CHAPTER VII

SCLERAL IMPLANTS OF INDOMETHACIN

7.1 INTRODUCTION

Drug delivery to the posterior segments of the eye

As mentioned earlier, the eye is generally divided into two parts; anterior and posterior segments. The anterior segment includes the cornea, iris, crystalline lens and ciliary body, while the posterior segment constitutes the vitreous body, retina and choroid. Topically applied drugs as eye drops usually penetrate the cornea and reach the anterior segments, hardly reach the posterior segment. Due to the poor penetration of the drugs to the posterior segments after topical instillation, diseases affecting the posterior segments of the eye are difficult to treat by this route of administration. Direct injection of drugs into the vitreous cavity is sometimes employed to achieve high drug concentration in the vitreous body and the retina. However, repeated injections are needed to maintain a drug concentration at the effective range for a certain period of time since the half-life of drugs in the vitreous body is relatively short. Repeated injections cause patient discomfort and may lead to vitreous hemorrhage, infection and lens or retinal injury. Systemic administration of drugs has been attempted to treat some vitreo-retinal diseases, a very small amount of drugs could reach the eye after systemic administration. A large systemic amount is required to obtain a therapeutic level of drug concentration in the eye. In addition, the blood-retinal barrier that is located at the level of retinal vascular endothelial cells and retinal pigment epithelium inhibits entry of certain drugs from systemic circulation into the retinal tissue. For the above reasons, drug delivery to the posterior segment of the eye has been challenging for both clinicians and basic scientists. A number of approaches for the delivery of drugs to the posterior segments of the eye have been developed in the last decade. These novel approaches include microparticle carriers such as liposomes, microspheres and nanospheres, vitreal and scleral implants using polymers, transcleral delivery system and targeting of drugs via systemic circulation.
Microparticles like liposomes or microspheres can be injected into the vitreous cavity with a fine needle (Moritera et al., 1991 and 1992; Veloso et al., 1997). They release drugs in the vitreous and maintain drug concentration at the therapeutic level for a certain period. Microspheres of biodegradable polymers like poly (lactic acid) or poly (glycolic acid) effectively deliver drugs to the vitreous body and the retina and can be tolerated by the ocular tissues. Drug release from the polymeric microspheres could be controlled by changing the molecular weight of the polymers or co-polymerization ratio of poly (lactide) and poly (glycolide). Speed of degradation of microspheres can be also controlled. These biodegradable polymeric microspheres have been shown to be effective for treatment of experimental proliferative vitreoretinopathy, intraocular inflammation such as uveitis, ocular infection such as cytomegalovirus (CMV) retinitis.

A small device that has a reservoir for drugs surrounded by semi-permeable polymeric membranes has been implanted in the vitreous at the parsplana (Smith et al., 1992; Sanborn et al., 1992). The device containing ganciclovir (GCV) has been approved for the treatment of CMV retinitis. This GCV device successfully treats vitreoretinal lesions in CMV infection. The device releases GCV for one to two years after the implantation. Other types of vitreal implants using biodegradable polymers have been studied for the vitreoretinal drug delivery, which are dealt in more detail elsewhere in this section. Scleral plugs of poly (lactic acids) and poly (glycolic acids) can deliver a number of drugs in the vitreous cavity after a simple implantation procedure (Kimura et al., 1994; Hashizoe et al., 1994; Yasukawa et al. 2000). The plug undergoes hydrolytic degradation during drug release and finally disappears.

The sclera is the outer coat of the eye ball, which is composed of mainly collagen fibers. Since the scleral collagen fibers have a relatively loose arrangement, the substances could diffuse through the sclera into the choroid and the retina. It was considered that the substance hardly reaches the retina even if it can diffuse through the sclera because it may be washed out by the fast and rich choroidal blood flow. However, recent studies reported that macromolecules such as bioactive proteins can reach the retina through the choroid after transcleral delivery (Ambatti et al., 2000; Lee et al., 1999). This delivery route is very promising because it is easily accessible and much less invasive than the vitreal injection or implants.

Targeting of drugs to the retinal tissues via systemic route has been studied with the use of special carriers. Heat-sensitive liposomes containing drugs are injected in the systemic circulation and the retinal vessels are heated up with transpupillary laser application. The
drugs are released from the liposomes in the heated retinal vessels at a very high concentration. This system can selectively deliver drugs in a desired area of the fundus by the selective application of laser photocoagulation (Ogura et al., 1991 and 1993).

Choroidal neovascularization is a vision threatening complication of many retinal diseases such as age-related macular degeneration, degenerative myopia and presumed ocular histoplasmosis. Because neovascular vessels show increased permeability and other unique characteristics different from the normal vasculature, several methods of drug targeting to the choroidal neovascularization have been investigated. One approach is the application of polymer conjugates with drugs. Drugs are conjugated with high molecular weight polymers and injected into the systemic circulation. Because of the big molecular size of the drug-polymer conjugate, the normal vasculature does not leak the conjugate outside of the vessels. However, abnormal vessels such as neovascularization have the increased permeability that leaks big molecules of the conjugates. Once the drug-polymer conjugate accumulates around the abnormal vessels, the conjugate stays in the tissue for a certain period and releases the drug. This phenomenon is known as the enhanced permeability and retention effect (Matsumura and Maeda, 1986; Muggia, 1999; Maeda et al., 2000). Using this effect, the drug could be targeted to the choroidal neovascularization in the form of polymer conjugates. Another approach is the antibody-mediated drug targeting. Recent studies reported that the endothelial cells of the new vessels in the neoplastic tumor expressed unique antigens specific to the newly growing endothelial cells. When the drug is combined with antibodies to these neovascular endothelial cell specific antigens, it will bind only these abnormal endothelial cells without affecting the normal vessels. Drug targeting to the choroidal neovascularization may also be possible with this approach (Burrows and Thorpe, 1994; Huang et al., 1997).

Gene therapy to treat certain retinal diseases is expected to be possible in the near future. The eye has been suggested to be an ideal tissue for gene therapy. This is because the eye ball is a closed chamber separated from the systemic circulation and readily accessible from the outside. Several modalities have been developed to deliver genes to the retinal cells using different kinds of vectors.
7.2 SYSTEM SPECIFIC REVIEW

7.2.1. Treatment approaches for uveitis and retinopathies

The development of newer, more sensitive diagnostic techniques and therapeutic agents renders urgency to the development of more successful ocular delivery system. Potent immuno suppressant therapy in transplant patients and the developing epidemic of Acquired Immuno Deficiency Syndrome (AIDS) have generated an entirely new population of patients suffering from virulent uveitis and retinopathies.

Uveitis is an inflammation of middle vascular tunic of the eye (uveal tract). It is a specific organ disease frequently considered as autoimmune. Uveitis can occur as an ocular manifestation of a variety of autoimmune diseases such as juvenile rheumatoid arthritis, Reiter’s syndrome, and inflammatory bowel diseases (Blagovevic, et al., 1970) and sarcoidosis (Lurhuma et al., 1976). When associated with Behcet's disease uveitis frequently leads to blindness (O’Connor, 1983).

Uveitis can be treated with topical or systemic steroids but frequently recurs after discontinuation of therapy (Stein et al., 1981). Complications of topical steroids use include cataract formation, poor wound healing, toxicity to corneal epithelium and increased intra-ocular pressure (Leopold and Gaster, 1988). Complications arising from systemic administration of steroids are varied and often extremely unpleasant (Gilman, 1990). To overcome the disadvantages of steroid administration (both topical and systemic) in the treatment of uveitis, non-steroidal anti-inflammatory drugs such as indomethacin have been investigated. In recent years, cyclosporine and cyclosporine A have been used to treat some forms of uveitis.

Cyclosporine is an effective secondary agent in the treatment of uveitis. Usually reserved for patients with advanced bilateral disease despite high doses of prednisolone, its main effect is on the recruitment and activation of T cells. It is believed to act by interfering with interleukin 2 (I-2) in the activation of T cell genes (Cornoelli-Piperno et al., 1999; Larson, 1990). Although CD4 lymphocytes are the main target, CD8 cells are also suppressed. Systemic administration is usually through the oral route as a suspension. Some of the most common side effects at a dose of 10 mg/kg include paraesthesias and hyperaesthesia (40%), hypertension (24%), epigastric burning (20%), hypertrichorism and gingivitis (20%) (Nussenblatt et al., 1996). Many patients require long term management, thereby increasing the risk of complications and making careful monitoring of their renal
function, blood pressure and surveillance for malignancy, being an important part of their management.

The extent of penetration after topical application has been the subject of some controversy with some groups reporting no significant permeation into the aqueous (BenErza and Maftzir, 1990) and others finding therapeutic levels in this compartment (Kaswan and Kaplan, 1988). No one has reported therapeutic levels in the vitreous or posterior uvea after topical application and unfortunately intermediate and posterior uveitic syndromes are those most likely to result in severe and irreversible vision loss (Nussenblatt, 1988). The National Eye Institute (USA) recommends that sustained local delivery would treat the disease effectively.

Cytomegalovirus (CMV) is the most common cause of viral retinitis in patients with AIDS, affecting approximately 25% of the patients (Holland et al., 1983; Rosenberg, et al., 1983; Palestine et al., 1984; Khadem et al., 1984; Freeman et al., 1984; Pepose et al., 1985; Culbertson, 1989). If left untreated blindness inevitably results (Henderly et al., 1987).

Intravenous Ganciclovir and foscarnet are effective in the treatment of CMV retinitis, but require frequent intravenous dosing. Serious dose-limiting side effects are associated with both drugs necessitating a two-week period of induction therapy followed by indefinite lower dose maintenance therapy. Retinitis normally reactivates while patients are on maintenance therapy with either drug, with a mean reactivation time of 56 days for Ganciclovir and 59 days for Foscarnet (Anonymous, 1992). Other problems associated with systemic administration include sepsis related to permanent indwelling catheters of long infusion times.

Intravitreal Ganciclovir injections provide a higher intraocular drug concentration than systemic therapy and reduce systemic exposure to the drug. The intravitreal half-life of Ganciclovir in the human eye necessitates frequent injection (at least once each week) to maintain therapeutic levels in the eye (Henry et al., 1987). Repeated intravitreal injections have an attendant risk of cataract formation, retinal detachment, cystoid macular edema, progressive retinal toxicity and endophthalmitis (Harris and Mathalone, 1989).

The main approaches investigated in the treatment of uveitis and CMV retinitis using sustained release ophthalmic formulations to internal structures of the eye are;
7.2.2. Intravitreal administration through Novel delivery vehicles:

7.2.2.1. Approaches for uveitis

Intravitreal injections of cyclosporine have been used in the rat model to treat EAU (experimental autoimmune uveitis) without significant blood levels (Nussenblatt et al., 1985). However, the intraocular half-life of cyclosporine would require multiple weekly injections, making such a delivery impractical (Pearson et al., 1996). Other studies in rabbits have demonstrated that intravitreal administration of 100 µg of cyclosporine is non-toxic to retinal structures (Grisolano, Jr. and Peyman, 1986).

7.2.2.2. Approaches for CMV retinitis

Although the intravenous administration of ganciclovir is used for the treatment of bilateral CMV retinitis and control of CMV infection of other sites (Kuperman et al., 1993), the high toxicity levels of this route has necessitated the search for direct placement of a device in the vitreous.

Akula et al., (1994) have studied treatment of CMV retinitis with intravitreal injections of liposome-encapsulated ganciclovir (GCV) in a patient with AIDS. To overcome the risk to and poor tolerance by end stage patients, GCV was encapsulated in liposomes, to increase the intravitreal retention of the drug, thereby decreasing the frequency of injections. The right eye of the patient was injected with liposome encapsulated ganciclovir and the left eye served as the control, receiving intravitreal free ganciclovir. The right eye showed no retinal hemorrhages or detachment; but vision declined initially, stabilizing later. Weekly examination showed neither progression of CMV retinitis nor new lesions in the right eye, but the left eye showed reactivation of CMV retinitis.

