CHAPTER 11
RESULTS AND DISCUSSION
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Most commonly available ophthalmic preparations are eye drops and ointments. But these preparations when instilled into the cul de sac of the eye are rapidly drained away from the ocular cavity due to tear flow and lacrimal nasal drainage. Only a small amount of drug is available for its therapeutic effect resulting in frequent dosing. This inefficient drug delivery into the eye occurs due to rapid tear turnover, lacrimal drainage and drug dilution by tears.

Different drug delivery systems have been developed cited in the literature in order to enhance the bioavailability and efficacy of the drug to the eye tissues and such delivery systems are sol to gel systems, ocular inserts, microspheres, nanoparticles etc. Among these systems ocular inserts provides the release of the drug for maximum duration of time i.e. 7 days in case of pilocarpine developed by Alza corporation. As these are the solid polymeric thin devices and remain in the cul de sac cavity of the eye for longer duration of time. Another drug delivery system is sol to gel system which when placed in the eye in solution converted into gel due to action of pH, ions or temperature of the eye condition. Such delivery systems can released the drug upto 24 hours. Other ophthalmic drug delivery systems like microemulsion, microparticulate type of system which enhanced penetration of drug to the eye tissues and thereby increases bioavailability. By using these ophthalmic drug delivery systems the older drugs used in the eye ailments can be utilized in a better way as these physical properties are changed by employing these novel techniques.

Topically applied ocular drugs have to reach the inner parts of the eye and transcorneal permeation is believed to be major route of drug absorption. Corneal absorption is much slower than elimination. Ocular bioavailability of drugs can be increased by increasing absorption rate or by decreasing elimination rate. The decrease in elimination rate can be achieved by modifying the ocular dosage form. Therefore to optimize topical ocular drug delivery system prolonged contact time with the corneal surface and better penetration through cornea is necessary.
In the present study we have developed ocular drug delivery systems like ocular inserts and sol to gel systems with improved bioavailability, better patient compliance and with no irritation effect.

In sol to gel systems bioavailability of the drug can be increased by increasing the viscosity of the preparation by using viscosity enhancing polymers. In case of ocular inserts, bioadhesive polymers can be used which increase ocular contact time of drug and also prolong the release of the drug. The use of such polymers proved to enhance the ocular bioavailability or therapeutic efficacy of the applied drugs and prolonged duration of action.

Hence in the present study ocular inserts and sol to gel systems were using model drug as Acetazolamide and Levobunolol hydrochloride. Polyvinyl alcohol as major polymer was used for the preparation of ocular inserts while gelrite and poloxamer 407 were used for the preparation of sol to gel systems. These provided improve bioavailability, better patient compliance and nonirritating effect on the eye tissues.

Characterization and Analytical methodology

Both drugs were characterized for melting point, solubility, and assay by UV spectrophotometric methods and for Thin layer chromatography (TLC) studies. The IR spectra were determined and compared with IR spectrum of the drug. The melting point of Acetazolamide (Acetazolamide) was found to be 260°C and for Levobunolol HCl (Levobunolol HCl) was found to be 207°C. The solubility was determined on the basis of visual observations and found comparable with the literature. The assay of drug as per B.P. for Acetazolamide was found to be 99.75% (purity of drug) and for Levobunolol HCl it was found to be 99.00% (purity of drug). In TLC studies, the Rf value of drug sample was similar to reference standard. Other identification tests like clarity, color etc. were found as per B.P. The IR spectra of both drug samples were found similar to IR reference spectra of drug in every respect i.e. position of the peaks for carboxyl, carbonyl, and bands for aromatic rings. On the basis of these studies, it was found that both supplied drug sample i.e. Acetazolamide and Levobunolol HCl thus obtained was an authentic one and purity of drug was on higher side i.e. 99.75% (Acetazolamide) & 99.00% (Levobunolol Hydrochloride) respectively.
On the basis of physical characteristics solubility, melting point, assay, TLC and IR spectrophotometric studies it was found that the supplied sample of Acetazolamide was authentic one.

The UV absorption spectrum of Acetazolamide and Levobunolol Hydrochloride exhibited λmax at 264nm in simulated tear fluid (STF) of pH 7.4 and Isotonic phosphate buffer (IPB) of pH 7.4 for Acetazolamide. While at 254nm in simulated tear fluid (STF) of pH 7.4 and isotonic phosphate buffer (IPB) of pH 7.4 for Levobunolol HCl. The comparison of UV absorption spectra before and after storage of Acetazolamide and Levobunolol Hydrochloride in Simulated Tear Fluid (STF) and Isotonic Phosphate Buffer (IPB) of pH 7.4 at different conditions were determined and no change in λmax in all temperature conditions. Slight change in absorption was seen in the samples kept under laboratory conditions not protected from light however negligible change in absorbance were seen in the samples kept in dark conditions for 24 hours.

