CHAPTER – VI

IN-SITU GELLING SYSTEMS

6.1 INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging endeavor facing the pharmaceutical scientists. This presents a constant challenge to the formulator to circumvent the various protective barriers of the eye without causing any permanent tissue damage.

Topical administration of drugs has been the treatment of choice for the diseases of the anterior segments of the eye. However, the conventional liquid ophthalmic formulations (eye drops) show low bioavailability because of spillage by overflow, dilution of drug by tear turnover, nasolacrimal drainage, systemic drug absorption and enzymatic metabolism (Lee et al., 1980). An increase in the dosing frequency or the use of highly concentrated solutions to compensate for the short ocular residence time is undesirable because of poor patient compliance and the twin risks of local and systemic toxicity. To increase the ocular bioavailability and duration of drug action various ophthalmic vehicles such as viscous solutions, ointments / gels or polymeric inserts have been used. The corneal contact time has been increased to varying degrees by these vehicles, but because of blurred vision (ointments), lack of patient compliance (inserts), and sticking of lids (gels), they have not been widely accepted. Therefore other systems, which combine the ease of administration of liquid dosage forms with the prolonged residence time of inserts, are being investigated actively, and a few have been commercialized.

From the point of view of patient acceptability, a liquid dosage form that can sustain the drug release and remain in contact with the cornea for extended periods of time is ideal. If the precorneal residence time of a drug could be improved from 5 minutes to say, a few hours, then improved local bioavailability, reduced dose concentration and dosing frequency and improved patient compliance may be achieved. Drug delivery systems based on the concept of *in-situ* gel formation should provide these properties. These delivery systems are made from polymers that exhibit sol-gel phase transition due to physico-chemical changes in their environment, in this case the cul-de-sac of the eye.
A variety of polymers with different mechanisms of sol-gel phase transitions have been investigated to develop in-situ gel forming systems. Among them are:

- Poloxamer 407, tetronics and ethyl (hydroxy ethyl) cellulose – whose solution viscosity increases upon increasing the temperature to that of eye (Miller and Donovan, 1982).

- Cellulose acetate phthalate (CAP) latex – coagulates when its acidic pH of 4.5 is raised by the tear fluid to 7.4 (Gurny et al., 1985).

- Carbopol solutions which are acidic and less viscous, transform to stiff gels upon increase in pH (Chandrasekaran et al., 1989).

- Alginates with high G content form three dimensional ionotropic hydrogel matrices, generally by the preferential interaction of Ca\(^{+2}\) ions of the tear fluid with the G moieties, resulting in the formation of homogenous gels (Grant et al., 1073).

- Gelrite\textsuperscript{®}, low acethyl gellan gum, like alginate, gels in the presence of Ca\(^{+2}\) ions in tears (Moorehouse et al., 1981).

Ideally, an in-situ gelling delivery system should be a low viscous, free flowing liquid to allow for uniform dosing into the eye as drops and the resulting gel formed following phase transition should be strong enough to withstand the shear forces in the cul-de-sac and should prolong the residence time in the eye. Thus, an in-situ gel forming system will have good patient compliance because it is easy to instill into the eye and the resultant gel formed erodes gradually, obviating the need for removal. Increase in the residence time brings about an improvement in the ocular bioavailability and the changes in the concentration and/or the molecular weight of the carriers/vehicles (polymers) affects the rheological behavior and hence the release profiles incorporated drugs from the gels. This offers flexibility in the design of in-situ gel forming systems with desirable rheological properties and drug release rates.

6.2 SYSTEM SPECIFIC REVIEW

Gellan was evaluated for applications in ocular sustained release devices (Sanzgiri et al., 1993). A methyl prednisolone (MP) ester of gellan (gellan–MP) was synthesized.
The various sustained release dosage forms evaluated were gellan–MP films, gellan films with physically incorporated MP and eye drops of MP suspended in a 0.6% w/w gellan dispersion in water. The control dosage form was suspension of MP in normal saline. MP concentrations in the tear fluid of New Zealand white rabbit were measured after ocular application of the dosage forms. In-vitro, the gellan-MP films released covalently bound MP in an approximate zero order pattern, whereas the release of physically incorporated MP from the gellan eye drops and films followed a square root of time relationship and anomalous kinetic, respectively. Compared with the MP suspension control, the gellan-MP films yielded an approximately 4 – fold higher area under tear fluid concentration versus time curve, but exhibited a tendency to slip out of the eye due to a higher degree of swelling. The area under tear fluid concentration of the films was approximately equal to that of the control, while that from the eye drops showed 2.6 fold increase than the control and also provided ease of administration.

A 0.6% Gelrite® vehicle has been compared to an equiviscous solution of hydroxyl ethyl cellulose (HEC) using timolol maleate as a drug probe (Rozier et al., 1989). In-vitro, release rates of timolol from HEC and Gelrite® gel were similar. In-vivo, the formation of the gel prolonged the pre-corneal residence time and increased ocular bioavailability of timolol in the cornea, aqueous humor, iris and ciliary body of albino rabbits.

The rheology of Gelrite® in-situ gels were studied (Calfors et al., 1998). A complimentary in-vivo study for determining precorneal contact times in humans and in rabbits was performed. The elastic modulii of the gels increased with increasing concentration of electrolytes. At physiological concentration of the electrolytes, the elasticity of the gels was independent of Gelrite® concentration. The human contact times increased up to 20 hours with decreasing osmolality of the formulations.

Gamma scintigraphic studies were carried out to compare isoviscous Gelrite® solution (0.6 %w/v) and HEC solution (0.5%w/v) with an isotonic saline solution in man. A significant retention of the Gelrite® formulation was found when compared to the HEC and saline solution, with the mean pre-corneal residence half life being 1089 ± 1485 seconds, 81± 89 seconds and 22 ± 19 seconds, respectively (Greaves et al., 1990).

Gunning et al., (1993) compared the ocular hypotensive activities of two potent topical carbonic anhydrase inhibitors, sezoamide (MK 417) and dorzolamide (MK507),
formulated in Gelrite® vehicle. Duration of action of both the compounds was slightly prolonged by the use of Gelrite® vehicle, when compared to earlier studies.

