Formulation Development and Characterisation of Gemifloxacin Mesylate and Loteprednol Etabonate Ophthalmic Ocuserts

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ABSTRACT
Gemifloxacin Mesylate is a fluoroquinolone antibacterial drug preferably used in the treatment of bacterial conjunctivitis. The addition of Loteprednol Etabonate enhances the anti-inflammatory activity of the developed formulation. The objective of the present work was to develop ocular inserts of Gemifloxacin Mesylate with Loteprednol Etabonate and thereby evaluate its potential as a sustained ocular delivery system. Poor bioavailability and poor therapeutic responses are associated with conventional ophthalmics solutions due to many pre-corneal constraints. These constrain trigger the researcher’s mind to formulate a controlled and sustained drug delivery system. Ocular inserts based on the solvent cast technique were formulated and characterized by in vitro drug release studies using a flow-through apparatus that simulated the eye conditions. Compatibility of Gemifloxacin Mesylate, Loteprednol Etabonate, polymer, and excipients was checked based on preformulation studies. Different combinations of Gemifloxacin Mesylate, Loteprednol Etabonate, Carbopol 974, 98 981, PEG 400, and glycerine were formulated by the solvent cast method and evaluated. Clarity, smoothness, surface pH, drug content, and in-vitro drug release study were the various parameters evaluated on the formulated ocusert. Formula GLE 74 fulfilled the needs of all organoleptic parameters and also the in-vitro release study. Based on in vitro correlation stability studies, it was concluded that this ocular inserts formulation could be a promising controlled release formulation.

INTRODUCTION
The eye serves as a portal for ocular drug delivery generally for local therapy to avoid the risk of eye damage from high blood concentrations of the drug. Constant challenges presented to the formulators were in understanding the anatomy, physiology and biochemistry of the eye, which is impervious to foreign substances. Poor bioavailability and poor therapeutic responses are associated with eye drop due to rapid preconeral elimination of the drug, which often results in patient compliance problems Barbu et al. (2005); Sreenivas et al. (2006). Design, construction and technology of ocular insert in the field of the controlled and sustained ocular delivery systems are gaining rapid improvement to over-
come conventional ocular dosage constraints (Attia et al., 1988; Bharath and Hiremath, 1999). Thus the aim of the present work was to formulate oclusert with a definite concentration of Gemifloxacin and Loteprednol Etabonate for the treatment of ocular conjunctivitis and compared for the sustained release of the active. The formulation was formulated with the objective of increasing the residence time of the drug, reducing the dosing frequency by combining with Carbopol 974, 980, 981, PEG 400, polyvinyl alcohol, and glycerine.

MATERIALS AND METHODS

Materials

Gemifloxacin Mesylate was obtained as a gift sample from Glenmark Pharmaceuticals, Solan, Himachal Pradesh and Loteprednol Etabonate was from Ajanta Pharma, Pvt, Ltd. Carbopol 974, 980 and 981 were gifted by Lubrizol Pvt, Ltd, Mumbai. PVA, PEG 400, Beta cyclodextrins used were procured from Hi-Media. Analytical grade chemicals were used for analytical purposes.

Methods

Preformulation Studies

Preformulation studies were performed on the pro- cured drug samples and excipients with respect to the description, melting point, solubility, IR spectra, ultraviolet (UV) spectroscopic studies, and Differential Scanning Calorimetry (DSC) (Keny and Shah, 2020; Kumar et al., 2013b).

UV Spectroscopy Study

Determination of wavelength of maximum absorption

Pure Gemifloxacin Mesylate and Loteprednol Etabonate were weighed separately and diluted in distilled water. The prepared solutions were scanned in the wavelength region of 200 – 400 nm. UV-visible spectrophotometer (UV- Shimadzu make) was used for scanning purposes.

Determination of Linearity and Range

25mg of Gemifloxacin Mesylate and 25 mg Loteprednol Etabonate were weighed separately and transferred to two different 25 ml volumetric flask, dissolved and diluted up to mark with water and methanol to give a stock solution having the strength of 1mg/ml and further diluted to get 0.1mg/ml. Aliquots of 0.25ml, 0.5ml, 0.75ml, 1.0ml, 1.25ml, 1.5ml, 1.75ml, 2.0ml, 2.25ml and 2.5ml of working standard solution of individual drugs were transferred to a series of 10 ml standard volumetric flask and diluted with Phosphate buffer pH 6.8 to get 2.5µg/ml, 5µg/ml, 7.5µg/ml, 10 µg/ml, 12.5µg/ml, 15µg/ml, 17.5µg/ml, 20 µg/ml, 22.5µg/ml and 25µg/ml of Gemifloxacin Mesylate and Loteprednol Etabonate individually. The resulting solution was estimated in a UV- spectrophotometer at 263.8nm and 245.8nm, respectively, for Gemifloxacin Mesylate and Loteprednol Etabonate against the blank solution prepared using methanol and phosphate buffer pH 6.8. A graph of concentration against absorbance was plotted, and Beer’s Lambert law was verified (Keny and Shah, 2020; Azharuddin et al., 2011).

