CHAPTER-2

Review of Literature
REVIEW OF LITERATURE

2.1 SOL-GEL SYSTEMS¹⁹:

Eye solution (homogeneous preparations) offers the assurance of greater uniformity of dosage and bioavailability. One major disadvantage of solutions is their relatively short residence time (corneal contact time) of the dosage form and it has been increased to varying degrees by including the viscous and semisolid vehicles in the dosage forms. Extended corneal contact times as well as sustained releases have been obtained with solid dosage forms such as ocluserts. However, poor patient acceptance and difficulties in administration have led ophthalmic researchers to seek other systems which would combine the ease of administration of liquid forms with the prolonged residence time of inserts.

On this concept, currently two drug delivery systems are explored and they are:

- Bioadhesive systems and
- Phase transition systems.

Gel forming solutions (phase transition systems) are liquid in the container and thus can be instilled as eye drops but gel on contact with the tear fluid and provide increased contact time with the possibility of improved drug absorption and increased duration of therapeutic effect. Phase transitions (liquid-gel) vary according to the particular polymer(s) employed and their mechanism(s) for triggering the transition to a gel phase in the eye. The mechanisms that make them useful for the eye take advantage of changes in temperature, pH, ion-sensitivity or ionic strength upon contact with tear fluid or due to the presence of proteins such as lysozyme in the tear fluid.
The pH sensitive systems may have limited use for drugs that require a neutral to slightly alkaline environment for stability, solubility, etc. A number of liquid-gel phase transition-dependent delivery systems have been researched and patented. Gel-forming ophthalmic solutions have been developed and approved by FDA for timolol maleate, which is used to reduce elevated intraocular pressure (IOP) in the management of glaucoma. Timolol maleate ophthalmic solutions, as initially developed, require twice-a-day dosage for most patients. With the gel-forming solutions, intraocular pressure-lowering efficacy was extended from 12 to 24 hours and thus required only once-a-day dosing. The first gel-forming product, timolol XE, uses the polysaccharide gellan gum and is reported to gel in situ in response to the higher ionic strength of tear fluid. The second product (Timolol maleate) uses the polysaccharide xanthan gum as the gelling agent and is reported to gel upon contact with tear fluid, at least in part due to the presence of tear protein lysozyme.

2.2 LITERATURE REVIEW ON PAST WORK OF SOL-GEL SYSTEMS AND NOVEL OPHTHALMIC DRUG DELIVERY SYSTEMS:

A basic concept shared by most scientists in ophthalmic research and development is that the therapeutic efficacy of an ophthalmic drug can be greatly improved by prolonging its contact with the corneal surface. Various approaches have been adopted for the purpose as enumerated in the following paragraphs.

Hong-Ru Lin and K.C.Sung20 (2000) developed a series of carbopol and pluronic based solutions as the 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 were evaluated. They found that, the optimum concentration of carbopol solution for the in-situ gel
forming delivery systems was 0.3% (w/w) and that for pluronic solution was 14% (w/w). The mixture of 0.3% carbopol and 14% pluronic solutions showed a significant enhancement in gel strength in the physiological conditions. The results demonstrated that, carbopol/pluronic solution had the better ability to retain drug and can be used as an in-situ gelling vehicle to enhance the ocular bioavailability.

Kumar S, Haglund BO and Himmelstein KJ21 (1994) explained that, poor bioavailability of ophthalmic solutions caused by dilution and drainage from the eye can be overcome by using in situ-forming ophthalmic drug delivery systems prepared from polymers that exhibit reversible phase transitions. In the present study, the rheological characterization of such a system, prepared by a combination of carbopol and methyl cellulose, was carried out at two different pH (4.0 and 7.4) and temperatures (25 and 37°C) by rotational cone and plate viscometry. An increase in pH from 4.0 to 7.4, or temperature from 25 to 37°C, resulted in an increase in viscosity, An increase in concentration of either carbopol or methyl cellulose, results in an increase in eta, tau, and yield point. Among the compositions studied, a solution containing 1.5% methyl cellulose 0.3% carbopol was found to have low eta, and formed a strong gel under simulated physiological conditions. Such a system can be formulated as drug containing liquid suitable for administration by instillation into the eye, which upon exposure to physiological conditions will shift to the gel (semi-solid) phase, thus increasing the precorneal residence time of the delivery system and enhancing ocular bioavailability.

Kumar S and Himmelstein KJ22 (1995) studied the modification of in-situ gelling behaviour of carbopol solutions by hydroxypropyl methyl cellulose. In their study, they explained that Aqueous solutions of carbopol [polyacrylic acid (PAA)] are low viscosity acidic solutions that transform into
gels upon an increase in the pH and, therefore, may be used as in situ gelling ophthalmic drug delivery systems. However, the amount of polyacrylic acid required in the solution to form stiff gels upon installation in the eye is not easily neutralized by the buffering action of tear fluid. A reduction in the polyacrylic acid concentration without comprising the in situ gelling properties as well as the overall rheological behavior of the system can be achieved by adding a suitable viscosity-enhancing polymer. The rheological properties of aqueous solutions containing polyacrylic acid and hydroxypropyl methylcellulose, a viscosity-enhancing polymer, evaluated as a function of temperature and pH, were similar to those of pure polyacrylic acid solutions; that is, both form low viscosity liquids at pH 4.0 and transform into stiff gels with plastic rheological behavior and comparable viscosities upon increasing the pH to 7.4. In addition, HPMC-PAA gels show slow in vitro release of incorporated timolol maleate. Thus, the HPMC-PAA combination demonstrates properties suitable for formulation as a liquid ophthalmic delivery systems, which upon instillation into the cul-de-sac of the eye can undergo in situ phase transition to form gels capable of sustained drug release.

Rozier A, Maruel C, Grove J and Plazonnet B23 (1989) studied that the use of 'Gelrite' a novel, ion-activated, in-situ gelling polymer for ophthalmic vehicles and its effect on bioavailability of timolol. They explained that, Gelrite solution, a novel ophthalmic vehicle, gels in the presence of mono or divalent cations. In the conjunctival sac 'ion-activation' of the sol-gel transition is accomplished by the lacrimal fluid. A 0.6% Gelrite vehicle has been compared to an equiviscous solution of hydroxyethyl cellulose (HEC) using timolol maleate as a drug probe. In vitro release rates of timolol from HEC and Gelrite gel were similar. In vivo, the formation of the gel prolonged precorneal residence time and increased ocular bioavailability of timolol in the cornea, aqueous humor and iris + ciliary body of albino rabbits.

