



Original Article

Formulation and Evaluation of *In-Situ* Ocular Gel of Gatifloxacin

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In-situ gelation is a process of gel formulation at the site of application, in which a drug product formulation that exist as a liquid has been transformed into gel upon contact with body fluid or at body temperature. The key objective of this study was to formulate and evaluate *in-situ* ocular gel of Gatifloxacin using HPMC, HPMC K15M, Carbopol 934 as polymers. *In-situ* ocular gel was prepared by different combination of drug and excipients. Formulation was evaluated in terms of pH, gelling capacity, viscosity, Drug content estimation and *in vitro* drug release etc. Results indicated that HPMC K15M and carbopol 934 in combination increase the precorneal residence time and prolong the drug release.

Keywords: Gatifloxacin, HPMC, HPMC K15M, Carbopol 934, *In-situ* gel.

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1. INTRODUCTION

In the recent year considerably attention has been focused on the development of new drug delivery system. For many decades treatment of an acute disease or chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms, including tablets, capsule, pills, suppositories, creams, ointment, liquids, aerosols and injectables as drug carriers. Even today, these conventional drug deliveries are the primary pharmaceutical products commonly seen in the prescription¹. Over the past 30 years greater attention has been focused on the development of controlled and sustained drug delivery system. Amongst the extensive research has been carried in

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 designing of polymeric drug delivery systems ². Basically there are three modes of drug delivery i.e.

1. Targeted Delivery: It refers to the systemic administration of drug carrier with the goal of delivering the drug to specific cell types, tissues or organ.
2. Controlled Delivery: it refers to the use of delivery with the objective of releasing the drug into the patient body at a predetermined rate, or at a specific time or with specific release profiles.
3. Modulated Delivery: Release of a drug delivery device refers to the release of the drug at a variable rate, controlled by environmental condition, bio feedback, sensor input or an external control device ³.

IN-SITU GELLING SYSTEM ⁴⁻¹¹:

In-situ gelling systems are viscous, mucoadhesive, polymer-based liquids that exhibit sol-to-gel phase transition with its favourable residence time on the ocular surface due to change in a specific physico-chemical parameter like temperature, ionic strength, ultra violet irradiation or pH. The effective dose administered can be altered by increasing the retention time of medication into the eye by using *in-situ* gel forming systems, thereby preventing the tear drainage. These systems can be injectable fluids that can be introduced into the body in a minimally invasive manner prior to solidifying or gelling within the desired tissue, organ, or body cavity. Injectable gel-forming matrices offer several advantages over systems shaped into their final form before implantation. When they are used to fill a cavity or a defect, their flowing nature enables a good fit. These have a characteristic property of temperature dependence, pH dependence and cation induced gelation. *In-situ* gels are administered by oral, ocular, rectal, vaginal, injectable and intra-peritoneal routes. Both natural and synthetic polymers can be used for the production of *in-situ* gels. *In-situ*-forming systems have been reported in the literature for various biomedical applications, including drug delivery, cell encapsulation, and tissue repair. The choice of a special Hydrogel depends on its intrinsic properties and envisaged therapeutic use. There is several possible mechanisms that lead to *in-situ* gel formation: solvent exchange, UV irradiation, ionic cross-linkage, pH change, and temperature modulation.

ADVANTAGES ^{4,12,8,9,13}:

- It overcome the side effects of pulsed dosing produced by conventional systems and reduce frequency of dosing.
- Less blurred vision as compared to ointment.
- Decreased nasolacrimal drainage of the drug which may causes undesirable side effects due to systemic absorption (i.e. reduced systemic side effects).
- Due to systemic absorption (i.e. reduced systemic side effects).
- The possibility of administering accurate and reproducible quantities, in contrast to already gelled

formulations and moreover promoting precorneal retention.

- Sustained, Prolonged drug release and maintaining relatively constant plasma profile.

REQUIREMENT OF IDEAL SYSTEM ^{4,7,13}:

Ideally, an *In-situ* gelling system should be a low viscous, free flowing liquid to allow for reproducible administration to the eye as drops and the gel formed following phase transition should be strong enough to with stand the shear forces in the cul-de-sac and demonstrated long residence times in the eye with its ability to release drugs in sustained manner will assist in enhancing the bioavailability, reduce systemic absorption and reduce the need for frequent administration leading to improved patient compliance.

