

Formulation and characterization of ofloxacin ophthalmic GEL

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Abstract

Ofloxacin is a fluoroquinolone broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria for the treatment of infections. Ofloxacin is being used as eye drops in the treatment of eye infections. Rapid elimination and poor bioavailability is the disadvantage associated with eye drops. An attempt was made to prolong the retention of ofloxacin in the eye by formulating into gel using HPMC K100, Carbopol 940 and Sodium Alginate individually and in combination and the prepared formulations were evaluated.

Keywords: Ofloxacin gel, Ophthalmic gels, Ocular drug delivery.

Introduction

Ophthalmic route of drug administration is the most interesting and challenging as the anatomy, physiology and biochemistry of the eye render this organ highly impervious to foreign substances. The eye is a unique organ containing several widely varied structures with different physiological functions. The conventional drug delivery such as suspension, ointment, solution show some drawbacks like increased pre-corneal drainage, blurred vision, low bioavailability low residence time. The absorption of drugs in the eye is severely limited by some protective mechanisms that ensure the proper functioning of the eye and by other concomitant factors like, drainage of the instilled solutions, lachrymation and tear turnover, metabolism, tear evaporation, non-productive absorption/adsorption, limited corneal area and poor corneal permeability, binding by the lachrymal proteins. A major goal in ocular therapeutics is to circumvent structural obstacles and protective mechanisms of the eye to elicit desired pharmacological response.

Most conventional ophthalmic dosage forms are simplistic. It is usual that water-soluble drugs are delivered through topical administration in an aqueous solution and water-insoluble drugs are administered topically as an ointment or aqueous suspension.

Poor ocular drug bioavailability is the result of ocular anatomical and physiological constraints, which include the relative impermeability of the corneal epithelial membrane, tear dynamics, nasolacrimal drainage and the high efficiency of the blood-ocular barrier. It is standard for only 1% or less of a topically applied dose to be absorbed across the cornea and thus reach the anterior segment of the eye. Pulse entry is a common and yet highly undesirable, pharmacokinetic characteristic associated with eye drops. The initial high drug concentration found in tears, followed by a rapid decline, poses a potential risk of toxicity and suggests a requirement for frequent dosing.

Therefore, it seems promising to develop a dosage form that can aim at increasing the bioavailability in the ocular region by increasing the retention time in the eye.

Materials and Method

Materials were obtained from commercial suppliers and were used. IR spectra were recorded in KBr discs on a Bruker analyzer.

Standard Graph of Ofloxacin: Standard graph of Ofloxacin was plotted in phosphate buffer pH 6.8 by making serial dilutions from the stock solution of 1mg/ml. Prepared solutions were analyzed at 292 nm using UV-visible spectrophotometer.

Drug-Excipient Compatibility Study: Excipients are integral components of almost all pharmaceutical dosage forms. The successful formulation of a stable and effective solid dosage form depends on the careful selection of the excipients, which are added to facilitate administration, to promote the consistent release and bioavailability of the drug and protect it from degradation. API and excipients were thoroughly mixed in predetermined ratio. FTIR studies were carried out to determine the compatibility of excipients with the drug.

Preparation of Gels: The weighed quantities of polymers were kept for swelling overnight in distilled water and dissolved (heated, if necessary) using a magnetic stirrer. Aqueous solution of Ofloxacin was added in to the polymeric solution with continuous stirring. Benzalkonium chloride was added to the resulting solution. The pH of the formulation was adjusted to 7.4 using 0.1 N NaOH/0.1 N HCl. The gel formulations are depicted in Table 1.

Table 1: Formulation of gels

| Formulation | F1 | F2 | F3 | F4 | F5 | F6 |
|-----------------------|--------|--------|--------|--------|--------|--------|
| Ofloxacin | 0.3g | 0.3g | 0.3g | 0.3g | 0.3g | 0.3g |
| HPMC K100 | - | 1g | - | 0.5g | - | 0.5g |
| Carbopol | 1g | - | - | 0.5g | 0.5g | - |
| Sod. Alginate | - | - | 1g | - | 0.5g | 0.5g |
| Benzalkonium Chloride | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Water q.s | 100 gm | 100 gm | 100 gm | 100 gm | 100 gm | 100 gm |

Sterilization of Prepared Formulations: Prepared solutions were sterilized by autoclaving at 121° C for 15 min and stored in aseptic conditions until use.

