml. The formulated drug was place in a well 5 ml with a diameter of 5 cm which was covered with the same dialysis membrane. Again 1 ml samples were taken at varying intervals; however the release medium was not replaced in these experiments since the volume difference was considered negligible. Below (Figure 14) is a schematic of the system:

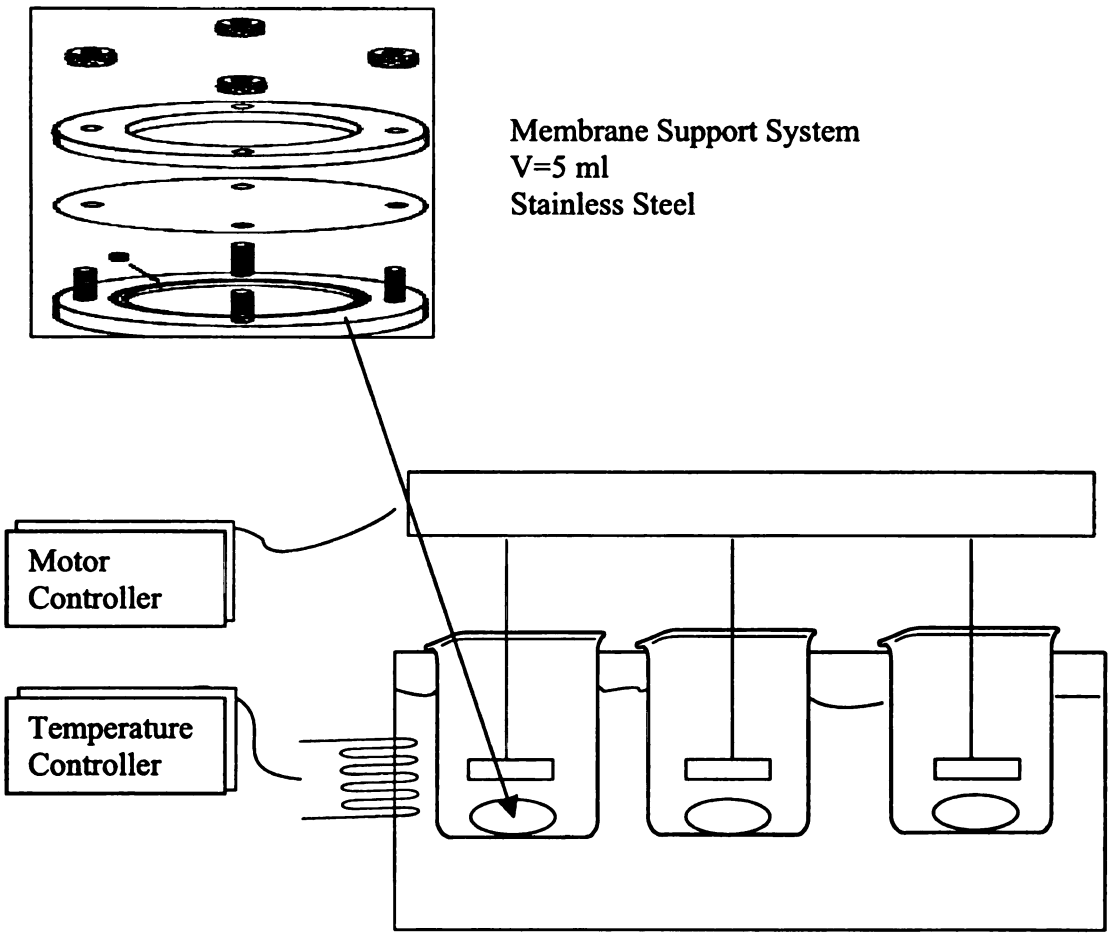


Figure 14. Membrane support (above) and dissolution bath for drug release studies

A Perkins-Elmer Lambda 900 Ultraviolet-Visible Spectrophotomer was used to determine the concentration of the drug in the drug release profiles. The strongest peak was at 290 nm and the absorbance there was used to determine the concentration. The UV/VIS integration time was 0.3600 s, and the slit width was set to 2.00 nm. Deionized water was used as the reference, since the tear solution did not contribute to the peak at 290 nm. The software used to obtain the data was UV Winlab for Lambda 900, version 2.90.02.

4.4 ESEM

An environmental scanning electron microscope was used to characterize the structure of the material. The instrument is an Electroscan 2020 environmental scanning electron microscope. For these samples, there was a beam voltage of 15 kV with an emission current of 49 uA. The water pressure was varied from 2 Torr to 9 Torr.

Chapter 5.Dicarboxy Matrix Synthesis and Characterization

5.1 Oxidation Methods:

First the method of oxidation was examined.

The following three methods were used with the native starch.

Table 4. Explanation of Oxidation Methods

		Reaction Time	Results
Method 1	1-step oxidation with sodium hypochlorite	24 hours	Completely water soluble product that is extremely hydroscopic in the presence of air. Also yellows when exposed to air.
Method 2	1-step oxidation with ozone	6 hours	Non-water soluble product that shows very little carboxyl peaks in IR.
Method 3a	2-step oxidation with sodium m-periodate followed by sodium chlorite	6 hours + 12-24 hrs	Gummy product that is soluble in water. Swells quickly when rewetted. Low yield because of over-oxidized starch.
Method 3b	Same as above, except that special care was taken to keep the dialdehyde from drying out in between reactions	3 hours + 6 hrs	Gummy product that is soluble in water. Swells quickly when rewetted.

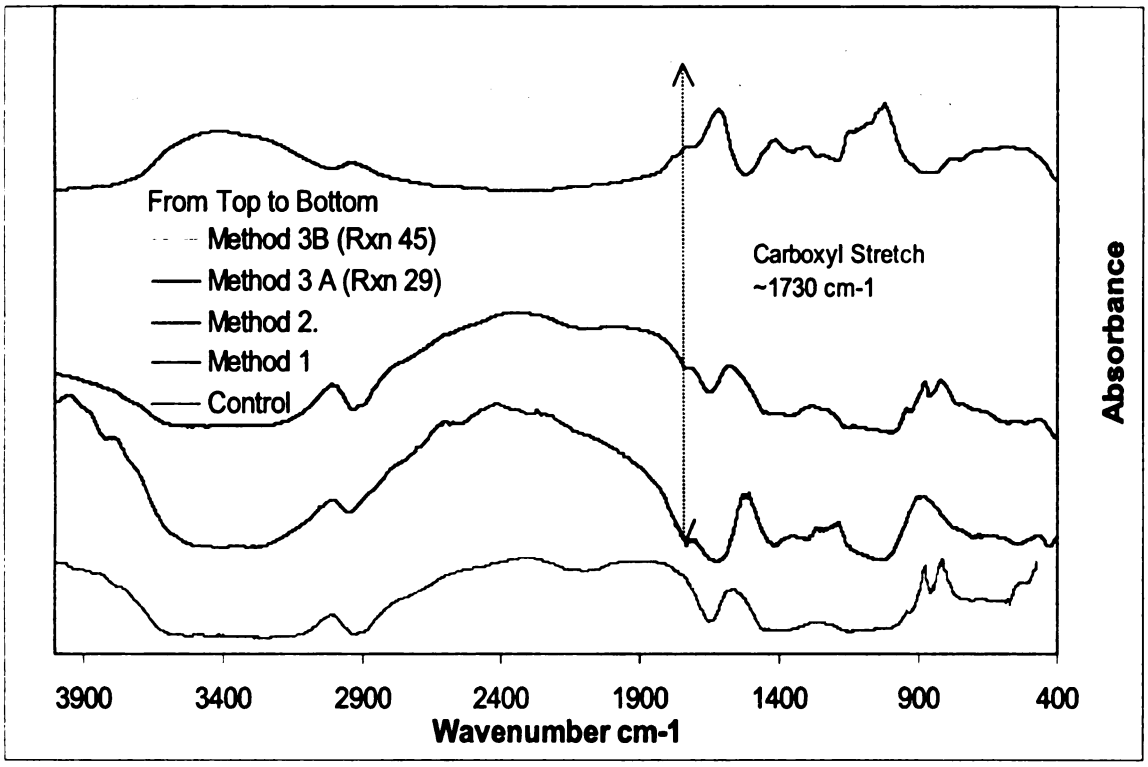


Figure 15. FTIR Comparison of oxidation methods

From the FTIR in Figure 15, the carbonyl stretch around 1740 cm^{-1} shows that the different methods had different impacts on oxidation. While not quantitative, a comparison can be made by referencing it to the neighboring 1620 cm^{-1} (C-OH) peak. The ozonated starch, shows a very slight shoulder around 1740 cm^{-1} indicating that there was some reaction. The hypochlorite method and Method 3a show that there is slightly more carbonyl present, but the peak is much smaller than the 1620 cm^{-1}. This leads to the possibility that the water solubility of the material may be due to hydrolysis of the starch as opposed to high carboxyl presence. As seen in the top peak, there is a high level of carboxyl and the peak is stronger than the 1620 cm^{-1} peak. The difference between the 3a and the 3b method, which in this case had the exact same reactant concentrations, indicates that the structure of the dialdehyde product before the second oxidation plays a

very important role in the subsequent oxidation. As seen in Figure 16, the additional peak at 1784 cm^{-1} indicates the presence of an anhydride which suggests the presence of the hemi-aldol structure. Specifically a strong anhydride of the structure R-COOCO-R shows a carbonyl stretch at 1790-1740 cm^{-1}. This would be consistent of the oxidization of the hemi-aldol structure.

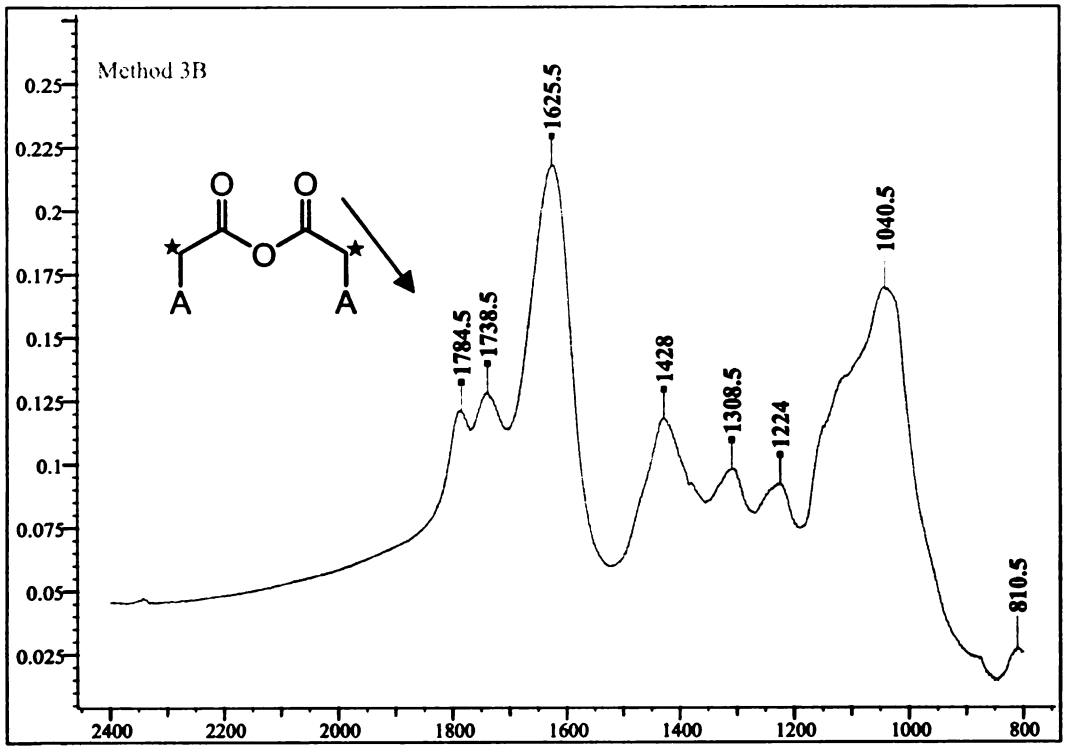


Figure 16. FTIR of a possible oxidized hemialdol structure

5.2 Polysaccharide Choice

Oxidation Method 3a was tried on different saccharides including native corn starch, waxy starch, cellulose, pretreated cellulose, xlyans and glucose. The native and waxy starch produced the best results. The cellulose produced similar results; but the reaction time was longer and required pretreatment with a strong acid. Accordingly, the starch

was used in subsequent reactions. The following chart summarizes the resultant products from each of these materials.

