Drug Delivery to the Posterior Segment of the Eye by Subconjunctival Injection

By

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DRUG DELIVERY TO THE POSTERIOR SEGMENT OF THE EYE BY SUBCONJUNCTIVAL INJECTION

Thomas Wai-Yip Lee

Under the Supervision of Professor Joseph R. Robinson

At the University of Wisconsin-Madison

Drug delivery to the posterior segment of the eye is one of the most challenging tasks. Due to physiological constraints, topical and systemic routes are usually not satisfactory for the delivery of a therapeutic agent to the back of the eye. So far, the most promising method is intravitreal injection but this method is too invasive to be used routinely.

Subconjunctival injection is proposed as a method/location for the delivery of therapeutic agents to the back of the eye. It is hypothesized that a properly designed delivery system can achieve therapeutic concentrations in the posterior segment of the eye for an extended period of time through this route. The utilization of pharmacokinetic models is useful in characterizing drug delivery systems. In the current study, the contribution of each penetration pathway for a subconjunctivally injected compound to enter the vitreous chamber was studied and it was found that direct penetration is the only significant pathway for a subconjunctivally injected compound to enter the vitreous chamber. With this piece of information, and some data from the literature, a simple but realistic pharmacokinetic model for subconjunctival injection was developed and validated. Based on this model, the influence of various factors on the drug level in the posterior segment of the eye were studied using computer simulation with an aim to designing a drug

delivery system for subconjunctival injection. It was found that a properly designed delivery system for subconjunctival injection should: (1) provide continuous drug release for an extended period of time, (2) slow down drug loss from the subconjunctival space and (3) preferable have solubilizing capacity.

Finally, Regel® was evaluated in vitro and in vivo since it has a number of unique features and it is believed that these features make it attractive as a potential system for subconjunctival injection. Although Regel® has generated some encouraging results, we still do not have sufficient evidence to accept the hypothesis that Regel® has the requisite properties to be a properly designed drug delivery system for subconjunctival injection. Further study has to be carried out to test this hypothesis.

To our Father in Heaven

TO OUR FATHER IN HEAVEN

Thank you for your salvation and adopt me as your son so that I can enjoy eternity with you

TO DR. J.R. ROBINSON

A man who has changed my life

TO MY FATHER, MOTHER, SISTER, BROTHER AND BROTHER-IN-LAW,

For their love and support

TO YEE-LAY

For her love, support, encouragement and prayers, which absolutely lighten up my life and make my life so beautiful

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CHAPTER 1

INTRODUCTION

I. History of Drug Delivery to the Posterior Segment of the Eye

Many eye diseases such as macular degeneration, diabetic retinopathy, endophthalmitis, etc. affect the posterior segment of the eye. In order to treat these ocular diseases, it is necessary to deliver therapeutic agents to the back of the eye in sufficient concentrations and in a timely manner. However, this is not an easy assignment (Lee, Pince et al. 1989).

The most popular route for ophthalmic delivery is topical, e.g., eye drops, gels, etc. However, therapeutic drug levels in the vitreous humor cannot typically be achieved after topical instillation. After topical application, the drug in the cul-de-sac is subject to a number of very efficient elimination mechanisms such as drainage, induced tear production, etc. In addition, the cornea has relatively poor permeability characteristics to both water soluble and sparingly soluble molecules (Lee 1993). Thus, typically, only 1% or less of a topically applied dose will be absorbed into the anterior segment and very minute amounts can move from there to the posterior segment. In short, delivery of a therapeutic agent to the back of the eye using the transcorneal pathway is neither efficient nor satisfactory (Lee, Pince et al. 1989).

Oral or IV administration is equally unsatisfactory because of the presence of a blood-aqueous (BAB) and blood-retinal barriers (BRB) which limits entry of most drugs into the eye from the blood (Cunha-Vaz 1979). Furthermore, even if they do penetrate, due to the dilution effect of blood, a high dose has to be administered in order to achieve a sufficient concentration in the vitreous and this may cause systemic toxicity. As a result, local administration of a drug is preferred.

Various attempts have been made to introduce a drug directly into the vitreous chamber. Direct intravitreal injection is the most promising method to ensure high drug concentration in the vitreous chamber. Cytomegalovirus retinitis (CMV) is the most common ocular disease associated with Acquired Immunodeficiency Syndrome (AIDS). Intravitreal injection of ganciclovir is the standard treatment for CMV. However, due to the short half-life of intravitreal ganciclovir, frequent injection is necessary to maintain therapeutic levels (Peyman and Ganiban 1995). In order to circumvent this problem, various dosage forms have been developed to sustain drug release in the vitreous chamber. Biodegradable microsphere/microcapsule is one of the strategies to prolong drug release. Herrero-Vanrell et al (Herrero-Vanrell, Ramirez et al. 2000) used microspheres to control release of ganciclovir. The in vitro release profile is very impressive (Figure 1.2). It provides controlled release for more than a month. However, one of the difficulties associated with microspheres is the sterilization step. Typically, sterilization is accomplished by gamma irradiation. However, for some polymers such as poly(ortho ester), gamma irradiation markedly degrades the polymer and formation of degradation products may cause biocompatibility problems (Zignani, Bernatchez et al. 1998). In order to avoid gamma irradiation as the sterilization step, polymers and drug loading have to be prepared using aseptic techniques and this typically greatly increases the cost.

Implantation of a delivery system is another approach to deliver a drug to the back of the eye. The only implantable system in the market is Vitrasert® which is a nondegradable system. In other words, the implanted system has to be surgically removed after all the incorporated drug has been released. Vitrasert® (Figure 1.3) provides controlled release of ganciclovir over a 7-

month period and this may be considered as an alternative to repeated intravitreal injections (Dhillon, Kamal et al. 1998).

A biodegradable implant system has also been developed to circumvent the "nonbiodegradable" nature of Vitrasert® so that no surgical removal is required. A scleral plug, which was made of PLGA, was developed to deliver therapeutic agents to the vitreous chamber (Figure 1.4) (Yasukawa, Kimura et al. 2001). The surgical procedure is relatively simple and it involves a 1mm-sclerotomy made by a 18G needle (Kunou, Ogura et al. 2000). A scleral plug with ganciclovir gives a therapeutic concentration in the vitreous humor for up to 28 weeks (Yasukawa, Kimura et al. 2001) (Figure 1.5). However, heating over 80°C is necessary to fabricate the scleral plug. Therefore, this delivery system may not be suitable for heat-labile drugs.

Generally, intraocular administration of drugs, suffers from a major drawback that it is invasive and not many patients can tolerate the procedure if multiple injections are required. Trauma may result due to damage to the retina, the lens, or extreme changes in intraocular pressure. Infection is another complication that needs to be considered. High drug concentration in the vitreous humor may also cause retinal toxicity. Obviously, intraocular administration, as well as topical and systemic delivery is not satisfactory in delivering a drug to the back to the eye (Maurice 1976). Therefore, a method which is minimally invasive but efficient enough to supply sufficient drug to the posterior segment of the eye is highly desirable.

II. Transcorneal Vs Transscleral Pathway

In terms of drug delivery to the anterior segment of the eye, the transcorneal pathway is considered to be the major route. Ahmed and Patton showed that (Ahmed and Patton 1985) timolol concentration in the anterior segment was dramatically decreased (40x for aqueous humor, 300x for cornea and 1300x for iris ciliary body) when corneal access was denied after topical instillation of a timolol eye drop. The cornea has a very unique multi-laminate structure (Figure 1.6) (Robinson 1993). The outermost epithelial layer, about 5-7 cell layers thick, is lipophilic and followed by the high water content stromal layer comprising 85-90% of the corneal thickness and lastly, the single cell layer thick endothelium. The endothelium is quite leaky so that it is not considered as a barrier in terms of permeability. For lipophilic compounds, they can partition into the lipophilic epithelium easily but their further movement will be hindered by the hydrophilic stroma. Therefore, in order to have efficient delivery using the transcorneal pathway, compounds should have a balance between their hydrophilic and lipophilic characters so that they can pass across both the epithelium and stroma effectively. Typically, the optimum partition coefficient has been reported in the range of 10 to 100, showing that lipophilic compounds are generally preferred (Schoenwald and Huang 1983). For the majority of compounds, the epithelium is the major barrier. Since the epithelium behaves like a typical biological membrane, there exists two major penetration pathways for a molecule to gain access to the interior of the eye, namely, paracellular and transcellular. Paracellular transport is limited by the presence of tight junctions between adjacent cells in the epithelium. Transcellular pathway involves partition into the lipophilic membrane. Unless a drug is extremely big and hydrophilic, it would probably opt for the transcellular pathway due to a much larger surface area.

The transscleral pathway, as the name implies, means transport of a molecule across the sclera. In fact, after topical application, an instilled drug has to diffuse across the conjunctiva before reaching the sclera. The conjunctiva is a highly vascularized mucous membrane that covers the anterior surface of the eye except the cornea. Generally speaking, the permeability of the conjunctiva is higher than that of the cornea (Hamalainen, Kananen et al. 1997) and at the same time the surface area of the conjunctiva is about fives times that of the cornea (Robinson 1993). Therefore, it may serve as an alternative pathway for molecules which are poorly absorbed via the cornea. However, the high vascularity of the conjunctiva may be a disadvantage in terms of drug delivery since a significant amount of an instilled drug reaching the conjunctiva will be carried into the systemic circulation. The transscleral route appears to be favored by drugs which are poorly absorbed by the cornea such as hydrophilic macromolecules. For example, inulin, which has a molecular weight of about 5000 daltons, as much as 40% of the absorbed amount is due to non-corneal penetration (Ahmed and Patton 1987). A more detailed description of the sclera will be discussed later. In general, the sclera is much more permeable than the cornea. (Prausnitz and Noonan 1998). It was shown that the permeability of the sclera is more sensitive to molecular weight rather than lipophilicity (Ahmed, Gokhale et al. 1987; Prausnitz and Noonan 1998). Therefore, it serves as an alternative pathway, especially for hydrophilic macromolecule, to enter the eye. Besides topical application, a more direct way to make use of the transscleral pathway is to place a drug in direct contact with the sclera, i.e., bypass the conjunctiva. Subconjunctival injection is one of the ways to achieve this goal.

III. Drug Delivery to the Posterior Segment of the Eye using Subconjunctival Injection

As mentioned earlier, many studies have shown that the sclera is much more permeable than the cornea (Prausnitz and Noonan 1998; Unlu and Robinson 1998) and this may serve as a potential pathway to deliver a drug to the back of the eye. Some efforts have been made to make use of this "noncorneal" pathway. Lee (Saha, Uchiyama et al. 1996; Lee 1998) has carried out extensive studies on drug delivery across the conjunctiva. His intention was to make use of the relatively permeable conjunctiva and sclera (transscleral) to deliver therapeutic agents to the back of the eye after topical application. Alternatively, drugs can be injected subconjunctivally to bypass the conjunctiva, which is also considered as a barrier in terms of permeability (Prausnitz and Noonan 1998).

Not many studies are available regarding drug delivery to the posterior segment of the eye by subconjunctival injection. Moreover, their primary focus is the outcome, i.e., how much drug can be detected in the vitreous after subconjunctival injection, rather than identifying penetration pathways and barriers. Therefore, overcoming the biological barriers associated with this route may be difficult and will need to be explored. Typically, after subconjunctival injection, the bioavailability in the vitreous is poor (Rootman, Tisdall et al. 1979; Tsuji, Tamai et al. 1988). However, for most cases, subconjunctival injection can yield a much higher bioavailability in the vitreous than IV or topical route. The peak levels and bioavailability achieved after subconjunctival injection of dacarbazine are 380 and 248 times that achieved after IV administration (Kalsi, Silver et al. 1991) and subconjunctival injection of 6-mercaptopurine

gave peak levels and bioavailability which is 10 and 6.4 times that achieved after IV administration (Gudauskas, Kumi et al. 1985). Cyclosporine also showed similar results (Kalsi, Gudauskas et al. 1991).

Lincoff et al (Lincoff, Kreissig et al. 2001) modified a balloon catheter which was originally used for retinal detachment to deliver cortisol sequentially into the subtenon's space (Figure 1.7). They showed that the peak vitreous cortisol level of the dosed eye is 20 times greater than that of the contralateral eye after a single injection (Figure 1.8). They also demonstrated a sustained vitreous drug level can be achieved after sequential injections (3-hour interval) (Figure 1.9). This indicates that it is possible to deliver a drug to the posterior segment of the eye via the transscleral pathway. Results from sequential injection also shows that it is possible to sustain the vitreous drug level by a properly designed delivery system which can provide sustained drug concentration at the absorption site (sclera). Another study by Amabti et al (Ambati, Gragoudas et al. 2000) confirms this speculation. They implanted an Alzet Osmotic Pump subconjunctivally (Figure 1.10) and used it to deliver IgG to the choroid and retina for 28 days. Although IgG cannot be found in the vitreous chamber due to its size (150kD), statistically significant higher drug level can be found in the choroid and retina compared with that of the contralateral eye (Figure 1.11). Although the Alzet Osmotic Pump is not a practical way to deliver a drug to the back of the eye from a clinical point of view, this study indicates that with a properly designed drug delivery system, it is possible to sustain the drug level in the posterior segment of the eye via the transscleral pathway.

IV. Fundamentals of Subconjunctival Injection

(i) *Potential Penetration Pathways for a Subconjunctivally injected compound to enter the Vitreous Chamber*

Conrad and Robinson (Conrad and Robinson 1980) carried out an extensive study on penetration of pilocarpine into the aqueous chamber after subconjunctival injection in rabbits. They concluded that reflux out of the puncture hole and subsequent transcorneal absorption was the dominant pathway for pilocarpine to enter the aqueous chamber, especially when the injection volume was over 100μL. However, they did not carry out a detailed study on the penetration pathways into the vitreous chamber after subconjunctival injection and so this aspect is still poorly understood.

Basically, there are three possible pathways for a subconjunctivally injected compound to enter the vitreous chamber (Figure 1.12): (1) direct penetration across the underlying tissue layers into the vitreous chamber; (2) absorption of the drug into the aqueous chamber and from there it moves backward via the space between the lens and ciliary body (lens zonule) to the vitreous chamber and (3) absorption of the drug into the general circulation and then recirculation back to the vitreous chamber. Pathway (iii) is easily identified since the drug level should be almost the same for both eyes when a drug is injected subconjunctivally into one of the eyes if this pathway is dominant. However, it is hard to distinguish pathway (i) and (ii) experimentally. One early study showed that there was direct penetration of hydrocortisone (McCartney, Drysdale et al. 1965) into the underlying layer after subconjunctival injection.

Therefore, pathway (i) is a likely penetration pathway. However, it is not appropriate to simply exclude the possible existence of pathway (ii) based on such evidence, especially when there exists a favorable concentration gradient to move a drug from the aqueous to the vitreous chamber, i.e., the concentration of a drug in the aqueous chamber is higher than that in the vitreous chamber (Tsuji, Tamai et al. 1988).

Pathway (ii) involves absorption of drugs into the aqueous chamber and from there it moves backward into the vitreous chamber according to a favorable concentration gradient. As mentioned earlier, reflux out of the puncture hole and subsequent transcorneal penetration is the major pathway for a subconjunctivally injected compound to enter the anterior chamber (Conrad and Robinson 1980). However, an injected drug may also enter the anterior segment using the transscleral pathway (Schoenwald, Deshpande et al. 1997) via the iris-ciliary body which is considered as a relatively leaky tissue (Cunha-Vaz 1979).

As mentioned earlier, contribution of systemic delivery to the total vitreous drug level after subconjunctival injection is believed to be insignificant due to the presence of the BRB and BAB (Cunha-Vaz 1979). Moreover, the dilution effect of blood further decreases the possibility that systemic delivery can play a major role in determining the vitreous drug level. The pathways for a subconjunctivally injected compound to enter the systemic circulation are poorly understood. However, it can be speculated that a drug may enter the systemic circulation via the highly vascularized conjunctiva and choroid. Moreover, blood vessels in the sclera also carry an administered drug into the systemic circulation.

The direct penetration pathway involves diffusion across a number of tissue layers under the injection site (sclera). An injected drug will diffuse across the following layers before

reaching the vitreous chamber. Sclera→ Choroid→ Retinal Pigment Epithelium (RPE)→ Retina→ Vitreous Chamber (Figure 1.13). More detailed descriptions of the anatomy and physiology of each tissue are as follows.

(a) Sclera

Structure (Foster and de la Maza 1994)

The sclera encloses about five-sixths of the eye. Its thickness varies from 0.3mm immediately behind the insertion of the rectus muscles to 1.0mm near the optic nerve. It measures 0.4-0.5mm at the equator, 0.6mm where the tendons of the rectus muscles attach, and 0.8mm adjacent to the limbus. The sclera is divided into three distinct layers (Figure 1.14). Episclera is the outer superficial layer which merges with the underlying scleral stroma. The episclera consists of loosely arranged collagen bundles intermingled with proteoglycans, glycoproteins, etc. The scleral stroma consists of collagen bundles with a few elastic fibers. The inner layer, lamina fusca, is the portion of the sclera that is adjacent to the uvea. A large number of melanocytes is present so that it has a faintly brown color.

Basically, collagen forms 75% of the dry weight of the sclera. Elastin forms less than 2% of the dry weight of the sclera. Proteoglycans constitute 0.7-0.9% of the dry weight of the sclera. The water content of the sclera ranges from 65 to 75%. The sclera appears opaque if the water content is maintained between 40 to 80% but becomes transparent if it falls below 40% or rises above 80%.

Function (Torczynski 1982)

The sclera protects the intraocular content from injury and mechanical displacement. The toughness of the sclera, coupled with intraocular pressure, resists deformation by the contracting muscles through the full range of ocular movement.

Relevance to Drug Delivery

Except for perforating vessels, the sclera is a relatively avascular tissue due to a low metabolic requirement because of the slow turnover rate of its collagen. This may be an advantage in terms of drug delivery since less drug will be carried away into the systemic circulation because of the limited blood supply in the sclera.

In terms of permeability, the sclera is much more permeable than the cornea (Ahmed, Gokhale et al. 1987) so that a noncorneal or transscleral pathway has been proposed for ocular drug delivery (Schoenwald, Deshpande et al. 1997). The transscleral pathway is particularly important for hydrophilic macromolecules which are poorly absorbed by the cornea such as inulin (Ahmed and Patton 1985). It was shown that macromolecules up to 150kDa can pass across scleral tissue (Ambati, Canakis et al. 2000). Typically, the permeability coefficients of the sclera to small molecular weight compounds (<500Da) are on the order of magnitude of 10^{-5}cm/sec or greater (Prausnitz and Noonan 1998). It was shown that permeability of the sclera is much more sensitive to molecular weight instead of lipophilicity (Ahmed, Gokhale et al. 1987; Prausnitz and Noonan 1998). A comprehensive mathematical model has been set up to predict permeabilities of the sclera to various chemicals (Edwards and Prausnitz 1998).

(b) Choroid

Structure (Richard 1992)

The choroid, together with the ciliary body and iris (the uvea) forms the middle of the three coats surrounding the eye ball. The choroid is located between the sclera and the retina and covers the inner portion of the eyeball, and merging anteriorly into the ciliary body and iris. It is connected to the sclera by a network of collagen fibers. The thickness of the choroid is reported to be 0.1-0.3mm.

Several layers can be identified in the choroid. Bruch's membrane is formed from collagen and elastic fibers and is about 2μm thick. It lies directly on the basement membrane of the retinal pigment epithelium and is partly connected with the basement membrane of choriocapillaries, which forms the next layer. Sattler's layer is the layer of the medium and smaller vessels. Haller's layer is formed by the larger vessels, arteries, and veins.

The choroid consists of a rich supply of blood vessels (Figure 1.15) which are embedded in a ground substance of loose connective tissue with collagen, elastic, and reticular fibers. Unlike retinal capillaries, choriocapillaries have fenestrations which are about 55-60nm in diameter which allows passive diffusion of fluids and small molecules (Spitznas 1974).

Function

Basically, the main function of the choroid is to nourish the outer layer of the retina. Nutrients and oxygen come out through large pores of the choriocapillaries and then pass through the Bruch's membrane and retinal pigment epithelium until they reach the sensory epithelial cells. The pores in the choriocapillaries can allow molecules with diameter as large as 1.9-5.8nm to pass through.

Another function of the choroid is to regulate the temperature of the eye. Due to its rich supply of capillaries, the choroid can serve as a source of heat to replace heat loss to the outer part of the eye. On the other hand, it can also cool off the eye under extreme environmental conditions.

Relevance to Drug Delivery

In terms of permeability, the choroid does not seem to be a barrier since a sizable amount of hydrocortisone can be found in both the choroid and retina after subconjunctival injection (McCartney 1965). Moreover, IgG, a 150kDa macromolecule, can be found in both the choroid and retina after subconjunctival administration (Ambati, Gragoudas et al. 2000).

However, fast blood flow of the choriocapillaries may impose a barrier for drug delivery. Its circulation volume is about 20 times greater than the retina and about 15-20 times greater than that of the brain cortex. With such a high blood flow, a significant amount of an administered drug reaching the choroid will be carried away into the systemic circulation. It has been shown that fluorescein level in the vitreous after subconjunctival injection was markedly increased when the choroidal circulation was locally closed down by pressure (Tsukahara and Maurice 1995). This may indicate choroidal circulation is important in removing drug after local administration.

(c) Retinal Pigment Epithelium (RPE)

Structure

The Retinal pigment epithelium (RPE) is a single layer of cells lying between the retina and choroid (Figure 1.16). The RPE is considered to be the outer barrier of the blood-retinal barrier (BRB) which prevents various substances in the blood from entering the eye. The RPE extends to the optic nerve margin posteriorly and to the ora serrata anteriorly, where it fuses with the anterior continuation of the sensory retina and continues forward as the pigmented epithelium (Newell 1992). The cells of the RPE contain varying amount of melanin to give it a granular appearance. The apical surface of the RPE faces the subretinal surface, in which the outer segments of the photoreceptor cells (rods and cones) are located. There are numerous microvilli present at the apical surface and they are involved in the phagocytizing of rod and cone outer segments (Zinn and Benjamin-Henkind 1982). The lateral surfaces of the apices of adjacent cells are bound together by tight junctions so that there is no intercellular space at the level of these junctions. These junctions and together with the nonfenestrated retinal blood vessels constitute the blood retinal barriers (BRB) (Newell 1992).

Function (Zinn and Benjamin-Henkind 1982)

Physically, the RPE serves as a barrier to protect the retina from unwanted molecules from the choroidal circulation. It is involved in adhesion of the retina to the RPE by its differential permeance (compared with the retina) to water (Kirchhof and Ryan 1993). Optically, the RPE absorbs light energy and reduce light scatter, improving resolution of images. Metabolically, the RPE is involved in phagocytosis of rod and cone outer segments and subsequent degradation by hydrolytic enzymes. It is involved in vitamin A metabolism. The RPE transports numerous metabolites and ions to and from the visual cells and choroidal circulation.

Relevance to Drug Delivery

As mentioned earlier, the tight junctions between adjacent cells prevent entry of any unwanted molecules from the choroidal circulation. However, these tight junctions also hinder transport of drugs across it. Studies show that penetration of carbon, trypan blue and fluorescein from the choroid stops at the level of the RPE (Cunha-Vaz 1979). Studies show that (Frambach, Valentine et al. 1988) the RPE is the only significant contributor to the TEER of the Sclera-Choroid-RPE layer. Removal of the RPE decreased the TEER from 350 to 2.3Ωcm^2 (Frambach, Valentine et al. 1988). Another study also showed that removal of retina-choroid from the sclera increased the permeabilities of the sclera by 5-10x (Ohotori and Tojo 1994; Tojo, Nakagawa et al. 1999) to small molecules. This evidence clearly shows that the RPE is a barrier for posterior delivery in terms of permeability.

In addition, the presence of multiple transport systems in the RPE may speed up elimination of drugs in the vitreous from the retina to choroid direction. Koyano et al (Koyano, Araie et al. 1993) showed that outward permeability of an excised retinal pigment epithelium-choroid to fluorescein is four times greater than inward permeability. Various inhibitors can decrease the outward permeability but not the inward permeability. This may indicate that passive diffusion is the major transport mechanism for influx and multiple carrier-mediated transport systems are involved in outflux. However, the substrate specificity of these active transport systems is poorly understood.

(d) Retina

Structure (Riordan-Eva 1995)

The retina is a thin, semitransparent, multilayered sheet of neural tissue that lines the inner part of the posterior two-thirds of the wall of the globe. It extends almost as far anteriorly as the ciliary body, ending at the oral serrata, a ragged edge. The outer surface of the retina is attached to the retinal pigment epithelium and is thus related to Bruch's membrane, the choroid, and the sclera.

The retina is divided into 9 layers (Figure 1.17), namely, from its inner aspect, (1) internal limiting membrane; (2) nerve fiber layer containing the ganglion cell axons passing to the optic nerve; (3) ganglion cell layer; (4) inner plexiform layer, containing the connections of the ganglion cells with the amacrine and biopolar cells; (5) inner unclear layer of bipolar and horizontal cells with photoreceptors; (6) outer plexiform layer, containing the connections of the bipolar and horizontal cells with the photoreceptors; (7) outer nuclear layer of photoreceptor cell nuclei; (8) external limiting membrane and (9) photoreceptor layer of rod and cone inner and outer segments. The retina receives its blood supply from two sources: the choriocapillaries immediately outside Bruch's membrane, which supplies the outer third of the retina, the inner two-thirds of the retina receives its blood supply from branches of the central retinal artery. The retinal blood vessels have a non-fenestrated endothelium, which forms the inner blood-retinal barrier.

Function (Hardy 1995)

Obviously, the main function of the retina is vision. Rods and cones in the photoreceptor layers are capable of transforming light stimulus into a nerve impulse that is conducted by the nerve fiber layer of the retina through the optic nerve and ultimately to the occipital visual

cortex. The macula is responsible for the best visual acuity and for color vision, and most of its photoreceptor cells are cones. The remaining retina, which is populated mostly by the rod photoreceptors, is mainly used for peripheral and night (scotopic) vision.

Relevance to Drug Delivery

Thorotrast particles can pass from the vitreous into the intracellular spaces of the retina (Smelser, Ishikawa et al. 1965). The movement of horseradish peroxidase is not stopped until it reaches the tight junctions of the retinal pigment epithelium (Peyman and Apple 1972; Peyman and Bok 1972). Therefore, the retina is not considered as a barrier in terms of permeability. However, the role of the retina in terms of drug metabolism is not well understood.

(e) Vitreous

Structure

The vitreous is a transparent connective tissue surrounded posteriorly by the internal limiting membrane of the retina and anteriorly by the posterior lens capsule. The spherical vitreous body has an average volume of about 4mL in human and 1.4mL in rabbit. The vitreous is a clear gel composed almost entirely of water (99%). The remaining 1% is collagen, hyaluronic acid, etc. Collagen is the major structural component of the vitreous and is organized as thin fibrils. There is heterogeneity in the distribution of collagen throughout the vitreous. The highest density is present in the vitreous base, followed by the posterior vitreous cortex anterior to the retina. The lowest density was found in the central vitreous. Hyaluronic acid (HA) has a

different distribution that is most abundant in the posterior cortical gel with a gradient of decreasing concentration as one moves centrally and anteriorly.

Function (Schepens and Neetens 1987)

The vitreous provides clarity for vision. The refractive index of vitreous is 1.3349, nearly the same as that of aqueous humor. The vitreous transmits 90% of light between 300 and 1400nm. The transparency of the vitreous is achieved by the extremely low concentration of macromolecular solutes. The vitreous also inhibits the proliferation of cells involved in the inflammatory, proliferative, and neovascular responses. The vitreous also assists in accommodation. The anterior vitreous presses on the posterior lens capsule, causing the lens to protrude forward into a more deeply curved lens-zonule diaphragm. The smaller diameter of the ciliary muscle sphincter would therefore result in an increase in axial lens thickness and a decrease in equatorial lens diameter. Another important function of the vitreous is to provide mechanical strength of the eyeball. The vitreous can protect against friction as well as high-frequency stress since it is viscoelastic. In addition, it is obvious that the vitreous serves as a metabolic repository for neighboring tissues. Although it is not well understood, it is believed that the vitreous may serve as a repository for amino acids destined for use by the retina. It may also serve as a repository for metabolic wastes such as lactic acid.

Relevance to Drug Delivery

The major concern of the vitreous as a barrier for posterior delivery is diffusion of drugs inside the vitreous since any administered drug has to diffuse across the vitreous before reaching the macula region or other disease target located in the various parts of the retina. The diffusion coefficients of dexamethasone sodium m-sulfobenzoate and ganciclovir in the vitreous are

$5.1x10^{-6}cm^2/s$ and $9.89x10^{-6}cm^2/s$ which are the same order of magnitude as in an aqueous solution ($7.0x10^{-6}cm^2/s$) (Ohotori and Tojo 1994; Tojo, Nakagawa et al. 1999). This may indicate that diffusion is not a problem in the case of small molecules.

There are too many controversies regarding the vitreous turnover. Some claimed that there is no flow within the vitreous and it acts like an unstirred water pool (Friedrich, Cheng et al. 1997). However, some people found that water movement is very active. The turnover rate is 50% every 10-15 minutes (Kinsey, Grant et al. 1942). A later study confirmed this finding (Bay 1974). Maurice (Maurice 1957) and Kinsey (Kinsey, Reddy et al. 1960) showed that diffusion is the main process influencing transvitreal movement of molecules for the size of glucose and lactic acid. This is in good agreement with another study (Moseley, Foulds et al. 1984) that the mean transit time from the retina to the mid vitreous is 32±2 minutes, and from the mid vitreous to the anterior chamber is 84±3 minutes and computation showed that such transport can be accomplished by diffusion alone. However, Fatt and Hedbys (Fatt and Hedbys 1970) postulated the existence of appreciable transvitreal bulk flow. Although this appeared to contradict earlier findings, Fatt (Fatt 1975) pointed out that whereas both diffusion and bulk flow occur in the vitreous, the former is eight times greater than the latter for a small ion. This appears to be true of any low molecular weight substances because the velocity of posteriorly directed bulk flow is too small to influence transport. Thus low molecular weight substances diffuse in response to a concentration gradient and high molecular weight substances and colloidal particles travel as a front with convective flow, as the diffusion constant is very small. According to the literature, the half-lives after intravitreal injection vary from 3 to 25 hours (Maurice 1976) which are much

longer than the turnover rate of water in the vitreous (15 minutes). Obviously, the water turnover in the vitreous does not play a significant role in drug elimination from the vitreous.

(ii) Elimination Pathways from the Vitreous Chamber

In contrast to the penetration pathways, the elimination pathways from the vitreous chamber are comparatively well understood. Three major pathways can be identified: (a) via the lens zonule into the posterior aqueous chamber and then out of the eye by the way of aqueous turnover; (b) directly across the retinal surface by simple diffusion. (Maurice and Mashima 1984) and (c) across the retinal surface by active transport mechanisms.

(a) Via Lens Zonule

If a compound cannot pass across the retinal surface easily, e.g., a hydrophilic compound, it can only leave the vitreous chamber anteriorly, where it will be carried away by aqueous turnover or diffusion across the iris surface. In this case, the rate-determining step will be the speed at which it can diffuse through the vitreous body and the lens zonule. Maurice (Maurice and Mashima 1984) derived an equation to evaluate elimination of a compound from the vitreous after intravitreal injection. Assume the drug cannot pass across the retinal surface and it can only leave the vitreous chamber by the anterior pathway (via the lens zonule), then

$$k_v C_v V_v = k_a C_a V_a \qquad (1.1)$$

$$k_v = k_a (V_a/V_v)(C_a/C_v) \qquad (1.2)$$

$$k_v = f/V_v(C_a/C_v) \quad (1.3)$$

where k_v=rate constant for elimination from the vitreous

k_a=rate constant for aqueous turnover

V_a=volume of aqueous humor

V_v=volume of vitreous humor

C_a=concentration in the aqueous humor

C_v=concentration in the vitreous humor

f= aqueous flow rate

By plotting k_v versus C_a/C_v, a straight line which is calibrated in terms of molecular weight can be obtained (Figure 1.18). Any deviation from the line may simply imply the presence of other elimination pathways (Na^+, Br^-). It can be seen from Figure 1.18 that the higher the molecular weight, the smaller is the diffusion coefficient which in turn gives a longer half-life in the vitreous. In other words, the rate of loss from the vitreous is controlled entirely by the rate of diffusion in the vitreous.

This method cannot be applied directly to evaluate elimination pathways after subconjunctival injection since it cannot be assumed that the drug in the aqueous after subconjunctival injection is solely coming from the vitreous humor. A significant amount of drug can enter the aqueous chamber from the subconjunctival space.

(b) Via Retinal Surface

When passage occurs across the retina, the diffusional path through the vitreous body is shortened and the small cross-sectional area of the lens zonule is replaced by a wide surface available for absorption. Therefore, the loss will be accelerated. The barrier (Blood-retinal Barrier, BRB) between the vitreous and the blood, located partly in the retinal vessels and partly in the retinal pigment epithelium (RPE), is impermeable to compounds which have high molecular weight or are highly charged or highly hydrophilic such as albumin, but pass others, such as small ions. Therefore, after intravitreal injection, hydrophilic compounds or compounds with high molecular weight tend to have longer half-lives than lipophilic compounds (Maurice 1976).

(c) Via Carrier-mediated Active Transport systems

It is known that both fluorescein and carboxyfluorescein are moved out of the vitreous by active carrier systems which involve multiple transport systems such as Na-K ATPase, etc. However, the substrate specificity of such systems is not known.

The rates of loss of fluorescein and iodopyracet are 28 and 23%/hr respectively. These rates are six times higher than the expected rates due to loss by the anterior route on the basis of their molecular weight. Not only k_v is changed but C_a/C_v is altered as well. Figure 1.18 showed that they deviate from the line corresponding to the anterior route significantly.

It is possible to slow the removal of these compounds by introducing competitive or metabolic inhibitors. When the transport system is blocked, not only the rate but the line in the k_v and C_a/C_v plot move closer to the line corresponding to the aqueous loss. In this case, loss via aqueous turnover becomes more important in elimination of the compounds from the vitreous.

It is interesting to note that the values of the elimination rate constant for various compounds lie within a narrow range no matter which pathway they are using. Typically, it ranges from 0.03 to 0.8hr^{-1} (Maurice 1976). Therefore, the elimination rate constant can be estimated with reasonable accuracy.

V. Experimental Approaches to Study Drug Delivery to the Posterior Segment of the Eye (Liaw and Robinson 1993; Urtti and Salminen 1993; Lee 1996)

(I) In Vitro

Basically, an in vitro model can evaluate drug transport across biological membranes/tissue layers, mechanism of transport (i.e., passive diffusion, carrier-mediated transport, endocytosis, etc.); and effect of formulation pH, tonicity and the presence of adjuncts such as chelating agents, preservatives on drug transport. Typically, either a tissue or cell monolayer is mounted between two diffusion cells. The amount of drug accumulated in the receiver chamber is plotted against time and the permeability coefficient determined. Although an in vitro model is very simple to use, and is an excellent tool to study drug transport across biological membranes, it does have a number of drawbacks. Firstly, there is an unrealistically long exposure of the absorbing surface to the drug and this may not be the case in vivo. Secondly, permeability is determined under pseudo steady-state conditions, which are not established in vivo. Most importantly, in vitro models neglect factors other than permeability such as drug loss to the systemic circulation, elimination of drugs from the subconjunctival space, etc. Moreover, before carrying out an in vitro transport study, the exact penetration

pathways have to be known. Otherwise, it is not possible to isolate a specific tissue or develop a cell culture system for drug transport study. However, little is known about the penetration pathways after subconjunctival injection. Therefore, in vitro models may not be useful for the current study.

(II) In Vivo

Typically, an in vivo model involves a pharmacokinetic study using animals. This method is beneficial at an early stage of studying a potential route for drug delivery since it gives a global view of the whole pharmacokinetic phenomenona such as absorption, elimination from the target, elimination from the absorption site, etc. However, it fails to give insight on a cellular or subcellular levels of drug delivery. Ideally, an in vivo study should be supplemented by an in vitro experiment.

Typically, 6-12 animals are used for each time point. Although a large number of animals are used for each time point, coefficients of variation on the order of 25-75% are not unusual.

VI. Rabbit as a Model

Due to a destructive sampling technique used in ocular drug delivery study, humans are rarely used as study subjects except during surgery. Therefore, an animal model has to be used to conduct ocular drug delivery studies. The animal model selected must be predictive of behavior of a drug as well as performance of the delivery system in humans. Over the years, rats, mice, pigs, monkeys, cats, chickens, guinea pigs and rabbits have been used. Of these, the albino rabbit

is the most widely used species in studying ocular drug delivery primarily because of ease of handling, low cost, comparable size of its eyes to human eyes, and extensive information about its ocular biochemistry, physiology, pharmacokinetics, pharmacodynamics, etc. However, care should be taken in extrapolating results from an animal study to humans since dissimilarities do exist between these two species (Table 1.1).

The major concern in using rabbits as an animal model to study ocular drug delivery is the difference in blinking rate. The higher precorneal loss in human is attributed to a higher blinking rate (6-7/min) rate. However, it is believed that this factor may not contribute significantly to the discrepancy between rabbit and human studies in the case of subconjunctival injection since a drug is injected into a space which is enclosed by the conjunctiva and the sclera. Therefore, removal of injected drug is less sensitive to the precorneal fluid dynamics, especially when a small volume (< 100μL) is injected.

Another concern is the much larger eye-to-body ratio in the rabbit compared to the human and this will in turn affect the contribution of systemic delivery to the total drug level in the vitreous. Although some studies showed that (Bodker, Ticho et al. 1993) the contribution of systemic delivery to the total drug level in the vitreous chamber of a rabbit is sizable after subconjunctival injection, it is believed that this contribution in human will be much smaller since the human has a much smaller eye-to-body ratio.

On the positive side, isolated rabbit and human scleras show similar permeabilities (Edelhauser and Maren 1988) and removal of the injected compound from the subconjunctival space also shows a similar pattern in both species (Maurice and Mashima 1984). Therefore, the

rabbit is an invaluable model in studying drug delivery to the posterior segment of the eye by subconjunctival injection.

Table 1.1: Comparison of Rabbit and Human Eye

	Rabbit	Human
Precorneal Area		
(i) Tear volume	7-8μL	7-8μL
(ii) Tear Turnover	8%/min(0.5μL/min)	16%/min (1.2μL/min)
(iii)Blink rate	4/hour	6-7/min
(iv) Drainage rate constant 50μL 25μL	 0.545/min 0.55/min	 1.40/min 1.6/min
(v) Protein content of tears	0.5%	0.7%
(vi) pH of lacrimal fluid	7.4	7.4
(vii) Buffering capacity of the lacrimal fluid	poor	Poor
(viii) Milliosmolarity of tear	305mOsm/L	305mOsm/L
Nonproductive absorption		
(i) Nictating membrane	present	Absent
(ii) conjunctiva/cornea area ratio	9	17
Cornea		
(i) area	1.5-2.0cm^2	1.04cm^2
(ii) Thickness	0.35-0.45mm	0.52mm
(iii) Permeability to hydrophilic compound	lower	Higher
(iv) Diameter	15mm	12mm
(v) Bowman's membrane	absent	Present
(vi) Number of epithelial layers	5-7	5-7
Sclera		
(i) Permeability compared with the cornea	Higher	Higher
(ii) Permeability compared between species	Similar	Similar
Intraocular Kinetics		
(i) Aqueous humor		
(a) Volume	0.25-0.3mL	0.10-0.25mL
(b) Protein concentration	0.55mg/mL	30mg/mL
(c) Turnover rate	1-2%/min (3.0-4.7μL/min)	1-2%/min (2.0-3.0μL/min)
(d) pH	7.2	7.1-7.3
(ii) Anterior uveal		
(a) Pigmentation in iris- ciliary body	absent	Present
(iii) Posterior segment		
(a) Volume	1.4mL	4mL
Drug metabolism		
(i) Esterase activity	-	1.6-4.2 fold of the rabbit

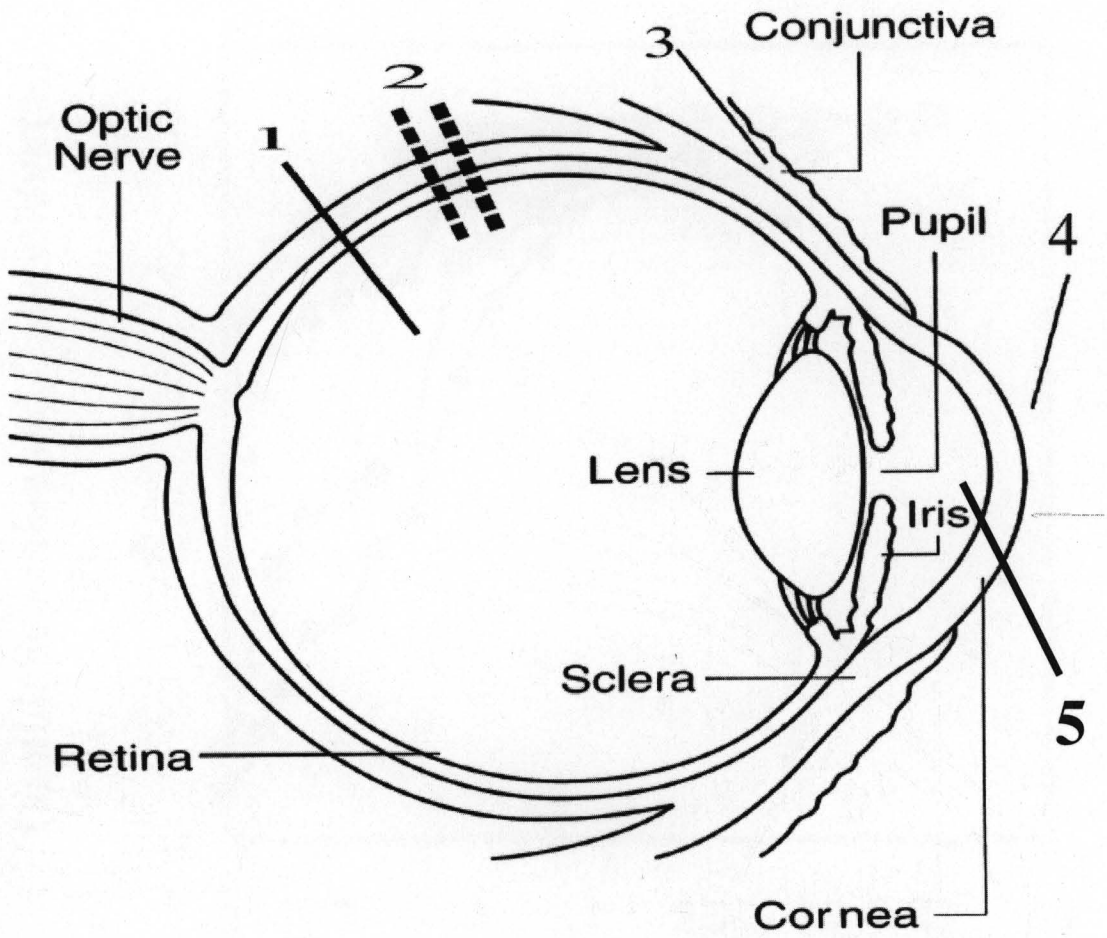

Figure 1.1: Different modes of administration

1. Intravitreal injection; 2. Implantation; 3. Subconjunctival injection; 4. Topical application; 5. Intracameral injection (Systemic delivery is not shown)

(Adapted from NEI 2000 with modifications)

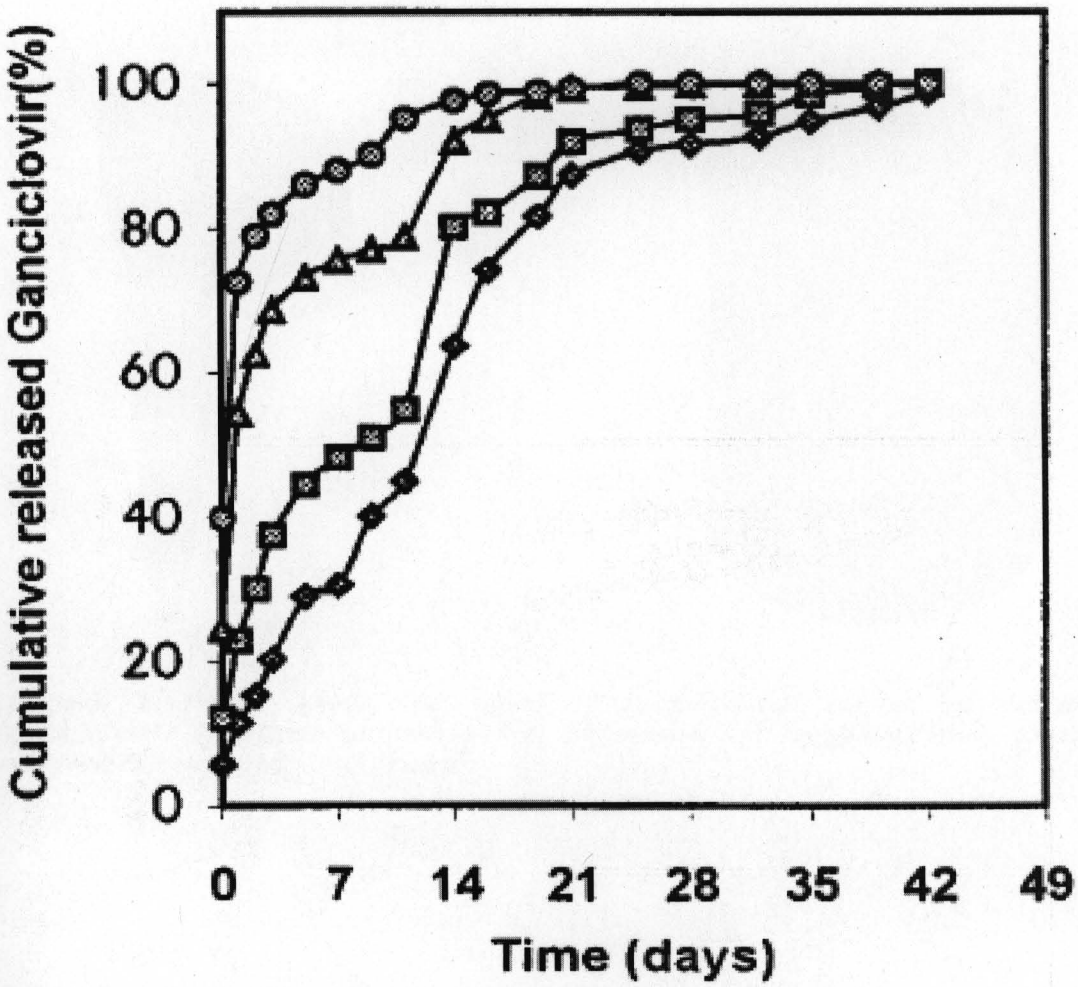

Fig. 1. In vitro cumulative ganciclovir released from 10-mg microspheres of different sizes (●53–106 μm microspheres:681 μg; ▲106–212 μm microspheres: 764 μg; ■ 212–300 μm microspheres: 845 μg and ♦ 300–500 μm microspheres: 864 μg) prepared using the evaporation solvent method from an O/O emulsion, with PLGA (50:50), inherent viscosity 0.41 dl/g. (Adapted from Ref. [57], R. Herrero Vanrell, L. Ramirez, A. Fernandez-Carballido, M.F. Refojo. Pharm. Res. 16 (2000).

Figure 1.2: Release profile of ganciclovir from microspheres

(Adapted from Herrero-Vanrell et al, 2000)

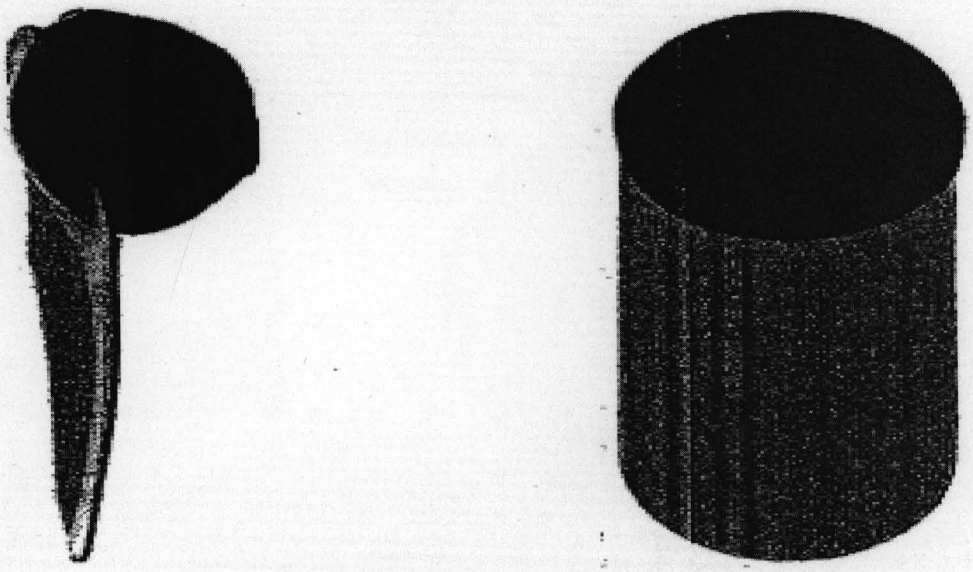

Figure 1. Front and side view of intravitreal sustained release ganciclovir implant (Vitrasert®). Dimensions: length=8 mm, thickness=2.5 mm, width=3.5 mm

Figure 1.3: Vitrasert®

(Adapted from Dhillon and Kamal, 1998)

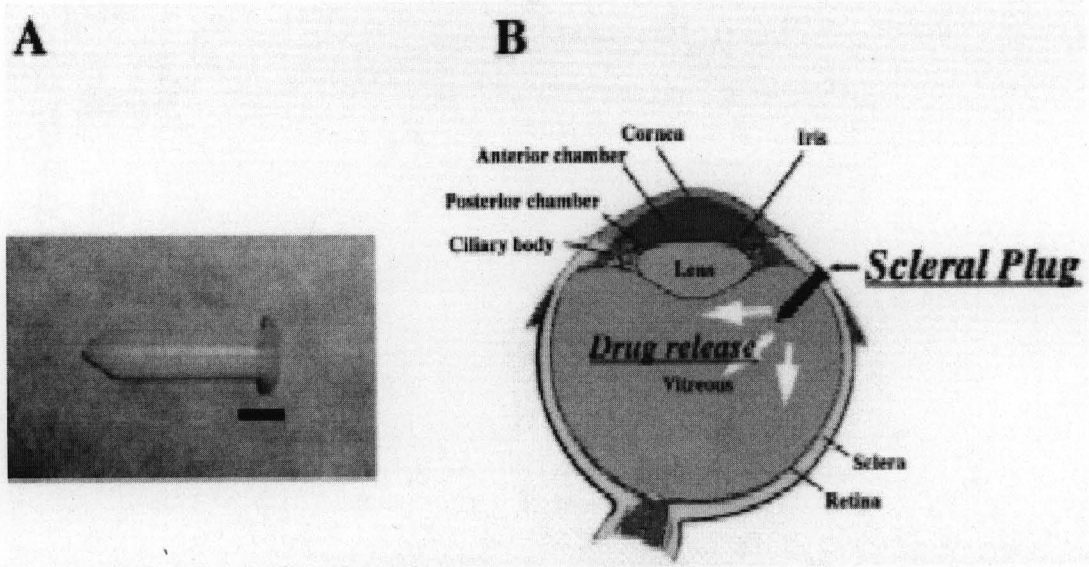

Fig. 2. Scleral plug made of biodegradable polymers. The plug weighs 8.5 mg and is 5.0 mm long. The scale bar indicates 1 mm (A). The scleral plug is implanted at the pars plana and gradually releases effective doses of drugs into the vitreous cavity with polymer degradation (B).