Evaluation

Physical parameters:

Clarity Test: The formulations were visually checked for the clarity by visual inspection under a good light against a black and white background, with the contents set in motion with a swirling action.

pH Determination: pH of each formulation was determined immediately after preparation by using digital pH meter which was previously calibrated by pH 4 and pH 7 standard buffers.

Determination of Percent Drug Content: Accurately weighed quantities of gels sample equivalent to 30 mg of ofloxacin were separately added to 100 ml of distilled water in stoppered conical flasks. The solution was filtered, diluted suitably and was assayed by a UV-Vis spectrophotometer for drug content at 292 nm.

Amount of drug = $\frac{\text{Concentration} \times \text{dilution factor}}{\text{volume of dissolution}}$

1000

Percent drug content = $\frac{\text{Actual amount present} \times 100}{\text{Amount expected}}$

In-vitro drug diffusion study: In-vitro drug release studies of the formulations were conducted using Franz diffusion cell apparatus. Egg membrane isolated from egg by placing in dil. HCl was used to study the in-vitro diffusion of the formulations. The receptor chamber was filled with freshly prepared buffer of pH 6.8. The donor compartment was loaded with 1gm of formulation. Aliquots of receptor medium were withdrawn and replenished with fresh medium at specific intervals of time and analysed spectrophotometrically for the drug content.

Sterility Test: The sterility test was carried out using three sets of agar medium where the first set was a negative control containing sterile media, second set was a positive control for this sterilized media inoculated with *Staphylococcus aureus* was used and third set was a test.

1gm of sterile optimized formulation was taken and this formulation was diluted with 100ml sterile water for injection, from this 10ml test solution was added to the medium and incubated for a period of 7 days at 20-25° C to find out growth of bacteria.

Antibacterial Activity: Agar diffusion method was used for the determination of antibacterial activity of formulations. Standard Petri dishes containing medium to a depth of 0.5 cm were used. The inoculum (0.5ml) was spread over the surface of agar and the plates were dried at 35°C for 15 min prior to placing the formulation. The bores of 0.5 cm diameter were prepared and 100mg of formulations were added in the bores. After incubation at 35°C for 24 hrs, the zone of inhibition around the bores was measured.

Results and Discussion

Construction of Calibration Curve of Ofloxacin: The data standard graph of Ofloxacin has shown good linearity over a concentration range of 0-10µg/ml with R² value of 0.9977. The equation was y=0.0782x. This was utilized in the estimation of Ofloxacin samples.

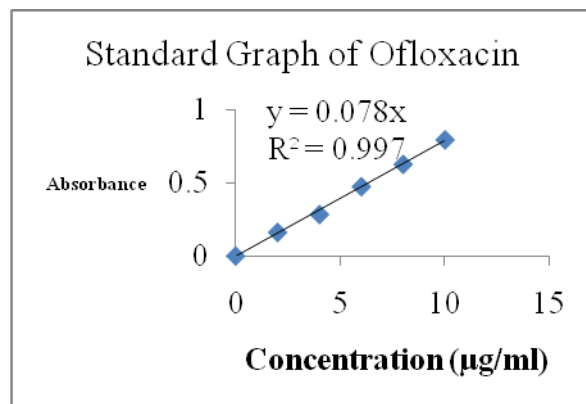


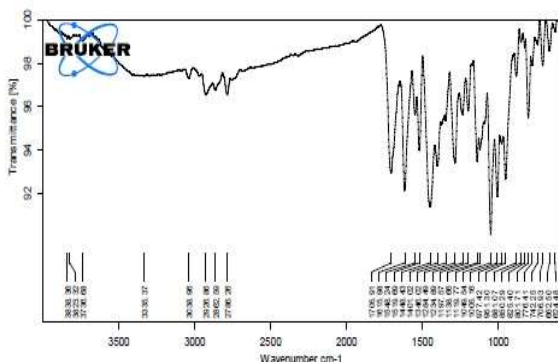
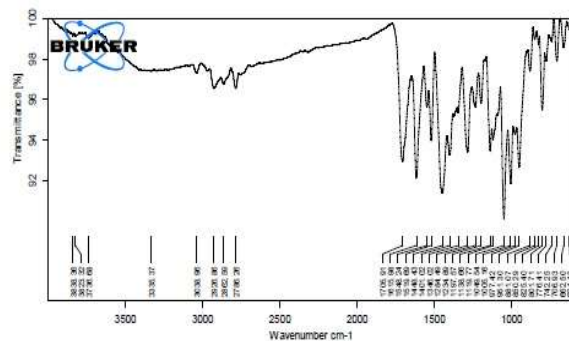
Fig. 1: Standard graph of Ofloxacin

Drug-Excipient Compatibility Study: Infrared spectroscopy studies of Ofloxacin alone and their physical mixture with HPMC, Carbopol 934 and Sodium alginate revealed that, Ofloxacin is compatible with all the polymers used.