The calibration curves of Acetazolamide and Levobunolol Hydrochloride in Simulated Tear Fluid of pH 7.4 were plotted for UV analysis of the said drugs in the respective media. The curves obeyed Beer-Lambert’s law in the selected concentration ranges. Also the calibration curves of Acetazolamide and Levobunolol HCl were plotted for HPLC analysis of the drug in the respective media. The curves obtained obeyed Beer-Lambert’s law in the selected concentration ranges.

The excipients which were used for making ocular inserts and sol to gel systems exhibited λmax other than λmax of the drug. Insignificant UV absorbance was seen at λmax of drug i.e. 264nm (Acetazolamide) and 254 nm (Levobunolol HCl). Therefore, excipients in delivery system did not interfere with drug in the delivery systems.

The assay methods developed for the estimation of drug in medicated ocular inserts containing Acetazolamide, Acetazolamide-HPβCD and Levobunolol HCl and sol to gel systems containing Acetazolamide, Acetazolamide-HPβCD and Levobunolol HCl were found to be accurate and reliable with a high percentage recovery.
Preformulation studies

The placebo films containing polymer Polyvinyl alcohol and Polyethylene glycol 400 as plasticizer were prepared by varying the quantities of Polyvinyl alcohol and taking different quantities of Polyethylene glycol 400 i.e. 4-28% w/w of polymer. These films were evaluated visually for brittleness, uniformity, transparency, flexibility etc. It was found that the film containing 4% and 8% of plasticizer were brittle while film containing 16%, 20%, 24% and 28% of plasticizer were sticky. Hence these were rejected. The films containing 12% of plasticizer were found to be suitable in which the polymer PVA was 0.100g, 0.150g, 0.200g, 0.250g and 0.300g per glass ring (diameter 5.5 cm). The film in which polymer was 0.01g per ring was having less strength. The selected films i.e. III, IX, X, XI & XII were further evaluated for thickness, folding endurance and % elongation at break. It was observed that the thickness increased as the concentration of polymer increases, while % elongation at break decreased and folding endurance increased. As the drug Acetazolamide is slightly soluble in water it was planned to prepare its complex with HPβCD and for this purpose it was considered that in some placebo films the Polyvinyl pyrrolidine should be included. Therefore in some selected formula Polyvinyl pyrrolidine was also included.

On the basis of preformulation studies for ocular inserts it was concluded that formulations III, IX, X, XI, XII, IIIC, IXC, XIC, XIIIC were considered suitable for incorporation of drug i.e. Acetazolamide and Levobunolol HCl.

Sol to gel systems were prepared by using poloxamer 407 and gelrite. Poloxamer 407 is converted into gel form due to temperature change i.e. at eye temperature. In case of gelrite the solution converted into gel in the presence of ions present in the eye. Therefore these principles were utilized to form sol to gel preparation of Acetazolamide, Acetazolamide-HPβCD complex and Levobunolol HCl. 0.6%, 4.3% and 0.3% concentration of Acetazolamide, Acetazolamide-HPβCD complex and Levobunolol HCl respectively were used for making sol to gel systems.

The use of sodium or other compound containing cations for adjusting isotonicity was avoided by the use of isotonic boric acid buffer of pH 4.7. The modified boric acid buffer was prepared in such a way that after incorporation of drug, polymers and preservatives the solution so formed was isotonic. The isotonicity calculation was carried using NaCl.
equivalent aspect by employing $I_{iso}$ value and molecular weight of drug and polymers. Methyl parabens and propyl parabens were selected as preservatives on the basis of market survey of these drugs.

The placebo formula were developed for poloxamer 407 in varying concentration i.e. 14%-24% and taking preservative and modified boric acid buffer of pH 4.7. Similarly gelrite placebo sol to gel formula were prepared by taking varying concentration of gelrite i.e. 0.2% to 1.2% and taking preservative and modified boric acid buffer pH 4.7.

From the results of Polaxamer based placebo sol to gel system evaluation for there physical characteristics before and after bringing to eye temperature (37 ± 0.2°C) it was found that the gels were more viscous unable to pour as the concentration of poloxamer was in more amount in the formula. Therefore, on the basis of clarity, transparency and consistency four placebo formula were selected i.e. P1, P1, P3 and P4. Similarly the physical characteristics of solution to gel system of gelrite were observed before and after addition of STF into it in order to convert sol to gel form. Again it was observed that more viscous gels were formed in case of the more content of gelrite in the formula and hence only those formula were selected which gave gels of good consistency i.e. G2, G3, G4 and G5.