Table 5. The results of the oxidation of different saccharides

Material	Comments	Periodate Oxidation Reaction time	Chlorite Oxidation Reaction Time	Results
Native Starch		6 hours		Good results, high dicarboxy content, material swells
Waxy Starch	Waxy pearl 1108	6 hours		Good results, high dicarboxy content, material swells
Cellulose	Sigmacell from Sigma-Aldrich	24 hours		Only small percentage was oxidized
Pretreated Cellulose	Sigmacell pretreated with phosphoric acid and sodium hydroxide	24 hours		Good results, high dicarboxy content, material swells
Glucose		24 hours		Material was over oxidized
Xylans		7 hours		No change in the material

5.3 Titration

Titration with sodium hydroxide was used to measure the amount of carboxyl groups present in the samples. All of the values reported are in terms of carboxyl groups/anhydrogluco ring. For example, 100% would indicate that every anhydrogluco ring has one carboxyl group present. Theoretically, the maximum value would be 300% since the C-2, C-3 and C-6 carbon could potentially contain a carboxyl group. Besides

actual content, the titration also could be used to quantify the reproducibility of the reaction.

Because of the heterogeneity of the material produced using Method 3a for oxidation, titration of those samples was not reproducible. A single sample would have values ranging from 10%-30%. This proved that the material was not being produced in a consistent manner and further confirms that other structures, such as hemi-aldols, were being formed. Table 4 shows the titration results for the material produced by Method 3B. The standard deviation of the titrating a sample in duplicate was from 0.01%-3.1%, which were acceptable values. Also it can be seen from the table that materials produced using the same periodate-to-starch ratio showed consistent carboxyl content. All of the data presented here were for reactions using 3 hours for the periodate reaction followed by 6 hours for the chlorus acid oxidation with waxy cornstarch as the starting material. Figure 14 graphically shows the relationship between the periodate ratio used and the resulting carboxyl content. A logarithmic dependence is can be explained by the fact that as more dialdehyde is the polysaccharide becomes more susceptible to acid hydrolysis breaking the chain into smaller molecular weight chains. These chains are removed during the washing of the material and therefore do not show up in the titration.

Table 6. Titration results

Sample	Periodate ratio%	COOH/ring	Std. Dev.
44	50	46.7%	0.01%
45	50	45.0%	0.02%
46	30	29.1%	0.03%
47	10	0.8%	0.1%
48a	30	27.8%	2.1%
48b	30	24.2%	1.8%
49a	20	15.3%	3.1%
49b	20	12.7%	-
50	50	42.7%	1.9%
51a	20	14.3%	1.5%
51b	20	13.6%	2.0%
52a	80	54.7%	1.5%
53a	100	58.2%	0.0%

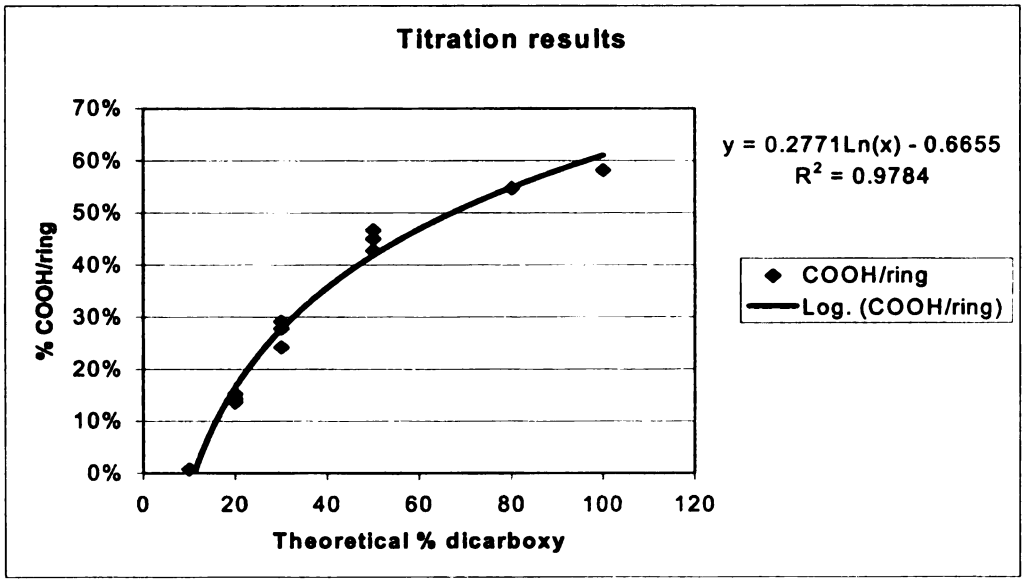


Figure 17. Carboxyl content versus periodate ratio

This data deviates from the data presented by Veelart, which shows a linear dependence as the stoichiometric amount is increased. This data may be explained by the fact that high amylopectin starch is being used. This highly branch material may be sterically hindering the oxidation as higher concentrations of periodate are used.

5.4 Periodate Oxidation Kinetics Data

Samples were taken during the periodate oxidation of starch and of cellulose at different varying time intervals. The UV spectrophotometer was used to analyze the samples since the periodate has a maximum peak at 223 nm. Data was used from previously obtained periodate data with new data and compared to the model presented by Veelaert and Narayan.

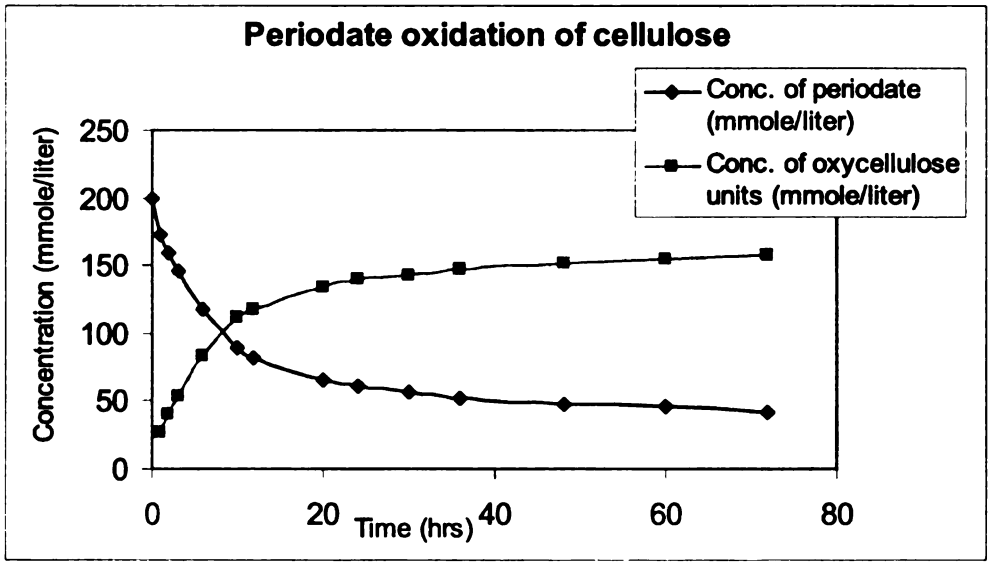


Figure 18. Periodate oxidation of cellulose kinetics

A close fitting relationship was found using this Veelaert's model. A Runge-Kutta differential equation solver set up on Excel was used to solve for the rate constants. Two

separate reactions one for cellulose and one for starch were compared to the model.

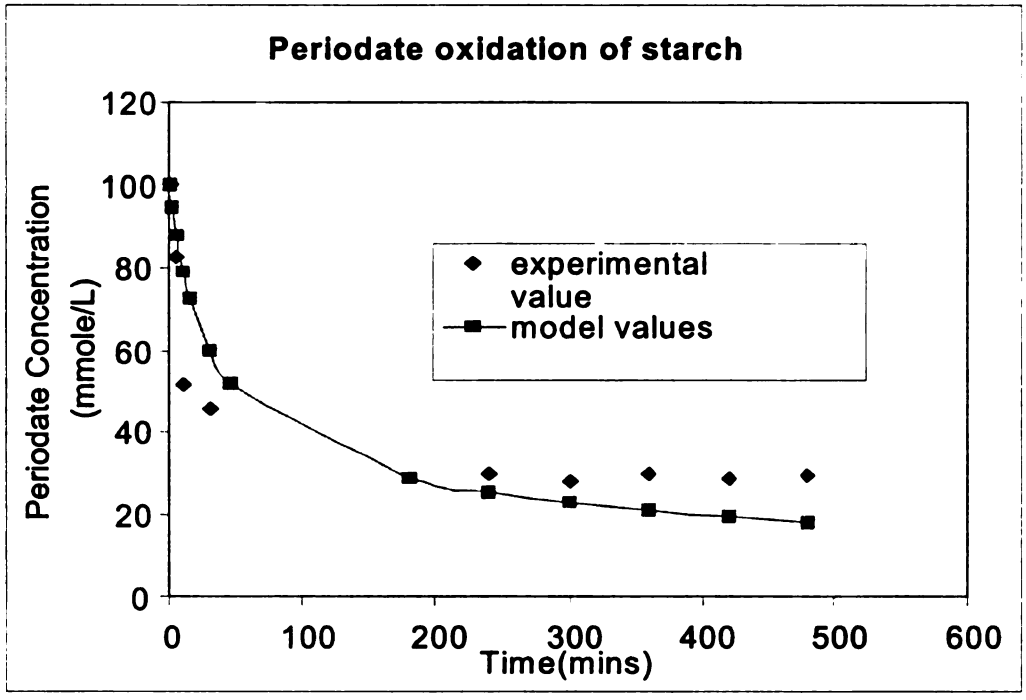


Figure 19. Periodate Oxidation of Starch Kinetics

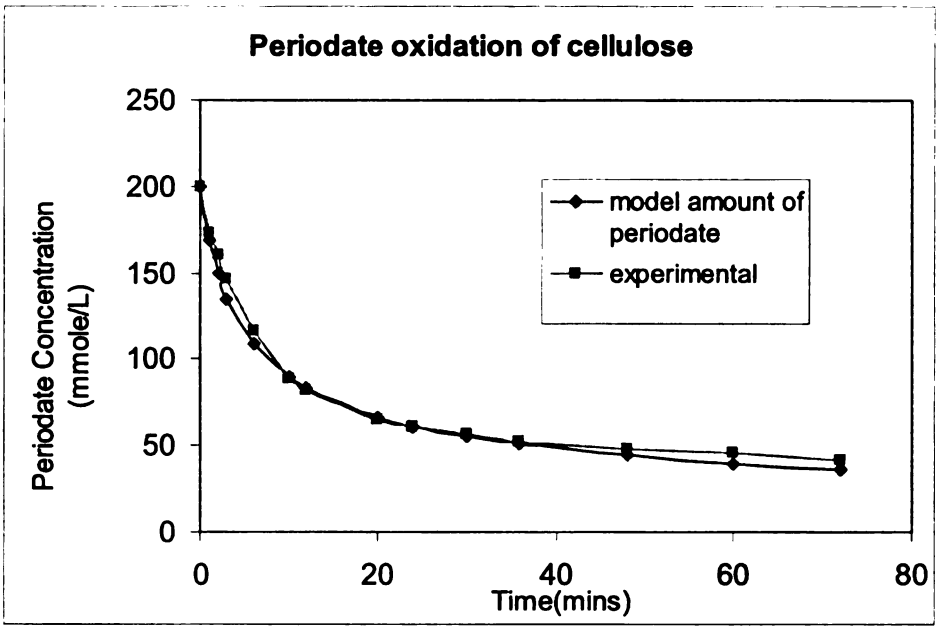


Figure 20. Comparison of actual and theoretical kinetic data

As can be seen in Figures 19 and 20, the models show a close relationship.

The rate constants for each are using the endpoint of three hours and the carboxyl content at that point. This introduces error because the assumption is that the dialdehyde is fully oxidized to carboxyl groups.