DSC

The thermal property of drug and excipients alone and in combination was studied using DSC (DSC-60 Shimadzu, TA-60 WS collection software). Endothermic and exothermic parameters of the drug and polymer were subsequently obtained.

IR

The FT-IR spectrums of the obtained sample were compared with the reference standard FT-IR spectrum of Gemifloxacin Mesylate and Loteprednol Etabonate by potassium bromide method (Kumar et al., 2013a).

Preparation of Ocusert

Preparation of Beta cyclodextrin and Loteprednol Etabonate complex

Loteprednol Etabonate being a poorly water-soluble drug, needs to be complexed with β-cyclodextrin to enhance its solubility. Six different molar ratios were prepared and evaluated. The solubility profile of the drug was checked, and the ratio to be used (Drug: β cyclodextrins) was finalized based on % cumulative drug release (Fialho and da Silva-Cunha, 2004).

Preparation of Ocusert of Gemifloxacin Mesylate and Loteprednol Etabonate

Petri dish with a 9 cm diameter was chosen and the area of the same calculated. Drug quantity was calculated depending on the area of the petri dish. The proportion of 1: 9 (carbopol: PVA) was kept for overnight soaking in 20ml of distilled water, followed by incorporation of drug and PEG 400 and glycerin with stirring on a magnetic stirrer for 6 hours as represented in Table 1. On completion of 6 hours, the preparation was poured in the mentioned Petri dishes and was dried at 50°C in a hot air oven for 4 hours. 1cm² x 1cm² areas of the prepared films were used for the evaluation purpose (Rajalakshmi et al., 2013).

Surface pH

The ocular insert should be non-irritating to the
eye and should be compatible with lachrymal fluid. The prepared films were allowed to swell in 0.1ml of double distilled water at room temperature for 30 minutes. The swollen films were placed under a digital pH meter, and the surface pH was determined (Parmar and Tank, 2013).

**Drug Content**

1cm x 1 cm film was cut and dissolved in 10 ml phosphate buffer pH 6.8. Further, 1ml is diluted to 10ml to be analyzed using a UV-visible spectrophotometer at the wavelength of 263.8 nm and 245.8 nm, respectively (Pawar et al., 2012).

**In-Vitro drug release study**

Franz Diffusion Cell was employed to determine the in vitro drug release. The set up was placed on the magnetic stirrer with a minimum stirring rpm closely relating to eye blinking movement and the room temperature was maintained. Semi-permeable membrane (dialysis membrane 50, HIMEDIA) was used as a drug release study. 1 ml sample receptor compartment was withdrawn at periodic intervals and subsequently replaced with 1ml Phosphate buffer. The withdrawn sample was analyzed and drug release was calculated (Chowdhury et al., 2017).

**Antimicrobial activity**

The cup-plate technique with an agar diffusion medium was used. The cup was bored at the center of the plate. The developed film and standard solution of the pure drug were taken separately into the soya bean casein digest medium earlier seeded with *Staphylococcus Aureus* organism. On placing the film and standard solution on the plate, they were incubated for a day at 37°C. Compared with the standard, the zone of inhibition (ZOI) was calculated (Mishra and Gilhotra, 2008).

**Sterility Testing**

The standard procedure from Indian Pharmacopoeia 1996 was employed to perform this test. Fluid thioglycolate and soya bean casein digest media were used. The formulated films were cut into two equal halves under laminar airflow and dropped in the two test tubes simultaneously. Both the media were checked for microbial growth by incubating at 37°C for seven days. The results were compared with positive and negative control samples (Pharmacopoeia, 1996).