Figure 1.4: Scleral plug

(Adapted from Yasukawa et al, 2001)

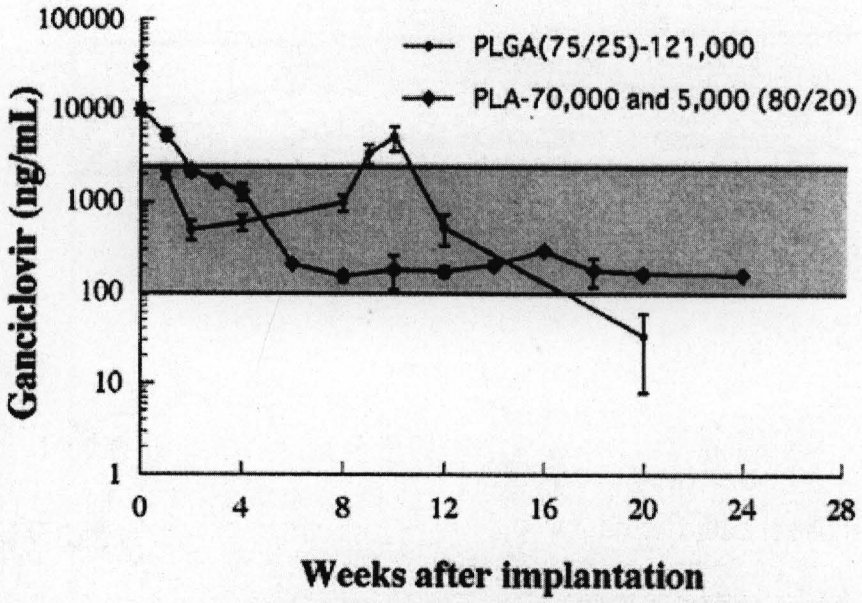

Fig. 7. Semilogarithmic plots of ganciclovir concentrations in the vitreous humor after implantation. The concentration was measured by high-performance liquid chromatography. The shaded area indicates a 50% effective dose (ED_{50}) of ganciclovir for human cytomegalovirus replication. The final drug burst can be suppressed by blending PLAs of different molecular weights. Data taken from Ref. [31].

Figure 1.5: In vivo performance of a scleral plug loaded with ganciclovir

(Adapted from Yasukawa et al, 2001)

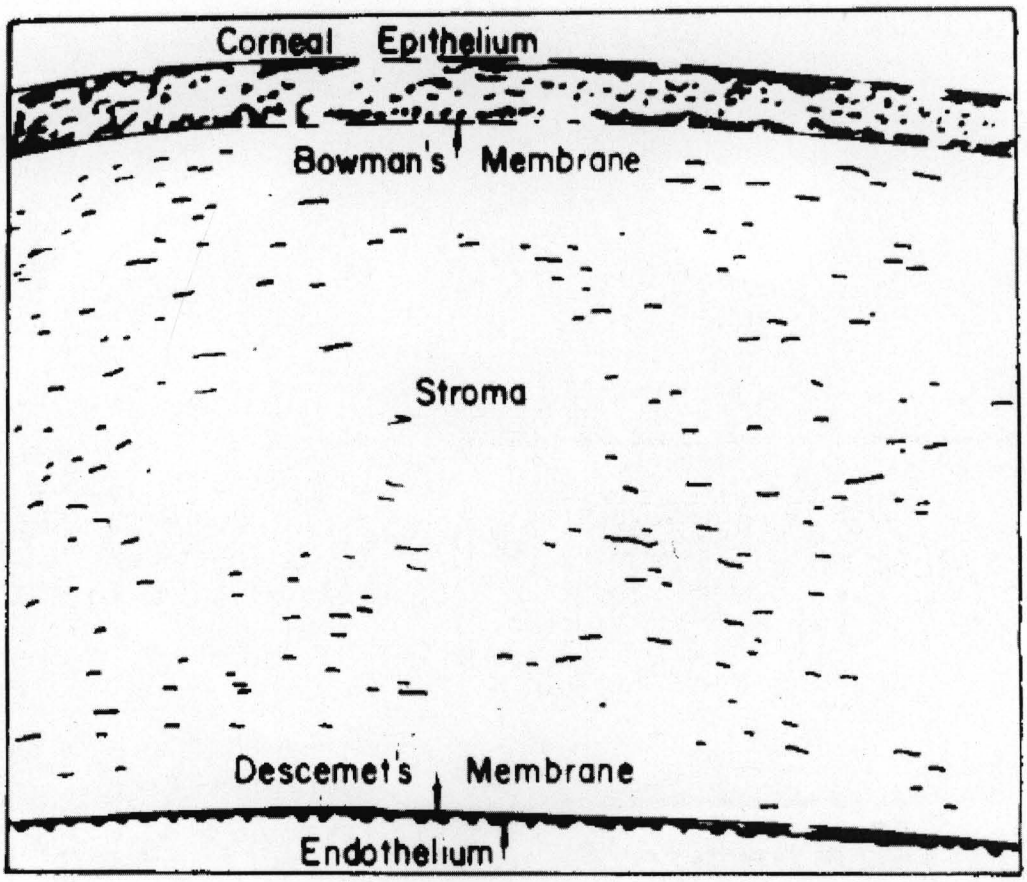

Figure 1.6: Structure of the cornea

(Adapted from Robinson, 1993)

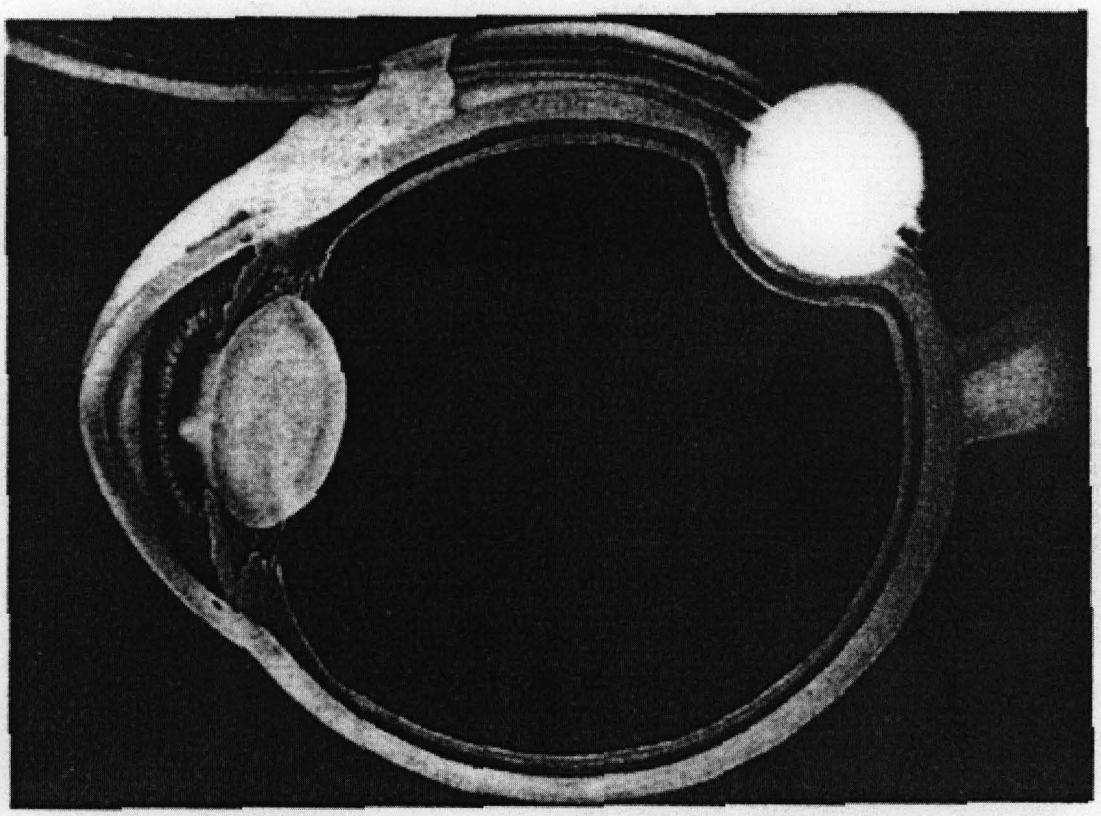

Fig. 1 Drawing of the double-lumen balloon catheter in place near the optic nerve. A fiberoptic in the second lumen glows from within the balloon.

Figure 1.7: A balloon catheter was implanted subtenonly to deliver cortisol to the vitreous chamber of the eye

(Adapted from Lincoff, Kreissig et al. 2001)

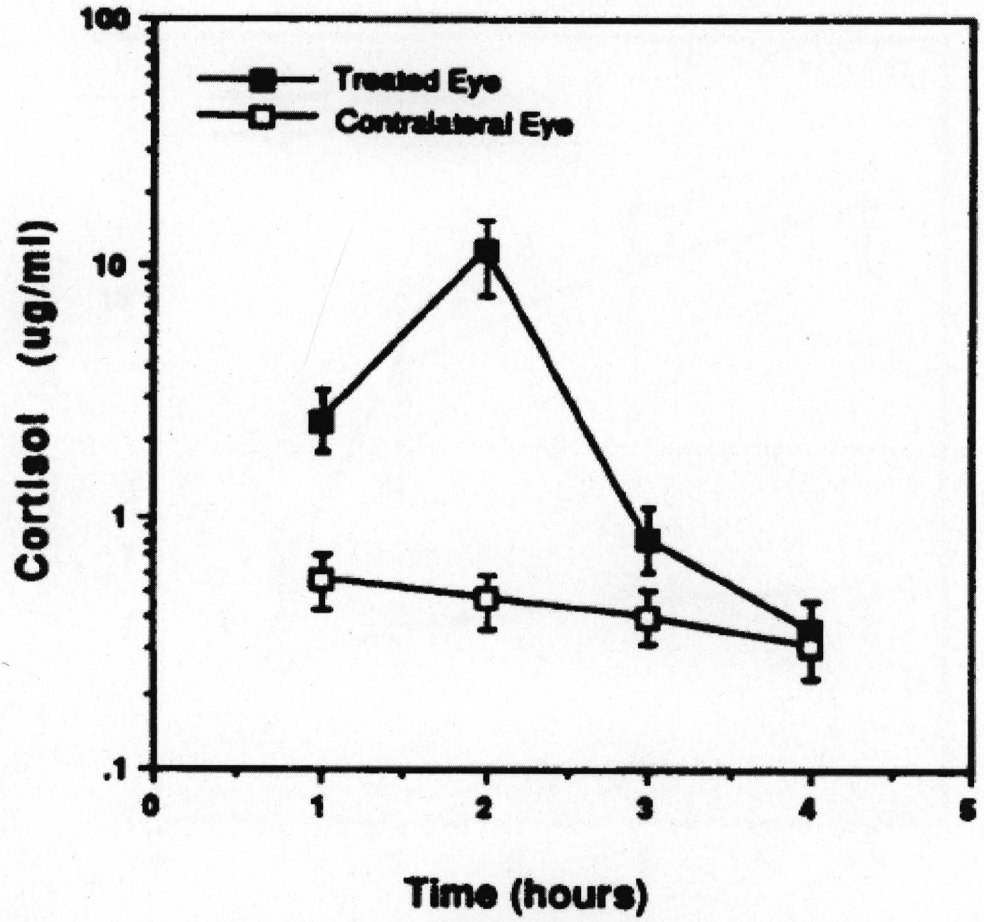

Fig. 4 Vitreous levels of cortisol after a single injection of 14 mg/kg of hydrocortisone sodium succinate through the balloon catheter.

Figure 1.8: Vitreous cortisol level after single administration via the catheter

(Adapted from Lincoff, Kreissig et al. 2001)

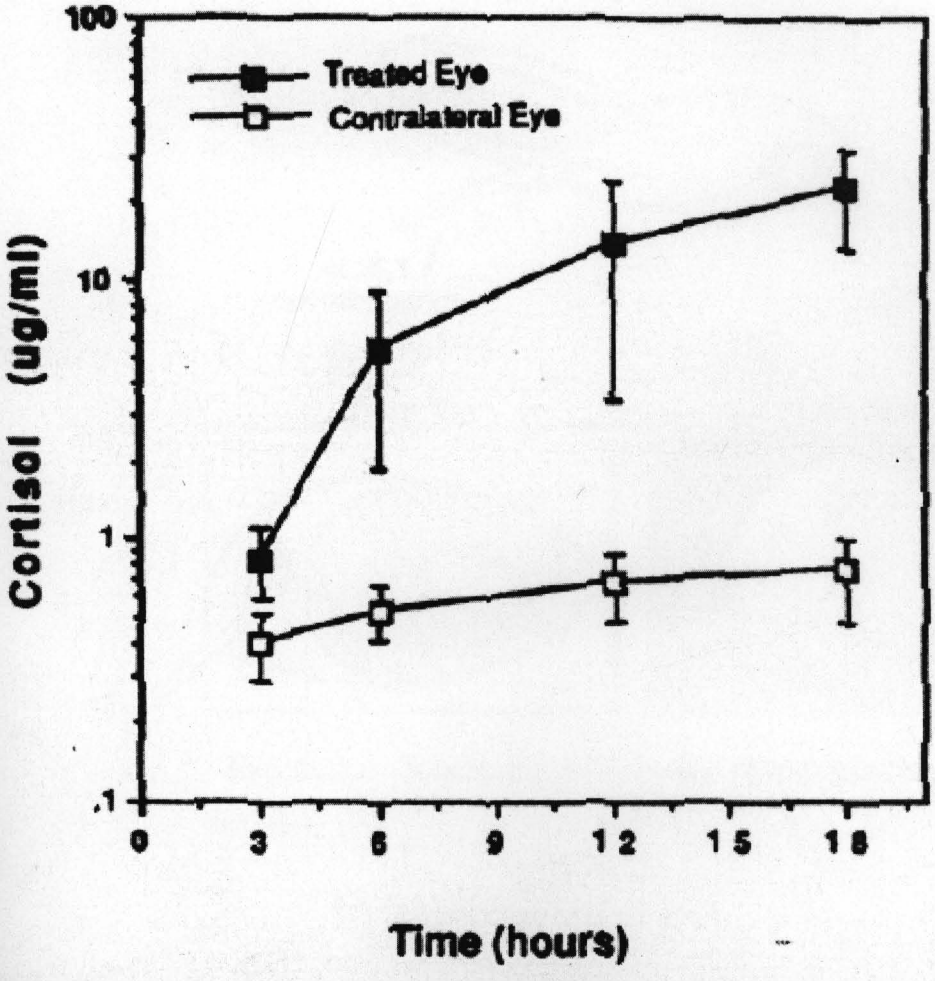

Fig. 7 Vitreous levels of cortisol after sequential injections of 14 mg/kg of hydrocortisone sodium succinate through the balloon catheter at 3 hour intervals.

Figure 1.9: Vitreous cortisol level after sequential administration (3-hour interval) via the catheter

(Adapted from Lincoff, Kreissig et al. 2001)

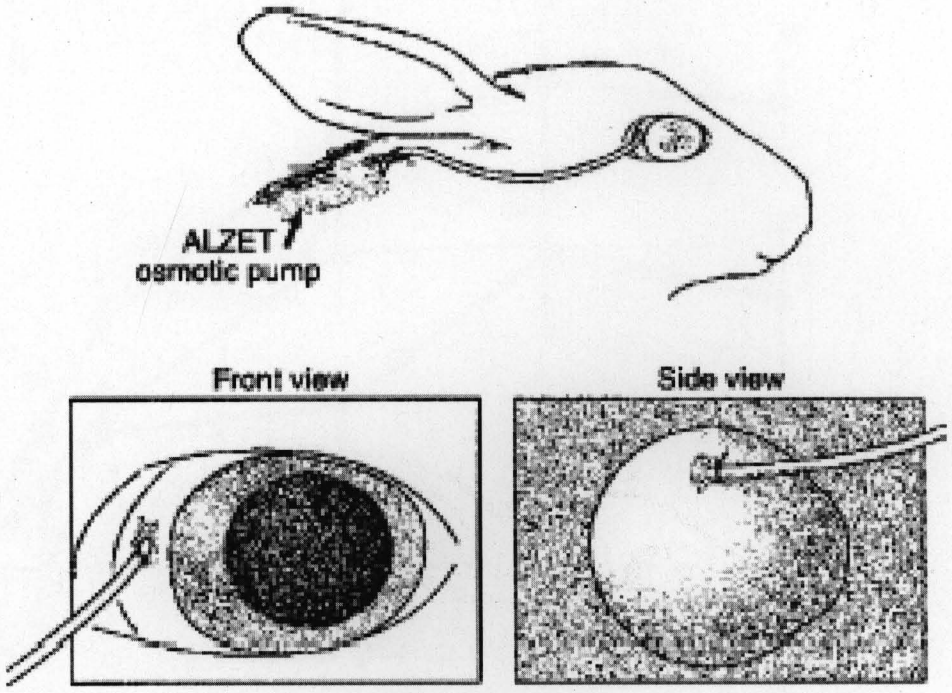

FIGURE 1. Schematic of osmotic pump placement.

Figure 1.10: Implantation of an Alzet Osmotic Pump

(Adapted from Ambati, Gragoudas et al. 2000)

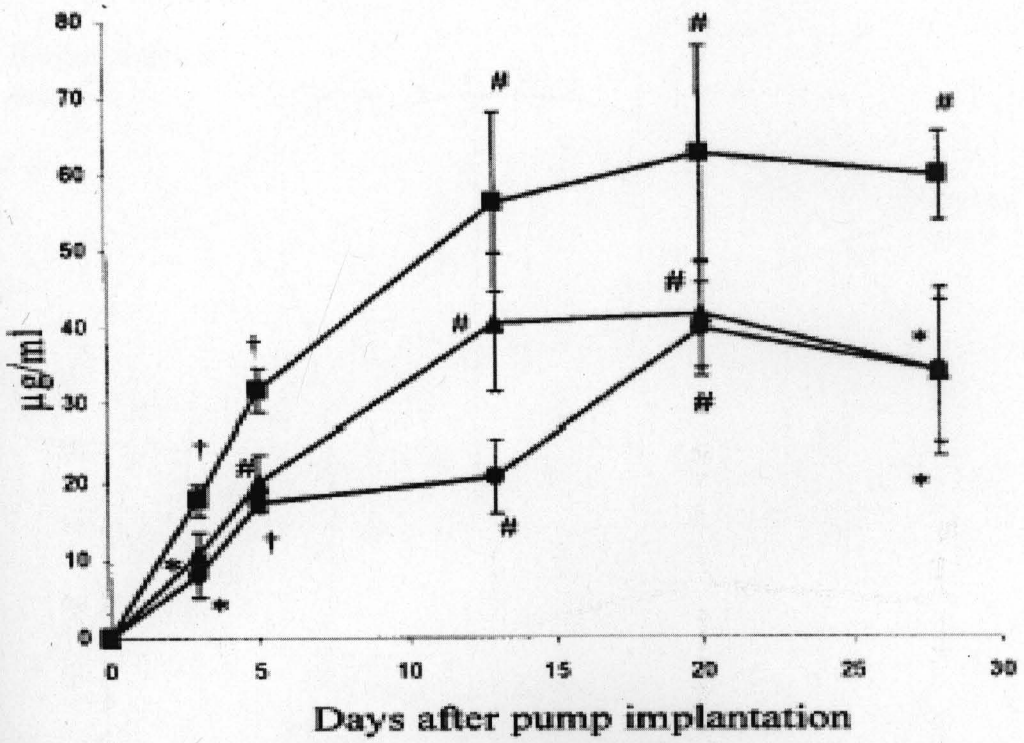

FIGURE 2. Concentration of FITC–IgG (1 mg/ml delivered at 2.5 µl/h) in the choroid (proximal hemisphere [■] and distal hemisphere [▲]) and the retina (●). *$P < 0.01$, #$P < 0.005$, †$P < 0.001$ versus day 0. $n = 4$ for all times.

Figure 1.11: IgG level in the choroid and retina after subconjunctival implantation of an Alzet Osmotic Pump

(Adapted from Ambati, Gragoudas et al. 2000)

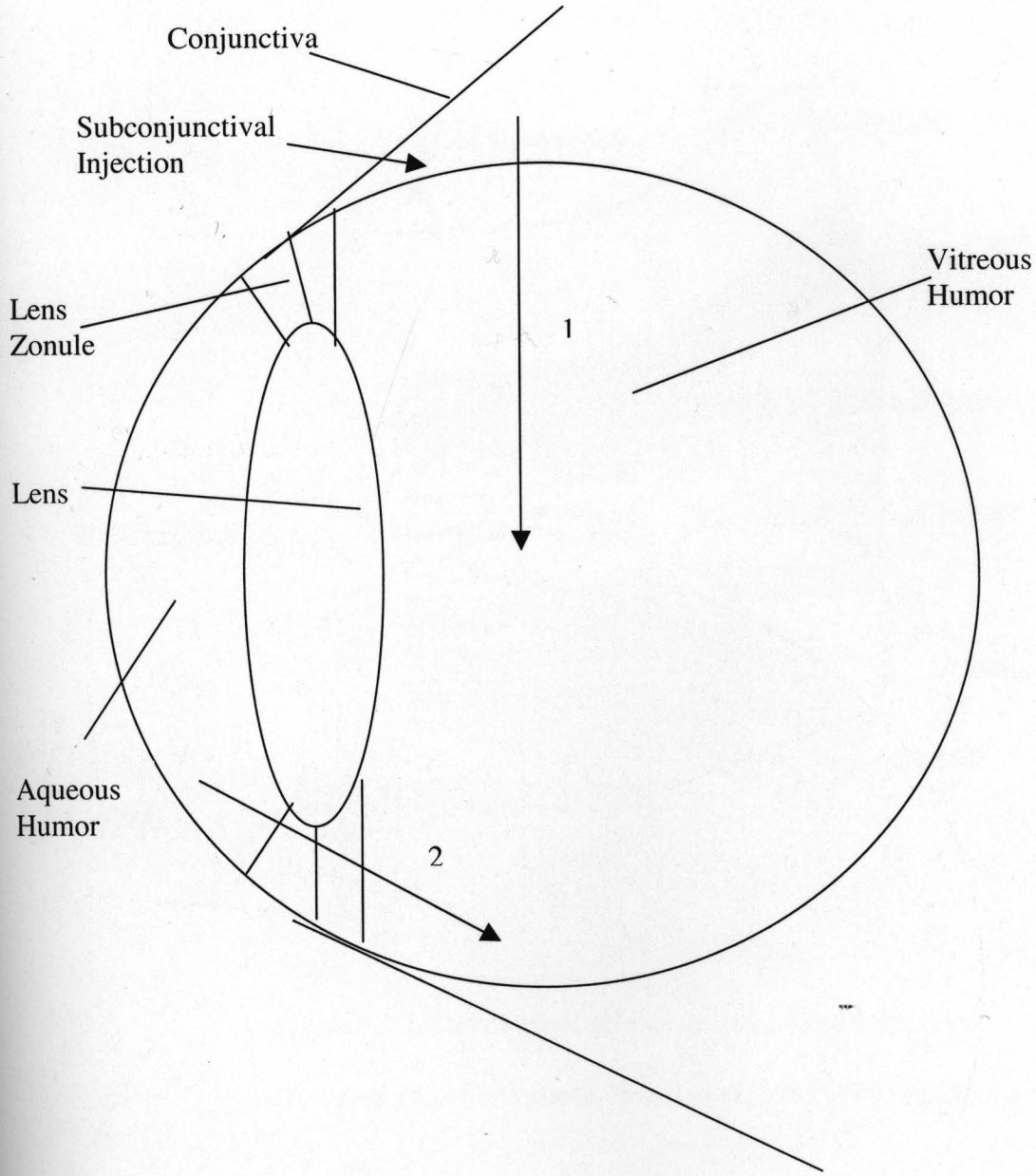

Figure 1.12: Penetration pathways after subconjunctival injection

1. Direct penetration pathway

2. Movement of a drug from the aqueous to the vitreous chamber

*Recirculation pathway is not shown

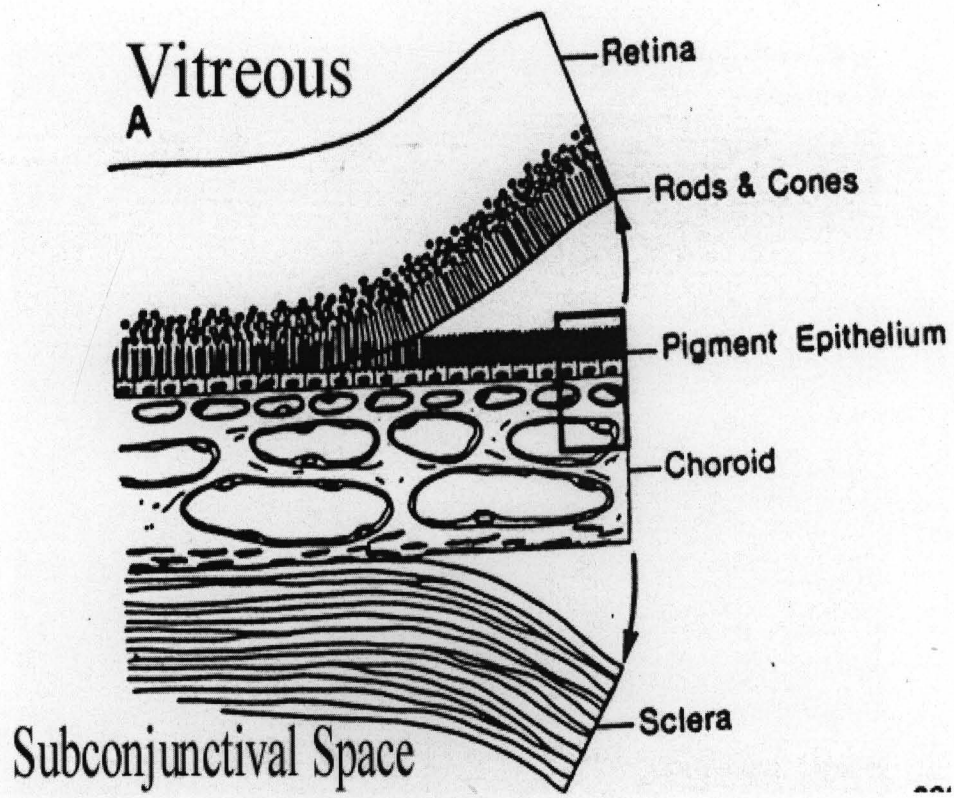

Figure 1.13: Direct penetration pathway at a cellular level

(Adapted from Gallemore, Huges et al. 1997 with modifications)

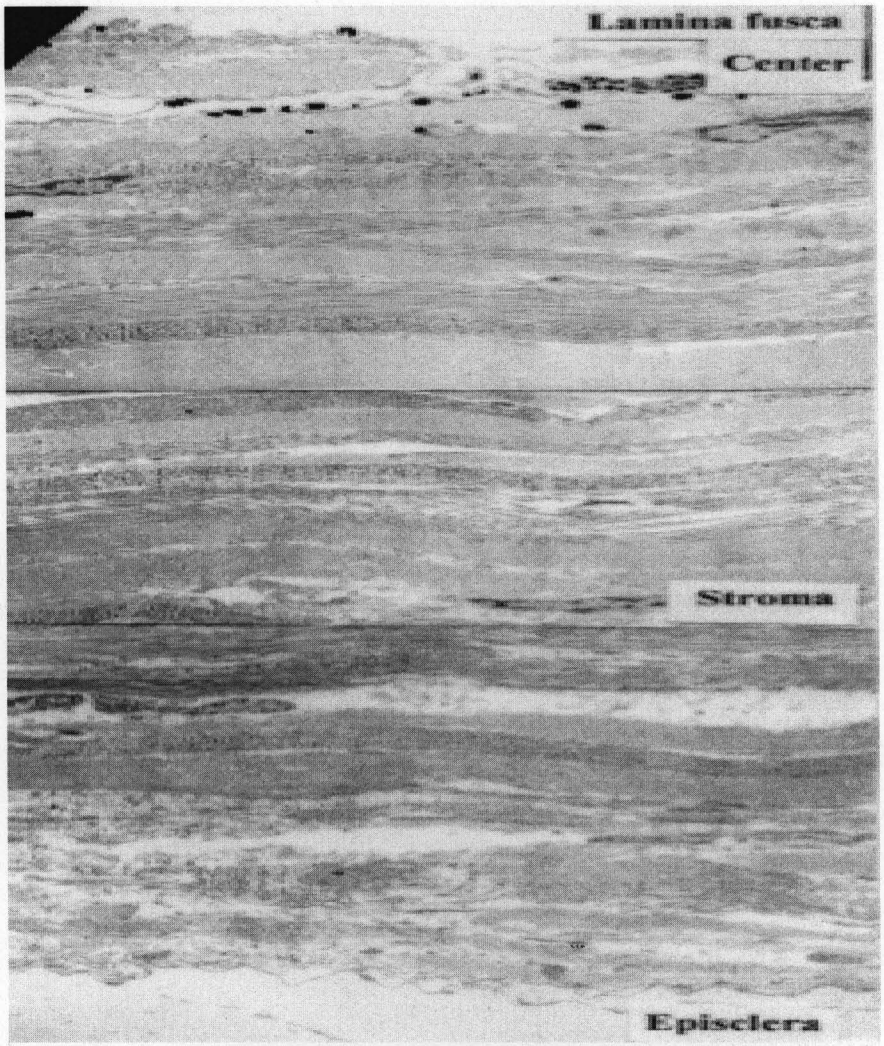

Fig. 1. Transmission electron micrographic montage of Rhesus monkey sclera depicting the tissue's three major layers — the lamina fusca, the scleral stroma, and the episclera. Though monkey sclera is significantly thinner than human, tissue from both species show comparable ultrastructure.

Figure 1.14: Structure of the sclera

(Adapted from Geroski and Edelhauser 2001)

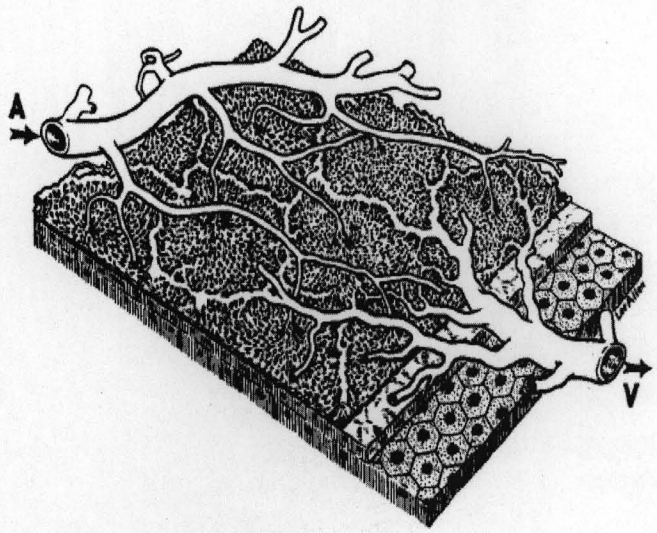

Fig. 12 Schematic representation of the choriocapillaris. A = artery, V = vein (from: Hayreh, 1974b)

Figure 1.15: The highly vascularized choroid on the top of the retinal pigment epithelium (RPE)

(Adapted from Richard 1992)

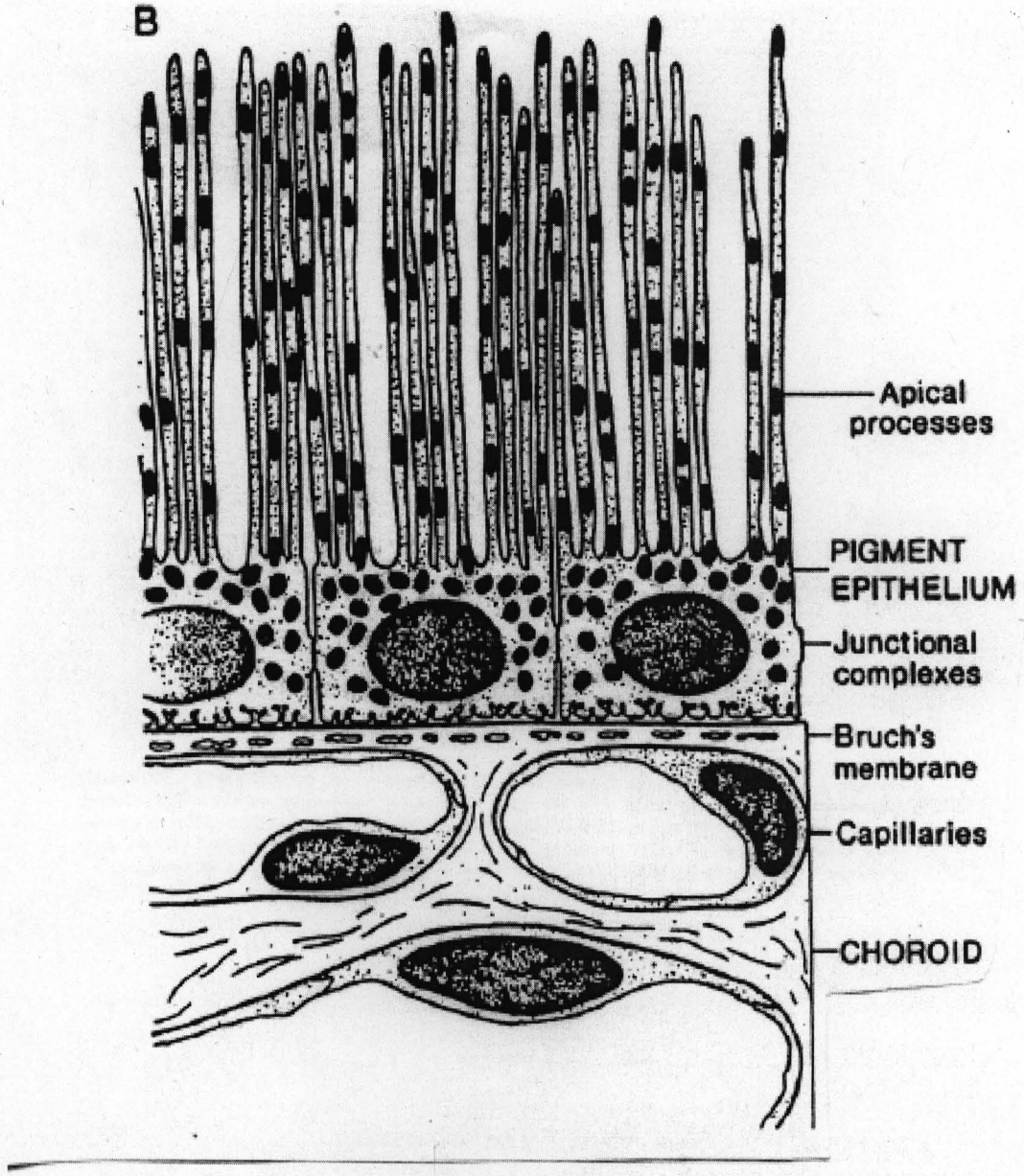

Figure 1.16: Structure of the choroid-RPE layer

(Adapted from Gallemore, Huges et al. 1997)

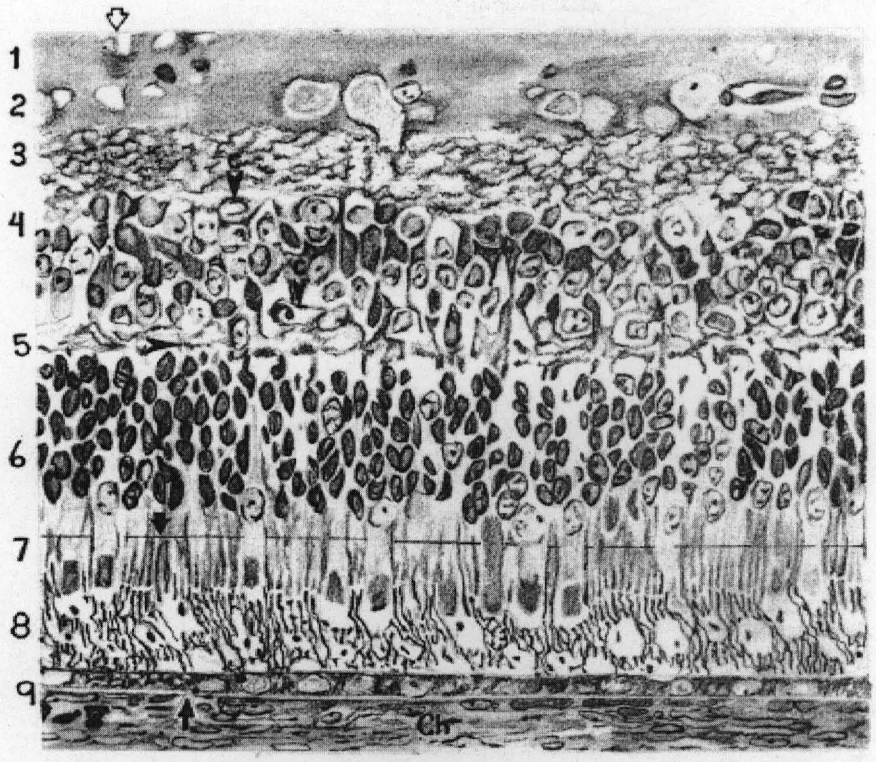

Figure 15. Illustration of light micrograph of retina. Bruch's membrane (arrow): 9 = pigment epithelium, 8 = rod and cone outer segments, 7 = rod and cone inner segments. External limiting membrane (solid thick arrow): 6 = nuclei of photoreceptors (outer nuclear layer), 5 = outer plexiform layer. Middle limiting membrane (arrow head): 4 = inner nuclear layer, 3 = inner plexiform layer, 2 = ganglion cell layer, 1 = nerve fiber layer. Inner limiting membrane (clear arrow): c = capillary; Ch = choroid.

Figure 1.17: Nine layers of the retina

(Adapted from Robinson 1993)

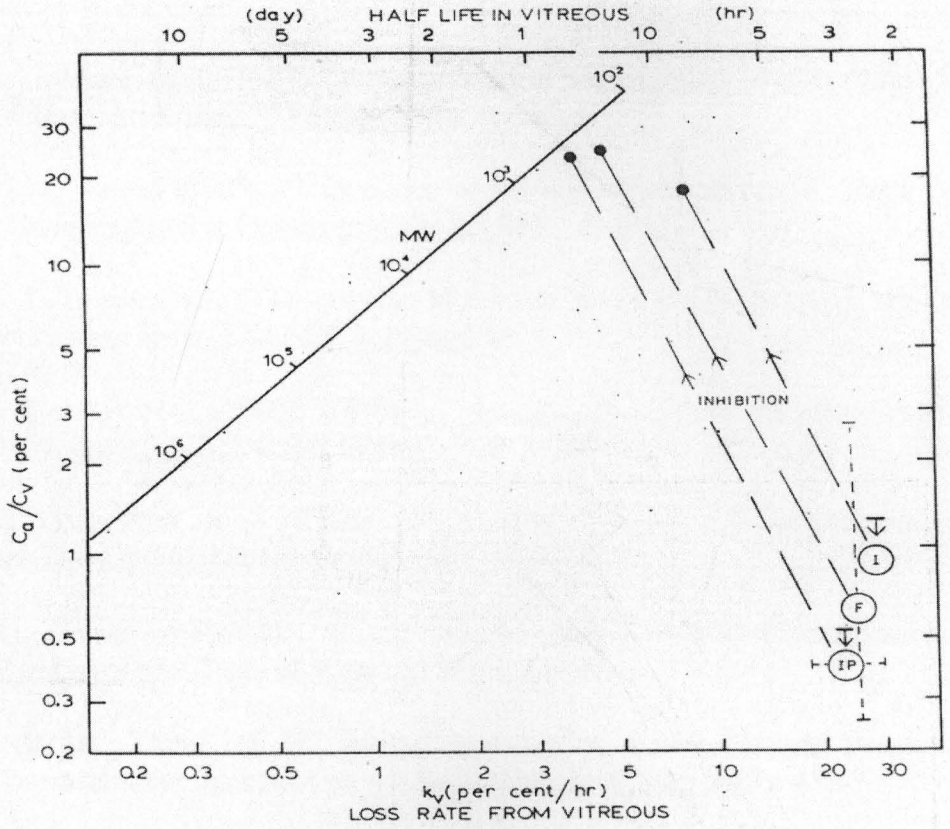

Figure 1.18: Relationship between the average concentrations in aqueous and vitreous humors, C_a/C_v, of substances actively transported across the retina after intravitreal injection and their rates of loss from the vitreous, k_v. The straight line represents a theoretical relationship for loss only through the anterior chamber. Experimental values for fluorescein, [^{131}I]iodopyracet, and [^{131}I] in the rabbits are represented by the symbols F, IP, and I. The data point for prostaglandin E_1 is almost identical to that for IP. The data were recalculated and replotted where necessary to find C_v and k_v. The limits shown for F and IP correspond to extreme values. The value of Ca was reported to be zero for IP and the arrow symbol corresponds approximately to the lower limit of sensitivity of detection. The points joined to the symbols by lines show the experimental values on inhibition of the transport mechanism.

(Adapted from Maurice 1976)

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CHAPTER 2

STATEMENT OF RESEARCH PROBLEM AND OBJECTIVES OF RESEARCH

I. Statement of Research Problem

As mentioned earlier, there is a need to deliver therapeutic agents to treat ocular diseases associated with the posterior segment of the eye such as macular degeneration, diabetic retinopathy, etc. However, various routes of administration such as topical, oral, IV, etc. fail to achieve therapeutic levels in the vitreous chamber. Intravitreal administration, being the most promising method, is too invasive to be used routinely.

Due to high permeability of the sclera, the transscleral pathway may serve as a potential pathway to deliver therapeutic agents to the back of the eye. The most direct method to make use of this pathway is to place a drug in direct contact with the sclera, i.e., subconjunctival injection.

Although subconjunctival injection has been used in clinical practice for more than 30 years, little is known about the penetration and elimination pathways as well as the major biological barriers associated with this route. In order to effectively deliver a therapeutic agent to the back of the eye via this route, it is essential to have a better and more fundamental understanding of this route with respect to the variables of drug delivery. The present research is trying to achieve this goal. Moreover, attempts will also be made to overcome various barriers by applying a properly designed drug delivery system.

II. Ultimate Objective

Our ultimate objective is to develop a drug delivery system which can be injected subconjunctivally to provide sufficient vitreal concentration for an extended period of time

(preferably from two weeks to a month). Such a system can be represented by a simple model (Fig. 2.1). An ideal drug delivery system is the one that can make $k_{release}$ to be the rate-limiting step so that the entire pharmacokinetics will be governed by $k_{release}$ (Lee and Robinson 2001). However, as mentioned earlier, subconjunctival injection is poorly understood. There may exist other factors which are predominant over $k_{release}$. In order to determine the feasibility of such a system, a better understanding of subconjunctival injection is necessary.

III. Global Hypothesis

Our global hypothesis is:

A properly designed drug delivery system can be injected subconjunctivally to provide sufficient vitreal concentration for an extended period of time (preferably from two weeks to a month).

IV. Specific Aims

Our main focus is to gain a theoretical and fundamental understanding of using subconjunctival injection to deliver a drug to the back of the eye. Therefore, our primary emphasis will be on the pharmacokinetic aspects of subconjunctival injection with respect to drug delivery. Other aspects of drug delivery at the cellular or subcellular levels such as permeability, specific transporters, etc. will not be covered in details.

With this global hypothesis, our specific aims are:

1. To gain an understanding of the penetration pathways after subconjunctival injection with an aim to eliminating unimportant pathways so that a simple pharmacokinetic model can be developed.

   Hypothesis: Direct penetration is the only significant pathway for a subconjunctivally injected compound to enter the vitreous chamber (posterior segment).

2. To develop a simple but realistic pharmacokinetic model for subconjunctival injection.

   Hypothesis: A simple but realistic model for subconjunctival injection can be constructed with reasonable accuracy.

3. Based on the pharmacokinetic model developed in (2), to gain insights on the effect of various modes of administration (simple injection, zero-order release system, first-order release system), solubility, release rates, magnitude of various rate constants, etc. on the vitreous level with an aim to determining the theoretically derived boundary conditions which will give a desired vitreal drug level for a desired period of time.

4. To evaluate performance of a properly designed drug delivery system (Regel®) for subconjunctival injection.

   Hypothesis: Regel® is a properly designed drug delivery system for subconjunctival injection.

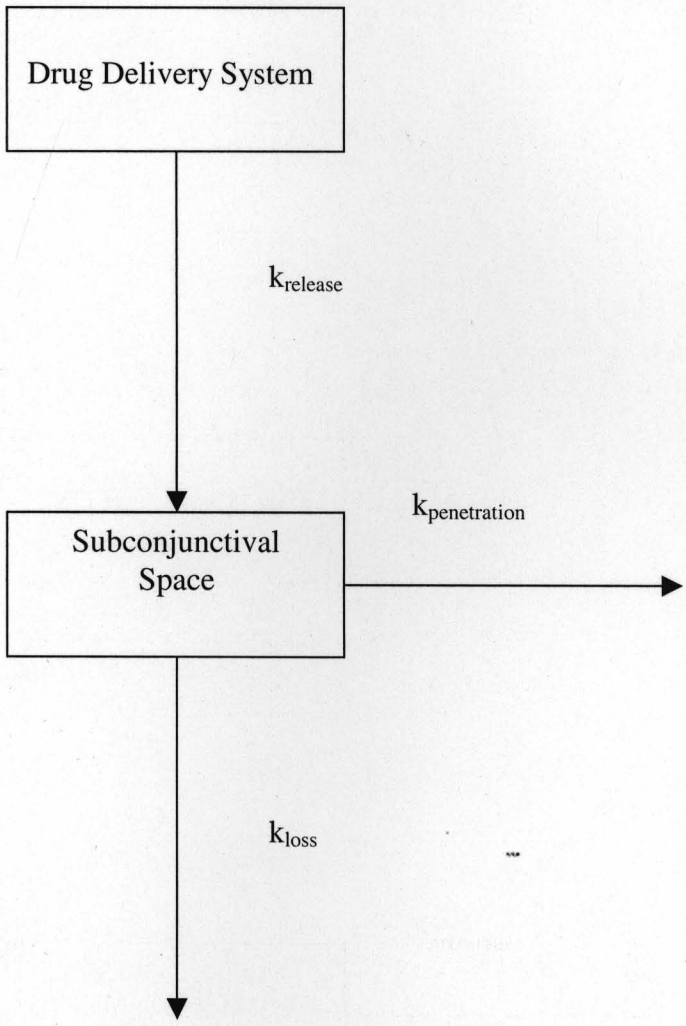

Figure 2.1: A simplified pharmacokinetic model for subconjunctival injection

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CHAPTER 3

PENETRATION PATHWAYS FOR A SUBCONJUNCTIVALLY INJECTED COMPOUND TO ENTER THE VITREOUS CHAMBER

I. INTRODUCTION

Effective drug delivery to the posterior segment of the eye is one of the most challenging tasks. One of the approaches to study drug delivery is to make use of a validated pharmacokinetic model. It is not uncommon for a drug to have more than one penetration pathway to move from the administration site to the target site. However, typically, not all the penetration pathways are equally important. The contribution of some pathways may be so small that they are practically non-existent. In other words, the performance of a model will not be significantly affected even though some pathways are removed from the model because they are pharmacokinetically unimportant. In order to develop a simple but realistic model, it is essential to have a clear understanding of the contribution of each penetration pathway. This is the main focus of this study.

Analogous to penetration into the anterior segment after subconjunctival injection (Conrad 1979), it is believed that there are three possible pathways for a subconjunctivally injected compound to enter the vitreous chamber, namely, (1) direct penetration across the underlying tissue layers into the vitreous chamber; (2) absorption of the drug into the aqueous chamber and from there via the space between the lens and ciliary body (lens zonule) to the vitreous chamber and (3) absorption of the drug into the general circulation and then recirculation back to the vitreous chamber. A compartmental representation of these three pathways is shown in Figure 3.1. Although pathway 3 is easily distinguishable from 1 and 2, it is difficult to separate pathway 1 and 2 experimentally.

Many studies have shown that a direct penetration pathway does exist (McCartney, Drysdale et al. 1965; Tsukahara and Maurice 1995). However, we cannot exclude the possibility of the existence of pathway 2. Although many studies have implied that pathway 2 is practically nonexistent, quantitative evidence is still lacking. In addition, basically, those studies were targeted at early time points (less than 2 hours) so that whether or not pathway 2 exists at a later time point is still an unknown. Therefore, the objective of the current study is to determine the relative contribution of each pathway to the vitreous level after subconjunctival injection. More specifically, we wish to (1) determine the contribution of the recirculation pathway to the vitreous level after subconjunctival injection (pathway 3); (2) give quantitative evidence on the transport of a molecule from the aqueous to the vitreous chamber (pathway 2) and (3) to give quantitative information on the existence of pathway 2 at later time points.

II. HYPOTHESIS

Direct penetration is the only significant pathway for a subconjunctivally injected compound to enter the vitreous chamber.

III. SPECIFIC AIMS

To gain an understanding on the contribution of each penetration pathway to the vitreous drug level after subconjunctival injection.

IV. EXPERIMENTAL

A. Materials

^{14}C-mannitol (specific activity=55mCi/mmol) and ^{14}C-inulin (specific activity=2.25mCi/g) were purchased from Sigma (St. Louis, MO) and NEN (Boston, MA) respectively. Ready Safe scintillation cocktail was purchased from Beckman Coulter, Inc (Fullerton, CA). All the chemicals were analytical grade and all water used in the experiments was distilled by a Barnstead Water Purification System (Barnstead PCS, Dubuque, IA).

B. Animals

New Zealand albino rabbits (either sex) weighing 1.8 to 2.3kg were purchased from Harlan Rabbitry (Madison, WI). The investigations utilizing rabbits described in this report conformed to the Principles of Laboratory Animal Care (NIH Publication #85-23, revised 1985). Lighting was maintained on a 12 hour cycle and animals were fed on a regular diet with no restrictions on the amount of food and water consumed.

C. Preparation of Dosing Solutions

Drug solutions were prepared using Sorenson's buffer at pH 7.4 made isotonic with sodium chloride. For ^{14}C-mannitol solution, the original solvent was removed by evaporation before addition of Sorenson's buffer to give a final concentration of 0.1μCi/μL which

corresponds to 1.82 x 10^{-3}M. 2mL of Sorenson's buffer was added to 50μCi of inulin which was further diluted to give a final concentration of 0.015μCi/μL which corresponds to 1.28 x 10^{-3}M. The reconstituted inulin was kept at –10°C and was used within two weeks.

D. Subconjunctival Injection

Rabbits were anesthetized with an IM injection of ketamine (35mg/kg) and xylazine (5mg/kg) fifteen to twenty minutes prior to dosing. Five minutes before drug application, the dosed eye received one drop of 0.5% tetracaine hydrochloride solution. Following topical anesthesia, 50μL of ^{14}C-mannitol solution was injected through the conjunctival membrane in the center of the upper quadrant bulbar conjunctival membrane using a microsyringe fitted with a 26G needle. Only one of the eyes was dosed with the other eye serving as a control. Left and right eye was alternated to minimize any left-to-right and right-to-left bias. Rabbits were put in restraining boxes immediately after injection and care was taken to make sure the rabbits were in an upright position but head movement was allowed. Blood samples were drawn via a mid-ear vein five minutes before sacrifice of the animal. At selected times from 10 minutes to 6 hours, rabbits were sacrificed with an overdose of sodium pentobarbital delivered through a marginal ear vein at a concentration of 100mg/kg. Immediately following death of the animal, aqueous humor and vitreous humor were collected for analysis. Six to twelve rabbits were used for each time point.