Table 7. Calculated rate constants for the periodate oxidation of starch

Sample	%dialdehyde	*k*(calculated)L/mmole/min	*k*(ave.) L/mmole/min	+/-
47	10	1.50E-08	1.50E-08	
49b	20	1.40E-07		
51a	20	1.25E-07	1.33E-07	8.0%
46	30	1.30E-07		
48a	30	1.20E-07	1.25E-07	5.7%
44	50	3.49E-07		
45	50	3.21E-07		
50	50	2.80E-07	3.17E-07	11.0%
52a	80	8.30E-07	8.30E-07	
53a	100	1.35E-06	1.35E-06	

5.5 Design of Experiments Optimization

Stat-Ease software, Design-Expert 6.0 was used to create a design of experiments to see how the initial periodate ratio affected the product. This was used to optimize the reaction to predict the most desirable product. These final results were used as the case that was scaled up in Chapter 8. Acid content, overall reaction yield and dispersibility were used to qualify the product. The titration results were used for the acid content. Because of the logarithmic relationship show in Figure 17, the exponential values of the carboxyl content were used. The yield was calculated by the looking at the percentage of the polymeric material left at the end of the reaction compared to the theoretical amount that could be produced, and dispersibility was rated on a scale of 0 to 3. On this scale 3 indicated that within 10 minutes of adding the material to water it appeared completely dispersed; 2 indicated that in that time frame the majority of the material was swollen and dispersed; 1 indicated that a majority of the material was not dispersed, but at the least the material had swollen considerably; and, 0 indicated that there was no visible hydration of the material within the 10 minute timeframe. This is an important factor to consider for manufacturing and formulating of a final product and to ensure that the drug can be uniformly distributed in the matrix. Carboxyl contents and reaction yield showed a statistically significant relationship to the periodate ratio used and the dispersibilty show a relationship with a $p=0.0761$.

Response: Yield

ANOVA for Response Surface Linear Model
Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	0.48	1	0.48	40.98	0.0002
significant					
A	*0.48*	*1*	*0.48*	*40.98*	*0.0002*
Residual	0.094	8	0.012		
Lack of Fit	*0.066*	*4*	*0.016*	*2.30*	*0.2204* *not significant*
Pure Error	*0.029*	*4*	*7.166E-003*		
Cor Total	0.58	9			

The Model F-value of 40.98 implies the model is significant. There is only a 0.02% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant.
In this case A are significant model terms.
Values greater than 0.1000 indicate the model terms are not significant.

Final Equation: Yield =+0.97444 -0.81743 * periodate ratio

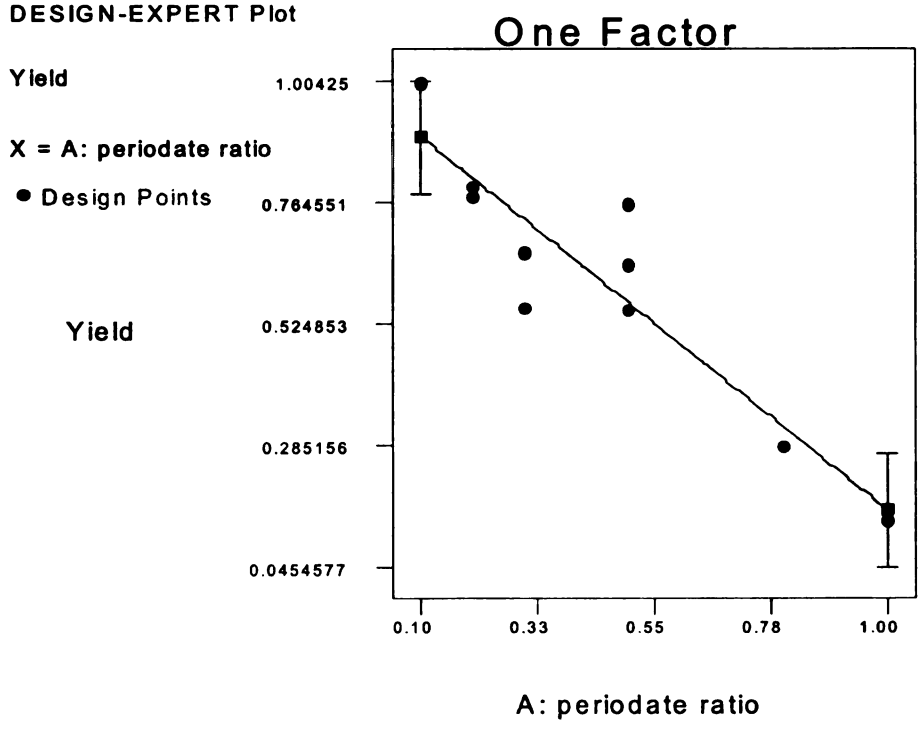


Figure 21. Relationship between the periodate ratio and the yield

Response:COOH %/ring

ANOVA for Response Surface Linear Model
Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	0.56	1	0.56	70.70	< 0.0001	
significant						
A	*0.56*	*1*	*0.56*	*70.70*	*< 0.0001*	
Residual	0.063	8	7.864E-003			
Lack of Fit	*0.061*	*4*	*0.015*	*28.71*	*0.0033*	*significant*
Pure Error	*2.117E-003*	*4*	*5.293E-004*			
Cor Total	0.62	9				

The Model F-value of 70.70 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant.
In this case A are significant model terms.
Values greater than 0.1000 indicate the model terms are not significant.

Final Equation : COOH %/ring = +1.03430 + 0.87633 * periodate ratio

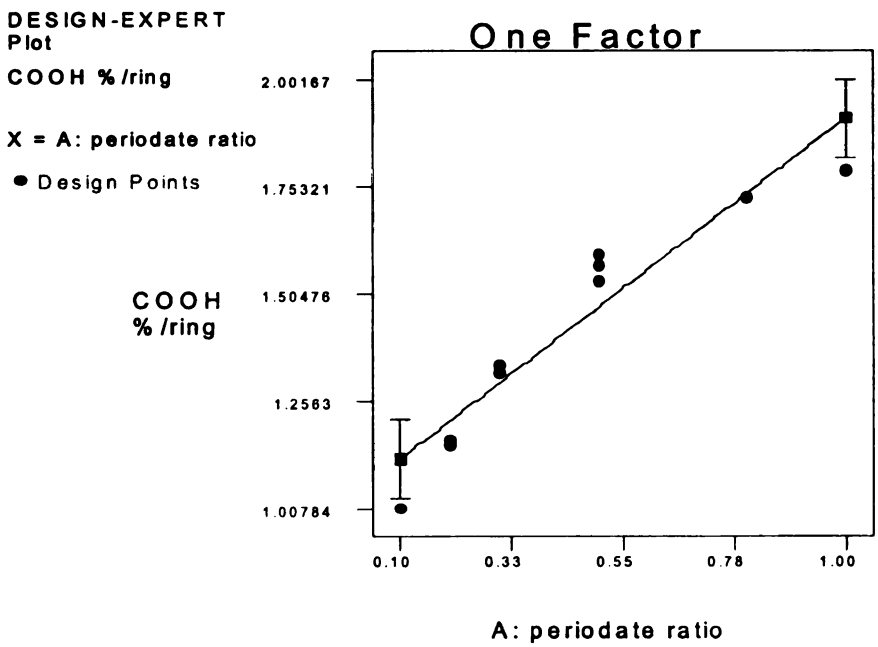


Figure 22. Relationship between the periodate ratio and the final material acid content

Response: Dispersibility

ANOVA for Response Surface Linear Model
Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	D	Square	Mean Value	F Prob > F
Model	3.45	1	3.45	4.15	0.0761
not significant					
A	*3.45*	*1*	*3.45*	*4.15*	*0.0761*
Residual	6.65	8	0.83		
Lack of Fit	*3.49*	*4*	*0.87*	*1.10*	*0.4641not significant*
Pure Error	*3.17*	*4*	*0.79*		
Cor Total	10.10	9			

The Model F-value of 4.15 implies there is a 7.61% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant.
In this case there are no significant model terms.
Values greater than 0.1000 indicate the model terms are not significant.

Final Equation: Dispersibility = +1.33978 + 2.18232 * periodate ratio

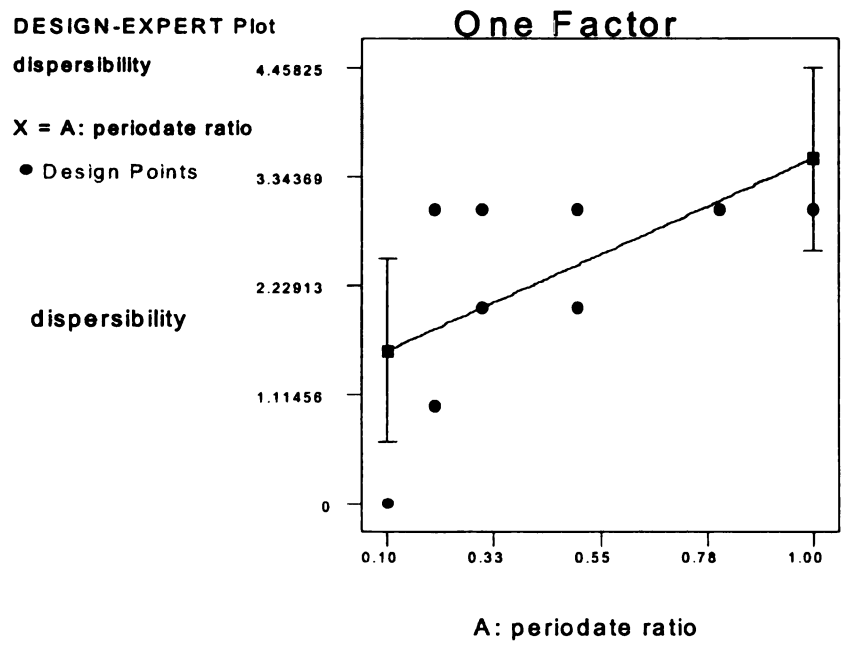


Figure 23. Relationship between the periodate ratio and the dispersibility of the material

Optimization Results

The following constraints were set to fine the optimal periodate ratio used.

Constraints

Name	Goal	Lower Limit	Upper Limit	Importance
periodate ratio	**is in range**	**0.1**	**1**	**3**
Yield	**maximize**	**0.136**	**1**	**3**
COOH %/ring	**is target = 1.398**	**1.007**	**1.789**	**3**

Solution

periodate ratio	Yield	COOH %/ring
0.42	0.634683	1.39854

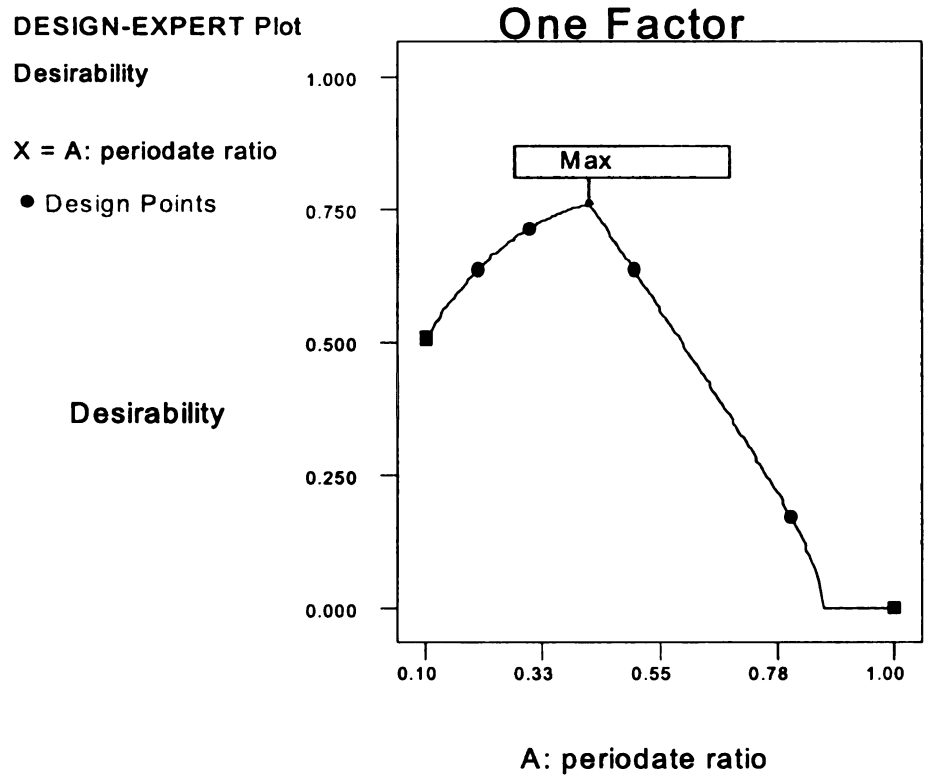
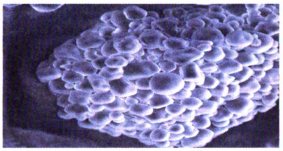


Figure 24. Optimization of the periodate concentration

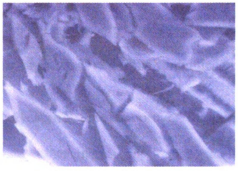
This design of experiments could be be expanded in the future for to incorporate the release data and calculated difussion coeffients to develop a predictive model with reactant molarity as the input and diffusion coefficients as the output.