A single application of 20% aqueous solution of GCV by trans-scleral Iontophoresis (1.0 mA for 15 minutes) in rabbit eyes (Lam et al., 1994) gave a vitreal/retinal level of ganciclovir at 74 ± 17 µg/ml at 2 hours as determined by HPLC. At 24 hours after iontophoresis, the vitreal/retinal level was above the therapeutic level at 4.2 ± 0.6 µg/ml. At 72 hours, there were still detectable levels in the vitreous/retina. Thus trans-scleral iontophoresis is able to deliver effective dose of GCV into the vitreous and multiple applications of iontophoresis should be examined as a possible means of CMV treatment.
A pharmacokinetic model of intravitreal drug delivery was developed for describing the elimination and distribution of GCV in the eye following intravenous polymeric delivery (Tojo et al., 1999). The model was based on Fick’s second law of diffusion and assumed a cylindrical vitreous body. The model parameters such as the diffusion coefficient and the partition coefficient of the drug in the vitreous body and its surrounding tissues were determined from in-vitro experiments using rabbit tissues. The time course of in-vivo mean concentration of GCV in the rabbit vitreous body agreed well with the profile calculated from the proposed pharmacokinetic model for both membrane controlled polymeric devices and biodegradable rod-matrix systems. The clinical vitreous concentration following implantation of the membrane controlled delivery system was of the same order of magnitude, but approximately four times lower than that predicted from the proposed model.

The intraocular safety and the anti viral treatment efficacy of the sustained lipid pro-drug of ganciclovir, 1-o-hexadecyl propanediol-3-phospho-ganciclovir (HDP-P-GCV) as an intra vitreal injectable drug system for CMV retinitis were evaluated by Cheng et al., (2000). HDP-P-GCV was formulated into liposomes. The antiviral activity was assessed by DNA reduction in-vitro and intraocular safety was assessed by ophthalmoscopy, electrophysiology, and histology after intravitreal injections, with resultant intravitreal concentration of 0.2, 0.632, 1.12 and 2 mM. The treatment efficacy was evaluated by simultaneous intravitreal injection of HDP-P-GCV and Herpes simplex type I (HSV-I) or by intravitreal injection of HDP-P-GCV at various times before HSV-I intravitreal inoculation. The IC_{50} (in-vitro) of HDP-P-GCV against HSV-I and human cytomegalovirus (H CMV) infected cell was 0.02 and 0.6 µM, respectively. In rabbits, HDP-P-GCV dispersed evenly and maintained a good vitreous clarity at all doses except 2mM final intravitreal concentration.

7.2.3. Intravitreal and scleral implants:

In the area of ocular drug administration, important efforts concern the conception and design of new biodegradable implantable systems to interior parts of the eye to prolong the residence time. The use of implants, which are solid devices to be placed trans-sclerally by minor surgery represent possibilities to achieve increased residence time. The use of biodegradable polymeric devices offers certain advantages over more conventional formulations. If drug release kinetics can be controlled, target tissue concentration of the drug can be maintained in the therapeutically appropriate range, and harmful side effects associated with intravitreal and intravenous administration can be reduced. Continuous long-
term administration can eliminate the discomfort associated with multiple dosing and improve patient compliance.

These potential advantages must be viewed in light of the disadvantages that if does not biodegrade; the device may require surgical removal. The implanted polymer must be biocompatible, causing no tissue irritation, and if it is biodegradable its breakdown products must be non-toxic. The device must be adequately designed to eliminate possible dumping of the dose. There are also problems associated with the removal or the shutting off of release from the implant.

Fig. 7.1 Cross sectional view of the eye, showing position of the intraocular implant

An implant has been designed for the long term intravitreal release of cyclosporine A. It bypasses the systemic circulation avoiding the side effects associated with cyclosporine A, while administering therapeutic doses of the medication to the eye over an extended period of time. The implant consists of a drug pellet coated with silicone attached to a PVA anchor strut. This design has been used to create implants that can release the drug at several rates, depending on the material used to coat the drug pellet. Experimental studies have shown that
devices releasing cyclosporine at a rate of 1.3μg / day can achieve intravitreal levels over a period of 6 month 500 ng/ml, or 5 times the therapeutic level needed to suppress T-cell activation (Pearson et al., 1996; Laferty, 1983). Systemic doses of atleast 5 mg/kg are usually necessary for intravitreal levels of 100 ng/ml (BenEzra et al. 1990).

Scleral implants of indomethacin with sodium alginate as carrier were evaluated in uveitis induced rabbit eye-model. The pharmacodynamic studies showed a marked improvement in the various clinical parameters; congestion, keratitis, flare, clot, aqueous cells and synechias, in the implanted eye when compared to the control eye in the rabbits (Balasubramaniam et al., 2001).

Non degradable drug delivery devices containing 2 or 15 mg of a synthetic corticosteroid, fluocinolone acetonide were constructed (Jaffe et al., 2000a). The long term in-vitro release rates of these devices were determined in protein free buffer or buffer containing 50% plasma protein. Fifteen mg devices were also implated into the vitreous cavities of rabbit eyes. Intravitreal drug levels, the amount of drug remaining in explanted devices, and the release rate of explanted devices were determined over a 1 year time period. Drug toxicity was assessed over this same period by slit lamp examination, indirect ophthalmoscopy, electroretinography and histological examination. The release rate increased by approximately 20% when devices were transferred from protein free buffer to buffer that contained protein. Vitreous level remained fairly constant over the 1 year period. No drug was present in the aqueous humor during this time period. Further, there was no evidence of drug toxicity. A pilot safety and efficacy trial of a novel and sustained drug delivery system containing fluocinolone acetonide to treat patients with severe uveitis was conducted (Jaffe et al., 2000b). The delivery devices designed to deliver the drug for at least 2.5 years were implanted through the parsplana into the vitreous cavity of seven eyes of 5 patients. All patients had severe uveitis at the time of device implantation. Before device implantation the patients underwent complete evaluation. After surgical placement of the implants the patients were re-examined at 1 week, 2 weeks, and 4 weeks and at 1 to 3 month intervals. All the eyes had stabilized or improved visual acuity after device implantation, and four of seven eyes had an improvement of three lines or more. After surgery, at the final visit, no eye had clinically detectable inflammation and all seven eyes had a marked reduction in systemic, topical, and peri-ocular anti-inflammatory medication use. Four eyes had increased IOP 6 weeks to 6 months after device implantation. IOP was controlled on topical medications and no patient experienced intraoperative complications.
Devices giving zero order release of GCV were implanted intravitrealy first in rabbits and then in eight patients with AIDS associated CMV retinitis as part of phase I clinical trial (Ashton et al., 1992). Steady state intravitreal GCV levels were obtained and elimination rate constants were calculated assuming first order pharmacokinetics. Normalizing for retinal surface area, distribution volume and anatomic volume, the retinal elimination rate constants were found to be 0.017 cm$^{-2}$ hr$^{-1}$ in rabbits and 0.015 cm$^{-2}$ hr$^{-1}$ in humans. The study indicated that rabbit eye could serve as a good model for studying IV pharmacokinetic and suggested a common elimination mechanism, which may be trans-vitreal.

Smith et al., (1992) developed devices that release GCV at rates of 2μg/ h and 5μg/ h in-vitro. When implanted into the vitreous of rabbit eyes, mean intravitreal GCV levels of 9mg/L and 16mg/L were maintained for more than 80 and 42 days, respectively. The devices were found to be well tolerated and may prove useful in the clinical management of CMV retinitis in patients with AIDS.

Scleral implants of GCV for the treatment of CMV retinitis were evaluated in rabbits (Kunou et al., 1995). The scleral implants were made of poly (DL-lactide) (PLA) or poly (DL-lactide-co-glycolide) (PLGA) and contained various amounts of GCV. The in-vitro release studies demonstrated a triphasic release pattern. The in-vivo release and biodegradation was studied using 25% GCV loaded implant made from PLGA (75/25) in pigmented rabbits. The GCV concentration in the range of ED$_{90}$ for human GCV was maintained in the vitreous for over 3 months and in the retina/choroid for over 5 months. The GCV concentration was greater in the retina/choroid than in the vitreous throughout the study. The scleral implant showed two phases of biodegradation. All the scleral implants separated into two pieces at the site of scleral penetration and displaced into the vitreous body 10 weeks after implantation. The disadvantage of the second burst in the late phase of release from the developed scleral implants was modified by blending PLA of two different molecular weights. Blending of PLAs of different molecular weights prevented the onset of the second burst and the GCV release profiles followed pseudo-zero order kinetics after the initial burst as resulting from a diffusional mechanism following Higuchi equation. Duration of the GCV release was also controlled by changing the blending ratio of high and low molecular weight PLA. Further, the GCV concentrations in the vitreous after implantation of the scleral implant with a blending ratio of 80/20 were maintained in the range of effective level for 6 months without a significant burst (Kunou et al., 2000).
A surgically implantable device for sustained intra vitreal release of GCV was reported (Sanborn *et.al.*, 1992). The device delivered GCV intraocularly over approximately 4 to 5 months. Eight patients with AIDS and associated CMV retinitis were recruited as part of phase 1 study. Thirteen eyes with active CMV retinitis underwent surgical implantation of the GCV device. All eyes showed resolution of the CMV retinitis; none showed progression. Surgical complications included mild vitreous hemorrhage, astigmatism and suprachoroidal placement of the device.

A randomized controlled clinical trial to assess the safety and efficacy of a 1μg/h GCV release implant in AIDS patients was conducted (Martin, *et.al.*, 1994). Patients with previously untreated peripheral CMV retinitis were randomly assigned either to immediate treatment with GCV implant or to deferred treatment. Standardized fundus photographs were taken at 2-week intervals and analyzed in a masked fashion. The GCV implant was found to be effective for the treatment of CMV retinitis.

Morley *et al.* (1995) reported their surgical experience in replacing empty ganciclovir implants in patients with AIDS related CMV retinitis. Nine eyes in eight patients received two or more implants and the average time before a second implant was needed was 6 months. CMV retinitis was controlled in all patients except one. Three patients required intermittent intravenous exogenous anti-CMV therapy, one for persistent CMV retinitis and two for systemic CMV infection. Visual acuity of 20/40 or better was maintained in five of eight patients, despite a long standing history of CMV retinitis.

Similarly Guembel *et al.* (1999) reported their surgical experience after sustained release GCV treatment, as well as replacing empty GCV implants in patients with AIDS and CMV retinitis. 79 eyes of 49 patients received 99 intravitreal GCV implants. Patients were examined monthly after implant surgery. Follow-up periods ranged from 6 to 128 weeks. At the first three week post-operative visit, 73 eyes (97.2%) of 46 patients exhibited stable conditions. In 6 eyes (3.8%) of 3 patients, further progression was noted due to resistance to GCV. The most common early complication was cystoid macular edema, observed in 7 eyes receiving implants. Retinal detachment was the most and common late complication in 11 eyes. In almost all eyes with CMV retinitis and retinal detachment, involvement of more than 25% of the retina was observed. Additional severe complications included extrusion of the first pellet in 2 eyes and cataract as a late complication in 5 eyes. A total of 28 eyes of 16 patients receiving a second implant did not experience significant three-line loss by the end of the follow-up period.
A single-suture technique for the placement of GCV implants was described by Jones and Weinberg (2000), whereby the wound, caused due to surgical placement was closed and the implant secured with a single running suture. The advantage of this method includes the efficiency and elimination of the exposed knots. Further the investigators observed no complications attributable to this technique.

7.2.4. Transscleral Iontophoresis

Transscleral iontophoresis for intravitreal introduction of pharmacological agents was first reported in 1943 by Von Sallaman, who reported very low intravitreal drug levels and concluded that this technique was not efficient. Contrary to this finding, studies by Burstein et al., (1985), Maurice (1986), Choi and Lee (1988) and Barza and co-workers (1986; 1987a; 1987b) suggested that transcleral iontophoresis could be a potentially useful noninvasive technique in the intravitreal delivery of ionizable drugs. Several factors that affect intravitreal drug levels, including intensity and duration of the applied current and the design of the application electrode have been identified. Barza et al. (1986) observed that, even though cefazolin sodium bears a single negative charge and ticarcillin disodium bears two negative charges, similar levels of cefazolin and ticarcillin were obtained under identical conditions.