E. Intracameral Injection

The procedure was exactly the same as the subconjunctival injection except 10μL of either ^{14}C-Mannitol or ^{14}C-Inulin was injected intracamerally with a microsyringe fitted with a 30G needle using a self-sealing technique (Langford, Berg et al. 1997). The rabbits were sacrificed at 0.5 hour in the case of mannitol and at 1.5 and 6 hour in the case of inulin and aqueous and vitreous samples were collected for analysis.

F. Scintillation Counting

10mL of Ready Safe was added to 150μL of aqueous humor and 300μL of vitreous humor. The samples were then stored for at least 24 hours in the dark to minimize chemiluminescence before counting with a Beckman LSC 6000IC (Beckmann Instruments, Arlington Heights, IL). Each sample was counted twice and the average disintegration per minute (dpm) were used to calculate the molarity of mannitol and inulin in the aqueous and vitreous humor.

V. RESULTS AND DISCUSSIONS

A. Indirect Evidence on the Insignificance of Pathway 2: Movement of a Drug from the Anterior Segment to the posterior Segment

There is indirect evidence that can invalidate the speculation that pathway 2 contributes significantly to the vitreous drug level after subconjunctival injection. Conrad and Robinson (Conrad and Robinson 1980) showed that the aqueous turnover of inulin (from 0 to 120 minutes)

in the rabbit after intracameral injection was within the range of normal aqueous turnover. This may imply an insignificant amount of inulin entered the vitreous. Otherwise, elimination of the inulin in the aqueous should be faster than aqueous turnover. Tsuji et al (Tsuji, Tamai et al. 1988) showed that subconjunctival injection of prednisolone to the lower subconjunctival space of the eye brought a statistically significant reduction in the aqueous level (at 30 minutes) because of the reduced contact time of the drug with the cornea. However, the change in the vitreous level was statistically insignificant. This may imply a direct penetration pathway, independent of the transcorneal pathway, which is the dominant path to the aqueous, governs the drug level in the vitreous after subconjunctival injection. Similar to Tsuji's argument, Ahmed and Patton (Ahmed and Patton 1985) demonstrated that topical application of inulin without "corneal" access brought a much lower aqueous level (70x lower) than with corneal access. However, the vitreous levels did not differ from each other significantly (t-test, $p<0.05$). On the other hand, topical application of timolol without corneal access brought about a 262-fold decrease in aqueous level and only a 3-fold decrease in the vitreous level. It is tempting to conclude that pathway 2 did play a role in the case of a small molecule like timolol. However, if the movement of the drug from the aqueous to the vitreous contributed significantly to the vitreous level, a 262-fold decrease in the aqueous level should bring a parallel decrease in the vitreous but this is indeed not the case.

From the above evidence, it is obvious that it is not likely that the drug enters the vitreous from the aqueous in significant amounts after subconjunctival injection, especially at early time points. However, we still lack quantitative evidence regarding the contribution of pathway 2,

especially, the existence of this pathway at later time points. Therefore, the objective of the current study is to try to address this issue.

B. Contribution of Pathway 2 at Early Time Points

The result of the subconjunctival injection study is summarized in Table 3.1 and 3.2. The vitreous drug level is higher than the aqueous drug level from 0 to 10 minutes (Table 3.1). Obviously, there does not exist a driving force to move the drug from the aqueous to the vitreous chamber within this period of time, therefore, it is not likely for pathway 2 to be important from 0 to 10 minutes. The aqueous drug level rises to a level which is higher than the vitreous drug level after the 0.5 hour time point (Table 3.2). This implies that pathway 2 may exist since there exists a favorable concentration gradient. Our result is in good agreement with the result obtained by Tsuji et al (Tsuji, Tamai et al. 1988). They also showed that the vitreous drug level was higher than the aqueous drug level at early time points after subconjunctival injection of prednisolone.

A number of important findings can be extracted from the intracameral injection results which are summarized in Table 3.3 and 3.4. Based on a simple observation at the 0.5 hour time point, it can be seen that a much higher aqueous drug level (133x aqueous drug level after subconjunctival injection) is produced after intracameral injection although only 1/5 of the subconjunctival dose was injected. At the same time, the vitreous drug level after subconjunctival injection is higher (7x vitreous drug level after intracameral injection) than that after intracameral injection. It is obvious that an insignificant amount of drug enters the vitreous

in the first 30 minutes after subconjunctival injection using pathway 2. The above conclusion can be strengthened by a quantitative analysis. Since the aqueous levels after intracameral and subconjunctival injections are not of the same order of magnitude, it is difficult to compare results. In order to compare results, we assume that after intracameral injection all the drug in the vitreous is coming from the aqueous humor (pathway 2). This is a reasonable assumption since the level in the contralateral eye is not significantly different from background dpm and this implies that absorption of the drug into the general circulation and then recirculation back to the eye is not a likely penetration pathway. With this assumption, we normalize the aqueous drug level obtained at 0.5 hour after intracameral injection of mannitol to a level equal to the aqueous drug level at 0.5 hour after subconjunctival injection. This normalization would bring a parallel decrease in the vitreous drug level which is equal to 1.93×10^{-11}M. This vitreous level is 970 times lower than the vitreous level after subconjunctival injection at the same time point (0.5 hour). Since the dpm of the vitreous samples after intracameral injection is very low, it may lead to a counting error as high as 20%. Thus, we can estimate that only 0.08-0.12% of the vitreous drug level after subconjunctival injection comes from the aqueous chamber.

C. Contribution of Pathway 2 at later time points

The contribution of pathway 2 after 30 minutes was studied using inulin as a model compound so that the extremely low vitreous level after intracameral injection cannot be explained in terms of rapid elimination and this can simplify interpretation of the results. Inulin is a hydrophilic macromolecule (MW=5000-5500 Daltons) which cannot pass through the retinal surface easily (Maurice 1976). Therefore, the only elimination pathway is diffusion across the

lens zonule (the interface between the vitreous chamber and the posterior anterior chamber). Typically, drugs leaving the vitreous using this pathway have very long half-lives due to the small surface area and longer diffusion distance. For example, gentamicin has a half-life of 20 hours after intravitreal injection. Since elimination would not be the reason for the low vitreous inulin level, it can be deduced that only very minute amounts of inulin can enter the vitreous chamber from 1.5 to 6 hours, at least to a level that cannot be captured by statistical testing (unpaired t-test, $p<0.05$). This implies that inulin failed to reach the vitreous in significant amounts even though 4.5 hours is allowed for it to diffuse (Table 3.4).

The insignificant contribution of pathway 2-movement of a drug from the anterior segment to the posterior segment may be explained in terms of convective flow of the aqueous humor. It is demonstrated that after intravitreal injection a hydrophilic compound, which cannot pass across the retinal surface easily, leaves the vitreous via the lens zonule and is then carried away by the flow of aqueous humor. Since there does not exist a membrane barrier between the vitreous body and the lens zonule as demonstrated elsewhere (Maurice 1976), it is generally accepted that diffusion from the vitreous chamber to the posterior aqueous chamber is the rate determining step in movement of the drug from the vitreous to the aqueous. Similarly, when movement of a molecule from the aqueous to the vitreous is considered, the speed at which it diffuses should be one of the rate-determining steps. However, the normal flow of aqueous humor has to be taken into consideration as well. Basically, before reaching the ciliary body and then the lens zonule, the molecule has to diffuse against the convective flow of aqueous humor. Schoenwald et al (Schoenwald, Deshpande et al. 1997) set up a diffusion model to exclude this possibility mathematically. He showed that the shortest pathway to move a molecule as big as

pilocarpine from the center of the pupil 5mm towards the ciliary body yielded a permeability coefficient of 7×10^{-43} cm/sec (Figure 3.4: from point X to point Y). In other words, if 1μg of the drug reached the center of the pupil, in one hour about 1×10^{-37} ng would reach the target point (5mm from the center of the pupil towards the ciliary body) and thus this transport is practically non-existent.

D. Contribution of Pathway 3: Systemic Recirculation

One of the potential penetration pathways of drug to the vitreous is absorption of the drug into the general circulation and then recirculation back to the eye. Similar to what Conrad and Robinson (Conrad and Robinson 1980) found in the case of penetration of pilocarpine into the anterior segment following subconjunctival injection, this pathway is unlikely to be a major penetration pathway for the drug to enter the vitreous chamber. Our result (Table 3.5) clearly shows that the vitreous level in the dosed eyes is always much higher than that of the corresponding contralateral eyes during the time course of the experiment. Moreover, the vitreous level in the dosed and contralateral eyes should peak at the same time if the recirculation pathway was the major determinant of the vitreous level. This is clearly not the case. This result is further supported by Tsuji et al's study (Tsuji, Tamai et al. 1988). They found that the vitreous level in the dosed eye peaked at 30 minutes while the contralateral eye peaked at 1 hour after subconjunctival injection of prednisolone. Our result and other evidence clearly shows that drug absorption into the general circulation is a means of drug loss rather than as a source to supply the subconjunctivally injected compound to the vitreous chamber of the eye.

VI. CONCLUSIONS

Based on our data and evidence from the literature, it is suggested that movement of the drug from the aqueous chamber backwards to the vitreous chamber after subconjunctival injection contributes minimally to drug levels in the vitreous. Although a minute quantity of the drug can enter the vitreous via this pathway, this pathway can be practically neglected as a potential pathway for drug delivery to the posterior segment of the eye after subconjunctival injection. This is an important finding because it indirectly implies that direct penetration is the predominant pathway for the drug to enter the vitreous after subconjunctival injection since the recirculation pathway is relatively insignificant as well. As a result, we accept our hypothesis that direct penetration is the only significant pathway for a subconjunctivally injected compound to enter the vitreous chamber.

Table 3.1: Mannitol levels in the vitreous are higher than those of the aqueous at 10 minute after subconjunctival injection, n=12

Time (Hour)	Dosed Aqueous x 10^9M	Dosed Vitreous x 10^9M	Ratio Aqueous/Vitreous of the dosed eye
0.17 (10 min)	99.1* (32.8)	112* (49.9)	0.88

*Not statistically different from each other (unpaired t-test, $p<0.05$)

**Number in parenthesis represents standard error of the mean

Table 3.2: Mannitol levels in the aqueous are higher than those of the vitreous from 0.5 to 6 hour after subconjunctival injection, n=6-12

Time (Hour)	Dosed Aqueous x 10^9M	Dosed Vitreous x 10^9M	Ratio Aqueous/Vitreous of the dosed eye
0.5	261 (84.7)	18.7 (11.3)	14.0
1	349 (122)	14.2 (7.15)	24.6
3	59.9 (14.1)	7.45 (0.99)	8.00
6	7.93 (0.77)	4.78 (0.80)	1.66

*There is a statistically significant difference between the aqueous and vitreous drug levels at each time point (unpaired t-test, $p<0.05$)

*Number in parenthesis represents standard error of the mean

Table 3.3: Comparison of subconjunctival and intracameral injections at 0.5 hour time point

Modes of Administration	Aqueous x 10^9M	Vitreous x 10^9M	Ratio Aqueous/Vitreous of the dosed eye
Subconjunctival Injection	261 (84.7)	18.7 (11.3)	14.0
Intracameral Injection	34800 (4000)	2.57 (1.41)	13600

*Number in parenthesis represents standard error of the mean

Table 3.4: Inulin Level in the aqueous and vitreous after intracameral injection at 1.5 and 6 hour time points, n=6-9

Time (Hour)	Aqueous x 10^5M	Vitreous x 10^9M	Ratio Aqueous/Vitreous of the dosed eye
1.5	0.58 (0.25)	6.59 (4.4)**	880
6	0.0171(0.00727)	1.81 (0.86)**	94.0

*Number in parenthesis represents standard error of the mean

**not statistically different from each other (unpaired t-test, $p<0.05$)

Table 3.5: The contribution of systemic delivery after subconjunctival injection

Time (Hour)	Dosed Vitreous x 10^9M	Contralateral Vitreous x 10^9M	Ratio of Dosed/Contralateral Vitreous
0.17	112 (49.9)	0.01 (0.00)	11200
0.5	18.7 (11.3)	0.34 (0.08)	55
1	14.2 (7.15)	0.54 (0.06)	26
3	7.45 (0.99)	1.44 (0.05)	5
6	4.78 (0.80)	1.22 (0.06)	4

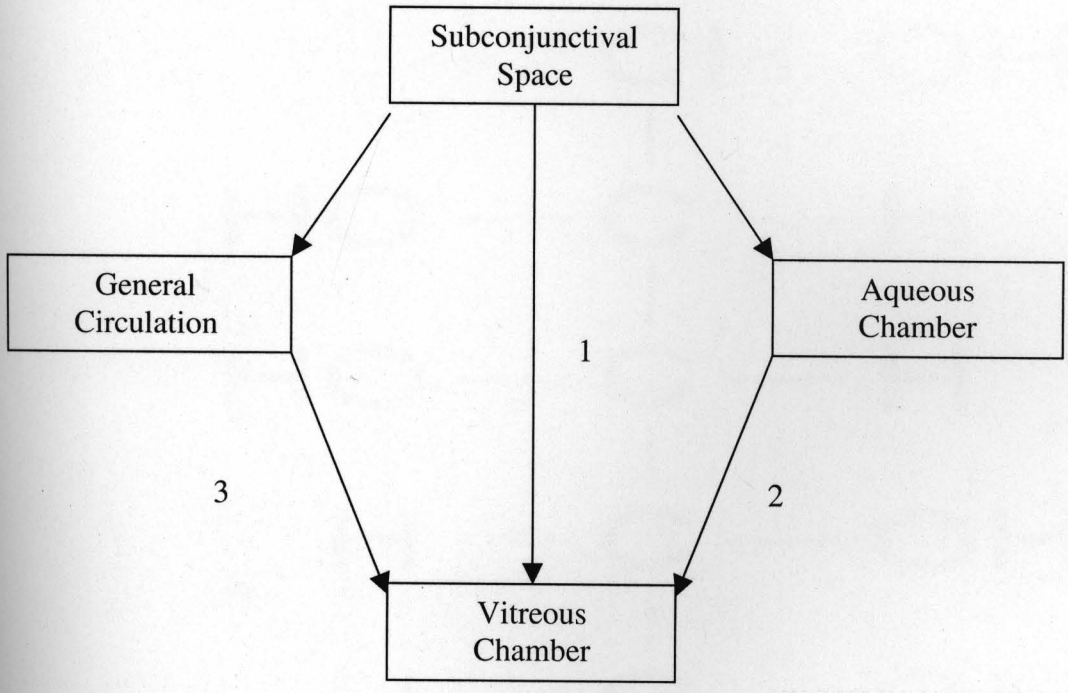

Figure 3.1: A simplified compartmental representation of the penetration pathways after subconjunctival injection

1: Direct Penetration across underlying tissue layers (e.g. sclera, choroid, etc.)

2: Movement of the drug from the aqueous chamber into the vitreous chamber

3: Absorption of the drug into the general circulation and then recirculates to the back the eye

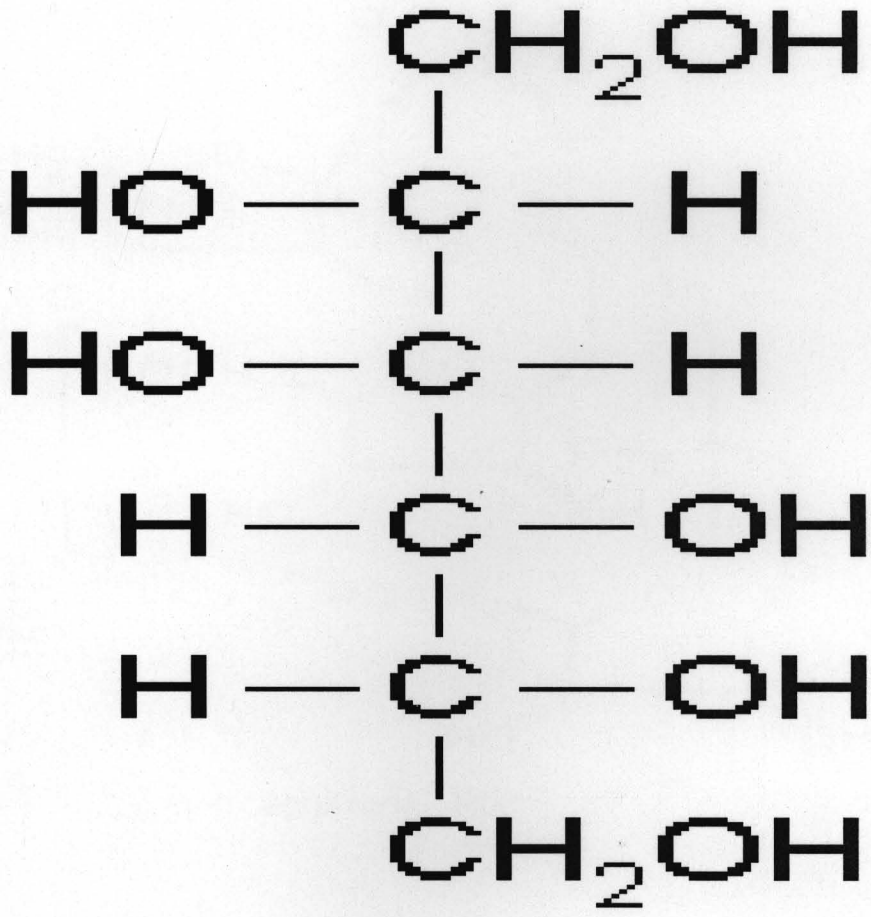

Figure 3.2: Chemical structure of mannitol (MW=182.2)

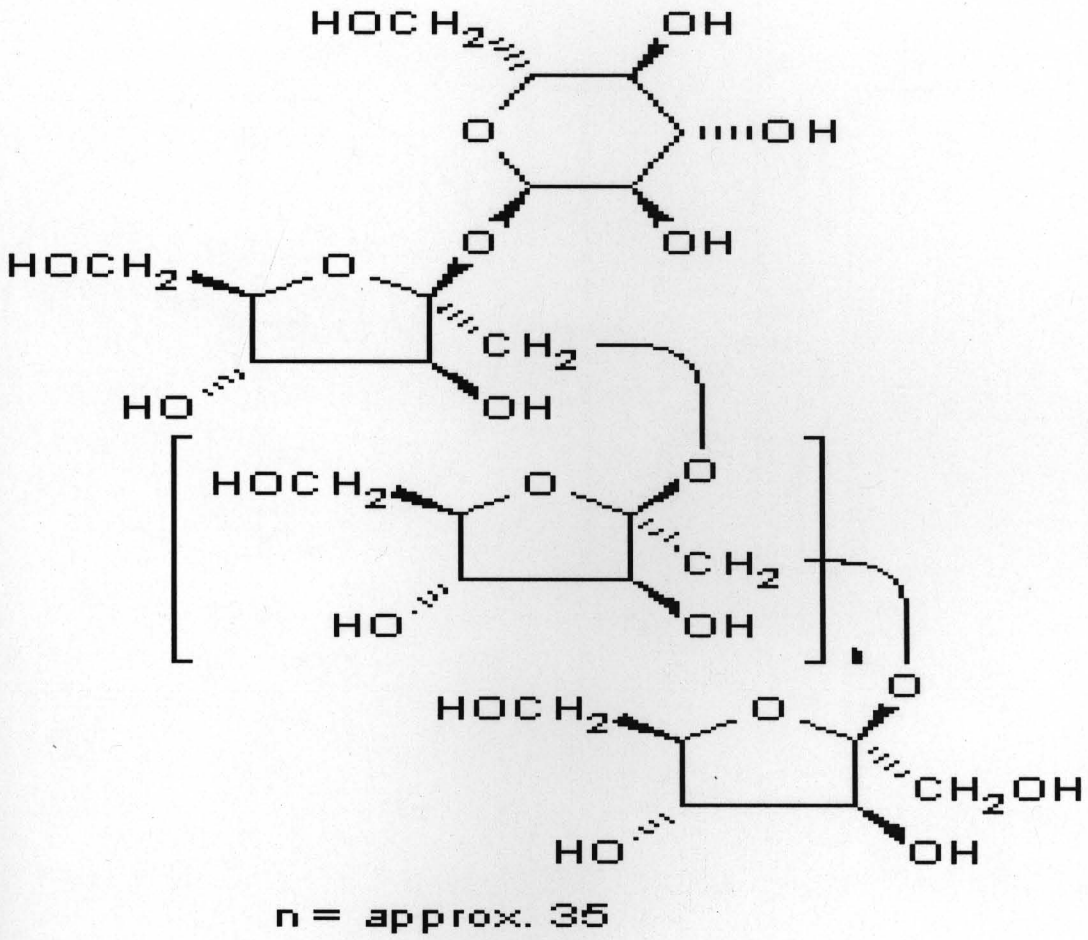

Figure 3.3: Chemical structure of inulin (MW=5000-5500)

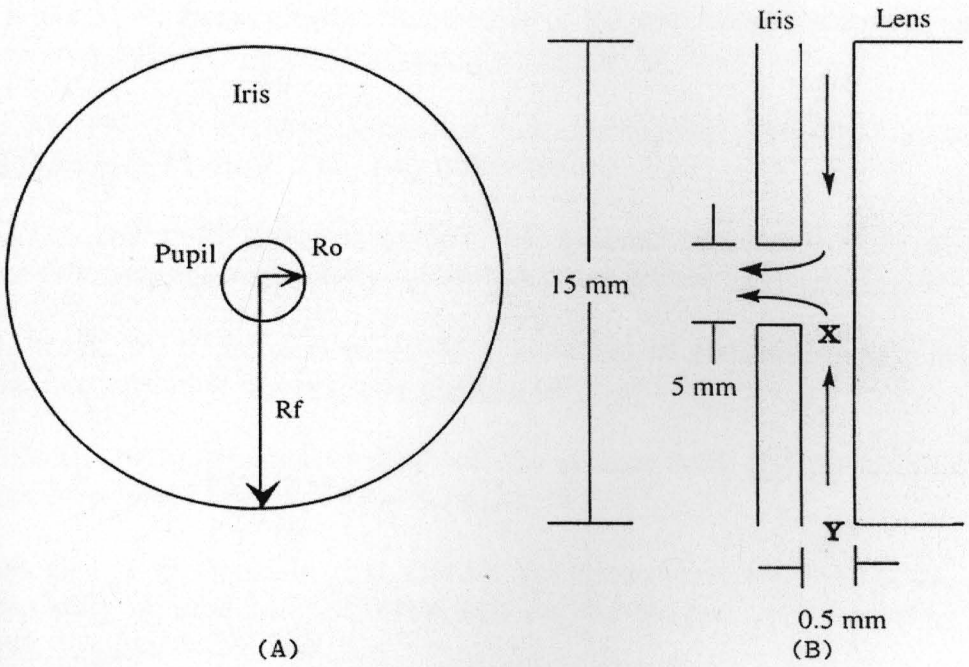

Figure 3.4: Diffusion model for a molecule in the aqueous chamber to move into the vitreous chamber

(Adapted from Schoenwald, Deshpande et al. 1997)

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CHAPTER 4

DEVELOPMENT AND VALIDATION OF A SIMPLE PHARMACOKINETIC MODEL TO STUDY DRUG DELIVERY TO THE POSTERIOR SEGMENT OF THE EYE USING SUBCONJUNCTIVAL INJECTION

I. INTRODUCTION

Subconjunctival injection is proposed as one of the potential routes to deliver therapeutic agents to the back of the eye. However, this route of administration is poorly understood. Previous studies (Gudauskas, Kumi et al. 1985; Kalsi, Gudauskas et al. 1991) only targeted the endpoint, i.e., whether or not a drug can enter the vitreous chamber after subconjunctival injection. Other important aspects such as penetration pathways, location of barriers, etc. are not known. The only pharmacokinetic model for subconjunctival injection was developed by Tsuji et al. (Tsuji, Tamai et al. 1988). However, the methods of modeling and parameter estimation were not stated and this limits its use. Therefore, further development of subconjunctival injection to be a practical way to deliver therapeutic agents to the back of the eyes requires a number of definitive studies.

Computer simulation is an invaluable tool to drug delivery (Slovin 1997) Pharmacokinetic modeling is particularly important since it can give insight on proper design of a dosage form. Norris et al. (Norris, Leesman et al. 2000) showed that pharmacokinetic modeling can give insight as to drug absorption from the GI tract such as dissolution-limited absorption, permeability-limited absorption, etc.

Grass et al (Grass, Bozarth et al. 1994) developed a pharmacokinetic model using Stella® to evaluate performance of a controlled release system. Stella® is a computer simulation software originally developed for business. The beauty of this software is that a pharmacokinetic model can be built and modified diagrammatically without advanced knowledge of mathematics and computer programming. A comprehensive pharmacokinetic model has been developed by

Grass and Lee (Grass and Lee 1993) using Stella® to predict drug disposition after topical instillation of a timolol eye drop into the precorneal area of the eye.

Although Stella® is simple and easy to use, lack of ability to perform parameter estimation by curve-fitting limits its use. Moreover, more advanced modeling tools such as sensitivity analysis is not yet available.

Some of the popular computer software, such as SigmaPlot® or KaleidaGraph®, allows parameter estimation by curve fitting and simulation when the equations are in an integrated form. In other words, the series of complicated differential equations have to be solved before simulation. However, it may not be easy to do this for a complicated pharmacokinetic model such as a four or five-compartmental model. It is highly desirable to have software that can simulate and curve fit even if the equations are in differential form.

ModelMaker 4.0 (Cherwell Scientific, succeeded by FamilyGenetix, Ltd., UK) is a modeling software which allows models to be built diagrammatically without advanced knowledge of computer programming. It also allows simulation or curve-fitting in differential form. This software is being used in the current study.

As mentioned earlier, pharmacokinetic modeling can give insight as to proper design of a dosage form. Therefore, the main goal of the current study is to develop and validate a simple but realistic model to study drug delivery to the posterior segment of the eye after subconjunctival injection. Our model may not be precise enough to describe details of the pharmacokinetic behavior of a subconjunctivally injected compound. However, it is reasonably accurate so as to give insight in terms of drug delivery. Based on this validated model, computer simulation can be run to obtain information on features of a drug delivery system that will work.

II. HYPOTHESIS

A simple but realistic pharmacokinetic model for delivery to the back of the eye via subconjunctival injection can be constructed with reasonable predictability in terms of drug delivery.

III. SPECIFIC AIMS

To construct and validate a simple pharmacokinetic model for subconjunctival injection.

IV. EXPERIMENTAL

A. Model Building

The starting pharmacokinetic model is based on the one suggested by Conrad and Robinson (Conrad 1979), with modifications, and is shown in Figure 4.1. However, preliminary studies have shown that the recirculation pathway as well as movement of a drug from the aqueous to the vitreous chamber is not likely to contribute significantly to the vitreous level after subconjunctival injection (Previous Chapter). Therefore, the model was simplified by removing the aqueous chamber and systemic circulation compartments. The simplified model is shown in Figure 4.2. This model shows that there is direct penetration from the subconjunctival space into the vitreous chamber and it is similar to the one used by Tsuji et al (Tsuji, Tamai et al. 1988) in

the modeling of subconjunctival injection of prednisolone with some modifications. The differential equations describing the model are shown in Appendix 4.1.

B. Estimation of Parameters and Validation of the Model

Our model was applied to a set of in vivo data generated by the subconjunctival injection of prednisolone at a dose of 2.61mg (Tsuji, Tamai et al. 1988). Pharmacokinetic rate constants were estimated by curve fitting using ModelMaker 4.0 (Cherwell Scientific, UK).

Validation was accomplished by administering the same drug at different doses. Consistency of the concentration-independent model would be evident if various concentrations of a drug generated a family of curves with similar slopes. In short, a valid model will give the same values to the rate constants at different doses.

V. RESULTS AND DISCUSSIONS

A. Parameter Estimation

Our first curve fitting was done by setting k_{10} (rate constant associated with drug loss from the subconjunctival space) to $1.1hr^{-1}$ ($1.83x10^{-2}min^{-1}$) since Tsuji et al found that (Tsuji, Tamai et al. 1988) the drug was eliminated from the conjunctiva in a first-order manner with a rate constant equal to $1.1hr^{-1}$. However, a poor fit resulted with $r^2=0.57$ (Table 4.1). Although the fitting was not very poor visually (Figure 4.3 and 4.4), fixing $k_{10}=1.83x10^{-2}min^{-1}$ generated an

unrealistically large k_{30} ($1.3hr^{-1}$). Therefore, it is not likely that this is an appropriate model for subconjunctival injection.

The curve fitting was re-run by letting k_{10} float and the results are summarized in Table 4.2. The fitting was much improved (r^2=0.83) by letting k_{10} float. However, the fitting at the late time points (Figure 4.5 and 4.6) is unsatisfactory. When the last two data points were ignored, a much better r^2 (>0.99) was obtained (Table 4.3 and Figure 4.7&4.8). This implies that our model can fit the experimental data perfectly at the early time points but deviates from the experimental data at late time points. The reason for the poor fit at late time points is not immediately apparent. Basically, two possibilities may slow drug elimination from the vitreous chamber, namely, protein binding and existence of a peripheral (distribution) compartment. Assuming prednisolone only binds to a single site of a protein in the vitreous, the unbound fraction which is subject to elimination from the vitreous can be expressed by the following equation (Gabrielsson and Weiner 1994).

$$f_u=1/(1+K_a[P]) \qquad (4.1)$$

where f_u=unbound fraction of the drug in the vitreous, K_a= association constant between the drug and the protein and P=total protein concentration in the vitreous. Since both K_a and P are constants, the above equation can be rewritten as

$$F_u=1/(1+A) \qquad (4.2)$$

where $A = K_a[P]$.

Curve fitting was re-run by assuming only unbound drug can leave the vitreous. The model and the equations describing the model are shown in Figure 4.9 and Appendix 4.2 respectively. The results of the curve fitting is shown in Table 4.4 and Figure 4.10&4.11. It is obvious that the current model fails to yield a satisfactory $r^2(=0.83)$ and it fails to fit the data at late time points as well. Therefore, this model is not likely to be the appropriate model for subconjunctival injection.

It may be argued that one binding site per protein molecule may not represent the real situation since Rosenthal analysis of binding of prednisolone to proteins in the vitreous show curvilinearity implying existence of two binding sites (Tsuji, Tamai et al. 1988). In this case, the bound fraction, C_b, can be expressed by the following equation:

$$C_b=[n_1P_1K_1/(1+K_1C_f)]+[n_2P_2K_2C_f/(1+K_2C_f)] \quad (4.3)$$

where n_1 and n_2 are number of binding sites on the proteins; K_1 and K_2 are association constants; P_1 and P_2 are the protein concentrations, C_f is the unbound drug concentration and C_b is the bound drug concentration. Tsuji et al (Tsuji, Tamai et al. 1988) estimated that $n_1P_1=0.0176\mu M$; $n_2P_2=0.124\mu M$; $K_1=45{,}200/\mu M$ and $K_2=0.160/\mu M$. Therefore, Equation (4.3) can be written as:

$$C_b=[795.52Cf/(1+45200C_f)]+[0.01984C_f/(1+0.16C_f)] \quad (4.4)$$

Since $[Drug]_{Total}=C_b+C_f$,

$$[Drug]_{Total} = [795.52C_f/(1+45200C_f)]+[0.01984C_f/(1+0.16C_f)]+C_f \quad (4.5)$$

Equation (4.5) can be converted into (4.6) by changing the units of those parameters from μM into ng/mL:

$$[Drug]_{Total} = [795.52C_f/(1+125.56C_f)]+[0.01984C_f/(1+4.44x10^{-4}C_f)]+C_f \quad (4.6)$$

It was determined that the second term of Equation (4.6), $[0.01984C_f/(1+4.44x10^{-4}C_f)]$, contributes minimally to the $[Drug]_{Vitreous}$ within the concentration range in our curve-fitting process. Therefore, Equation (4.6) can be simplified into Equation (4.7):

$$[Drug]_{Vitreous} = [795.52C_f/(1+125.56C_f)]+C_f \quad (4.7)$$

C_f can be re-written as

$$C_f = ([Drug]_{Vitreous}-6.344)+(40.246336-12.3694[Drug]_{Vitreous}+[Drug]_{Vitreous}^{2})^{1/2} \quad (4.8)$$

Curve fitting was re-run by incorporating Equation (4.8) into our model. The equations describing the model are shown in Appendix 4.3 respectively. The results of the curve fitting is shown in Table 4.5 and Figure 4.12&4.13 and an excellent correlation coefficient was obtained (>0.99).

Another possibility that cannot be excluded is the existence of a peripheral (distribution) compartment (Figure 4.14). In fact, existent of a reservoir compartment was also observed for the anterior segment of the eye (Makoid and Robinson 1979). The exact location of this reservoir in the posterior segment of the eye is not known but it is likely to be the lens. Due to its impermeability and thickness, it is generally accepted that it is highly unlikely for a drug to pass across the lens and enter the vitreous chamber from the anterior chamber. However, there may be a chance that a drug in the vitreous chamber partitions into the lens and then partitions back out later (Ahmed, Francoeur et al. 1989) and this may account for the deviation from the predicted value for the late time points. In fact, Kojima et al (Kojima, Shui et al. 1995) showed that prednisolone can partition into the lens and stay there for very long period of time.

The curve fitting was re-run by incorporating the peripheral distribution compartment into our model and is shown in Figure 4.14 and the equations describing the model are shown in Appendix 4.4. The results are summarized in Table 4.6. Figure 4.15 and 4.16 showed that the fitting for the late time points was greatly improved by incorporation of a reservoir compartment. Similar to the protein binding model, an excellent correlation coefficient was obtained (>0.999).

Since both protein binding and peripheral model give excellent correlation coefficients, model selection cannot be made by using this parameter. Additional statistical testing has to be carried out in order to choose the most appropriate model.

Since the two models are not nested, the popular likelihood ratio test (F-test) cannot be used in our case (Lindsey and Jones 1998). However, AIC (Akaike Information Criterion) and SC (Schwarz Criterion) (Schwarz 1978; Koehler and Murphree 1988) can be used to determine which model is better for the current situation. A brief description of AIC and SC can be found

in Appendix 4.5 and the results of these two tests are summarized in Table 4.7. For these two tests, the model with lowest (highest negative) value is the better model. It is clear that both tests showed that the distribution model is a better model.

Another piece of evidence to support the distribution model can be obtained by comparing the simulated data generated by two models and the experimental data. Figure 4.17 showed the comparison between the simulated data generated by the two models at dose=2.61mg and 26.1mg. Two important pieces of information can be extracted from this graph: (1) The distribution model gives a lower vitreous level at late time points at the dose=2.61mg. However, at the dose=26.1mg, the situation is exactly opposite, namely the distribution model gives a higher level at late time points; and (2) The higher the dose, the earlier the deviation. Suppose the distribution model is the correct model but the protein binding model is used to fit the data, what is expected to happen according to Figure 4.17 is that the experimental data points will lie above the line generated by the protein binding model after the peak at the dose=26.1mg. For the dose=2.61mg, there is no deviation before 400 minute but a slight deviation will exist after that. This is exactly happening in our case (Figure 4.18). Therefore, distribution model is the most appropriate model for subconjunctival injection.

The magnitude of the rate constants can give insights as to the appropriateness of our model. $k_{30}=1.14\times10^{-2}min^{-1}$ falls within a reasonable range for a small lipophilic molecule (Graham and Peyman 1974; Maurice 1976; Scholes, O'Brien et al. 1985; Cochereau-Massin, Marrakchi-Benjaafar et al. 1994). $k_{10}=1.28\times10^{-1}min^{-1}$ is much larger than the rate constant associated with elimination of the drug from the conjunctiva. This may be due to the fact that the conjunctiva actually acts as a sponge to absorb any injected fluid (Conrad 1979). Once injected

into the subconjunctival space, the injected dose will not come out unless the conjunctiva is squeezed. In other words, the drug in the conjunctiva is not available for absorption and it is not surprising that depletion of the drug from the subconjunctival space is faster than its elimination from the conjunctiva. According to our experience, a bleb is formed right after a subconjunctival injection. This bleb will become progressively smaller with time. Typically, after subconjunctival injection of 50μL, the bleb will become very small after 45 to 60 minutes. Assuming only 1/100 of the injected dose is left in the subconjunctival space after 45 minutes, one can estimate that the elimination rate constant is approximately $0.102min^{-1}$ which is of the same order of magnitude that we got from curve-fitting. By considering other factors that may eliminate the drug, such as drainage by replacement of the drug by inflammatory fluid (there may exist a mild inflammatory reaction right after injection) (Conrad 1979), uptake into the conjunctiva, etc, the value that we got from curve-fitting is within a reasonable range. Another study (Levine and Aronson 1970) showed that disappearance of soluble radiopaque medium after subconjunctival injection took place in about an hour also supports our k_{10} value. By considering $F=k_{13}/k_{13}+k_{10}$, drug bioavailability can be estimated to be 0.06% which also falls within a reasonable range for subconjunctival injection (Maurice, Velilla et al. 1999).

In order to ensure a global minimum was achieved in our curve-fitting process, curve fitting was performed by different combination of initial estimates and different values of initial estimates either gave essentially the same value to each rate constant in Table 4.6 or a poor r^2 and an unrealistically high k_{30}. Therefore, it is very likely that the global minimum was achieved.

B. Model Validation

The validity of the model (distribution model) was checked by comparing the curves generated by our model and experimental data at two additional doses (0.261mg and 26.1mg). Comparisons of predicted and experimental data at the dose of 0.261mg and 26.1mg are shown in Figure 4.19 and 4.20 and Figure 4.21 and 4.22 respectively. It is apparent from Figure 4.23 that curves obtained at three different doses have similar slopes and there is reasonable agreement between predicted and experimental data. However, there is no experimental data beyond 400 minutes for the dose=0.261mg and 26.1mg. Therefore, a definitive validation at the late time points is not possible and this may be one of the limitations of our model. However, this limitation does not jeopardize the usefulness of our model. As demonstrated in the next chapter, the contribution of the distribution compartment to the steady state vitreous drug level is insignificant in the case of a drug delivery system. As a result, our model should be reasonably accurate to be used as a tool to study drug delivery to the posterior segment of the eye by subconjunctival injection.

VI. CONCLUSIONS

A validated pharmacokinetic model was developed for subconjunctival injection. Therefore, we can accept our hypothesis that a simple but realistic pharmacokinetic model can be developed for subconjunctival injection. Based on this validated model, computer simulation can

be performed to study various factors that affect drug levels in the vitreous after subconjunctival injection and such study will be addressed in Chapter 5.

Table 4.1: Estimation of pharmacokinetic parameters by curve-fitting by fixing $k_{10}=1.83x10^{-2}min^{-1}$

Rate constants	
k_{13}	$1.77x10^{-5}$ min^{-1}
k_{30}	$2.16x10^{-2}$ min^{-1}

$R=0.75$

$R^2=0.57$

Table 4.2: Estimation of pharmacokinetic parameters by curve-fitting by letting k_{10} float

Rate constants	
k_{10}	1.96×10^{-1} min^{-1}
k_{13}	1.07×10^{-4} min^{-1}
k_{30}	1.20×10^{-2} min^{-1}

$R=0.91$

$R^2=0.83$

Table 4.3: Estimation of pharmacokinetic parameters by curve-fitting by letting k_{10} float and ignoring the last two data points

Rate constants	
k_{10}	1.60×10^{-1} min^{-1}
k_{13}	9.49×10^{-5} min^{-1}
k_{30}	1.26×10^{-2} min^{-1}

$R=0.99$

$R^2=0.99$

Table 4.4: Estimation of pharmacokinetic parameters by curve-fitting by incorporating a protein binding term

Rate constants	
k_{10}	1.95×10^{-1} min^{-1}
k_{13}	1.06×10^{-4} min^{-1}
k_{30}	1.34×10^{-2} min^{-1}
A	1.19×10^{-1}

$R=0.91$

$R^2=0.83$

Table 4.5: Estimation of pharmacokinetic parameters by curve-fitting by incorporating a protein binding term with two binding sites

Rate constants	
k_{10}	1.17×10^{-1} min^{-1}
k_{13}	8.15×10^{-5} min^{-1}
k_{30}	1.48×10^{-2} min^{-1}

$R=0.997$

$R^2=0.994$

Table 4.6: Estimation of pharmacokinetic parameters by curve-fitting by incorporating a distribution compartment

Rate constants	
k_{10}	1.28×10^{-1} min^{-1}
k_{13}	8.51×10^{-5} min^{-1}
k_{30}	1.14×10^{-2} min^{-1}
k_{34}	2.72×10^{-3} min^{-1}
k_{43}	3.32×10^{-4} min^{-1}

$R=0.999$

$R^2=0.999$

Table 4.7: The results of AIC and SC Tests

	Protein Binding	Peripheral (Distribution)
AIC	-2.5	-18.3
SC	-1.91	-17.3

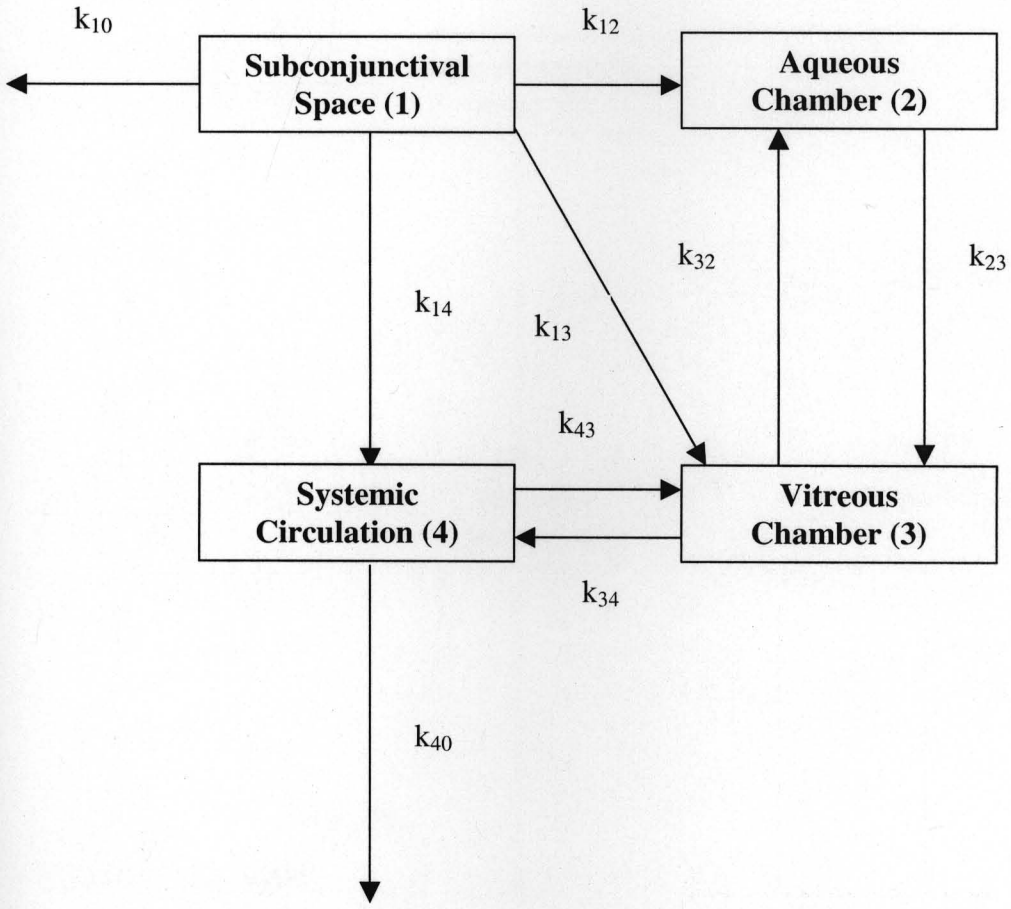

Figure 4.1: A comprehensive pharmacokinetic model for subconjunctival injection

(Adapted from Conrad 1979 with modifications)

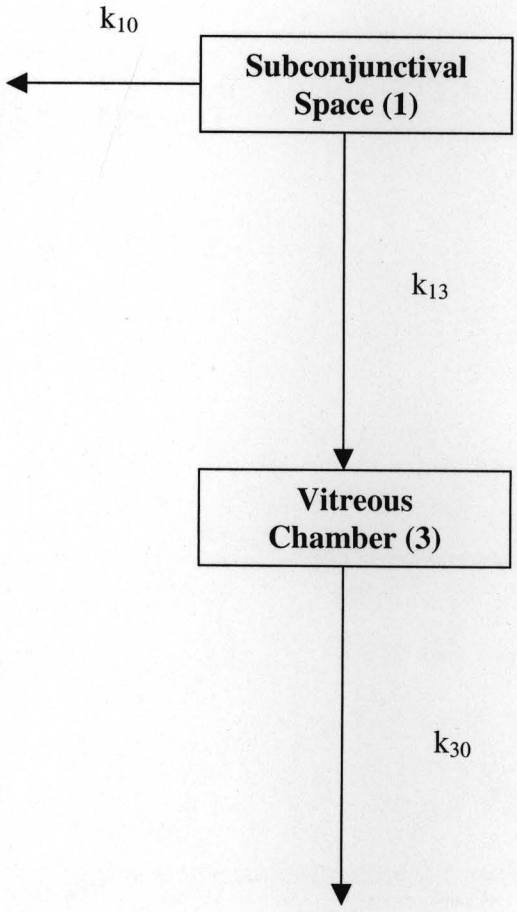

Figure 4.2: A simplified pharmacokinetic model for subconjunctival injection

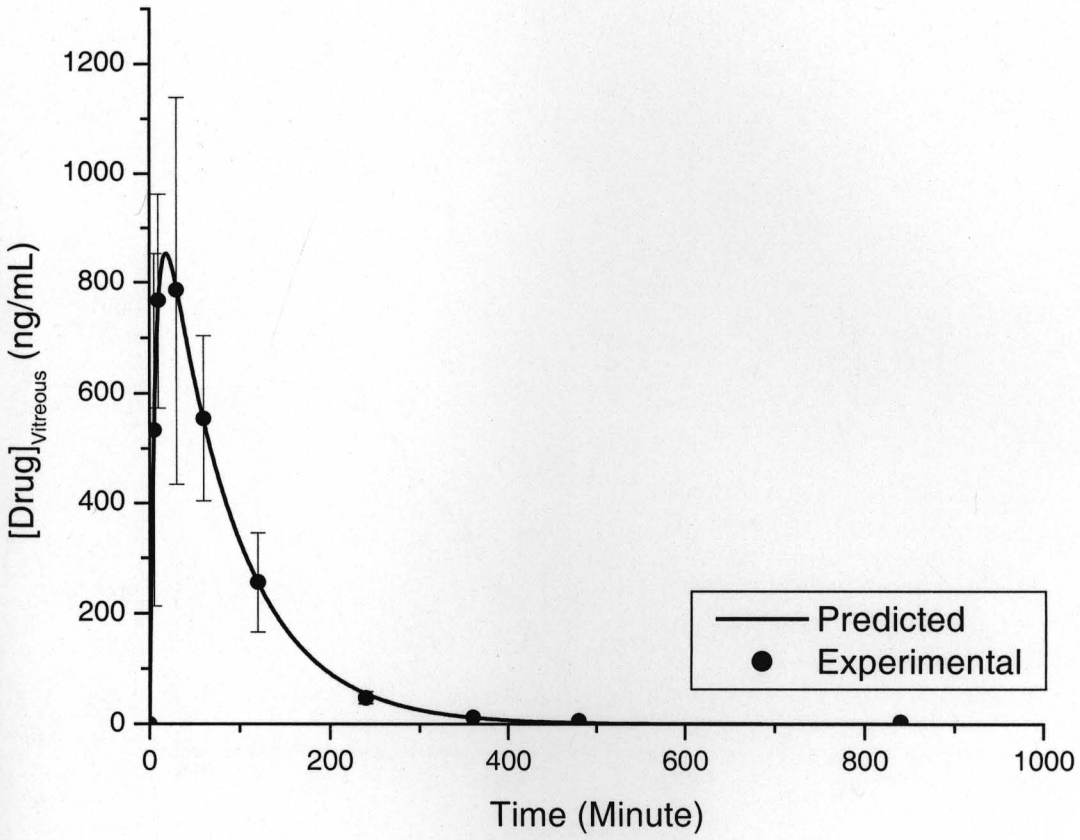

Figure 4.3: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 2.61mg by fixing $k_{10}=1.83x10^{-2}$ min^{-1}

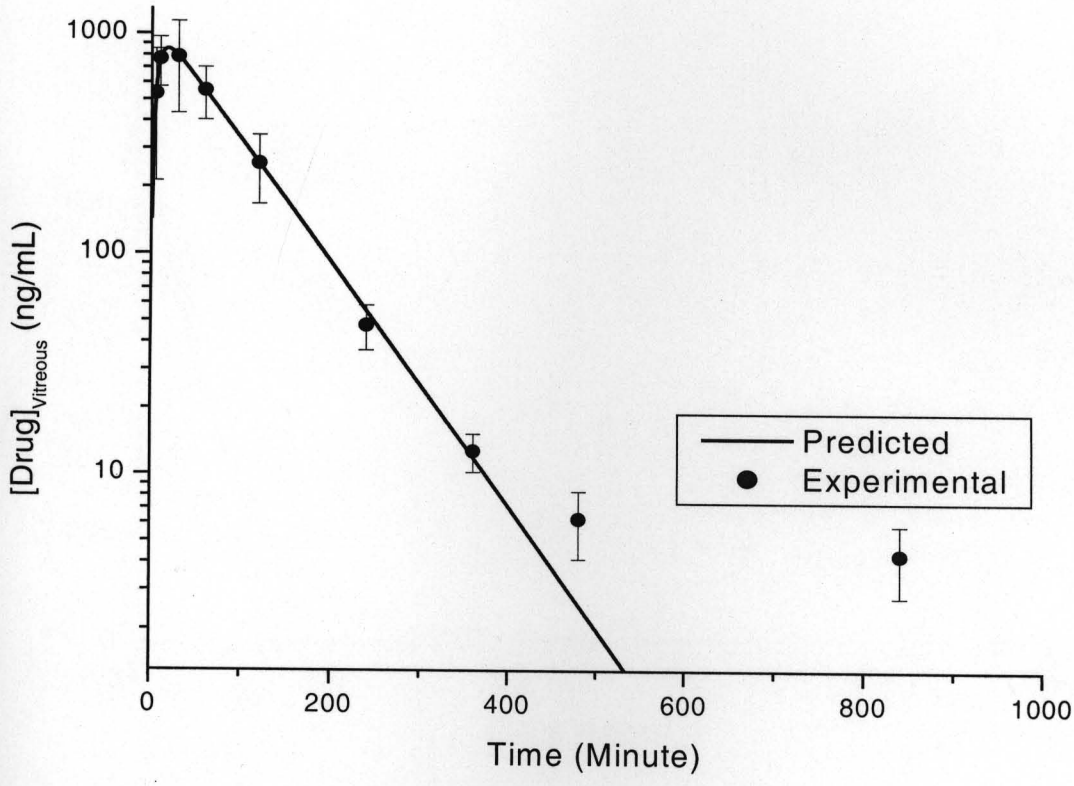

Figure 4.4: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 2.61mg by fixing $k_{10}=1.83x10^{-2}$ min^{-1} (Log scale)