The viscosity of the gels after conversion form solution was also determined using a temperature controlled brookfield viscometer. The poloxamer bases system was examined at varying temperature, while the gelrite based system after the addition of varying amount of STF. On the basis of these studies again the solution to gel system is selected under the physical characteristic gave satisfactory results.

In the view of the above findings from preformulation studies the four formulas i.e. P1, P2, P3 and P4 were selected for poloxamer sol to gel systems. On similar lines the formula which were selected for gelrite based solution to gel systems were G2, G3, G4, G5 and G6.

**Preparation of ocular drug delivery systems**

**Ocular inserts**

Three types of ocular inserts were prepared i.e. containing Acetazolamide, Acetazolamide-HPβCD and Levobunolol HCl. The drug Acetazolamide was calculated
for one ocular inserts and ocular inserts containing polyvinyl alcohol as polymer and Polyethylene glycol 400 as plasticizer were prepared and evaluated for physical characteristics, tensile strength, thickness, % elongation at break. The ocular inserts which gave satisfactory results were selected for further study. These were coded as IID, IXD, XD, & XID.

Similarly the ocular inserts were also prepared using Acetazolamide-HPβcd complex (1:1 ratio prepared by freeze drying technique), Polyvinyl alcohol as polymer, Polyethylene glycol 400 as plasticizer and Polyvinyl pyrrolidone as solubilizer. These were also evaluated for similar parameters as given above and those gave satisfactory results were selected i.e. IIIC, IXC, XC & XIC. In each ocular inserts the drug content was 0.480mg (equivalent to 0.128 mg of complex).

Levobunolol HCl containing ocular inserts was also prepared in similar manner employing 0.240mg of drug per ocular insert. The optimized ocular inserts contained drug, Polyvinyl alcohol and Polyethylene glycol 400 and these were termed as III, IXL, XL and XIL.

All the above ocular inserts were prepared for assuming the release of drug upto in a constant manner into the eye tissues over a period of 24 hrs.

On the basis of physical characterization of complex prepared 1:1 complex of Acetazolamide-HPβcd complex prepared by freeze drying technique were selected for incorporation into the ocular inserts. From the results of physical characteristics, tensile strength, thickness, % elongation at break studies on selected ocular insert containing Acetazolamide, Acetazolamide-HPβcd complex and Levobunolol HCl it was found that formulations IID, IXD, XD, XID (Acetazolamide), IIIC, IXC, XC, XIC (Acetazolamide-HPβcd complex) and III, IXL, XL and XIL (Levobunolol HCl) were found to be suitable for incorporation of drug.

Sol to gel systems

Under the preformulation studies two types of placebo sol to gel systems were prepared containing poloxamer 407 and gelrite respectively. Among various formula only those were selected which gave satisfactory physical characteristic and viscosity. In the gelrite based placebo four formulas selected i.e. G2, G3, G4 and G5. On the similar parameters
again four formulas were identified among the various placebo sol to gel systems of poloxamer viz. P1, P2, P3 and P4.

Six types of medicated sol to gel formulations were prepared by taking above placebo formula and incorporating Acetazolamide, Acetazolamide-HPβcd complex and Levobunolol HCl in the concentrations of 0.6%, 4.327% and 0.3%, respectively.

The final formula's which were prepared were given for Acetazolamide containing ocular inserts as P1-A, P2-B, P3-C, P4-D, P1-AA, P2-BB, P3-CC and P4-DD (for Poloxamer based sol to gel systems), G2-A, G3-B, G4-C, G5-D, G2-AA, G3-BB, G4-CC & G5-DD (for gelrite based sol to gel systems) and for Levobunolol HCl containing ocular inserts as P1-L, P2-L, P3-L, P4-L (for Poloxamer based sol to gel systems) and G2-L, G3-L, G4-L and G5-L (for gelrite based sol to gel systems).

All these medicated sol to gel formulations were isotonic as the quantity of all ingredients including drug were planned in advance in the calculation part in order to obtain isotonic sol to gel systems. All these preparations were packed in plastic eye containers immediately after the preparation and stored at low temperature for poloxamer based preparations and at room temperature for gelrite based formulations. All these medicated sol to gel systems were subjected to in-vitro release studies in search of optimized formula.

In-vitro release studies

Ocular inserts

Ocular inserts which were prepared in preparation solutions were subjected to in-vitro release of the drug using flow through apparatus. The flow through apparatus was designed in the laboratory which consisted jacketed flask, jacketed flow through cell, peristaltic pump, regulating device for controlling flow and temp.