Hommer et al. (1995) compared the ocular hypotensive effect of 0.25% timolol in Gelrite®, once daily (TG) to that 0.25% timolol solution, twice daily (TS). The results of this study supported the hypothesis of comparable hypotensive effect at peak and trough TG and TS. Furthermore, TG had an acceptable tolerability profile. The incidence of blurred vision and foreign body sensation was higher in TG.

Hartmann (1996) investigated the effect of artificial human tears containing calcium or magnesium ions on the rheological behavior of 0.6% gellan gum ophthalmic preparations, the release of pilocarpine hydrochloride in-vitro and their miotic effect in rabbits. Pure 0.6% gellan gum showed pseudoplastic flow properties and nearly no thixotropy. The addition of artificial tears led to plastic flow properties and an increase in thixotropy, compared with an aqueous pilocarpine formulation. The release properties of the gellan gum formulations were significantly decreased on addition of artificial tears and more so on adding Ca$^{2+}$ or Mg$^{2+}$ ions to artificial tear fluid. A significant prolongation of drug effect was not measurable in-vivo when Ca$^{2+}$ or Mg$^{2+}$ were applied before instilling the gellan gum-pilocarpine formulation.

Rozier et al. (1997) developed a functionality test ensuring the consistency of the dosage form’s gelling property and reproducible pharmacological effect. The rupture strength of the gel was shown to be a reliable indicator of the ocular drug bioavailability in albino rabbits. The test parameters susceptible to influence the test results were indentified, evaluated and optimized. The influence of the raw material characteristics and of the processing parameters on the final product gel strength were determined and optimized and the finished product specifications also established.

Gelrite® was tested in humans for its efficacy as an ophthalmic vehicle by a noninvasive fluorometric technique. Fluorescein was used as the tracer and its concentration in the anterior chamber was used as the principle measure of bioavailability. The gel afforded a two-fold increase in the penetration of fluorescein compared with an isotonic buffer solution (Maurice and Srinivas, 1992).

The formulation and evaluation of an ophthalmic delivery system of an antibacterial agent, ofloxacin, based on the concept of pH-triggered in-situ gelation was
investigated (Srividya et al., 2001). Carbopol® 940 was used as the gelling agent in combination with HPMC (Methocel E50LV), which acted as a viscosity enhancing agent. The developed formulations were therapeutically efficacious, stable, and non-irritant and provided sustained release of the drug over an 8 hour period.

In order to reduce the total polymer content and improve the gelling properties, (Joshi et al., 1993) first used the combination of polymers in the delivery system. The main idea is that aqueous composition reversibly gelled in response to simultaneous variations in at least two physical parameters, such as pH, temperature, and ionic strength can be formed by using a combination of polymers, which exhibit reversible gelatin properties.

The rheological characterization of an in-situ system prepared by a combination of carbopol and methylcellulose (MC) was carried out at two different pH (4.0 and 7.4) and temperatures (25 and 37 °C) (Kumar et al., 1994). The studies indicated a pseudoplastic behavior and an increase in pH from 4.0 to 7.4, or temperature from 25 to 37 °C, resulted in an increase in viscosity, shear stress and yield point, and the magnitude of changes being highest when both the temperatures were altered simultaneously. An increase in concentration of either carbopol or MC, or an increase in MC molecular weight resulted in an increase in shear stress, viscosity and yield point. Among the compositions studied, a solution containing 1.5% MC and 0.3% carbopol was found to have low viscosity and formed a strong gel under simulated physiological conditions.

The rheological properties of an aqueous solution containing Carbopol (974 NF) and HPMC, were evaluated as function of temperature and pH and were found to be similar to those of pure Carbopol (974 NF) solutions (Kumar and Himmelstein, 1995). In addition the Carbopol –HPMC gels decreased the in-vitro release of incorporated timolol maleate.

Lin and Sung (2000) characterized a series of carbopol and pluronic-based solutions as in-situ gelling vehicles for ophthalmic drug delivery. The rheological properties, in-vitro release as well as in-vivo pharmacological response of various polymer solutions, including carbopol, pluronic and carbopol/pluronic solution, were evaluated. It was found that the optimum concentration of the carbopol solution for the in-situ gel forming delivery system was 0.3 % (w/w) and that for pluronic solution was 14% (w/w).
The mixture of 0.3% carbopol and 14% pluronic showed a significant enhancement in gel strength in the physiological condition; this gel mixture was also found to be free flowing at pH 4.0 and 25°C. The rheological behaviors of carbopol/pluronic solution were not affected by the incorporation of pilocarpine hydrochloride. Both the \textit{in-vitro} release and \textit{in-vivo} pharmacological studies indicated that the carbopol/pluronic solution had better ability to retain drug than the carbopol or pluronic solutions alone.

Pluronic F127 (PF 127) based formulations of timolol maleate (TM) aimed to enhance its ocular bioavailability were developed (EL-Kamel, 2002). The effect of isotonicity agents and PF 127 concentration on the rheological properties of the prepared formulations was examined. In an attempt to reduce the concentration of PF127 without compromising the \textit{in-situ} gelling capabilities, various viscosity enhancing agents were added to PF 127 solution containing 0.5% TM. The viscosity and the ability of PF 127 gels to deliver TM, \textit{in-vitro}, in absence and presence of various viscosity enhancing agents were also evaluated. At the used concentration some of the examined isotonicity agents had effect on the viscosity of TM gel. However, the viscosity of gel increased as the PF 127 concentrations increased. The slowest drug release was obtained from 15% PF 127 formulations containing 3% methyl cellulose. \textit{In-vivo} studies showed that the ocular bioavailability of TM, measured in albino rabbits increased by 2.5 and 2.4 fold for 25 % PF 127 gel formulation and 15% PF127 containing 3% methyl cellulose, respectively, compared with 0.5% TM aqueous solution.