Transscleral iontophoretic delivery of dexamethasone to rabbit eyes was attempted by Lam et al. (1989). They suggested that a high dose of dexamethasone could be delivered by this technique.

The foregoing description of the routes and problems of drug administration in uveitis and retinopathies can be summarized as below

1. Intravenous and intravitreal injections

- Frequent Administration
- Poor drug penetration into ocular tissues
- Dose related bone marrow depression
- Cataract formation
- Retinal detachment
- Endophthalmitis
- Reactivation
2. Intravitreal non-erodible implants, Liposomes.

- Extended duration of drug release, but surgical implantation under general anesthesia.
- Device removal necessary
- Visual disturbances
- Low drug loading and poor physico-chemical stability of Liposome

3. Iontophoresis (very limited investigation)

- One iontophoretic application is effective for only 48-72 hours.
- Only institutionalized patients eligible
- Possible iontophoretic burns on long term usage.

4. Scleral implants

- Displacement of device due to breaking of device.
- Drug release, if not well modulated, may result in multiple burst releases with short periods of slow release.

7.3. OBJECTIVE AND PLAN OF STUDY

As already indicated NSAIDs like indomethacin have been investigated as a safer alternative to steroid administration in the treatment of uveitis, as the topical and systemic administration of steroids often cause unpleasant complications.

In the present study the following types of indomethacin-loaded implants were formulated and characterized.

- Sodium alginate based compressed implants.
- Ethyl cellulose based film type implants.
- Gellan based film type implants.

The study was planned as follows/

Sodium alginate compressed implants

- Preparation of implants using sodium alginate alone and in combinations with HPMC by direct compression.
- Characterization of the prepared implants for their physico-chemical properties.
- Evaluation of drug release from the implants using 4 different in-vitro methods.
Ethyl cellulose based film type implants

- Preparation of EC implants by casting.
- Characterization of the prepared implants for their physico-chemical properties.
- Evaluation of drug release using a static dissolution setup.
- Pharmacodynamic evaluation of the implants in uveitis induced rabbit eye model.

Gellan based film type implants

- Preparation of implants by casting.
- Characterization of the prepared implants for their physico-chemical properties.
- Selection of a suitable plasticizer for gellan films through determination of void volume of drug free gellan films.
- Morphological evaluation of the implants by SEM.
- Evaluation of drug release kinetics using the static dissolution setup.
- Pharmacodynamic evaluation of the implants in uveitis induced rabbit eye model.
- Pharmacokinetic evaluation of the implants in normal rabbit eye model.

7.4. MATERIALS

Sodium alginate : Loba Chemie Pvt. Ltd., Mumbai, India
Gellan : Sigma, USA
Ethyl cellulose : C. D. H., Mumbai, India.
Indomethacin : Jagsonpal Pharmaceuticals, New Delhi,
HPMCK-100 : Dow Chemicals, USA
Di-butyl phthalate : Loba Chemie Pvt. Ltd., Mumbai
Di-ethyl phthalate : Loba Chemie Pvt. Ltd., Mumbai,
Calcium chloride.2H₂O : Qualigens Fine Chemicals, Mumbai,
Propylene glycol : S. D. Fine Chemicals, Mumbai, India
PEG 200 : S. D. Fine Chemicals, Mumbai, India
PEG 400 : S. D. Fine Chemicals, Mumbai, India
Glycerol : S. D. Fine Chemicals, Mumbai, India
Potassium dihydrogen-o-PO₄ : Qualigens Fine Chemicals, Mumbai,
Sodium hydroxide : S. D. Fine Chemicals, Mumbai, India
Agar : Hi-media Pvt. Ltd., Mumbai, India
Methanol : Qualigens Fine Chemicals, Mumbai,
Bovine Serum Albumin : Spectrochem Ltd., Mumbai, India
7.5. METHODS

7.5.1. Calibration curve of indomethacin in phosphate buffer pH 7.4.

Stock solution (1 mg / ml) of indomethacin was prepared by dissolving 100 mg indomethacin in 10-15 ml of methanol and the volume was made up to 100 ml with phosphate buffer pH 7.4. From this stock solution, graded dilutions were made to obtain standard solutions of 5-50 µg/ml range using phosphate buffer pH 7.4. The absorbance of the solutions was noted at 319.5 nm on a UV spectrophotometer (UV-1601, Shimadzu, Japan). A calibration curve was constructed, which was utilized for both assay procedures and in- vitro drug release studies.

7.6. SODIUM ALGINATE COMPRESSED IMPLANTS

7.6.1. Fabrication of implants

Indomethacin and sodium alginate alone and in combinations with HPMC with or without calcium chloride were mixed together well in a mortar and slugged on a single punch machine using 12mm diameter punches. The resultant slugs were broken and sieved through sieve #60 and #100. The granules passed through #60 and retained over #100 were compressed directly using 2x7.5mm punches to yield track-field type implants.

7.6.2. Evaluation of implants

7.6.2.1. Weight, drug content uniformity, friability and hardness.

For uniformity of weight, ten implants from each batch were weighed individually and their average weight was determined.

For determination of uniformity of drug content, 6 implants from each batch were weighed individually and dissolved in 50 ml of Phosphate buffer pH 7.4. The resultant solution was filtered through G2 glass filter. An aliquot of the filtrate was diluted suitably and analyzed for indomethacin content at 319.5nm (Shimadzu, UV-1601, Japan).

Friability and hardness were tested using Roche's friabilator and Monsanto hardness tester using 6 and 10 implants, respectively.

The release studies from the prepared implants were studied using 4 different methods.
7.6.2.2. *In-vitro* dissolution studies using Agar diffusion method:

10 ml of 1 and 2% w/v sterilized agar solution was poured on petri dishes of 90 mm diameter, aseptically and allowed to set. A circular hole was made in the center of the agar plates and the implant was placed at the center of the bore and covered with the agar plug. The implants were removed at pre-determined time intervals and dispersed in phosphate buffer pH 7.4 and filtered. The filtrate was diluted with the same buffer to a known volume and an aliquot was used for determining the residual drug content in the implant, spectrophotometrically (319.5 nm). The agar gel was dissolved in hot phosphate buffer pH 7.4 and analyzed for indomethacin content. A blank (without implant) was run concurrently to account for absorbance due to agar gel.

7.6.2.3. *In-vitro* dissolution studies using USP apparatus

USP apparatus I was used with 500 ml of phosphate buffer pH 7.4 as the dissolution medium. The basket was rotated at 50 rpm and the temperature was maintained at 37±1°C. 5 ml aliquots were withdrawn at pre-determined time intervals and replaced with an equal volume of the pre-warmed buffer. The samples were analyzed for indomethacin content at 319.5 nm, spectrophotometrically.

7.6.2.4. *In-vitro* dissolution studies by static method

Individually weighed implants were placed in stainless steel mesh holder of dimensions 2×4×6 mm and suspended in amber colored vials containing 10 ml of phosphate buffer pH 7.4 as the dissolution medium. The vials were placed in a vial holder, to prevent dislodging, fitted in a water bath thermostated at 37±1°C. At pre-determined time intervals the dissolution medium was completely withdrawn and replaced with a fresh 10 ml portion of the pre-warmed buffer to ensure sink conditions. The withdrawn samples were analyzed for indomethacin content as described earlier.

7.6.2.5. *In-vitro* dissolution studies on flow through apparatus

The studies were conducted using a dissolution cell, which consisted of two circular plates of 3.8 cm diameter and 1.2 cm thickness, made of acrylate. The plates were held together by means of 3 screws. The bottom plate had a groove of 1.7 cm diameter and 6mm deep, fitted with #80 mesh for supporting the implant. An outlet tube was provided for collecting the eluate. The top plate had a hole for the inlet of the dissolution medium (phosphate buffer pH 7.4). The entire setup was connected from the top by a silicone tubing
of 1mm internal diameter to a peristaltic pump. The flow rate of the medium was maintained at 0.8 ml/hr and the eluate was collected in amber colored vials as a function of time and analyzed for indomethacin content at 319.5 nm. The schematic representation of the apparatus and the detailed procedure are dealt with in Chapter V.

7.6.2.6. Statistical Evaluation

Experimental results are expressed as mean ± standard deviation (S.D.). The student ‘t’ test was performed to determine the level of significance. Differences were considered to be statistically significant at P<0.05

7.6.3. RESULTS AND DISCUSSION

The formulation variables and the physico-chemical characteristics of the various batches of the prepared implants are shown in Tables 7.1 and 7.2, respectively. Thickness, weight and drug content varied within ± 5%.

7.6.3.1. In-vitro dissolution studies

A number of methods are reported in the literature for studying the in-vitro release of drugs from scleral/vitreal implants. For example, Kunou et al (1995 and 2000) studied the in-vitro release from PLGA scleral implants of GCV by incubating the implants in 2 ml of phosphate buffer solution in a shaking water bath at 37°C and Balasubramaniam et al (2001 and 2002) reported a static method for studying the in-vitro release of indomethacin from film type scleral implants of indomethacin. As there are a lot of variations in the different methods used by various investigators, an attempt was made in the present study to evaluate the dissolution profiles of the prepared (compressed) implants using 4 different methods. In all the cases effect of parameters like particle size of the drug, HPMC concentration and calcium chloride concentration were studied.

The drug release was independent of the particle size of the drug from all the methods except the static method, where a significant difference (P<0.05) was observed (Figs 7.2-7.6). However, an increase in the concentration of both HPMC (Figs 7.7-7.11) and calcium chloride (7.12-7.16) caused a significant decrease (P<0.05) in the amount of drug released from all the methods.

Drug release from all the methods followed square root of time kinetics, as evidenced by r values, which were always higher for Q Vs t^{1/2} than for Q Vs t. Further, the release exponent (n) values were also predominantly suggestive of matrix diffusion kinetics,
excepting the agar diffusion method, wherein the ‘n’ values suggested the prevalence of an anomalous (erosion) mechanism in addition to the swelling controlled (matrix) diffusion.

Though various factors are likely to be responsible for the decrease in drug release with an increase in HPMC concentration from all the methods, some general trend seems to appear.

HPMC partially hydrates on the outer surface to form a skin, as a pseudogel, controlling the swelling of the implants, overall dissolution rate and consequently the drug availability. Once the protective gel layer is formed, two rate mechanisms predominate (Majumdar et al., 2001). Firstly, the pseudogel permits additional water to penetrate into the device, extending the gel layer into the implant. Secondly, the outer gel layer fully hydrates and begins to be dissolved by the fluids. For sparingly soluble drugs like indomethacin, the dissolution rate is primarily dependent on diffusion (due to swelling) and to some extent on erosion, which in turn is dependent on viscosity. Thus increasing the concentration of HPMC increases the viscosity of the resultant gel thus resulting in slower drug release.

Cross linking of sodium alginate involves the interaction between cations and G residues in sodium alginate, resulting in the formation of an ‘egg box’ structure (Grant et al., 1973). With increasing concentration of the cross-linker (calcium chloride), more calcium ions will be available for cross-linking, which results in the formation of a dense calcium alginate matrix that can bring about a decrease in drug release.

The ability of sodium alginate to swell when contacted with aqueous dissolution medium results in an increase in the porosity of the matrix, thus facilitating the mobilization of the water molecules into the polymer matrix. In case of sodium alginate implants containing calcium chloride, the water present in the dissolution medium dissolves the calcium chloride resulting in an in-situ cross-linking of sodium alginate, which is an instantaneous phenomena. In case of the agar diffusion and the continuous flow through methods, the amount of aqueous medium that contacted the implant was significantly less and the time of contact between the implant and the dissolution medium was comparatively higher than the other two methods. Hence the cross-linking of the sodium alginate matrix in these two methods would be progressive and more uniform in comparison to the static method, wherein the presence of relatively more dissolution medium would have resulted in more rapid and incomplete cross-linking. In case of USP dissolution method the very fact that the implant disintegrated upon swelling would have resulted in formation of isolated cross-linked mass.
In case of the agar diffusion method an increase in the concentration of agar from 1 to 2\% resulted in a significant decrease (P<0.05) in the amount of drug released. Increase in the concentration of agar from 1 to 2\% resulted in an obvious increase in the strength of the gel formed and a consequent decrease in the amount of available aqueous medium, as the water molecules would be entrapped within the gel structure, resulting in decreased rate of diffusion of the drug from the implants to the surrounding medium. It has been reported earlier that the drug diffusion coefficients from HPMC and sodium alginate matrices strongly dependent on the water content of the system (Siepmann et al., 1999).