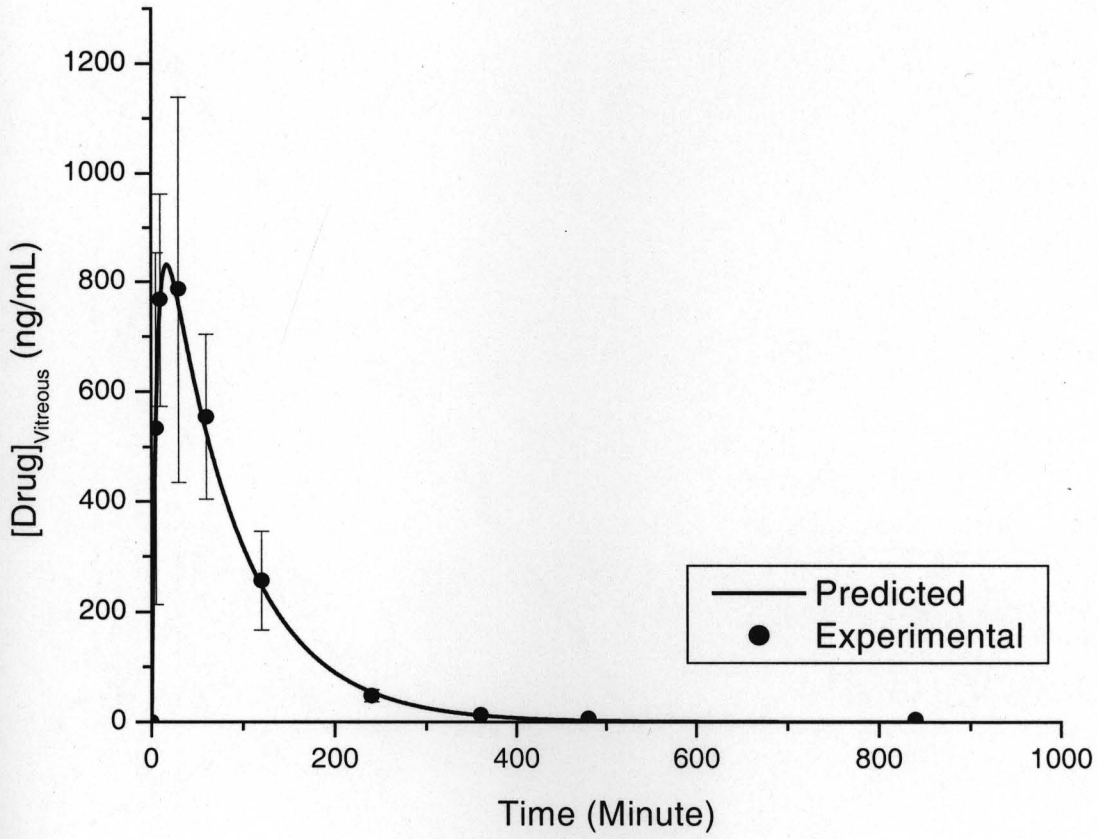

Figure 4.5: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 2.61mg by letting k_{10} float

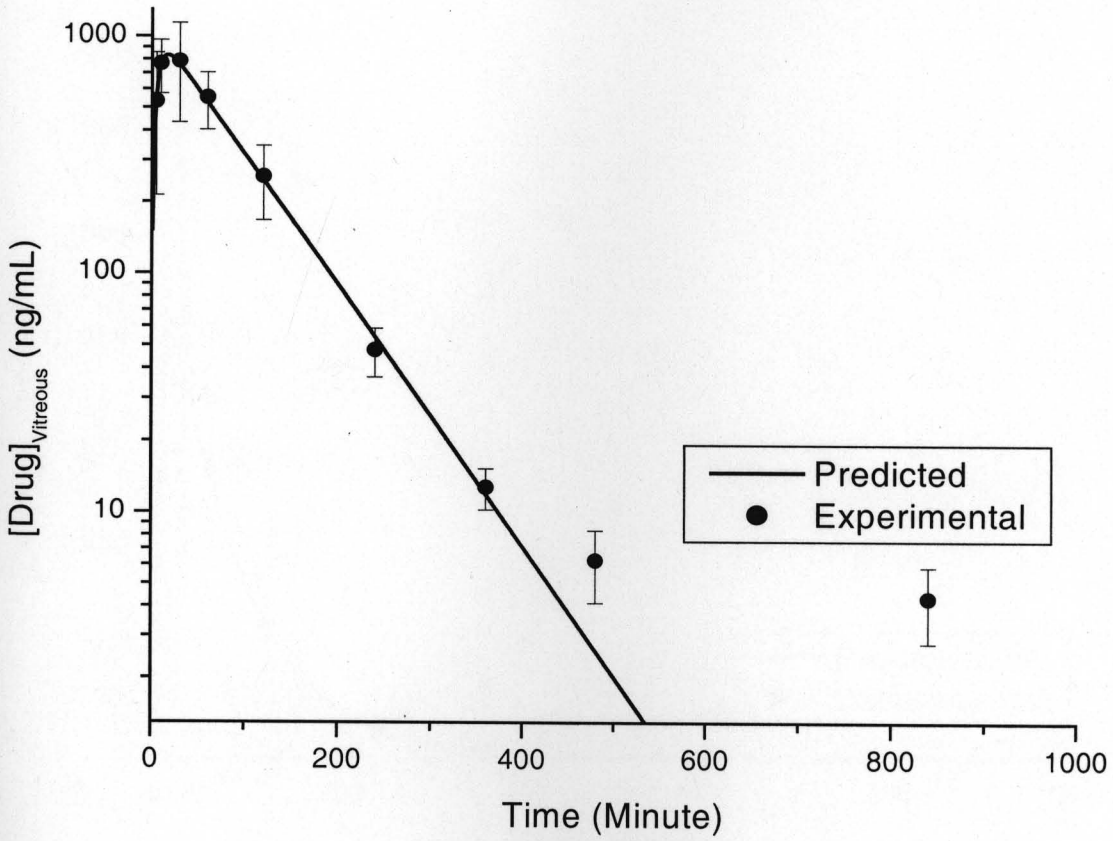

Figure 4.6: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 2.61mg by letting k_{10} float (Log scale)

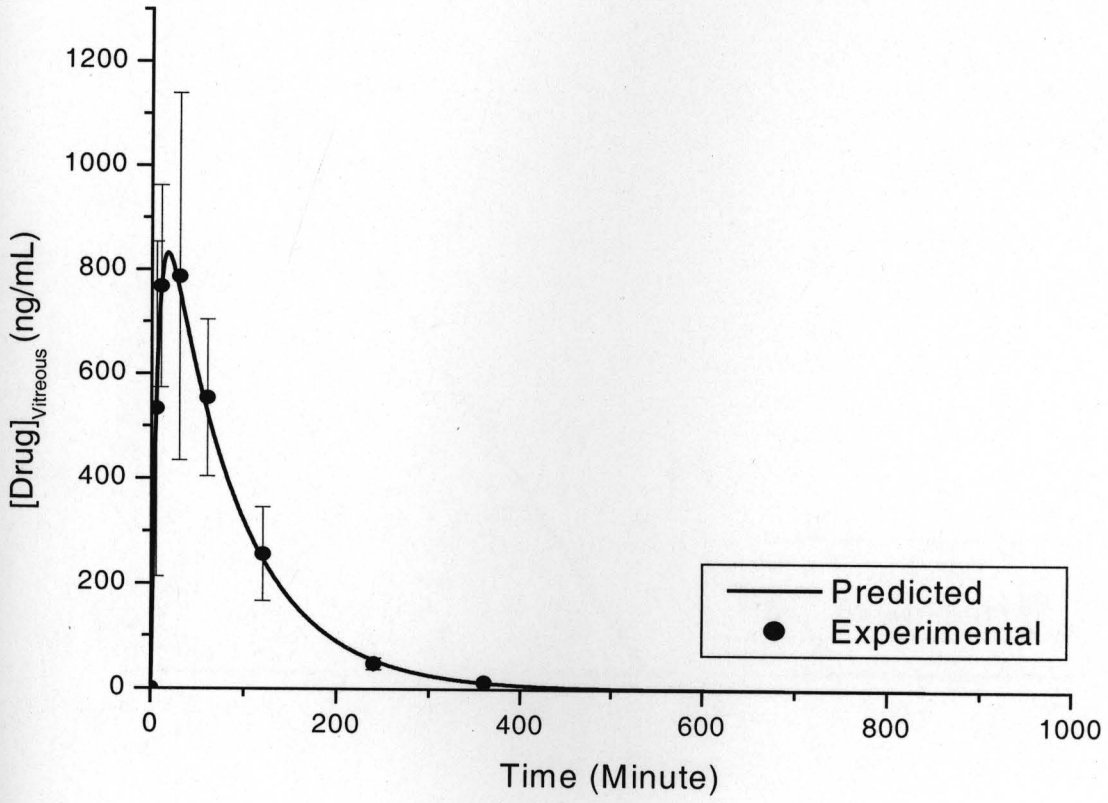

Figure 4.7: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 2.61mg by ignoring the last two points

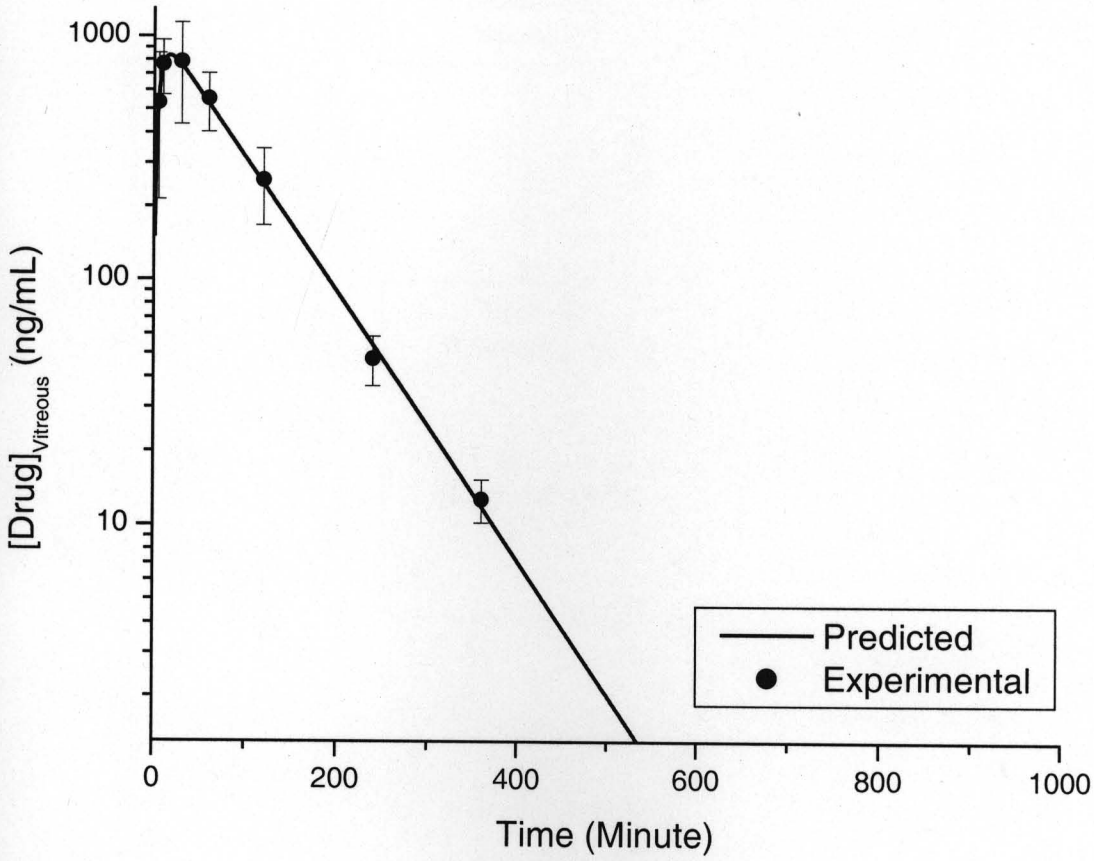

Figure 4.8: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 2.61mg by ignoring the last two points (Log scale)

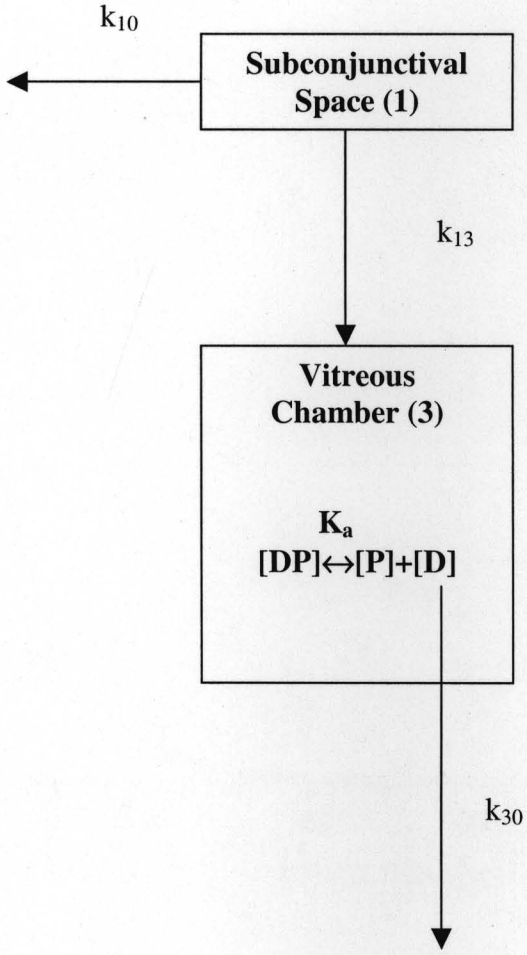

Figure 4.9: A pharmacokinetic model for subconjunctival injection with protein binding [P]=protein, [D]=free drug and [DP]=drug-protein complex, K_a=association constant

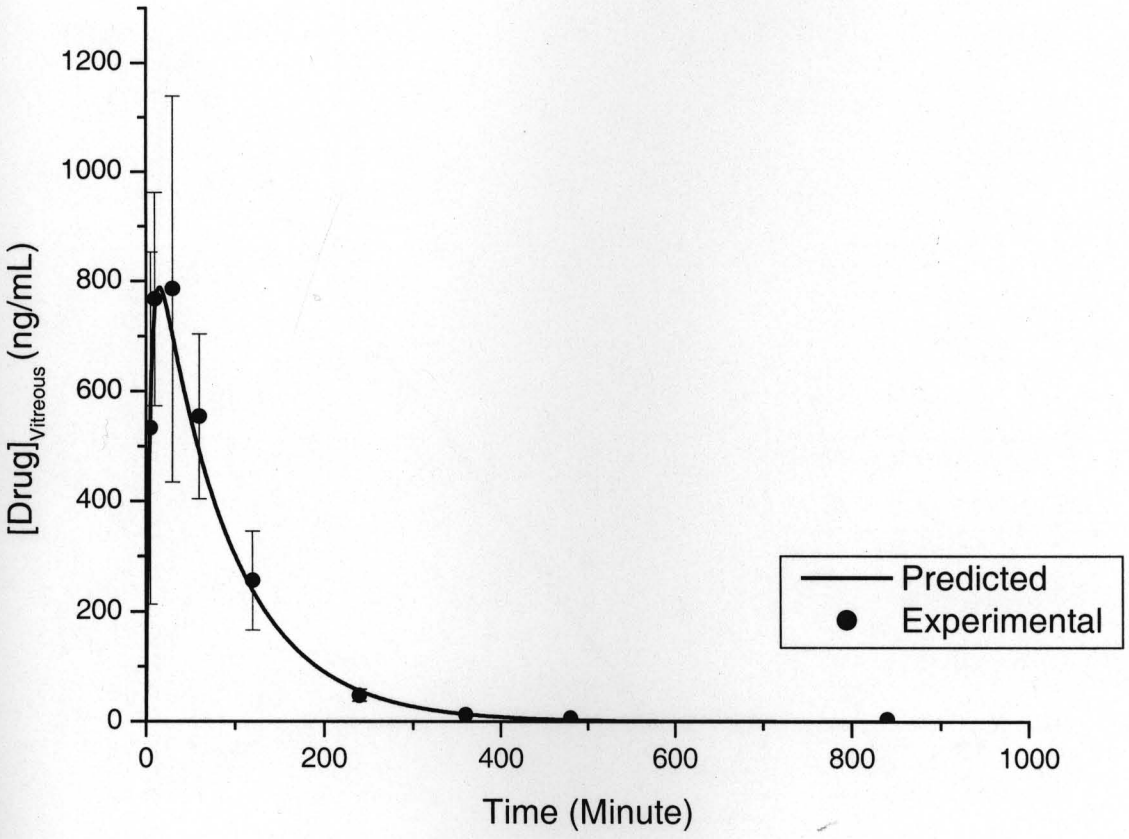

Figure 4.10: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 2.61mg by incorporating a protein binding term

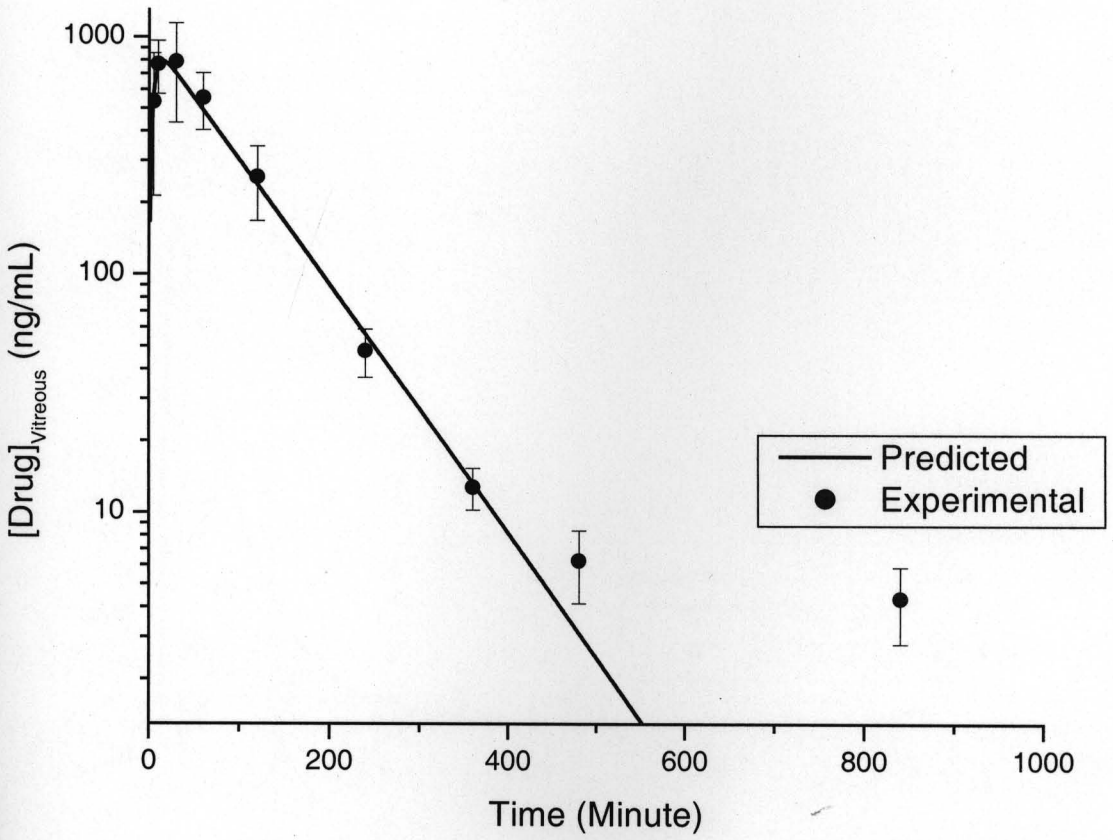

Figure 4.11: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 2.61mg by incorporating a protein binding term (Log scale)

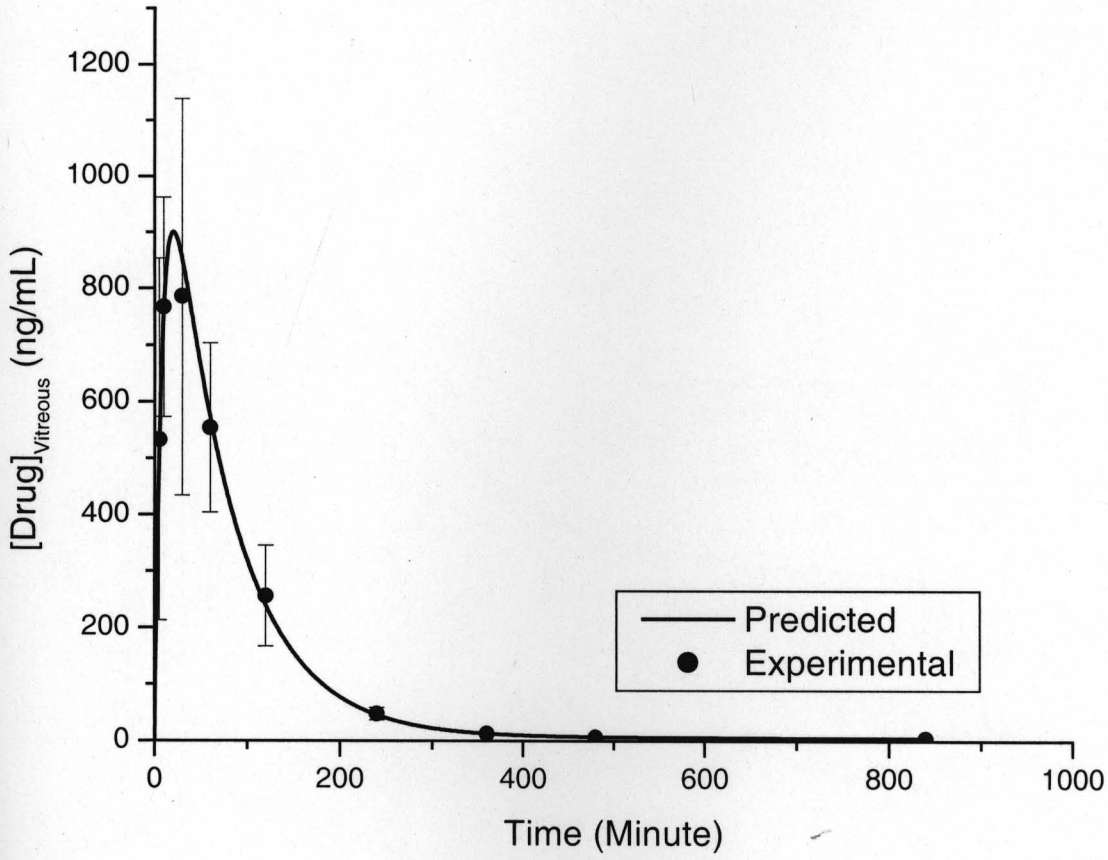

Figure 4.12: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 2.61mg by incorporating a protein binding term with two binding sites

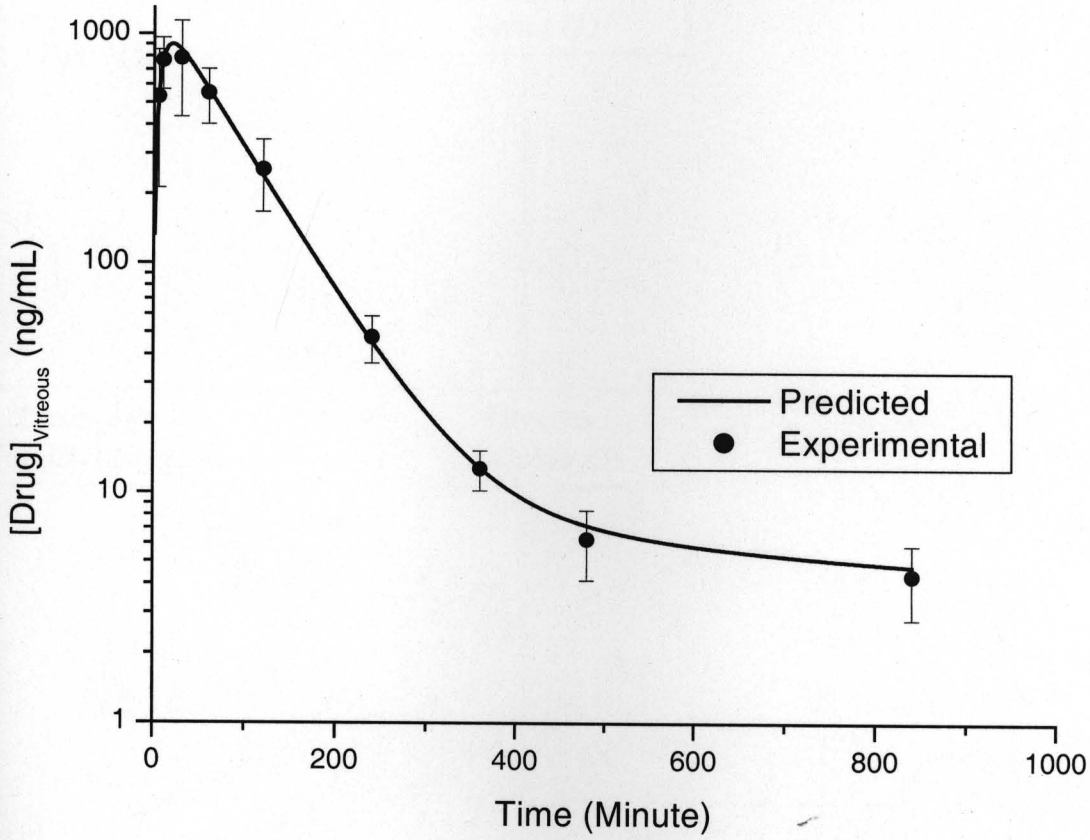

Figure 4.13: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 2.61mg by incorporating a protein binding term with two binding sites (Log scale)

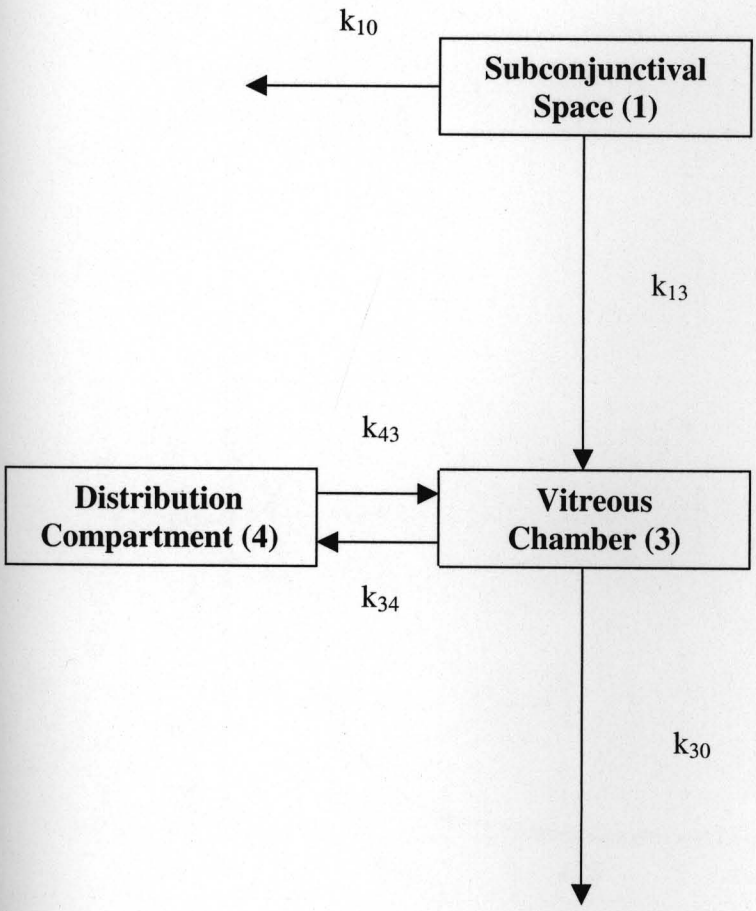

Figure 4.14: A pharmacokinetic model for subconjunctival injection with incorporation of a reservoir compartment

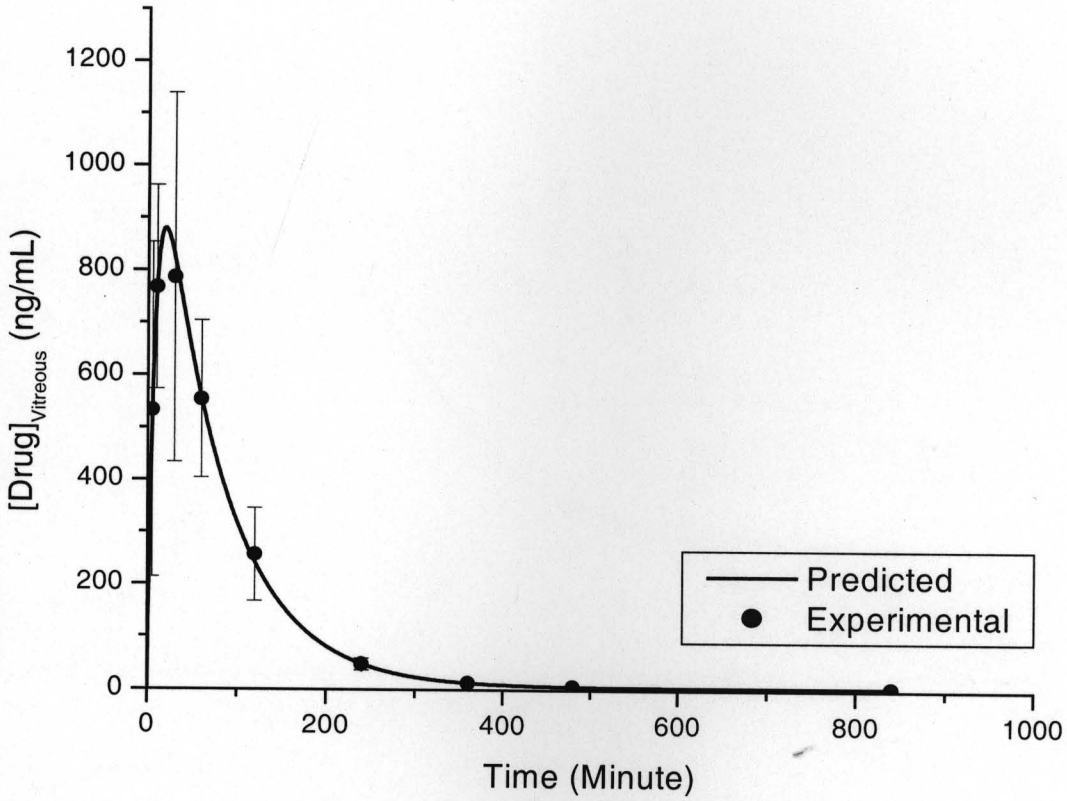

Figure 4.15: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 2.61mg by incorporating a reservoir compartment

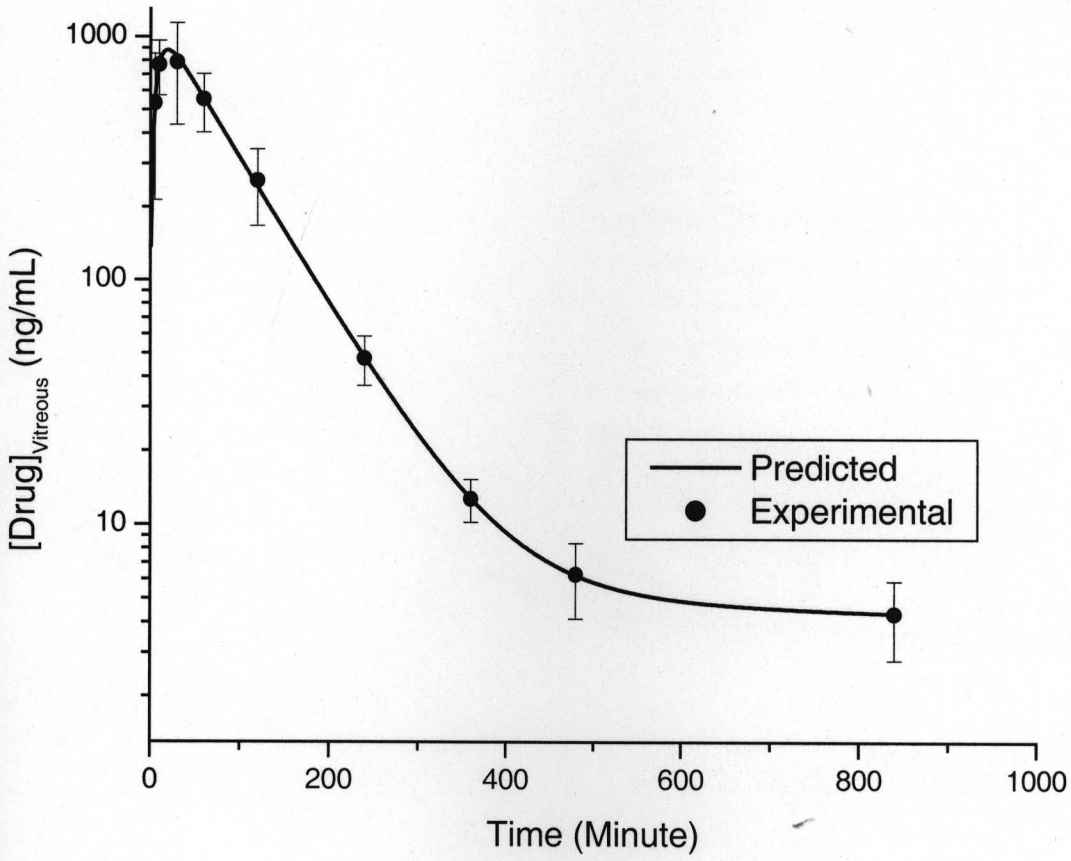

Figure 4.16: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 2.61mg by incorporating a reservoir compartment (Log scale)

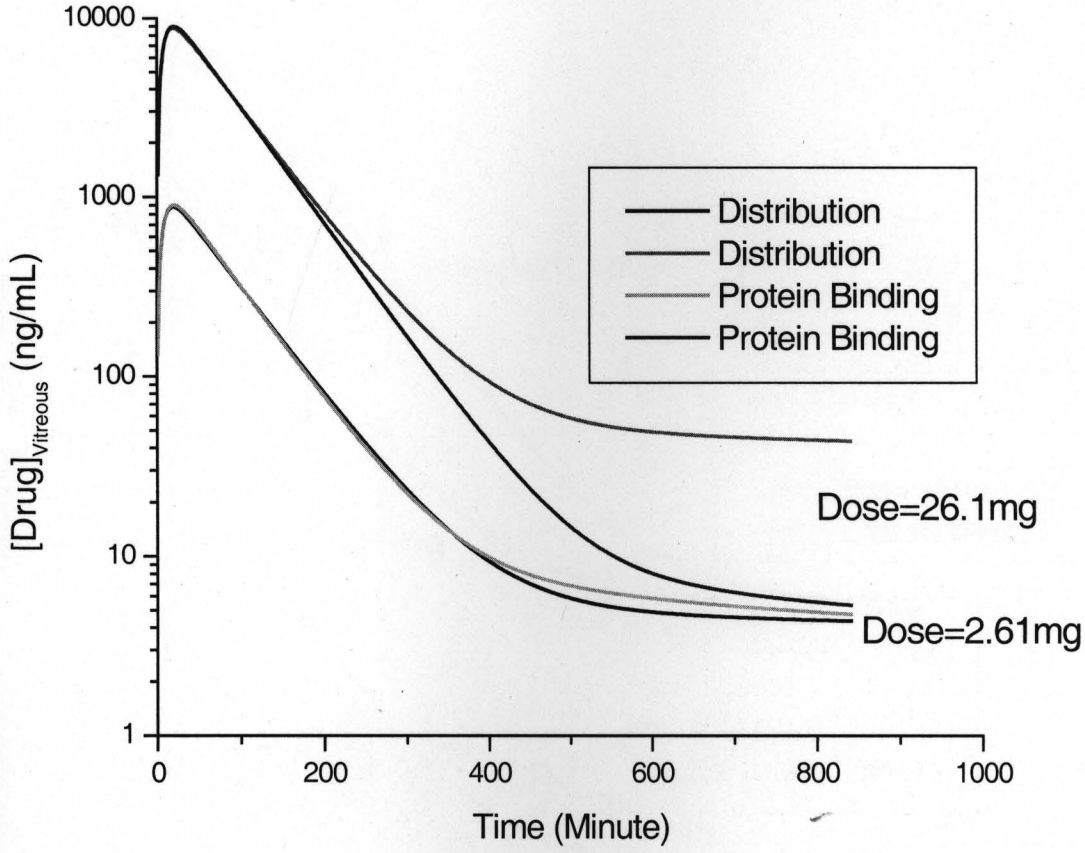

Figure 4.17: Comparison of simulated vitreous drug level after subconjunctival injection of prednisolone by two different models

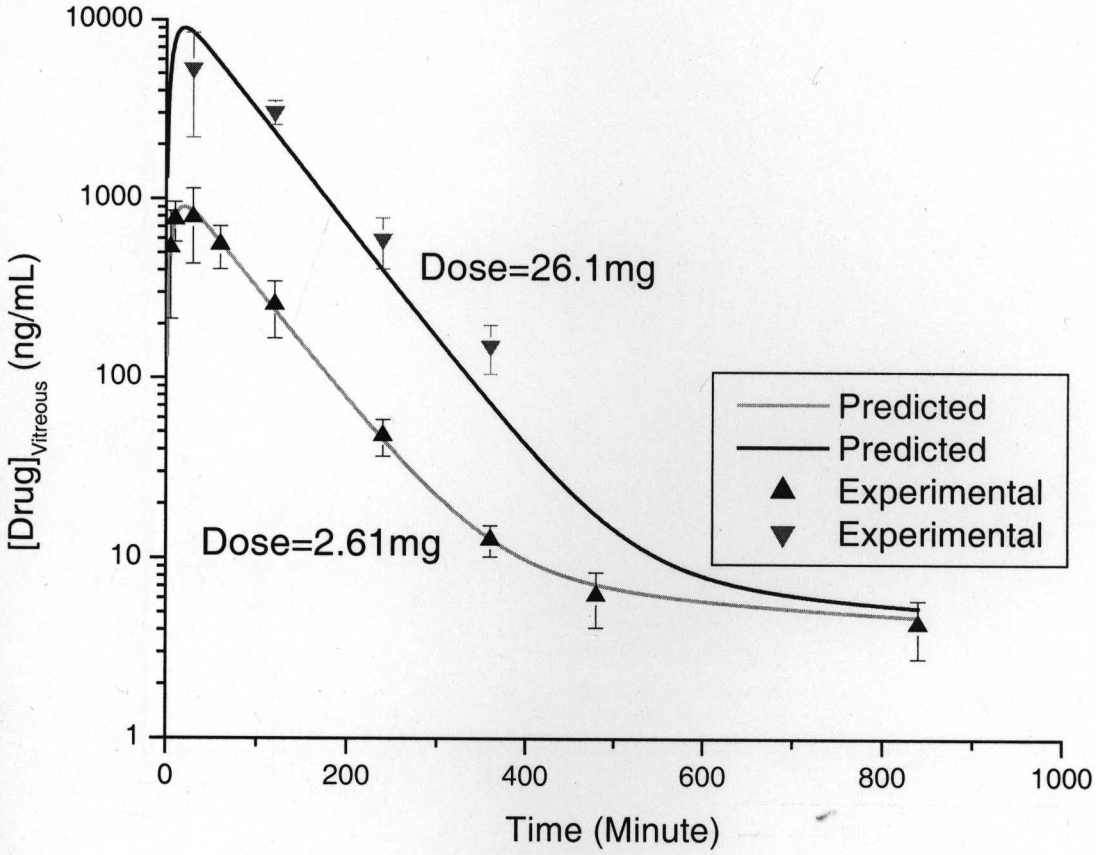

Figure 4.18: Comparison of experimental and simulated vitreous drug level after subconjunctival injection of prednisolone generated by the protein binding model

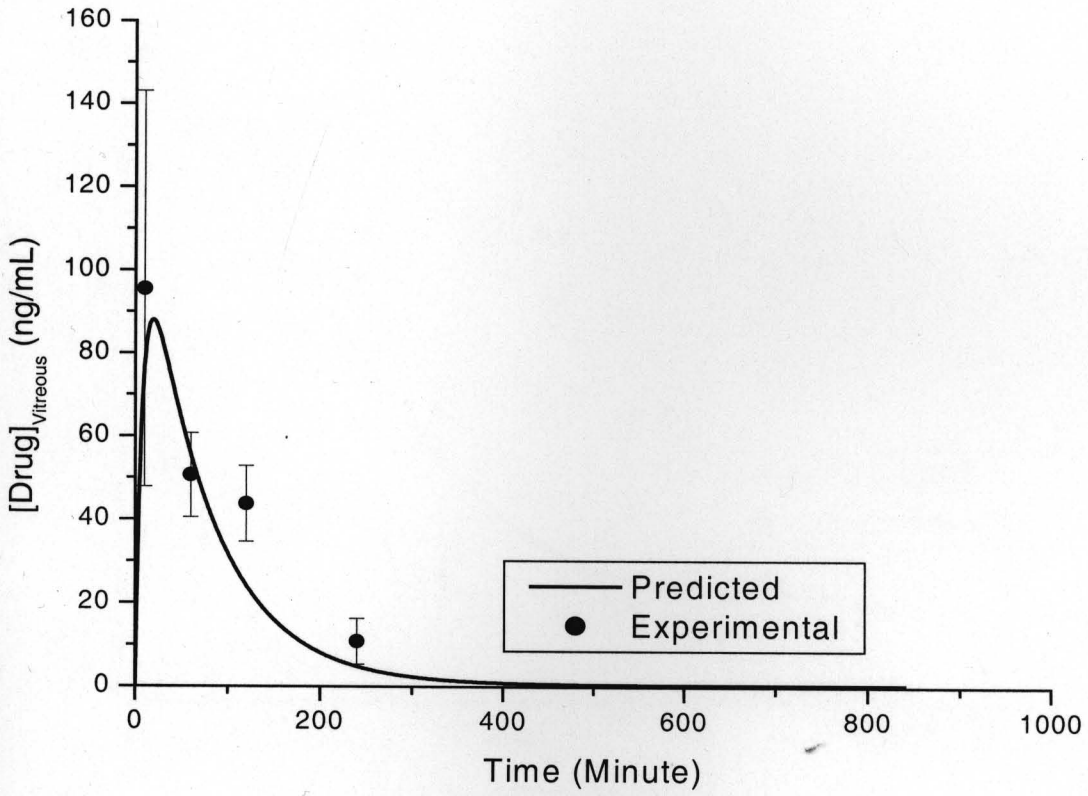

Figure 4.19: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 0.261mg

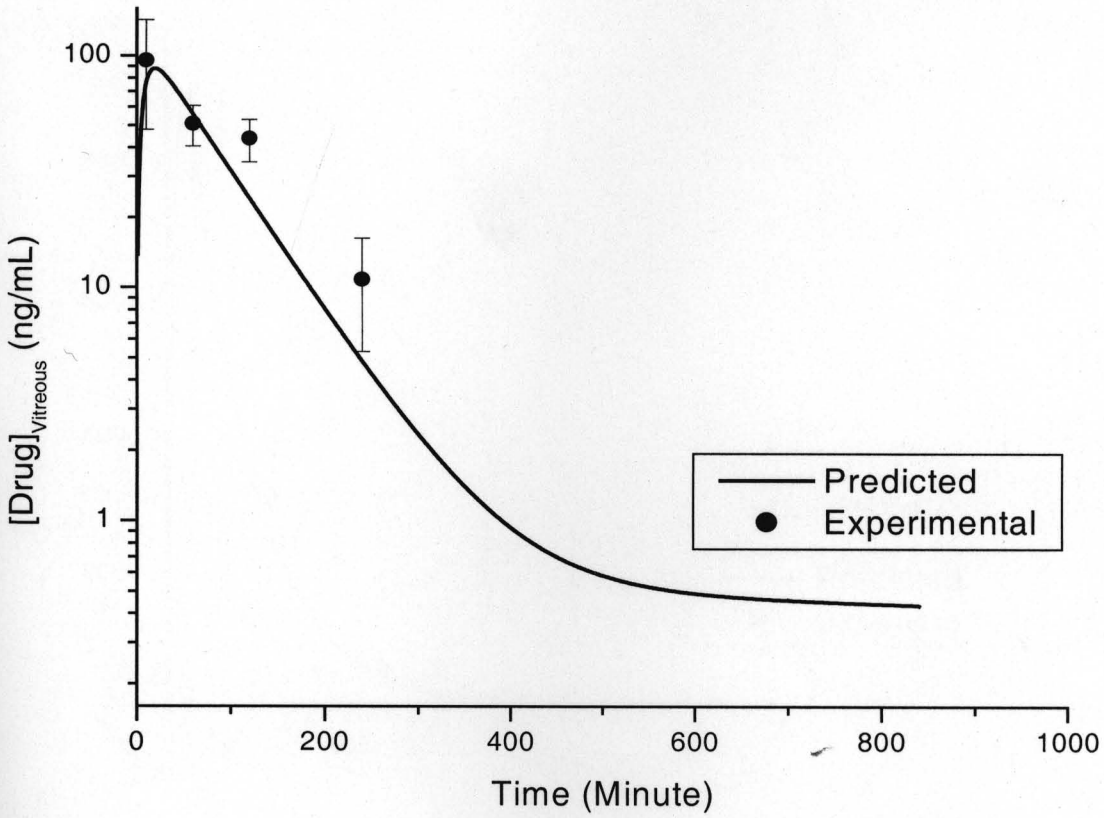

Figure 4.20: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 0.261mg (Log scale)

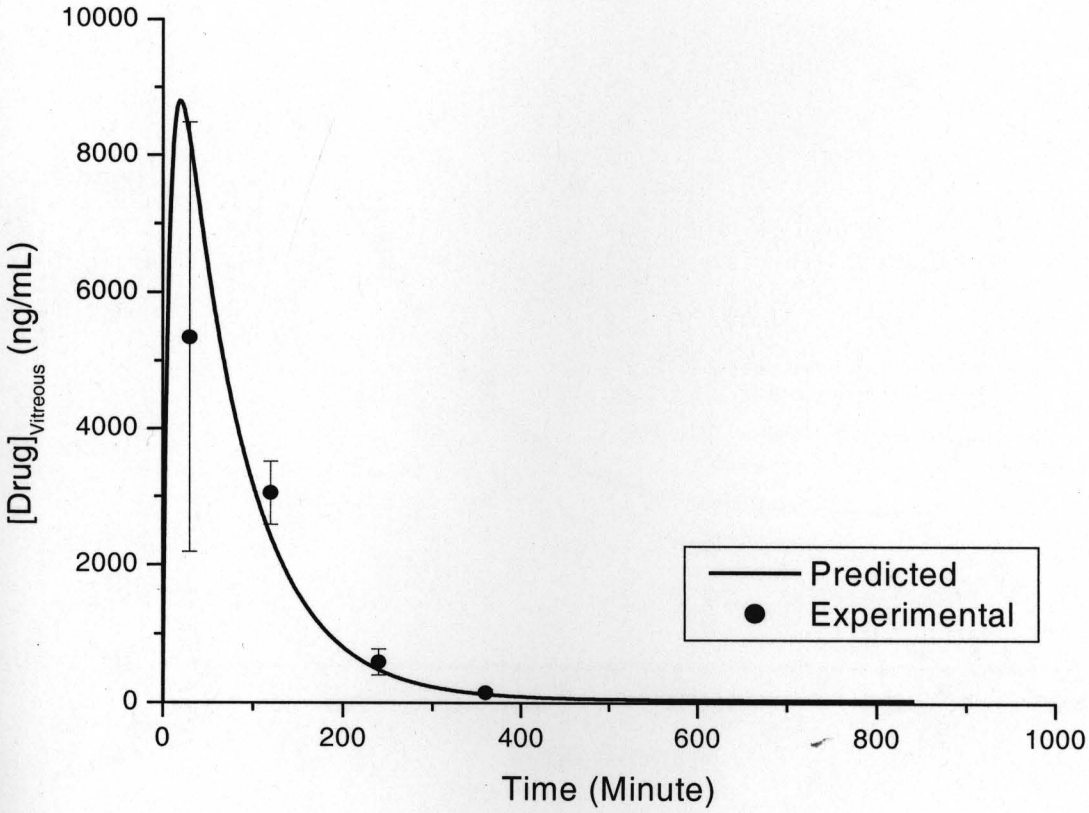

Figure 4.21: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 26.1mg

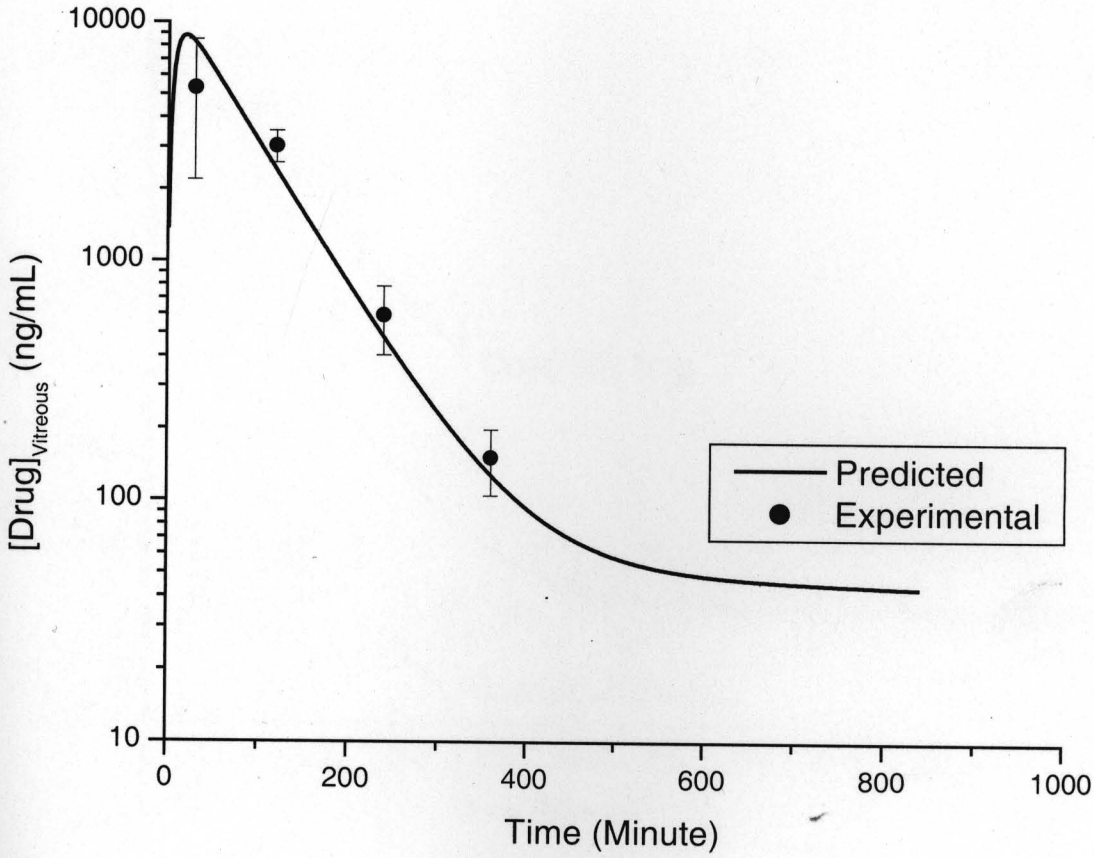

Figure 4.22: Comparison of predicted and experimental vitreous drug level after subconjunctival injection of prednisolone at a dose of 26.1mg (Log scale)

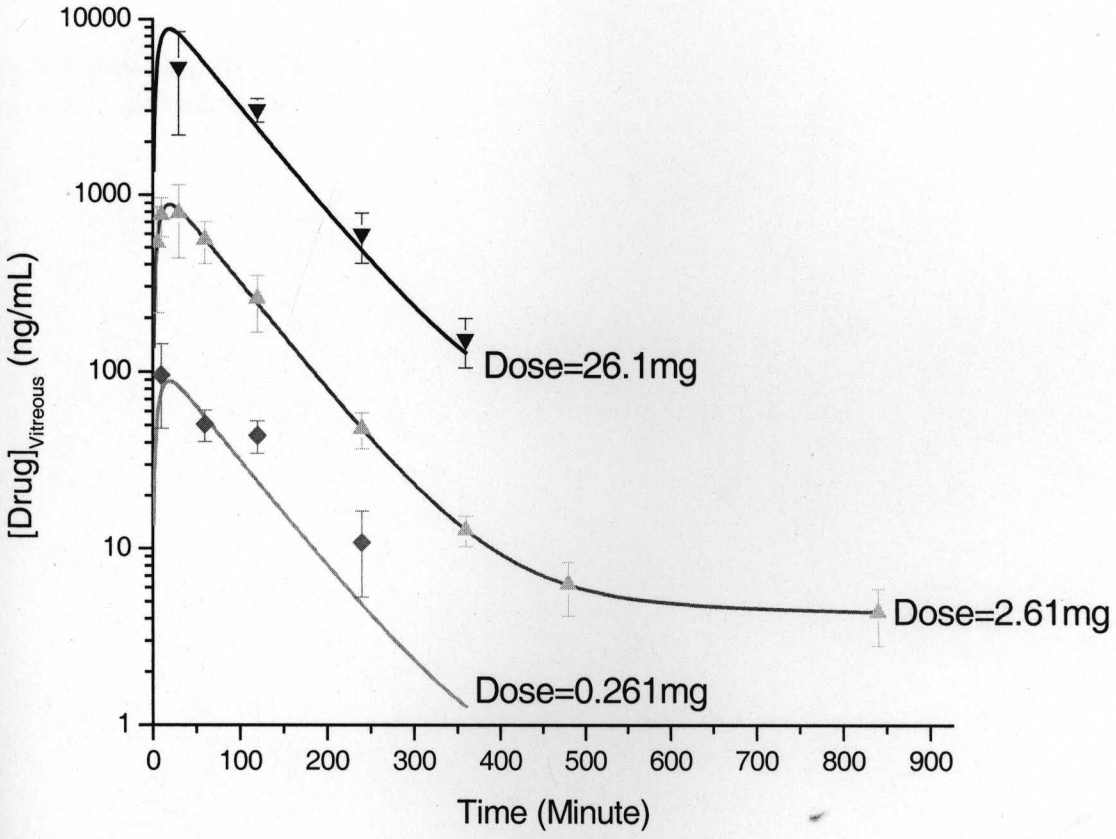

Figure 4.23: Time course of vitreous drug level after subconjunctival injection of prednisolone at three different doses (Solid lines present predicted data and the symbols represent experimental data)

Appendix 4.1: Differential Equations describes the model in Figure 4.2

$d(\text{Drug in 1})/dt=-(k_{10}+k_{13})(\text{Drug in 1})$
$d[\text{Drug}]_3/dt=k_{13}(\text{Drug in 1})/1.5-k_{30}[\text{Drug}]_3$

Appendix 4.2: Differential Equations describes the model in Figure 4.9

$d(\text{Drug in 1})/dt=-(k_{10}+k_{13})(\text{Drug in 1})$
$d[\text{Drug}]_3/dt=k_{13}(\text{Drug in 1})/1.5-k_{30}[\text{Drug}]_3][1/(1+A)]$

Appendix 4.3: Differential Equations describes the model with two binding sites in the protein binding process

$d(\text{Drug in 1})/dt=-(k_{10}+k_{13})(\text{Drug in 1})$

$d[\text{Drug}]_3/dt=k_{13}(\text{Drug in 1})/1.5-0.5k_{30}[([\text{Drug}]_3-6.344)+ (40.246336-2.3694[\text{Drug}]_3+[\text{ Drug}]_3^2)^{1/2}$

Appendix 4.4: Differential Equations describes the model in Figure 4.14

$d(\text{Drug in 1})/dt=-(k_{10}+k_{13})(\text{Drug in 1})$
$d[\text{Drug}]_3/dt=k_{13}(\text{Drug in 1})/1.5-k_{30}[\text{Drug}]_3+k_{43}[\text{Drug}]_4-k_{34}[\text{Drug}]_3$
$d[\text{Drug}]_4/dt=k_{34}[\text{Drug}]_3-k_{43}[\text{Drug}]_4$

Appendix 4.5: Definition of Akaike Information Criterion (AIC) and Schwarz Criterion (SC) (Bourne 1995)

AIC=nln(WSS)+2m

SC=nln(WSS)+mln(n)

where WSS=weighted sum of square of the residuals
m=number of parameters
n=number of data points

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CHAPTER 5

THEORETICAL FORMULATION OF A DRUG DELIVERY SYSTEM FOR SUBCONJUNCTIVAL INJECTION (COMPUTER SIMULATION)

I. INTRODUCTION

Computer simulation is an invaluable tool in studying drug delivery (Slovin 1997; Worakul 2001). Pharmacokinetic modeling is particularly important in this aspect since it can give insight on proper design of a dosage form. Norris et al (Norris, Leesman et al. 2000) showed that pharmacokinetic modeling can give insight on drug absorption from the GI tract such as dissolution-limited absorption, permeability-limited absorption, etc.