In case of Acetazolamide four formulations were tested i.e. III, IX-D, X-D & XI-D. In these preparations all the graphs did not show straight lines which suggested that the release of drug was due to first order rate kinetics however in the log % of drug remaining vs time curves almost straight lines were observed up to 12 hrs and then somewhat curve lines up to 24 hrs. These also suggested first order release. Less coefficient of variation were observed in case of first order release rate constant as compared to zero order rate constant indicating the release due to first order rate kinetics.
The first order release rate might occurred due to the matrix nature of ocular insert. In the cumulative amount of release vs time curves constant release occurred in case of formulation III-D as compared to other preparations and 98.31% of drug was released upto 24 hrs. Preparation X-D gave first release and 96.16% of drug was released in 24 hrs. In preparation XI-D the release was slow and 85.43% of drug was released in 24 hrs. The release was occurred in the order of X-D> IX-D> III-D>XI-D and it indicated that when polymer concentration was more the release rate decreased and when polymer concentration was less, the faster release rate occurred. On these observations the preparation III-D was found to be optimum i.e. it release the drug upto 24 hrs in a constant manner with first order release in total 98.31% of drug released in 24 hrs.

In case of ocular insert prepared by using Acetazolamide-HPβed complex the faster release rate occurred in all formula and total drug release was less than 24 hrs. In case of X-C the release were very constant but drug was release quickly i.e. all drug was released in 10 hrs. On preparation IX-C constant release occurred upto 10hrs and then release decreased upto 12 hrs. In 12 hrs it gave 97.34 %. In the preparation III-C release occurred upto 14 hrs, however 14 hrs regimen was not found suitable. In preparation XI-C the release also occurred in a constant manner upto 14 hrs. As per the dosage regimen preparation IX-C was selected as it gave release upto 12 hrs. the release rate constants for the preparation was following as first order rate kinetics as less coefficient of variation was observed in case of first order rate constant as compared to zero order rate constant. The faster rates might be due to the presence of soluble complex of the drug with cyclodextrin. In case if formulation X-C burst effect was observed and this may be due to less amount of polymer and higher solubility of drug in the complex form. It was also observed that as concentration of polymer increased the release decreases accordingly. On the basis all above aspects the preparation no IX-C was observed as optimum one. This type of ocular insert gave constant release upto 12 hrs only.

The ocular insert containing Levobunolol HCl (LVB HCl) gave faster release in case of preparation X-L and release occurred upto 98.88% in 14 hrs, while the preparation XI-L gave release upto 24 hrs and 98.53% of drug was release in 18 hours. In these preparation it was also observed when the polymer concentration was more in ocular inserts the release rate decreased accordingly. The curves formed were observed in
all cases which indicated first order release kinetics. This was further proved by calculating coefficient of variations for first order and zero order release rate constants. The less values of coefficient of variation in case of first order release rate constant as compared to zero order rate constant indicated first order release rate kinetics of drug from ocular insert. This might be due to the matrix nature of the ocular inserts. The preparation XI-L was found to be optimum. In the overall release rate constant first and zero order release again it was observed again less coefficient of variation values were observed in case of first order kinetic in case of all preparation. This further also indicated the release due to first order.

On the basis of above observations, results it was concluded that three preparation were found optimum i.e. III-D containing Acetazolamide, IX-C containing Acetazolamide-HPβcd complex & XI-L containing Levobunolol HCl respectively. It was also found that Acetazolamide which is having low solubility when complex with cyclodextrin gives faster and controlled released rate as compared with given alone. These suggested importance of Acetazolamide-HPβcd complex in the formulation.

**Sol to gel preparations**

The sol to gel preparation of polaxamer and gelrite as polymers were subjected to release of drug by using modified paddle over disk apparatus, in STF of pH 7.4 at 37±0.5°C. 

**Polaxamer based sol to gel**

In the sol to gel preparations of Acetazolamide the release was faster in case of preparation having less concentration of Polaxamer while it was slower when concentration of Polaxamer increased. The preparation P3-C which contains 18% polaxamer gave constant release up to 12 hrs and 97.22% of drug was released. In the sol to gel systems containing Acetazolamide-HPβcd complex faster release were observed in all the cases. Which might be due to the complex of the drug. In PI-AA the entire drug released in 7 hrs while P3-CC gave the release of drug up to 11 hrs. Further increase of polaxamer was not warranted in the preparation section as good gels was not observed in the case of 20% polaxamer solution. Hence the preparation P3-CC was found optimum one.
In the graph of cumulative % of drug release vs time for sol to gel systems of Levobunolol HCl. The release rates were faster in case of less concentration of polaxamer preparation P4-L which contain 20% polaxamer was found to be optimum as it gave release of drug upto 12 hours and 97.02% of drug was released.