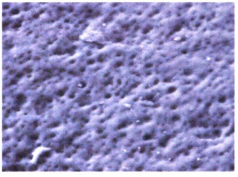
5.6 ESEM

As seen in the ESEM images below (Figures 25), there is a difference between native starch and the dicarboxy starch. It can be observed that the oxidation process destroys the granular structure of the starch, releasing the amylose and amylopectin from the structure creating a smooth and flexible material. The ESEM is run under vacuum so it impossible to observe the hydrated structure. However, the swelling and subsequent dehydration of the material can be observed while the material is first wetted and the vacuum chamber comes to equilibrium.

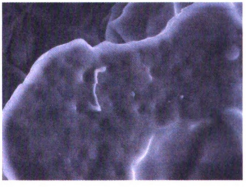


Native Starch





Air Dried Dicarboxy Starch



Rehydrated Dicarboxy Starch

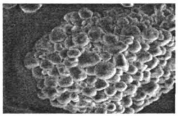
Figure 25. ESEM images of starch, air dried dicarboxy starch and hydrated dicarboxy starch

Chapter 6. Synthesis of Grafted Dicarboxy Starch and Characterization

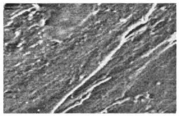
6.1 Background

Starch grafted with polycaprolactone (PCL) was made by the above synthesis to show that a similar material with different hydrophobic groups could be made. This has the potential of increasing the solubility and delivering drugs that are poorly water-soluble. This is very important as there are many gene therapies being developed currently that are high effective for their intended purpose but have very low bioavailability.

Polycaprolactone is approved for medical uses for sutures and is being investigated in many other medical applications including tissue engineering, bone imaging, drug delivery, and cardiac grafts(Domb, Kost et al. 1997) . PCL was tested in long-term release studies such as the release of contraceptives and the release of cancer therapies. To fully improve the functionality of the drug delivery system the PCL must be grafted to the starch allowing for a flexible hydrophilic backbone chain with the PCL attached at random intervals. The thermodynamic incompatibility between starch and synthetic polymers makes it difficult to graft these polymers on the backbone using a mechanical extrusion process. To improve the compatibility between starch and the polyester phases, starch is plasticized using common plasticizers such as glycerol, ethylene glycol or sorbitol in a twin-screw extruder to form thermoplastic starch (TPS). The plasticization process breaks the hydrogen bonds and disrupts the granular crystalline organization. It further releases the amorphous polymer chains with $\alpha 1 \rightarrow 4$ and $\alpha 1 \rightarrow 6$ linkages. Figure 26 below shows the ESEM pictures of starch and plasticized starch.



Starch



TPS

Figure 26. ESEM of native starch compared to maleated thermoplastic starch

To be able to graft the copolymer to the backbone, grafting maleic anhydride to the starch backbone forms a reactive carboxyl group at the end of the C-6 carbon (Figure 27). This allows for the polyester to be added in transesterfication reaction to that carbon (Figure 28). For this application PCL was chosen as the polyester because of its accepted use in medical applications.

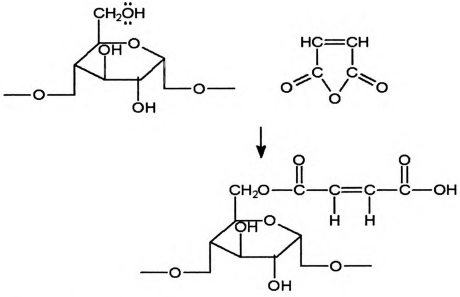


Figure 27. Maleation of starch

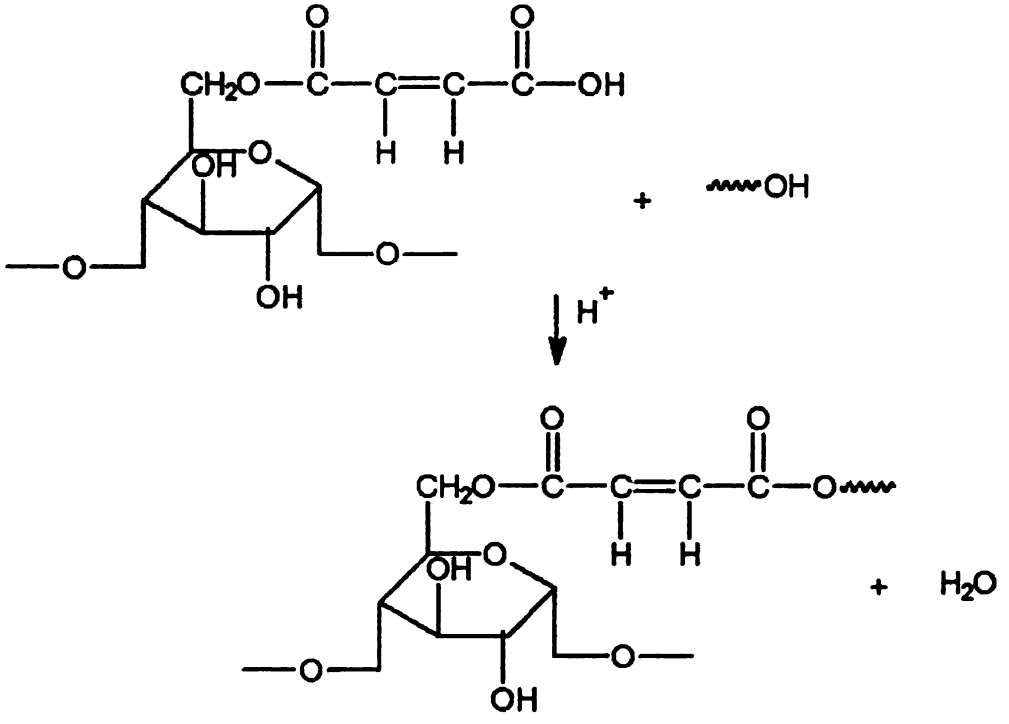


Figure 28. Transesterfication reaction with maleated starch

PCL, marketed under the trade name TONE polymers, was obtained from Dow Chemicals. TONE polymers are homopolymers of ε-caprolactone, a seven-member ring compound. The chemical structure of the PCL polyester is shown in Figure 29.

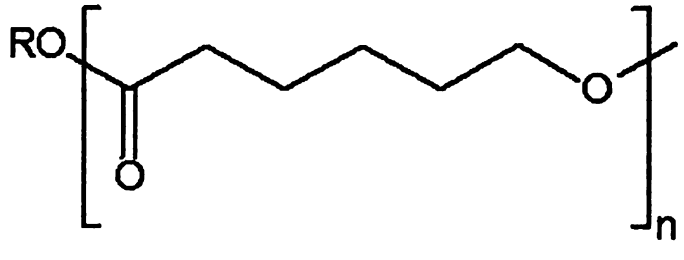


Figure 29. Chemical structure of polycaprolactone

For these experiments, lower molecular weight was used; TONE P-737 and P-757 polymers of M_n 32,000 g/mol and 43,000 g/mol respectively..

6.2 Results

An FTIR confirmed the formation of the maleated thermoplastic starch (MTPS) in Figure 30. The strong double bond of the maleic acid group can be seen at the1703 cm^{-1} peak.

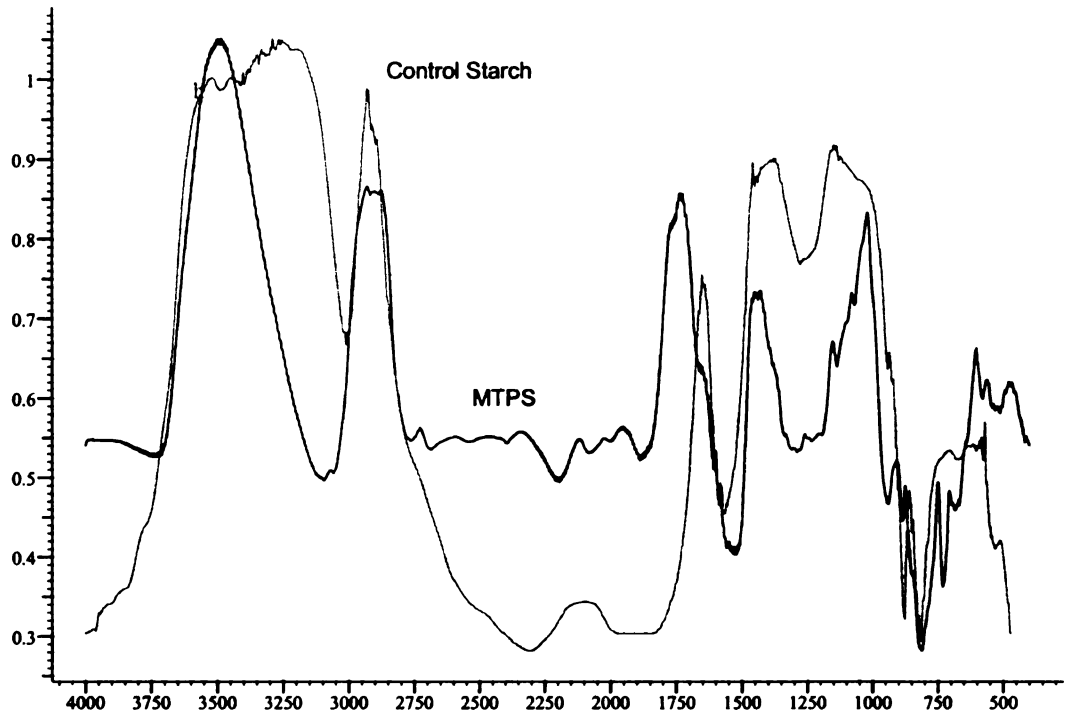


Figure 30. FTIR of Starch and MTPS

The PCL/MTPS was able to be oxidized slightly and form a water-soluble fraction. However the yield of that product was less then 0.1% and is was not able to be characterized. While there is potential with this reaction, improvements in the reaction setup would have to be tailored to this reaction, such as a more efficient way to mill the material before reacting.

Chapter 7. Drug Release Profiles

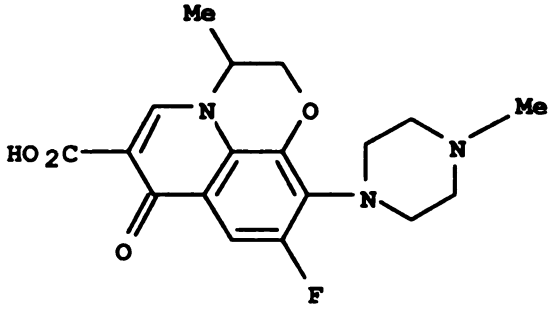


Figure 31. Structure of ofloxacin

Ofloxacin is an antibacterial agent belonging to the fluoroquinolone family with a molecular weight of 361.37. Of the available fluoroquinolones, ofloxacin is one of only usually given as a single agent and has been shown to have the best aqueous humor penetration. As an ophthalmic formulation, ofloxacin is formulated as a 0.3% w/v solution and goes by the trade name OCUFLOX ®. According to Allergan's prescribing information packet, OCUFLOX solution is unbuffered and formulated with a pH of 6.4 (range - 6.0 to 6.8). It has an osmolality of 300 mOsm/kg. Ofloxacin is a fluorinated 4-quinolone which differs from other fluorinated 4-quinolones in that there is a six member pyridobenzoxazine ring from positions 1 to 8 of the basic ring structure.

7.1 Drug Release Results

The drug release profiles were studied using a Perkin Elmer's Lambda 900 ultraviolet-visible spectrophotomer. The absorbance spectrum for the drug ofloxacin can be seen in Figure 32.