Making use of Stella®, Grass and Lee (Grass and Lee 1993) studied the effect of release rate of a timolol device on the aqueous drug level based on a validated pharmacokinetic model. They found that the aqueous drug level increased with release rate. However, a release rate of 1μg/min or higher will cause the dosage form to be depleted of the drug after two hours if only 125μg was incorporated. This piece of information is invaluable in designing a dosage form.

As mentioned earlier, pharmacokinetic modeling can give insight on proper design of a dosage form. Therefore, the main goal of the current study is to investigate various factors that can influence vitreous drug levels after subconjunctival injection, based on the validated pharmacokinetic model developed in the last chapter.

II. SPECIFIC AIMS

To study the effect of various rate constants, different methods of administration and other factors, such as solubility, etc. on the vitreous drug level with the ultimate aim to providing information for designing an effective drug delivery system.

III. EXPERIMENTAL

A. Model

The model used in our computer simulation was the one developed and validated in Chapter 4. This simple model is adequate to describe the pharmacokinetics of subconjunctival injection with reasonable accuracy and is shown in Figure 5.1.

B. Parameter Assignment

The magnitude of the rate constants obtained from Chapter 4 was used in our simulation unless otherwise specified. The value for each model parameter is summarized in Table 5.1.

C. Methods of Administration

(i) Simple Injection

A single dose of 2.61mg is injected subconjunctivally. For multiple injections, it is assumed that the frequency of injection is one injection per week.

(ii) Zero-order Release System

A system is required that can deliver a drug at a rate of 15.5μg/hr for 7 days.

(iii) First-order Release System

A 2.61mg dose can be released at a rate of $0.04hr^{-1}(k_1)$ and only 1/1000 of the dose is left after 7 days. For multiple injections, it is assumed that the frequency of injection is one injection per week.

D. Simulation Software

ModelMaker 4.0 (Cherwell Scientific, UK) was used in our computer simulation. This software allows a model to be constructed diagrammatically and simulation can be carried out in differential form. In other words, complicated differential equations need not to be solved. Only minimal knowledge of mathematics and computer programming is required for the process. The differential equations describing the model is shown in Appendix 5.1.

IV. RESULTS AND DISCUSSIONS

A. Limitations of the Model

It is believed that our model is also applicable to other compounds since any drug is subject to the same penetration and elimination pathways after subconjunctival injection (Conrad 1979; Tsuji, Tamai et al. 1988). Of course, the magnitude of each rate constant will be altered according to physicochemical properties of the injected compound.

Ideally, with a properly designed drug delivery system, the pharmacokinetics will be governed by the release rate only if $k_{release} << k_{absorption}$ (Lee and Robinson 2001). In this case, the physicochemical properties of a compound will not have a large influence on the drug level since

the major determining factor is $k_{release}$. If this is indeed the case, our model can be easily applied to other compounds without the need to estimate the magnitude of the rate constants associated with that particular compound since $k_{release}$ is the major determinant.

However, this case may not be easily achieved in the case of subconjunctival injection due to the presence of a parallel loss pathway (discussed later). As can be seen later, $k_{absorption}$, which is believed to be related to the physicochemical properties of a compound, is one of the major determinants of the vitreous drug level. In other words, it is necessary to have adjustments in the magnitude of each rate constant in order to apply our model to a particular compound. However, to the authors' knowledge, our study is the first systematic study on subconjunctival injection. There does not exist any study to try to address the correlation between physicochemical properties such as partition coefficient and $k_{absorption}$ for subconjunctival injection. Therefore, our model may not be easily applied to other compounds without an estimation as to the magnitude of the rate constants. In order to circumvent this problem, computer simulation was run based on a certain range of k_{13} and k_{30} estimates based on the information from the literature. Therefore, boundaries of the rate of drug delivery to the subconjunctival space that will result in therapeutic vitreal levels can be set up based on these ranges of rate constants. This piece of information is invaluable in determining whether it is feasible to achieve therapeutic levels in the vitreous using subconjunctival injection and a proper design of a dosage. For example, after computer simulation, if the rate of delivery has to be >1g/hr in order to achieve therapeutic vitreal levels, it may indicate that subconjunctival injection is not a practical way to deliver a drug to the back of the eye since it is highly unlikely to have a

system with such a high release rate and at the same time has sufficient drug loading to last for 7 days.

B. Criteria for Therapeutic Concentration

Since therapeutic concentrations for most drugs are in the range of ng/mL to μg/mL (Shargel and Yu 1999), this can be used as a criterion to determine the possibility of achieving a therapeutic level in the vitreous after subconjunctival injection. It is further assumed that pharmacological/therapeutic effects can be triggered once therapeutic drug levels are achieved and other complicated PK-PD relationships such as post-antobiotic effects will not be considered. One of the purposes of running computer simulation is to gain some insight as to the magnitude of the relevant parameters in order for a therapeutic concentration to be achieved in the vitreous. As mentioned earlier, it is not easy to know the exact magnitude of a particular parameter for a particular compound without running any in vivo experiment and so prediction of the vitreous drug level based on physicochemical properties of a compound is very difficult. However, it is still desirable to have quantitative information on these parameters. Another purpose of running computer simulation is to gain an understanding of the effect of a particular parameter on the vitreous drug level with an aim to overcoming various barriers by changing the magnitude of some of the parameters by use of a delivery system.

C. Flip-flop Pharmacokinetics

For drug delivery to the anterior segment of the eye using topical instillation, the simplest pharmacokinetic model is the single compartment model as shown in Figure 5.2. Drug concentration in the cornea can be expressed by the following equation:

$$C=(FD/V_d)[k/(k-K)](e^{-Kt}-e^{-kt}) \quad (5.1)$$

where F is the fraction of dose absorbed, D is the dose, k and K are absorption and elimination rate constants respectively, and V_d is the apparent volume of distribution (Worakul and Robinson 1997). However, typically, the true absorption rate constant is much smaller than the elimination rate constant and this leads to the classical flip-flop model (Shargel and Yu 1992). In other words, the terminal slope of a flip-flop model represents the absorption rate constant instead of the elimination rate constant. What stops this flip-flop behavior is incorporation of a parallel loss pathway at the precorneal area (Figure 5.3) and the model then becomes "well behaved".

With incorporation of a parallel loss pathway, the corneal drug concentration can be represented by (Makoid, Sieg et al. 1976)

$$C=(FDk_{12}/V_d)[1/(k_{12}+k_{10}-k_{23})][e^{-k23t}-e^{-(k12+k10)t}] \quad (5.2)$$

Typically, the true absorption rate constant is 100 to 1000 times smaller than the apparent rate constant (Makoid, Sieg et al. 1976; Grass and Robinson 1984).

Analogous to drug delivery to the anterior segment, there exists a parallel loss pathway in the case of drug delivery to the posterior segment of the eye using subconjunctival injection.

Typically, the bioavailability in the vitreous after subconjunctival injection is in the range from 0.01 to 0.1%, indicating the true absorption rate constant is 1,000 to 10,000 times smaller than the apparent absorption rate constant.

There are a number of interesting phenomena associated with this parallel loss pathway. Firstly, analogous to the peak time after topical instillation, the peak time after subconjunctival injection shows a similar pattern (See Figure 5.3 for definition of rate constants)

$$T_p=\ln(k_{12}+k_{10}/k_{23})/(k_{12}+k_{10}-k_{23}) \quad (5.3)$$

Since $k_{10}>>>k_{12}$,

$$T_p=\ln(k_{10}/k_{23})/(k_{10}-k_{23}) \quad (5.4)$$

This equation shows that T_p is relatively insensitive to both k_{12} and k_{23}. As a result, T_p would be similar for drugs with different chemical structures due to the predominance of k_{10} in determining T_p. This is exactly the case for subconjunctival injection. Prednisolone (Tsuji, Tamai et al. 1988), cyclosporine (Kalsi, Gudauskas et al. 1991), dacarbazine (Kalsi, Silver et al. 1991), 6-mercaptopurine (Gudauskas, Kumi et al. 1985), mannitol (Chapter 3), etc., all show T_p about 30 minute or earlier.

The effect of the three rate constants, k_{10}, k_{13} and k_{30}, on the vitreous drug level for our model (Figure 5.1) is shown in Figure 5.4a&b, 5.5a&b and 5.6a&b respectively. Basically, all three rate constants have an influence on the vitreous drug level. As mentioned earlier, k_{30} does

not have a big influence on T_p and C_{max} (Figure 5.6). Decreasing k_{30} by 10-fold only brings about a 1.5 times increase in C_{max}. The major role of k_{30} is in sustaining the vitreous drug level. However, after a drug enters the vitreous chamber, there will be no control over the elimination rate constant. In other words, it is technically impossible to change the magnitude of k_{30} by formulation means. Figure 5.5 also shows an agreement as to what is predicted, namely k_{13} does not have a big influence on T_p. Roughly speaking, doubling the magnitude of k_{13} doubles the C_{max} but the terminal slopes show a similar pattern. Figure 5.4 shows that k_{10} has a big influence on T_p, and C_{max}. Figure 5.4a shows that there is a dramatic increase in the vitreous level once k_{10} is $0.06min^{-1}$ or smaller. This can be explained by using $F=k_{13}/k_{13}+k_{10}$. Figure 5.7 shows that there is a large increase in F when k_{10} becomes $0.06min^{-1}$ or smaller.

In short, if only C_{max} need to be changed, k_{13} is the best parameter to be altered by the means of formulation. On the other hand, if both C_{max} and t_{max} profile need to be changed, modification of k_{10} would be the best choice. Decreasing k_{30} will sustain the vitreous drug level but this parameter is very difficult to be changed by formulation means.

D. Simple Subconjunctival Injection

The vitreous drug level after single and multiple injections is shown in Fig. 5.8 and 5.9 respectively. It is obvious that simple subconjunctival injection only gives a very brief vitreal level and the vitreous drug level falls to a very low level before the next dose. At the same time, there is a very large fluctuation between C_{max} and $C_{min.}$ Therefore, more frequent injections have to be made, i.e. more than one injection in a week, in order to maintain the vitreous drug level above the therapeutic concentration for an extended period of time. In this case, patient

compliance may be a problem. Therefore, the major challenge lies in how to sustain the vitreous drug level. Moreover, a high peak level may cause retinal toxicity. This situation happens in both single and multiple injection. Therefore, this will make simple subconjunctival injection less attractive in clinical practice to treat chronic eye diseases. Obviously, a drug delivery system which is capable of maintaining a sustained vitreous drug level is required to avoid extreme peak and trough vitreous levels.

The dosing concentration in our simulation is 2.61mg in 50μL, i.e., 52.2mg/mL. In the case of a poorly soluble compound such as triamcinolone acetonide, progesterone, paclitaxel, etc., it is not possible to prepare such a high dosing concentration. To take triamcinolone acetonide in Sorenson's buffer at pH 7.4 as an example, it has a solubility of about 10μg/mL at room temperature (Lee and Robinson 2001). Fig. 5.10 clearly shows that it is impossible to create a vitreous level higher than 1ng/mL. It is obvious that drug solubilization is very important in preparing a dosage form for subconjunctival injection. Of course, a poorly soluble compound can be formulated as a suspension and injected into the subconjunctival space provided that there is enough fluid in the subconjunctival space to dissolve the drug. However, the environment of the subconjunctival space is poorly understood and this issue needs to be further explored. Therefore, in order to make subconjunctival injection a practical route to deliver a therapeutic agent to the back of the eye, it is necessary to have a system which can sustain the vitreous drug level and also solubilize a poorly soluble compound.

E. Zero-order Release System

It is expected that a zero-order release system will give a steady vitreous drug level in the vitreous chamber. This is clearly the case in our computer simulation (Figure 5.11).

Another interesting point is that the existence of a reservoir has no effect on the steady vitreous drug level after application of a zero-order release system (Figure 5.12). Although the reservoir compartment affects the time to reach steady state, it has no effect on the steady state vitreous drug level. Therefore, the model in Figure 5.1 was simplified into a two-compartment model as shown in Figure 5.13. This model will be used for further analysis for simplicity.

As mentioned earlier, there is a very important feature associated with the introduction of a parallel loss pathway. There is a strong dependence of the vitreous drug level on k_{13} (absorption rate constant). With a well-behaved pharmacokinetic model ($k_a >> k_e$) (See Figure 5.14), k_a (k_{13} in the case of subconjunctival injection) does not play a role in determining the drug level at the target site since the slowest step, namely, drug release from the dosage form becomes the dominant factor in determining the pharmacokinetics (Figure 5.15). However, our pharmacokinetic model shows a strong dependence of the vitreous drug level on k_{13} (Figure 5.16) and this indicates that k_0 and k_{30} are not the only determinants of the vitreous drug level and k_{13} also plays a significant role. This is more obvious when the integrated equation is considered (Appendix 5.2):

$$[Drug]_3=[k_{13}k_0/(1.5k_{10})][1/k_{30}-(1/k_{30}+k_{10})e^{-k30t}] \qquad (5.5)$$

This equation shows that k_{13} will have an influence on the vitreous drug level no matter what value that k_0 is going to take. Therefore, the release has to be adjusted according to k_{13} and k_{30} for a particular compound.

As mentioned in Chapter 2, our ultimate objective is to develop a drug delivery system whose release rate is the rate-limiting step. In other words, the pharmacokinetics is mainly controlled by the rate of delivery to the subconjunctival space. However, based on the current finding, this is an extremely difficult task. Unless a delivery system is able to decrease k_{10} to a level low enough so that k_0 becomes rate-limiting again. Otherwise, k_{13}, which is a function of the physicochemical properties of a compound, will be one of the determinants of the pharmacokinetics after subconjunctival injection.

Although the magnitude of the rate constants will be drug-dependent, it is still desirable to set up boundaries on the rate of delivery in order to achieve therapeutic vitreal levels by assuming the rate constants fall within certain ranges. This piece of information is invaluable in determining whether subconjunctival injection is a practical way to deliver therapeutic agents to the back of the eye.

Assuming $k_{10}=1.28\text{x}10^{-1}\text{min}^{-1}$ is a constant since it is related to the sponge-like nature of the conjunctiva but not the physicochemical properties of a compound. According to the results obtained after intravitreal injection (Maurice 1976; Cobo and Forster 1981; Scholes, O'Brien et al. 1985; Huang, Blumenkranz et al. 1988; Miller, Madu et al. 1992; Mochizuki, Yamashita et al. 1992; Pang, Branchflower et al. 1992; Mochizuki, Torisaki et al. 1993; Cochereau-Massin, Marrakchi-Benjaafar et al. 1994; Hughes, Krishnamoorthy et al. 1996; Leeds, Henry et al. 1997), k_{30} ranges from $5\text{x}10^{-4}$ (hydrophilic compound) and $1.33\text{x}10^{-2}\text{min}^{-1}$ (lipophilic compounds)

depending on the elimination pathways. It is demonstrated that after intravitreal injection a hydrophilic compound, which cannot pass across the retinal surface easily, leaves the vitreous chamber via the lens zonule and then is carried away by the flow of aqueous humor. Because of the greater surface area of the retina and the shorter diffusional path, lipophilic compounds, which are able to use the retinal pathway, have a greater k_{30} than hydrophilic compounds which leave the vitreous via the "anterior" pathway. For k_{13}, there is limited information on drug bioavailability in the vitreous after subconjunctival injection. It is assumed that it is of the order of 0.01-0.1%. Since $F=k_{13}/k_{13}+k_{10}$, k_{13} can be estimated to be in the range of $1.28x10^{-5}$-$1.28x10^{-4}$ min^{-1}.

Basically, four cases will be considered in determining the boundaries on the rate of delivery, namely,

Case 1: Good penetration and fast elimination ($k_{13}=1.28x10^{-4}min^{-1}$, $k_{30}=1.33x10^{-2}min^{-1}$)

Case 2: Poor penetration and slow elimination ($k_{13}=1.28x10^{-5}min^{-1}$, $k_{30}=5x10^{-4}min^{-1}$)

Case 3: Poor penetration and fast elimination ($k_{13}=1.28x10^{-5}min^{-1}$, $k_{30}=1.33x10^{-2}min^{-1}$)

Case 4: Good penetration and slow elimination ($k_{13}=1.28x10^{-4}min^{-1}$, $k_{30}=5x10^{-4}min^{-1}$)

The effect of release rates on the vitreous drug level for these four cases are shown in Figure 5.17-5.20. In general, when release rate >10μg/hr, the vitreous drug level will be >1ng/mL except case 3: poor penetration and fast elimination. In Case 3, increase the release rate to 20μg/hr is necessary to bring the vitreous drug level to >1ng/mL. However, according to the physiology of the eye, Case 3 and Case 4 are less likely to exist. As mentioned in Chapter 1, the direct penetration pathway involves movement of a drug across the sclera->choroids->RPE->retina->vitreous. It is generally believed that the RPE is the major barrier since it has tight

junctions. As a result, a lipophilic compound can pass across it faster due to adoption of transcellular pathway. On the other hand, as mentioned earlier, a lipophilic compound tends to have a shorter half-life in the vitreous since it can leave the vitreous by the way of the retinal pathway. Similarly, a hydrophilic compound will have poorer penetration but it will stay longer in the vitreous. Therefore, under normal circumstance, Case 1 and Case 2 are very likely to exist but not Case 3 and 4. In summary, if the release rate >10μg/hr, it is very likely that the vitreous can reach 1ng/mL level. However, it should be stressed that this number is likely a overestimate since a drug delivery system may reduce the k_{10} (drug loss from the subconjunctival space). In reality, a much slower rate is needed to bring the vitreous drug level to >1ng/mL.

On the other hand, therapeutic concentrations of some drugs, e.g., antibiotics, are >1μg/mL. It is highly desirable to determine the release rate in order to maintain such a high concentration in the vitreous for an extended period of time. To take Case 1: Good penetration and fast elimination ($k_{13}=1.28\times10^{-4}min^{-1}$, $k_{30}=1.33\times10^{-2}\ min^{-1}$) as an example since it is likely to happen in the real situation. Assume the maximum dose that can be injected is 15mg and it is further assumed that the injection volume is 0.15mL. Therefore, a 100mg/mL dosing concentration is prepared. If we want to have a system that can have an extended release for four weeks, the maximum release rate is equal to (15mg/28Day)/24hour/Day)=22,321ng/hr. Figure 5.22 shows that it is impossible to reach >1μg/mL for such a system. Figure 5.23 shows that the minimum release rate in order to achieve >1μg/mL is 1200μg/hr. In other words, 1200μg/hr x 24hr x 28 Day =0.8064g is needed for 28 days. It is not very likely that such a high dose can be injected. Therefore, if the therapeutic concentration of a drug is >1μg/mL, subconjunctival

injection may not be a practical way to deliver that drug to the back of the eye unless k_{10} can be reduced by a drug delivery system by 100-fold (Figure 5.24).

Verification of our boundary conditions is not possible at this moment. The authors tried to validate these boundary conditions by using an Alzet Osmotic Minipump but the experiment was constrained by technical difficulties.

Another advantage of using a drug delivery system is that it can diminish the requirement for solubility since it is not necessary to keep the whole injected dose in solution at the same time. What is only required is to keep the released drug in solution in the subconjunctival space for absorption. To take case 1 as an example. For a zero-order release system, a release rate=10μg/hr gives a vitreous drug level about 8ng/mL (Figure 5.17) and the drug level in the subconjunctival space is about 1300ng/mL which is equivalent to 1.3μg (Figure 5.25). Therefore, only 1.3μg of drug is needed in solution. On the contrary, at least 20μg dose is needed in order to bring the vitreous drug level to >8ng/mL after simple injection (Figure 5.26). Assuming the injection volume is 50μL, the solubility of a compound has to be 0.4mg/mL or higher and it is very difficult to have such a high solubility for a poorly soluble compound.

F. First-order Release System

A first-order release system gives a vitreous level between that following a simple subconjunctival injection and injection of a zero-order release system (Figure 5.27). It gives a lower peak level than that after a simple subconjunctival injection. However, it is not as efficient as a zero-order release system in sustaining the vitreous drug level. However, analogous to a

simple subconjunctival injection, a first-order release system suffers from a major drawback that it gives a very short duration. Basically, the vitreous drug level falls into a very low level before the next dose. Therefore, the major challenge is to sustain the vitreous drug level.

Since both k_{13} (absorption rate constant) and k_{30} (elimination from the vitreous chamber) are drug-dependent, it is not easy to manipulate them by means of formulation. However, there exists two parameters which are easily manipulated by formulation, namely, dose and k_1 (first-order release rate constant). Increasing dose can maintain the vitreous level above a certain level for longer periods of time. However, the peak concentration is also increased and this is not desirable for drugs with a narrow therapeutic window (Figure 5.28). On the contrary, decreasing k_1 (first-order release rate constant) can be one of the strategies to lessen fluctuation and to prolong the vitreous drug level provided that the minimum effective concentration can be reached since reducing k_1 also lowers the peak level (Figure 5.29).

Analogous to a zero-order release system, a first order system has a definite advantage over simple injection in terms of solubility requirement. In order to have both peak and trough of the vitreous drug level to be higher than 8ng/mL, a dose of 4mg is required (Figure 5.30) and this dose gives a peak drug level in the subconjunctival space roughly equal to 6μg (Figure 5.31). It is obvious that it is easier to keep the 6μg of drug in solution than prepare a dosing solution to be >0.4mg/mL as used for simple injection (Figure 5.26).

V. CONCLUSIONS

After our computer simulation study, the following conclusions can be drawn:

1. Existence of flip-flop phenomenon
    (a) leads to a very early peak time
    (b) gives a similar peak times for chemically-diversified compounds
    (c) the vitreous drug level is not only governed by k_0 and k_{30}, but k_{13} also plays a significant role.
2. Simple subconjunctival injection is not practical since frequent injection is required to keep the vitreous drug level above the therapeutic concentration for an extended period of time.
3. A drug delivery system can diminish the requirement for solubility but it is still desirable to keep a drug in solution form so that a higher dose can be administered.
4. Zero-order release
    (a) gives a steady vitreous drug level
    (b) if the release rate is 10μg/hr or higher, it is likely to bring the vitreous drug level to the 1ng/mL level. However, validation of this boundary condition is not possible at this moment
    (c) subconjunctival injection may not be a practical way to deliver a compound to the back of the eye if the therapeutic concentration of a compound is >1μg/mL, unless k_{10} can be reduced by 100x by using a drug delivery system.
5. First-order release
    (a) increasing the dose can increase the peak level
    (b) decreasing k_1 can prolong the vitreous drug level.

Table 5.1: Summary of the values of the rate constants used in computer simulation

Rate Constant	Description	Value
k_{10}	Elimination of a drug from the subconjunctival space after subconjunctival injection	$1.28 \times 10^{-1} min^{-1}$
k_{13}	Absorption of the drug into the vitreous chamber from the subconjunctival space	$8.51 \times 10^{-5} min^{-1}$
k_{30}	Elimination of a drug from the vitreous chamber after subconjunctival injection	$1.14 \times 10^{-2} min^{-1}$
k_{34}	Movement of a drug from the vitreous chamber to a reservoir compartment	$2.72 \times 10^{-3} min^{-1}$
k_{43}	Movement of a drug from the vitreous chamber to a reservoir compartment	$3.32 \times 10^{-4} min^{-1}$

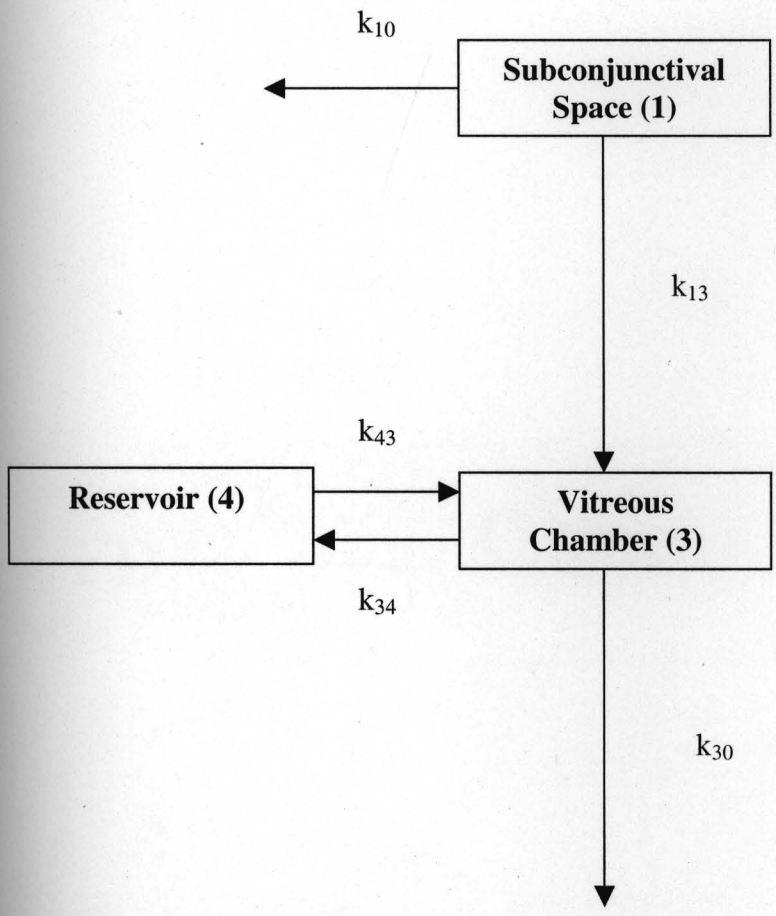

Figure 5.1: The pharmacokinetic model for subconjunctival injection

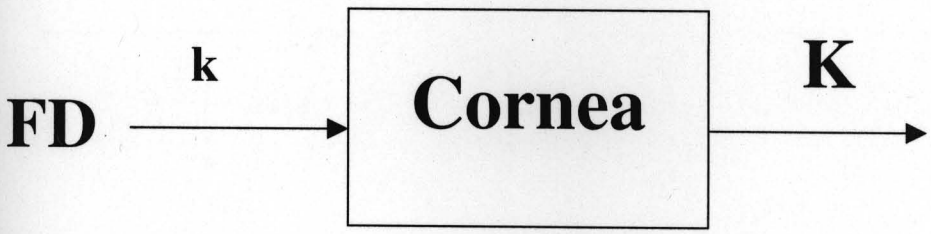

Figure 5.2: One-compartment model for topical instillation

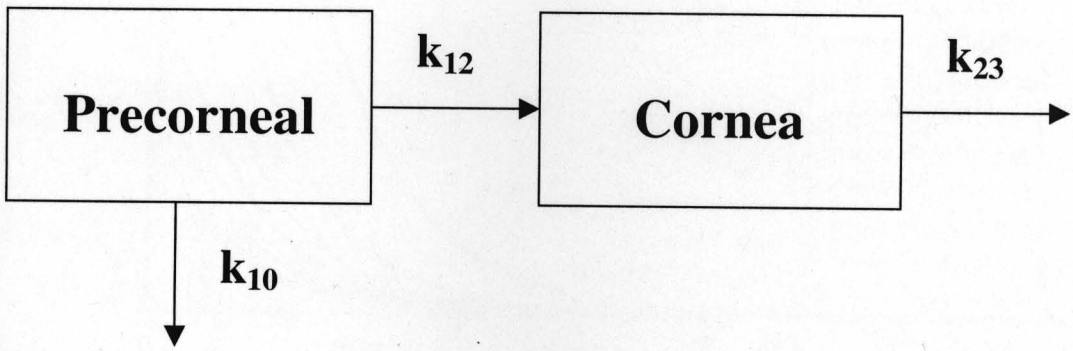

Figure 5.3: A two-compartment model with incorporation of a parallel loss pathway for topical instillation

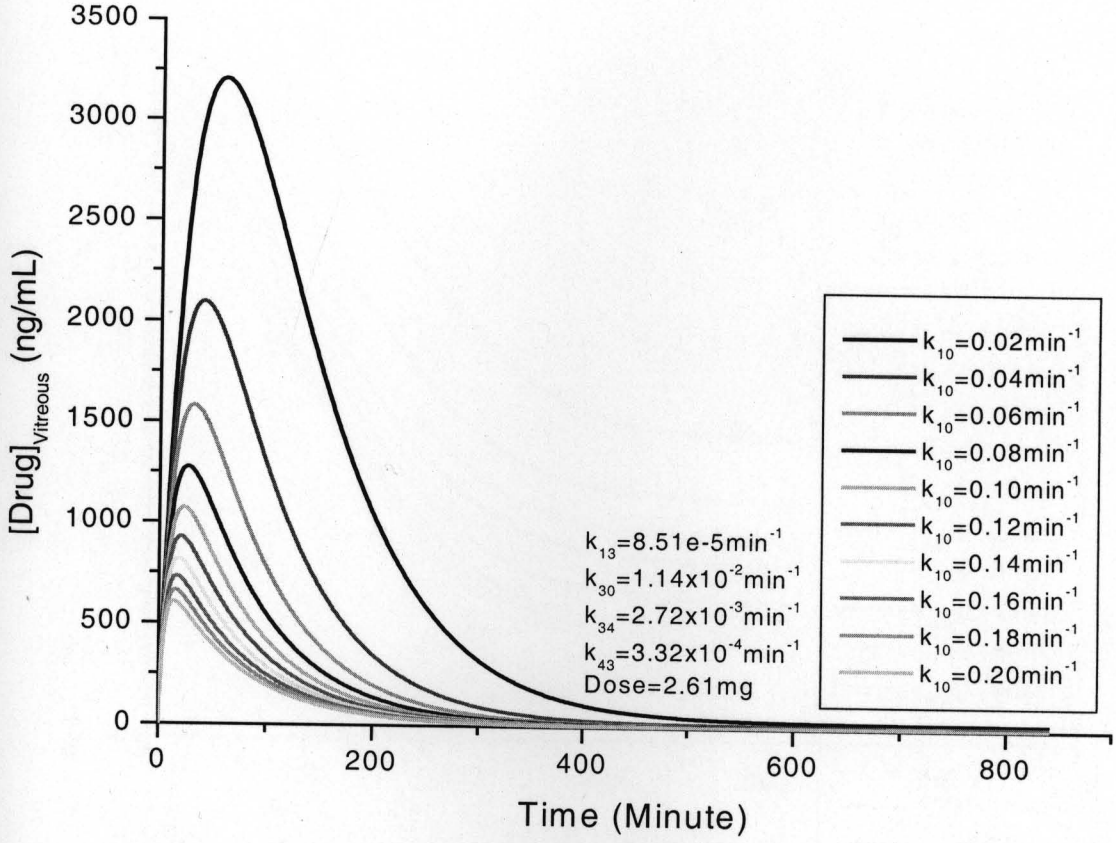

Figure 5.4a: Effect of k_{10} on the vitreous drug level after simple subconjunctival injection

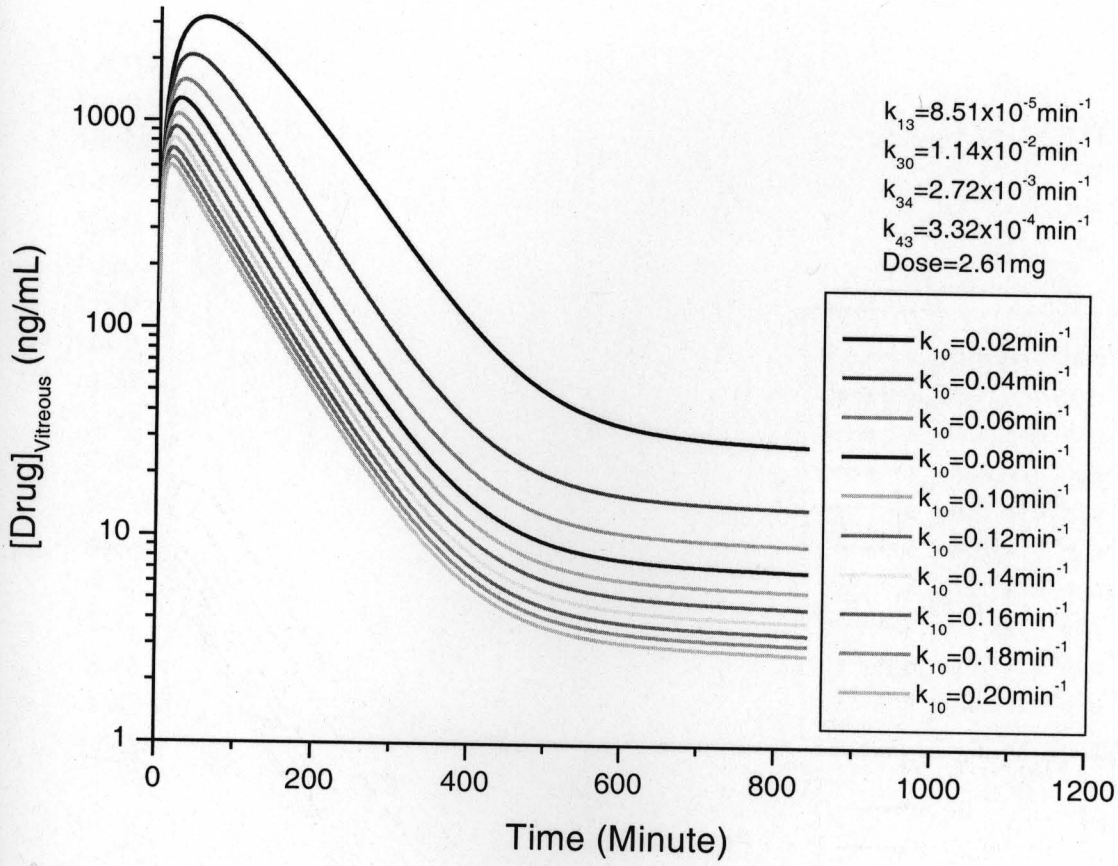

Figure 5.4b: Effect of k_{10} on the vitreous drug level after simple subconjunctival injection (Log scale)

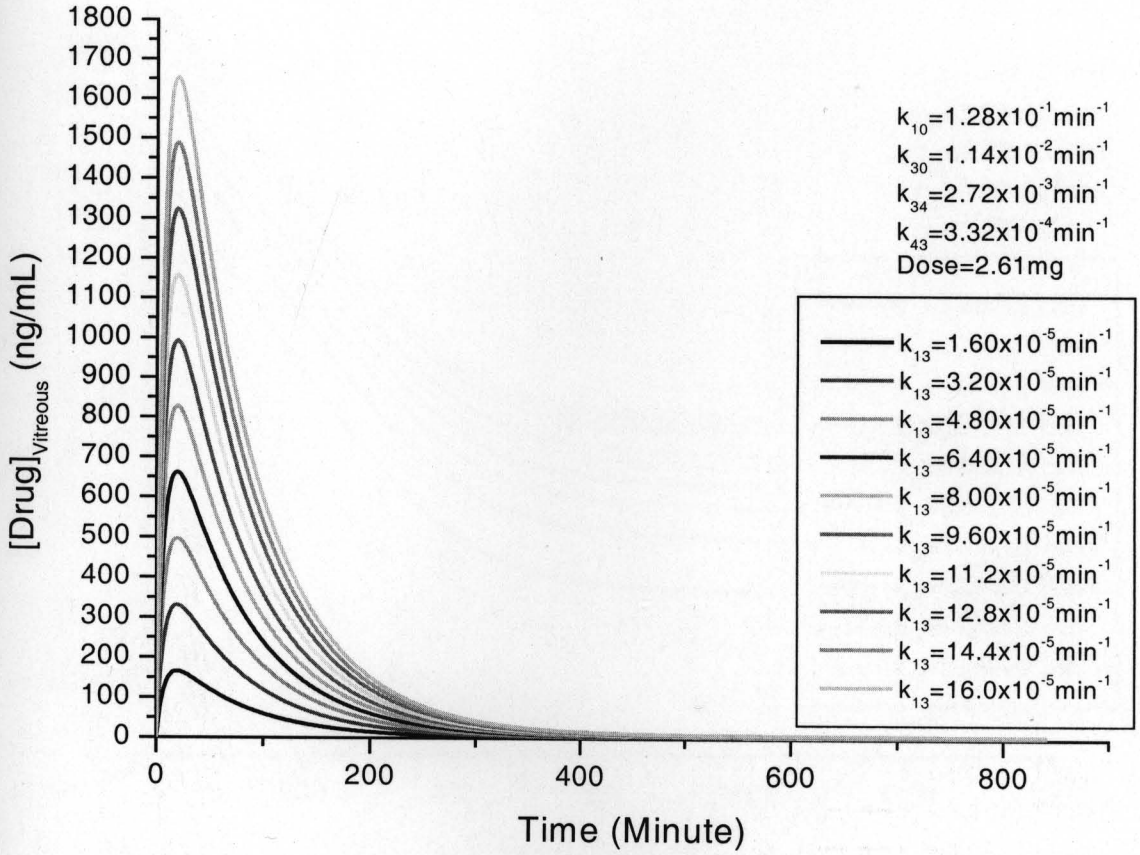

Figure 5.5a: Effect of k_{13} on the vitreous drug level after simple subconjunctival injection

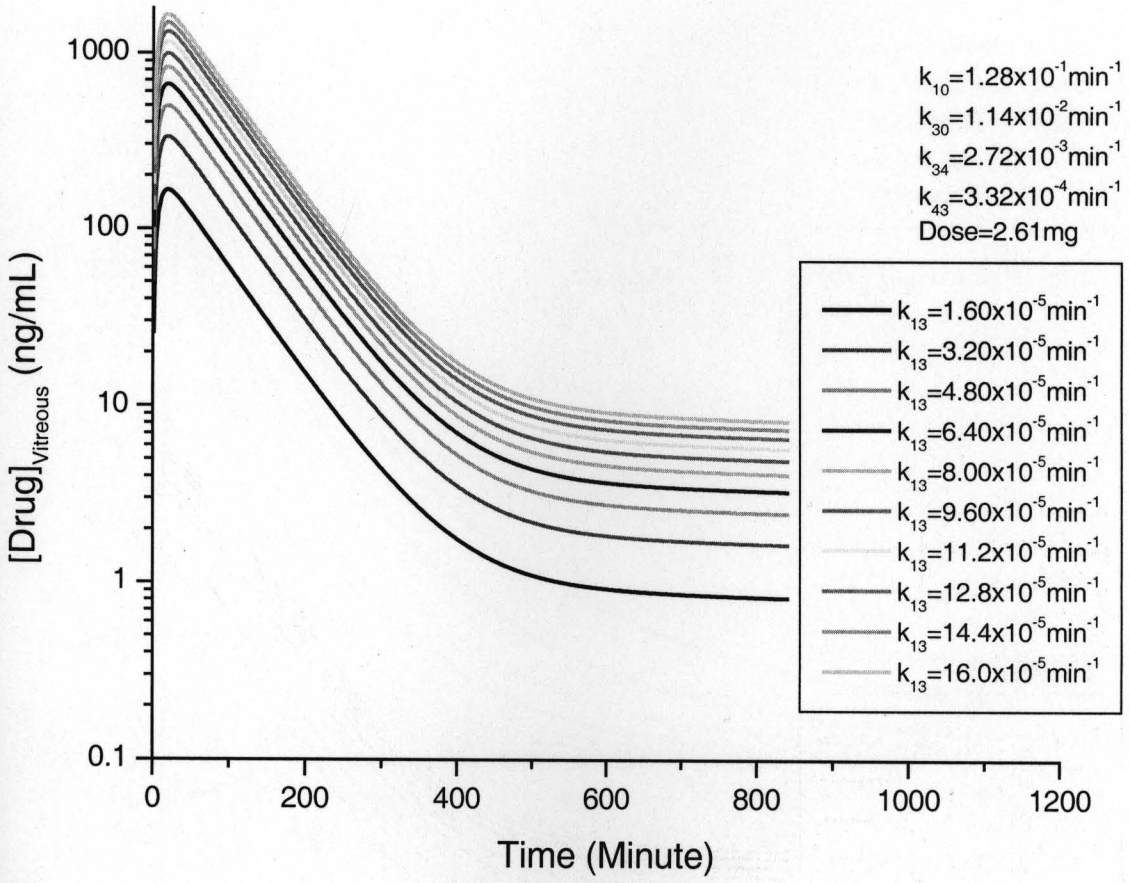

Figure 5.5b: Effect of k_{13} on the vitreous drug level after simple subconjunctival injection (Log scale)

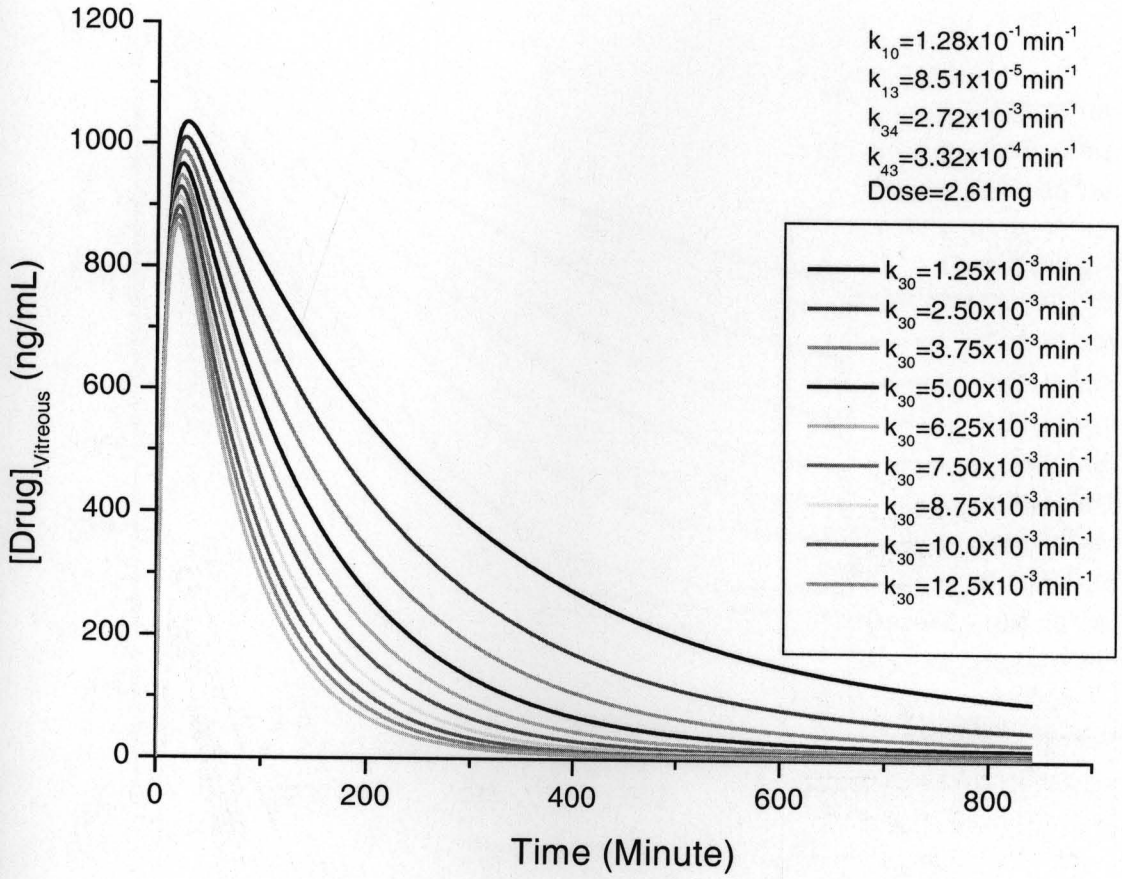

Figure 5.6a: Effect of k_{30} on the vitreous drug level after simple subconjunctival injection

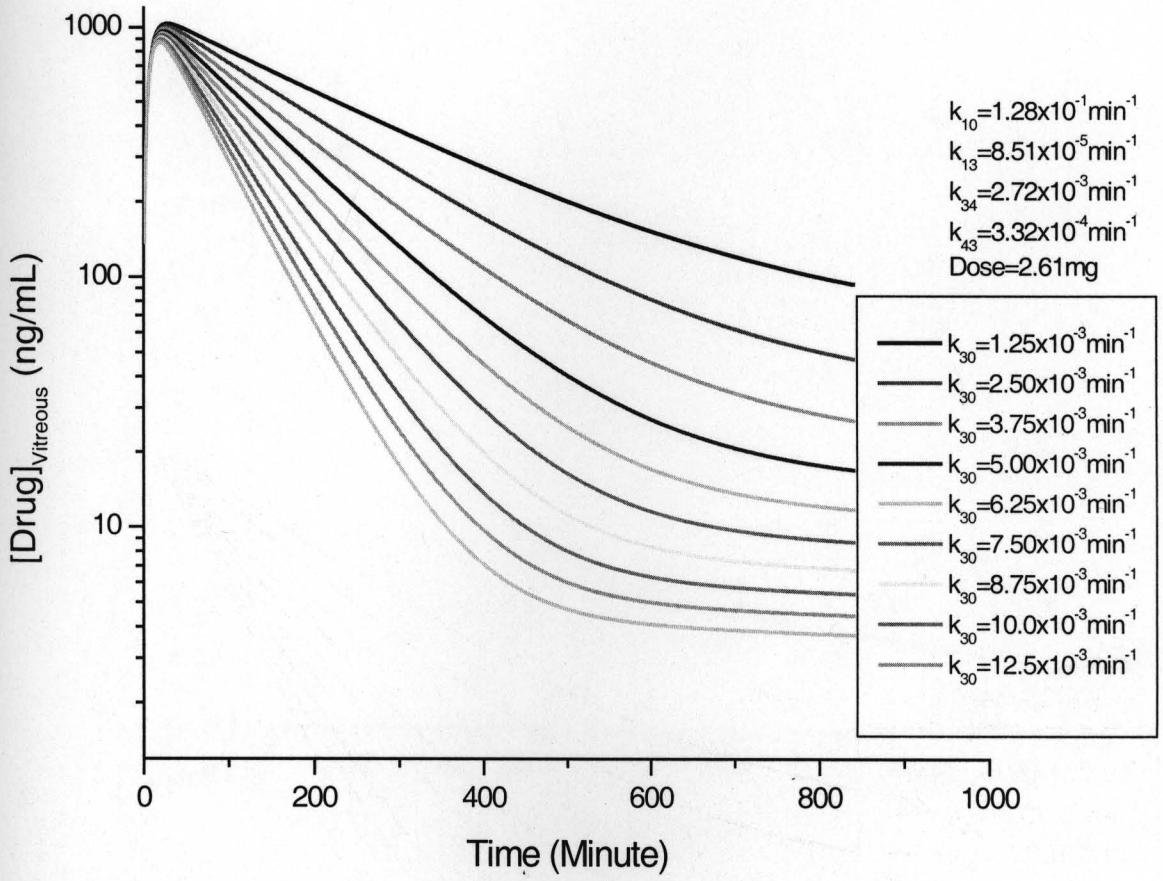

Figure 5.6b: Effect of k_{30} on the vitreous drug level after simple subconjunctival injection