In all the above formulations the curves were observed in the graphs of plotted between cumulative % of drug released vs time which indicated the first order release rate kinetics. This was further supported by observing less coefficient of variation in case of first order release rate constants as compared to zero order release rate constants. This was further evident from the graph between loss % of drug remaining vs time as almost straight curves were observed.

In view of the above observation and conclusions the sol to gel system which were found optimum were P3-C, P3-CC and P4-L for Acetazolamide, Acetazolamide-HPβcd complex and LVB-HCl respectively. Formulation having Acetazolamide-HPβcd complex shows faster release rate when compared with Acetazolamide alone. This is due to complex formation of Acetazolamide with HPβcd which increase solubility of Acetazolamide.

Gelrite based sol to gel systems

The sol to gel preparation of Acetazolamide containing gelrite as polymer showed the release of drug in a constant manner. The preparation i.e.G2-A containing 0.4% of gelrite showed faster release as compared to the preparation G4-C containing 0.8% of gelrite. This was due to the effect of gelrite concentration on the release rate of the drug i.e more polymer concentration slower the release rates. Preparation (G5-D) containing 0.7% gelrite was found to be optimum as produced constant release of drug upto 12 hrs & 98.52% of drug released.

The preparation containing Acetazolamide-HPβcd complex showed slightly faster release as compared to the above preparations containing Acetazolamide only. This effect might be due to the presence of water soluble complex in the sol to gel formulations. In the preparation (G2-AA) which contained 0.4% gelrite, burst effect was observed & all the
drug release in about 7 hrs. The preparation G4-CC i.e. containing 0.8% gelrite gave more constant release up to 12 hrs and it appeared to be optimum one.

Levobunolol HCl containing sol to gel system of gelrites also produced the similar effects i.e more release in the preparation containing less concentration of gelrite and release decreased as the concentration of gelrite was increased. The preparation G4-L containing 0.8% gelrite gave constant release up to 12 hrs and 97.95% of drug was released.

In all the above preparation first order release rates were observed. On the basis of above observations, results it was concluded that three preparation were found optimum i.e. G5-D containing Acetazolamide, G4-CC containing Acetazolamide-HPβcd complex & G4-L containing Levobunolol HCl respectively.

Existing marketed preparation for Acetazolamide is 0.1% eye drop solution and for Levobunolol HCl is 0.05% eye drop solution. There are no products in the market like ocular inserts and sol to gel preparations of Acetazolamide and Levobunolol HCl prepared by using polaxamer and gelrite as polymers. From the results of the in-vitro release studies it has been surprisingly found that products prepared by using these polymers released the drug at controlled rate thereby overcoming the problem of existing marketed preparation.

Physicochemical characterization, preservative efficacy and Transcorneal Permeation studies
The optimum sol to gel formulation of Acetazolamide (P3-C and G5-D), Acetazolamide-HPβcd complex (P3-CC & G4-CC) and Levobunolol HCl (P4-L & G4-L) were observed for clarity, pH, viscosity and mucoadhesive strength (after conversion into gel). All the sol preparations were clear liquid and this parameter was comparable with tears. Slightly acidic pH was observed in all cases 4.7 to 5.5 as it was desirable on the stability point of view. The viscosities of all the preparation offer conversion to gel were satisfactory. The Bioadhesive strength was also found satisfactory in all preparations. This might be due to the gel nature and it was having advantage i.e. more retention into the eye and hence more sustained release over the desired period of time.
Preservative efficacy of the sol to gel system was determined as per LP. method using three types of microorganisms viz. S. aureous, P. aeruginosa and E.coli. It was observed that in few preparations the viable count increased for some microorganism i.e. in case of G5-D, G4-L, P3-CC and P4-L. However none of the formulation showed increase of the viable count not more than 0.1% of the initial count. As per the interpretation the preservatives combination present in all six sol to gel preparation was effective. The preservative system used was effective in the product as the concentration of the viable bacteria was not more than 0.1% of the initial concentration by the 14th day. Also the concentration of each test organism remain below these designated levels remain during remainder of the 28 day period

Transcorneal permeation studies of then optimized sol to gel preparation was performed using buffalo eye cornea collected from slaughter house. It was found that the drug permeated with zero order rate kinetics as almost straight curves were observed in all cases when amount of drug permeated was plotted against time. More amount of drug were permeated in case of preparation G4CC, G4D, P3C, P3CC as compared to the preparation containing Levobunolol HCl (G4-L & P4-L). This was also confirmed by the values of apparent permeability coefficient i.e. higher values for sol to gel preparation of Acetzolamide and Acetzolamide-HPβl complex. This might be due to the more lipophilic and less hydrophillic nature of Acetzolamide as compared to Levobunolol HCl.