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Katarina Lindell and Sven Engstrom\textsuperscript{24} (1993) designed a thermogelling drug delivery system composed of a cellulose ether (ethyl (hydroxyethyl) cellulose – EHEC), an ionic surfactant and water was thoroughly characterized in the presence of timolol maleate with respect to phase and rheological behaviour, as well as \textit{in vitro} drug release. The phase studies reveal that gelling systems may be formed with 0.34\% (w/w) timolol maleate, and that the gelling behaviour is sensitive to the surfactant concentration and ionic strength of the solution. The release of timolol maleate from the gels is retarded compared to a non-gelling EHEC system. The release rate is about equal for systems with 1 and 2\% (w/w) EHEC, implying that the release is controlled by a low convection in the gels and not by any drug-polymer interaction.

Srividya B, Rita M, Cardoza and Amin PD\textsuperscript{25} (2001) developed sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. In this study, they demonstrated that, the poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precomeal elimination of the drug may be overcome by the use of in situ gel-forming systems that are instilled as drops into the eye and undergo a sol-gel transition in the cul-de-sac. The present work describes the formulation and evaluation of an ophthalmic delivery system of an antibacterial agent, ofloxacin, based on the concept of pH-triggered in situ gelation. Polyacrylic acid (carbopol 940) was used as the gelling agent in combination with hydroxypropyl methyl cellulose (Methocel E50LV) which acted as a viscosity enhancing agent. The developed formulation was therapeutically efficacious, stable, non-irritant and provided sustained release of the drug over an 8h period. The developed system is thus a viable alternative to conventional eye drops.
Florence Thermes, Annouk Rozier, Bernard Plazonnet and Jeffrey Grove\(^\text{26}\) (1992) studied the effect of polyacrylic acids (bioadhesive polymer) on the ocular bioavailability of timolol. They have prepared ophthalmic formulations by using polyacrylic acid as bioadhesive polymer and evaluated for its effect on the ocular distribution of timolol. Ocular bioavailability of 0.5% timolol was measured in cornea, aqueous humor and iris+ciliary body, of albino rabbits and was compared to that of 0.5% timolol in isoviscous solutions of polyvinyl alcohol (PVA), polyacrylic acid (PAA) and timolol-polyacrylic acid salt (PAA salt). Ocular bioavailability of timolol was increased by each of the viscous solutions. These increases assessed by measurement of AUC (0-4h) in cornea, aqueous humor and iris+ciliary body ranged from 1.4 to 2.8 fold. The largest increases were obtained with the non-mucoadhesive polymer PVA. The bioadhesive PAA polymers modified the concentration versus time profiles of timolol and gave the highest concentrations in iris+ciliary body at later sampling times.

Edsman K, Carlfors J, Petersson R\(^\text{27}\) (1998) evaluated poloxamer as an in situ gel for the ophthalmic use. In their study, they explained that the contact time of a vehicle on the cornea is of utmost importance for ocular drug delivery. In the present study rheological measurements were performed to study the gel and the 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. 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 1h. The poloxamer system did not seem to be
promising as an ophthalmic in situ gel due to the strong concentration
dependence of the sol-gel transition temperature combined with the dilution
that occurs in the eye.

Jeffrey Grove, Michel Durr, Marie-Paule Quint and Bernard Plazonnet\textsuperscript{28} (1990) studied the effect of vehicle viscosity on the ocular bioavailability of L-653, 328. They have investigated the effect of viscosity of an ophthalmic vehicle on ocular drug penetration. Ocular concentration of L-652, 698 have been measured using HPLC and fluorescence detection, in the cornea, aqueous humor and iris+ciliary body of rabbits after instillation of 1% solutions of L-653, 328 in 0.2, 0.25, 0.3, 0.35, 0.4 and 0.5% hydroxyethyl cellulose (HEC). Maximum drug concentrations in all three ocular sites increased concomitantly with increase in viscosity. The correlation coefficients between ocular bioavailability, assessed by AUC (0-4h), and with HEC viscosity were 0.93, 0.96 and 0.83 in cornea, aqueous humor and iris+ciliary body, respectively. When isoviscous solutions containing polyvinyl alcohol were examined, ocular bioavailability was similar in cornea and aqueous humor but reduced by 50% in the iris+ciliary body when compared to the equivalent HEC solution.

Thilek Kumar M, Bharathi D, Balasubramaniam J, Kant S and Pandit JK\textsuperscript{29} (2005) designed pH induced in-situ gelling systems of indomethacin for sustained ocular delivery. They studied that the bioavailability and ocular residence time exhibited by the topical conventional liquid ophthalmic formulations because of spillage by overflow, dilution of drug by tear turnover, nasolacrimal drainage and systemic absorption may be overcome by the use of in-situ forming systems that are instilled as liquid drops into the cul-de-sac of the eye, where they transform into a gel or semisolid phase. The present work describes the formulation and evaluation of an ophthalmic delivery system of an anti-inflammatory drug. Indomethacin for the treatment of uveitis
based on the concept of pH induced in-situ gelation. The carbopol solutions, which are acidic and less viscous, transform into stiff gels upon increase in pH of eye as the gelling agents and its combination with hydroxypropyl methyl cellulose-K₁₅M, a well known ocular viscosity enhancing agent. The enhanced therapeutic efficacy and sustained release of indomethacin over 8 hours period \textit{in vitro} make them an excellent candidate for in-situ gelling ocular delivery systems.

Abhilash AS, Jayaprakash S, Nagarajan M and Dhachinamoorthi D³⁰ (2005) designed timolol maleate ocluserts using different polymers such as hydroxypropyl methyl cellulose, ethyl cellulose, Eudragit RL100 and Eudragit RS 100 at various concentrations. The \textit{in vitro} release of the drug from the formulations was studied using commercial semi permeable membrane. The physicochemical parameters of ocluserts were evaluated. A zero order release formulation I (drug reservoir with 1.25% hydroxypropyl methyl cellulose and 1.25% ethyl cellulose and 2% hydroxypropyl methyl cellulose as rate controlling membrane) was subjected to \textit{in vivo} studies. The expected zero order release for one day was observed in formulation I (drug reservoir with 1.25% hydroxypropyl methyl cellulose and 1.25% ethyl cellulose and 2% hydroxypropyl methyl cellulose as rate controlling membrane).

Patel MM, Seth MN and Dave DJ³¹ (1994) developed prolonged release ophthalmic preparations containing timolol maleate in the form of viscous solutions and gels using methyl cellulose, polyvinyl alcohol, hydroxypropyl methyl cellulose, carbopol 940 p and carbopol 934 p. The formulations were evaluated for drug content and drug release profile \textit{(in vitro)} and data generated were subjected to linear regression. All formulations were subjected to autoclaving but no significant loss of drug was found. The formulation containing carbopol 934p in 0.5% concentration was found to be the most
promising for prolonged action. This formulation was subjected to accelerated stability testing and no significant degradation of drug was found. Drug release profile remained unchanged after 25 days storage of formulation at different temperatures.

