Design and Evaluation of Polymeric Ocular Drug Delivery System

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The objective of the present study was to prepare ocular inserts of Gatifloxacin. The inserts were fabricated by solvent casting technique, with an aim by achieving once a day administration in the treatment of conjunctivitis. Inserts were evaluated for film thickness, weight variation, drug content, percentage moisture absorption and loss. In-vitro drug release studies were done using bi-chambered donor receiver compartment model. The optimized formulations were subjected to in-vivo studies using rabbits as an animal model and stability studies to assess the effectiveness of the formulations. Finally in-vitro and in-vivo correlation was established. In-vitro drug release data was treated according to zero, first, Korsmeyer Peppas and Higuchi kinetics to access the mechanism of drug release. Formulations were found to be uniform in physicochemical parameter with a fewer variations. Plasticizer was found to influence in mechanical properties as well as modify the drug release rate of the films. Prepared ocular inserts exhibited desired release within 24 h and found to be strongly revealing the efficacy of in vitro–in vivo correlation. From stability studies inserts were remained stable both physically and chemically. No burst effect but a prolonged drug release was observed from all formulations. Thus it achieves target such as increased residence time, prolonged drug release, reduction in frequency of administration and may improve the patient compliance.

Key words Gatifloxacin; conjunctivitis; ocular insert; in-vitro in vivo release; stability study

In the recent years, great efforts are being directed towards the refabrication of existing drug molecules in a fashion, capable of solving problem related to poor water solubility, poor bioavailability, dosing problem, stability, toxicity etc. This trend of working has led to the development of new drug delivery system. Eye, as a portal for drug delivery is generally used for the local therapy as against systemic therapy in order to avoid the risk of eye damage from high blood concentrations of drug, which are not intended for eye.1) Most of the ocular treatments call for the topical administration of ophthalmically active drugs to the tissues around the ocular cavity. Several types of dosage forms can be applied as the delivery systems for the ocular delivery of drugs. The most prescribed conventional ocular dosage forms for the delivery of drugs are eye drops, eye ointments and suspensions have major disadvantages like poor bioavailability due to rapid precorneal elimination, normal tears turnover and conjunctival absorption, frequent instillation of concentrated medicament, side effects due to systemic absorption of drugs, blurred vision due to presence of viscous vehicles.2) The present study aims at formulating ocular inserts using biodegradable polymers to overcome the drawbacks of conventional eye preparations.

The ocular insert presents valuable assets such as increasing contact time, reduced number of administrations, possibility of providing a prolonged drug release and thus a better efficacy, reduction of systemic side effects and thus reduced adverse effects.3) In order to improve drawbacks associated with conventional dosage form, it is desired that an alternative way of administration is needed to enhance the bioavailability of drug. Ocular inserts of polymeric materials which can release the drug at preprogrammed rate4) without interference with the normal vision can serve this purpose. Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientists. Although very few ophthalmic formulations containing bioadhesive or penetration enhancer are commercially available in the market, research in this area has provided a new impetus and dynamism, as never before, for the development of modified or novel ophthalmic formulations, with the promise of new and exciting directions in the field of formulations technology.5) Eye is a unique organ and drug administration is a challenging task. Eye is prone to number of diseases; some of them are blepharitis, conjunctivitis, ophthalmia neonatorum, trachoma, iritis, and corneal ulceration. Bacteria are the causative pathogens for a large number of eye disorders. The anatomy and physiology of the eye render this organ exquisitely impervious to foreign substances.6) The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. The development of newer, more sensitive diagnostic techniques and therapeutic agents render urgency to the development of maximum successful and advanced ocular drug delivery systems.

The eye drops are easy to instill but suffer from the inherent drawbacks, that the majority of the medication it contains are immediately diluted in the tear film as soon as the eye drop solution is instilled into the cul-de-sac and is rapidly drained away from the precorneal cavity by constant tear flow, a process that proceeds more intensively in inflamed eyes than in the normal eyes, and lachrymal nasal drainage. Therefore only a very small fraction of the instilled dose is absorbed into the target tissues (e.g., 1.2% is available to the aqueous humor) and relatively concentrated solution is required for instillation to achieve an adequate level of therapeutic effect. The frequent periodic instillation of eye drops becomes necessary to maintain a continuous sustained level of medication.7) This gives the eye, a massive and unpredictable dose of medication and unfortunately the higher the drug concentration in the eye drop solution, the greater the amount of drug lost through lachrymal nasal drainage system. Subsequent absorption of this drained drug, if it is high enough, may result in undesirable systemic side effects.8) Suspension types of pharmaceutical dosage forms are formulated with relatively water insoluble drugs to avoid the intol-
erable high toxicity created by saturated solutions of water-soluble drugs. However, the rate of drug release from the suspension is dependent upon the rate of dissolution of the drug particles in the medium, which varies, constantly in its composition with the constant inflow and outflow of lachrymal fluid.