Studies have been carried out on a number of pluronic polyols with the aim of determining factors which influence the transition temperature of the hydrogels. The sol-gel transition temperatures, Tm, were measured for aqueous solution of the polyols with and without additives such as sodium chloride, potassium chloride, urea, ethanol, sodium sulfate, and sodium dodecylsulfate (Vadnere et al., 1984). A linear relationship was found between the logarithm of the pluronic polyol concentration and the reciprocal of the sol – gel transition temperature. Although no linear relationship was observed between log molecular weight and the reciprocal of the gel-sol transition temperature for all polymers, such a relationship does seem to exist among the polymers having the same ratio of poly (oxypropylene) to poly(oxyethylene) units per mole of polymer (P/E ratio). All the pluronic polyols studied showed endothermic enthalpy change for the sol-gel process. These results were substantiated with data from calorimetric studies.
The distribution of a model 16-mer oligothymidylate (pdT16) in several ocular tissues (cornea, conjunctiva, sclera, iris, lens, aqueous and vitreous humors) was determined after instillation in the eye of various dosage forms in a rabbit model. Radiolabelled pdT16 was applied as a simple solution, a 27% poloxamer 407 gel, a suspension of liposomes or liposomes dispersed within a 27% poloxamer 407 gel. pdT16 concentrations were measured in the tissues and fluids by radioactivity counting at the intervals of 10 minutes, 2 hours and 24 hours. When the pdT16 solution was used, the highest concentrations were observed in the conjunctiva and the cornea, while a substantial amount of drug was also present in the sclera. Low concentrations were measured in the iris. Using the same treatment protocol, the two liposomal formulations delivered low amounts of pdT16 to all ocular tissues, and particularly to the conjunctiva and the cornea. The poloxamer gel provided higher tissue concentrations of pdT16 than liposomes but lower than those observed with the solution except 10 minutes after administration in the iris where the amount of pdT16 was higher when administered under the gel form (Bochot et al., 1998).

Rheological measurements were performed to study the gel and sol-gel transition of an in-situ gel, poloxamer 407. The rheological measurements and a small in-vivo study of ocular residence times in humans were used to evaluate poloxamer as an ocular vehicle (Edsman et al., 1998). An increasing concentration of poloxamer resulted in a slightly increasing elasticity of the gels and a decreasing sol-gel transition temperature. The contact time increased with increasing concentration of poloxamer, which could be explained and correlated, with the rheology of poloxamer solutions/ gels mixed with simulated tear fluid. The maximum contact time for the preparations studied was about 1 hour.

Gurny et al. (1993) used the gamma scintigraphy technique to monitor the ocular residence time of an ophthalmic preparation based on cellulose acetate phthalate (30%w/w, viscosity 50 mPas). The gelled system constituted an in-situ microreservoir of high viscosity. The pre-corneal residence time (half life) in rabbits was 400 seconds when compared to 40 second for a solution. Pilocarpine formulated with cellulose acetate phthalate (CAP) maintained a constant miosis in the rabbit for up to 10 hours when compared to 4 hours with eye drops. This system is however, characterized by a high
polymer concentration (30 %w/w CAP) and the low pH of the instilled solution may be a discomfort for the patient.

Ruel et al., (2000) investigated the physical properties of a chitosan-glycerophosphate (GP) thermosensitive solution that gels at 37 °C and evaluated the in-vitro release profiles of different model compounds. The gelation rate was dependent on temperature and on the chitosan deacetylation degree. The solution containing 84% deacetylated chitosan could be stored for 3 months at 4 °C without apparent change in viscosity. The in-vitro release profiles of the model compounds were dependent on the presence of GP in the chitosan solution, on their molecular weight and on the presence of lysozyme in the release media.

A thermogelling drug delivery system composed of cellulose ether [ethyl (hydroxyethyl) cellulose-EHEC], an ionic surfactant and water was characterized in the presence of timolol maleate with respect to phase and rheological behavior, as well as in-vitro drug release (Lindell and Engstrom, 1993). The phase studies revealed that gelling systems may be formed with 0.34% (w/w) timolol maleate, and that the gelling behavior was sensitive to the surfactant concentration and ionic strength of the solution. The release of timolol maleate from the gels was retarded compared to a non-gelling EHEC system.

The orally administered acetazolamide has a limited use in glaucoma due to the systemic side effects associated with its use. It has been reported to show little effect on the intra ocular pressure (IOP) of human and rabbit eyes upon topical application, probably owing to its poor bioavailability and instability at pH > 5.0. In order to enhance the bioavailability of the drug, contact time between the drug molecules and the ocular surface was increased using high viscosity water soluble polymers (PVA and HPMC) and by incorporating acetazolamide in an in-situ forming ophthalmic drug delivery system (Kaur et al., 2000). Moreover, a penetration enhancer (EDTA) was also used in these formulations to increase extent of absorption of the drug. Acetazolamide at a concentration of 10% was used and the formulations (eyes drop suspensions) were evaluated for their in-vitro release pattern. The effect of these formulations on the IOP in normosensitive conscious rabbits was also investigated. These formulations were found to be therapeutically effective with a peak effect at 2 hours. A fall in IOP of up to 46.4% was observed with repeated administration of one of the formulation containing PVA, EDTA and Tween 80.
Thermoreversible gels formed in-situ by aqueous solutions of an enzyme-degraded xyloglucan polysaccharide were evaluated as sustained release vehicles for the ocular delivery of pilocarpine hydrochloride (Miyazaki et al., 2001). In-vitro release of pilocarpine from gels formed by warming xyloglucan sols (1.0, 1.5 and 2.0%w/w) to 34°C followed root time kinetics over a period of 6 hours. The miotic responses in rabbit following administration of xyloglucan sols were compared with those from in-situ gelling pluronic F127 sols and from an aqueous buffer solution containing the same drug concentration. Sustained release of pilocarpine was observed with all gels, the duration of miotic response increasing with increase of xyloglucan concentration.

Cohen et al. (1997) demonstrated that an aqueous solution of sodium alginate can gel in the eye without the addition of external calcium ions or other bivalent/polyvalent cations. The extent of alginate gelation and consequently the release of pilocarpine depended on the % G residues in the polymer backbone. Alginates with G contents of more than 65%, such as Manugel, DMB, instantaneously formed gels upon their addition to simulated lacrimal fluid, while those having low G contents, such as Kelton LV, formed weak gels at a relatively slow rate. In-vitro studies indicated that pilocarpine was released slowly from the alginate gels, over a period of 24 hours and the release occurred mostly via diffusion from the gels. IOP measurements of rabbit eyes treated with 2%(w/v) pilocarpine nitrate, in solution or in the in-situ gel forming formulations composed of the high G content alginate, indicated that it significantly extended the duration of pressure reducing effect of pilocarpine to 10 hours as compared to 3 hours when pilocarpine nitrate was delivered as a solution.