Saettone MF, Chetoni P, Mariotti Bianchi L, Giannaccini B, Conte U, Sangalli ME\textsuperscript{32} (1995) studied the controlled release of timolol maleate from coated ophthalmic mini tablets prepared by compression. In their work, ophthalmic inserts (mini tablets) for sustained release of timolol were prepared by a standard compression and coating technique. An adequate control of the \textit{in vitro} drug release from the devices could be obtained by adjusting the type and amount of acrylic polymer coating.

Manvi FV, Soppimath KS and Gadad AP\textsuperscript{33} (1997) developed timolol maleate ocular inserts. They have prepared timolol maleate circular ophthalmic inserts by solvent casting, using cellulose acetate as polymer with polyethylene glycol 600 (PEG 600) and diethylphthalate (DEPT) as plasticizers in two different concentrations. An \textit{in vitro} method was designed and utilized for the study. Inserts release the drug as a function of square root of time. A clear vision was available that plasticizer systems were influencing their effect on drug release i.e., PEG 600 enhanced the drug release in comparison with DEP. Release was directly proportional to the amount of drug loaded. \textit{In vivo} release of the inserts was determined in rabbits. \textit{In vitro} methods simulate \textit{in vivo} conditions, which was confirmed by strong positive correlation (+0.856) between the two. Results indicate the inserts can control drug release and might improve ocular bioavailability and reduce toxicity of timolol maleate.

Rai AK, Vyas SP and Chitme HR\textsuperscript{34} (2004) designed reverse micelles of timolol maleate for controlled ocular delivery. They explained that, the reverse
micelle is one of many models thought to have properties more nearly resembling the biological cellular environment, than does the traditional dilute-solution biochemical reaction system. The reverse micellar ocular system was prepared using cetyltrialammonium bromide, span 60 alone and in combinations in organic solvent of isopropylpalmitate: isopropyl myristate (50:50). The designed systems were characterized for drug content and the process variables that affect the percent drug payload and release profiles of drug. The effect of hydration, temperature and ionic strength on drug payload on reverse micellar systems had been considered for the present study. Systems were evaluated for in vitro performance. The drug release was recorded to follow approximate first order release kinetics. On the basis of in vitro characterization the selected systems were evaluated for in vivo activity. It was observed that the micellar systems exhibited prolonged and controlled biological response of timolol maleate.

Gang Wei, Hui Xu, Ping Tian Ding, San Ming Li and Jun Min Zheng (2002) studied thermosetting gels with modulated gelation temperature for ophthalmic use. They developed a thermosetting gel with a suitable phase transition temperature by combining Pluronic analogs and to examine the influence of incorporating mucoadhesive polysaccharide, sodium hyaluronate (HA-Na), on the ocular retention of the gel. Dynamic rheological method and single photon emission computing tomography (SPECT) techniques were used to ex/in vivo evaluate the thermosetting gels, respectively. An optimized formulation containing 21% F127 and 10% F68 increased the phase transition temperature by 9°C as evaluated by elasticity modulus compared to that of individual 21% F127 solution. Rheological behaviors of the Pluronic solutions showed that the combined Pluronic formulation was free flowing liquid below 25°C and converted to a firm gel under the physiological condition. Furthermore, this formulation possessed the highest viscosity both before and
after tear dilution at 35°C. Gamma scintigraphic data demonstrated that the clearance of the thermosetting gel labeled with $^{99m}$Tc-DTPA was significantly delayed with respect to the phosphate buffered solution, and at least a threefold increase of the corneal residence time was achieved.

Saettone MF, Torracca MT, Pagano A, Giannaccini B, Rodriguez L and Cini M (1992) prepared pilocarpine coated polymeric ophthalmic inserts by extrusion for its controlled release. A series of cylindrical ophthalmic inserts based on mixtures of PVA, glyceryl behenate and different polymers (xanthan gum, jota-carrageenan, hydroxypropyl methyl cellulose, hyaluronic acid), and containing pilocarpine nitrate (PiN) were prepared by extrusion, and were subsequently coated with a mixture of Eudragit RL and RS. The inserts had the following characteristics: diameter, 1.5mm, length, 3mm; weight, 7 mg; PiN content, 1.16 mg. The applied coating was 4% of the inserts’ weight. The inserts were submitted to release tests in vitro, and to miotic activity tests in rabbits. The uncoated inserts released 50% of the drug within 20-30 minutes, with predominantly diffusive kinetics. The release profiles of the four types of uncoated inserts were essentially similar. The coated units released 50% PiN in 3-5h, depending on the core composition. Zero-order release kinetics were observed in the case of three of the four types of coated inserts. Release was incomplete in all cases: this was due, as shown by equilibrium dialysis tests, to PiN binding by the polymers. The uncoated inserts, when tested for miotic activity in albino rabbits, showed little or no sustained activity, and moderate AUC increases with respect to an aqueous solution of the drug. Conversely, the coated inserts showed miotic activity profiles indicating a prolonged-pulse or sustained release (9-10 h duration, shift of the peak time to 120-240 minutes, over 3-fold increase in AUC over the aqueous solution). The in vitro/in vivo relationships, the effects of different core compositions and coating thickness, and the possible mechanism governing release from the coated
inserts are discussed. This preliminary study indicates the possibility of realizing, using relatively simple techniques and common pharmaceutical materials, ocular delivery devices showing substantially improved properties when compared with traditional ophthalmic vehicles.

Soltan A Monem, Fadel M Ali and Medhat W Ismail\textsuperscript{37} (2000) investigated the possibility of using liposomes as an ophthalmic drug delivery carrier for the lipophilic drug pilocarpine hydrochloride in normal and glaucomatous pigmented rabbits. The intraocular pressure of rabbits was measured, using a Shiotz tonometer, as a function of time after topical administration with free drug, neutral and negatively charged multilamellar vesicles (MLVs) encapsulating pilocarpine hydrochloride. The results showed that administration with neutral MLVs displayed the most prolonged effect with respect to negatively charged MLVs and free drug. The efficiency of MLVs encapsulating pilocarpine hydrochloride measured using spectrophotometric technique was found to be 96\% in our modified preparations. The storage stability of MLVs encapsulating pilocarpine hydrochloride was investigated by measuring phase transition and size distribution using light scattering technique. The results show that liposomes encapsulating pilocarpine hydrochloride have kept their integrity and physiochemical properties for at least 15 months, which makes them suitable for commercial use.