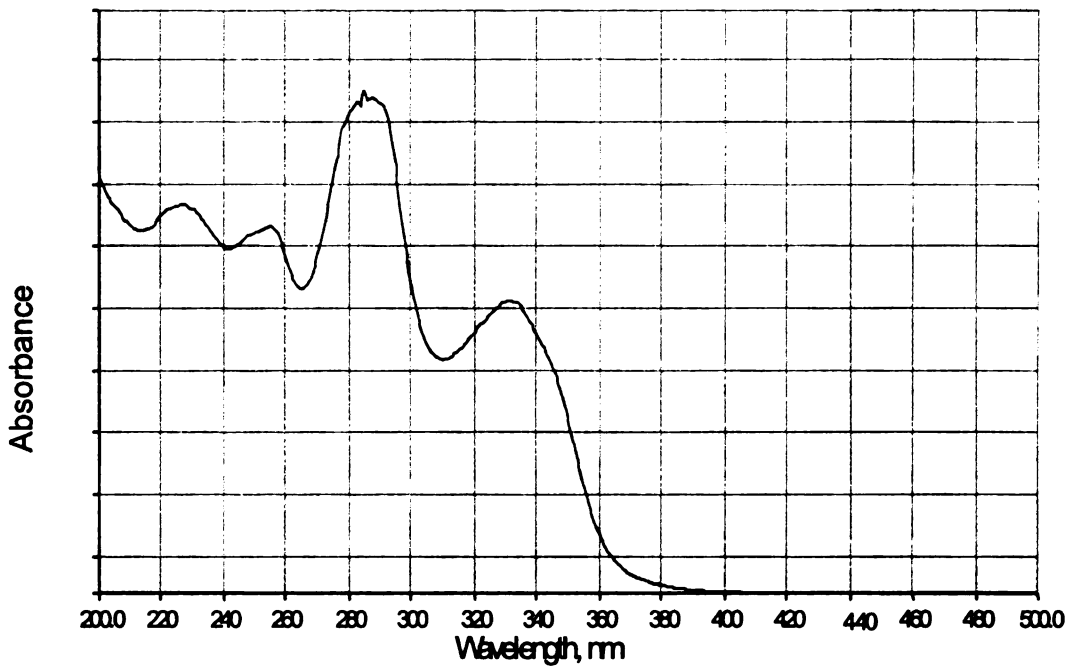


Figure 32. UV-VIS Curve for Ofloxacin

The strongest peak at 290 nm was used to determine concentration of ofloxacin as compared to a calibration curve. The absorbance at concentrations of 0.0036% w/v to 0.00075%w/v was found to linearly dependent and measurable using the parameters described in the analytical technique chapter. Some of the samples had to be diluted to 1 part sample to 2 parts plain tear solution to have samples in a measurable range. A concentration method was developed to read the output only the absorbance at 290 nm. This eliminated the need of developing full spectra for all of the samples. The calibration confirmed that absorbance was linear with concentration from a range of absorbance from 0-2.5

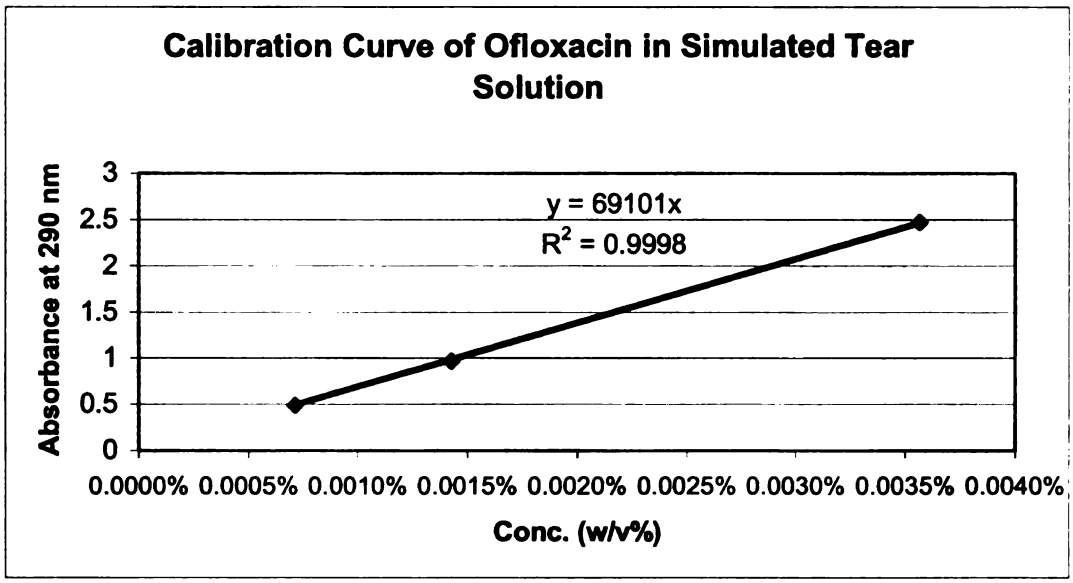


Figure 33. Calibration Curve for Ofloxacin

The drug release profiles were conducted using two different set-ups a more equipment became available. The setup are explained in Chapter 4 and here will be referred to as the stir plate method and the USP transdermal method.

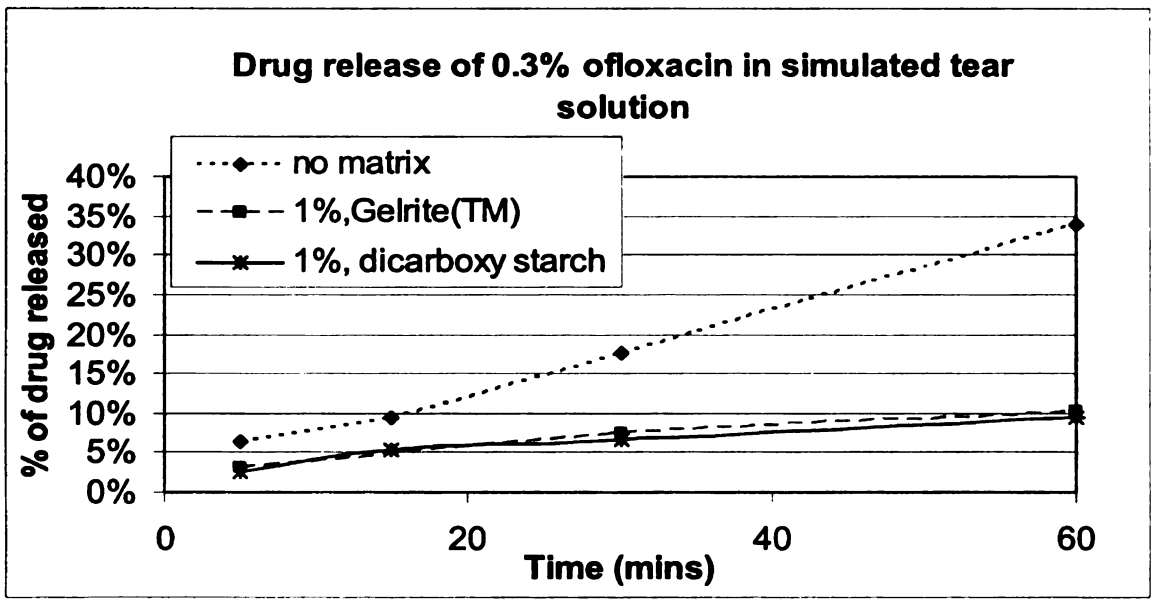


Figure 34. Comparison of dicarboxy starch to gelrite

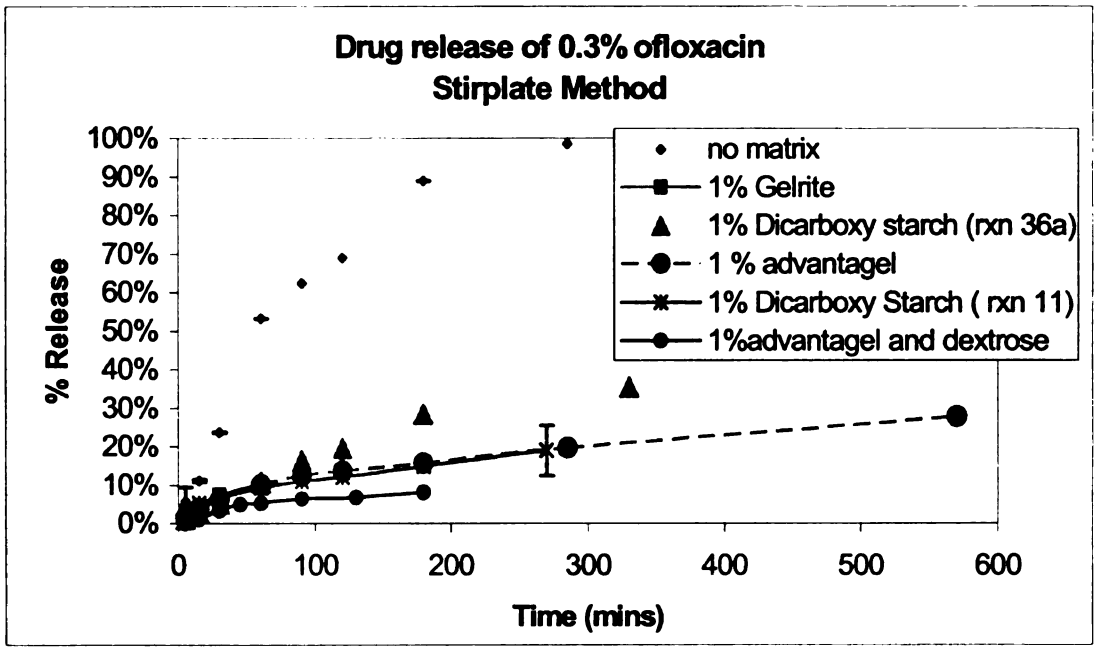


Figure 35. Drug release of various materials

Stir plate method results

Initially the release of the dicarboxy starch was compared to that of Gelrite to see if it exhibited similar release properties. As seen in Figure 34, over a period of one hour the dicarboxy starch and the Gelrite release the ofloxacin in a similar manner.

Results from the USP method

Figure 25 shows the release profiles from three different dicarboxy starches (20% dicarboxy, 50% dicarboxy and 80% dicarboxy) compared to the release profile of Gelrite. All were formulated using 1wt% of the matrix in a phosphate buffer, pH =7.4. There is no statistical different between the release profiles of the materials with different carboxy concentrations. This is important because the optimization of the material in the previous chapter assumed that all the materials would have the same release profile.