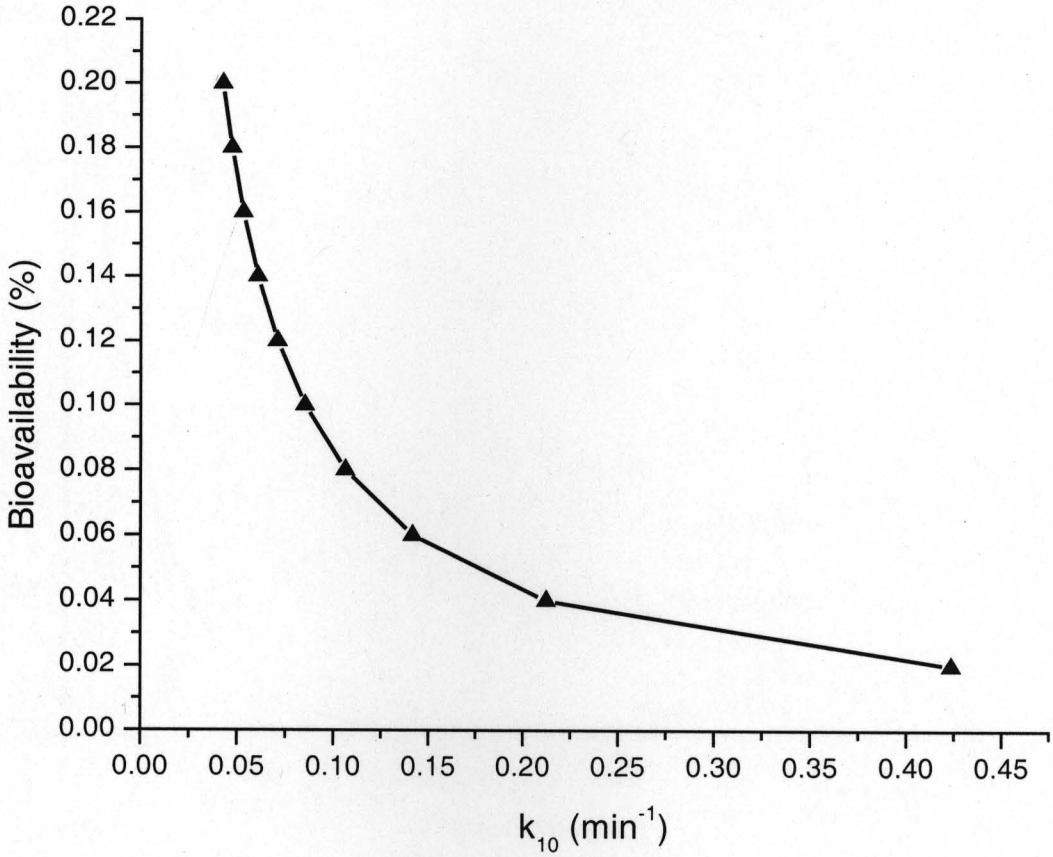

Figure 5.7: Effect of k_{30} on the bioavailability

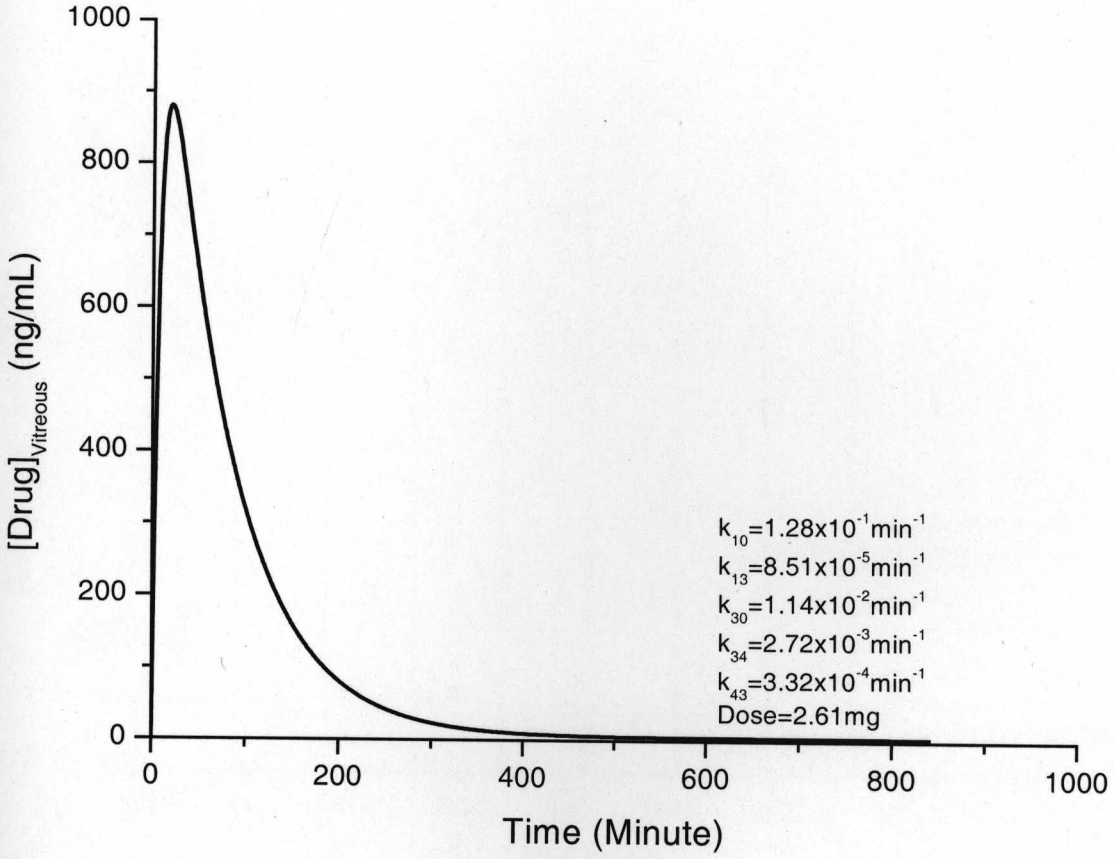

Figure 5.8: Time course of vitreous drug level after a single subconjunctival injection

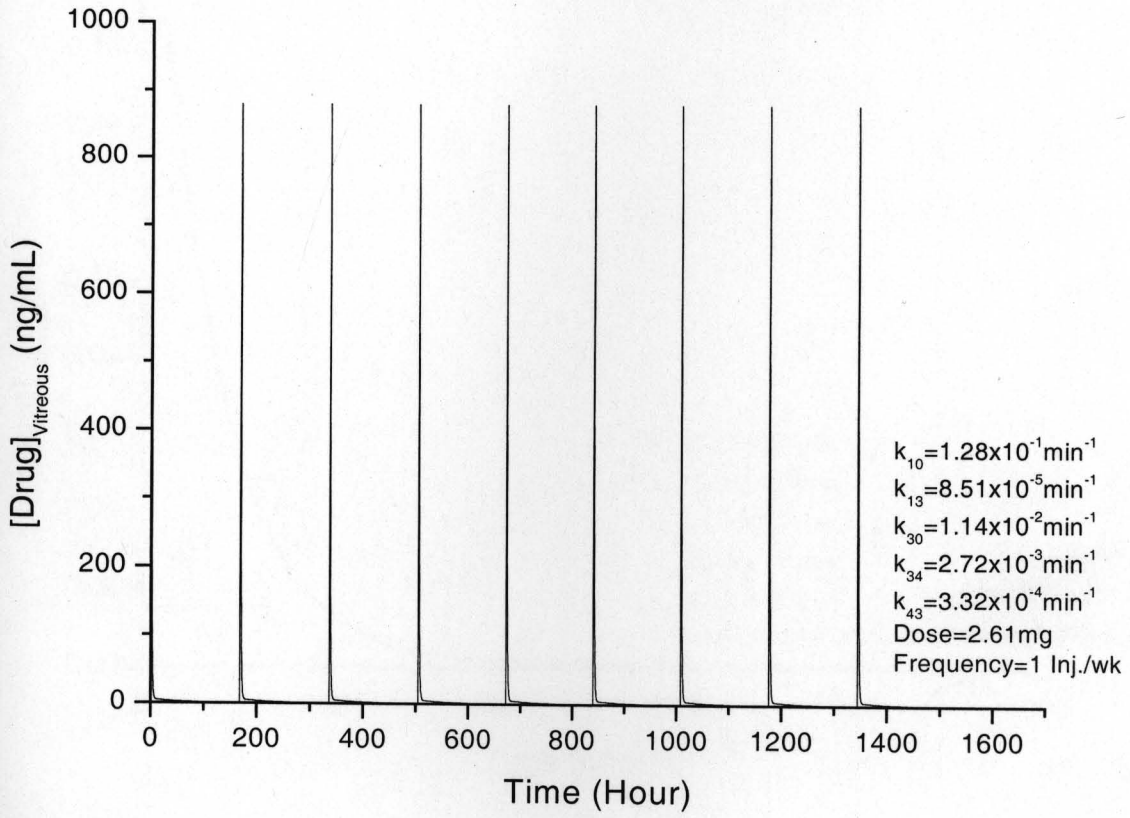

Figure 5.9: Time course of vitreous drug level after multiple subconjunctival injections

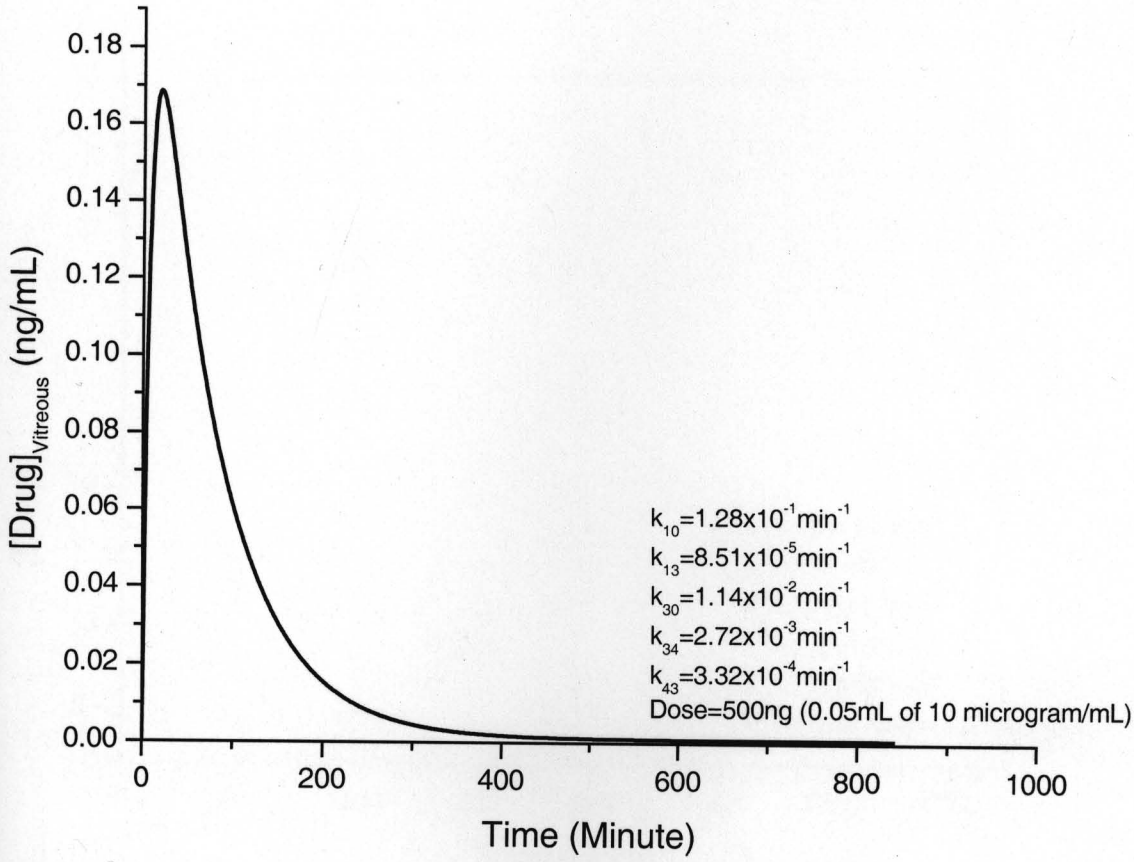

Figure 5.10: Time course of vitreous drug level after subconjunctival injection of a poorly soluble compound

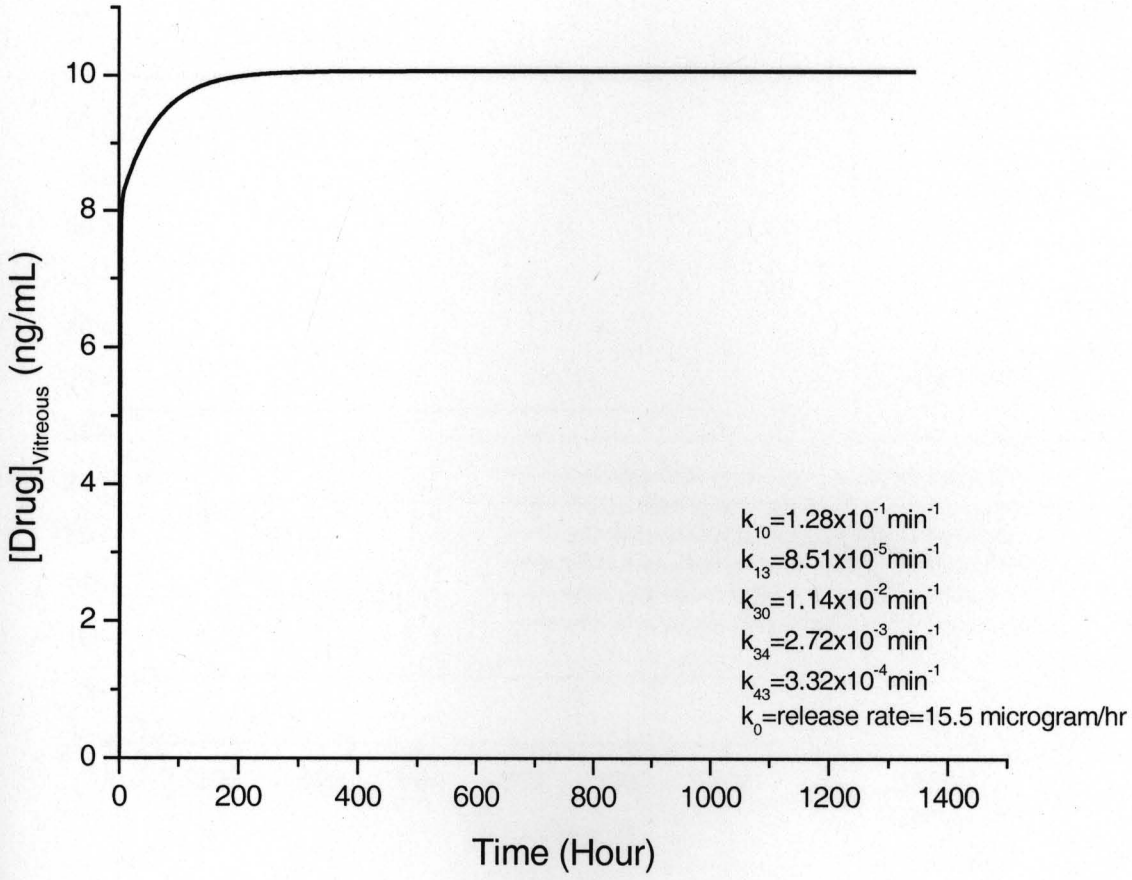

Figure 5.11: Time course of vitreous drug level after subconjunctival injection of a zero-order release system (assumed unlimited supply of drug from the system)

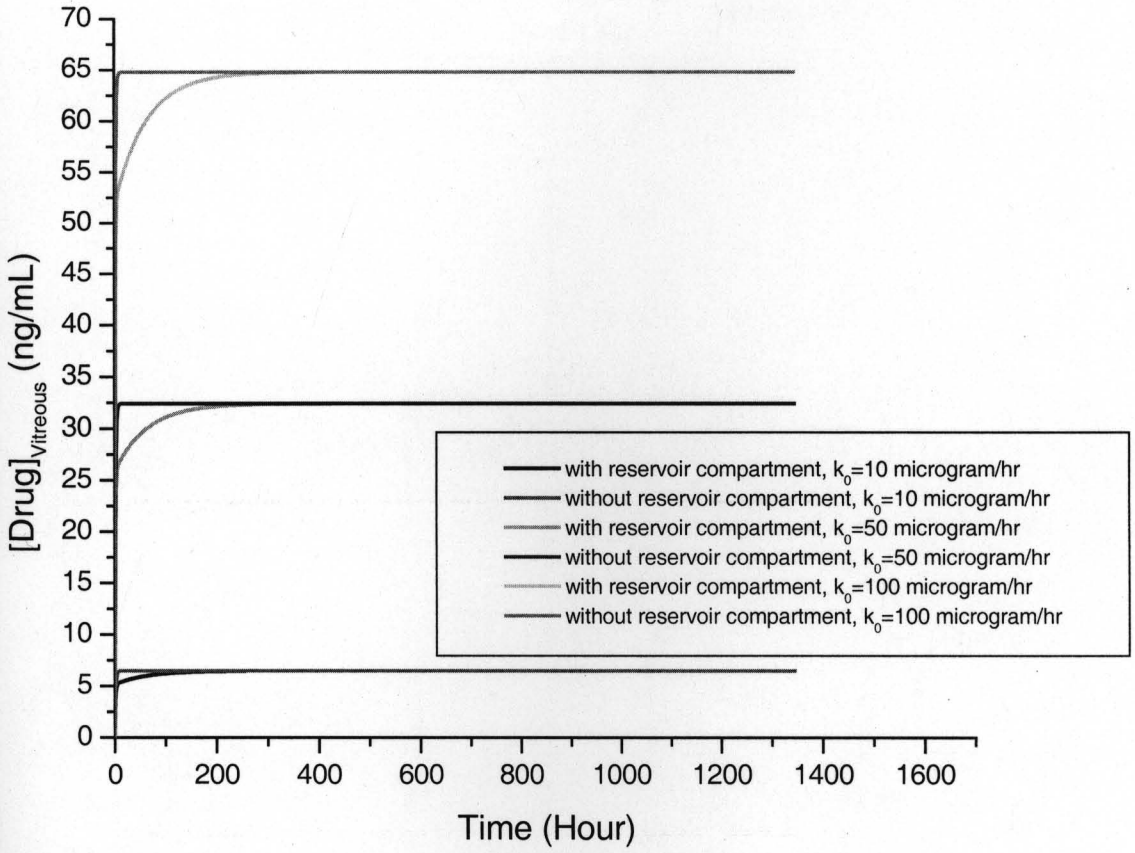

Figure 5.12: Lack of effect of reservoir compartment on the steady state vitreous drug level

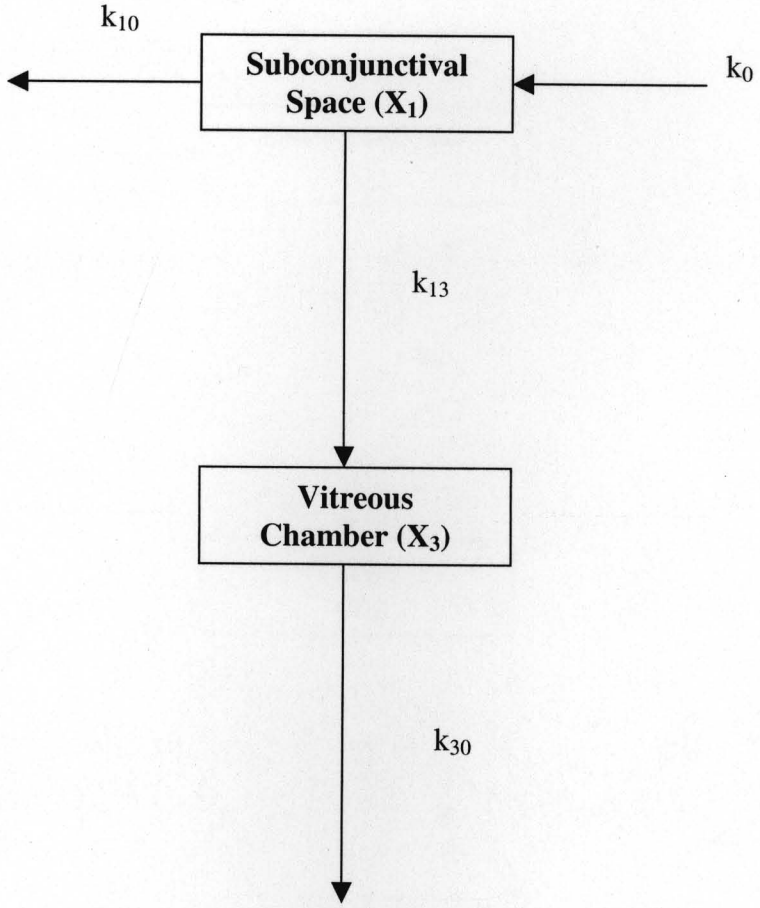

Figure 5.13: The simplified pharmacokinetic model for subconjunctival injection for evaluation of a zero-order release system

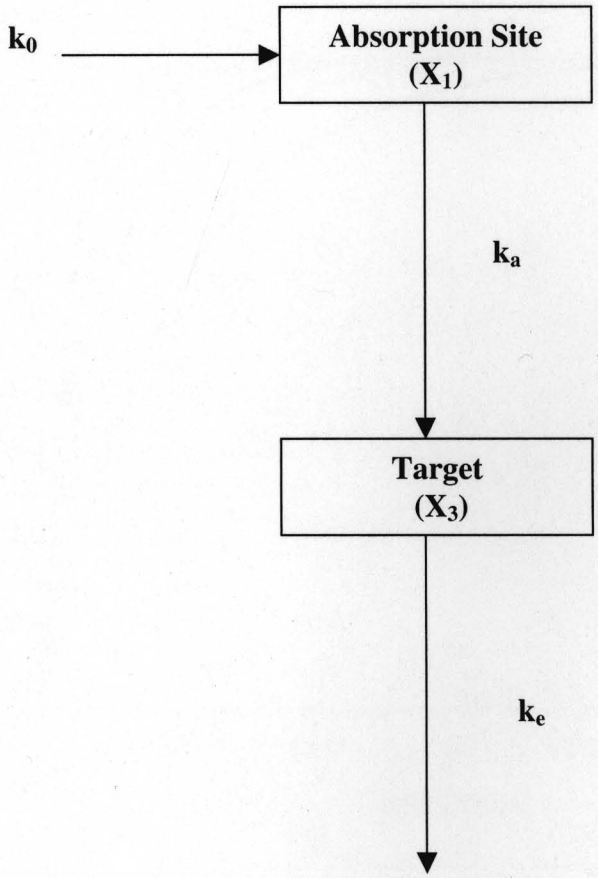

Figure 5.14: A well behaved pharmacokinetic model

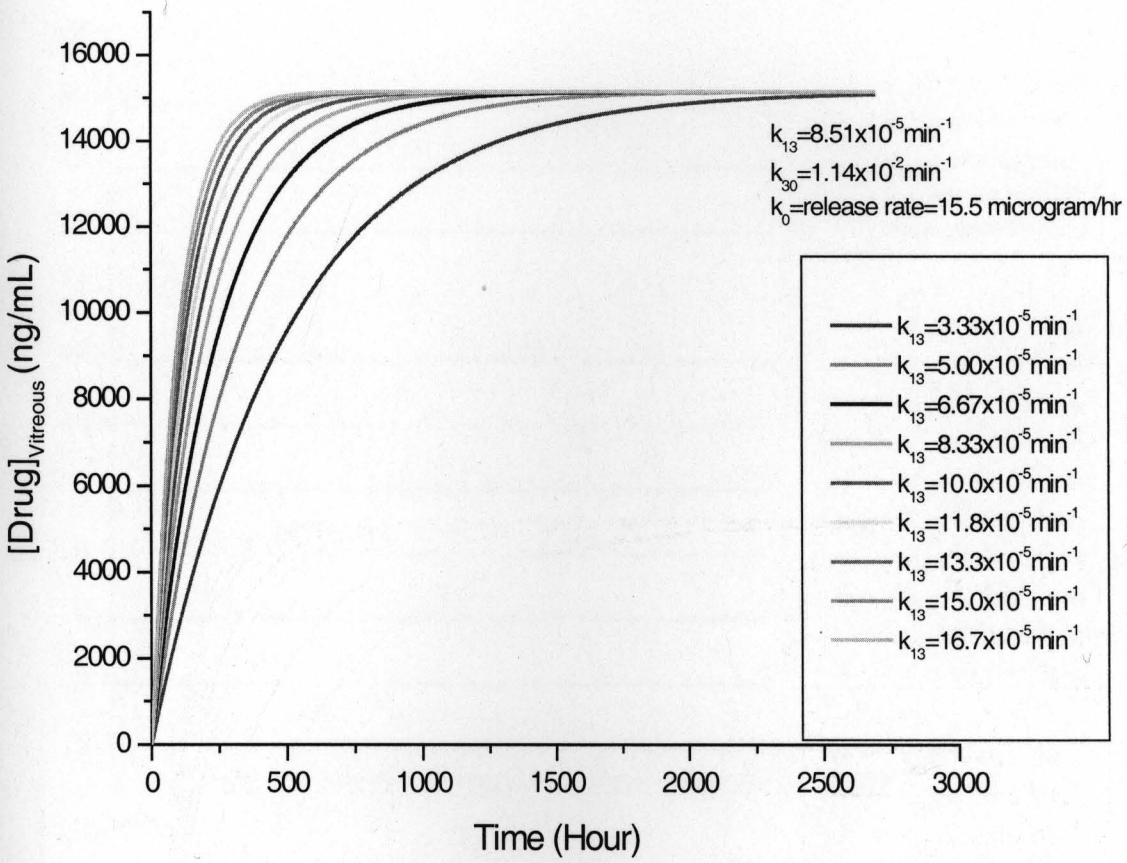

Figure 5.15: Insensitivity of the steady state vitreous drug level to the change in k_{13} for a well behaved model

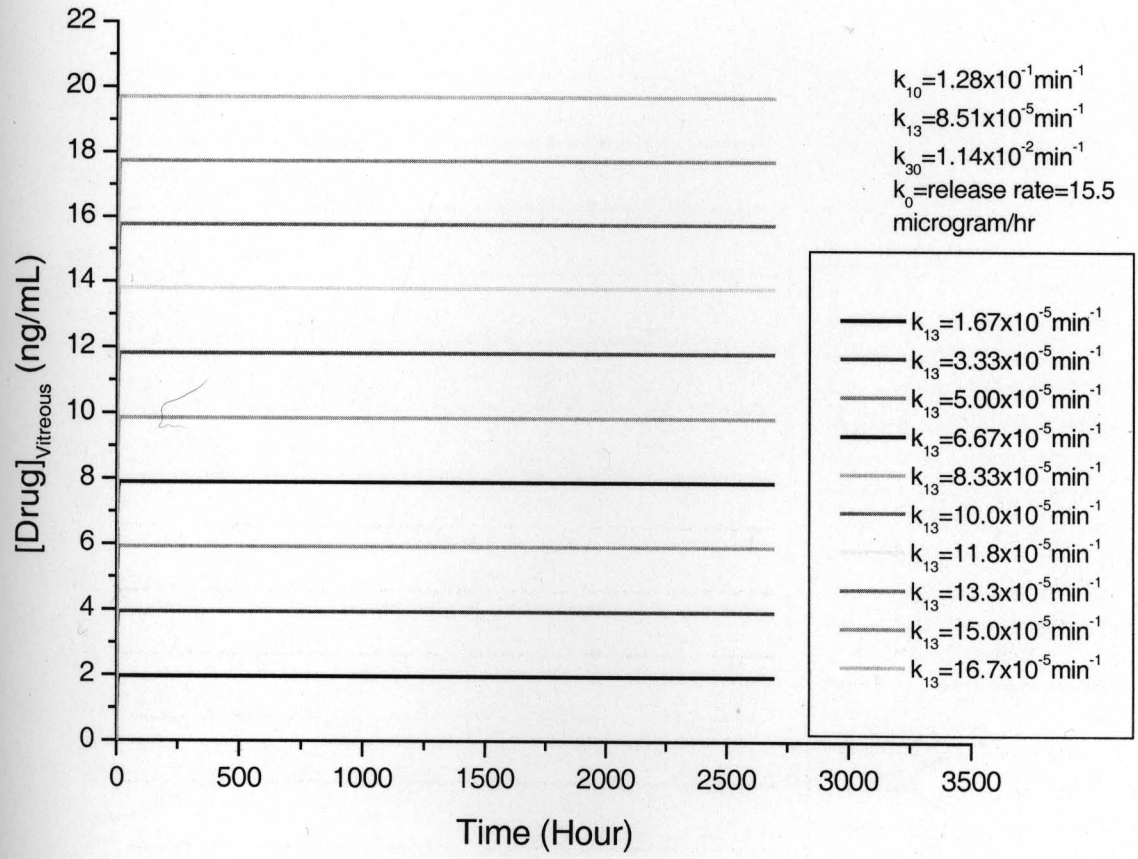

Figure 5.16: Strong dependence of the steady state vitreous drug level to the change in k_{13} for a model with a parallel loss pathway

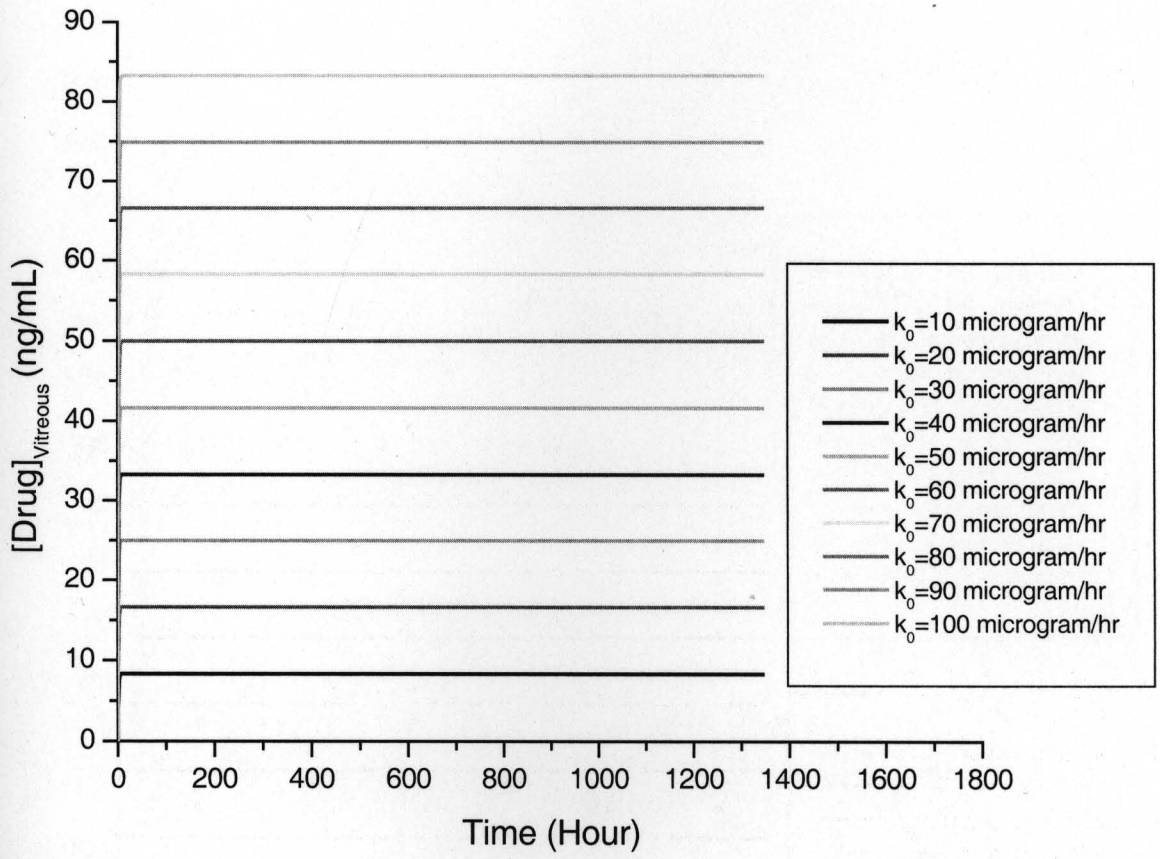

Figure 5.17: Effect of release rate on the vitreous drug level for case 1: Good penetration and fast elimination

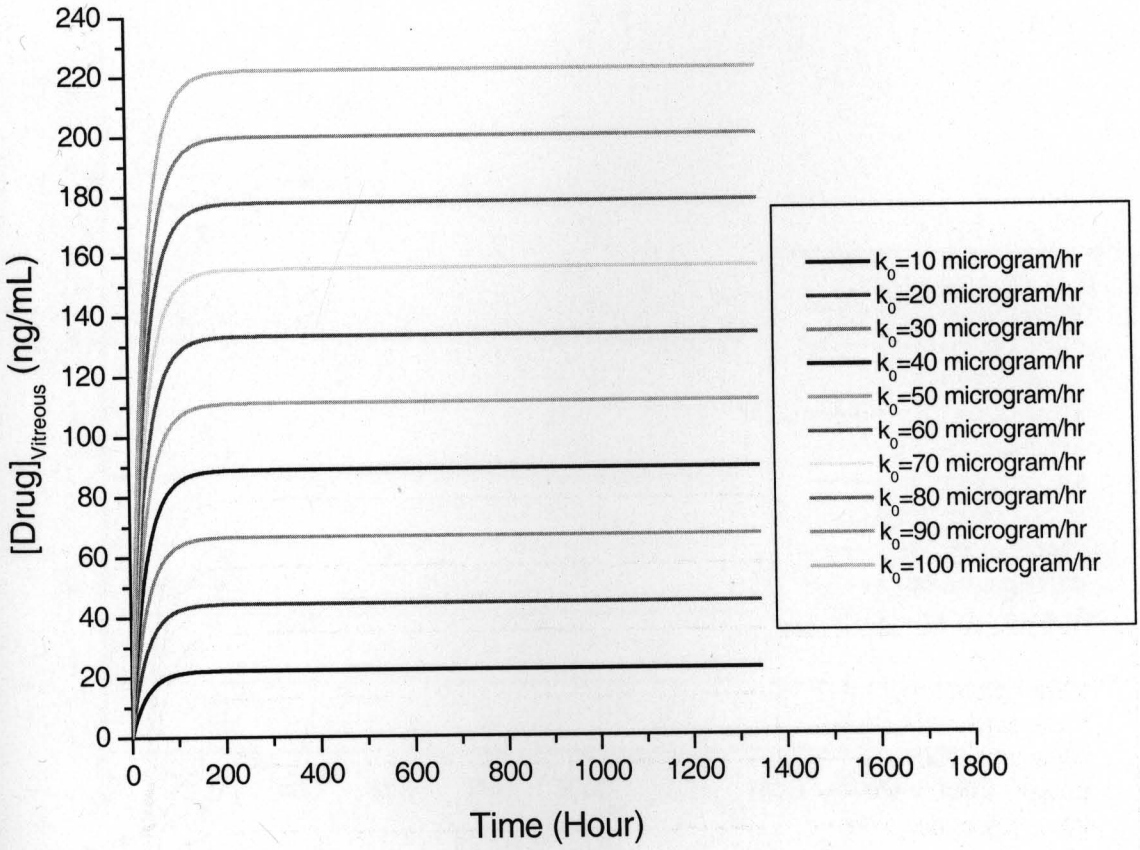

Figure 5.18: Effect of release rate on the vitreous drug level for case 2: Poor penetration and slow elimination

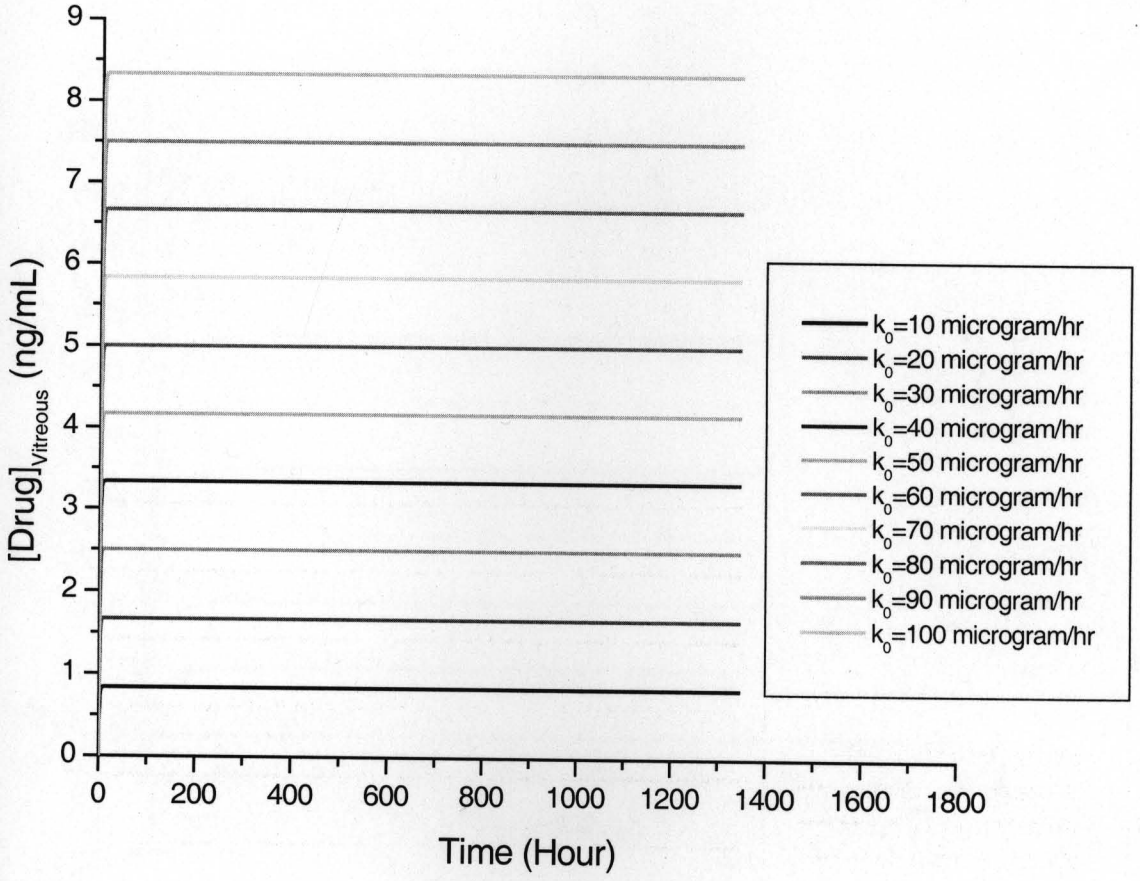

Figure 5.19: Effect of release rate on the vitreous drug level for case 3: Poor penetration and fast elimination

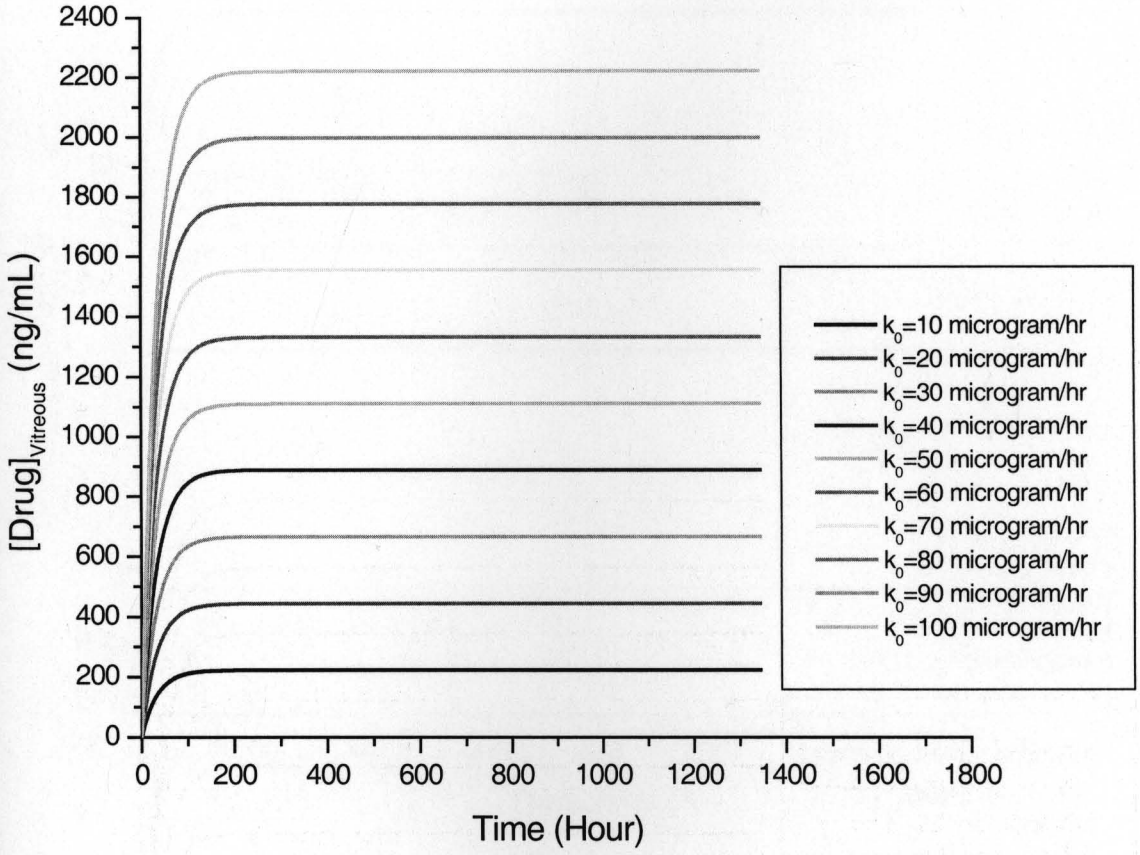

Figure 5.20: Effect of release rate on the vitreous drug level for case 4: Good penetration and slow elimination

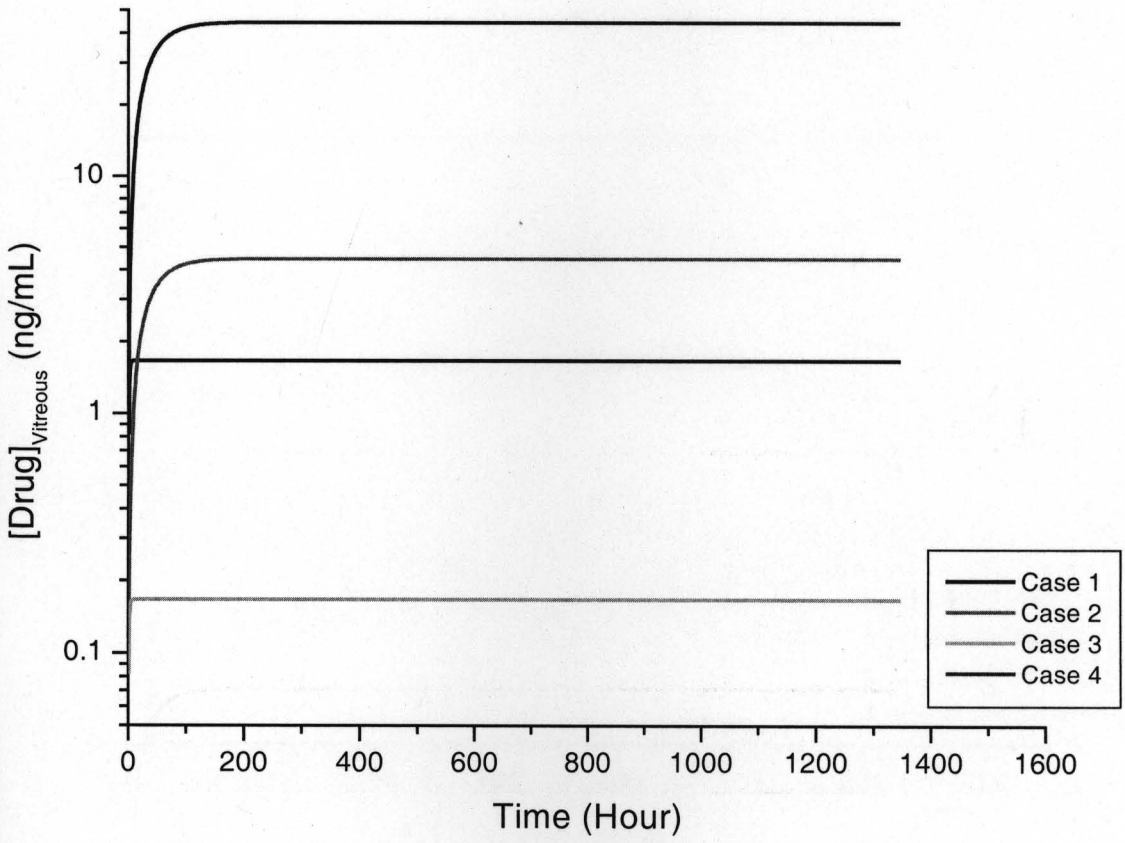

Figure 5.21: Comparison of four cases by setting k_0=2μg/hr

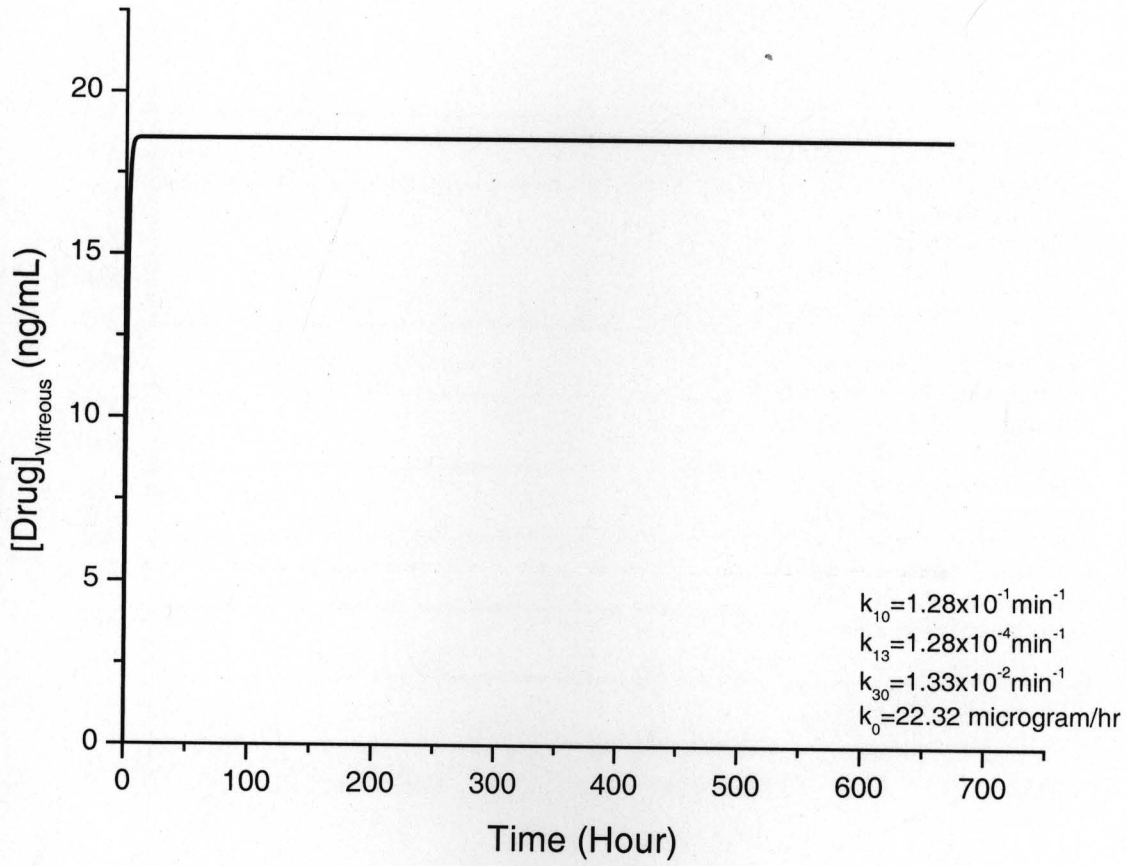

Figure 5.22: Time course of vitreous drug level for Case 1: Good penetration and fast elimination by setting k_0=22.32μg/hr

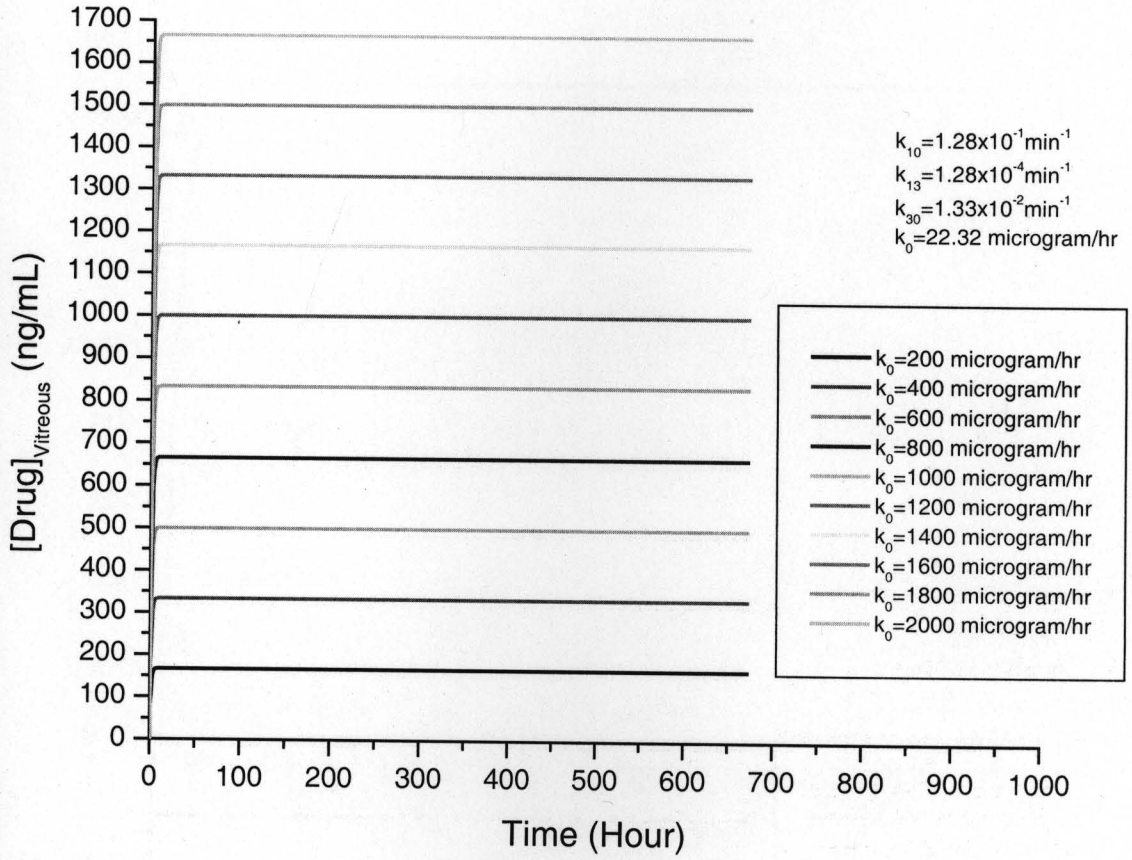

Figure 5.23: Determination of k_0 in order to reach >1μg/mL for Case 1

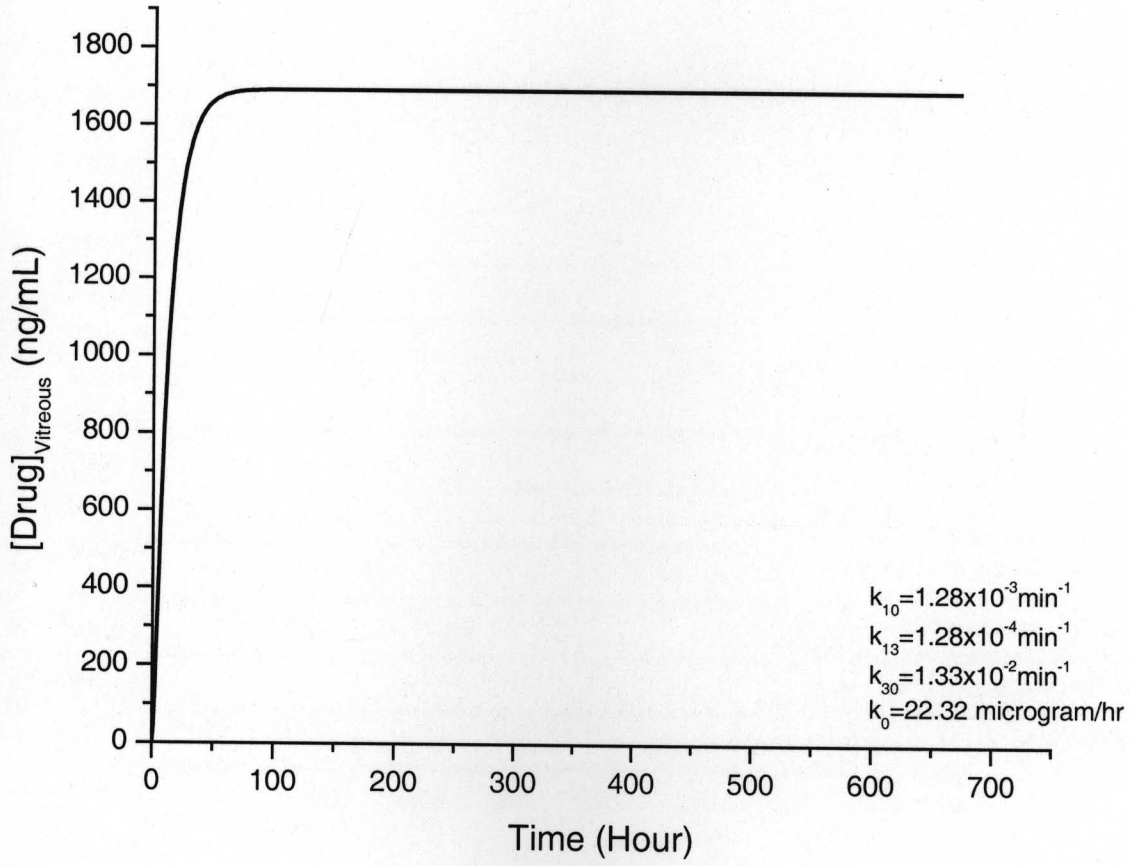

Figure 5.24: Time course of vitreous drug level for Case 1 with k_{10} reduced by 100x