6.3 OBJECTIVE AND THE PLAN OF STUDY

The objective of the present work was to develop an ion activated in-situ gelling system of MOXI, a fluoroquinolone derivate used in external infections of the eye using gellan alone and in combinations with sodium alginate, and indomethacin, and NSAID used as an alternative to steroids for treatment of uveitis and cystoids macular edema using gellan, which would undergo gelation when instilled into the cul-de-sac of the eye and provide sustained release of the drug(s) during treatment of the ocular ailments.
The proposed work was planned as follows;

- Formulation of the gelling systems
- Physico-chemical evaluation (drug content, viscosity, gelling efficiency and sterility).
- *In-vitro* drug release studies (using dialysis method and a modified method for MOXI system and the modified method for indomethacin).
- Pharmacodynamic evaluation (antibacterial efficacy using rabbit eye model of MOXI system and uveitis induced rabbit eye model for indomethacin systems) of the fabricated systems.

### 6.4 MATERIALS

<table>
<thead>
<tr>
<th>Material</th>
<th>Supplier</th>
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<tbody>
<tr>
<td>MOXI</td>
<td>Ranbaxy labs, Gurgaon, India</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Jagsonpal Pharmaceuticals Ltd., New Delhi, India</td>
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<td>Sodium Alginate</td>
<td>Loba Chem Pvt Ltd., Mumbai, India</td>
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<tr>
<td>Bovine Serum Albumin</td>
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<td>γ–Globulin</td>
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<td>S.D Fine Chemical Ltd., Mumbai, India</td>
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<td>D-Glucose</td>
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<td>Glacial Acetic Acid</td>
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</tr>
<tr>
<td>Methanol</td>
<td>Qualigens Fine Chemicals, Mumbai, India</td>
</tr>
</tbody>
</table>
6.5 METHODS

6.5.1 Preparation of Acetate Buffer

13.6 gms of sodium acetate was dissolved in distilled water pH was adjusted to 5.0 by addition of 6 ml of glacial acetic acid. The volume was adjusted to 100 ml using distilled water.

6.5.2 Preparation of Simulated Tear Fluid, Composition 1 (STF1) (Srividya et al., 2001)

<table>
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<th>Ingredient</th>
<th>Quantity</th>
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<tr>
<td>Sodium Chloride</td>
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<tr>
<td>Sodium Bicarbonate</td>
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<tr>
<td>Calcium Chloride (2H₂O)</td>
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</tr>
<tr>
<td>Distilled Water (q.s.)</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

The above ingredients were weighed accurately and dissolved separately in small portions of distilled water. All the solutions were then mixed and the volume was made up to 100 ml with distilled water.

6.5.3 Preparation of Simulated Tear Fluid, Composition 2 (STF2) (Van Haeringen, 1981)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine Serum Albumin (Fraction V)</td>
<td>0.268g</td>
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<tr>
<td>Lysozyme</td>
<td>0.268g</td>
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<tr>
<td>γ Globulin</td>
<td>0.134g</td>
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<td>D-Glucose</td>
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<tr>
<td>Sodium Chloride</td>
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<tr>
<td>Calcium Chloride (2H₂O)</td>
<td>0.008g</td>
</tr>
<tr>
<td>Distilled Water (q.s.)</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

The ingredients were weighed accurately and dissolved in small amount of distilled water. Upon stirring a clear solution was obtained and finally the solution was made up to required volume with distilled water.
6.5.4 Calibration Curve of the Drugs in STF1

MOXI

100 mg of MOXI was accurately weighed and dissolved in 100 ml of 1% v/v glacial acetic acid and the volume was made up with STF1 to 100 ml. From this stock solution, graded dilutions were made to obtain standard solution of 5 - 30µg/ml range using STF1. The absorbance of the solution was noted at 291 nm on a UV spectrophotometer (shimadzu-1601, Japan). A calibration curve was constructed by plotting the absorbance against concentration. A straight line with $r^2$ value of 0.9998 was obtained. The calibration curve was utilized for both assay procedure and in-vitro drug release studies.

Indomethacin

Stock solution of indomethacin (1mg/ml) was prepared by dissolving 100 mg of indomethacin in 10 ml of methanol and the volume was made up to 100 ml with STF1. From this stock solution, graded dilutions were made to obtain standard solutions for 5-30µg/ml range with STF1. The calibration curve was constructed at 318.5 nm, as described for MOXI. A straight line with $r^2$ value of 1.0 was obtained.

6.5.4. A X-ray Diffraction (XRD)

X-ray diffractograms of gellan, MOXI, indomethacin and physical mixtures of gellan and MOXI; gellan and indomethacin (1:1 and 4:1 ratios) were recorded using Shimadzu XD-DI X-ray diffractometer under following conditions; Ni-filtered Cu-Kα radiation, 40 KV, 30 mA current and scan speed of 2.5 °/min in terms of 2θ angle.

6.5.5 Preparation of Formulations

MOXI System

Gellan alone and its combinations with sodium alginate, with or without sodium citrate (20%w/w, with respect to gellan) were dissolved in hot acetate buffer (70° C. prepared in fresh water for injection under laminar flow) pH 5.0 by continuous stirring at 40° C. Required quantity of MOXI to give a final drug concentration of 0.3% w/v was added to the polymeric solution and stirred until dissolved. The formulations were filled in
clean 10 ml amber colored glass vials, capped with rubber closures and sealed with aluminum caps. The formulations, in their final pack were terminally sterilized by autoclaving at 121°C and 15 p.s.i pressure for 20 minutes. The sterilized formulations were stored in refrigerator (4-8 °C) until further use.

**Indomethacin Systems**

Gellan with or without sodium citrate (10-40%w/w, with respect to gellan) was dissolved in hot phosphate buffer (composition and preparation dealt in Chapter V) pH 7.4 as described for MOXI systems. Required quantity of indomethacin to give a final drug concentration of 1% w/v was added to the polymeric solution and stirred until dissolved. The formulations were sterilized and stored as described for MOXI systems.