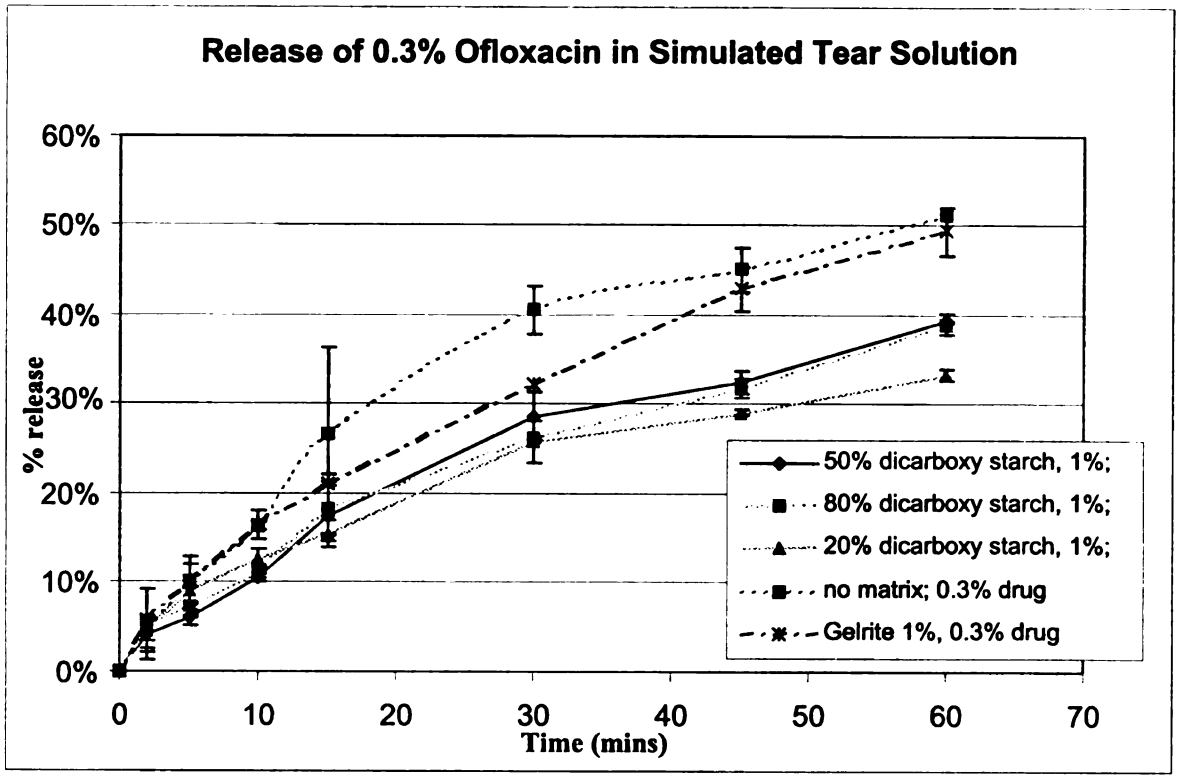


Figure 36. Release profiles using USP dissolution system

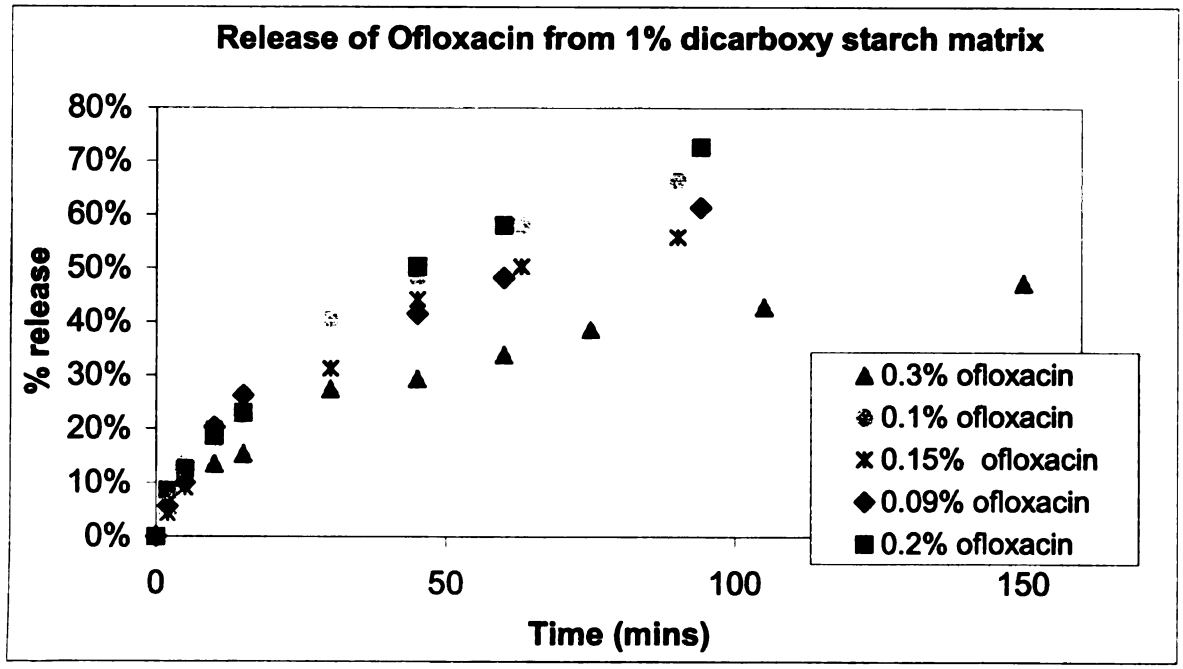


Figure 37. Release with varying drug concentration

7.2 Modeling the Diffusion Method

The data obtained from the USP dissolution method was used compared to the models predicted by the Higuchi Model presented in Chapter 2. As indicated by the model the release of the drug from a swollen hydrogel is diffusion controlled and should follow a square-root time dependence when the percentage released is less than 60%. As seen in Figure 38, the drug release is consistent with that model because all experimental data for each of the matrices can be fitted with a linear fit with a R^2 value greater than 0.97. The varying slopes of the lines indicates that there are different apparent diffusion coefficients for each of the different materials. This confirms the fact that material can be engineered to change the release profiles. The release profile with no matrix appears linear when plotted against the square root of time, however there is a higher linear correlation when it is plotted against time which is consistent with standard diffusion through a membrane [Saltzman].

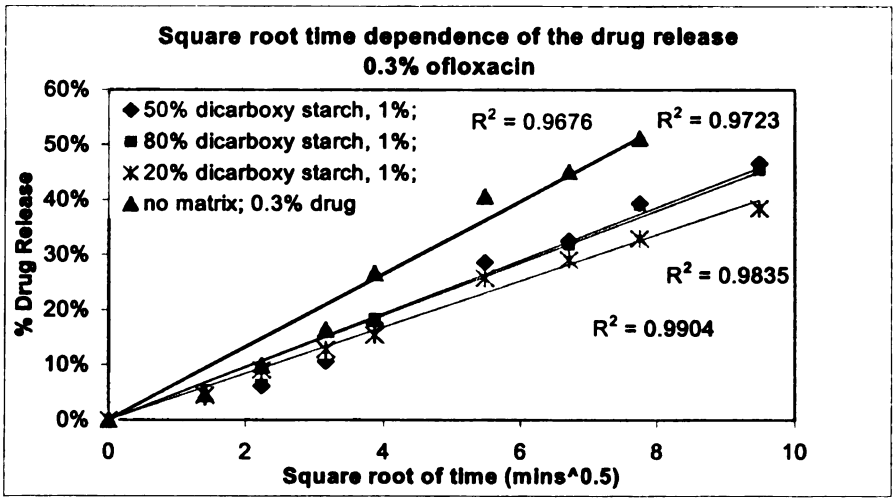


Figure 38. Square root time dependence of the ofloxacin

Following the Higuchi model presented in Chapter 2, for the same material, surface area and volume, the diffusion coefficient should be able to be calculated by changing the concentration of the drug. To calculate this, a formula with 1% of the 20% dicarboxy

starch was made up with varying concentrations ofloxacin. These runs were conducted once each. The percent released was plotted against the square root of time and the slopes of the lines were found. According to Higuchi model when rearranged for the dimensionless percent released, the slope of the line, y, should be equal to:

$$Y=2A(D_m/\pi)^{0.5} \qquad (8)$$

Following this equation the slope of the lines showed be equal for the same matrix and release area. As seen if Figure 39, the slopes vary for each of the release profiles. Since each of these runs were conducted only once, the difference could be due to the number of runs. The 0.3% formulation may becoming close to the solubility limit of the drug in which case the Higuchi model presented would deviate. If the values for the 0.3% value are removed, the average of the slope of the lines becomes 0.0665 ± 9% which is considered a reasonable deviation. Using that number an apparent diffusion coefficient can be calculated with the answer being 0.000225 cm^2/s.

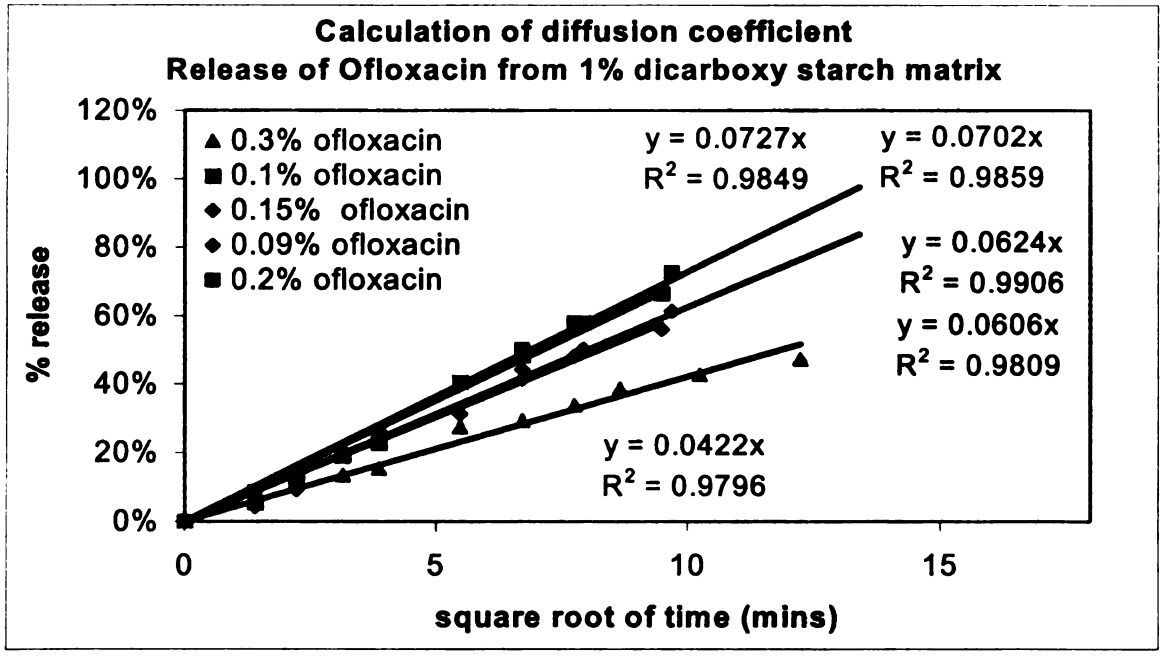


Figure 39. Calculation of diffusion coefficient

In conclusion, it appears that the release profiles can be modeled using the Higuchi equation. Monitoring the drug concentration with the UV-visible spectrophometer provides a good means to analyze the release profile.

Chapter 8. Conclusions and Recommendations

8.1 Conclusions

- This engineered dicarboxylate material can be characterized by FTIR and titration methods to analyze the amount of added carboxylate groups.
- The dicarboxy starch is water dispersible and shows drug release profiles consistant of hydrogel matrices. This would make it suitable for an ocular drug delivery system.
- Using the diffusion model presented, the material could be engineered to have exhibit desired diffusion properties.
- This material has the potential to be used in a variety of drug delivery applications including topical and depot ocular, medicated wound dressing.
- Carboxylated polysacchrides with grafted biodegradable hydrophobic branches can be produced, however those reactions are not currently optimized.
- From observation, this material appears to have a good ability to adhere to biological surfaces. The mucoadhesive properties need to be evaluated further, but there are is the potential for muccoadhesive applications such as nasal drug delivery.
- This material is worth pursuing for commercial applications.

8.2 Kinetic considerations and scale up

Below in Figure 40, a large-scale batch process for the production of the dicarboxy starch is proposed. For the situation were this would be used to to produce enough material to be formulated at a 1wt.% for a major product such as a glaucoma treatment the following would be suggested.

Table 8. Reaction Specifications

Formulated product required/annually	200000	kg
Assume 10,000,000 bottles & 20 ml each		
Amount of dicarboxy starch needed/annually	2000	kg
# of batch runs annually	50	
Amount/batch needed for 42% dialdehyde	40	kg
Yield =	63.47%	
Reactants/ batch		
Starch	60.9	kg
Sodium m-periodate	33.8	kg
Water	6282.4	kg
Acetic Acid	13.6	kg
Hydrogen Peroxide	45.1	kg
Na-EDTA	0.8	kg
Sodium Chlorite	42.5	kg

Reactor 1 would be a 500 gallon stainless steel jacketed reactor. Water would be used to heat the reactor to 40° C. The second reactor would be a stainless steel 1250 gallon reactor. Chlorine byproduct will have to be controlled and quenched accordingly. The ethanol/water washwater could be recycled using a basic distillation column.

Special considerations that would have to be addressed include:

- A suitable filter that would ionically repel the material or atleast, not attract it

- The iodate can be reoxidized electrolytically to periodate or can be oxidized to paraperiodate using sodium hypochlorite which then will release the metaperiodate ion.

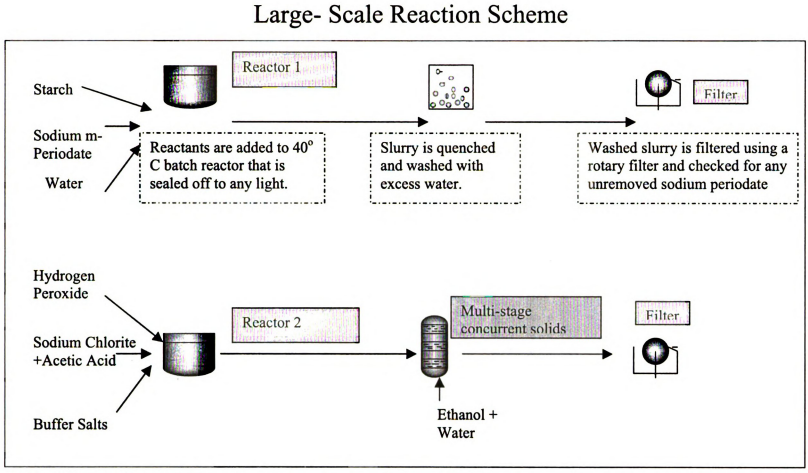


Figure 40. Large-scale reaction scheme

Chapter 9. Examination of business potential

9.1 Overview of market

Market for Drug Delivery Systems

Pharmaceutical companies are facing intense pressure to develop new and better drugs. R&D expenditures grew from $18 billion in 1990 to $43.8 billion in 2000, reflecting this competitive pressure. Drug delivery technologies are increasingly recognized as a critical strategic tool, allowing companies to make their drugs easier to administer and more effective. Currently, within the $337 billion global pharmaceutical market, products incorporating drug delivery systems constitutes 13%. The overall market for advanced drug delivery systems is expected to jump from $16.28 billion in 2000 to $27.35 billion in 2005. The figure below depicts the trends for increased use of drug delivery for both new and existing drugs[1].