The therapeutic efficacy of an ophthalmic drug can be greatly improved by prolonging its contact with the corneal surface. In order to achieve this, viscosity enhancing agents are added to eye drop preparations or the drug is formulated in a water insoluble ointment formulation to sustain the duration of intimate drug–eye contact. Unfortunately, these dosage forms give only marginally maximum sustained drug–eye contact than eye drop solutions and do not yield a constant drug bioavailability. Repeated medications are still required throughout the day.13)

Gatifloxacin, a fourth generation fluoroquinolone is used to treat bacterial conjunctivitis.9) Gatifloxacin is a drug of choice as antibacterial in the treatment of bacterial infections and used as eye drops and eye ointments in the market. Newer delivery systems are being explored to develop comprehensive and controlled release strategy. Some of the newer, sensitive and successful ocular delivery systems like inserts, biodegradable polymeric systems, collagen shields are being developed in order to attain better ocular bioavailability and sustained action of ocular drugs. Utilization of the principle of controlled release as embodied by ocular inserts therefore offers an attractive alternative approach to the difficult problem of prolonging pre-corneal drug residence time. Hence an ocular inserts is made up of polymeric materials chitosan and gellan gum which releases the drugs at a programmed rate for a specific period of time without interfering with normal vision having additional desired advantages.10)

The mucoadhesive polysaccharide chitosan for enabling increased precorneal drug residence times. This cationic polymer was expected to slow down drug elimination by the lachrymal flow both by increasing viscosity and by interacting with the negative charges of the mucus.10) Gellan gum is a linear anionic heteropolysaccharide12) natural hydrophilic, biodegradable and biocompatible polymer, characterized by prolonged release due to the formation of hydrogen bond with drug.4) Polymers confer some adhesive properties to films due to its hydrophilicity. The use of release rate modification agent may either decrease or increase the release of the drug in the range of multiple orders preferably up to a ten fold change. Release rate modification agents which are hydrophilic such as polyethylene glycol may increase the re-

Fabrication of Ocucersets Polymer (in different concentrations) was dissolved in simulated tear fluid of pH 7.4 to form the drug reservoir by using magnetic stirrer in a beaker to get different concentrations of each polymer.14) Drug was added in required concentration (0.3%, w/w). Plasticizer polyethylene glycol 400 (PEG-400) was then incorporated to above solution under stirring condition. After complete mixing the solution was poured in a clean petriplate (Anumbra). Films were prepared using petriplates containing mercury as a substrate. After drying at room temperature for 24h, circular rings of 8 mm diameter each containing 4 mg of the drug were taken out. To accommodate these variables, 8 batches of cast films were fabricated.

Physicochemical Evaluation. 1. Preformulation Studies Preformulation studies were carried out in order to find out the drug excipients compatibility. The samples of drug and excipients were intimately mixed, in equal parts and screened by IR and TLC after storage under accelerated conditions of temperature and humidity.15)

2. Thickness of Film The thickness of the film was measured by using micrometer at three different points (Mitutoyo, Japan) and the mean value was calculated.16) The standard deviation of thickness was computed from the mean value.

3. Drug Content Uniformity To check the uniformity of the drug in the circular film, three inserts were taken out from each film. Each insert was then placed in volumetric flask containing 100 ml of phosphate buffer pH 7.4 and shaken to extract the drug from film.10) One milliliter of above resulting solution was withdrawn, after suitable dilution with phosphate buffer pH 7.4 and analyzed spectrophotometrically in the Lambert Beer’s range 2—12 μg/ml. The absorbance of the solution was measured by UV-visible spectrophotometer at 286 nm and against blank. The drug content was determined from the standard curve of drug. The mean and standard deviation of drug content of three randomly selected films were calculated. The same procedure adopted for all the batches and drug content was noted.

\[
\text{amount of drug in one insert} = \frac{As \times G_L}{Gr}
\]

Where, \(As\) = absorbance of sample solution, \(G_L\) = conc. of drug in standard solution, \(Gr\) = absorbance of standard drug solution.