2. PRELIMINARY STUDIES

In the preliminary studies to prepare the *in situ* gel of gatifloxacin the two polymers are used carbopol 934 and carbopol 940 to prepare the different formulations. Different concentrations of carbopol 934 and carbopol 940 are used to prepare the formulations. Then various evaluation parameters are performed like pH, gelling capacity and viscosity. The concentration range of carbopol 934 and carbopol 940 is used between 0.1 – 0.4 %w/v. The concentration of gatifloxacin is 300 mg in all formulations. HPMC K15M is used in concentration 0.1-0.5 %w/v. and HPMC is used in concentration 0.1%w/v in all formulation.

Table 1: Preliminary studies

| Sr. No. | Evaluation parameter | Carbopol 934 | | | | | Carbopol 940 | | | | |
|---------|---------------------------|--------------|-----|-----|-----|-----|--------------|-----|-----|-----|-----|
| | | F1 | F2 | F3 | F4 | F5 | F1 | F2 | F3 | F4 | F5 |
| 1 | pH | 6.5 | 6.7 | 6.6 | 6.9 | 6.7 | 6.8 | 6.5 | 6.9 | 6.7 | 6.8 |
| 2 | Gelling capacity | + | ++ | ++ | +++ | +++ | + | + | ++ | ++ | +++ |
| 3 | Viscosity in cps (50 rpm) | 30 | 100 | 110 | 135 | 70 | 25 | 70 | 100 | 105 | 45 |

Notice : + Gels after few minute, dissolve rapidly, ++ Gelation immediate, remain for few hours, +++ gelation immediate remains for extended period.

PREPARATION OF pH- TRIGGERED IN-SITU GELLING SYSTEMS ^{15,16}:

Accurately weighed 0.1g of HPMC was dispersed in 50ml of purified water, HPMC K15M was added, carbopol 934 was sprinkled over this solution and allowed to hydrate overnight. The solution was stirred with an overhead stirrer and buffer salts (Disodium hydrogen phosphate, citric acid) were dissolved in the solution. Gatifloxacin was dissolved in appropriate dissolution media such water, benzylkonium chloride (BKC) was added to the polymer solution. Purified water was then added to make up the volume to 100ml and prepared formulation were sterilized in an autoclave at 121°C for 20 min.

Table 2: Formulations of *in situ* gels.

| Ingredient (g) | Concentration | | | | | | | | | | | |
|-----------------------------|---------------|------|------|------|------|------|------|------|------|------|------|------|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| Gatifloxacin | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| HPMC | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| HPMC K 15M | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 | 0.3 | 0.3 | 0.3 | 0.4 | 0.4 | 0.4 | 0.4 |
| Carbopol 934 | 0.1 | 0.2 | 0.3 | 0.4 | 0.1 | 0.2 | 0.3 | 0.4 | 0.1 | 0.2 | 0.3 | 0.4 |
| Benzalkonium chloride | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Disodium hydrogen phosphate | 1.12 | 1.12 | 1.12 | 1.12 | 1.12 | 1.12 | 1.12 | 1.12 | 1.12 | 1.12 | 1.12 | 1.12 |
| Citric acid | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |

All above concentrations in grams.

EVALUATION OF pH – TRIGGERED IN-SITU GELLING SYSTEM:

1) Determination of visual appearance and clarity:

The appearance and clarity were determined visually against a white and black back ground for presence of any particulate matter.

2) pH:

pH is one of the most important parameter involved in the ophthalmic formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulation should have pH range in between 5 to 7.4. The developed formulations were evaluated for pH using control dynamics digital pH meter.

3) % Drug Content:

Uniform distribution of active ingredient is important to achieve dose uniformity. The drug content was determined by diluting 1 ml of formulation to 100 ml with simulated tear fluid pH 7.4. Aliquot of 5 ml was withdrawn and further diluted to 25 ml with STF. Gatifloxacin concentration was then determined at 285 nm by using UV-VIS spectrophotometer.

4) Rheological studies:

Viscosity of instilled formulation is an important factor in determining residence time of drug in eye. The viscosity determination of prepared formulations was carried out using Brookfield viscometer LVDV-E with spindle 64. Viscosity of sample was measured at different angular velocities between 20-200 rpm.

5) Gelling capacity:

All prepared formulations were evaluated for gelling capacity and viscosity in order to identify the compositions suitable for use as *in situ* gelling systems. The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 37° C and visually assessing the gel

formation and noting the time for gelation and time taken for the gel formed to dissolve.

The flow behavior with “+” sign indicates that the vehicle is in the liquid form which show gels slowly and dissolves rapidly.