Lisbeth R Hume, Hyeyoun K Lee, Luca Benedetti, Yeshwant D Sanzgiri, Elizabeth M Topp and Valentino J Stella\textsuperscript{38} (1994) studied the ocular sustained delivery of prednisolone using hyaluronic acid benzyl ester films. They have used four polymers HYAFF 11 p25 (25\% benzyl ester, 75\% sodium salt), HYAFF 11 p50 (50\% benzyl ester, 505 sodium salt), HYAFF 11 p75 (75\% benzyl ester, 25\% sodium salt) and HYAFF 11 (100\% benzyl ester). The
polymer with the lowest degree of esterification, HYAFF 11 p25, was the most hydrophilic and released drug faster than those with higher degrees of esterification, HYAFF 11 and HYAFF 11 p75, which are generally less hydrophilic. Tear fluid prednisolone concentrations were measured in rabbits after administration of the test films. Areas under the tear fluid concentration versus time curves (AUC$_{0-8h}$) were calculated for all the dosage forms, from the time of dosing to 8 hr postdosing. The HYAFF 11 p25 films provided higher initial concentrations which rapidly declined below 30 µg/ml, 2 h post-dosing. Concentrations for the HYAFF 11 p75 film dropped below 30 µg/ml, 3 h post-dosing. The HYAFF 11 films provided the best results with sustained concentrations between 45 and 75 µg/ml for the 8 hr study period. The results showed that sustained delivery of prednisolone to the eye may be achieved with the use of hyaluronic acid esters.

Bharath S, Hiremath SR$^{39}$ (1999) developed ocular delivery systems of pefloxacin mesylate. They have prepared ocular films of pefloxacin mesylate with the objectives of reducing the frequency of administration, to improve patient compliance, obtaining controlled release and greater therapeutic efficacy in the treatment of eye infections such as conjunctivitis, keratitis, keratoconjunctivitis, corneal ulcers etc. Polymers such as hydroxypropyl cellulose, hydroxypropyl methylcellulose, polyvinyl pyrrolidone and polyvinyl alcohol were used in different ratios to prepare the ocular films. They were evaluated for drug content which varied from 96-104%. Those which consisted of flexible and transparent films were subjected to in vitro release studies. The formulations which prolonged the release for eight hours were selected. The average weight and thickness of these were found to be 38.92-49.71 mg and 31.68-46.08 microns, respectively. The intactness of the formulations was confirmed by infrared and thin layer chromatographic studies. In vivo studies carried out in the eyes of rabbits showed controlled release upto 8-9h. There
was a good correlation between the in vitro and in vivo data \( r = 0.97-0.995 \). A minimum of 1 Mrad was found to be necessary for the sterilization of ocular films by gamma radiation. They were found to be stable at temperatures below 45°C.

Deshpande S.G., Satish Shirolkar\(^\text{40}\) (1999) designed sustained release ophthalmic formulations of pilocarpine. The bioavailability of drugs from conventional ophthalmic formulations is low. To optimize the therapy, sustained release ophthalmic dosage forms are warranted. Hydrogels such as sodium carboxymethyl cellulose, hydroxypropyl methyl cellulose. Carbopol-gel, carbopol 941 and lutrol FC-127 increase the duration of action of various drugs. Gels containing pilocarpine were prepared and evaluated by measuring the intensity and duration of miotic response in albino rabbits. Carbopol 940 gels, being the best of those prepared were studied further for the effect of its concentration and of additives (benzalkonium chloride, phenyl mercuric nitrate, chlorbutol and disodium edetate), autoclaving at 121°C for 30 min and sterilization with gamma rays (2.5 Mrad) on the end product.

Grass GM, Cobby J, Makoid MC\(^\text{41}\) (1984) studied the ocular delivery of pilocarpine from erodible matrices. They examined the feasibility of sustaining the release of a water-soluble drug, pilocarpine, to the tear film. Both gels and dried films were utilized as drug delivery systems. In vitro studies demonstrated significant prolongation of drug release from these systems as compared with simple aqueous or viscous solutions. The in vitro results were supported by in vivo miosis studies in albino rabbits.

Fresta M, Panico AM, Bucolo C, Giannavola C, Puglisi G\(^\text{42}\) (1999) studied the potential of liposomes as an in-vivo ophthalmic drug delivery system for acyclovir was investigated. The drug-membrane interaction was evaluated by means of differential scanning calorimetry analysis. These
experiments showed that acyclovir is able to interact with both positively and negatively charged membranes via electrostatic or hydrogen bonds. No interaction was observed with neutral membranes made up of dipalmitoylphosphatidylcholine. Different liposome preparation procedures were carried out to encapsulate acyclovir. The drug encapsulation mainly depends on the amount of water which the liposome system is able to entrap. In the case of multilamellar vesicles, charged systems showed the highest encapsulation efficiency. No particular difference in the encapsulation efficiency was observed for oligolamellar vesicles prepared with the reverse-phase evaporation technique. Oligolamellar liposomes showed the highest acyclovir encapsulation parameters and had release profiles similar to those of multilamellar liposomes. In-vivo experiments using male New Zealand albino rabbits were carried out to evaluate the aqueous humour concentration of acyclovir bioavailability. The most suitable ophthalmic drug delivery system was oligolamellar systems made up of dipalmitoylphosphatidylcholine-cholesterol-dimethyldioctadecyl glycerole bromide (7:4:1 molar ratio), which presented the highest encapsulation capacity and were able to deliver greater amounts of the drug into the aqueous humour than a saline acyclovir solution or a physical liposome/drug blend.

Nagarsenker MS, Vaishali Y Londhe and Nadkarni GD\textsuperscript{43} (1999) prepared liposomal formulations of tropicamide for ocular delivery. Tropicamide, a mydriatic, cycloplegic drug was entrapped in liposomes. Liposomes were investigated by laser counting studies, transition electron microscopy and differential scanning calorimetry for characterization. The precorneal clearance of liposomes was compared with solution by \(\gamma\)-scintigraphy in the rabbit. The neutral liposomes failed to demonstrate significant enhancement in precorneal retention in comparison with aqueous solution. The potential of liposomes as an ophthalmic drug delivery system
was investigated by comparing pupil dilatory effect of tropicamide by topical instillation, in the rabbit eye, of the solution and various drug-loaded liposomal forms i.e., neutral liposomes, positively charged liposomes and neutral liposomes dispersed in 0.25% 9w/v) polycarbophil gel. The positively charged liposomal formulation and liposomes dispersed in polycarbophil gels were found to be more effective than neutral liposomal dispersion when data were statistically treated at 5% level of significance.

Calvo P, Vila Jato JL, Alonso MJ\textsuperscript{44} (1996) designed several colloidal systems namely nanoparticles, nanocapsules and nanoemulsions as ocular drug delivery systems. They have investigated the capacity of colloidal systems for increasing the corneal penetration of drugs. The three systems differed in their inner structure and composition, but they had a similar size (200-250 nm) and a negative superficial charge (-16 to -42 mV). Indomethacin, which was used as a model drug, was dispersed at a molecular level within the colloidal systems, no chemical interaction between the polymer and the drug being detected. Release of the encapsulated indomethacin occurred very rapidly upon high dilution in a buffered medium and was independent of the composition of the system. The in vitro corneal penetration of the encapsulated indomethacin was more than 3-fold that of the commercial eye drops. This increased penetration was similar for the three formulations investigated, which therefore excludes the influence of the inner structure or chemical composition of the colloidal systems on the corneal penetration of indomethacin. Thus, it could be stated that the main factor responsible for the favorable corneal transport of indomethacin is the colloidal nature of these carriers rather than their inner structure or composition.