**6.5.6 Evaluation of the Formulations**

**6.5.6.1 Drug content Uniformity**

The vials (n=3) containing the preparations (MOXI and indomethacin systems) were shaken for 2-3 minutes and 100µl of the preparation was transferred aseptically to sterile 25 ml volumetric flasks with a micropipette and the final volume made up with STF1. The concentration of MOXI and indomethacin were determined at 276 nm and 318.5 nm, respectively, (Shimadzu, UV-1601, Japan).

**6.5.6.2 Gelation Studies**

The gelation studies were carried out in gelatin cells, fabricated locally using Teflon®. The cells were cylindrical reservoirs capable of holding 3 ml of the gelation solution (STF). Within the cells located at the bottom was a 250-µl transparent plastic cup to hold the gel sample in place after its formation. The studies were carried out using STF1 and STF2, which simulated the divalent cation content and both the protein and divalent cation content of the tear fluid, respectively.

100 µl of the preparation (MOXI and indomethacin systems) was carefully placed into the cavity of the cup using a micropipette and 2 ml of the gelation solution (compositions 1 or 2) was added slowly. Gelation was detected by visual examination.
6.5.6.3 Rheological Studies

Viscosity determinations of the prepared formulations (both MOXI and indomethacin) were carried out on a cone (0.8°) and plate geometry viscometer (Brookfield) using spindle cp 40. The viscosity of the sample solutions were measured at different angular velocities at a temperature of 37 ± 1°C. A typical run comprised changing angular velocity from 0.5 to 100 rpm at a controlled ramp speed. After a wait of 0.1 minute (6 seconds) 0.5 rpm, the velocities were increased to 100 at the same controlled ramp speed with similar wait at each rpm. The hierarchy of the angular velocity was reversed at the same ramp speed with a similar wait of 0.1 minute. The average of the two readings was used to calculate viscosity. The evaluations were conducted in triplicate.

6.5.6.4 Test for sterility

The sterilized formulations were incubated for 14 days with nutrient agar medium (aerobic organisms) and the thioglycollate medium (anaerobic) organisms at 30-35°C. At the end of 14 days, the incubated formulations were checked for growth.

6.5.6.5 In-vitro release studies

The drug release kinetics from the prepared formulations (MOXI and indomethacin systems) was studied using a modified method reported earlier (Lin and Sung, 2000). 2 ml of the test solution was placed in circular plastic cup (2.5 cm internal diameter and 1.2 cm in depth). This was in turn placed on an inverted USP basket kept inside a 250 ml beaker. 200 ml of dissolution medium (STF1) was added and stirred with a star headed magnetic bead. Temperature of 37 ± 1°C was maintained throughout the study. 5 ml samples were withdrawn at regular time intervals and replaced with an equal volume of the pre-warmed medium. The samples were analyzed for MOXI and indomethacin content at 276 nm 318.5 nm, respectively using a UV spectrophotometer (Shimadzu-1601, Japan).

6.5.6.6 Stability studies

Selected sterilized formulations (both MOXI and indomethacin systems) were stored at room temperature, 4°C and at 45°C for a period of 3 months. The formulations were evaluated for drug content, gelling efficiency, viscosity and in-vitro drug release.
6.5.6.7 Antimicrobial efficacy Studies (For MOXI systems)

This was determined by the agar diffusion test employing the cup-plate technique. Marketed eye drop of MOXI (Standard preparation) and the developed formulations, diluted suitably with acetate buffer pH 5.0 (test preparations) were poured into cups bored into sterile nutrient agar previously seeded with *Pseudomonas aeruginosa* ATCC 27853 (*P. aerugionosa*) and *Staphylococcus aureus* ATCC 25923 (*S. aureus*). After allowing diffusion of the solutions for 2 hours, the agar plates were incubated at 37±0.5°C for 24 hours. The zone of inhibition (ZOI) measured around each cup was compared with that of control. The entire operation except the incubation was carried out in a laminar flow unit. Each solution was tested in triplicate. Both positive and negative controls were maintained throughout the study.

6.5.6.8 *In-vivo* rabbit eye (Pharmacodynamic) study

**MOXI Systems**

Albino rabbits of either sex weighing 1.5 to 1.8 kgs were used for the single dose study. The animals were acclimatized to the animal room conditions, sanitized and maintained at 24°C. The animals were placed in restraining boxes during the experiment, which allowed their heads to move freely but their eye movements were not restricted. Between experiments, the rabbits were housed singly and allowed food and water.

Suspensions of *S. aureus* and *P. aeruginosa* were prepared to give 0.5 McFarland standards (preparations given in Chapter V). The standard is said to have been achieved when the absorbance of the prepared suspensions of the microorganism matched with that of a barium sulphate 0.5 McFarland standards at 625 nm. Ideally the absorbance should be between 0.08 and 0.1.

Three rabbits were used of each of 10, 25 and 40 µl of 0.5 McFarland suspensions of *S. aureus* and *P. aeruginosa* instilled in both the eyes of each rabbit. After allowing the growth to proceed to the log phases (approx. 18 hours) swabs from both the eyes were taken at periodical time intervals up to 24hrs and streaked on sterile nutrient agar plates and incubated at 37 ± 0.5°C for 24 hours and checked for growth. This study helped to arrive at an inoculum volume that was capable of maintaining a growth up to 24 hours for conducting the *in-vivo* study. The selected inoculum dose was then instilled in the right
eye of each of 5 rabbits and the left eye served as the positive control. The formulations (two drops each of GC₃ and GC₇) were then instilled in the right eye of the rabbit and cotton swabs from both the eyes were taken at 2, 4, 8, and 24 hours.