Out of the methods studied, the agar diffusion method and the continuous flow through method seem to have the potential to prolong the drug release from the compressed implant and also simulate to certain extent the in-vivo conditions as far as the placement of the device is concerned. The static method, which was successfully utilized for evaluating drug release kinetics from film type implants (Balasubramaniam et al., 2001; Balasubramaniam and Pandit, 2002) was not suitable for evaluating drug release from the compressed implant, since the mesh holder, in which the implant was placed acted as an impediment to uniform swelling of the implant, resulting in irregular swelling during the course of the study.
Table 7.1 Composition of sodium alginate based implants

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>sodium alginate (mg)</th>
<th>Calcium chloride (mg)</th>
<th>HPMC (K-100) (mg)</th>
<th>Indomethacin (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSI_1</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>BSI_2</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>BSI_3</td>
<td>12.5</td>
<td>-</td>
<td>12.5</td>
<td>5</td>
</tr>
<tr>
<td>BSI_4</td>
<td>10</td>
<td>-</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>BSI_5</td>
<td>20</td>
<td>5</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>BSI_6</td>
<td>15</td>
<td>-</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>BSI_7</td>
<td>17.5</td>
<td>7.5</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>BSI_8</td>
<td>15</td>
<td>10</td>
<td>-</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 7.2 Physico chemical properties of prepared implants

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Weight (mg ± S.D.)</th>
<th>Thickness (mm ± S.D.)</th>
<th>Hardness * (kg/cm²)</th>
<th>Friability (%) ± S.D.</th>
<th>DCU** (%) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSI1</td>
<td>29.2 ± 0.48</td>
<td>0.54 ± 0.001</td>
<td>7.5 ± 0.48</td>
<td>0.90 ± 0.01</td>
<td>97.5 ± 0.56</td>
</tr>
<tr>
<td>BSI2</td>
<td>28.5 ± 0.67</td>
<td>0.52 ± 0.08</td>
<td>7.5 ± 0.34</td>
<td>0.94 ± 0.04</td>
<td>98.4 ± 0.23</td>
</tr>
<tr>
<td>BSI3</td>
<td>30.1 ± 0.44</td>
<td>0.55 ± 0.004</td>
<td>8.0 ± 0.18</td>
<td>0.92 ± 0.02</td>
<td>99.8 ± 0.35</td>
</tr>
<tr>
<td>BSI4</td>
<td>29.7 ± 0.18</td>
<td>0.54 ± 0.006</td>
<td>7.5 ± 0.66</td>
<td>0.92 ± 0.05</td>
<td>98.7 ± 0.58</td>
</tr>
<tr>
<td>BSI5</td>
<td>30.5 ± 0.18</td>
<td>0.58 ± 0.004</td>
<td>8.0 ± 0.48</td>
<td>0.92 ± 0.03</td>
<td>100.01 ± 0.14</td>
</tr>
<tr>
<td>BSI6</td>
<td>29.8 ± 0.44</td>
<td>0.55 ± 0.006</td>
<td>7.0 ± 0.81</td>
<td>0.90 ± 0.04</td>
<td>97.9 ± 0.14</td>
</tr>
<tr>
<td>BSI7</td>
<td>28.9 ± 0.81</td>
<td>0.53 ± 0.004</td>
<td>7.5 ± 0.11</td>
<td>0.94 ± 0.03</td>
<td>99.4 ± 0.21</td>
</tr>
<tr>
<td>BSI8</td>
<td>29.7 ± 0.74</td>
<td>0.54 ± 0.002</td>
<td>7.5 ± 0.41</td>
<td>0.90 ± 0.02</td>
<td>99.6 ± 0.48</td>
</tr>
</tbody>
</table>

*Hardness kg/cm² (Monsanto hardness tester)

** Drug content uniformity
Table 7.3 Kinetic parameters of drug release from prepared implants, 1% agar diffusion method

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>$r (Q Vs t)$</th>
<th>$r (Q Vs t^{1/2})$</th>
<th>$K^a$ (µg/mm$^2$h$^{-1/2}$)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSI$_1$</td>
<td>0.934</td>
<td>0.971</td>
<td>69.3</td>
<td>0.787</td>
</tr>
<tr>
<td>BSI$_2$</td>
<td>0.934</td>
<td>0.700</td>
<td>72.9</td>
<td>0.793</td>
</tr>
<tr>
<td>BSI$_3$</td>
<td>0.940</td>
<td>0.973</td>
<td>56.45</td>
<td>0.817</td>
</tr>
<tr>
<td>BSI$_4$</td>
<td>0.928</td>
<td>0.962</td>
<td>37.71</td>
<td>0.871</td>
</tr>
<tr>
<td>BSI$_5$</td>
<td>0.980</td>
<td>0.992</td>
<td>64.04</td>
<td>0.814</td>
</tr>
<tr>
<td>BSI$_6$</td>
<td>0.945</td>
<td>0.975</td>
<td>48.59</td>
<td>0.845</td>
</tr>
<tr>
<td>BSI$_7$</td>
<td>0.931</td>
<td>0.959</td>
<td>50.41</td>
<td>0.768</td>
</tr>
<tr>
<td>BSI$_8$</td>
<td>0.951</td>
<td>0.978</td>
<td>41.64</td>
<td>0.759</td>
</tr>
</tbody>
</table>
Table 7.4 Kinetic parameters of drug release from prepared implants, 2% agar diffusion method

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>r (Q Vs t)</th>
<th>r (Q Vs t&lt;sup&gt;1/2&lt;/sup&gt;)</th>
<th>K&lt;sub&gt;a&lt;/sub&gt; (µg/mm&lt;sup&gt;2&lt;/sup&gt;h&lt;sup&gt;-1/2&lt;/sup&gt;)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSI&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.965</td>
<td>0.985</td>
<td>62.3</td>
<td>0.814</td>
</tr>
<tr>
<td>BSI&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.966</td>
<td>0.986</td>
<td>65.7</td>
<td>0.748</td>
</tr>
<tr>
<td>BSI&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.970</td>
<td>0.988</td>
<td>37.15</td>
<td>0.871</td>
</tr>
<tr>
<td>BSI&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.937</td>
<td>0.970</td>
<td>26.91</td>
<td>0.864</td>
</tr>
<tr>
<td>BSI&lt;sub&gt;5&lt;/sub&gt;</td>
<td>0.973</td>
<td>0.990</td>
<td>44.63</td>
<td>0.779</td>
</tr>
<tr>
<td>BSI&lt;sub&gt;6&lt;/sub&gt;</td>
<td>0.969</td>
<td>0.989</td>
<td>35.46</td>
<td>0.781</td>
</tr>
<tr>
<td>BSI&lt;sub&gt;7&lt;/sub&gt;</td>
<td>0.961</td>
<td>0.979</td>
<td>45.93</td>
<td>0.818</td>
</tr>
<tr>
<td>BSI&lt;sub&gt;8&lt;/sub&gt;</td>
<td>0.941</td>
<td>0.972</td>
<td>35.70</td>
<td>0.864</td>
</tr>
</tbody>
</table>
Table 7.5 Kinetic parameters of drug release from prepared implants, USP dissolution apparatus

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>( r (Q Vs t) )</th>
<th>( r (Q Vs t^{1/2}) )</th>
<th>( K^a (\mu g/mm^2 h^{1/2}) )</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSI_1</td>
<td>0.9572</td>
<td>0.9874</td>
<td>147.17</td>
<td>0.615</td>
</tr>
<tr>
<td>BSI_2</td>
<td>0.9554</td>
<td>0.9854</td>
<td>142.12</td>
<td>0.583</td>
</tr>
<tr>
<td>BSI_3</td>
<td>0.9875</td>
<td>0.9986</td>
<td>103.93</td>
<td>0.644</td>
</tr>
<tr>
<td>BSI_4</td>
<td>0.9874</td>
<td>0.9988</td>
<td>89.34</td>
<td>0.722</td>
</tr>
<tr>
<td>BSI_5</td>
<td>0.9728</td>
<td>0.9959</td>
<td>108.30</td>
<td>0.575</td>
</tr>
<tr>
<td>BSI_6</td>
<td>0.9897</td>
<td>0.9985</td>
<td>99.76</td>
<td>0.649</td>
</tr>
<tr>
<td>BSI_7</td>
<td>0.9678</td>
<td>0.9946</td>
<td>106.28</td>
<td>0.592</td>
</tr>
<tr>
<td>BSI_8</td>
<td>0.9738</td>
<td>0.9966</td>
<td>97.92</td>
<td>0.607</td>
</tr>
<tr>
<td>Batch Code</td>
<td>$r$ (Q Vs t)</td>
<td>$r$ (Q Vs $t^{1/2}$)</td>
<td>$K^a$ (µg/mm²h⁻¹/²)</td>
<td>n</td>
</tr>
<tr>
<td>------------</td>
<td>-------------</td>
<td>----------------------</td>
<td>---------------------</td>
<td>-----</td>
</tr>
<tr>
<td>BSI₁</td>
<td>0.8971</td>
<td>0.9662</td>
<td>31.62</td>
<td>0.591</td>
</tr>
<tr>
<td>BSI₂</td>
<td>0.8858</td>
<td>0.9592</td>
<td>35.25</td>
<td>0.426</td>
</tr>
<tr>
<td>BSI₃</td>
<td>0.8744</td>
<td>0.9525</td>
<td>25301</td>
<td>0.470</td>
</tr>
<tr>
<td>BSI₄</td>
<td>0.8542</td>
<td>0.9402</td>
<td>28.56</td>
<td>0.704</td>
</tr>
<tr>
<td>BSI₅</td>
<td>0.8830</td>
<td>0.9570</td>
<td>34.30</td>
<td>0.456</td>
</tr>
<tr>
<td>BSI₆</td>
<td>0.8981</td>
<td>0.9681</td>
<td>30.55</td>
<td>0.564</td>
</tr>
<tr>
<td>BSI₇</td>
<td>0.8781</td>
<td>0.9476</td>
<td>31.72</td>
<td>0.455</td>
</tr>
<tr>
<td>BSI₈</td>
<td>0.9126</td>
<td>0.9733</td>
<td>29.77</td>
<td>0.549</td>
</tr>
</tbody>
</table>
Table 7.7 Kinetic parameters of drug release from prepared implants, continuous flow apparatus method

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>$r$ (Q Vs t)</th>
<th>$r$ (Q Vs $t^{1/2}$)</th>
<th>$K^a$ (µg/mm$^2$h$^{-1/2}$)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSI_1</td>
<td>0.9715</td>
<td>0.9935</td>
<td>34.29</td>
<td>0.551</td>
</tr>
<tr>
<td>BSI_2</td>
<td>0.9733</td>
<td>0.9948</td>
<td>35.09</td>
<td>0.519</td>
</tr>
<tr>
<td>BSI_3</td>
<td>0.9797</td>
<td>0.9963</td>
<td>33.07</td>
<td>0.691</td>
</tr>
<tr>
<td>BSI_4</td>
<td>0.9734</td>
<td>0.9949</td>
<td>35.35</td>
<td>0.682</td>
</tr>
<tr>
<td>BSI_5</td>
<td>0.9671</td>
<td>0.9939</td>
<td>29.10</td>
<td>0.548</td>
</tr>
<tr>
<td>BSI_6</td>
<td>0.9858</td>
<td>0.9953</td>
<td>31.13</td>
<td>0.731</td>
</tr>
<tr>
<td>BSI_7</td>
<td>0.9741</td>
<td>0.9966</td>
<td>28.87</td>
<td>0.651</td>
</tr>
<tr>
<td>BSI_8</td>
<td>0.9706</td>
<td>0.9960</td>
<td>26.74</td>
<td>0.674</td>
</tr>
</tbody>
</table>
Fig 7.2: Effect of particle size by agar diffusion method (1% agar)
Fig. 7.3: Effect of particle size by agar diffusion (2% agar)
Fig. 7.4: Effect of particle size on drug release (USP method)
Fig. 7.5: Effect of particle size on drug release (Continuous flow)
Fig. 7.6: Effect of particle size on drug release (static model)
Fig. 7.7: Effect of HPMC concentration on drug release (1% agar)
Fig. 7.8: Effect of HPMC concentration on drug release (2% agar)
Fig. 7.9: Effect of HPMC concentration on drug release (USP method)
Fig. 7.10: Effect of HPMC concentration on drug release (Continuous flow)
Fig. 7.11: Effect of HPMC concentration on drug release (Static model)
Fig. 7.12: Effect of Calcium chloride concentration on drug release (1% agar)
Fig. 7.13: Effect of Calcium chloride concentration on drug release (2% agar)
Fig. 7.14: Effect of Calcium chloride concentration on drug release (USP Model)
Fig. 7.15: Effect of Calcium chloride concentration on drug release (Continuous flow)
Fig. 7.16: Effect of Calcium chloride concentration on drug release (Static model)
ETHYL CELLULOSE BASED FILM TYPE SCLERAL IMPLANTS OF IINDOMETHACIN