4. Weight Variation Test The weight variation test was carried out by weighing three inserts individually using digital balance (Shimadzu Inc., Japan). The mean weight of insert was noted.17) The standard deviations of weight variation were computed from the mean value.

5. Percentage Moisture Absorption The percentage moisture absorption test was carried out to check physical stability or integrity of the inserts maintaining high humidity. Three inserts were weighed individually from each batch and placed in desiccators, which maintained high relative humidity (RH) at about 75±5% RH using an excess amount of salt in solution. After 3 d the inserts were taken out and reweighed.18) The percentage moisture absorption was calculated using the formula,

\[
\text{percentage moisture absorption} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100
\]

6. Percentage Moisture Loss The percentage moisture loss was carried out to check integrity of the film at dry condition 33±5% RH. Three inserts from each formulation were taken for the study. Inserts were weighed individually and kept in a desiccators containing anhydrous calcium chloride. After 3 d, the inserts were taken out and reweighed.19) The percentage moisture loss was calculated using the formula,

\[
\text{percentage moisture loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100
\]

7. Folding Endurance This was determined by repeatedly folding the film at the same place until it broke. The number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance. This also gives an indication of brittleness.15)

8. In-Vitro Drug Release Studies The in-vitro drug release studies were carried out by using bi-chambered donor receiver compartment model designed by using commercial semi permeable membrane of transparent and regenerated cellulose type (Sigma Dialysis Membrane). The insert was placed inside the donor compartment. In order to simulate the tear volume, 0.7 μl of phosphate buffer pH 7.4 was placed and maintained at the same level throughout the study in the donor compartment. The semipermeable membrane was used to mimic in-vitro conditions like corneal epithelial bar-
The drug content was analyzed at 286 nm against reference standard phosphate buffer pH 7.4. The spectra recorded were taken as qualitative in order to assess the changes in pick, patterns of curve etc. No major differences were observed in the IR spectra of the pure drug and the medicated formulations. The results of the compatibility studies indicated that there was no chemical interaction between the drug and the excipients in the ocular inserts.

Interaction studies were carried out to ascertain any kind of interaction of the drug with the excipients used in the formulation of ocular inserts. For this purpose, the optimized formulations F1, F2, F5 and F6, placebo formulation and the pure drug were subjected to the assay, UV, IR and TLC analyses. The principle spot in TLC obtained with the test solution was similar in position, colour and size to the size chromatogram obtained with the reference standard of the drug. Rf value of 0.4 was obtained with the medicated formulation and drug reference standard. The UV absorption maximum for the pure drug and the medicated formulations was found to be at 286 nm. The spectra recorded were taken as qualitative in order to assess the changes in pick, patterns of curve etc. No major differences were observed in the IR spectra of the pure drug and the medicated formulations. The results of the compatibility studies indicated that there was no chemical interaction between the drug and the excipients in the ocular inserts.

Thickness of the formulations was almost uniform and it was found to be in the range of 0.132 (0.05) to 0.174 (0.02) in mm. Thickness of the film was higher in case of formulation containing gellan gum as a polymer. Formulation F5, F6, F7 and F8 had a greater film thickness than F4, F3, F2 and F1. Therefore moisture vapor transmission was greater in latter case. The average area of the film was 0.502 cm². The standard deviation was noted for all the batches.

The drug content of the formulations was determined according to procedure described above. For the various formulations drug contents were found to be uniform and were found in the range of 3.83 to 3.98 mg per film. No significant difference in drug content was noted when increase in polymer concentration. Cumulative percentage drug release, percentage drug retained of each insert during in-vitro release studies and in-vivo release studies based on the mean content of the drug present in the respective inserts.

The weight of all the formulations was found to be uniform with their low standard deviation values. For each formulation the weight was taken in triplicate on a digital balance. The mean value of weight was found to vary between 12.33 (0.12) and 13.21 (0.13) in mg. Weight of inserts was found to be higher by increasing polymer and plasticizer concentration.

The percentage moisture absorption was noted for all the formulations in triplicate. Percentage moisture absorption found to be in between 10.43 (0.03) to 14.35 (0.04). Formulation F7 showed high moisture absorption which may be attributed to a high concentration of gellan gum. Polymers present in the above formulations are hydrophilic in nature and can be expected to absorb water. There was very high percentage moisture absorption at the humid condition. However, there was less or no change in the integrity of the film.
at that condition which was observed by its physical appearance.