The flow behavior with “++” sign indicates that the vehicle is in the liquid-gel like form and flows less readily which shows gelation immediate and remains for few hours.

The flow behavior with “+++” sign indicates that the sample is in the gel form and is very difficult to flow which also shows immediate gelation and gel remains for extended period of time.

6) *In vitro* drug release studies:

The *in-vitro* release of gatifloxacin from the formulation prepared was studied through cellophane synthetic membrane using diffusion cell. The dissolution medium used was artificial tear fluid freshly prepared (pH 7.4) The cellophane membrane previously soaked overnight in the dissolution medium was tied with the help of thread to one end of a specifically designed glass cylinder (opens at both end). A 1 ml volume of formulation was accurately pipette into this assembly. The cylinder was attached to a metallic drive shaft and suspended in 50 ml of dissolution medium maintained at 37± 1° C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Aliquots each of 1 ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with the receptor medium if needed and analyzed by a UV-Visible spectrophotometer at 285 nm using receptor medium (STF) as a blank.

7) Sterility test:

The sterility test was performed according to Indian Pharmacopoeia. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically transferred to fluid thioglycolate medium (20 ml), and soyabean- casein digest medium (20 ml) separately. The liquid was mixed with the media. The inoculated media were incubated for not less than 14 days at 30° C to 35° C in the case of fluid thioglycolate medium and 20° C to 25° C in the case of soyabean- casein digest medium.

3. RESULTS AND DISCUSSION

Drug Analysis

a) Physical appearance

Physical appearance of Gatifloxacin was examined by various organoleptic properties colour pale yellow, Crystalline powder, odourless.

b) Determination of melting point

Capillary fusion method was used to determine the melting point of Gatifloxacin. The melting point was recorded and compared to literature value. Experimental value 182°-188°C, Literature value 180°-188°C

c) Standard calibration curve of gatifloxacin using simulated tear fluid (STF):

STF having the composition of sodium chloride 0.67gm, sodium bicarbonate 0.20gm, calcium chloride 0.008gm in 100ml distilled water was prepared. Accurately weighed 100mg gatifloxacin was dissolved in minimum amount of 0.2M NaOH solution and volume was made up with 100 ml STF to get the stock solution of 1000µg/ml. the absorbance of these solution was measured at 285 nm by UV-Vis spectrophotometer. Absorbance shown in Figure 1

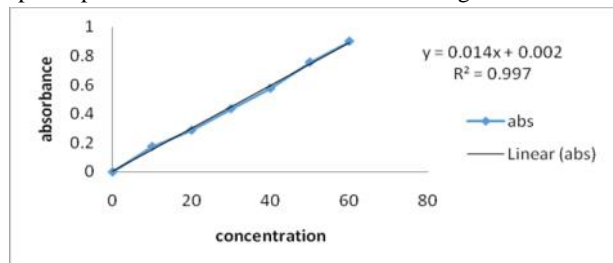


Fig 1: Calibration curve of gatifloxacin in simulated tear fluid

DRUG POLYMER INTERACTION STUDIES

d) Fourier transform infrared analysis:

The main application of FTIR spectrophotometry is the determination of identity of a compound by means of spectral comparison with that of an authentic sample and verification of the presence of functional groups in an unknown molecule. The sample was mounted in FTIR compartment and taken scan at wave length 4000 cm⁻¹ to 400 cm⁻¹. For analysis, IR spectra of the pure drug have been performed & no major differences were observed in the absorption peak pattern.

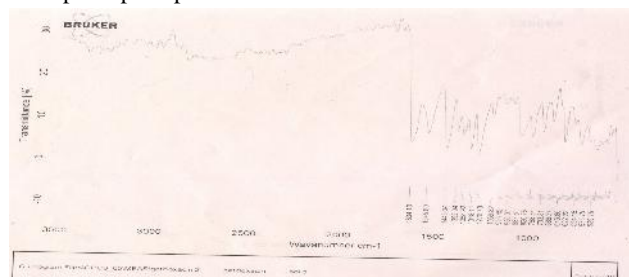


Fig 2: FTIR spectra of Gatifloxacin

Table 3 : Interpretation of IR spectra of Gatifloxacin

| Sr. No. | IR Spectrum | Groups | Peaks |
|---------|--------------|--------|-----------|
| 1. | Gatifloxacin | O-H | 3000-2975 |
| | | C-H | 1600-1635 |
| | | C=O | 1400-1459 |
| | | C-C | 1315-1393 |

Visual Appearance And Clarity:

Clarity of all the formulations were found to be satisfactory. Terminal sterilization by autoclaving had no effect on the clarity and other physiochemical properties of the formulations. The haziness that was observed after autoclaving was found to be disappeared and original clarity was gained after overnight standing.

pH:

The pH of all the formulations was found to be satisfactory and was in the range of 6.5 - 7.1. the formulations were liquid and at the pH formulated. Terminal sterilization by autoclaving had no effect on pH.