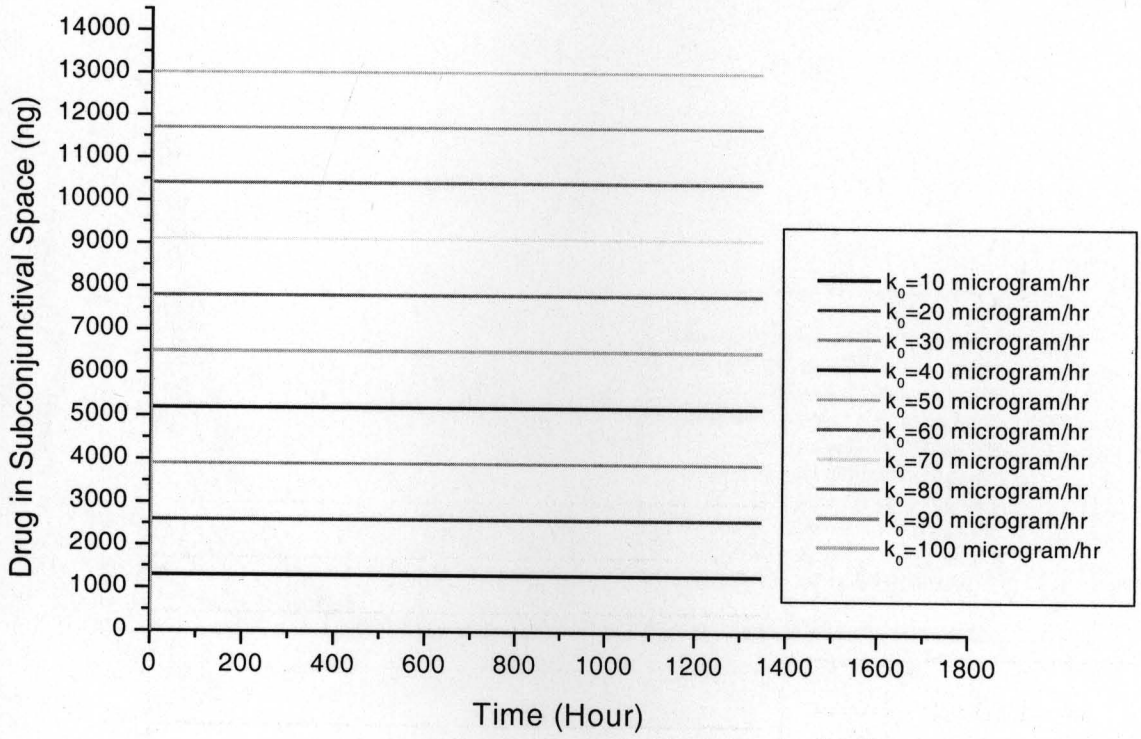

Figure 5.25: Effect of release rate on the drug level in the subconjunctival space for Case 1: Good penetration and fast elimination

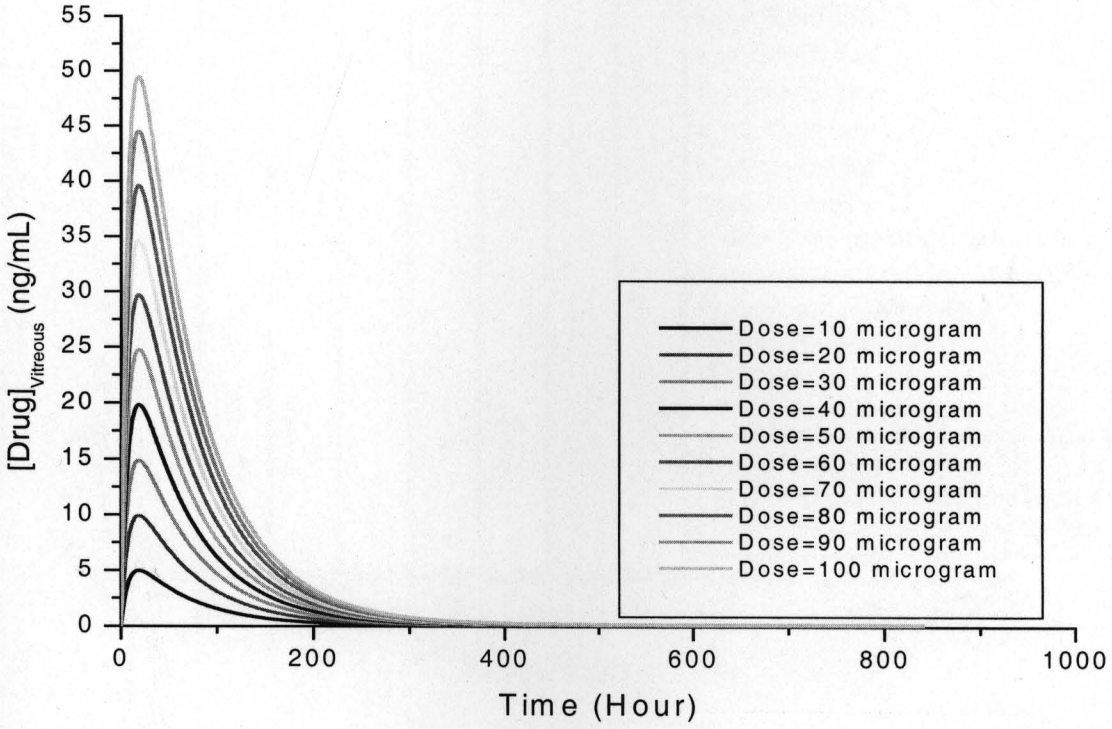

Figure 5.26: Effect of changing dose on the vitreous drug level after simple injection for case 1: Good penetration and fast elimination

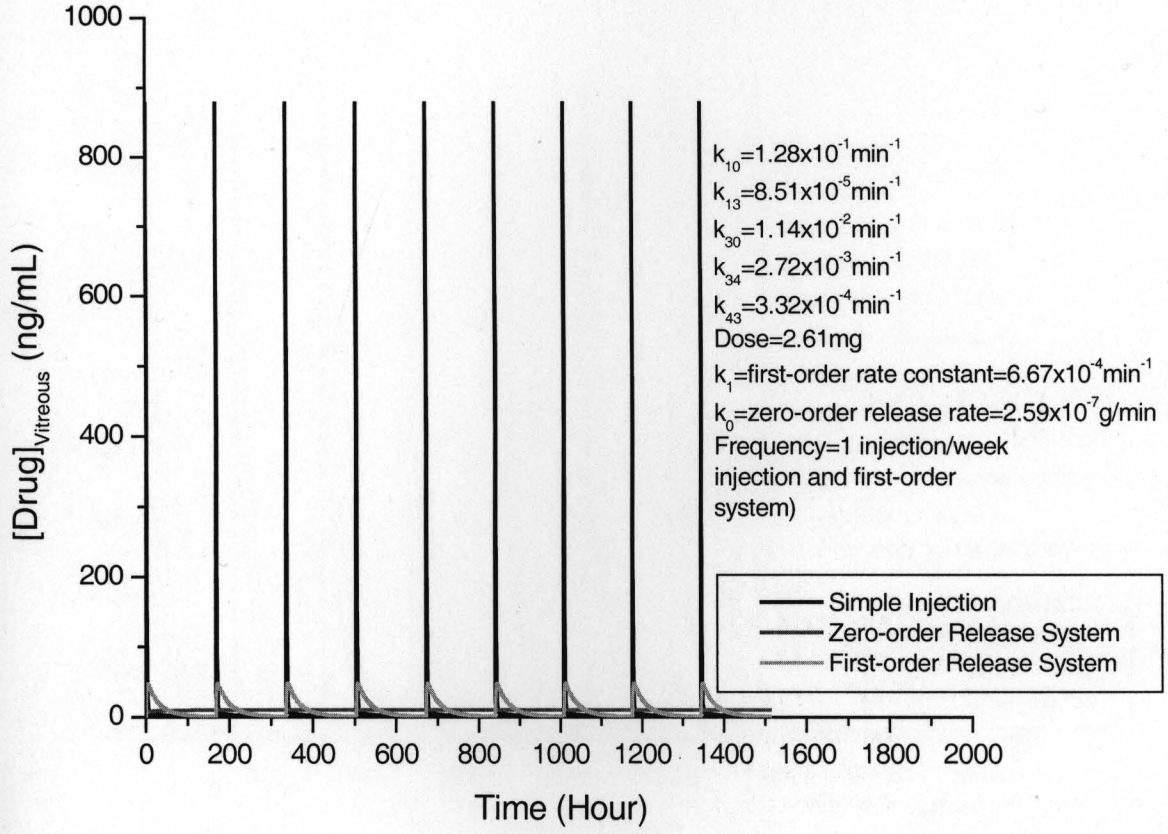

Figure 5.27: Comparison of vitreous drug level after three different methods of administration

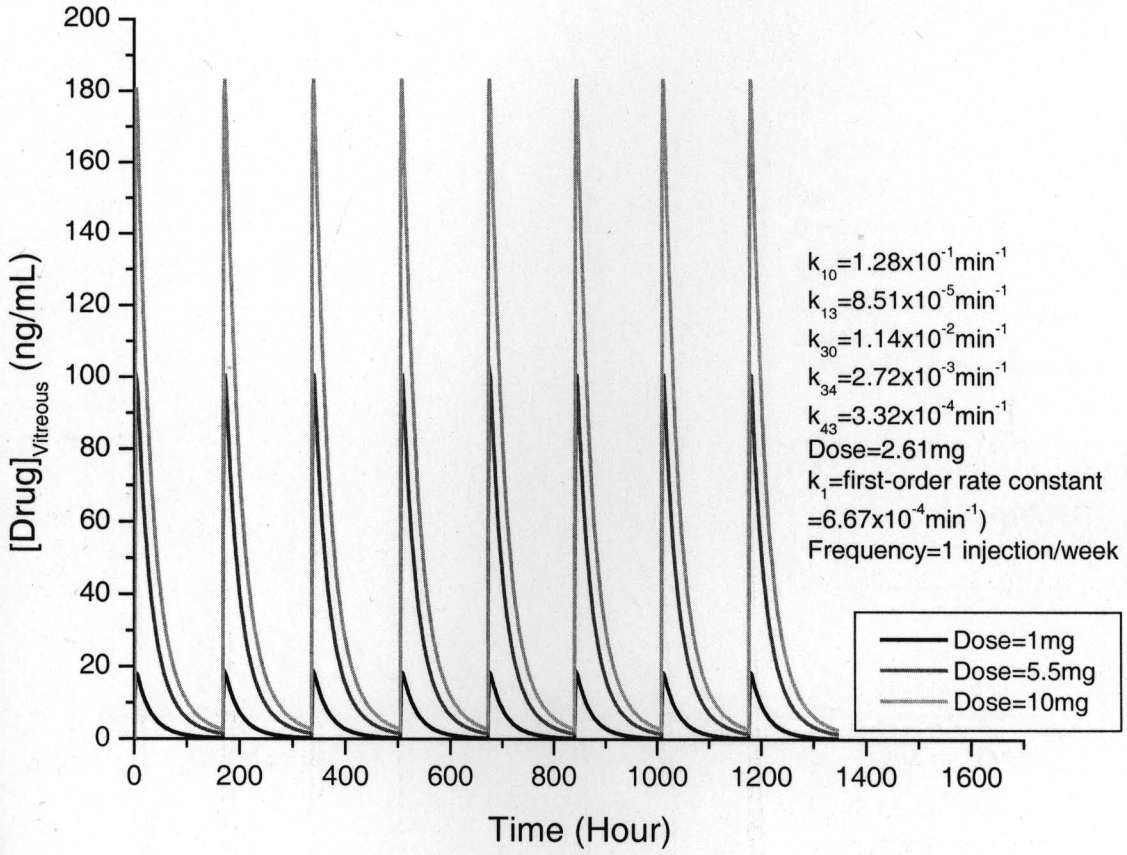

Figure 5.28: Effect of changing dose on the vitreous drug level after subconjunctival injection of a first-order release system

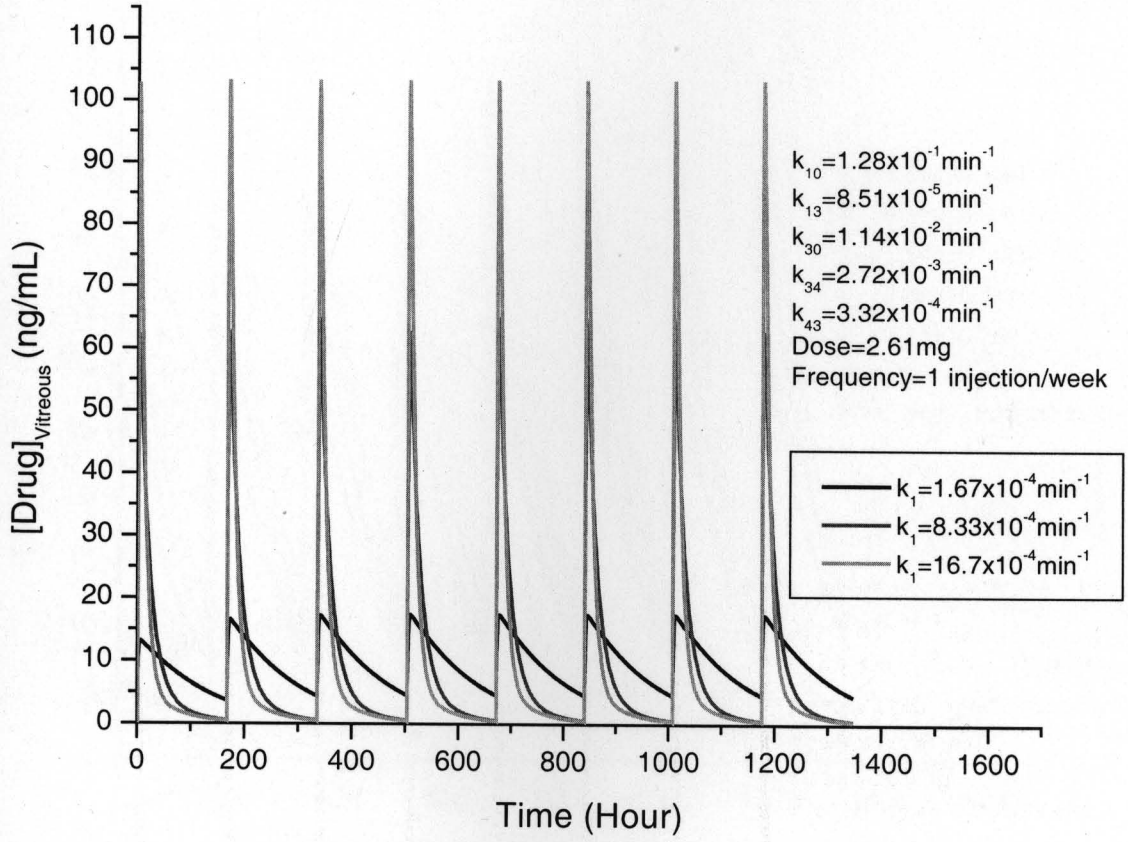

Figure 5.29: Effect of k_1 (first-order release rate constant) on the vitreous drug level after subconjunctival injection of a first-order release system

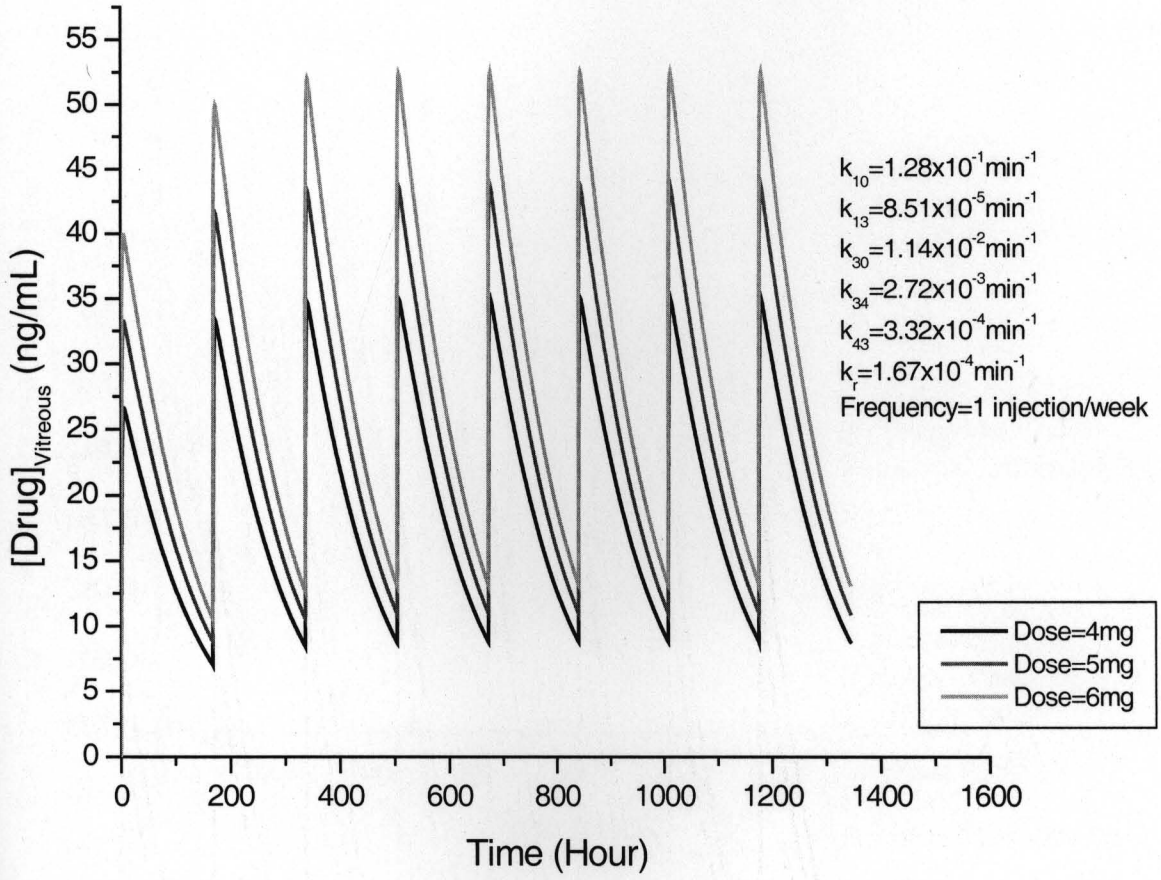

Figure 5.30: Effect of changing dose on the vitreous drug level after injection of a first-order release system for case 1: Good penetration and fast elimination

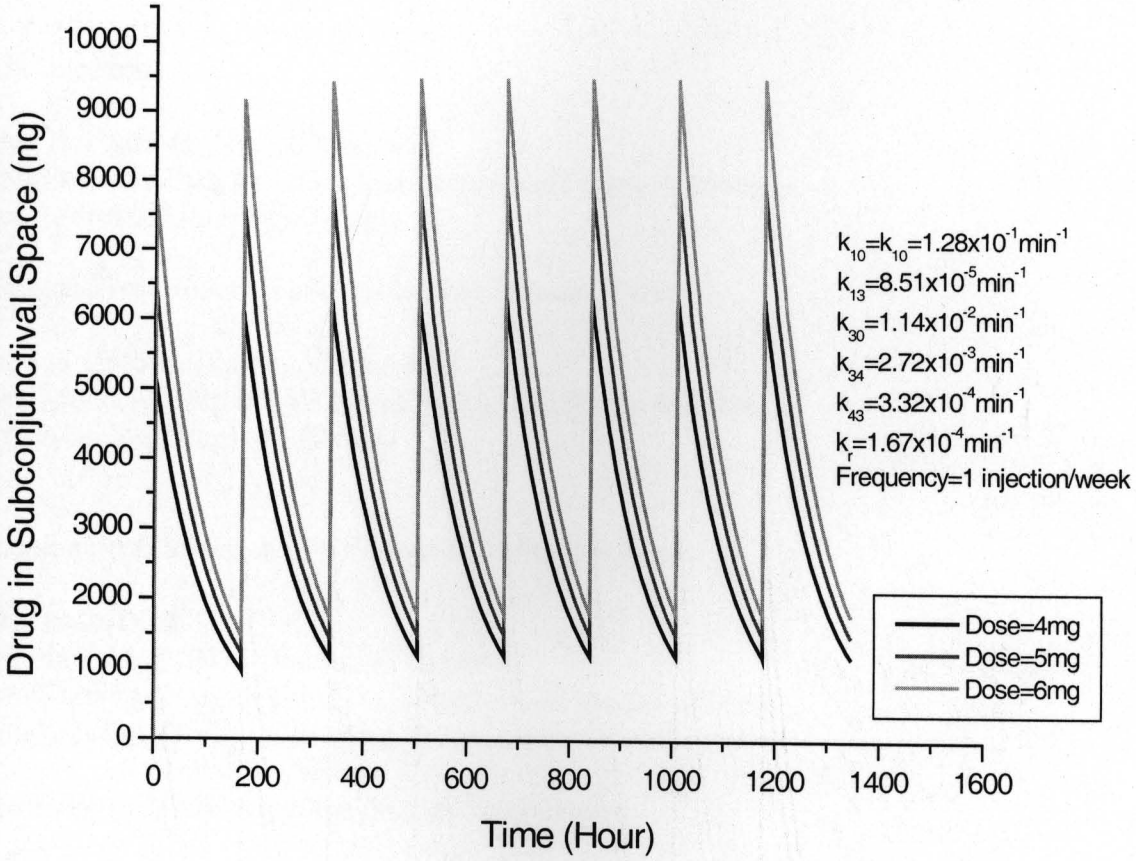

Figure 5.31: Effect of changing dose on the drug level in the subconjunctival space after injection of a first-order release system for case 1: Good penetration and fast elimination

Appendix 5.1: Differential Equations describes the model in Figure 5.13

Single Injection:

$d(\text{Drug in 1})/dt=-(k_{10}+k_{13})(\text{Drug in 1})$
$d[\text{Drug}]_3/dt=k_{13}(\text{Drug in 1})/1.5-k_{30}[\text{Drug}]_3+k_{43}[\text{Drug}]_4-k_{34}[\text{Drug}]_3$
$d[\text{Drug}]_4/dt=k_{34}[\text{Drug}]_3-k_{43}[\text{Drug}]_4$

Subconjunctival Injection of a Zero-order Release System:

$d(\text{Drug in 1})/dt=k_o-(k_{10}+k_{13})(\text{Drug in 1})$
$d[\text{Drug}]_3/dt=k_{13}(\text{Drug in 1})/1.5-k_{30}[\text{Drug}]_3+k_{43}[\text{Drug}]_4-k_{34}[\text{Drug}]_3$
$d[\text{Drug}]_4/dt=k_{34}[\text{Drug}]_3-k_{43}[\text{Drug}]_4$

Subconjunctival Injection of a First-order Release System:

$dDDS/dt=-k_1DDS$
$d(\text{Drug in 1})/dt=k_1DDS-(k_{10}+k_{13})(\text{Drug in 1})$
$d[\text{Drug}]_3/dt=k_{13}(\text{Drug in 1})/1.5-k_{30}[\text{Drug}]_3+k_{43}[\text{Drug}]_4-k_{34}[\text{Drug}]_3$
$d[\text{Drug}]_4/dt=k_{34}[\text{Drug}]_3-k_{43}[\text{Drug}]_4$

DDS=the amount of drug in the drug delivery system

Appendix 5.2

Differential equations describe the pharmacokinetic model for Figure 4.2 after subconjunctival injection of a zero-order release system are as follows:

$dX_1/dt=k_0-(k_{10}+k_{13})X_1$ (1)

$d[X_3]=k_{13}X_1/1.5-k_{30}[X_3]$ (2)

where k_0 is the zero-order release rate. Consider Equation (1), Laplace-Transforming Equation (1) will give

$sY-Y(0)= k_0/s-(k_{10}+k_{13})Y$ (3)

Since $X_1(0)=0$,

$sY= k_0/s-(k_{10}+k_{13})Y$ (4)

$(s+ k_{10}+k_{13})Y=k_0/s$ (5)

$Y=(k_0/s)[1/(s+ k_{10}+k_{13})]$ (6)

Let $(1/s)[1/(s+ k_{10}+k_{13})]=[A/s+B/(s+ k_{10}+k_{13})]$ (7)

$As+Ak_{10}+Ak_{13}+Bs=1$ (8)

$(A+B)s+A(k_{10}+ k_{13})=1$ (9)

$A=1/(k_{10}+ k_{13})$ (10)

Since A=-B,

$B=-1/(k_{10}+ k_{13})$ (11)

Substitute Equations (10) and (11) into (7) and combine with (6) to give

$Y= [k_0/(k_{10}+ k_{13})](1/s-1/(s+k_{10}+k_{13})]$ (12)

$X_1= [k_0/(k_{10}+ k_{13})][(1-e^{-(k10+k13)t}]$ (13)

Substitute Equation (13) into (2) to give

$$d[X_3]=[k_{13}k_0/(k_{10}+ k_{13})][(1-e^{-(k10+k13)t}]/1.5-k_{30}[X_3] \quad (14)$$

Laplace-Transforms Equation (14) into

$$sY-X_3(0)= [k_{13}k_0/1.5(k_{10}+ k_{13})] [1/s-1/(s+k_{10}+k_{13})]-k_{30}Y \quad (15)$$

Since $X_3(0)=0$,

$$(s+ k_{30})Y=[k_{13}k_0/1.5(k_{10}+ k_{13})] [1/s-1/(s+k_{10}+k_{13})] \quad (16)$$

$$Y=[k_{13}k_0/1.5(k_{10}+ k_{13})] [1/s-1/(s+k_{10}+k_{13})][(1/(s+ k_{30})] \quad (17)$$

$$Y=[k_{13}k_0/1.5(k_{10}+ k_{13})]\{[1/s][1/(s+k_{30})]-[1/(s+k_{10}+k_{13})][1/(s+k_{30})] \quad (18)$$

$$\text{Let } A_1/s+B_1/(s+k_{30})= [1/s][1/(s+k_{30})] \quad (19)$$

$$A_1s+A_1k_{30}+B_1s=[1/s][1/(s+k_{30})] \quad (20)$$

$$(A_1+ B_1)s+A_1k_{30}=1 \quad (21)$$

$$A_1=1/k_{30} \quad (22)$$

$$\text{Since } A_1=-B_1 \quad (23)$$

$$B_1=-1/k_{30} \quad (24)$$

$$\text{Let } A_2/(s+k_{10}+k_{13})+B_2/(s+k_{30})=[1/(s+k_{10}+k_{13})][1/(s+k_{30})] \quad (25)$$

$$A_2s+A_2k_{30}+B_2s+B_2k_{10}+B_2k_{13}=1 \quad (26)$$

$$(A_2+B_2)s+A_2k_{30}+(k_{10}+k_{13})B_2=1 \quad (27)$$

$$A_2=-B_2 \text{ and} \quad (28)$$

$$A_2k_{30}+(k_{10}+k_{13})B_2=1 \quad (29)$$

Substitute Equation (28) into (29) to give

$$-B_2k_{30}+(k_{10}+k_{13})B_2=1 \quad (30)$$

$$(k_{10}+k_{13}-k_{30})B_2=1 \quad (31)$$

$$B_2=1/(k_{10}+k_{13}-k_{30}) \quad (32)$$

$A_2=-1/(k_{10}+k_{13}-k_{30})$ (33)

Substitute Equations (22), (24), (32) and (33) into (18) to give

$Y=[k_{13}k_0/1.5(k_{10}+k_{13})]\{(1/k_{30})[1/s-1/(s+k_{30})]-[1/(k_{10}+k_{13}-k_{30})][1/(s+k_{30})-1/(s+k_{10}+k_{13})]\}$ (34)

$Y=[k_{13}k_0/1.5k_{30}(k_{10}+k_{13})][1/s-1/(s+k_{30})]-\{k_{13}k_0/[1.5(k_{10}+k_{13})](k_{10}+k_{13}-k_{30})][1/(s+k_{30})-1/(s+k_{10}+k_{13})]\}$ (35)

$[X_3]=[k_{13}k_0/1.5k_{30}(k_{10}+k_{13})](1-e^{-k30t})-\{k_{13}k_0/[1.5(k_{10}+k_{13})](k_{10}+k_{13}-k_{30})]\}[(e^{-k30t}-e^{-(k10+k13)t}]$(36)

Since $k_{10}>>>k_{13}$ and k_{30}

$[X_3]=[k_{13}k_0/(1.5k_{30}k_{10})](1-e^{-k30t})-\{k_{13}k_0/(1.5k_{10}^2\}[(e^{-k30t}-e^{-(k10+k13)t}]$ (37)

At $t\to\infty$,

$[X_3]=[k_{13}k_0/(1.5k_{30}k_{10})](1-e^{-k30t})-[k_{13}k_0/(1.5k_{10}^2](e^{-k30t})$ (38)

$[X_3]=[k_{13}k_0/(1.5k_{10})][1/k_{30}-(1/k_{30}+k_{10})e^{-k30t}]$ (39)

where X_1=Drug in the Subconjunctival Space

X_3=Drug in the Vitreous Chamber

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CHAPTER 6

EVALUATION OF REGEL® AS A DRUG DELIVERY SYSTEM FOR SUBCONJUNCTIVAL INJECTION (A PILOT STUDY)

I. INTRODUCTION

As mentioned in Chapter 5, simple subconjunctival injection is not a practical way to deliver a therapeutic agent to the posterior segment of the eye since frequent injection is required to maintain the vitreous drug level above the minimum therapeutic concentration for an extended period of time. Various in vivo studies from the literature also show the same conclusion (Tsuji, Tamai et al. 1988; Simpson, Gilbert et al. 2002). In other words, a drug delivery system is required to sustain drug release.

Various dosage forms have been developed for intraocular delivery such as microspheres (Herrero-Vanrell and Refojo 2001), implants (Yasukawa, Kimura et al. 2001), etc. but none of them were designed specifically for subconjunctival injection.

Regel® is a unique biodegradable triblock copolymer (PLGA-PEG-PLGA). It is a solution at low temperature and becomes a gel at body temperature (gelation temperature $\approx 15^{\circ}C$ at 23% w/w). Moreover, it can sustain drug release (from two weeks to two months) and has excellent solubilizing capacity for poorly soluble compounds. Moreover, it is biodegradable and there is no need to remove the empty drug delivery system from the body after all the drug has been released. Typically, the injected gel will be completely resorbed in one to two months (Zentner, Rathi et al. 2001).

In terms of drug delivery to the posterior segment of the eye using subconjunctival injection, apparently, Regel® carries a number of advantages. Firstly, it can sustain drug release so that frequent injection is not required. Drugs can either be solubilized or suspended in a Regel® system. Most importantly, Regel® is a solution at low temperature and becomes a gel at

body temperature. Therefore, it can be injected at low temperature so that surgical implantation is not required. Once inside the subconjunctival space, it will becomes a gel and stay there. According to Chapter 5, there exists a parallel loss pathway associated with subconjunctival injection due to rapid "removal" of drugs from the subconjunctival space. With this "gelling" system, it is very likely that removal of the injected drug can be slowed down. In this chapter, the possibility of using Regel® as a drug delivery system will be examined and our focus will be on the pharmacokinetic aspect. Other aspects such as biocompatibility will not be considered, although subcutaneous injection of Regel® is biocompatible (Zentner, Rathi et al. 2001).

II. HYPOTHESIS

Regel® is a properly designed drug delivery system for subconjunctival injection and it is able to provide prolonged vitreal levels.

III. SPECIFIC AIMS

To evaluate in vitro and in vivo performances of Regel® as a drug delivery system for subconjunctival injection.

IV. EXPERIMENTAL

A. Materials

Regel® was kindly supplied by MacroMed, Drug Delivery Technology (Sandy, UT). Dexamethasone (Purity >98%) was purchased from Steraloids (Newport, NY). All other chemicals were in analytical grade. Solvents used in HPLC were in HPLC grade. Milli Q water (Millipore, Bedford, MA) was used throughout the study unless otherwise specified.

B. In Vitro Evaluation of Regel®

(i) Solubility of Dexamethasone in 23% w/w Regel®

0.02g of dexamethasone was added to 8mL of 23% w/w Regel® solution to make a 2.5mg/mL preparation. The suspension was stirred at 4°C. A 2mL aliquot was taken out after one and three days. The sample was filtered using a 25mm glass prefilter (Nalgene, Rochester, NY) to remove large particles followed by a Cameo 13mm 0.45μm Nylon filter (MSI, Westboro, MA). 0.1mL of the filtrate was added to 5mL of acetonitrile and then 0.1mL of this solution was added to 0.9mL of 30% acetonitrile in water and 25μL was injected into the HPLC. The solubility of dexamethasone was assessed in triplicate.

(ii) Drug Loading

23% w/w Regel® solution was prepared in water. The preparation was stirred at 4°C until complete dissolution occurred to give a clear solution.

Dexamethasone was gone through a 425μm screen (American Scientific Products, Mcgaw Park, IL). Drug-Regel® preparation was made by dispersing dexamethasone powder in a Regel® solution. The preparation was stirred overnight at 4°C. The calculated concentration prepared was 75mg/mL and the actual drug concentration is 65±1mg/mL as determined by HPLC.

(iii) Drug Release from Regel®

Drug release from Regel® was assessed in triplicate using a modified USP Type I Dissolution Apparatus (USP 1995). Drug-Regel® preparation was placed in a holder which was the screw cap of a 2mL borosilicate glass vial (Fisher Scientific, Hanover Park, IL). The lining of the cap was removed before use. 0.15mL of the Drug-Regel® preparation was injected into the holder using a 1mL tuberculin syringe fitted with a 25G needle. The holder was stored at 35±1°C for 10 minutes to allow gelation to occur. After that, the holder with the gelled Drug-Regel® preparation was placed inside a basket for the dissolution apparatus (Model 2000 Dissolution System, Distek, North Brunswick, NJ) The dissolution medium was 10mM phosphate buffer at pH 7.2-7.6. The dissolution chamber was sealed to minimize evaporation and the system was kept at 35±1°C throughout the experiment. The basket was rotated at 100±3rpm. At different time periods, 1mL aliquot of the release medium was taken out for analysis. The

removed sample was replaced by the same volume of fresh release medium. The removed samples were subjected to analysis by HPLC.

(iv) HPLC Assay

The HPLC system consisted of a Hitachi L7100 Quaternary Pump (Hitachi, Ltd., Naperville, IL), a HP 1050 Series UV-Vis Detector, a HP 1050 Series Autosampler and a HP 3395 Integrator (Hewlett Packard, Germany). For solubility study, the column used was a Phenomenex μ-bondapak C18 Reverse Phase 150x3.9mm, 10μm column. The mobile phase consisted of 70% water and 30% acetonitrile. The λ_{max} for detection was 240nm and the flow rate was 1mL/min and 25μL of the sample was injected into the HPLC system. The retention time for dexamethasone was about 3.0 minute. The lowest Level of Quantification (LOQ) was determined to be 0.25μg/mL. For the release study, the column used was an Agilent Zobrax Eclipse XDB-C18 Reverse Phase 100x4.6mm, 3.5μm column with a Agilent Eclipse XDB Reverse Phase Guard Column, 12.5x4.6mm. The mobile phase consisted of 60% water and 40% acetonitrile. The λ_{max} for detection was 240nm. The flow rate was 0.5mL/min and 25μL of the sample was injected into the HPLC system. The retention time for dexamethasone was about 6.1 minute. The lowest level of quantification (LOQ) was determined to be 0.25μg/mL.

C. In Vivo Evaluation of Regel®

(i) Integrity of the Regel® in the Subconjunctival Space

Integrity of Regel® was checked at 0 hour, 1.5 hours, 2, 7, 14 and 28 days after subconjunctival injection by taking photos using a Minolta Dimage F100 digital camera (Minolta, NJ).

(ii) Pharmacokinetic Study

New Zealand White Albino Rabbits, either sex, 1.8-2.3kg, were purchased from Harlan Spraque Dawley (Indianapolis, IN). The investigations utilizing rabbits described in this report conformed to the Principles of Laboratory Animal Care (NIH Publication #85-23, revised 1985). Lighting was maintained on a 12 hour cycle and animals were fed on a regular diet with no restrictions on the amount of food and water consumed.

Rabbits were anesthetized with an IM injection of ketamine (35mg/kg) and xylazine (5mg/kg) fifteen to twenty minutes prior to dosing. Five minutes before drug application, the eye which was dosed received one drop of 0.5% tetracaine hydrochloride solution. Following topical anesthesia, 0.15mL of the Drug-Regel® suspension was injected subconjunctivally in upper quadrant (10-15mm from the limbus) using a tuberculin syringe using a 25G needle. Only one of the eyes was dosed with the other eye serving as a control. Left and right eye was alternated to minimize any left-to-right and right-to-left bias. At selected times, rabbits were sacrificed with an overdose of sodium pentobarbital delivered through a marginal ear vein at a concentration of 100mg/kg. Blood sample was obtained using intracardiac puncture five minutes before sacrifice of the animal. Immediately following death of the animal, the whole eyeball was then enucleated. All the biological samples were stored at –80°C until analysis. Four to six rabbits were used for each time point.

(iii) Quantification of Dexamethasone in Biological Samples

Dexamethasone in biological samples was quantified by ELISA (Neogen, Lexington, KY). The assays operate on the basis of competition between the drug and the drug-enzyme conjugate for a limited number of specific binding sites on a precoated plate. First, the sample or control was added to the microplate and then the diluted drug-enzyme conjugate was added and the mixture was incubated at room temperature for two hours. During this incubation the drug in the sample or the drug-enzyme conjugate bound to the antibody immobilized in the microplate wells. After incubation, the plate was washed three times to remove any unbound sample or drug-enzyme conjugate. The presence of bound drug-enzyme conjugate was recognized by the addition of K-Blue Substrate (Neogen, Lexington, KY). After 30 minute substrate incubation, the reaction was halted with the addition of red stop solution. The extent of color development is inversely proportional to the amount of the drug in the sample which was monitored by a Hyperion Microplate Reader I (Miami, Florida) at 650nm. A calibration curve from 0.2ng/mL to 15ng/mL was prepared for each ELISA assay. The biological samples were appropriately diluted so that their concentrations fell within this range.

V. RESULTS AND DISCUSSIONS

Regel® was chosen as a drug delivery system in our study since (1) it can solubilize a poorly soluble compound; (2) it can provide continuous drug release for an extended period time; (3) it can slow elimination of injected drugs from the subconjunctival space by its high viscosity

in the gel state and (4) it is biodegradable. These features are very important in designing a delivery system for subconjunctival injection as concluded in Chapter 5. Therefore, it is worthwhile of carrying out a pilot study in addressing the performance of Regel® in vitro and in vivo.

A. In Vitro Evaluation of Regel®

(i) Solubility of Dexamethasone in 23% w/w Regel®

Solubility of dexamethasone in 23% Regel® w/w in water is shown in Figure 6.1. Since the solubilities after one and three days are not statistically different from each other (unpaired t-test, $p<0.05$), it is believed that the equilibrium (or close to equilibrium) solubility was achieved after one day and it was determined to be about 0.8mg/mL. It can be concluded that 23% Regel® in water increases the solubility of dexamethasone by 8-10x (Cohen 1973). Compared with paclitaxel (>2000x) and cyclosporin (400x) (Zentner, Rathi et al. 2001), Regel® is not very efficient in solubilizing dexamethasone. The reason for that is not well understood since the structure-property relationships in terms of solubilization has not been explored for Regel®. For a closely related tri-block copolymer, PEG-PLGA-PEG, it was found that the polymer forms micelles in both the solution and gel state (Jeong 1999). It is not clear whether Regel® forms a similar micellar structure and other aggregate-like structures. For the PLA-PEG diblock copolymeric micelles, it was found that partition coefficient of a compound can partially account for its solubilization phenomenon (Hagan, Coombes et al. 1996). Sudan black B (log P=7.6) can

incorporate more effectively into the PLA-PEG polymeric micelles than testosterone (log P=3.35). Since paclitaxel is more hydrophobic than dexamethasone (log P=1.49) (Nagarajan, Barry et al. 1986), this may be the reason that Regel® cannot solubilize dexamethasone as effectively as paclitaxel. In addition, it was found that aromatic compounds can be solubilized more effectively in PEO-PPO polymeric micelles than aliphatic compounds (Nagarajan, Barry et al. 1986). In our case, dexamethasone (Figure 6.2) does not have any aromatic rings and it has only unsaturated bonds whereas paclitaxel (Figure 6.3) has three aromatic rings, this may account for the current finding that Regel® solubilizes paclitaxel more effectively than dexamethasone. In fact, a similar phenomenon was also observed in the case of Sudan black B and testosterone with PLA-PEG diblock copolymeric micelles (Hagan, Coombes et al. 1996).

Although Regel® is not very efficient in solubilizing dexamethasone in the solution state, it is believed that the solubility will be greatly increased in the gel state. The reasons for this are two-fold. Firstly, increasing temperature generally increases the solubility of a compound. Secondly, dehydration of PEG at high temperature (Kabanov and Alakhov 2002) will make the whole system more hydrophobic and this may enhance the solubilizing capacity of Regel®. However, the solubility of dexamethasone in the gel state is not known. To the authors' knowledge, there does not exist any promising technique to accomplish such an assignment.

(ii) Drug Loading

Since Regel® is not effective in solubilizing dexamethasone in the solution state, our dosage form was prepared by suspending dexamethasone solid particles in Regel® solution. One of the advantages of using Regel® is that it can be loaded with insoluble drug powder. Regel® is a

relatively good suspending agent compared with water. Although a rigorous physical stability study has not been carried out, significant sedimentation of dexamethasone in Regel® solution was not observed up to 12 hours and the sedimented powder was easily redispersed by shaking. For our drug release study and pharmacokinetic study, the calculated dosing concentration was 75mg/mL and the actual drug loading was 65±1mg/mL which is 650x the solubility of dexamethasone in water.

(iii) Drug Release from Regel®

The time course of release of dexamethasone from Regel® is shown in Figure 6.4. It is clearly shown that there is a continuous release of dexamethasone for up to 60 days. Since our dosage form was prepared by suspending an excess amount of drug in Regel® solution which forms a gel at high temperature (>15°C), this is analogous to suspending drug particles in a ointment base. It can be speculated that the release kinetics of dexamethasone from Regel® may be represented or closely related to Higuchi equation. In the case of a nonbiodegradable polymeric slab system, the Higuchi equation can be expressed as follows:

$$dQ/dt=(A/2)[(2DC_sC_0)/t]^{1/2} \qquad \text{for } C_0>>C_s \qquad (6.1)$$

where Q is the cumulative amount of drug release and A=total area of the slab, C_0=the initial drug loading, C_s=the solubility of the drug in the polymeric matrix and D=diffusion constant of the drug molecule in the polymer matrix. The integrated form of this equation can be expressed as:

$$Q=kt^{1/2} \quad (6.2)$$

where $k=A(2DC_sC_0)$. It is obvious that when Q is plotted against $t^{1/2}$, a straight line will be obtained. Therefore, our data is replotted using Higuchi equation and the result is shown in Figure 6.5. It is found that our release kinetics agrees reasonably well with Higuchi equation for the early portion of the release profile but deviates significantly from it after roughly 10 days. This may indicate diffusion alone cannot account for the entire release kinetics and other factors may be involved. As mentioned earlier, Regel® is a biodegradable polymeric system and it is degraded by hydrolysis. Therefore, it is believed that the deviation from Higuchi release kinetics at the late time points is due to polymer erosion. In fact, release of paclitaxel from Regel® also shows a biphasic pattern and Zentner et al claims that diffusion is responsible for the early portion of the release profile and the latter portion of the profile is due to both diffusion and polymer erosion (Zentner, Rathi et al. 2001). By assuming polymer degradation is a first-order process, Baker (Baker 1987) introduced a modified Higuchi equation to describe drug release from a biodegradable polymer.

Higuchi equation (Equation 6.1) can also be expressed by the following equation:

$$dQ/dt=(A/2)[(2PC_0)/t]^{1/2} \quad (6.3)$$

where P=the permeability of the polymer to the drug molecules. For a biodegradable system, P increases with time since the matrix is loosened by polymer degradation. However, the change in

permeability with time is difficult to measure. Therefore, it is assumed that the permeability is proportional to the number of hydrolyzed bonds. Thus,

$$P/P_0=(\text{initial number of bonds/number of bonds remaining})=N/N-Z \quad (6.4)$$

where P_0=original permeability, P=the permeability after Z cleavages and N=initial number of bonds. Assuming the bond cleavage is a first-order process, then

$$dZ/dt=k(N-Z) \quad (6.5)$$

and

$$kt=\ln(N/N-Z) \quad (6.6)$$

where k is the first-order rate constant. Equation (6.6) and (6.4) are combined and substituted into Equation (6.3) to give

$$dQ/dt=A/2\{2P_0[\exp(kt)C_0]/t\}^{1/2} \quad (6.7)$$

which can be simplified as

$$dQ/dt=K\{[\exp(kt)]/t\}^{1/2} \quad (6.8)$$

where Q is the cumulative amount of drug released and $K=(A/2)(2P_0C_0)^{1/2}$ and k are constants. This equation shows that drug release cannot be represented in terms of t or $t^{1/2}$ in a simple linear relationship. Therefore, curve fitting was performed using this differential equation and the results are summarized in Figure 6.6. An excellent fit ($r^2>0.99$) is obtained and this may indicate that the release kinetics of dexamethasone from Regel® is controlled by both diffusion and polymer erosion.

B. In Vivo Evaluation of Regel®

(i) General Observation and Integrity of Regel® after Subconjunctival Injection

(a) Integrity of Regel® after Subconjunctival Injection

After subconjunctival injection of Regel®, a well defined bleb was formed (Fig. 6.7) and this bleb can be seen for up to 28 days (Figure 6.12) indicating the integrity of the Regel® can be maintained in the subconjunctival space for up to 28 days. Since our experiment terminated at Day 28, it can be speculated that Regel® can remain in the subconjunctival space for more than a month.

(b) Morphology of Regel® after Subconjunctival Injection

Another interesting phenomenon is that the bleb formed at the time of injection eventually disappeared by spreading over the sclera (Figure 6.7-6.12). The reason for the spreading is not

well understood but it is likely due to the blinking action of the rabbit. Another possibility is that the backpressure developed in the conjunctiva (the injected Regel® causes the conjunctiva to lift up to accommodate the injected dose) pushes the injected Regel® to adapt a more flattened shape.

(c) Increase in Injection Volume by using Regel®

In the case of simple subconjunctival injection, the backpressure developed in the conjunctiva will push the injected fluid out of the puncture hole. It was found that a significant amount of injected pilocarpine was pushed out of the puncture hole into the precorneal area when the injection volume was greater than 100μL (Conrad 1979). However, due to its high viscosity (Zentner, Rathi et al. 2001), the formed gel cannot be pushed through the puncture hole and there was no problem in keeping the whole 150μL dose in the subconjunctival space in our study. The developed backpressure in the conjunctiva will finally push the formed gel to adapt a flattened shape as mentioned earlier. Therefore, one of the advantages of Regel® is its capability to increase the injection volume. However, how large it can go is still a mystery. Using fibrin glue as a drug delivery system, Simpson et al (Simpson, Gilbert et al. 2002) injected 300μL into the subconjunctival space without leakage. Therefore, the major concern is probably the discomfort generated by the injection. We believe that the larger the injection volume, the greater the backpressure experienced by the conjunctiva (due to stretching of the conjunctiva) and the greater the discomfort.

(d) Maximized Contact of the Injected Drug with the Sclera (Minimized Contact of the Injected Drug with the Conjunctiva) by using Regel®

The semisolid nature of Regel® at body temperature can reduce dispersion of the injected drug into other ocular tissues that are not involved in absorption to the posterior segment of the eye. In other words, contact of the drug with the sclera can be maximized. As mentioned earlier, the "sponge-like' nature of the conjunctiva tends to absorb any injected fluid and this leads to a very fast elimination of the drug from the subconjunctival space (Chapter 5). Regel® can certainly circumvent (or minimize) this problem due to its high viscosity in the gel state.

(e) Biocompatibility of Regel® after Subconjunctival Injection

Although a rigorous biocompatibility study has not been carried out, our first impression on the biocompatibility of Regel® in the rabbit eye is that it is well tolerated. A small degree of bleeding might occur at the time that an injection was made due to puncture of the conjunctiva by a needle but the bleeding stopped after 1 to 2 minutes and no sign of inflammation was observed. This excellent biocompatibility may be due to the flexible nature of the Regel®. As mentioned earlier, the injected Regel® spread over the sclera instead of remaining as a bleb.