7.7.1. Fabrication of implants

Implants containing ethyl cellulose, indomethacin and dibutyl phthalate (DBP) or diethyl phthalate (DEP) as plasticizers were prepared using chloroform as the solvent. Ethyl cellulose was dissolved in chloroform and the calculated quantities of indomethacin and plasticizers were incorporated in the resultant solution followed by stirring and degassing. The resulted solution was degassed and casted on Scotchpak® liner and dried at room temperature (30° C) under an inverted funnel for 24 hours to get films of 40 cm² area. The films were removed and cut into implants of 1 × 5 mm (0.36 to 0.49mm thickness), dried at 40° C in an oven to constant weight (to ensure complete removal of chloroform) and stored, in amber colored glass vials in a desiccator, until further use. These dimensions of the implants were observed earlier to be suitable for scleral implantation in the rabbit eye (Balasubramaniam et al., 2001). The compositions of the various batches of implants –BEC₁ to BEC₈ are shown in Table 7.8.

7.7.2. Evaluation of the implants

7.7.2.1. Thickness, weight and drug content uniformity

The thickness of the implant was measured at 5 different randomly selected spots with a screw gauge. For uniformity of weight, ten implants from each batch were weighed individually and their average was determined.

For determination of uniformity of drug content, 6 implants from each batch were weighed individually and dissolved in 50 ml of phosphate buffer pH 7.4 the resultant dispersion was filtered through G2 glass filter. An aliquot of the filtrate was diluted suitably and analyzed for indomethacin content at 319.5 nm (Shimadzu, UV-1601, Japan).

7.7.2.2. Percent moisture (absorbed/loss)

A modification of the American Standard Test Method, test no. D570-59T, was used for the testing of moisture absorption/loss of implants (Kanig and Goodman, 1962).

The implants were conditioned by placing them in an oven at 40° C and for the drying time that had been used originally in drying the wet patches. This step was carried out to ensure uniformity of the patches within each group before testing. The conditioned samples were accurately weighed, kept in a constant humidity chamber (humidity of 80.5% at 20-
30°C. After 24 and 48 hours the implants were removed and weighed again. The experiments were carried out in triplicate. Percent moisture absorption was calculated by means of the following formula

\[
\text{Percent moisture absorption} = \frac{(\text{Wt of exposed film} - \text{Wt of conditioned film})}{\text{Wt of conditioned film}} \times 100.
\]

7.7.2.3. Swelling index

Weighted implants were placed in a stainless steel wire mesh holder of dimensions 2×8×8 mm and the system was accurately weighed and placed inside vials containing 10 ml of phosphate buffer pH 7.4. The holder was removed at pre-determined time intervals, dried and weighed and the swelling index was then calculated using the formula (Vyavahare et al., 1990)

\[
\text{Swelling index} = \frac{\text{SW}_2 - \text{SW}_1}{\text{SW}_0}
\]

Where

SW\(_1\) is the weight of the holder,

SW\(_2\) is the weight of the swollen implant and holder and

SW\(_0\) is the initial weight of the implant.

Swelling index is indicated herein after as equilibrium water uptake (EWU).

All the implants retained their original shape at the end of the studies (8 hours). The experiments were carried out in triplicate.

7.7.2.4. Surface pH

Surface pH of the implants were determined by allowing them to swell in closed petri dish at room temperature for 30 min in 0.1 ml of double distilled water. The swollen devices were removed and placed on pH paper to determine the surface pH. After 60 s the color developed was compared with the standard color scale.
7.7.2.5. In-vitro release studies

Weighed implants were placed in a stainless steel wire mesh holder of dimensions 2×4×6 mm and suspended in amber colored vials containing 3 ml of phosphate buffer pH 7.4, as the dissolution medium. The vials were stoppered and placed in the vial holder (to prevent dislodging) fitted in a water bath, thermostated at 37 ± 1°C. At pre-determined time intervals, the dissolution medium was completely withdrawn and replaced with a fresh 3 ml portion of the pre-warmed buffer, to ensure sink condition. The samples were analyzed for Indomethacin content at 319.5 nm, after appropriate dilutions. The studies were conducted in triplicate for 48 hours.

7.7.2.6. Pharmacodynamic studies

A total of 6 albino rabbits weighing 1-2 Kg (1.68 ± 0.74) were used for this study. Prior to the commencement of the study the animals with observed ocular abnormalities were excluded after thorough examination. The animals were housed in individual cages, and the experiments were conducted in a sanitized room at a temperature maintained around 24° C. Uveitis was induced in both eyes of each rabbit by an intra-vitreal injection (30 g needle) of a sterile solution of BSA (0.5 ml / eye of 50μ/ml sterile solution). Two days after the intra-vitreal injection of BSA, the eyes of the individual rabbits were observed by slit-lamp examination for the induction of uveitis. The following clinical parameters - congestion, keratitis (keratopathy), flare, aqueous cells, clot and synechias were evaluated and scored as described in Chapter VI.

Based on the pre-treatment scores of the above descriptors, the eye (left or right) showing more severe uveitis was selected for placing the implant.

The animals were lightly anaesthetized with ether. The eye into which the implant was to be surgically placed was anaesthetized by instillation of one drop of procaine hydrochloride (0.5% w/v solution).

A fornix based conjunctival flap was raised. After hemostasis was achieved, a partial thickness scleral pocket was made by a crescent knife 4 mm behind the limbus and the implant was placed. The scleral pocket was then closed with 6.0’ silk suture. The site of the operation was treated with an antibiotic solution (eye drop) to prevent secondary infection for 2 days.
The animals were examined periodically for improvement in the clinical parameters and after 10 days post-implantation, the suture was opened to retrieve the remaining implant, if any, to assay the remaining drug in the implant. Additionally, the tissues around the implantation site were examined for any evidence of redness, swelling and/or other pathological signs.

7.7.2.7. Statistical Evaluation

Experimental results are expressed as mean ± standard deviation (S.D.). In case of multiple comparisons of groups, analysis of variance (ANOVA) was performed. The student’s t-test was also performed to determine the level of significance. Differences were considered to be statistically significant at P<0.05.

7.7.3. RESULTS AND DISCUSSION

The composition of the various batches of implants and their physico-chemical properties are depicted in Tables 7.8 and 7.9, respectively. Ethyl cellulose alone yields very tough films of excellent tensile strength, flexibility and elongation characteristics; yet such films lack suppleness (Rekhi and Jambhekar, 1995). Thus the addition of a plasticizer was necessary for obtaining drug-loaded films with sufficient pliability to facilitate uniform subdivision of implants of the desired size (1×5 mm). Initially, three drug loadings were tried viz., 0.5, 0.6 and 0.75 mg of indomethacin per implant. Implant containing 0.75 mg/implant showed crystallization of the drug over the implant and considerable burst effect. Hence, implants containing 0.5 mg indomethacin were considered to be suitable for this study.

The surface pH of the prepared implants ranged from 7 to 7.4, indicating that the implants did not have an irritation potential (Sampath Kumar et al., 2001), as it is identical with pH of normal ocular fluids.

The moisture absorbing capacity of the implants was generally low in all cases under the conditions applied in the study. The % moisture absorbed by the prepared implants showed only a marginal increase with an increase in the time of exposure to humidity conditions (Table 7.9). Further, the % moisture absorbed was found to be inversely proportional to EC concentration present in the implants. This could be attributed to the pronounced hydrophobic nature of EC matrix (Donbrow and Friedman, 1975). Moreover the nature and concentration of plasticizer used also influenced the amount of moisture absorbed. Due to hydrophilic nature of DEP, such implants absorbed relatively more moisture than the corresponding implants containing DBP.
The results indicated that the extent of swelling of the implants was influenced by the concentration of ethyl cellulose present in the implants, as the EWU for the batches (BEC₃, BEC₆, and BEC₈- composition shown in Table 7.8) containing 10% (w/v) of ethyl cellulose was not attained during the course of the experiment (8 hrs). Comparison of the swelling characteristics of batches BEC₁ and BEC₄ indicate that the EWU for batch BEC₄ was attained at 4 hours while that of BEC₁ at 6 hours (Table 7.10). The presence DEP might be responsible for the observed rapid rate of swelling of batch BEC₄ than BEC₁. Further, the extent of swelling of the implants seemed to increase with decrease in the concentration of the respective plasticizer(s).

The drug release profile from all batches followed Higuchi type kinetics (Table 7.11) as evidenced by $r^2$ values for $Q$ Vs $\sqrt{t}$ and $Q$ Vs $t$.

All the implants remained intact at the end of dissolution studies. This indicated that the drug release mechanism was diffusion through the plasticized film. This observation was similar to that observed by Moroni and Ghebre-Selassie (1995) and Opota et al. (1999) on the release of diphenhydramine from tablets and microgranules containing poly (oxyethylene) homopolymers and ethyl cellulose, respectively.

The results indicated that the amount of drug release decreased progressively with an increase in EC concentration, irrespective of the nature of plasticizer used (viz. DBP and DEP). The observed effect is due to an increase in film thickness with increase in EC concentration. The calculated ‘n’ values obeyed Fickian diffusion at 5% w/v EC concentration, whereas at higher concentrations (7.5 and 10% w/v) an anomalous mechanism prevailed.