The functional groups present in the drug were identified. The wavenumbers of drug were compared with final formulated product IR spectrum. The resulted peaks revealed that there was no significant disturbance in the principle peaks of pure drug Ofloxacin. From the interpretation it was understood that there was no major shifting in the frequencies of Ofloxacin which indicated that there is no chemical interaction in the formulations. This further confirms the integrity of pure drug and compatibility of it with excipients.

Table 2: Principle IR Peaks of Ofloxacin

| Ofloxacin | Ofloxacin + excipients | Functional group |
|-----------|------------------------|------------------|
| 2926 | 2932 | OH group |
| 1004 | 1005 | C-F |
| 2785 | 2786 | CH ₃ |
| 1709 | 1705 | C=O |
| 3037 | 3038 | C-H(AROMATIC) |

**Fig. 2: FTIR of Ofloxacin****Fig. 3: FTIR of ofloxacin with polymers
Evaluation of Ophthalmic Gel Formulation
Physical parameters**

Clarity: On careful visual inspection against dark and white background, all the prepared ophthalmic gel formulations were found to be free from any suspended particulate matter.

pH Determination: The pH of the prepared gels was found to be in the range of 7.40-7.53 with a standard deviation of 0.025 to 0.197. All the formulations have shown a pH with not much deviation.

Drug Content of Ofloxacin: The drug content of the prepared gels was found to be in the range 96-99 indicating the application of the present method for the preparation of gels with high content uniformity.

Table 3: Physical parameters and drug content of the formulations

| S. No | Formulation code | Appearance | Observed pH (\pm S.D.) | Assay |
|-------|------------------|------------|---------------------------|------------------|
| 1 | F1 | Clear | 7.40 \pm 0.025 | 97.61 \pm 0.92 |
| 2 | F2 | Clear | 7.52 \pm 0.134 | 96.01 \pm 0.23 |
| 3 | F3 | Clear | 7.51 \pm 0.178 | 98.33 \pm 1.48 |
| 4 | F4 | Clear | 7.40 \pm 0.197 | 95.16 \pm 0.36 |
| 5 | F5 | Clear | 7.53 \pm 0.115 | 99.32 \pm 0.25 |
| 6 | F6 | Clear | 7.43 \pm 0.145 | 98.31 \pm 0.38 |

In-vitro Drug Diffusion Study: The percentage drug release observed from the in vitro diffusion studies are shown in Table 4. Out of the formulations containing 1% of polymer, by the end of six hours, formulation F1 with carbopol as polymer has shown good release characteristics than those with HPMC K 100 and Sodium alginate.

When the combination of polymers were used, the F5 containing Carbopol and Sodium alginate each at 0.5% and F6 formulation containing HPMC K 100 and Sodium alginate each at 0.5% have shown a decrease in drug release may be due to hindrance of release by interaction of two polymers used in combination.

The formulation F4 containing HPMC K 100 and Carbopol each at 0.5% as polymer has shown a release of 98.89% by the end of 6th hour. Thus it is considered as optimised formulation as it has shown maximum drug release by the end of six hours.

Table 4: Cumulative percentage of drug release

| Time (hrs) | Cumulative % drug released | | | | | |
|------------|----------------------------|------------------|------------------|------------------|------------------|------------------|
| | F1 | F2 | F3 | F4 | F5 | F6 |
| 0.5 | 21.60 \pm 0.84 | 17.21 \pm 0.62 | 19.14 \pm 1.43 | 24.14 \pm 1.30 | 12.60 \pm 1.08 | 14.06 \pm 0.99 |
| 1 | 34.71 \pm 0.61 | 26.01 \pm 0.30 | 31.30 \pm 0.58 | 46.96 \pm 3.28 | 21.46 \pm 0.79 | 19.31 \pm 1.39 |
| 2 | 45.52 \pm 1.07 | 36.17 \pm 0.64 | 40.98 \pm 1.35 | 62.37 \pm 1.57 | 29.41 \pm 0.53 | 30.7 \pm 0.52 |
| 3 | 51.84 \pm 0.96 | 44.53 \pm 0.94 | 47.32 \pm 2.27 | 72.01 \pm 2.43 | 37.21 \pm 0.73 | 39.96 \pm 0.63 |
| 4 | 69.51 \pm 0.96 | 54.58 \pm 0.88 | 61.18 \pm 1.28 | 88.71 \pm 1.28 | 45.90 \pm 0.57 | 46.71 \pm 1.13 |
| 5 | 82.88 \pm 1.45 | 59.66 \pm 0.93 | 69.84 \pm 0.43 | 92.52 \pm 1.12 | 47.35 \pm 0.40 | 52.66 \pm 0.97 |