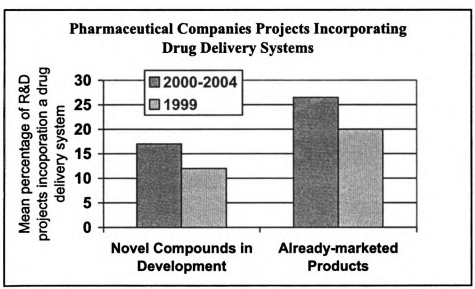


Figure 41. Drug delivery growth

[1] Findlay G, Kermani F. Making Drug Delivery Alliances Successful. CMR International. 2000. www.cmr.org/drugrpt.html

Market for Ocular Therapeutics

There is strong agreement among industry analysts that ocular therapeutics represents substantial business opportunity. Major medical insurance programs cover the expense of topical ocular prescription drugs, with appropriate co-pays applied.

Glaucoma, Antibiotics and Anti-Allergy Therapeutics. Projected growth in the ophthalmic market for topical products for glaucoma, antibiotics and anti-allergy therapeutic areas – our initial focus - is for these markets to approximately double by 2010 to over $8 billion (from $3.6 billion in 2000). For example, according to estimates from Aciont, the current market size for anti-inflammatories to treat intermediate and posterior uveitis is $600million[2]. The glaucoma market is the only one in which gelling ocular products have been introduced. Merck's Timoptic XE® was introduced first, followed by Alcon (Falcon). The market positioning for both has been that the gel increases efficacy and decreases systemic exposure as a major advantage. After the introduction of Xalatan® and other similar prostaglandin glaucoma drugs with superior intraocular pressure (IOP) lowering, market share for timolol and other glaucoma medications declined. However, Timoptic XE still sells well and had an 18% increase in sales 3Q 2003 relative to 3Q 2002.[2]

Retinal Degenerative Disease Therapeutics. Biopolymer Innovations' (BI) secondary targets include treatments of such retinal degenerative diseases as age- related macular degeneration (AMD) and diabetic retinopathy (DR). The incidence of these diseases is

[2] Merck & Company, Inc. , financial disclosure, third quarter, 2003.

expected to increase substantially with the aging of the U.S. and world population. While there are currently few effective treatments on the market, the pharmaceutical companies are directing large amounts of research and development funds to these areas; many more drugs are expected in the relative near-term. The growth in age-related macular degeneration (AMD) and diabetic retinopathy (DR) drugs is expected to grow exponentially to about $10 billion in 2010 from $800 million in 2000. According to estimates from Aciont, the current market size for chronic macular edema associated with diabetes is approximately $1.2 billion, for anti-angiogenic activity to treat "wet form" age related macular degeneration is $1.2 billion, and anti-angiogenic activity to treat diabetic retinopathy is $1 billion[3].

International market growth

Growth trends in economically advanced countries are similar to those in the US. Our pharmaceutical partners will vary on their relative market size and distribution reach in international locations; yet international market growth in general will be robust. Third world countries, in contrast, are still focused largely on the prevention of blindness (the most common cause of blindness is Vitamin A deficiency). Some opportunity may exist in third world markets for the development of an inexpensive glaucoma agent (timolol reformulation, for example).

[3] [2]http://www.aciont.com/products.htm

COMPETITION PROFILE

There are three companies that currently offer an *in situ* gelling drug delivery system: Merck, Falcon, and DelSite. Their products along with other products in development are compared in the chart below.

- *Merck.* Merck currently has the only true *in situ* forming ocular gelling product on the market. Gelrite®, registed to Merck, is a naturally occurring polysaccharide and is used in their product Timoptic.

- *Falcon (Alcon).* Falcon's "gel forming solution" is not a true *in situ* forming gel and therefore does not increase bioavailability

- *DelSite.* DelSite markets an *in situ* forming gel for multiple applications and routes of administration. The polymer is a naturally occurring acidic polymer derived from aloe vera. No topical ocular applications are described.

Listed below are companies that are working on ocular drug delivery technologies across the spectrum of eye conditions and diseases, and that could possibly have programs similar to the ocular gelling technology of BI. None of these companies is publicly stating that *in situ* forming gels for ocular applications are under development. Also, none has announced that it is working with modified biopolymers in the area of ocular

topical delivery (or depot delivery). The competitors were compared on the following three criteria, described below:

- Synthetic or Natural. In general, "natural" means that the products are based on materials that are common to the body, with known metabolism pathways. This suggests high biocompatibility with the body, and the material would not be seen as a foreign substance. A naturally occurring substance at low dosages will have no harmful effects, leading to quicker FDA approvals.

- Easy to Use. Will the product potentially increase patient compliance, or at least not decrease it? Some new technologies are difficult to use and potentially decrease compliance – they must be administered by a doctor or are very disconnected to the current way people treat eye diseases. The ability to reduce dosing frequency was also considered.

- Broad Application. Broad application is defined as: modifiable to conform to characteristics of the drug to create the optimum formulation; the drug formulation/ reformulation process is quick and simple; it is easy to manufacture.

OcularGel™ performs very well on all three criteria:

- Natural Product
    - It is based on materials the body can recognize and metabolize.
- Easy to Use
    - It canbe formulated in current drugs without changing the way drugs are administered.
    - Can decrease the frequency of dosing
- Broad application
    - Can be engineered to conform to the drugs properties
    - Can be used in other routes of administration besides ocular
    - Is simple to manufacture, ship and formulate

Table 9. Competitors in the area of ocular drug delivery

Company Name	Technology Name	(A)lliances/ (P)arent Co.	Synthetic or Natural	Easy to Use	Broad application
Currently in the market:					
CP Kelco	Gelrite™	A: Merck	Synthetic	Yes	No
Falcon	"Gel forming solution"		Natural	Yes	No
Delsite Biotechnologies (TX)	GelSite™	P: Carrington Laboratories Inc.	Natural	Yes	Yes but not for ocular applications
Under development or very little market share:					
InSite Vision (CA)	DuraSite	A: Bausch&Lomb; Japan's SSP Co.	Synthetic	Yes	Yes
Oculex Pharmaceuticals Inc. (CA)		A: Allergan	Synthetic	No	No
Oxigene	CA4P	A: Arizona State; U of Lund; Baylor, U of Florida	Synthetic	-	No
Escalon Medical (PA)	Ocufit	A: West Pharmaceutical Services	Synthetic	No	No
BioSante Pharmaceuticals, Inc. (IL)	CAP Nanoparticles		Synthetic	No	Yes
Lipocore	Galacticles Ophthalmic		Natural	No	Yes
Retinapharma (PA)	PhotoTarget ™ System	A: John Hopkins	Synthetic	Yes	No

CUSTOMER PROFILE

Pharmaceutical companies are relying increasingly on novel drug delivery technologies to differentiate their products,to extend product life cycles, and to sustain their high growth and profitability. Trends are for large pharmaceutical companies to develop strong alliances with drug delivery partners, including acquisitions, as drug delivery represents an area outside their core research competence. Some of the specialized firms work in partnership with the pharmaceuticals,and others independently develop their platforms with the goal of selling the platform or licensing the technology to major pharmaceutical companies once development is complete.

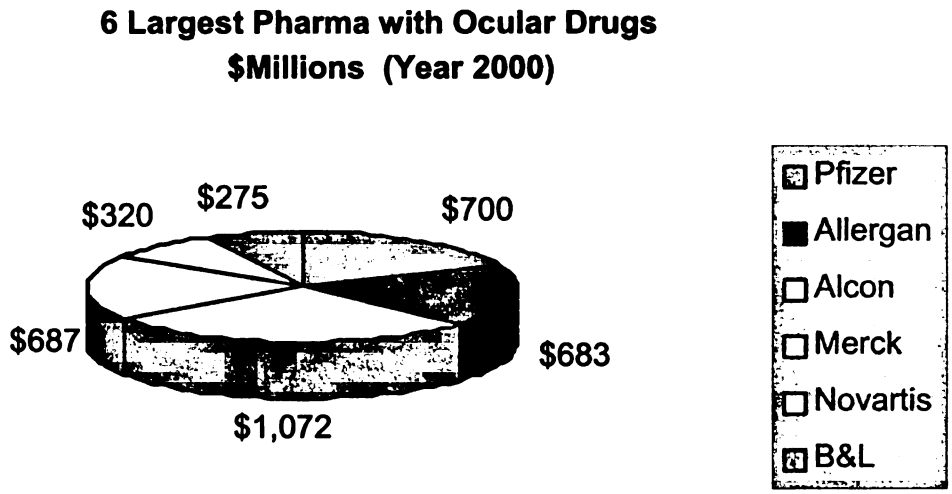


Figure 42. Largest Pharmaceutical Companies with Ocular Drugs

Our customers are pharmaceutical companies with treatments for glaucoma, eye infections, allergies, dry eye and inflammation. The customer base consists of major manufacturers - Pfizer, Merck, Alcon, Allergan, Novartis, and Bausch & Lomb - and a

number of smaller manufacturers and generic drug companies. The current market shares for ocular drugs for all major companies are illustrated below.

BI will pursue discussions and negotiations with the major pharmaceutical companies with treatments in its initial target therapeutic areas – glaucoma, antibiotics, and anti-allergy therapeutics – once the initial proof of concept is complete. Our initial partnership will involve joint development to ensure we maximize our learning about the platform's characteristic and performance, and that it is performing well in its initial application. We also anticipate that this initial project will be for an ocular drug that is currently on the market, or one that was previously marketed that could benefit greatly by the OcularGel™ drug delivery system. The advantage of starting with a known product is that the formulation issues related to the incorporation of OcularGel™ will be simpler and more straightforward. Subsequent partnership relationships will remain in the initial target areas, yet could include projects involving new drugs.

The following chart shows major pharmaceuticals companies with ocular treatments in BI's initial target therapeutic areas, as representative of our potential customer base.

Table 10. Customer Profile

Company	Drug	Chemical	FDA Approval	Patent Expiration	Use /Mechanism	Predicted Product Revenue ($MM) FY 2003
Pharmacia / Pfizer	Xalatan	Latanoprost	1996	2006-2011	Open Angle Glaucoma - Prostaglandin F2Alpha	$1,051 $623 (2003)[4]
Novartis	Visudyne	Verteporfin	2000	2007-2016	Macular Degeneration	
Novartis	Zaditor				Anti-allergy	
Allergan	Alphagan	Brimonidine	2001	2012-2015	Open Angle Glaucoma - Alpha 2 Stimulator	$249 $286.8[5]
Allergan	Lumigan	Bimatoprost	2001	2012	Open Angle Glaucoma - Prostaglandin F2Alpha	$181.3[5]
Fujisawa	Rescula	Isopropyl Unoprostone	2000	2008-2011	Open Angle Glaucoma - Prostaglandin F2Alpha	$7
Alcon	Patanol				Anti-allergy	$252[6]
	Ciloxan Vigamox		2004 Vigamox	2004 (Ciloxan)	Anti-infection	
Alcon	Travatan				Glaucoma	$135[6]
Merck	Trusopt/ Cosopt	Dorzolamide	1994	2003-2008	Glaucoma - Carbonic Anhydrase Inhibitor	$460
Merck	Timoptic/ Timoptic XE	Timolol	1982-1993	2006	Glaucoma - Non selective beta blocker	$159
	Chibroxin				Anti-infection	
Bausch & Lomb	OTC & prescript.					$467.9[7]
Senju	OTC					18040[8]

[4] http://www.pfizer.com/are/investors_reports/annual_2003/financial2003.pdf, after the purchase of Pharmacia

[5] http://www.shareholder.com/AGN/EdgarDetail.cfm?CompanyID=AGN&CIK=850693&FID=892569-04-307&SID=04-00#'_102', page 24

[6] http://media.corporate-ir.net/media_files/NYS/ACL/reports/2003ar.pdf, page 29

[7] http://www.bausch.com/us/vision/about/investor/pdfs/2003annual.pdf, page 23

9.2 Path to commercialization

Product Development: Timeframes and Milestones

The phases of product development are depicted in Table 1 and described below.