Nigel M Davies, Guangji Wang and Ian G Tucker\textsuperscript{45} (1997) formulated hydrocortisone/hydroxypropyl β-cyclodextrin solution for ocular drug
delivery. They have investigated the effect of hydroxypropyl β-cyclodextrin (HP-β-CD) on the aqueous solubility and chemical stability of hydrocortisone (HC) was investigated with an ultimate aim of formulating a stable topical ophthalmic solution of HC. The ocular bioavailability following topical administration to rabbits of the aqueous formulation of HC was then compared to that of a suspension formulation having an equivalent HC concentration. The aqueous solubility of HC was markedly increased upon addition of HP-β-CD due the formation of a soluble 1:1 inclusion complex. The apparent association constant of the HC/HP-β-CD complex determined by phase-solubility analysis was estimated to be 0.636 mM⁻¹. The decomposition of HC in pH 7.4 phosphate buffer followed pseudo first-order kinetics having rate constants of 13.6×10⁻³ and 1.70×10⁻³ h⁻¹, respectively, in the presence and absence of disodium edetate. Complexation with HP-β-CD increased the chemical stability of HC with the respective pseudo first-order rate constants of decomposition being reduced to 6.73×10⁻³ and 0.90×10⁻³ h⁻¹. The ocular bioavailability following topical administration to rabbits of a tritium labelled 1% HC solution formulation of the HC/HP β-CD complex was lower than that of a 1% suspension formulation. A significant reduction (p<0.05) of between 25 and 40% was apparent in the cornea, aqueous humour, iris and sclera.

Deepika Aggarwal and Indu P Kaur⁴⁶ (2005) studied an improved pharmacodynamics of timolol maleate from a mucoadhesive niosomal ophthalmic drug delivery system. They have prepared chitosan (REVTMbio-1) or carbopol (REVTMbio-2 and 3) coated niosomal timolol maleate (0.25%) formulations by reverse phase evaporation and compared in terms of in vitro release and intraocular pressure lowering pharmacodynamic effect. The in vitro release phase of timolol (91% release in 2h) was extended significantly by its incorporation into niosomes and further by the polymer coating (40-43% release up to 10h). The developed formulations were evaluated for their
pharmacodynamics in albino rabbits, by measuring intraocular pressure (IOP) using a non-contact pneumatonometer, and were compared to a marketed in situ gel forming solution of timolol. REVTMbio-1 formulation showed a more sustained effect of up to 8h (vis a vis 6h for carbopol-coated niosomes). TMS in comparison showed effect for only 2 h though the peak effect was slightly more (14%). Lowering of intraocular pressure in the contralateral eye (20-40% as compared to 100% in case of TMS), considerably reduces with REV and REVbio formulations indicating lesser systemic side effects. Moreover, the results of REV TMbio-1 formulation containing 0.25% of timolol maleate compared well with the 0.5% marketed gel formulation, indicating our formulation to be significantly better considering that similar effect is obtained at half the concentration. The later becomes especially important in context to the cardiovascular side effects associated with ocular timolol maleate therapy.

Masayo Higashiyama, Katsuhiro Inada, Akira Ohtori and Kakuji Tojo\(^{47}\) (2004) studied the improvement of the ocular bioavailability of timolol by sorbic acid. The ocular bioavailability of timolol increased in sorbic acid solution due to ion pair formation. Its octanol/ water partition coefficient also increased, suggesting the formation of a more lipophilic complex. The concentration of timolol in rabbit aqueous humour was determined after instillation of timolol opthalmic solution containing sorbic acid. When the molar ratio of sorbic acid to timolol was two or higher, the concentration of timolol in the aqueous humor was higher than with timolol alone. In the presence of sorbic acid the maximal aqueous humor concentration and the area under the curve were more than two-fold higher than those of Timoptol, a timolol maleate opthalmic solution, and similar in value of TIMOPTIC-XE, a gel forming opthalmic solution. To investigate the transcorneal absorption mechanism, \textit{in vitro} permeation profiles across the intact and de-epithelialyzed cornea were analyzed on the basis of the bilayer diffusion model. The partition
coefficient in the epithelium was about twice as high in the presence of sorbic acid than with timolol alone, although the diffusion coefficient in the epithelium did not change. We conclude that the improved ocular bioavailability in the presence of sorbic acid is due to increased partitioning of timolol in the corneal epithelium.

Trueblood JH, Rossomondo RM, Wilson LA, Carlton WH\textsuperscript{48} (1975) in their study, they have evaluated corneal contact times of ophthalmic vehicles by microscintigraphy. Lacrimal microscintigraphy, in conjunction with a recently developed computer system, was used to evaluate the corneal contact time of three ophthalmic vehicles in 18 humans. The percentage of a radioactively labeled vehicle remaining over the cornea after 90 seconds was 2.9\% plus and minus 2.2\% for saline, 4.3\% plus and minus 2.4\% for polyvinyl alcohol, and 8.8\% plus and minus 4.1\% for hydroxypropyl methylcellulose.

Norman S Levy and Cynthia Alsbury\textsuperscript{49} (1994) studied the evaluation of timolol in gellan gum – a new vehicle to extend its duration of action. Timolol, a nonspecific $\beta$-adrenergic antagonistic used for the treatment of elevated intraocular pressure is usually applied every 12 hours. To reduce its frequency of application, while intraocular pressure lowering is achieved, requires enhancement of its duration of action. The residence time of the drug was increased with a vehicle that changes from a sol to gel on contact with the tear film thereby extending the time for drug absorption. An increase in the vehicle’s viscosity is one of the important variables to extend the residence time of the drug on the surface of the cornea. Vehicles that change from a sol to a gel under the proper ionic conditions can increase in viscosity as they come in contact with the tear film. Gellan gum (Gelrite) is an extracellular microbial polysaccharide elaborated by Pseudomonas elodea. It is an anionic heteropolysaccharide that forms a clear transparent gel in the presence of...
cations at temperatures below 70°C. It is useful vehicle for the delivery of timolol to the eye. The ionic strength and the concentration of gellan gum in the ophthalmic preparation are such that the solution can be delivered through the tip of an ocumeter and will form a gel on the corneal surface after contact with the precorneal tear fluid.

Saisivam S, Vijaya Muthu Manikandar R and Nagarajan M50 (1999) designed ciprofloxacin hydrochloride ocuserts using different polymers in various proportions and combinations. The in vitro release of the drug from the formulations was studied using a commercial semipermeable membrane. The physico-chemical parameters of the ocuserts were evaluated. A zero order release formulation VI (drug reservoir with 2% HPMC and 6% EC as rate controlling membrane) was subjected to in vivo studies using rabbits. The results indicated a good correlation between in vitro and in vivo studies. The expected release for an extended period of 24 hours was observed in formulation VI (Drug reservoir with 2% HPMC and 6% EC as rate controlling membrane).