**Indomethacin Systems**

A total of 6 albino rabbits weighing 2 - 2.5 kg (2.18 ± 0.34) were used for the study. Prior to the commencement of the study the animals observed with 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 (0.5ml/eye of 50 µ/ml sterile solutions). Two days after the intra-vitreal injections 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 follows

**Congestion**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No congestion</td>
</tr>
<tr>
<td>+</td>
<td>Slight to moderate circum-corneal congestion</td>
</tr>
<tr>
<td>++</td>
<td>Marked circum-corneal ciliary congestion</td>
</tr>
<tr>
<td>+++</td>
<td>Marked circum-corneal, diffuse episcleral and conjunctival congestion</td>
</tr>
<tr>
<td>++++</td>
<td>Marked circum-corneal, diffuse episcleral and conjunctival congestion with edema</td>
</tr>
</tbody>
</table>

**Keratitis**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No inflammation</td>
</tr>
<tr>
<td>+</td>
<td>Slight diffuse stromal edema</td>
</tr>
<tr>
<td>++</td>
<td>Moderate epithelial and stromal edema with thickening and folds in Descemet’s Membrane</td>
</tr>
<tr>
<td>+++</td>
<td>Diffuse epithelial and stromal edema, and folds in Descemet’s membrane: peripheral vascularisation.</td>
</tr>
<tr>
<td>++++</td>
<td>Severe edema of the stroma</td>
</tr>
</tbody>
</table>

**Flare**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Complete absence</td>
</tr>
</tbody>
</table>
Based on the pre-treatment scores of the above descriptors, the eye (left or right) showing more severe uveitis was selected for instilling the formulations (prepared formulations and standard dispersion).

6.5.6.9 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 ‘t’ test was also performed to determine the level of significance. Differences were considered to be statistically significant at p<0.05.

6.6 Results and Discussion

The X-ray diffractograms of the samples recorded did not reveal any possible interactions between gellan and the two drugs in the physical states (fig. A - G). The
composition of the various batches of the fabricated MOXI and indomethacin eye drops are shown in Tables 6.1 and 6.2 respectively. In case of MOXI preparations increasing the concentration of gellan, in preparations containing only gellan, beyond 0.0625% caused gellan upon cooling to 40 °C (during stirring). This observation was quite interesting since gellan at a concentration of 0.6% was used earlier (Sanzgiri et al., 1993; Rozier et al., 1989) to prepare eye drops of timolol maleate and methyl prednisolone. The ionic content of the vehicle used; in this acetate buffer pH 5.0 and the presence of HCl in MOXI could have contributed to the gelatin of gellan when used beyond 0.0625% (used in this study). In case of combination systems with sodium alginate, the concentration of gellan was kept constant at 0.03% and the concentration of sodium alginate was varied so as to give a maximum of 1% polymer concentration, since increase in total polymer concentration beyond 1% resulted in gelation during formulation. However, in case of indomethacin system gellan up to 0.5% w/v was used. Increasing the concentration beyond 0.5%w/v resulted in gelation.

6.6.1. Evaluation of Formulations

The physico-chemical properties of the prepared MOXI and indomethacin formulations are shown in Tables 6.3 and 6.4 respectively. The drug content, clarity and pH of the formulations were found to be satisfactory and the formulations were liquid at both room temperature and when refrigerated. All the formulations were sterile.

The two main pre-requisites of an *in-situ* gelling system are viscosity and gelling capacity (speed and extent of gelation). The formulation should have an optimum viscosity that will allow easy instillation into the eye as a liquid (drops), which then undergo a rapid sol to gel transition due to ionic interaction. Moreover, the *in-situ* formed gel should preserve its integrity without dissolving or eroding for a prolonged period of time to facilitate sustained release of the drug to the ocular tissues. All the formulations showed instantaneous gelation when contacted with the gelation fluids (STF 1 and 2). However, the nature of the gel formed depended upon the polymer concentration. In case of MOXI formulations batch GC1 showed the weakest gelation, due to the presence of minimal amount of gellan (0.01%). The nature of the components of the gelation medium did not seem to influence the nature of gel formed ascertaining the gelation occurred primarily due to the presence of cation in the fluid.
6.6.2 Rheological Studies

The formulations (MOXI and indomethacin) exhibited pseudoplastic behavior as evidenced by shear thinning and an increase in the shear stress with increase in the angular velocity. The viscosity was directly dependent on the polymeric content of the formulations. The viscosity of the MOXI formulations containing sodium alginate showed around 10-15% reduction on autoclaving, while the formulations containing gellan alone (both MOXI and indomethacin formulations) did not show any change in viscosity after autoclaving. The concentration of sodium alginate was adjusted in such a way that the difference in the viscosity before and after sterilization was compensated. Addition of sodium citrate in gellan formulations reduced the viscosity significantly (P<0.05; t-test) in comparison to the corresponding batch without sodium citrate in both the systems (Figs 6.1-6.4).

The administration of ophthalmic preparations should influence as little as possible the pseudo-plastic character of the pre-corneal film (Van Ootegaham, 1993). Since the ocular shear rate is very large ranging from 0.03s⁻¹ during inter blinking periods to 4250-28500s⁻¹ during blinking (Bothner et al. 1990), viscoelastic fluids with a viscosity that is high under conditions of low shear rate and low under conditions of high shear rate are often preferred.

6.6.3 In-vitro release studies

The gelling studies showed that the nature of gelation of the formulations with STF of either composition 1 or 2 was similar and STF1 was selected as the dissolution medium to avoid interference by the protein components used in STF2 during spectrophotometric analysis of the release study samples for MOXI and indomethacin content. First sampling was done 1 minute after the gelling system came in contact with the dissolution medium in order to account for the drug release before the complete formation of the gel and also to evaluate the effect of increasing polymer concentration on the nature of the gel formed. The results showed that the amount of drug released in the first minute decreased with increasing polymer concentration (Figs 6.5-6.7) and this trend continued for the entire duration of the study. The initial burst release of MOXI and indomethacin from the prepared systems could be explained by the fact that these systems were formulated in aqueous vehicle. The matrix formed on gelation was already hydrated and hence hydration and water permeation would no longer limit the drug release. A similar release pattern for
pilocarpine, wherein the initial fast release (burst effect) decreased with an increase in polymer concentration is reported (Cohen et al 1997) from alginate systems. These results also correlated well with the results of the gelation study. The formulations containing sodium citrate (GC₄ and GC₅ in MOXI systems and GI₆ to GI₀ in indomethacin systems) showed significantly higher release (P<0.05; t-test) than the corresponding batches without sodium citrate, apparently due to the formation of a less stiffer gel resulting in faster diffusion of the drugs from the gel to the dissolution medium.