Packaging, sterilization and interaction studies

The selected optimized ocular inserts were packaged in aluminium foils using a strip packaging machine and these were evaluated for leak test, water vapour transmission test and removability test. The packaging was found satisfactory as all test were passed. These ocular inserts were sterilized by gamma radiation at 2.5 mm rad dose and further evaluated for assay and other parameters before and after sterilization. It was found that same assay results contained and there was no change in color etc. Therefore it was concluded that gamma radiation affect the drug or other excipients chemically. In the test
for sterility the test was passed as per I.P. procedure which indicated that sufficient gamma radiation dose was achieved and hence products were sterilized effectively.

Poloxamer based sol to gel preparations were packed in plastic eye drop bottles and sterilized by gamma radiation as autoclaving was not found suitable due to sensitivity of poloxamer towards temperature. The test before and after sterilization i.e. assay and physical appearance revealed that there was no any effect of radiation on the assay of the drug and an stability of sol to gel formulations. The preparation were found sterile as the test for sterility passed as per I.P.

In case of gelrite based sol to gel systems aseptic procedure was adopted i.e. gelrite and preservatives in buffer was sterilized by autoclaving and drug in buffer was passed through bacteria proof membrane filter then these two portions were mixed together and packed in already sterilized eye bottles. This procedure was adopted to avoid exposure of the drug towards the heat which occurred in autoclaving process. The test for sterility was performed as per I.P. and it was passed.

The results of the interaction studies were performed for the samples stored for 1 month. It was performed by observing assay, physical appearance, UV scanning, IR and TLC studies. In the assay almost the whole amount of drug which was added to formulation was noticed in ocular inserts and sol to gel systems. The preparations did not change in physical appearance. The pattern of peaks and UV absorption of the drug extracted from the formulation was almost found matching with the spectrum of pure drug as evident from UV spectra. Similarly IR spectra were also matching in terms of peaks and patterns for the extracted drug from the formulation with that of pure drug. In TLC studies no extra peaks were seen on the plates for the drug extracted from the formulations and single spot only for drug at Rf value of pure drug was seen. On the basis of the above observations it was revealed that the preparation were not having any interaction between the drug and excipients in case of ocular inserts and sol to gel preparations.

Irritation studies

The optimized ocular inserts and sol to gel preparations were subjected to irritation studies in order to assess any irritating effect upon application in the eyes. It has been found that in the literature that chorioallantoin membrane test on Hen eggs can be used for irritation studies as it simulates the test is performed on the eye. The various
parameters like haemorrhage, coagulation and hyperaemia were determined in the score forms and mean and cumulative scores were determined for them. The cumulative scores of the optimized formulation were 0.33, 0.66, 0.66, 0, 0.33, 0.66, 0.33, 0.66, 0.33, 0.33 and 2.49 for the preparations HID, IX-C, XI-L (ocular inserts) P3-C, P3-CC, P4-1. (Polaxamer based sol to gel systems), G5-D, G4-CC, G4-L (Gelrite based sol to gel systems), Acetazolamide (Marketed solution) and Levolunolol HCl (Marketed solution) respectively.

As per this method the score 0 - 0.9 is said to be practically non irritant or 1 - 4.9 is slight irritant, 5 - 8.9 is moderate irritant and 9-21 is said to be strong irritant. From the above observation it was obtained that none of the optimized ocular preparations i.e. ocular inserts and sol to gel preparations were having not more than 0.9 score and hence all of these found practically non irritant.

Pharmacodynamic studies

The pharmacodynamic studies on ocular inserts and sol to gel systems were performed on albino rabbits weighing 1.5 to 2.0 kg for the measurement of IOP after the application of preparations on the rabbit eyes using schiotz tonometer. These study was performed at Govt college of pharmacy, Karad (M. S.) after due permission from the animal ethical committee. Sterilized optimized preparation were used for the study.