As expected the amount of drug release decreased significantly ($P<0.05$) when the concentration of the plasticizer(s) increased from 20 to 25% (w/w), possibly due to an interaction between the polymer and plasticizer networks resulting in lower mobility of the polymer and plasticizer (Guo, 1994). The drug release from the batches containing 20 and 25% DEP was comparatively higher than that from the corresponding batches containing DBP as plasticizer (Fig. 7.17). This could be attributed, firstly to the more hydrophilic nature of DEP in comparison to DBP. Secondly, DEP shows superior plasticizing effect on ethyl cellulose, since it lowers the glass-transition temperature of ethyl cellulose to a greater extent than DBP and finally imparts a higher intrinsic viscosity to EC solution in comparison with DBP.
Two batches (BEC\textsubscript{3} and BEC\textsubscript{6}) of the fabricated implants were selected to study the influence of type of plasticizers on the resolution of induced uveitis in rabbit eyes. Six characteristics of uveitis were evaluated pre- and post-treatment with the fabricated implants. The results (Table 7.12) showed that the type of plasticizer did not influence the resolution of the clinical parameters of uveitis, since both these batches resolved the parameters to similar extent. The implanted sites were exposed after 10 days of placement. The implants had survived at the implanted site in all the cases. Residual drug assays in these implants indicated that 38.05 ± 3.69\% of indomethacin was left behind in batch BEC\textsubscript{3} and 26.42 ± 3.61\% was left in batch BEC\textsubscript{6}. In spite of higher amounts of drug released from BEC\textsubscript{6}, due to the presence of more hydrophilic DEP, the resolution in clinical parameters of these two batches were similar. The implants did not loose their integrity, when retrieved from the implanted site after 10 days. The results further suggested that these implants have the potential to survive and release the drug for prolonged periods of time.
Table 7.8 Composition of Ethyl cellulose based implants

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Ethyl cellulose (conc. % w/v)</th>
<th>Drug / Implant (mg)</th>
<th>Plasticizer conc. (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEC₁</td>
<td>5</td>
<td>0.5</td>
<td>25 (DBP)</td>
</tr>
<tr>
<td>BEC₂</td>
<td>7.5</td>
<td>0.5</td>
<td>25 (DBP)</td>
</tr>
<tr>
<td>BEC₃</td>
<td>10</td>
<td>0.5</td>
<td>25 (DBP)</td>
</tr>
<tr>
<td>BEC₄</td>
<td>5</td>
<td>0.5</td>
<td>25 (DEP)</td>
</tr>
<tr>
<td>BEC₅</td>
<td>7.5</td>
<td>0.5</td>
<td>25 (DEP)</td>
</tr>
<tr>
<td>BEC₆</td>
<td>10</td>
<td>0.5</td>
<td>25 (DEP)</td>
</tr>
<tr>
<td>BEC₇</td>
<td>7.5</td>
<td>0.5</td>
<td>20 (DEP)</td>
</tr>
<tr>
<td>BEC₈</td>
<td>10</td>
<td>0.5</td>
<td>20 (DBP)</td>
</tr>
</tbody>
</table>

The drug sieved through #100 was used in all cases
<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Thickness (mm)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Weight Uniformity (mg)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DCU (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Moisture absorption (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24 hrs</td>
</tr>
<tr>
<td>BEC₁</td>
<td>0.36 ± 0.02</td>
<td>2.84 ± 0.35</td>
<td>99.17</td>
<td>10.24</td>
</tr>
<tr>
<td>BEC₂</td>
<td>0.391 ± 0.03</td>
<td>3.08 ± 0.18</td>
<td>99.65</td>
<td>9.78</td>
</tr>
<tr>
<td>BEC₃</td>
<td>0.49 ± 0.02</td>
<td>3.86 ± 0.28</td>
<td>99.82</td>
<td>6.68</td>
</tr>
<tr>
<td>BEC₄</td>
<td>0.368 ± 0.02</td>
<td>2.98 ± 0.25</td>
<td>97.61</td>
<td>10.48</td>
</tr>
<tr>
<td>BEC₅</td>
<td>0.374 ± 0.01</td>
<td>3.06 ± 0.31</td>
<td>99.26</td>
<td>9.62</td>
</tr>
<tr>
<td>BEC₆</td>
<td>0.458 ± 0.03</td>
<td>3.18 ± 0.44</td>
<td>99.88</td>
<td>7.18</td>
</tr>
<tr>
<td>BEC₇</td>
<td>0.41 ± 0.02</td>
<td>3.07 ± 0.22</td>
<td>97.98</td>
<td>8.88</td>
</tr>
<tr>
<td>BEC₈</td>
<td>0.47 ± 0.01</td>
<td>3.68 ± 0.21</td>
<td>100.67</td>
<td>7.08</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.D. (n = 5 for thickness and n = 10 for mass uniformity)

<sup>b</sup> DCU Drug content uniformity
Table 7.10 Swelling index of Ethyl cellulose implants

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Time (hr)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>BEC₁</td>
<td>0.005 ± 0.0008</td>
<td>0.0054 ± 0.0004</td>
<td>0.0082 ± 0.001</td>
<td>0.0099 ± 0.0004</td>
<td>0.013² ± 0.0012</td>
<td>0.0078 ± 0.0006</td>
</tr>
<tr>
<td>BEC₂</td>
<td>0.0022 ± 0.0018</td>
<td>0.0027 ± 0.0008</td>
<td>0.0048 ± 0.0001</td>
<td>0.0061 ± 0.0021</td>
<td>0.019² ± 0.0004</td>
<td>0.0094 ± 0.0021</td>
</tr>
<tr>
<td>BEC₃</td>
<td>0.0013 ± 0.0001</td>
<td>0.0018 ± 0.001</td>
<td>0.0019 ± 0.001</td>
<td>0.0031 ± 0.0002</td>
<td>0.0045 ± 0.0004</td>
<td>0.0047 ± 0.0003</td>
</tr>
<tr>
<td>BEC₄</td>
<td>0.0052 ± 0.0012</td>
<td>0.0061 ± 0.0008</td>
<td>0.018² ± 0.0007</td>
<td>0.010 ± 0.0015</td>
<td>0.0082 ± 0.0009</td>
<td>0.0089 ± 0.0002</td>
</tr>
<tr>
<td>BEC₅</td>
<td>0.0023 ± 0.0012</td>
<td>0.003 ± 0.0008</td>
<td>0.0044 ± 0.0012</td>
<td>0.0071 ± 0.0021</td>
<td>0.021² ± 0.0014</td>
<td>0.010 ± 0.0019</td>
</tr>
<tr>
<td>BEC₆</td>
<td>0.0021 ± 0.0021</td>
<td>0.0029 ± 0.0007</td>
<td>0.0039 ± 0.0009</td>
<td>0.0045 ± 0.0018</td>
<td>0.0054 ± 0.0011</td>
<td>0.0059 ± 0.002</td>
</tr>
<tr>
<td>BEC₇</td>
<td>0.0013 ± 0.0014</td>
<td>0.0024 ± 0.0014</td>
<td>0.0033 ± 0.0009</td>
<td>0.0041 ± 0.0007</td>
<td>0.0048 ± 0.0009</td>
<td>0.0055 ± 0.0017</td>
</tr>
<tr>
<td>BEC₈</td>
<td>0.0011 ± 0.0012</td>
<td>0.002 ± 0.0014</td>
<td>0.0022 ± 0.0001</td>
<td>0.0031 ± 0.004</td>
<td>0.0048 ± 0.002</td>
<td>0.0051 ± 0.003</td>
</tr>
</tbody>
</table>

² Equilibrium water uptake values

Values are represented as mean ± S.D. (n=3)
Table 7.11 Kinetic parameters of drug release from prepared implants

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>$r^{2a}$ (Q Vs t)</th>
<th>$r^{2b}$ (Q Vs $t^{1/2}$)</th>
<th>$K^a$ (mg/mm²·h⁻¹/²)</th>
<th>$T_{50%}$ (hr)</th>
<th>$n^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEC₁</td>
<td>0.766</td>
<td>0.915</td>
<td>0.150</td>
<td>15.05</td>
<td>0.536</td>
</tr>
<tr>
<td>BEC₂</td>
<td>0.760</td>
<td>0.908</td>
<td>0.091</td>
<td>30.64</td>
<td>0.705</td>
</tr>
<tr>
<td>BEC₃</td>
<td>0.739</td>
<td>0.894</td>
<td>0.0819</td>
<td>-</td>
<td>0.644</td>
</tr>
<tr>
<td>BEC₄</td>
<td>0.776</td>
<td>0.917</td>
<td>0.0155</td>
<td>7.95</td>
<td>0.573</td>
</tr>
<tr>
<td>BEC₅</td>
<td>0.815</td>
<td>0.938</td>
<td>0.118</td>
<td>-</td>
<td>0.685</td>
</tr>
<tr>
<td>BEC₆</td>
<td>0.803</td>
<td>0.931</td>
<td>0.144</td>
<td>-</td>
<td>0.593</td>
</tr>
<tr>
<td>BEC₇</td>
<td>0.791</td>
<td>0.922</td>
<td>0.135</td>
<td>31.46</td>
<td>0.658</td>
</tr>
<tr>
<td>BEC₈</td>
<td>0.823</td>
<td>0.934</td>
<td>0.110</td>
<td>-</td>
<td>0.644</td>
</tr>
</tbody>
</table>

* $r^{2a}$ According to zero order
* $r^{2b}$ According to matrix diffusion
* $K^a$ Release exponent

- 50% drug release was not attained during the course of the study
Table 7.12 Pharmacodynamic Studies of the prepared EC based implants

<table>
<thead>
<tr>
<th>Batch</th>
<th>Animal No</th>
<th>Treated Eye</th>
<th>Clinical parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Congestion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pr-T</td>
</tr>
<tr>
<td>BEC3</td>
<td>1</td>
<td>R*</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>R</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L*</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>R</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L*</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>R*</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>R*</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>R</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L*</td>
<td>4+</td>
</tr>
</tbody>
</table>
Fig. 7.17: Effect of Pasticizers on drug release from implants
with varying concentration of EC
7.8. GELLAN BASED FILM TYPE IMPLANTS OF INDOMETHACIN

7.8.1. Preparation of implants

Ocular implants of indomethacin were prepared by casting, using gellan as the carrier plasticized individually with glycerol, PG, PEG 200 and 400 at 10% w/w concentration. Based on initial screening glycerol and PG were selected for full scale studies.

Implants with different concentrations of gellan and of glycerol and PG (Table 7.13) were prepared using distilled water as the solvent. Gellan was dissolved in hot distilled water (75°C) and the calculated quantity of indomethacin, to give a drug load of 1 mg/implant was incorporated into it, followed by further stirring for 14-16 hours (at 45°C) and degassing. The resultant dispersion was casted on leveled glass moulds (6.5 × 6.5 × 0.8 cm) and dried in an oven at 50°C for 20–22 hours. The films were removed and cut into implants of 1 × 5 mm (0.48 to 0.81 mm thickness) using a surgical scalpel and stored in amber colored glass vials in a desiccator until further use. These dimensions of the implants were observed earlier to be suitable for scleral implantation in the rabbit eye (Balasubramaniam et al., 2001).

7.8.2. Preparation of cross-linked implants

Gellan produces three dimensional networks in the presence of mono and divalent cations by formation of co-ordinates by cross-linking. The characteristics of the cross-linked polymer are outlined elsewhere.

Cross-linking by method I

The parent patch (prepared by casting as described earlier) was placed in petri dishes of 90 mm diameter and 25 ml of calcium chloride solution of different concentrations (5, 10, 15 and 20% w/v) was poured separately over each patch, such that the film remained immersed in the solution. After pre-determined time intervals (1, 2, 4 and 12 hours) the film was inverted in the cross-linking solution and left for a further similar duration (Al-Musa et al., 1999) so as to give effective cross-linking times of 2, 4, 8 and 24 hours, respectively. The film was then removed, dried at 40°C in an oven and cut into implants and stored as described earlier.
Cross-linking by method II

Graded volumes (1, 2 and 3 ml) of calcium chloride solution (4, 8 and 12 % w/w of calcium chloride) were added to aqueous dispersions of gellan-plasticizer-drug and stirred for further 2 hours and casted as described earlier. The dried films were cut into 1 × 5 mm implants and stored in amber colored glass vials till further use.

7.8.3. Preparation of drug free films (placebo)

Placebo films containing gellan (2.5% w/v) with 10, 20, 30 and 40% of plasticizer (glycerol, PG, PEG 200 and 400) were prepared by casting as described. The dried films were used to derive a correlation between the theoretical and experimentally determined plasticizer concentration and to determine the effect of increasing plasticizer concentration on the void volume of gellan films.

7.8.4. Evaluation of the implants

7.8.4.1. Thickness, weight and uniformity of drug content

The thickness of the implants was measured at 5 different randomly selected spots with a screw gauge. For uniformity of weight, 10 implants from each batch were weighed individually and their average determined. For determination of uniformity of drug content, 6 implants from each batch were weighed individually and dissolved in 50 ml of phosphate buffer pH 7.4. The resultant solution was filtered through a G-2 glass filter. An aliquot of the filtrate was suitably diluted and analyzed for indomethacin content at 319.5 nm (Shimadzu, UV-1601, Japan).

7.8.4.2. Physical appearance

Three pieces of films from each parent patch (1×1 cm) were immersed in 20 ml of phosphate buffer pH 7.4 and stored at 37° C for a period of 2 weeks. The physical appearance of these films were observed.