**Isotonicity evaluation**

Tissue damages if the tonicity of the film is not maintained, hence the isotonicity of the film is a mandatory parameter. Sodium chloride solutions of three different concentrations namely hypertonic (HT – 3%w/v), hypotonic (HP - 0.2%w/v) and isotonic (IS - 0.9%w/v) concentrations were prepared. Four clean slides were taken and labeled as HT (hypertonic), HP (hypotonic), IS(isotonic) and Test. A drop of blood with heparin (1% w/v) was taken to prevent coagulation, further placed on all slides. An optimized film drop was placed on a test slide and all four sides were covered with a coverslip and checked using a 45x magnification microscope. Morphology of RBC’s was studied (Rathore, 2011).

**Antibacterial Activity**

The serial dilution method was employed to carry out the microbiological assay. The test organism employed was *Staphylococcus aureus*. Two samples for testing were coded as A (film) and B (pure sample) for minimum inhibitory concentration (MIC). The concentration of pure drug taken was 5mg/ml. 51μl of this drug solution contains 256μg of the drug.

Series of 14 test tubes were taken and numbered as 1 – 14. To 1st test tube, 2000μl of broth was added. And 1000μl broth was added from test tube 2nd to 14th. 51μl of broth from test tube 1st was withdrawn and discarded and replaced with drug solution corresponds to 256μg of the drug. 1000μl of the content from the 1st test tube was transferred to 2nd and so on.

This procedure is repeated until the second last test tube contains an amount of drug corresponding to 128μg, 64μg, 32μg, 16μg, 8μg, 4μg, 2μg, 1μg, 0.5μg, and 0.25μg. The last test tube serves as a negative control. 10μl of *S.aureus* broth is added in each tube except negative and kept for incubation at 37°C for 24 hours. Further, MIC was calculated, and results were tabulated (Prakash et al., 2009).

**Short term Stability Studies**

Short term stability study was carried out for a period of 3 months on the formulated products at room temperature. Samples were withdrawn for each month till three months and analyzed for visual appearance, pH and drug content (Gevariya et al., 2009).

**RESULTS AND DISCUSSION**

Results of preformulation studies performed on drug and excipients were represented in Table 2 and IR spectra of pure drugs and excipients were plotted and compared with standard samples. Drugs and excipients were found to be compatible with each other, as represented in Table 3.

**DSC**

DSC was employed to understand the thermal
### Table 1: Composition of Ocusert

| Ingredients                  | GLE74 | Ingredients                  | GLE80 | Ingredients                  | GLE81 |
|------------------------------|-------|------------------------------|-------|------------------------------|-------|
| Gemifloxacin Mesylate        | 19 mg equivalent to 0.3 mg Gemifloxacin | Loteprednol Etabonate: βeta Cyclo Dextrin | 40 mg equivalent to 0.3 mg Loteprednol Etabonate |       |
| Carbopel 974                 | 60 mg | Carbopel 980                 | –     | Carbopel 981                 | –     |
| Poly Vinyl Alcohol           | 540 mg | Poly Ethylene Glycol 400     | 0.5 ml | Glycerin                     | 25 mg |
| Distilled Water              | 20 ml | Distilled Water              |       | Distilled Water              |       |

### Table 2: Preformulation study on Drug and Excipients

| Observed parameters | Gemifloxacin Mesylate | Loteprednol Etabonate |
|---------------------|-----------------------|------------------------|
| Description         | Gemifloxacin is an off-white, amorphous solid form. The mesylate salt is a white to light brown solid | It is white – of the white amorphous powder |
| Melting Point       | 234.5 °C              | 222.5 °C               |
| Solubility          | Freely soluble 0.21 mg/ml distilled water, freely soluble in DMSO, DMF, Ethanol and Methanol | Insoluble in water (0.0336 mg/ml), freely soluble in DMSO, DMF, Methanol and sparingly soluble in Ethanol |

### Table 3: Interpretation of IR Spectra

| Functional Groups | Drug A | Drug B | A + B + Carbopol 974 | A+B+ Carbopol 980 | A+B+ Carbopol 981 |
|-------------------|--------|--------|----------------------|-------------------|-------------------|
| O-H               | 3248.13| 3419.79| 3421.72              | 3421.72           | 3421.72           |
| N-H               | 3431.8 | –      | 3246.20              | 3246.20           | 3246.20           |
| Aromatic C-H      | 2929.87| 2941.44| 3066.82              | 3066.82           | 3064.89           |
| Aliphatic C-H     | 2821.86| 2875.86| 2931.80              | 2931.80           | 2927.94           |
| C=O (Ketone)      | 1724.36| 1714.72| 1724.36              | 1718.58           | 1724.36           |
| C-F               | 1376.53| 1107.14| 1367.53              | 1367.53           | 1367.53           |