Gellan at a concentration of 0.03% w/v was present in the formulations containing varying proportion of sodium alginate (GC₆ - GC₉). Comparison of the release profile of GC₂ (containing only gellan) with those of GC₆ - GC₉ indicate that the burst effect was considerably reduced (P<0.05), thus indicating the additive effect of the formed calcium alginate on gel formation and consequently on drug release. The release indices (n) of the MOXI formulations studied ranged from 0.48 to 0.57 and that of indomethacin between 0.52 and 0.59, indicating square root time release kinetics.

The eye drops formed an opaque matrix immediately on addition to the dissolution medium, due to the cation interaction in the STF1. Hence the release of the drugs from these matrices would be possibly influenced by diffusion and/or by erosion of the matrices. The combinations of these processes seemed to result in the overall diffusion – controlled release kinetics and indicated by the n values. These results are in accordance with that reported earlier (Sanzgiri et al. 1993) for methyl prednisolone from gellan eye drops.

The in-vitro release study conditions may be very different from those likely to be encountered when instilled into the eye. However, the results showed that the formed gels had the ability to retain MOXI and indomethacin for the duration of the study (8 hours). In the cul-de-sac, the gels would probably undergo faster dissolution due to the shearing action of the eyelid and eye ball movements.

6.6.4 Stability Studies

The stability studies were carried as per the ICH Guidelines. The study was undertaken for 6 months by storing the ocusert under refrigerator and ambient conditions (25° C / 60 % RH, and 40° C / 75 % RH). The amount of drug released at various time intervals was subjected to two way analysis of ANOVA to determine the level of significance. Formulations GC₃ and GC₇ from MOXI system and formulation GI₅ from
indomethacin systems, were used for pharmacodynamic and stability studies. The preparations were found to be clear with no significant change in drug content, gelling capacity and *in-vitro* release. However, a slight decrease in viscosity (to the extent of <5%) was observed when the formulations was stored at 45° C for 3 months.

### 6.6.5 Antimicrobial efficacy studies (MOXI system)

Formulations were selected in such a way to study the effect of increasing gellan concentration (GC2, GC3), sodium citrate (GC2, GC5) and sodium alginate concentration (GC7, GC9). The ZOI values for the prepared formulations were either on par or higher than the ZOI values of the standard preparation in most of the cases (table 6.5). Overall the ZOI value against *P. aeruginosa* was higher than that against *S. aureus*. The higher ZOI values obtained for the formulations in comparison to the standard could be attributed to the slow and prolonged diffusion of the drug from the polymeric solution due to its higher viscosity.

### 6.6.6. *In-vivo* rabbit eye study

**MOXI Systems**

Varying aliquots of the 0.5 McFarland standard suspensions (10, 25 and 40 µl) were instilled in both eyes of the rabbit and checked for growth over 24 hours. It was observed that the time period of growth was directly proportional to the inoculum volume of the organisms. For 10 µl of the inoculum growth occurred over 8 hour; for 25 and 40 µl it occurred over 16 and 24 hours, respectively. Therefore 40 µl was selected as the inoculum dose for further studies.

The tested formulations showed markedly improved effect when compared to the marketed (standard) eye drop. The developed formulations were able to prevent growth of both *S. aureus* and *P. aeruginosa* till 24 hours (Table 6.6). Growth was observed in all the animals after 2 hours post instillation of the formulations, when infected with *S. aureus*, while the formulations were successful in inhibiting the growth for the entire duration of the study in all the animals infected with *P. aeruginosa*. Repeated dose study was not attempted since the aim of the study was to develop a suitable formulation for once daily application. The formulations used for the *in-vivo* study were seen to form a translucent gel immediately after instillation into the eye. Gross examination of the ocular tissues showed that the formulations did not cause undue irritation and no leakage of the gelled material was seen from any part of the eye.
Indomethacin system

The pharmacodynamic evaluation of the indomethacin formulations in uveitis induced rabbit eye model is shown in Table 6.7 (A and B).

In case of the eyes treated with the standard dispersion, no significant improvement in the clinical parameters was observed after initial 4 hours (where improvement was observed in some parameters) indicating the need for frequent dosing to produce optimum therapeutic effect, whereas the in-situ gelling formulation (batch GI5) showed improvements in the clinical parameters up to 24 hours post instillation suggesting the adequacy of the prepared systems to sustain drug release with minimal loss due to drainage.

MOXI and indomethacin were successfully formulated as in-situ gelling systems using gellan. Combining gellan with sodium alginate in case of MOXI system did not offer any advantage (as regards therapeutic efficacy) over the formulations based on gellan alone. Both the formulated systems provided sustained release of the drug over an 8 hours period in-vitro and the developed formulations were devoid of any deleterious effect on the ocular tissues. The formulations demonstrated correspondingly better therapeutic efficacy. Hence these systems can be viewed as viable alternative to conventional eye drops by virtue of their ability to enhance pre- corneal residence time and consequently the ocular bioavailability. The ease of administration coupled with the ability to provide sustained release could probably result in less frequent administration, thus enhancing patient compliance.

Table 6.1 Composition of the prepared in–situ gelling MOXI formulations

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Gellan (%w/v)</th>
<th>Sodium citrate (%w/w gellan)</th>
<th>Sodium Alginate (%w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC1</td>
<td>0.015</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GC2</td>
<td>0.032</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GC3</td>
<td>0.0625</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GC4</td>
<td>0.0625</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>GC5</td>
<td>0.032</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>GC6</td>
<td>0.03</td>
<td>-</td>
<td>0.22</td>
</tr>
</tbody>
</table>
Mannitol 5% was used as isotonic agent

**Table 6.2 Composition of the Prepared Indomethacin Gelling Formulations**

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Gellan (%w/v)</th>
<th>Sodium citrate (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI₁</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>GI₂</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>GI₃</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td>GI₄</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>GI₅</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>GI₆</td>
<td>0.5</td>
<td>10</td>
</tr>
<tr>
<td>GI₇</td>
<td>0.5</td>
<td>20</td>
</tr>
<tr>
<td>GI₈</td>
<td>0.5</td>
<td>30</td>
</tr>
<tr>
<td>GI₉</td>
<td>0.5</td>
<td>40</td>
</tr>
</tbody>
</table>

Indomethacin 1% w/v was added in the cases and Mannitol 5% was used as this isotonic agent.
Table 6.3: Physico-chemical properties of the prepared MOXI gelling systems