7.8.4.3. Void volume and comparison of theoretical and experimental plasticizer content

Drug free films were prepared with 10, 20, 30 and 40% w/v of plasticizer (glycerol, PG, PEG 200 and 400) by casting and 1×1 cm square pieces of film were punched out from the parent film and weighed. This was taken as the initial weight (W_i). The films were then dropped into amber colored vials containing 10 ml of distilled water and allowed to stand for 14 hours. The films were then taken out, dried on tissue paper and reweighed (W_f). These
films were next placed inside an oven at 45° C and weighed periodically until their weight became constant (Wc). The experimental plasticizer ratio (content) was obtained by \((W_i - W_c) / W_i\) and the plasticizer volume \((V_p)\) was obtained by \((W_i - W_c) / \text{density of the respective plasticizers}\). The total volume of water \((V_w)\) in the dried film was obtained by \((W_f-W_c) / 1\) (the density of water being 1 g/cm³) and finally the void volume was obtained from the following relationship \((V_w-V_p)/W_c\) (Guo, 1994).

7.8.4.4. Scanning Electron Microscopy (SEM)

The morphological characteristics of placebo films (containing 2.5% gellan, 10 and 40% PG); implants (containing 15% PG, indomethacin and gellan 2.5%) in the dry state and after 24 and 120 hours of dissolution and implants subjected to cross-linking by method I for 2 and 8 hours with 10% calcium chloride were examined by SEM (Super III A model, International Scientific Instruments, Militpas, CA).

7.8.4.5. In-vitro release studies

Weighed implants were placed in a stainless steel wire mesh holder of dimension 2×4×6 mm and suspended in amber colored vials containing 3 ml of phosphate buffer pH 7.4 as the dissolution medium. The vials were stoppered and placed in a water bath thermostated at 37 ± 1° C. At pre-determined time intervals the dissolution medium was completely withdrawn and replaced with a fresh 3 ml portion of the pre-warmed buffer, to ensure sink conditions. The samples were analyzed for indomethacin content at 3 19.5 nm, after appropriate dilutions.

7.8.4.6. Pharmacodynamic studies

A total of 6 albino rabbits weighing 2-3 kg (2.68 ± 0.84) were used for the present study. Prior to the commencement of the study the animals with observed ocular abnormalities were excluded after thorough examination. The animals were housed in individual cages, and the experiments were conducted in a sanitized room at a temperature maintained around 24° C. Uveitis was induced in both eyes of each rabbit by an intra-vitreal injection (30 g needle) of a sterile solution of BSA (0.5 ml / eye of 50μ/ml sterile solution). Two days after the intra-vitreal injection of BSA, the eyes of the individual rabbits were observed by slit-lamp examination for the induction of uveitis. The following clinical parameters — congestion, keratitis (keratopathy), flare, aqueous cells, clot and synechias were evaluated and scored as described in Chapter VI.
Based on the pre-treatment scores of the above descriptors, the eye (left or right) showing more severe uveitis was selected for placing the implant. The implantation procedure was followed as described earlier for ethyl cellulose implants.

The animals were examined periodically for improvement in the clinical parameters and after 21 days post-implantation, the suture was opened to retrieve the remaining implant, if any, to assay the remaining drug in the implant. Additionally, the tissues around the implantation site were examined for any evidence of redness, swelling and/or other pathological signs.

7.8.4.7. Pharmacokinetic studies

The study was conducted in normal healthy rabbits without inducing uveitis. A total of 12 rabbits (3 each per data point), weighing 2-3 Kg (2.77 ± 0.61) were used for the study. Prior to the commencement of the study the animals with observed ocular abnormalities were excluded after thorough examination. The animals were housed as described earlier. The cross-linked implant (G17), which showed better therapeutic efficacy for prolonged periods, was selected for the study. The implant was placed in both the eyes of the rabbits by the surgical procedure described earlier. At periodic time intervals the implant was retrieved and the drug content in the retrieved implants were determined as described. The percentage of indomethacin absorbed was calculated from the amount remaining in the implant after retrieving from the site of implantation.

7.8.4.8. Statistical Evaluation

Experimental results are expressed as mean ± standard deviation (S.D.). In case of multiple comparisons of groups, analysis of variance (ANOVA) was performed. The student’s test was also performed to determine the level of significance. Differences were considered to be statistically significant at P<0.05.

7.8.5. RESULTS AND DISCUSSION

The composition of the various batches of the prepared implants is shown in Table 7.13. Gellan based implants of indomethacin were formulated with 10% of glycerol, PG, PEG 200 and 400 to select a suitable plasticizer (in terms of drug release).
Experimental and theoretical plasticizer levels in the drug-free gellan films (Fig. 7.18) of all the plasticizers were very consistently similar. The effect of the plasticizers on the void volume of the gellan films is shown in Fig. 7.19. The void volume per unit weight of the polymer was found to decrease more predominantly (P<0.05) beyond 10% of the PEGs, but for glycerol and PG, the decrease in void volume was observed only when their respective concentrations were increased beyond 20%.

Normally, at low concentrations of the plasticizer, the void volume would be gradually occupied by the plasticizer molecules and would decrease with the plasticizer level continuously. However, when the plasticizer level was above 10% for PEG 200 and 400 and above 20%, for glycerol and PG, the void volume had been totally occupied by the respective plasticizer molecules, and a further increase in the plasticizer concentration did not change the void volume. Consequently, the observed decrease in the void volume is due to the aggregation of the plasticizer channels. The SEM of gellan films plasticized with 10 and 40% PG are shown in Fig. 7.20a and b. The morphology of the films containing 10 and 40% of PG changed significantly with increase in PG levels. At 40%, PG was found to aggregate (Fig 7.20b) together in the polymer film.

The films prepared with 10% of the above-mentioned plasticizers were of sufficient suppleness to facilitate the subdivision of the films into implants of the desired size. Drug release from glycerol containing implant was higher (P<0.05) when compared to other plasticizers, while it was the least from PG containing implant (Fig. 7.21). The release from the implants containing the PEGs was almost identical to one another and did not differ from the release profiles of the PG containing implants. Though the release studies and the void volume determination were in favor of the two PEGs, drug-free gellan films prepared with the PEGs showed the formation of white deposits at all concentrations studied upon storage at 37°C for 1 month, and hence the PEGs were omitted from further studies. This phenomenon was absent in both glycerol and PG containing gellan films and these two plasticizers were selected for further studies.

**Physical appearance and Surface pH**

The physico-chemical properties of the implants are shown in Table 2. All the films remained flat during the course of the experiments. The surface pH of the prepared implants ranged from 7 to 7.4 indicating that the implants did not have an irritation potential, being identical with the pH of normal ocular fluids (Balasubramaniam *et al.*, 2001; Balasubramaniam and Pandit, 2002).
Effect of plasticizer

Most polymers require a plasticizer to improve their handling and processing properties. Gellan, at the concentrations used in the study gave tough films of excellent flexibility, yet they lacked suppleness. Thus a plasticizer was necessary for obtaining drug-loaded films with sufficient pliability to facilitate uniform subdivision of implants of the desired size (1×5 mm). Hence drug release studies from unplasticized implants were not undertaken.

Fig 7.22a shows the effect of glycerol concentration on drug release. The results indicated that during the first 4 hours, drug release from the implants containing 10% glycerol (G1) was higher than from the implants containing 15 and 20% of glycerol, but at the end of 120 hours, drug release was proportional to glycerol concentration, being around 97% for 20% glycerol (G3) followed by 90% (G2) and 82% (G1) for 15 and 10% of glycerol, respectively.

The decrease in the drug release with increasing glycerol concentration during the initial phase of the study (first 4 hours) could be attributed to antiplasticization phenomena, as was evidenced by the void volume values determined at the end of 24 hours with drug free gellan films containing 10 and 20% glycerol (Fig. 7.19). The film with 10% glycerol showed a void volume of 0.412 cm³/g and that containing 20% showed a void volume of 0.410 cm³/g. The almost similar void volumes of the films even with a two-fold increase in the glycerol concentration are indicative of the antiplasticization effect (Guo, 1994) of glycerol on gellan. However, this antiplasticization effect responsible for the initial phase of drug release was not operational throughout the period of the release studies. The increase in drug release from the batches containing 15 and 20% glycerol during the later stages could be explained on the following lines.

Swelling indices of these batches showed that optimum swelling or equilibrium water uptake was not attained within 24 hours, thereby indicating the capacity of the gellan matrix to swell further on prolonged exposure to the dissolution medium (phosphate buffer pH 7.4). This ability of the gellan matrix to swell, consequently results in the increase in porosity of the matrix, thus mobilizing the plasticizer (glycerol) molecules to a greater extent within the matrix. The mobilization of the plasticizer molecules causes partial solubilization of the drug in the plasticizer. The presence of solubilized drug in the implant further enhances pore formation, owing to local release of the drug and subsequent speedier penetration of the dissolution medium into the device, ultimately resulting in the rapid leaching of the drug.
molecules. The extent of solubilization of indomethacin (7.3 mg/ml and 8.4 mg/ml at 15 and 20% glycerol concentration) was found to increase with increase in glycerol concentration, thus explaining the ultimate change in the pattern of drug release.

The results were much more consistent for implants containing PG (Fig 7.22b). There was no evidence of the antiplasticization phenomenon and an increase in the concentration of PG resulted in a significant increase (P<0.05) in drug release. The addition of more PG enhanced the aqueous permeability of the gellan matrix, leading to faster release of the drug.

Effect of gellan concentration

Four different concentrations of gellan viz; 2, 2.5, 3 and 4% w/v were used to study the effect of gellan concentration on drug release from the implants. Increasing the concentration of gellan beyond 4% caused instantaneous gelation during casting, resulting in films with non-uniform thickness.

Drug release decreased (P<0.05) with increase in gellan concentration. The implants containing the least gellan concentration (2% w/v - batch G9) released its total drug content in 54 hours while in the other cases the release extended up to 120 hours (Fig. 7.23).

Increasing the concentration of gellan caused an increase in the viscosity of the polymeric solution, resulting in more uniform distribution of the drug within the matrix. In case of the batch with 2% w/v gellan, the proportion of surface associated drug was noted to be higher upon visual examination, indicating that a decrease in gellan concentration resulted in less uniform distribution of the drug, thus causing the exceptionally high initial rate of release of the drug. Further, the diffusional path length, which the drug has to transverse, decreases progressively with decrease in polymer concentration (Thanoo et al., 1992) resulting in decrease in the film thickness and significantly higher (P<0.05) release from the batch G9.

Effect of cross-linking

Cross-linking by method I (Surface cross-linking)

Surface cross-linking of the parent patch was achieved by a procedure reported by Al-Musa et al., (1999) using calcium chloride solution (5, 10, 15 and 20%) for treatment periods of 2, 4, 8 and 24 hours.
Gellan forms three-dimensional networks due to the formation of co-ordinates by cross-linking with cations. Calcium (Ca\(^{2+}\)) was selected as a coordinating cation because of its relatively high gelation inducing ability and as well as its safety (Simon, 1992). As the concentration of calcium chloride increased there was a significant (\(P < 0.05\)) reduction in drug release, as evidenced by the K values: 0.0194 for non-cross linked (G\(_5\)) and 0.0168, 0.0138, and 0.0126 for cross-linked (G\(_{12}\), G\(_{13}\) and G\(_{14}\)) batches. However, when the concentration of calcium chloride was increased to 20% (G\(_{15}\)), only a marginal increase (\(P > 0.05\)) in drug release rate compared to G\(_{14}\) was observed, which was rather surprising (Fig. 7.24). The method of cross-linking employed in the present study seems to be responsible for the observed result. In this method, the films were exposed to the cross-linking solution from both the surfaces. Short exposure (2 hours) to cross-linking solution could have resulted in the termination of the cross-linking process before the Ca\(^{2+}\) could travel from one surface of the film to the other. Thus, it is quite likely that cross-linking starts at the exposed surface of the film, yielding a nearly completely cross-linked surface, and resulting in decreased size and number of voids. Consequently, fewer cross-linking ions succeed in reaching the deeper layers, causing slowing down of ionic movement. This process continues till no further penetration is possible, leading to incomplete cross-linking of the inner layers (Balasubramaniam \textit{et al.}, 2001).

An increase in the time of contact of the film with the cross-linking solution could solve the problem of incomplete cross-linking. Thus in order to maximize the cross-linking of the gellan matrix with Ca\(^{2+}\), the films were subjected to increased durations of cross-linking, viz., 4 hours (G\(_{16}\)), 8 hours (G\(_{17}\)) and 24 hours (G\(_{18}\)). A calcium chloride concentration of 10% was selected to be the most suitable based on the obtained results (Fig 7.24).