Phase 1 -Proof of Concept

A modified starch gel has been completed that can increase the residence time on the corneal epithelium. As described earlier, it is known that modified polysaccharides, when applied topically to the eye, are not significantly irritating. Gellan gum is a good example, since this polymer is similar in structure to OcularGel™. Current activity involves (see Table 1 below):

- Characterization (final) of the reaction conditions to produce OcularGel™
- Demonstration of increased ocular bioavailability with the compound timolol
- Characterization of reactions conditions continues, as does evaluation of new reactions
- Demonstration of safety in rabbit and dog, again while further characterization of reactions conditions continues

We are confident that we will not experience any significant technology development obstacles. The key possible obstacles, and the reasons for our confidence, are as follows:

[8] http://www.senju.co.jp/english/part/e-statistics.html 2001 revenue in Japanese currency

- Inability to produce OcularGel™ consistently. This is very unlikely as the chemistry is well understood, and the gelling properties demonstrated.
- Lack of an increase in ocular bioavailability. This is very unlikely as this effect is a property of all gels.
- Significant irritation in rabbit and/or dog GLP safety studies. This is very unlikely as polysaccharides with very similar structure have been demonstrated to not be irritating in similar tests.

Phase 2 – Applications Development

After the concept has been proved to be completely safe and effective, six to eight drugs will be tested extensively with OcularGel™ to determine the best potential commercialization partners. The tested needed for FDA approval to test in humans will be conducted at this time.

- Extensive *in vitro studies* to determine the best partners
- Extensive formulation studies
- Bioavailability testing in dogs and primates

Phase 3- Commercialization

BI will pursue three methods of commercialization of OcularGel™ for prescription drugs. These methods and their time frames are described below:

1. Licensing of OcularGel™ for use with Current and/or Previously Marketed Products.

BI will target drugs that will realize important benefits through incorporation of OcularGel™, particularly related to their safety and efficacy. The process of reformulation by integration of OcularGel™ into existing products is identical for aqueous based products (solutions) or suspensions, with the exception of specific pH and solubility requirements for a given drug. While each case might be slightly different, for the most part the reformulation will simple involve the addition of OcularGel™ at 0.5 – 5% by weight.

Integration of OcularGel™ into an existing product with patent protection on its active pharmaceutical ingredient (API) will be done in partnership with the original manufacturer. In the case of off patent drugs, which are readily available through commercial sources, the integration of OcularGel™ can be done solely by BI, or in partnership with the original manufacturer or a generic manufacturer. There are a large number of such products, from antibiotics to glaucoma agents. Selection of these targets will be made based on market size and share, and potential profitability.

In the cases of BI pursuing sole development/formulation of a product, it will seek a partnership for marketing and distribution.

2. Licensing of OcularGel™ for use with Products under Development

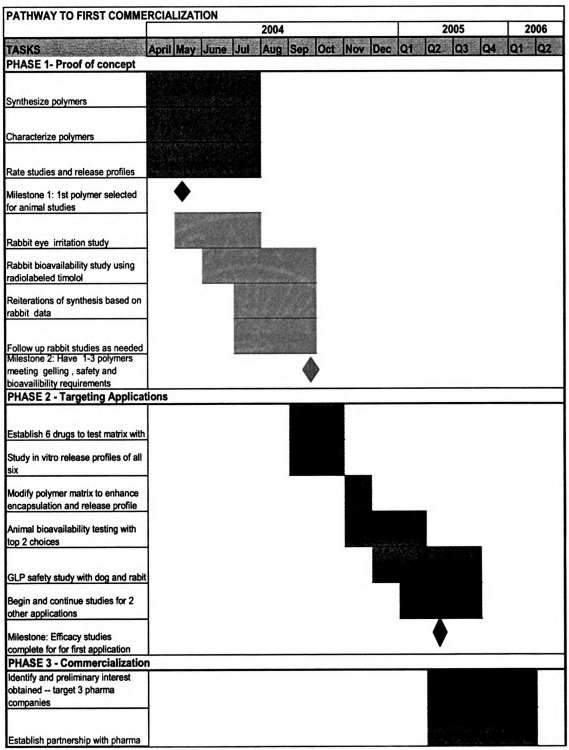
Integration of OcularGel™ into products under development will be done in partnership with the original manufacturer. A key variable in these projects is the time needed for FDA approval, which varies significantly by application. For example, products to treat age-related macular degeneration (AMD) or diabetic retinopathy would receive very rapid approval and the inclusion of OcularGel™ would not be an issue because of the immediate need for a product on the market where none exists currently. Products to treat serious infections may also be on a "fast track" to approval because of the severity of condition. On the other hand, treatments for ocular inflammation or glaucoma would require more extensive safety data because there are currently adequate drugs on the market to treat these conditions.

3. Direct Sale of OcularGel™ Platform.

The entire OcularGel™ platform could be sold directly. A major manufacturer would pursue this strategy to have proprietary use of OcularGel™ in all of its products, and to prevent OcularGel's™ use in competitive products. Merck is an example of such a manufacturer. In this relationship, BI would work directly with the partner to continue to improve the platform, as the partner requires. Pursuit of this commercialization route would likely end BI's involvement in starch based gels, as defined by the patent. BI would pursue development of depot and other platforms.

Table 11 below shows the timeframes and milestones for the Product Development Plan.

Table 11. Path to commercialization



Product Development Plan

We envision our product evolution pathway in the following stages:

Short-term goals (one to three years)

- *Reformulate existing IP protected drugs for glaucoma, infection, inflammation, and allergy.* Described earlier under "Product development milestones and timeframes" reformulation of existing drugs represents the quickest commercialization route.
- *Reformulate off patent drugs.* Also described earlier under "Product development milestones and timeframes".
- *Formulate with new products under development*
- *Reformulate over the counter (OTC) preparations.* This will not generate large of amounts of revenue for the company, but it could represent a way to generate a larger market recognition for our company.

Long-term goals (three to six years)

- *Develop depot IP for retinal diseases* – age-related macular degeneration (AMD) and diabetic retinopathy (DR). This is at the concept stage of development. The theoretical properties of esters of OcularGel™ may be ideal for local depot injections.
- *Develop Non-Ocular Drug Delivery.* This is at the concept stage of development
- *Gene Targeting.* This is at the concept stage of development

We anticipate the following time frames for accomplishing the stages above:

Table 12. Projected Product Development Timeline

	2004	2005	2006	2007	2008	2009	2010
Existing drugs	→	→	→				
Off patent drugs	→	→	→				
New drugs			→	→	→		
OTC			→	→	→		
Non-ocular drug delivery				→	→	→	→
Retinal diseases				→	→	→	
Gene targeting				→	→	→	

Proprietary rights

The materials under development are proprietary and will be protected under patents. The proof of concept research is occurring at Michigan State University (MSU), and the patents will be filed through the university. A composition of matter patent will protect the technology platform, and that is currently patent pending. A licensing arrangement has been negotiated with MSU in which Biopolymer Innovations will have exclusive rights to all of the patents around this particular drug delivery platform, including future inventions.

Technology for aspects of modifying the hydrophobic/hydrophilic balance of the material that will be licensed to BI for drug delivery applications are covered in eight patents by R. Narayan.

Governmental approvals

As OcularGel™ is not an active ingredient, i.e., it is an excipient, the FDA approval process is rapid compared to having a new active agent approved. The FDA gives only guidelines for approval of excipients in topical ocular formulations.

Permission will be needed from the FDA for evaluation of the OcularGel™ product in humans. The preclinical studies conducted by Biopolymer Innovations are designed to meet FDA standards for this approval process. This requires a Good Laboratory Practice (GLP) study in a dog and rabbit, for duration of one month, at higher dosing frequency and gel concentration levels compared to anticipated levels in humans. This study will be completed at an appropriate contract research organization (CRO).

As described earlier in Table 11, we anticipate that OcularGel™ will be ready for human trials starting in early 2005. The duration of that testing should be completed by will be dependent on the partnering drug but could take as little as six months if the drug has already been approved by the FDA.

Production

There are two production processes involved – first to produce OcularGel™, and second to produce the drug with the gel incorporated.

It is assumed that larger pharmaceutical companies will want to oversee the production of OcularGel ™ once is licensed to them. However, if desired, BI will commercially produce OcularGel™ at a Good Manufacturing Practice (GMP) approved manufacturing

site and commercial production of the pharmaceutical product incorporating OcularGel™ will be conducted by the licensee using their own GMP facilities.

Bibliography

StatRef medical reference.

Besemer, A. C., A. E. J. deNooy, et al. (1996). "Novel methods for the selective oxidation of carbohydrases." Abstracts of Papers of the American Chemical Society **212**: 21-Cell.

Bhardwaj, T., M. Kanwar, et al. (2000). "Natural gums and modified natural gums as sustained-release carriers." Drug Development and Industrial Pharmacy **26**(10): 1025-1038.

Burrows, J., J. Tsibouklish, et al. Drug Delivery to the Eye. **2003**.

Cohen, S. (1998). Ophthalmic delivery System. US, Teva Pharmaceutical Industries.

Davies, N. (2000). "Biopharmaceutical Considerations In Topical Ocular Drug Delivery." Clinical and Experimental Pharmacology and Physiology **27**: 558-562.

DeNooy, A. E. J., A. C. Besemer, et al. (1997). "New polyelectrolytes by selective oxidation of polysaccharides." Zuckerindustrie **122**(2): 126-127.

Domb, A. J., J. Kost, et al. (1997). Handbook of biodegradable polymers. Australia ; United States, Harwood Academic Publishers.

Engineering, L. o. R. R. (1983). Conversion of cellulose and xylan into glycols. NSF Grant No. 8022426AI. R. Narayan.

Fan, Q. C., D. Lewis, et al. (2001). "Characterization of cellulose aldehyde using fourier transform infrared spectroscopy." Journal of Applied Polymer Science **82**: 1195-1202.

Fechner, P. U., K. D. Teichmann, et al. (1998). Ocular therapeutics : pharmacology and clinical application. Thorofare, NJ, SLACK Inc.

Floor, M., A. P. G. Kieboom, et al. "Oxidation of cellulose and starches. Calcium complexation of 2,3 dicarboxy glucans." 63-77.

Gurney, R. (1986). Ocular therapy with nanoparticles. Polymeric Nanoparticles and Microspheres. P. Guiot and P. Couvreur, CRC Press.

Jarvinen, K., T. Jarvinen, et al. (1995). "Ocular Absorption Following Topical Delivery." Advanced Drug Delivery Reviews **16**(1): 3-19.

Jose, J. G., K. A. Polse, et al. (1984). Optometric pharmacology. Orlando, Grune & Stratton.

Kaur, I. P. and M. Kanwar (2002). "Ocular preparations: The formulation approach." Drug Development and Industrial Pharmacy **28**(5): 473-493.

Kim, U. J., S. Kuga, et al. (2000). "Periodate oxidation of crystalline cellulose." Biomacromolecules **1**(3): 488-492.

Lin, H.-R. and K.-C. Sung (2003). Ophthalmic drug delivery formulations and method for preparing the same. USPTO. US.

Mindel, J. S. Bioavailability. Biomedical Foundations of Ophthalmology: Chapter 22.

Nadkarni, S. and S. Yalkowsky (1993). "Controlled delivery of Pilocarpine. 1. In Vitro Characterization of Gelfoam Matrices." Pharmaceutical Research **10**(1): 109-112.

Ni, Y. and K. Yates (2002). In-situ gel formation of pectin. uspto. US.

Peppas, N. A. (1986). Hydrogels in medicine and pharmacy. Boca Raton, Fla., CRC Press.

Rozier, A., J. Mazuel, et al. (1989). "Gelrite: A novel, ion-activated, in-situ gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol." International Journal of Pharmaceutics **57**: 163-168.

Saltzman, M. (2001). Drug Delivery: Engineering Principles for Drug Therapy. New York, Oxford University Press.

Urtti, A. (1995). Kinetic Aspects in the Design of prolonged action ocular drug delivery systems. Advances in Pharmaceutical Sciences. D. Ganderton, Jones, T., and McGinity, J. London, Academic Press. **7**.

Veelaert, S., D. Dewit, et al. (1994). "An Improved Kinetic-Model for the Periodate-Oxidation of Starch." Polymer **35**(23): 5091-5097.