The percentage moisture loss was determined in triplicate. When the formulations were kept at very dry condition, the moisture loss had been occurred. Formulation F1 showed the maximum amount of moisture loss 7.64 (0.22) and formulation F7 had shown less amount of moisture loss 6.19 (0.24). Percentage moisture loss was decreased by increase in film thickness. The lower standard deviation value in all the formulations indicated that, the integrity of the film was maintained at dry conditions and it was viewed by observing the inserts after percentage moisture loss test.

The recorded folding endurance for all batches was greater than 200, which was considered satisfactory and reveals good film properties.

**In-Vitro Drug Release Studies**

*In-vitro* drug release studies were carried out in triplicate. For different time interval samples were withdrawn and cumulative percentage drug release was calculated. Cumulative percentage drug release and cumulative percentage drug retained were calculated on the basis of mean amount of Gatifloxacin present in the respective inserts. Formulation F8 shows a maximum cumulative percentage drug release of 94.98 at the end of 24 h, followed by the formulations F4 (94.42), F7 (93.31), F3 (92.32), F5 (90.29) F1 (88.45), F6 (88.27) and F2 (87.21). Therefore, it is probable that drug released from both the formulations F8 and F4 due to the higher concentration of plasticizer and minimum concentration of polymer. Figure 1 shows plot of cumulative percentage drug release as a function of time for all the eight formulations of Gatifloxacin ocular inserts. Where as, zero order plot of cumulative percentage drug release shows the regression coefficient values as 0.997, 0.998, 0.996, and 0.998 for the formulations F1, F2, F6 and F5 respectively. The zero order curves alone are not sufficient to predict zero order since each curves, albeit straight, had a different slope. Hence to confirm the exact mechanism of drug release from inserts, the data were computed and fitted as per Higuchi and Korsemeyer Peppas diffusion model. The release of drug from the inserts follows zero order kinetics. Regressions values $r$ suggested that the curves were fairly linear. Slope values were computed from graph. The $n$ value suggested that the formulations F2 and F6 follow zero order kinetics ($n=1.0$) whereas F1 and F5 follow Fickian diffusion ($n=0.5$). The zero order rate constant, slope value and their respective $r$ values are given in Table 3. The formulations F1, F2, F5 and F6 were found to extend drug release up to 24 h respectively fulfilling the criteria of once a day therapy and hence chosen for further studies such as *in-vivo* release studies. Incorporation of PEG-400 in all the formulations alters the release of Gatifloxacin and thus therapeutic levels of the drug could be achieved. This is because PEG-400 in addition to plasticizer. The programmed release is due to the formation of hydrogen bonds between the drug and polymer which has helped in rate controlled release of drug. The higher concentration of plasticizer resulted in rapid hydration and drug release, whereas by decreasing the plasticizer concentration and increasing the polymer concentration was responsible for prolonged release of Gatifloxacin. Results indicated that at the end of 24 h, the *in-vitro* drug release of formulation F2 which contains higher concentration of chitosan and minimum concentration of plasticizer was sustained the drug release and could be a better polymer in comparisions with gellan gum.

**In-Vivo Release Studies**

The *in-vivo* release studies were performed using albino rabbits in triplicate. The four formulations F1, F2, F5 and F6 were sterilized and subjected for *in-vivo* release studies. For different time interval withdrawal cumulative amount of drug release was calculated by subtracting drug remaining from mean content of respective insert. Figure 2 shows the plot of cumulative percentage of drug release as a function of time for four formulations. Cumulative percentage drug release from *in-vivo* studies were tried to correlate with the *in-vitro* drug release of formulations F1, F2, F5 and F6. The correlation values were found to be 0.9737, 0.9879, 0.9752 and 0.9871 respectively and found to be fairly linear, as indicated by their good regression value. Therefore it was ascertained that, the drug release form the F2 and F6 could followed either near zero or zero order release and F1 and F5 followed Fickian diffusion. Figure 3 shows plot of *in-vitro vs. in-vivo* cumulative percentage drug release correlation. The linearity was found in all the