The results showed that as the cross-linking time was increased there was reduction in the percent of drug released from the implants. A cross-linking time of 8 hours was found to be optimum, as it significantly (\(P < 0.05\)) reduced the drug release (Fig. 7.25), beyond which reduction in drug release was only marginal. The SEM of the non cross-linked implants and implants cross-linked with 10% calcium chloride for 2 and 8 hours are shown in Fig. 7.26 (a-c). Comparison of Figs. 7.26b and c clearly indicates that the increase in the cross-linking time had resulted in effective and more complete cross-linking of the inner layers, as evidenced by the formation of more uniformly dense structures throughout the film.

The main draw back of exposing the films to the cross-linking solution was that around 7-10% of the total drug load leached into the cross-linking solution. This was compensated by
adding the equivalent amount of the drug (in addition to what was already added) that was lost, while preparing the parent films.

**Cross-linking by method II (In situ cross-linking)**

To overcome the disadvantage of incomplete cross-linking encountered with method I, *in-situ* cross-linking was tried as an alternative, wherein calcium chloride solution was added after dispersing the drug in the gellan-plasticizer solution. One advantage of this method over surface cross-linking is that it is more quantitative than the former, since the entire amount of Ca\(^{2+}\) added to the polymer solution takes part in the cross-linking process of the polymeric network.

The gel strength of gellan is weaker at cation concentrations well below the stoichiometric equivalence of 0.5 for Ca\(^{2+}\) (Atkin *et al*., 2000). Accordingly, for a 25 mg/ml of gellan solution (used to prepare 2.5% w/v of gellan film) a Ca\(^{2+}\) concentration of 18.75 mM will give the stoichiometric equivalence [cation/carboxylate ratio] of 0.5.

In this study three concentrations of calcium chloride viz., 4% (6.8 mM), 8% (13.6 mM) and 12% (20.4 mM) with stoichiometric equivalence of 0.18, 0.36 and 0.54, respectively were used. Increase in calcium chloride concentration beyond 12% w/w resulted in instantaneous gelation of the gellan-drug dispersion. Out of the three concentrations studied, the first two concentrations (4 and 8% w/w) were well below the stoichiometric equivalence of 0.5, while the third was just above the stoichiometric equivalence.

The overall results indicated that though there was a marked reduction (P<0.05) in drug release compared to the non cross-linked batch (G0), batches cross-linked with 4 and 8% of calcium chloride showed almost identical drug release (Fig. 7.27). The formation of a comparatively weaker gel and hence, weaker cross-linking, at these concentrations, could be attributed to the observed result. However, at 12% Ca\(^{2+}\) a stiffer gel is formed, which results in more significant retardation of drug release.

**Kinetic analysis**

The kinetic analysis of drug release data revealed that the drug release was governed by matrix diffusion in all the cases, as the \(r^2\) values for Q Vs \(t^{1/2}\) were higher than values for Q Vs t. Further, the SEM of the implants (Figs 7.28 a and b) shows the formation of channels in the swollen gellan matrix, which is further indicative of the predominantly diffusion controlled mechanism as the formation of these ‘channels’ or ‘pores’ facilitates the diffusion
of the drug. Moreover, the release co-efficient (n) values ranged from 0.358 to 0.602 indicating Non-Fickian type of drug release (Vazquez et al., 1992).

**Pharmacodynamic studies**

The utility of cross-linking (G17) was studied *in-vivo* in comparison to non cross-linked implant (G5) through resolution of induced uveitis in rabbits. Six characteristics of uveitis were evaluated pre and post treatment (Tables 7.16 A and B). The data showed that the cross-linked implant resolved these parameters to a greater extent than the non-reinforced implant. After 3 weeks of placement of the implant, the cross-linked implants were present in all the three animals while retrieval was possible only from one of the three animals implanted with the non-reinforced implant. While all the retrieved cross-linked implants retained their original shape with evidence of swelling, the non-reinforced implant had lost its integrity completely and only a swollen, rubbery mass could be observed. Assay of these implants (procedure as described under determination of drug content uniformity) revealed that the cross-linked implants had delivered 86.84 ± 1.24% of their total drug load in three weeks, while no drug was detectable in the remnants of the non-reinforced implant.

**Pharmacokinetic study**

The result of the kinetic study is shown in Fig. 7.29. All the implants were retained at the implanted site until the time of retrieval. The implant survived at the site for a period of around 4 weeks (26 days), the duration of the study. No untoward side effect such as redness or ulceration after removal of implants was observed in the eyes of the rabbits. Around 66% drug was absorbed during the first 12 days. However, this initial spurt in release was not observed from the *in-vitro* study of the same batch (G17). This difference could be due to the fact that a simulation of *in-vivo* conditions, as regards to the presence of proteins and other enzymes were not attempted in the *in-vitro* studies. However, the kinetic study demonstrated the propensity of the prepared implant to sustain the drug release for prolonged periods of time *in-vivo*.

The indomethacin implant provided an initial phase of high *in-vitro* release followed by a phase of moderate release. Cross-linking with calcium chloride was effective in sustaining the release of indomethacin *in-vitro*. Out of the two types of cross-linking methods studied, surface cross-linking (method I) was found to be more suitable, in spite of the drawback of incomplete cross-linking, since in the cross-linking method II the concentration of Ca²⁺ could not be increased well beyond the stoichiometric equivalence of 0.5, which may
have resulted in the formation of a more stiff gel and consequentially could have resulted in more significant decrease in drug release.

The cross-linked implant survived for a period of around 4 weeks (pharmacodynamic studies) in- vivo, releasing around 90% of the drug load, thus showing the potential to release the drug for extended periods of time. The observations of the study show that these implants are likely to have no tendency to form fragments unlike the poly lactide co-glycolide (PLGA) implants reported earlier (Kunou et al., 1995). Since the gellan-based device formed a gelled mass in presence of calcium ions, which eroded slowly, device fragmentation and displacement into the vitreous is unlikely to occur in the gellan-based implants.

Though the implants were studied in a small group of animals and the distribution kinetics to the various ocular tissues were not monitored the results indicate the potential effectiveness of the implant. Further investigations to prolong the retention time beyond that reported here will certainly be fruitful.

A polymeric implantable system should be designed to not only deliver the drugs at a desired rate but should also be compatible with the tissue (Kamath and Park, 1993). The performance of a material, if biocompatible, should not cause any deleterious effect or toxic response even for a long-term application. Since no references exist in the literature on the biocompatibility of gellan as an ocular implant material, hence this aspect needs to be evaluated by further studies. However, in our studies based on a small number of observations, we did not observe any pathological and / or structural changes in the ocular tissues surrounding the implanted site.
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<th>Plasticizer (%w/v)</th>
<th>Cross linker concentration</th>
<th>Cross linking time (hr)</th>
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<td>G3</td>
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<td>Glycerol (20%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>PG (10%)</td>
<td>-</td>
<td>-</td>
</tr>
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<td>PG (15%)</td>
<td>-</td>
<td>-</td>
</tr>
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<td>PG (20%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>PEG 200 (10%)</td>
<td>-</td>
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<td>PEG400 (15%)</td>
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<td>PG (15%)</td>
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<td>PG (15%)</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>G21</td>
<td>2.5</td>
<td>PG (15%)</td>
<td>-</td>
<td>12</td>
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</table>

*Method one in text  **Method two in text
Table 7.14 Physico chemical properties of prepared implants

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Thickness (mm ± S.D.)</th>
<th>Weight (mg ± S.D.)</th>
<th>DCU (% ± S.D.)</th>
<th>Surface pH</th>
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<tbody>
<tr>
<td>G1</td>
<td>0.49 ± 0.017</td>
<td>3.14 ± 0.24</td>
<td>97.99 ± 1.00</td>
<td>7.0</td>
</tr>
<tr>
<td>G2</td>
<td>0.53 ± 0.015</td>
<td>3.28 ± 0.12</td>
<td>98.82 ± 2.09</td>
<td>7.4</td>
</tr>
<tr>
<td>G3</td>
<td>0.53 ± 0.019</td>
<td>3.31 ± 0.52</td>
<td>97.38 ± 2.03</td>
<td>7.4</td>
</tr>
<tr>
<td>G4</td>
<td>0.48 ± 0.025</td>
<td>3.04 ± 0.26</td>
<td>97.18 ± 0.70</td>
<td>7.2</td>
</tr>
<tr>
<td>G5</td>
<td>0.49 ± 0.021</td>
<td>3.24 ± 0.34</td>
<td>101.87 ± 2.50</td>
<td>7.0</td>
</tr>
<tr>
<td>G6</td>
<td>0.50 ± 0.030</td>
<td>3.34 ± 0.08</td>
<td>99.51 ± 0.75</td>
<td>7.4</td>
</tr>
<tr>
<td>G7</td>
<td>0.51 ± 0.020</td>
<td>3.18 ± 0.25</td>
<td>98.42 ± 1.35</td>
<td>7.2</td>
</tr>
<tr>
<td>G8</td>
<td>0.50 ± 0.024</td>
<td>3.26 ± 0.12</td>
<td>99.14 ± 0.97</td>
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</tr>
<tr>
<td>G9</td>
<td>0.42 ± 0.014</td>
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<td>G11</td>
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<td>0.48 ± 0.018</td>
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</tr>
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</tr>
<tr>
<td>G14</td>
<td>0.49 ± 0.020</td>
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<tr>
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<td>3.46 ± 0.18</td>
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</table>

*Hardness kg/cm² (Monsanto hardness tester)

** Drug content uniformity

 Thickness (mm ± S.D.)
Table 7.15 Kinetic parameters of drug release from prepared implants

| Batch Code | $r^2$  
(Q Vs t) | $r^2$  
(Q Vs t$^{1/2}$) | K  
(mg/mm$^2$h$^{1/2}$) | n |
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<tr>
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<td>0.0189</td>
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<td>0.961</td>
<td>0.0202</td>
<td>0.527</td>
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<tr>
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<td>0.947</td>
<td>0.993</td>
<td>0.0119</td>
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<td>0.976</td>
<td>0.0194</td>
<td>0.524</td>
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<td>0.992</td>
<td>0.0127</td>
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<td>0.989</td>
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### Table 7.16A Pharmacodynamic Studies of the Prepared Gellan-Based Implants

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Pr.T- Pretreatment scores
### Table 7.16B: Pharmacodynamic Studies of the prepared Gellan based implants (Contd)

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**Clinical Parameters**

- **Pr:** Present; **A:** absent; **ND:** not detectable, i.e., score > ++++; **Pr-T:** pretreatment scores

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Fig. 7.18: Correlation between theoretical and experimental plasticizer concentration
Fig. 7.19: Effect of increasing plasticizer concentration on void volume
Fig. 7.20: SEM of drug free gellan films plasticized with (a) PG 10% and (b) PG 40%
Fig. 7.21: Effect of different plasticizers on drug release

Effect of different plasticizer on drug release

Mean amount/unit area (mcg/mm²)

Time in hours

- G1
- G1
- C7
- G6
Fig. 7.22A: Effect of Glycerol concentration on drug release
Fig. 7.22B: Effect of propylene glycol on drug release
Fig. 7.23: Effect of gellan concentration on drug release
Fig. 7.24: Effect of calcium chloride concentration on drug release

(Cross-linking by method I)
Fig. 7.25: Effect of cross-linking time (cross-linking method I) on drug release
Fig. 7.26: SEM of gellan implants (a) non cross-linked; (b) cross-linked with 10% calcium chloride (for 2 hours) and (c) cross-linked with 10% calcium chloride (for 8 hours)
Fig. 7.27: Effect of calcium chloride concentration on drug release
(Cross-linking by method II)
Fig. 7.28: SEM of gellan implants after (a) 24 hours of dissolution and (b) 120 hours of dissolution
Fig. 7.29: Percentage of drug absorbed from the cross-linked implant in normal rabbit eye
7.9 REFERENCES


Barza, M., Peckman, C. and Baum, J., Ophthalmology, 93, 133 (1986).