It was observed that the IOP reduced slowly in all the preparations of ocular inserts as well as in eye drop treated eyes, upto 100 min and then it increased in case of marketed eye drops while in case of ocular inserts it almost remained constant upto 5 hrs. This study provided a clue that the effect of eye drops remained for a short period i.e. below 5 hrs of the study and it was assumed that they will provide the effect beyond 5 hrs, this effect might be due to slow and persistant release of the drug from the ocular insert as the release of drug is planned for 24 hrs. The study was not performed upto 24hrs as normotensive rabbits were used and it was not feasible long study due to animal ethical considerations.
In case of sol to gel systems the effect of eye drops on IOP reduction was again up to 100 minutes and then it increases in IOP was obtained. Which might be due to swiping effect of eye drop due to lacrimal drainage. However in case of sol to gel the reduction in IOP was seen up to about 100min which persisted further up to 5 hrs with slight increase in IOP. This might be due to the slow and persistent/sustained release of drug into the eye. It was assumed that it show further persistent effect up to 12 hrs as the release of drug in these preparation was planned for 12 hrs.

From the significant t-test it was observed that the % reduction in IOP for ocular inserts and ophthalmic sol to gel preparations were significant in all cases as compared to marketed preparations at 3hr and 5hrs. However non significant results were obtained at 1.5 hrs. This might be due to that eye drops also acted in a similar manner as the ocular inserts and sol to gel preparation up to 1.5 hrs. But as time pass the eye drops swept away and IOP which was reduced earlier again increased quickly and reach to the normal value. But in case of ocular inserts and sol to gel systems there was sustain release of drug and the IOP which was reduced persisted as the release of the drug from these preparation were planned for 24 hrs and 12 hrs in case of ocular inserts and sol to gel preparation respectively.

The results of in-vivo release of drug from ocular inserts was determined on optimized formulation i.e. III-(for Acetazolamide), IX-C (for Acetazolamide-HPβed complex) and XI-L (for Levobunolol HCl) on the albino rabbits eyes. These type of study was not feasible for sol to gel system as it was difficult to measure the remaining amount of drug in the gel, as it is difficult to remove the remaining gel from the eye at an appropriate time interval as compared to solid dosage form i.e. ocular insert.

It was observed that in case of ocular inserts (IX-C) the release of drug was observed up to 10 hrs and it was found that at up to 10hrs and could be up to 12 hrs but in slow manner and could not be predicted accurately in these study as it was difficult to remove the remaining part of ocular insert at 12th hour as it was eroded. This might be due to the presence of complex of the drug in the formulation and presence of polyvinyl pyrrolidine which act as solubilizers. The release of the drug was almost constant and due to the first order release rate from the matrix as evident due to less coefficient of variation when first order release kinetics was applied as compared to zero order. In case of ocular inserts
(HI-D and XI-L) results showed that continuous and sustain release of drug upto 20hrs which could be upto 24hr could not be concluded as remains of the ocular insert was not found at 22th hours. In both cases the release rate from the matrix was obtained for 1st order as less coeff of variation for 1st order release rate kinetics as compared to zero order release rate kinetics.

The scatter diagrams were plotted by using cumulative % of drug released (from *in-vivo* studies) and *in-vitro* cumulative % of drug released (from *in-vitro* release studies data taken from *in-vitro* studies section) show positive and high correlation in all three types of ocular inserts. Slight lag time was observed towards the *in-vivo* release in all three ocular inserts preparations as compared to *in-vitro* release. This might be due to the different environment of the eye (like less amount of lacrimal fluid, presence of proteins and enzymes) and absorption process of the drug side by side. These factors were not present in *in-vitro* experimentations. Due to these factors initially less amount of drug was released as compared to *in-vitro* data.

From the above studies and observations it was concluded that the optimized ocular inserts gave good results in *in-vivo* studies with little variations as planned earlier for their release rates, duration of drug release and duration of action etc.

**Stability studies:**

Accelerated stability studies on optimized ocular insert and sol to gel preparation were performed. The shelf life of the ocular inserts was found to be 2.32, 2.46 and 2.44 for products III D, IX C and XI L. These values were comparable with the marketed dosage forms of these drugs. The higher shelf life in case of ocular inserts might be due to presence of drug in solid state. In case of sol to gel systems, the shelf life were 3.26, 2.30 and 2.10 for polaxamer based sol to gel systems P3-C, P3-CC and P4-L. Also the shelf life were 2.30, 2.44 and 2.10 for gelrite based sol to gel systems G5-D, G4-CC and G4-L. In case of marketed eye drops of Acetazolamide and Levobunolol HCl, the shelf life written on the containers are 2.0 and 2.5 years. The value shelf life obtained in all sol to gel preparations was almost comparable with marketed eye drops. Hence these results of short life of sol to gel preparation were satisfactory as the drug is present in liquid in sol to gel preparation.
As per ICH guidelines for stability purpose Zone III is selected as India comes under (climatic zone III) and is having storage condition 40°C & 75% RH. It was observed that none of the preparation showed not more than 5 % degradation in six month. Therefore the tentative shelf life of all the products could be assigned more than 2 yrs. From the observation and results of stability studies, it was concluded that the optimized preparation of ocular insert and sol to gel system were stable under room temperature condition and were having sufficient shelf life.