Jayaprakash S, Cibi Chacko James, Maria NS Gerald Rajan, Saisivam S and Nagarajan M51 (2000) developed ketorolac tromethamine ocuserts using different polymers such as hydroxypropyl methyl cellulose, polyvinyl pyrrolidone, methyl cellulose and ethylcellulose at various concentrations. The in vitro release of the drug from the formulations was studied using commercial semi-permeable membrane. The physicochemical parameters of ocuserts were evaluated. A zero order release formulation 3 (drug reservoir with 3% HPMC and 4% EC as rate controlling membrane) was subjected to in vivo studies. The expected zero order release for one day was observed in formulation 3 (drug reservoir with 4% HPMC and 3% EC as the rate controlling membrane).
Maitra, Amarnath et al\textsuperscript{52} (2001) relates to sustained release and long residing ophthalmic formulation having thermosensitivity, mucoadhesiveness, hydrogel properties and small particle size. The said formulation comprises of micelle solution of random block co-polymer having a hydrophobic component and a hydrophilic component of general formula \((X+Y+Z).\text{sub.m.}\), wherein \(m\) is an integer greater than 2\(X\) is a monomer which will provide hydrogel formation properties of the copolymer to reduce the irritability of the eye and is selected from vinyl group of compounds \(Y\) is a monomer, which will provide thermosensitivity properties of the copolymer having a general formula \(R.\text{sub.1}—R.\text{sub.2N}—(C=O)—CH=\text{CH.}\text{sub.2—, R.\text{sub.1}=a proton or C.\text{sub.nH.}\text{sub.2n+1 in which n may have the value from 3 to 6 and R.\text{sub.2}=alkyl group having chain length of C.\text{sub.3 to C.\text{sub.6Z is a monomer, which will provide mucoadhesiveness and pH-sensitive properties to the co-polymer and is selected from acrylate based monomers at least one hydrophobic drug with the said block co-polymer solution. The invention also provides a process of preparing said formulation.}

Maitra et al\textsuperscript{53} (2001) relates to sustained release and long residing ophthalmic formulation having thermosensitivity, mucoadhesiveness, hydrogel properties and small particle size. The said formulation comprises micelle solution of random block co-polymer having a hydrophobic component and a hydrophilic component of general formula \(-(X+Y+Z—).\text{sub.m.}\), and at least one hydrophobic drug with the block co-polymer solution. The invention also provides a process of preparing said formulation.

Higashiyama et al\textsuperscript{54} (2001) prepared eye drops containing a beta-blockers such as a cartelol hydrochloride, which improved in the penetration of the beta-blocker into the eye and the retention thereof in the eye tissues by the
incorporation of a C$_{3-7}$ fatty acid such as sorbic acid to the eye preparations.

Schultz et al$^{55}$ (2000) developed a drug delivery system for antiglaucomatous medications utilizing a polymer hydrogel which can absorb an ophthalmic medication, which can then be transferred into the ocular fluid of the eye.

Schultz et al$^{56}$ (2002) prepared polymeric hydrogel contact lenses containing an anti-glaucoma medication, such as beta-adrenergic receptor antagonist, e.g., timolol maleate or an alpha-adrenergic receptor agonist, e.g., brimonidine tartrate, and methods of fabrication and uses thereof. A medication is passively transferred into a contact lens by absorption from a dilute aqueous solution. Such treated lenses are contacted with the ocular fluid of an individual to treat glaucoma.

Xia, Erning; et al$^{57}$ (2000) developed a reversible gelling system for ocular drug delivery. The invention provides an ophthalmic aqueous composition for topical administration, comprising: (a) a block copolymer of propylene oxide and ethylene oxide in concentration sufficient to provide viscosity of less than about 25 centipoise at ambient temperature and viscosity of from about 25 to about 55 centipoise when applied topically to a patient; (b) hydroxypropyl methylcellulose in concentration sufficient to improve the durability of the gel formed by the block copolymer. The invention further provides a method for administering ophthalmic pharmaceuticals.

Sawaya; et al$^{58}$ (1996) described ophthalmic aqueous gel formulation and related methods. An ophthalmic aqueous gel formulation containing at least one pharmaceutically active substance, purified water, and an amount of gelling agent effective to form an aqueous gel. The gel has a viscosity of from
75,000 to 3,000,000 centipoise and does not contain an oil phase. The pharmaceutically active substance is solubilized in the formulation. The gelling agent consists essentially of cellulose or a water soluble cellulose derivative.

A.H. El-Kame\textsuperscript{59} (2002) developed pluronic F 127 based formulations of timolol maleate to enhance its ocular bioavailability. The effect of isotonicity agents and PF 127 concentrations on the rheological properties of the prepared formulations was examined. In an attempt to reduce the concentration of PF 127 without compromising the in situ gelling capabilities, various viscosity enhancing agents were added to PF 127 solution containing 0.5% timolol maleate. The viscosity and the ability of PF 127 gels to deliver timolol maleate, 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 timolol maleate gel. However, the viscosity of gel increased as the PF 127 concentrations increased. The viscosity of formulations containing thickening agents was in the order of PF-MC 3%>PF–HPMC 2%>PF–CMC 2.5%>PF 127 15%. The slowest drug release was obtained from 15% PF 127 formulations containing 5% methylcellulose. In vivo study showed that the ocular bioavailability of timolol maleate, measured in albino rabbits, increased by 2.5 and 2.4 fold for 25% PF 127 gel formulation and 15% PF 127 containing 3% methylcellulose, respectively, compared with 0.5% timolol maleate aqueous solution.

Susan C. Miller and Maureen D. Donovan\textsuperscript{60} (1982) designed poloxamer 407 gel for use as a vehicle for ophthalmic drug delivery. Pilocarpine nitrate was incorporated into 25% poloxamer gel and the formulation was administered topically to rabbit eyes. The ocular activity of pilocarpine was assessed using the pharmacological response of miosis. The change in
pupillary diameter versus time curve was compared to that obtained with an aqueous pilocarpine solution, dosed under similar conditions. As indicated by the miotic response, the gel formulation appeared to enhance the activity of pilocarpine when compared to the aqueous solution.

Robert Gurny et al\textsuperscript{61} (1985) have prepared polymeric dispersions in the nanometer size range by emulsification techniques. These systems are based on the mechanism of drug absorption onto the surface of colloidal particles (0.3 \( \mu \text{m} \) average particle size), which show a good biocompatibility. These colloidal or near colloidal dosage forms have low viscosity and can accommodate solid content up to 40\% w/w. The features are that these systems provide a prolonged therapeutic effect, that they can be applied as easily as existing eye drops and that they show a good tolerance, which should result in improved patient compliance.