Table 2. Physicochemical Evaluation of Formulated Ocuserts

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Thickness $^{(a)}$ (mm)</th>
<th>Content uniformity $^{(a)}$ (mg)</th>
<th>Weight $^{(a)}$ (mg)</th>
<th>Percentage moisture loss $^{(a)}$</th>
<th>Percentage moisture absorption $^{(a)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.132 (0.05)</td>
<td>3.874 (0.03)</td>
<td>12.33 (0.12)</td>
<td>7.64 (0.22)</td>
<td>10.43 (0.03)</td>
</tr>
<tr>
<td>F2</td>
<td>0.147 (0.02)</td>
<td>3.989 (0.01)</td>
<td>12.47 (0.18)</td>
<td>7.26 (0.12)</td>
<td>10.62 (0.01)</td>
</tr>
<tr>
<td>F3</td>
<td>0.156 (0.02)</td>
<td>3.827 (0.03)</td>
<td>12.62 (0.06)</td>
<td>7.47 (0.34)</td>
<td>11.75 (0.04)</td>
</tr>
<tr>
<td>F4</td>
<td>0.149 (0.06)</td>
<td>3.879 (0.08)</td>
<td>12.56 (0.08)</td>
<td>7.40 (0.23)</td>
<td>11.44 (0.05)</td>
</tr>
<tr>
<td>F5</td>
<td>0.166 (0.03)</td>
<td>3.952 (0.09)</td>
<td>12.38 (0.04)</td>
<td>6.73 (0.14)</td>
<td>12.13 (0.06)</td>
</tr>
<tr>
<td>F6</td>
<td>0.171 (0.01)</td>
<td>3.987 (0.03)</td>
<td>12.88 (0.01)</td>
<td>6.27 (0.27)</td>
<td>12.49 (0.04)</td>
</tr>
<tr>
<td>F7</td>
<td>0.174 (0.02)</td>
<td>3.976 (0.06)</td>
<td>13.21 (0.13)</td>
<td>6.19 (0.24)</td>
<td>14.35 (0.04)</td>
</tr>
<tr>
<td>F8</td>
<td>0.168 (0.04)</td>
<td>3.982 (0.02)</td>
<td>13.01 (0.16)</td>
<td>6.84 (0.26)</td>
<td>13.82 (0.06)</td>
</tr>
</tbody>
</table>

$^{(a)}$ Indicates average of three reading. The S.D. values are given in the parentheses.

Fig. 1. *In-Vitro Drug Release Studies*
four formulations but formulation F2 gave a good correlation and better linearity. The in-vitro/in-vivo correlation for formulation F2 was strong and productive. There was no drag out of circular inserts at the time of experiment which suggests that the particular diameter (8.0 mm) was suitable as ocular inserts. The absence of redness in the rabbit eye suggests that the formulated ocusert dose not produce any irritation. On the basis of in-vitro and in-vivo studies, it could be revealed that Gatifloxacin, a potent antibacterial agent, could be successfully administered as controlled release ocular inserts for the treatment of bacterial keratitis and conjunctivitis.

**Stability Studies** From the accelerated stability studies, performed at elevated temperature and humidity revealed that no significant changes were observed in film thickness, weight or folding endurance. Ocular inserts could be stored safely at study storage conditions. However, storage temperature not in excess of 40 °C and moisture-proof packing are recommended to ensure stability of formulation. Overall the degradation is less than 5%, a tentative shelf-life of more than years may be assigned to the formulations as per the ICH guidelines.

**Conclusion**

From the experimental results it can be concluded that:

The formulations based on chitosan and gellan gum are able to form ocular inserts, well tolerated by the rabbit eye. This insert has the potential to provide an effective and time-constant drug concentration in the aqueous, with a reduced number of applications. A similar potential was also shown by the insert based on gellan gum which showed the additional, remarkable advantages of increasing the availability in the cul-de-sac. Prepared ocular inserts exhibited zero order kinetics, shows strong and productive in-vitro/in-vivo correlation. Incorporation of plasticizers in all the formulations enhances the permeability of drug and thus therapeutic level could be achieved. Absence of redness in the rabbit’s eye suggests that the formulated ocusert does not produce any irritation. Therefore, the use of bioadhesive such as chitosan and gellan gum considerably prolongs the corneal contact time, sustained the drug release whereas incorporation of the plasticizer like PEG-400 modified the release rate. Combining these two approaches would practically assure an increase of the bioavailability. Stability studies performed showed no significant changes in the inserts which suggest that the inserts were stable. The above findings open new prospects for ocular application. However, more exhaustive preclinical and clinical studies need to be performed to provide further information and insight into these approaches.

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**References**