In future these stable optimized formulations can be subjected for pharmacokinetic studies in human subjects, toxicity of formulation can be done by in-vitro SIRC cell line. Also delivering genetic material to targeted site can be delivered using developed formulation.
Summary & Conclusion

Formulation of ocular inserts:

The ocular inserts of Acetazolamide, Acetazolamide-HPβcd complex and Levobunolol HCl were prepared using Polyvinyl alcohol as polymer and polyethylene glycol 400 as plasticizer and the optimized formula was obtained on the basis of several studies and extensive experimentation. The optimized formula III-D, IX-C and XI-L for Acetazolamide, Acetazolamide-HPβcd complex and Levobunolol HCl respectively release the drug upto 2 hrs. The formulations designed have shelf life of 2.32, 2.46 and 2.44 yrs for formulations III-D, IX-C and XI-L and complied with all other test like irritation test and test for sterility. These pharmacological effect upto desired time period of 24 hrs in the experiments conducted with cul-de-sac cavity of rabbits eye.

In-situ gel preparations:

Poloxamer based systems

Ocular sol to gel preparation of Acetazolamide, Acetazolamide-HPβcd complex and Levobunolol HCl were prepared using Poloxamer as polymer and methyl paraben and propyl paraben as preservatives. The optimized formula was developed on the basis of various studies like physical appearance, viscosity, bioadhesive strength, irritation studies, stability, preservative efficacy etc. The optimized formula's P3-C, P3-CC and P4-L release the drug upto 12 hours. Formulations were stable, passed the test for preservative efficacy, irritation test and test for sterility. When placed in cul-de-sac cavity of rabbits eye produced pharmacological effect upto desired time period of 12 hrs.

Polaxamer based systems

In these preparations gelrite was used as gelling agent in place of polaxamer systems along with for preparation of Acetazolamide, Acetazolamide-HPβcd complex and Levobunolol HCl formulations. The optimized formula was developed on the basis of various studies like physical appearance, viscosity, bioadhesive strength, irritation studies, stability, preservative efficacy etc. The optimized formula's G5-D, G4-CC and G4-L release the drug upto 12 hours. Formulations were stable, passed the test for preservative efficacy, irritation test and test for sterility and produced desired pharmacological effect when placed in cul-de-sac of rabbits eye upto desired 12 hrs.
In the present work, three different sets of formulations have been developed with an objective of increasing the stability of the active medicament acetazolamide and increasing its residence time at the site of action for better efficacy and bioavailability. The new knowledge so developed in formulation development, design of flow through apparatus and application of these systems to other antiglaucoma drugs has been patented under two Indian patent applications entitled “Improved release of antiglaucoma drugs through novel ocular delivery systems” (1034/DEL/2007) and “Improved release through ocular delivery systems” (1035/DEL/2007) filed at Delhi Patent office. The patents have been filed after extensive patentability search using patent database search making use of Scifinder, STN etc. Whereas independent claim 1 for patent application entitled “Improved release of antiglaucoma drugs through novel ocular delivery systems” is

1. A modified release ophthalmic composition for the treatment of glaucoma comprising pharmaceutically active ingredients, hydroxy propyl beta cyclodextrin and pharmaceutically acceptable excipients, wherein the acetazolamide and hydroxy propyl beta cyclodextrin are present in the form of complex

and that of independent claim 1 for patent application entitled “Improved release through ocular delivery systems” is

1. A modified release ophthalmic composition for the treatment of glaucoma comprising pharmaceutically active ingredients, hydroxy propyl beta cyclodextrin, hydrophilic polymer and/or hydrophobic polymer, and optionally consist of solubilizers.

During prosecution of both of these patent applications, invention can be narrowed down by amending the claims based on the comments by the examiners. Specification and dependent claims also disclose a class of ophthalmic drug which possess bioavailability problems due to their low solubility. The patent application also covers preparation of oculosert and sol to gel system containing genetic material, which can deliver therapeutic gene to target tissue and thereby replace the deficient gene from the diseased cell. The polymers which is used for the preparation of such dosage form includes but not limiting to poly(lactic acid) (PLA) and poly(lactide-Co-glycolide) (PLGA) and their copolymers etc. These polymeric materials deliver the genetic material through microspheres, Niosomes, matrix systems containing genetic material, sol to gel system, and the like.