(i) Pharmacokinetics

(a) Intersubject Variation

There exists a large intersubject variation (Figure 6.13) associated with our pharmacokinetic data. Other studies using subconjunctival injection also have the same problem (Tsuji, Tamai et al. 1988; Simpson, Gilbert et al. 2002). The reason for this is not known. According to our experience, it is very difficult to place the injection needle at the same place every time the injection is made and this may cause a large intersubject variation. Another factor that needs to be considered is the speed at which the plunger was pushed at the time that the injection is made. The speed may affect the initial spreading of Regel® which in turn affects the area of the Regel® in direct contact with the sclera. Therefore, this may be another source of error. Thus, in order to make subconjunctival injection a practical route for drug delivery to the posterior segment of the eye, it is necessary to standardize the injection technique so that a more consistent injection pattern can be developed. Since different locations of the sclera have different thicknesses, this may serve as a starting point to look for an optimum injection site (Geroski and Edelhauser 2001).

(b) Pharmacokinetic Study

Due to limited data points and large intersubject variation associated with them, it may not be possible to analyze our in vivo data using the pharmacokinetic model developed in Chapter 4. However, our data can still give insight as to performance of Regel® in vivo.

The drug level in the vitreous in dosed and contralateral eyes and plasma after subconjunctival injection is shown in Figure 6.13 and 6.14 respectively. The main goal of this study was to investigate whether Regel® can provide a prolonged drug level in the vitreous. The first question is whether Regel® can maintain its integrity in vivo since it is not very likely that it

can provide sustained release in the subconjunctival space if its integrity cannot be maintained. As mentioned earlier, the integrity of Regel® can be maintained for up to 28 days. The next question is whether Regel® is able to provide a continuous release. Our data showed that dexamethasone can be detected in both vitreous chamber and plasma for up to 28 days. Therefore, it is very likely that Regel® can provide a prolonged drug level by continuous release of dexamethasone. At the same time, Table 6.1 shows that the drug level in the vitreous of the dosed eye is statistically significantly higher than that of the contralateral eye at all time points except Day 7 which was not determined due to statistical limitation (Wilcoxon Matched Pair Test, $p<0.05$, One-tailed) (DeMuth 1999; Glover and Mitchell 2002). This indicates that the drug present in the vitreous of the dosed eye cannot be explained by the systemic recirculation pathway since all the drug in the vitreous of the contralateral eyes is attributed to this pathway. Therefore, there exists a local entry pathway for the drug to enter the vitreous chamber of the dosed eye, namely, the direct penetration pathway as shown in Chapter 3.

It is speculated that Regel® may be able to decrease drug load to the systemic circulation because of the inability of the conjunctiva to absorb the "gel" at body temperature. Tsuji el al (Tsuji, Tamai et al. 1988) found that subconjunctival injection of 2.61mg of prednisolone in rabbits resulted in $C_{max}\approx3\mu g/mL$ in the plasma. In our study, 9.75mg of dexamethasone, injected subconjunctivally, resulted in $C_{max}\approx50ng/mL$ in the plasma. Thus our injected dose is about 3.7x that used in Tsuji et al's study but resulted in much lower plasma drug concentration. This may indicate that Regel® is capable of reducing drug load to the systemic circulation. However, it may be that prednisolone and dexamethasone are subject to different extents of metabolism and

protein binding in rabbits. Therefore, the existing evidence is not sufficient to draw a definitive conclusion. Further studies are required to confirm this speculation.

VI. CONCLUSIONS

After completion of the current study, the following conclusions can be drawn:

1. Regel® 23% w/w in water increases the solubility of dexamethasone by 8-10x in the solution state but its solubility in gel state is not known.
2. There is a continuous drug release from Regel® for up to 60 days in vitro and its release is governed by both diffusion and polymer erosion.
3. Integrity of Regel® can be maintained for up to 28 days in the subconjunctival space of rabbits and Regel® is reasonably biocompatible.
4. Prolonged drug level can be maintained in the vitreous chamber of the dosed eye after subconjunctival injection of Regel® and this drug level cannot be explained by the systemic recirculation pathway. A direct penetration pathway is suggested for the drug to enter into the vitreous chamber after subconjunctival injection.
5. There exists indirect evidence that Regel® can greatly reduce drug load to the systemic circulation and this can potentially decrease systemic side effects. Further studies have to be carried out to confirm this speculation.

Although Regel® has generated some encouraging results, we still do not have sufficient evidence to accept the hypothesis that Regel® is a properly designed drug delivery system for

subconjunctival injection to deliver drugs to the vitreous chamber. A more detailed description of suggested future studies will be discussed in Chapter 8.

Table 6.1: Comparison of Vitreous Drug Level in the Dosed and Contralateral Eyes using Wilcoxon Matched Pair Test ($p<0.05$, one-tailed)

Time	Vitreous in the Dosed Eye	Vitreous in the Contralateral Eye	Statistically significant difference between dosed and contralateral Vitreous
1.5 Hour	19.14 (7.13)	0.46(0.09)	Significant
Day 2	199.64 (97.80)	2.03(0.29)	Significant
Day 7	9.63 (1.00)	5.89(1.47)	-*
Day 14	99.85 (41.30)	1.22 (0.46)	Significant
Day 28	14.27 (10.98)	0 (0)	Significant

*statistical testing is not performed due to small sample size (n=4) at Day 7

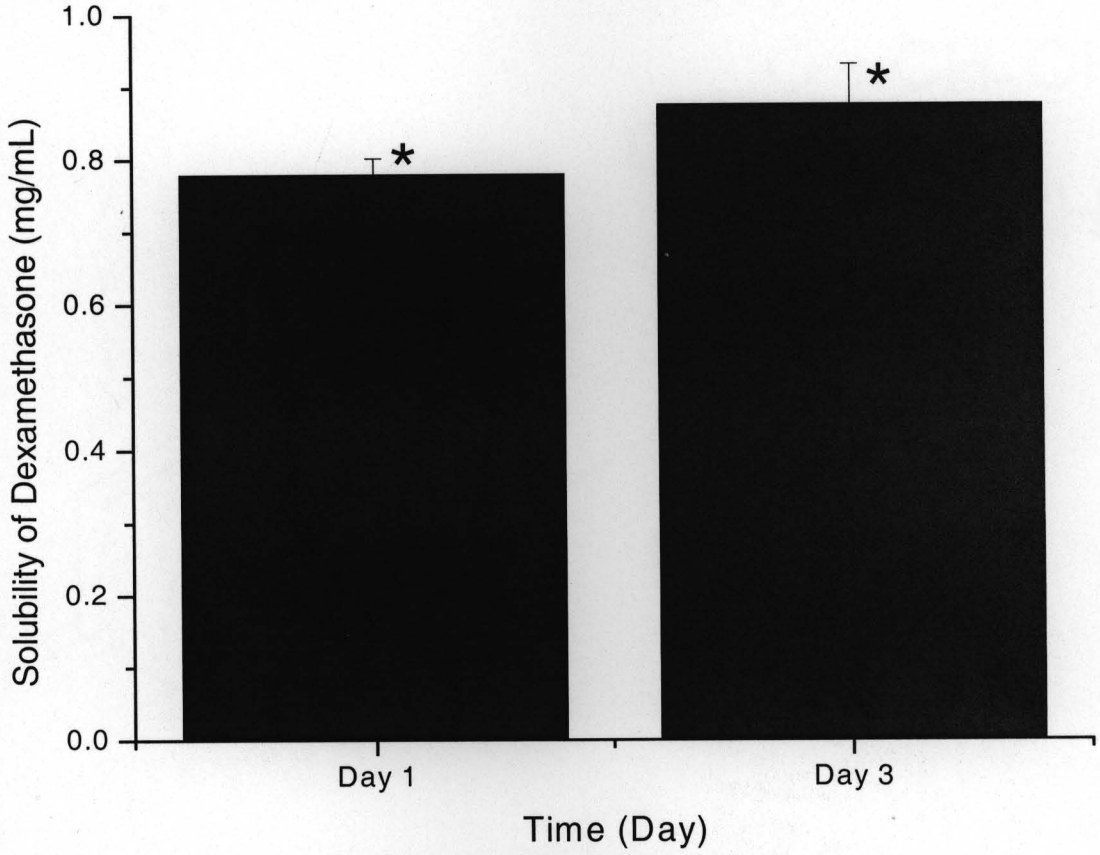

Figure 6.1: Solubility of dexamethasone in 23% w/w Regel® solution in water

*not statistically different from each other (unpaired t-test, $p<0.05$)

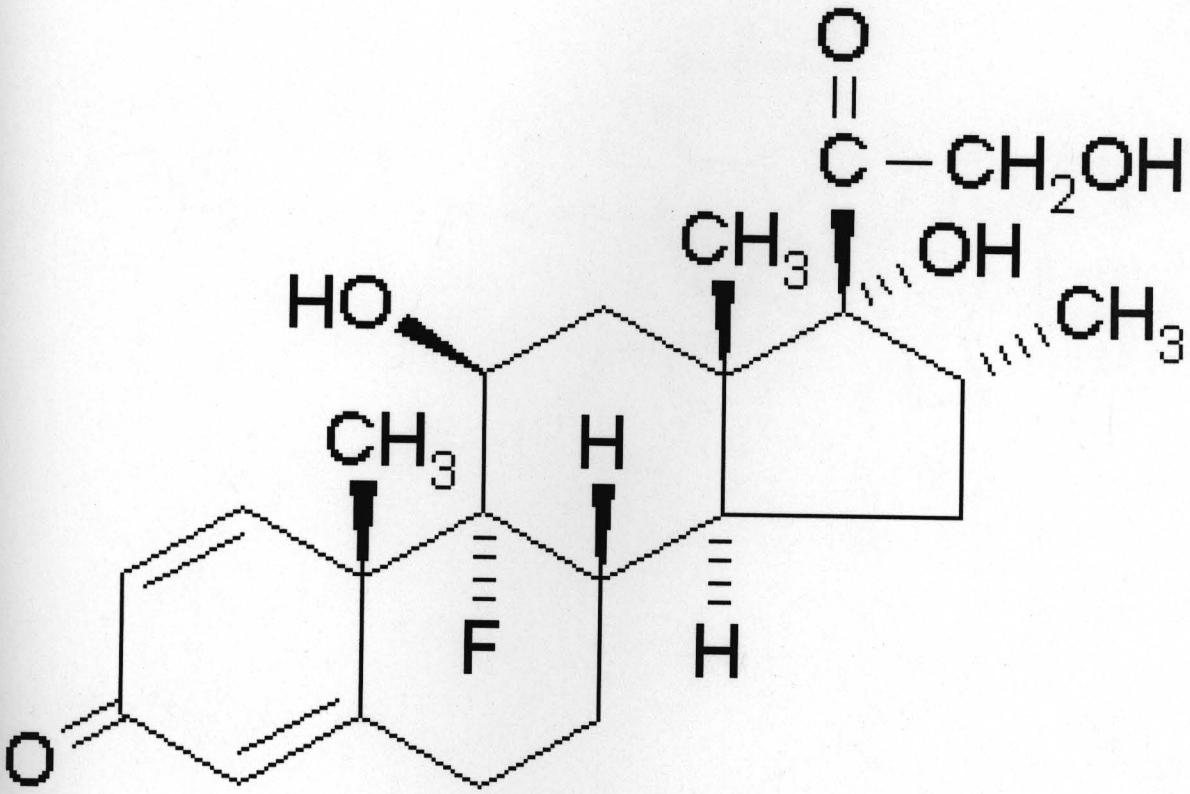

Figure 6.2: Structure of dexamethasone

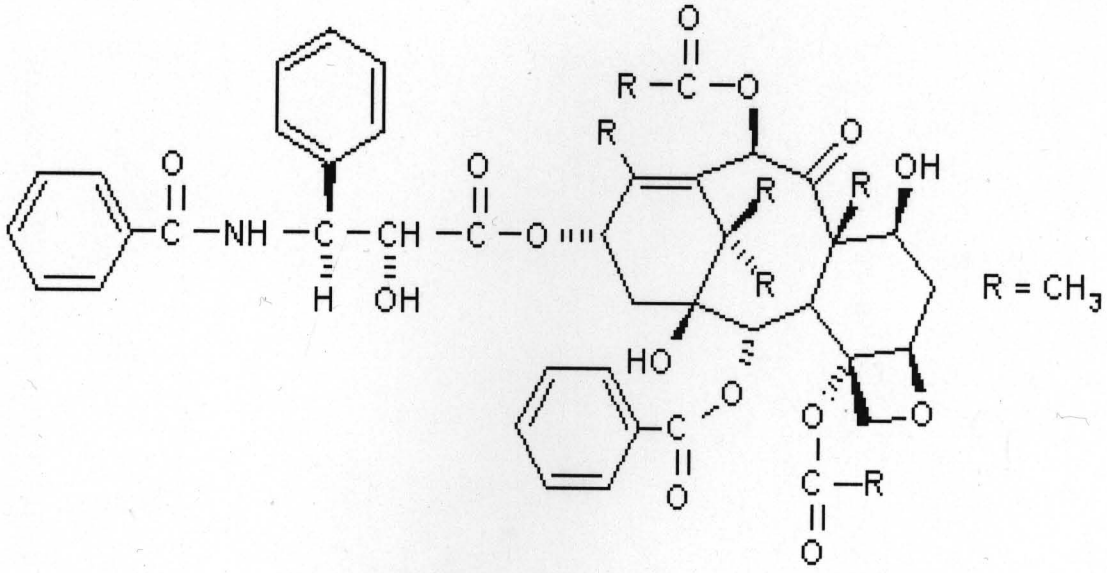

Figure 6.3: Structure of paclitaxel

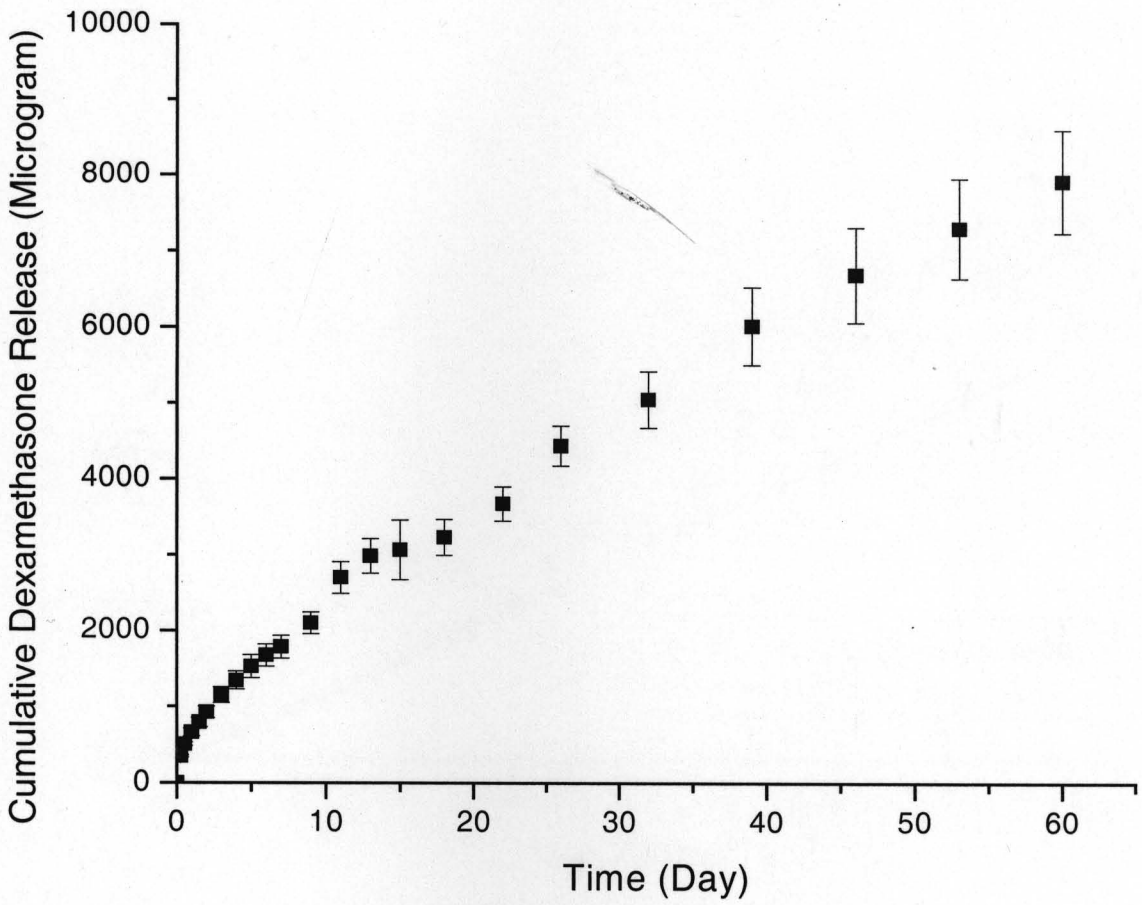

Figure 6.4: Release of dexamethasone from Regel® with time

(Error bar represents standard deviation, n=3)

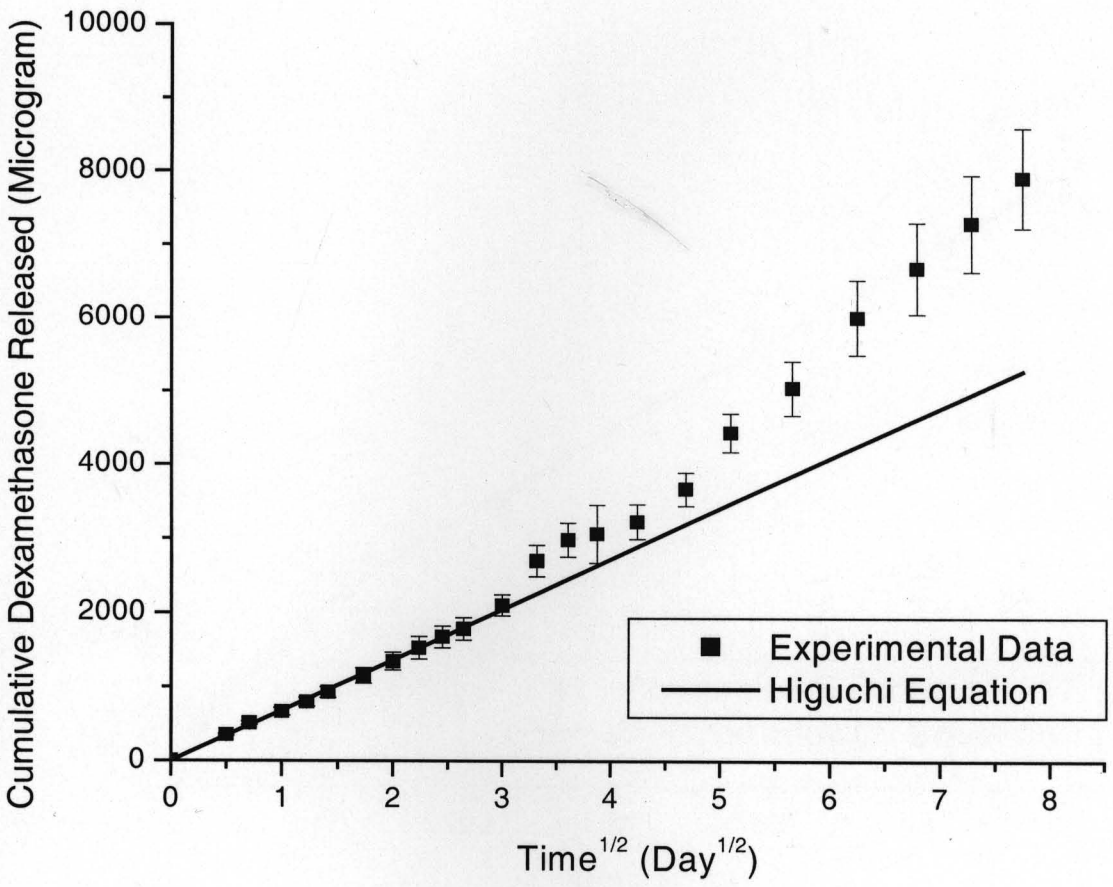

Figure 6.5: Comparison of experimental data with the Higuchi equation

(Error bar represents standard deviation, n=3)

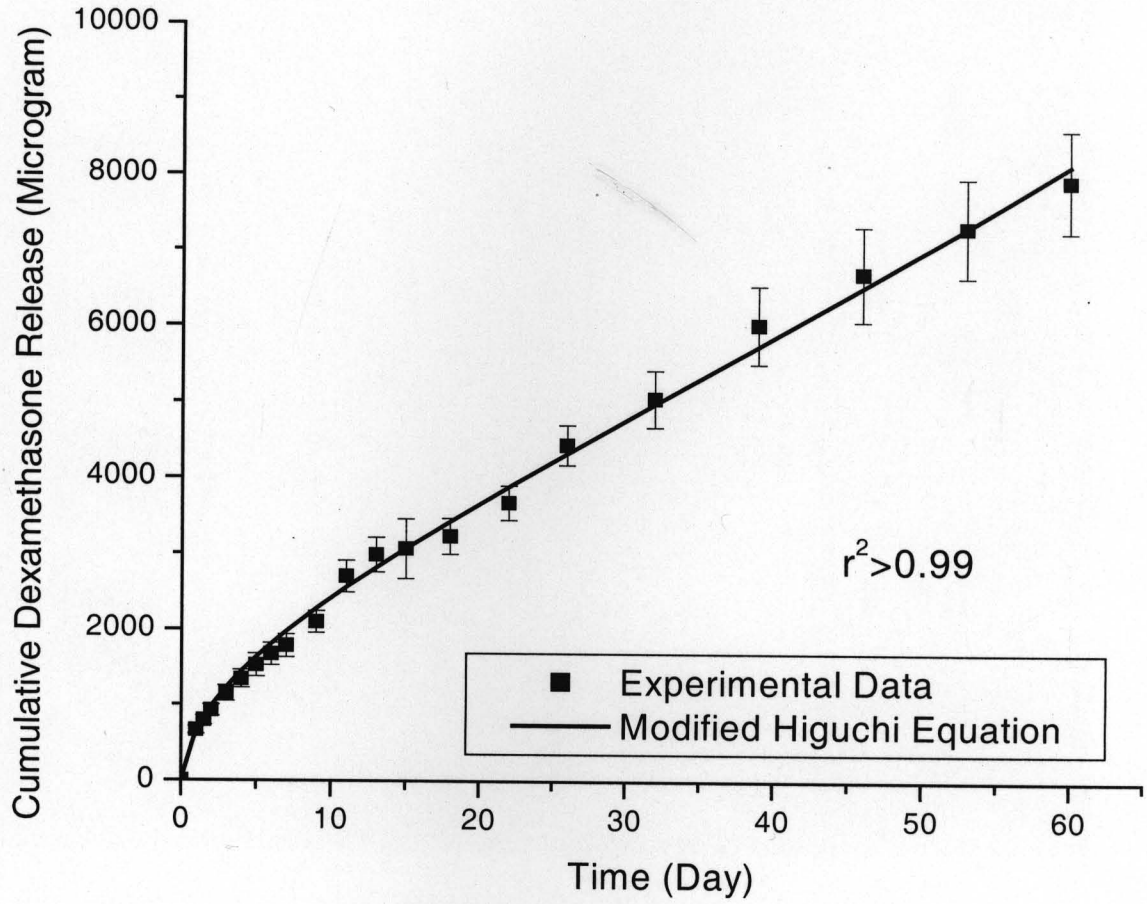

Figure 6.6: Comparison of experimental data with modified Higuchi equation

(Error bar represents standard deviation, n=3)

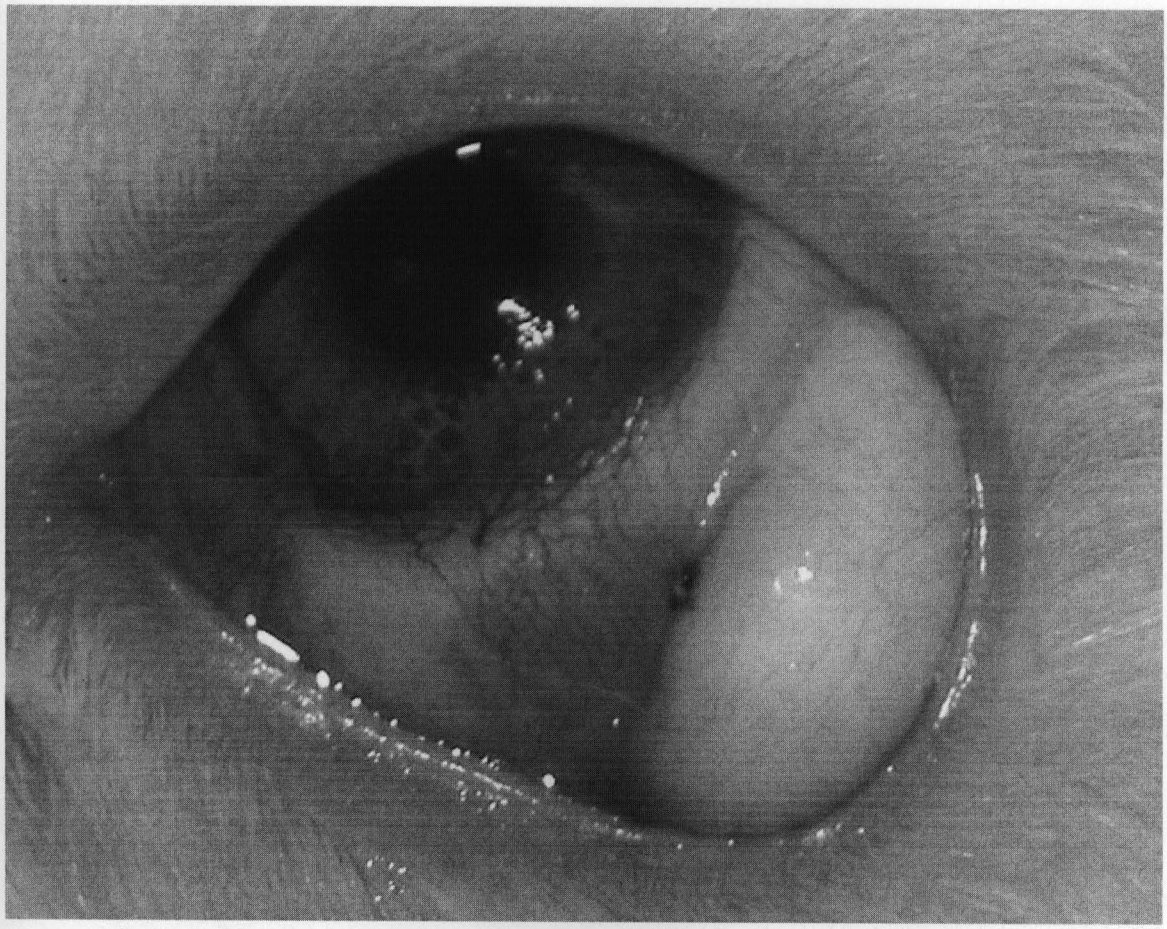

Figure 6.7: Regel® immediately after injection

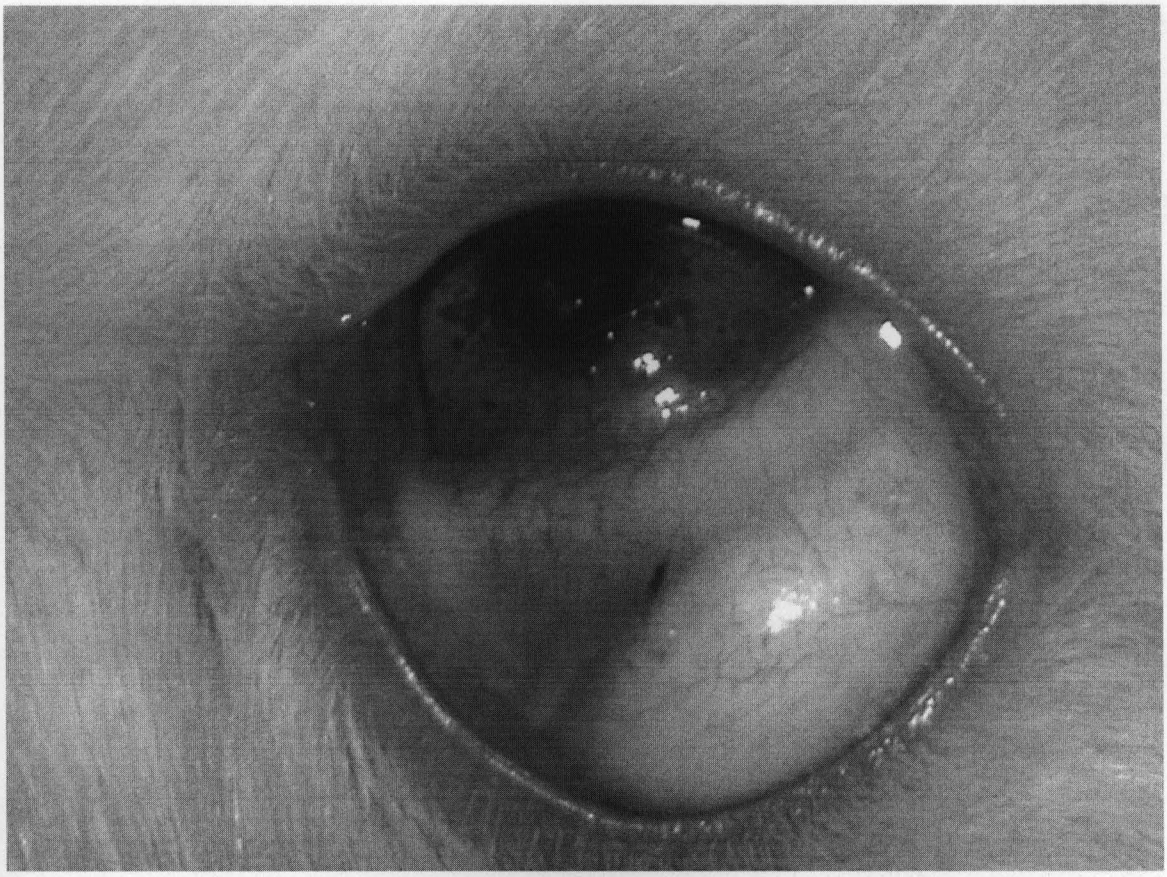

Figure 6.8: Regel® after 1.5 hour

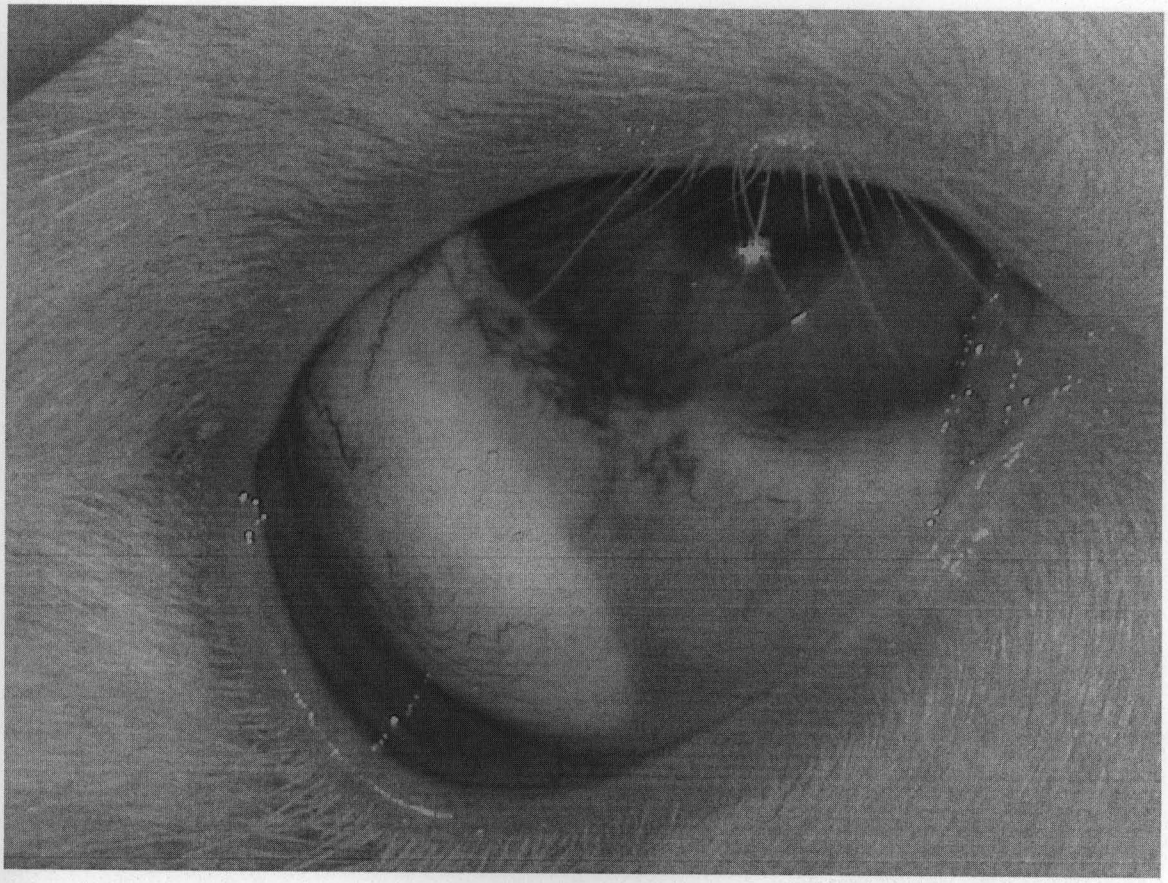

Figure 6.9: Regel® after 2 days

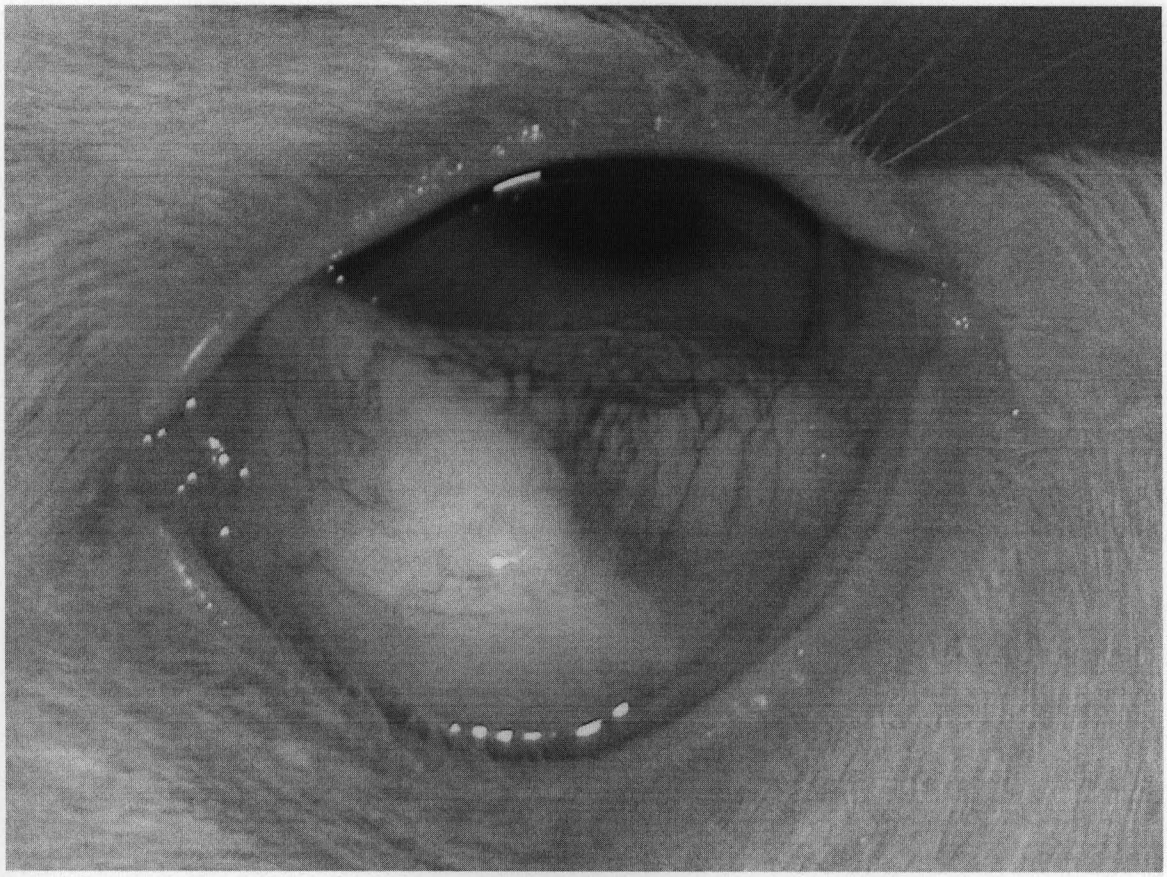

Figure 6.10: Regel® after 7 days

Figure 6.11: Regel® after 14 days

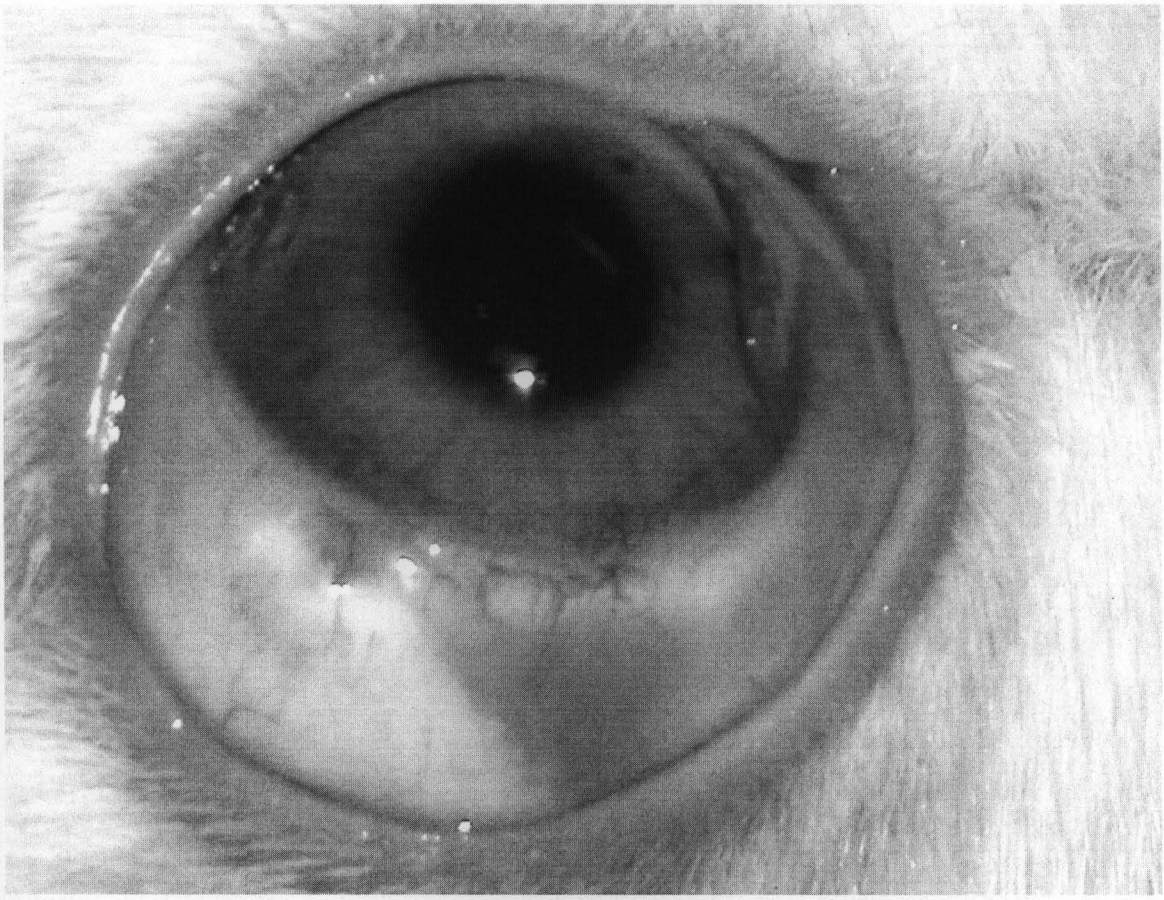

Figure 6.12: Regel® after 28 days

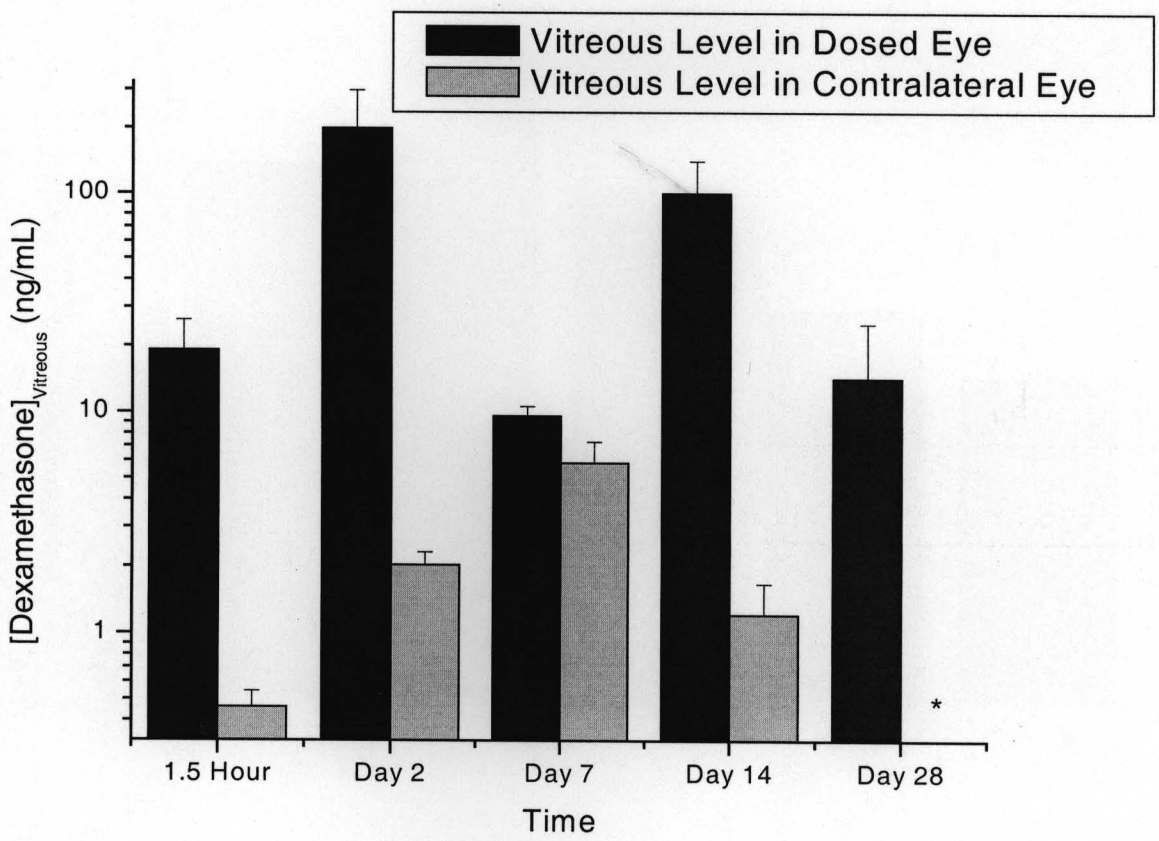

Figure 6.13: Vitreous dexamethasone level after subconjunctival injection of Regel®

*Drug level in the vitreous of the contralateral eye at Day 28 is below detection limit

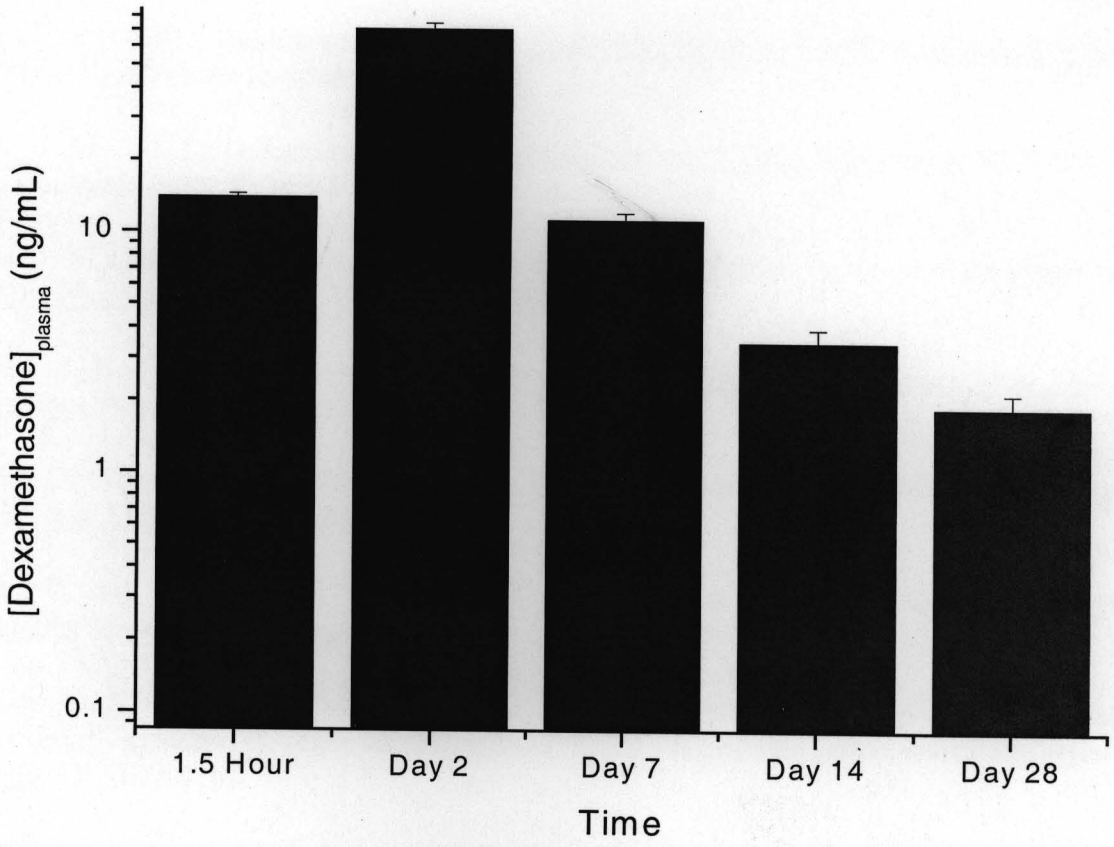

Figure 6.14: Plasma dexamethasone level after subconjunctival injection of Regel®

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CHAPTER 7

CONCLUDING REMARKS

After completion of the current study, our findings can be summarized as follows:

1. Direct penetration is the only significant pathway for a subconjunctivally injected compound to enter the vitreous chamber. Although there exists a favorable concentration gradient to move a drug from the aqueous to the vitreous chamber, this pathway contributes minimally to the total drug level in the vitreous chamber after subconjunctival injection.
2. A simple pharmacokinetic model for subconjunctival injection was developed and validated. It was found that the distribution model is more appropriate than the protein binding model in describing the pharmacokinetics after subconjunctival injection.
3. Analogous to drug delivery to the anterior segment of the eye, the pharmacokinetic model developed in Chapter 4 behaves as a flip-flop model due to rapid removal of an injected dose from the subconjunctival space. Therefore, the vitreous drug level will be governed by drug release rate from a dosage form, absorption rate constant and elimination constant. In order to "force" the vitreous drug level to be determined by the drug release rate only, it is necessary to decrease removal of an injected drug from the subconjunctival space (parallel loss pathway) and/or increase the magnitude of the absorption rate constant.
4. Based on the pharmacokinetic model developed in Chapter 4, computer simulation was carried out to investigate the effect of various factors on the vitreous drug level. It was found that simple subconjunctival

injection is not a practical way to deliver therapeutic agents to the posterior segment of the eye because frequent injection is required to maintain a therapeutic level for an extended period of time. In other words, an appropriately designed drug delivery system is required to sustain the vitreous drug level.

5. A drug delivery system can diminish the requirement for solubility but it is still desirable to keep a drug in solution form so that a higher dose can be administered.
6. A zero-order release system gives a steady vitreous drug level. If the release rate is 10μg/hr or higher, it is likely to bring the vitreous drug level to the 1ng/mL level. However, validation of this boundary condition is not possible at this moment.
7. Subconjunctival injection may not be a practical way to deliver a compound to the back of the eye if the therapeutic concentration of a compound is >1μg/mL, unless k_{10} can be reduced by 100x by using a drug delivery system.
8. Regel® increases the solubility of dexamethasone by 8-10 fold and can also provide continuous release for up to 60 days. The rate of drug release is governed by both diffusion and polymer erosion.
9. Prolonged drug level can be maintained in the vitreous chamber of the dosed eye after subconjunctival injection of Regel® and this drug level cannot be explained by the systemic recirculation pathway. Therefore, a

direct penetration pathway is suggested for the drug to enter into the vitreous chamber after subconjunctival injection.

10. There exists indirect evidence that Regel® can greatly reduce drug load to the systemic circulation and this can potentially decrease systemic side effects. Further study has to be carried out to confirm this speculation.

CHAPTER 8

SUGGESTED FUTURE STUDY

Our current study is the first systematic study on using subconjunctival injection to deliver therapeutic agents to the posterior segment of the eye. Although it is not possible to answer all questions from the results of this study, it gives some guidance of using subconjunctival injection for posterior delivery as well as limitations on subconjunctival injection. In order to ascertain whether subconjunctival injection is a practical way to deliver a drug to the back of the eye, the following studies are suggested:

1. As mentioned in Chapter 5, due to the presence of a parallel loss pathway, k_{13} ($k_{absorption}$), which is believed to be determined by the physicochemical properties of a compound, is one of the major determinants of the vitreous drug level after subconjunctival injection. However, there does not exist any study in addressing the correlation between k_{13} and the physicochemical properties of a compound such as partition coefficient, etc. This piece of information can widen the applicability of our model to compounds with different physicochemical properties.

2. As mentioned in Chapter 5, based on our computer simulation, A zero-order release system gives a steady vitreous drug level. If the release rate is 10μg/hr or higher, it is likely to bring the vitreous drug level to the ng/mL level. However, validation of this boundary condition is not possible at this moment. We tried to validate these boundary conditions by using an Alzet Osmotic Minipump but the experiment was constrained by technical difficulties. This experiment should be repeated by using a more appropriate surgical technique. The result of this study can provide more reliable information on the

rate of drug delivery to the subconjunctival space in order to attain therapeutic vitreal levels.

3. Our in-vivo in evaluation of Regel® is only a pilot study. Although Regel® generated very encouraging results, a number of very important pieces of information are still missing. Therefore, the in vivo evaluation of Regel® study should be repeated with more data points and more animals per data point so that the data can be analyzed by our pharmacokinetic model. The key is to determine the extent that Regel® can reduce the magnitude of k_{10}. There may be a chance Regel® can reduce k_{10} to the extent that k_{13} (absorption rate constant) can compete with it and this may make the model become "well-behaved' instead of a flip-flop model. If this can occur, the vitreous drug level will be governed by only k_{30} (elimination rate constant) and the release rate. In this case, Regel® is nearly an "ideal" system for subconjunctival injection.

4. A pharmacokinetic study using simple subconjunctival injection of dexamethasone should be carried out so that a more direct comparison with Regel® can be accomplished, e.g., to show the capability of Regel® to reduce drug loss to the systemic circulation. Although a similar study was found in the literature (Bodker, Ticho et al. 1993), limited data points and difference in injection volume, techniques, etc. limit its usefulness.
5. Biocompatibility study of Regel® should be carried out at a histological and cellular level so that the safety of Regel® in the eye can be ensured.

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