Yeshwant D et al\textsuperscript{62} (1993) developed various systems for ophthalmic sustained delivery of methyl prednisolone are 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 a suspension of MP in normal saline. In vitro release of MP from the test dosage forms was a determined in a pH 7.4 phosphate buffer at 32°C using a rotating bottle apparatus. MP concentrations in the tear fluid of New Zealand white rabbits 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 kinetics, 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, AUC\textsubscript{0-8h}, but exhibited a tendency to slip out
of the eye due to high degree of swelling. The in vivo release from films containing physically incorporated MP showed higher variability and provided mean AUC$_{0-8h}$ values approximately equal to control values. The gellan eye drops containing MP yielded 2.6 fold higher AUC$_{0-8h}$ values than the control and also provided ease of administration. Gellan solutions might thus provide a versatile vehicle for ocular sustained release of drugs. The results also show that the gellan-MP ester can be used to increase the residence time of methylprednisolone in the tear fluid of rabbits.

Balasubramaniam J et al$^{63}$ (2003) formulated in-situ gelling systems of ciprofloxacin hydrochloride for sustained ophthalmic delivery based on the concept of ion-activated in-situ gelation. Gelrite, gellan gum, a novel ophthalmic vehicle that gels in the presence of mono or divalent cations, present in the lacrimal fluid was used alone and in combinations with sodium alginate as the gelling agent. The developed formulations were therapeutically efficacious and provided sustained release of the drug over an 8-hr period in vitro.

2.3 **IN VIVO EVALUATION OR THERAPEUTIC EFFICACY OF OPHTHALMIC DRUG DELIVERY SYSTEMS (INTRAOCULAR PRESSURE MEASUREMENT):**

The ultimate and final appraisal of the integrity and efficacy of a formulation would be the estimation of the *in vivo* activity. The goal of this research being the development of a controlled release drug delivery system of timolol maleate for the treatment of glaucoma. The formulation was tested for its ability to lower the intraocular pressure.

To study the reduction in intraocular pressure, one has to induce intraocular pressure and the literature suggests the following different methods.
Sears and Sears\textsuperscript{64} (1974) induced glaucoma in rabbits by intraocular injection of $\alpha$-chymotrypsin.

Drago et al\textsuperscript{65} (1997) induced the increase of intraocular pressure in rabbits by a subconjunctival injection of betamethasone-21-phosphate (4 mg/ml) in both eyes every week for 4 weeks and they studied the effects of beta-blockers combined with pilocarpine by measuring intraocular pressure in conscious rabbits using an electronic pneumatonometer after surface anesthesia with one drop of 0.4% benoxinate solution.

Malena et al\textsuperscript{66} (1997) induced ocular hypertension in rabbits by weekly subconjunctival injection of betamethasone suspension into the left eye and measured intraocular pressure with a manometrically calibrated pneumatonograph.

Santafe et al\textsuperscript{67} (1999) induced ocular hypertension in rabbits by oral administration of 60 ml/Kg tap water and measured the effect of topical diltiazem on intraocular pressure.

Balaban et al\textsuperscript{68} (1997) studied the mechanism of vasopressin effects on intraocular pressure in anesthetized rats after cannulation of the anterior chamber of the eye and infusions of artificial cerebrospinal fluid or arginine vasopressin solutions.

Mermoud et al\textsuperscript{69} (1994) described an animal model of uveitic glaucoma. Uveitic was induced in Lewis rats by injection of S-antigen.

Morrison et al\textsuperscript{70} (1998) produced scarring of the aqueous humor outflow pathways by unilateral episcleral vein injections of hypertonic saline in Brown Norway rats and measured intraocular pressure and optical nerve damage after topical treatment with artificial tears, 0.5% betaxolol or 0.5% apraclanidine.
Ueda et al (1998) described an experimental glaucoma model in the rat induced by laser trabecular photocoagulation after an intracameral injection of India ink.

Gu et al (2000) induced experimental glaucoma in the right eyes of Wistar albino rats by intracameral injection of India ink followed by laser trabecular photocoagulation 4 days later. The left eye served as control. Drugs were injected intraperitoneally just before trabecular photocoagulation. Five days later, 3% fast blue was injected into both superior colli and culi. The eyes were enucleated another 3 days later and flat mounts of the retinas were prepared. Labeled ganglion cells were counted in the area 1mm away from the optical disc.

Wang et al (1997) induced glaucoma in one eye of cynomolgus monkeys by repeated organ laser photocoagulation or diode photocoagulation of the midtrabecular meshwork to study the effects of 5-methylurapidil.


Gelatt (1977) derived the method to induce glaucoma in rabbits. The procedure is – male New Zealand rabbits weighing about 2 Kgs are pretreated with 10 mg/Kg i.p. indomethacin to prevent the otherwise immediate onset of inflammation and then slightly anesthetized with pentobarbital to eliminate any nystagmus. The right eye in anesthetized topically with 2% lidocaine. The
anterior chamber is cannulated with a 30-gauge needle attached to a reservoir set at a pressure of 25 mm Hg. Then a second cannula, 32-gauge, is introduced into the anterior chamber near the limbus and directed to the posterior chamber through the pupil. A sterile isotonic saline solution (0.5ml) containing 150 units of α-chymotrypsin is irrigated through the cannula into the posterior chamber. Care is taken to avoid the injection of any enzyme into the corneal stroma. Both cannulae are then removed without significant loss of aqueous humor. The eyes are examined at daily intervals for the first week, then on alternate days for the second week, and then weekly for the duration of the experiments. Intraocular pressure is measured with a tonometer adapted for rabbit eyes.

Hester DE et al\textsuperscript{77} (1987) studied the effects of on intraocular pressure of three sub-conjunctivally injected steroids, betamethasone cortisone and triamcinolone. All three produced elevations in intraocular pressure above controls and the most consistent elevation was observed with triamcinolone. Their findings suggest that subconjunctival injections of steroids in rabbits are a viable alternative to topical application and may prove to provide a more consistent and reproducible model for the study of steroid-induced ocular hypertension.

Bonomi L et al\textsuperscript{78} (1978) developed an animal model and in their study they induced intraocular pressure by subconjunctival injections of 4 mg repository betamethasone, repeated over three weeks, produced a sustained increase of intraocular pressure in 96% of the treated rabbits. The well reproducible and sensitive to antiglaucoma drugs. They developed an animal model which is very suitable for testing the pressure lowering effect of drug on ocular hypertension and glaucoma.
2.4 OCULAR SAFETY OR EYE IRRITATION STUDIES:

Assessment of the ocular irritation potential of the ophthalmic drug delivery system represents an extremely important step in the development process of such systems. The evaluation of the assessment procedures can be traced through the literature and also the understanding of the mechanism of ocular response to irritants based upon the examination of conjunctiva, cornea or iris. Significant advances have been made in the methods of assessment of ocular irritation resulting in greater reliability, reproducibility and predictability. Excellent reviews have appeared in recent years with regard to the evaluation procedures utilized.