| | | | | | | |
|---|------------|------------|------------|------------|------------|------------|
| 6 | 90.48±0.84 | 64.46±0.91 | 76.76±1.17 | 98.89±1.13 | 50.12±1.79 | 61.37±1.17 |
|---|------------|------------|------------|------------|------------|------------|

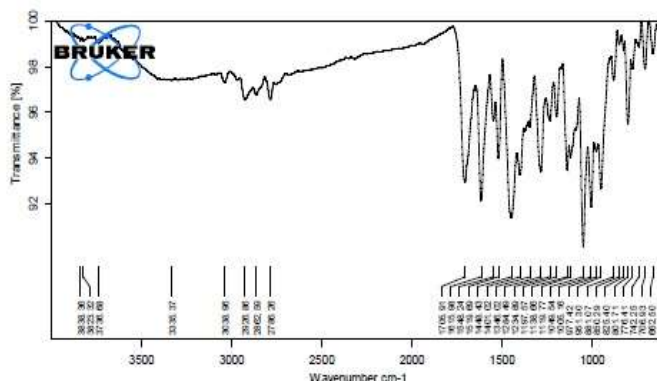


Figure 4: Cumulative drug release of F1-F6 formulations

Sterility test: The formulations showed no evidence of microbial growth when incubated for more than 7 days and cleared the sterility test.

Table 5: Sterility test data of prepared gels

| Formulation | Days of incubation | | | | | |
|-------------|--------------------|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| F1 | - | - | - | - | - | - |
| F2 | - | - | - | - | - | - |
| F3 | - | - | - | - | - | - |
| F4 | - | - | - | - | - | - |
| F5 | - | - | - | - | - | - |
| F6 | - | - | - | - | - | - |

*-ve sign indicates no growth.

Antibacterial Activity: Antimicrobial efficacy study was performed on formulations using Gram +ve *S. aureus* and Gram -ve *E. coli* organism. Clear zones showing inhibited zone of growth were observed. The zones of inhibition of the formulations were shown in the Table 6. The study indicated Ofloxacin retained its antimicrobial activity when formulated as gel against both selected *S. aureus* and *E. coli*.

Table 6: Zone of Inhibition of prepared In situ gels

| Microorganism | Zone of Inhibition(mm) | | | | | | |
|------------------|------------------------|------|------|------|------|------|------|
| | Standard (Pure Drug) | F1 | F2 | F3 | F4 | F5 | F6 |
| <i>S. aureus</i> | 29 | 28 | 28 | 27.5 | 25.5 | 28.5 | 26 |
| <i>E. coli.</i> | 30 | 27.5 | 28.5 | 29.5 | 27.5 | 28 | 27.5 |

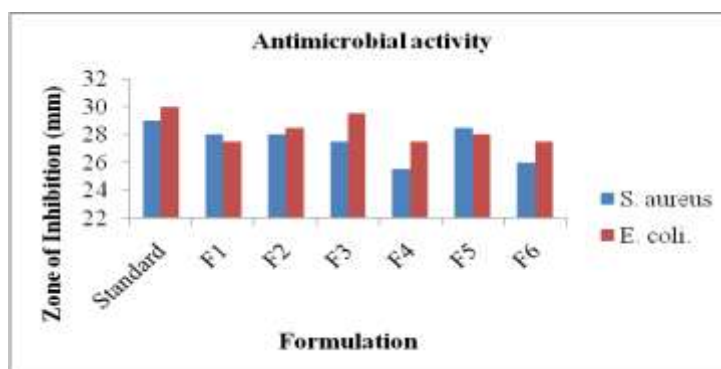


Fig. 5: Zone of Inhibition of prepared In situ gels

Conclusion

Ophthalmic gels of ofloxacin were prepared by using the polymers HPMC K100, Carbopol and Sodium Alginate alone and in combination. Comparative drug release was estimated. Of all the formulations, the formulation having HPMC K100 and Carbopol at 0.5% each has shown a sustained release of drug for the estimated six hours of time.

Therefore the combination of HPMC K100 and Carbopol was found to be optimized for ophthalmic gel of ofloxacin for a six hour release.

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