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>DCU(±S.D)</th>
<th>Gelling Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>STF1</td>
</tr>
<tr>
<td>GI1</td>
<td>98.48 ± 1.14</td>
<td>+</td>
</tr>
<tr>
<td>GI2</td>
<td>99.64 ± 0.38</td>
<td>++</td>
</tr>
<tr>
<td>GI3</td>
<td>99.18 ± 0.66</td>
<td>+++</td>
</tr>
<tr>
<td>GI4</td>
<td>98.81 ± 0.41</td>
<td>++</td>
</tr>
<tr>
<td>GI5</td>
<td>99.08 ± 0.63</td>
<td>++</td>
</tr>
<tr>
<td>GI6</td>
<td>98.64 ± 0.34</td>
<td>+++</td>
</tr>
<tr>
<td>GI7</td>
<td>98.11 ± 0.51</td>
<td>+++</td>
</tr>
<tr>
<td>GI8</td>
<td>100.08 ± 0.18</td>
<td>+++</td>
</tr>
<tr>
<td>GI9</td>
<td>100.11 ± 0.71</td>
<td>+++</td>
</tr>
</tbody>
</table>

+ - Gels after few minutes
++ - Gels immediately but remains for a few hours (Less Stiffer);
+++ - Gelation immediate and remains for extended periods & formed gels are stiffer

DCU - Drug content uniformity
Table 6.4: Physico-chemical Properties of the prepared indomethacin Gelling Systems

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>DCU(±S.D)</th>
<th>Gelling Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI1</td>
<td>99.64±0.86</td>
<td>+++</td>
</tr>
<tr>
<td>GI2</td>
<td>98.18±0.48</td>
<td>+++</td>
</tr>
<tr>
<td>GI3</td>
<td>99.09±0.51</td>
<td>+++</td>
</tr>
<tr>
<td>GI4</td>
<td>99.43±0.68</td>
<td>+++</td>
</tr>
<tr>
<td>GI5</td>
<td>98.78±0.76</td>
<td>+++</td>
</tr>
<tr>
<td>GI6</td>
<td>98.81±0.88</td>
<td>+++</td>
</tr>
<tr>
<td>GI7</td>
<td>99.66±1.04</td>
<td>+++</td>
</tr>
<tr>
<td>GI8</td>
<td>99.84±0.91</td>
<td>+++</td>
</tr>
<tr>
<td>GI9</td>
<td>98.93±0.43</td>
<td>+++</td>
</tr>
</tbody>
</table>

+++: Forms stiff gel instantaneously, which is retained for prolonged periods.
Table 6.5: Antimicrobial Efficacy of the Prepared Gelling Systems

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Zone of Inhibition (cm) (%Efficiency)</th>
<th>Std</th>
<th>GC2</th>
<th>GC3</th>
<th>GC5</th>
<th>GC7</th>
<th>GC9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
<td>1.4 (100)</td>
<td>1.4 (100)</td>
<td>1.2 (85.7)</td>
<td>1.2 (85.7)</td>
<td>1.4 (100)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2.0</td>
<td>2.0 (100)</td>
<td>2.2 (110)</td>
<td>1.8 (90)</td>
<td>1.8 (90)</td>
<td>2.2 (110)</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>2.6</td>
<td>3.2 (123.07)</td>
<td>3.0 (115.38)</td>
<td>2.8 (107.69)</td>
<td>2.8 (107.69)</td>
<td>2.6 (100)</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.8</td>
<td>2.0 (111.11)</td>
<td>2.2 (122.22)</td>
<td>1.8 (100)</td>
<td>1.8 (100)</td>
<td>2.0 (111.11)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2.4</td>
<td>2.6 (108.33)</td>
<td>3.0 (125)</td>
<td>2.4(100)</td>
<td>2.4(100)</td>
<td>2.6 (108.33)</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>3.4</td>
<td>3.6(105.88)</td>
<td>3.6 (105.38)</td>
<td>3.4 (100)</td>
<td>3.2 (94.1)</td>
<td>3.4 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Std- Standard (Marketed eye drop of MOXI)

GC2, GC3, GC5, GC7, GC9 -Prepared formulations whose compositions are shown in table1.

Values in parenthesis indicates the percentage efficiency, calculated from (ZOI of test/ZOI of standard) x100
Table 6.6: *In-vivo* anti-microbial activity of the marketed and prepared MOXI formulation in rabbit eye (n=5)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2h</td>
<td>4h</td>
</tr>
<tr>
<td>Std</td>
<td>-5</td>
<td>+5</td>
</tr>
<tr>
<td>GC3</td>
<td>+5</td>
<td>-5</td>
</tr>
<tr>
<td>GC7</td>
<td>+5</td>
<td>-5</td>
</tr>
</tbody>
</table>

+5 - Indicates growth of microorganisms in the animals

-5 – Indicates absence of growth of microorganisms in all animals.
### Table 6.7A Pharmacodynamic studies of indomethacin gelling systems

<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>0 4h 8h 12h 24h</td>
</tr>
<tr>
<td><strong>Gl5</strong></td>
<td>1</td>
<td>R*</td>
<td>++++</td>
</tr>
</tbody>
</table>
Table 6.7 B: Pharmacodynamic Studies of Indomethacin Gelling Systems

<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>Clot</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4h</td>
<td>8h</td>
</tr>
<tr>
<td>GI5</td>
<td>1</td>
<td>R*</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>R*</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>R</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>L*</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Std</td>
<td>1</td>
<td>R*</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>R*</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>L</td>
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Fig. 6.1: Viscosity of gellan based MOXI in situ gelling system
Fig. 6.2 Viscosity of MOXI formulations containing combinations of gellan and sodium alginate
Fig 6.3: Viscosity of in-situ gelling preparations of indomethacin

(Effect of gellan concentration)
Fig. 6.4: Effect of sodium citrate concentration on rheological behavior of gelling systems of indomethacin
Fig. 6.5: In vitro drug release from gellan based MOXI formulations
Fig. 6.6: In vitro release from combination of gellan-sodium alginate

MOXI formulations
Fig. 6.7: In vitro release of the prepared gelling systems of indomethacin
6.7 REFERENCES