### Table 4: Absorbance of Gemifloxacin Mesylate and Loteprednol Etabonate

| Conc.(μg/ml) | Absorbance at 263.8nm | Absorbance at 245.8nm |
|--------------|-----------------------|-----------------------|
| 0            | 0                     | 0                     |
| 2.5          | 0.233                 | 0.09                  |
| 5            | 0.434                 | 0.171                 |
| 7.5          | 0.647                 | 0.263                 |
| 10           | 0.861                 | 0.345                 |
| 12.5         | 1.03                  | 0.429                 |
| 15           | 1.279                 | 0.53                  |
| 17.5         | 1.461                 | 0.622                 |
| 20           | 1.700                 | 0.688                 |
Table 5: Absorbance v/s concentration plot for Loteprednol Etabonate beta cyclodextrin complex

| Ratio            | Absorbance | Concentration (μg/ml) |
|------------------|------------|-----------------------|
| Pure Drug        | 0.034      | 0.01                  |
| 1: 0.5 ratio     | 0.093      | 0.027                 |
| 1: 1 ratio       | 0.078      | 0.022                 |
| 1: 1.5 ratio     | 0.053      | 0.015                 |
| 1: 2 ratio       | 0.050      | 0.014                 |
| 1: 2.5 ratio     | 0.045      | 0.013                 |
| 1: 3 ratio       | 0.038      | 0.011                 |

Table 6: Evaluated parameters of formulation

| Formulation code | Surface texture | Thickness (mm)* | Weight (mg)* | Tensile strength (g/cm²)* | % Drug Content (±SD*) |
|------------------|-----------------|-----------------|--------------|----------------------------|-----------------------|
|                  |                 |                 |              |                            | Drug A | Drug B |
| GLE 74           | Smooth          | 0.115 ± 0.03    | 188 ± 0.02   | 410 ± 0.08                 | 96     | 93     |
| GLE 80           | smooth          | 0.111 ± 0.01    | 184 ± 0.04   | 415 ± 0.03                 | 70     | 93     |
| GLE 81           | smooth          | 0.113 ± 0.02    | 186 ± 0.06   | 420 ± 0.05                 | 90     | 80     |

Table 7: % Cumulative drug diffusion profile

| Time (h) | GLE 74 | GLE 80 | GLE 81 |
|----------|--------|--------|--------|
|          | Drug A | Drug B | Drug A | Drug B | Drug B | Drug B |
| 01       | 16.45  | 10.99  | 29.51  | 18.20  | 14.39  | 8.62   |
| 02       | 11.48  | 20.17  | 37.98  | 53.00  | 14.86  | 29.72  |
| 03       | 16.40  | 24.29  | 37.73  | 64.46  | 18.47  | 32.42  |
| 04       | 37.37  | 47.15  | 44.79  | 72.76  | 48.38  | 85.32  |
| 05       | 68.50  | 63.26  | 51.59  | 80.59  | 63.09  | 89.86  |
| 06       | 96.15  | 75.39  | 61.33  | 89.71  | 74.37  | 98.82  |
| 07       | 98.37  | 99.87  | 97.33  | 103.58 | 100.70 | 100.47 |

Table 8: Zone of Inhibition value

| Formula                  | Zone of inhibition |
|--------------------------|--------------------|
| Negative Control         | –                  |
| Positive Control         | –                  |
| Gemifloxacin Mesylate    | Present            | 3.2 cm            |
| Loteprednol Etabonate    | Absent             | 0 cm              |
| GLE 74                   | Present            | 4.0 cm            |
| GLE 80                   | Present            | 3.9 cm            |
| GLE 81                   | Present            | 3.8 cm            |
Table 9: MIC determination of Film GLE 74 and pure drug

| Concentr. (µg/ml) | 128 | 64  | 32  | 16  | 8   | 4   | 2   | 0.5  | 0.25 | 0.125 | NC | MC | PC |
|-------------------|-----|-----|-----|-----|-----|-----|-----|------|------|-------|----|----|----|
| Turbidity in GLE 74 | -   | -   | -   | -   | -   | -   | +   | +    | -    | -     | +  |    |    |
| Turbidity in pure drug | -   | -   | -   | -   | +   | +   | +   | +    | +    | -     | -  | +  |    |