Friedenwald, Hughes and Hermann given the procedure for the objective measurement of injuries to rabbit eyes. The procedure which consists essentially of dividing the overall effect into distinct elements, the extent or seriousness of which may easily be graded.

John H. Draize, Geoffrey Woodard and Herbert O. Calvery modified the interpretation of Friedenwald procedure slightly and extended the same principle to the evaluation of other physiological effects. In the "modified Draize technique" they have transformed qualitative observations of physiological effects to reasonably quantitative objective measurements and also they have applied the principle of assigning numerical values to physiological phenomena in order to obtain data easily subject to arithmetical interpretation. Out of the various methods prescribed, the "modified Draize technique" for evaluation of ocular irritation scoring has been considered as the official method in this Federal Hazardous Substance Act, USA.

Efficacy studies of ocular drugs and formulations are not conducted in the intact human eye. Obviously because of the inability to sample tissues or fluids without risking severe injury to the eye. Therefore, albino rabbits are
used frequently in toxicological, pharmacological and biopharmaceutical research in ophthalmology even though it possesses some ocular anatomical dissimilarities to the human eye.

**Table: Scale of weighted scores for grading the severity of ocular lesions**

### I] Cornea

<table>
<thead>
<tr>
<th>A</th>
<th>Opacity – Degree of density (area which is most dense taken for reading)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scattered or diffuse area – details of iris clearly visible</td>
</tr>
<tr>
<td></td>
<td>Easily discernible translucent areas, details of iris slightly obscured</td>
</tr>
<tr>
<td></td>
<td>Opalescent areas, no details of iris visible, size of pupil barely discernible</td>
</tr>
<tr>
<td></td>
<td>Opaque, iris invisible</td>
</tr>
<tr>
<td>B</td>
<td>Area of cornea involved</td>
</tr>
<tr>
<td></td>
<td>One quarter (or less) but not zero</td>
</tr>
<tr>
<td></td>
<td>Greater than one quarter – less than one-half</td>
</tr>
<tr>
<td></td>
<td>Greater than one-half less than three quarters</td>
</tr>
<tr>
<td></td>
<td>Greater than three quarters up to whole area</td>
</tr>
</tbody>
</table>

Score equals A x B x 5. Total maximum = 80

### II] Iris

<table>
<thead>
<tr>
<th>A</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)</td>
</tr>
<tr>
<td></td>
<td>No reaction to light hemorrhage; gross destruction (any one or all of these)</td>
</tr>
</tbody>
</table>

Score Equals A x 5. Total possible maximum = 10

### III] Conjunctivae

<table>
<thead>
<tr>
<th>A</th>
<th>Redness (refers to palpebral conjunctiva only)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vessels definitely injected above normal</td>
</tr>
<tr>
<td></td>
<td>More diffuse, deeper crimson red, individual vessels not easily discernible</td>
</tr>
<tr>
<td></td>
<td>Diffuse beefy red</td>
</tr>
<tr>
<td>B</td>
<td>Chemosis</td>
</tr>
<tr>
<td></td>
<td>Any swelling above normal (includes nictitating membrane)</td>
</tr>
<tr>
<td></td>
<td>Obvious swelling with partial eversion of the lids</td>
</tr>
<tr>
<td></td>
<td>Swelling with lids about half closed</td>
</tr>
<tr>
<td></td>
<td>Swelling with lids about half closed to completely closed</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C</th>
<th>Discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any amount different from normal (does not include small amount observed in inner canthus of normal animals)</td>
</tr>
<tr>
<td></td>
<td>Discharge with moistening of the lids and hairs just adjacent to the lids</td>
</tr>
<tr>
<td></td>
<td>Discharge with moistening of the lids and considerable area around the eye</td>
</tr>
</tbody>
</table>

Score (A + B + C) x 2. Total maximum = 20
The albino rabbit is readily available, docile, easily handled and relatively inexpensive. A large number of studies have been conducted in which ocular irritative effects in the rabbit eye accurately predict responses in human\textsuperscript{80}. When an uncomfortable preparation is applied to the rabbit eye, the animal quickly begins to lacrimate and either blinks frequently or holds its eyelids shut. The adult rabbit eye is nearly as large as the human eye. In addition, the nictating membrane which is well vascularized and contains many lymphatic nodules may be rapidly moved halfway across the inner surface of the cornea to facilitate removal of foreign objects\textsuperscript{81}.

The rabbit blinks less frequently than humans. Drainage rate studies\textsuperscript{82} have shown that the lacrimal turnover rate in man is twice as fast, which is due to the fact that whereas man has two lacrimal drainage ducts or puncta on the inner margin of each eyelid. The rabbit eye has only a single puncta located on its inner lower lid margins. The corneas of the rabbit and the human differ slightly in that the rabbit cornea is devoid of Bowman’s membrane and is somewhat thinner. Inspite of these differences, the rabbit ocular kinetics may resemble those of humans closely enough for predictive use in comparing formulations.

Albino rabbits are currently used to test the ocular toxicity and irritation of ocular formulations because of the rabbit has a large eye, both corneal surface and bulbar conjunctival area are large and easily observed and the iris is unpigmented allowing ready observation of the iridial vessels\textsuperscript{83}. The primary differences between rabbit and human with respect to ophthalmic studies are decreased tearing in rabbits\textsuperscript{84}, differences in the structure of Bowman’s membrane and a slower re-epithelization of the rabbit cornea\textsuperscript{85}.

Draize rabbit eye test is the only widely used assay for the effect of substances on the eye. It is suggested that the mechanism of action in the
Draize test and in the human eye irritation threshold (EIT) involves passive transfer of the compound to a biophase that is quite polar, is a strong hydrogen bond base, a moderate hydrogen bond acid and quite hydrophobic. The biophase does not resemble water or plasma, but resembles an organic solvent such as N-methyl formamide. In a comprehensive review of the Draize test, it was noted that the anatomy and biochemistry of the rabbit eye are not the same as those of the human eye and that there were numerous physiological reasons including low tear production, blink frequency and ocular surface area, that such a test on rabbits might not adequately predict human effects. Yark and Steiling (1998) stressed the need to validate the Draize test against controlled human eye data, but noted that "there are no adequate human data".

What comparisons have been made between the effects on rabbits and the effects on humans have been confined to consumer products that are a mixture of various chemicals. Freeburg et al. (1986) examined four such products and showed that the low-volume Draize test correlated with effects on the eyes of humans better than did the normal volume Draize test. Allgood (1989) also matched the low-volume Draize test against human experience for four shampoos, and Griffith (1989) compared Draize data to consumer eye accident data for soaps and detergents. Roggehand et al. (2000) studied the effect of very low volumes (1-3 μl) of a liquid detergent and a dish washing liquid on the eyes of rabbits and human volunteers. They observed that, the irritation responses in rabbits were greater than those in man and suggested that the low-volume Draize test could be used to assess eye irritation hazards in man.