NC- Negative Control, MC – Media Control, PC – Positive Control

Figure 1: Thermal Analysis of Beta Cyclodextrin

Figure 2: Thermal Analysis of Loteprednol Etabonate

Figure 3: Thermal Analysis of Loteprednol Etabonate + Beta Cyclodextrin

Figure 4: Gemifloxacin Mesylate Calibration Curve

Figure 5: Loteprednol Etabonate Calibration Curve

Figure 6: Graph of absorbance v/s concentration
properties of Loteprednol Etabonate and Beta cyclodextrins. Due to glass transition, endothermic peaks were observed for Loteprednol Etabonate at 240.1°C, Beta cyclodextrins at 121.1°C, and complex of Loteprednol Etabonate with Beta cyclodextrins at 108.8°C, 232.4°C respectively as shown in Figure 1, Figure 2 and Figure 3.

Determination of Linearity and range

The linear calibration curve was obtained for Gemifloxacin Mesylate and Loteprednol Etabonate in the concentration range of 2.5-20 μg/ml at λ max of 263.8 nm and 245.8 nm, respectively.

It followed Beer’s Lambert’s Law with a regression coefficient (R²) value of 0.999 for both Gemifloxacin Mesylate and Loteprednol Etabonate as represented in Table 4 and Figure 4 and Figure 5.

Preparation of Beta cyclodextrin and Loteprednol Etabonate complex

Loteprednol Etabonate was complexed with β-cyclodextrins in six different molar ratios before incorporating in the ocusert development. The solubility profile of the drug was checked and the ratio of 1:0.5 (Drug: β cyclodextrins) was finalized based on % cumulative drug release, as shown in Figure 6 and Table 5.

Drug Content

The Drug content was determined based on the UV-simultaneous estimation method developed for the combined dosage form. Other evaluated parameters of the prepared ocuserts with respect to surface pH, tensile strength, thickness are recorded in Table 6.

In-vitro Release Study

It was performed using Franz Diffusion Cell and it was found that Formulation GLE 74 gave the best results compared to the other two formulations. The values are shown in Table 7 and graphical representation in Figure 7 and Figure 8.

Antimicrobial activity

Measurement of ZOI by cup plate method

The zone of inhibitions of the formulated films was compared with that of the pure drug against positive and negative control. Readings of this study
were tabulated, and images on ZOI’s were depicted in Table 8 and Figure 9.

**Sterility Testing**

When the formulations were incubated for prescribed time and temperature, no turbidity was observed. This indicates it passes the test for sterility, as shown in Figure 10.

**Isotonicity evaluation**

Isotonicity test proved that the optimized film produces no change in the blood cells, neither bulging nor shrinking, as shown in Figure 11. This proves the formulated film was isotonic in nature.

**Antibacterial Activity**

Below the concentration of 0.5 μg/ml for the film and 8μg/ml for the pure drug, the turbidity was present, which indicates the growth of the organism. The MIC concentration was found to be 0.5 μg/ml for the film and 8μg/ml for the pure drug, as shown in Table 9.

**Short term Stability Studies:**

Stability studies proved that the formulations GLE 74, GLE 80 and GLE 81 showed no significant variations. There was a slight decrease in drug content but were not significant. The variation in drug content can be attributed to moisture content. Thus from the results, we can interpret that films can be stored at room temperature for the short term.

**CONCLUSION**

The formulated ocular films prove to be a novel drug delivery system with a promising approach in achieving greater drug absorption in comparison to the conventional ocular drops. The results concluded that film GLE 74 was the best amongst the three formulations in terms of drug content, in vitro drug release and anti-microbial activity. The optimized film showed no interactions between drugs, excipients and also beta-cyclodextrin complex when characterized by IR and DSC studies. Hence a combination of Gemifloxacin and Loteprednol Etabonate as an ocular film serves as a boost for the researchers and boon to the patients in the future.

**Abbreviations**

GLE 74 represents Gemifloxacin Mesylate and Loteprednol Etabonate in carbopol 974

GLE 80 represents Gemifloxacin Mesylate and Loteprednol Etabonate in carbopol 980

GLE 81 represents Gemifloxacin Mesylate and Loteprednol Etabonate in carbopol 981.

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**Authors Contribution**

Swati Mayur Keny’s corresponding author for this publication, working as an Assistant Professor, has carried out all the above research work on the premises of PES’s Rajaram and Tarabai Bandekar College of Pharmacy under the guidance of her Ph.D. guide Dr.Ketan Shah, Professor, Parul Institute of Pharmacy and Research, Vadodara, Gujarat.

**Conflict of Interest**

All authors have none to declare

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