% Drug Content:

Table-4 below shows the percent drug for formulations F1, F2, F3. The drug content was found to be in acceptable range for all the formulations.

Table 4: % Drug content of formulations

| Formulation | % Drug content |
|-------------|----------------|
| F1 | 95.81% |
| F2 | 97.20% |
| F3 | 99.76% |

Rheological studies:

Table 5 shows the viscosity values obtained for formulations F1, F2, F3 using Brookfield viscometer LVDV-E with spindle no. 64 at different angular viscosity. The results obtained from the rheological study revealed that the viscosity decreases with as the angular viscosity increases. Generally a viscosity value in the range of 15-50 cps significantly improves the contact time in the eye. The rheological profile of prepared in situ gelling system of Gatifloxacin is shown in Figure 3.

Table 5: Rheological Profile of In-situ Gelling Systems

| S No. | Angular velocity (rpm) | F1 | F2 | F3 |
|-------|------------------------|-----|-----|-----|
| 1 | 20 | 110 | 240 | 270 |
| 2 | 50 | 70 | 170 | 195 |
| 3 | 80 | 52 | 161 | 172 |
| 4 | 100 | 31 | 143 | 152 |
| 5 | 150 | 24 | 111 | 129 |
| 6 | 200 | 15 | 49 | 100 |

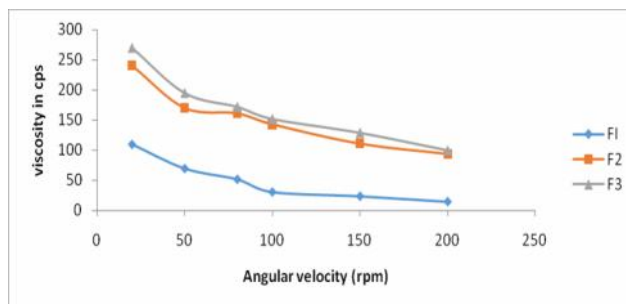


Fig 3: Plot showing rheological profile of in situ gelling systems

Gelling capacity:

All the formulations gelled instantaneously on addition to the simulated tear fluid and extended for few hours. The *in-situ* formed gel should preserve its integrity without dissolving or eroding for prolonged period to facilitate sustained release of drugs locally.

In-vitro drug release studies:

The three *in-situ* gelling formulations of Gatifloxacin F1,F2,F3 were subjected to in vitro release studies for 8 hours. These in-vitro release studies were carried out using simulated tear fluid (STF) of pH 7.4 as the dissolution medium. *In-vitro* drug release profile shown in Table 6

Table 6: In-vitro drug release profile of Gatifloxacin

| Time (in hrs) | % cumulative drug release (F1) | % cumulative drug release (F2) | % cumulative drug release (F3) |
|---------------|---------------------------------|---------------------------------|---------------------------------|
| 1 | 6 | 7 | 10 |
| 2 | 19.72 | 23.74 | 25.60 |
| 3 | 28.31 | 34.81 | 38.30 |
| 4 | 49.86 | 50.69 | 55.06 |
| 5 | 64.84 | 69.88 | 71.33 |
| 6 | 73.90 | 79.23 | 87.10 |
| 7 | 82.91 | 88.34 | 95.57 |
| 8 | 86.67 | 93.01 | 98.97 |

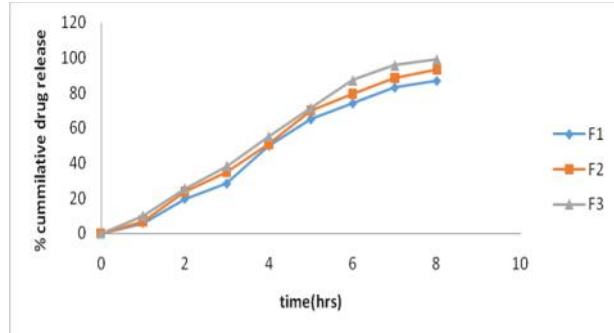


Fig 4: Plot showing In-vitro drug release profile of Gatifloxacin

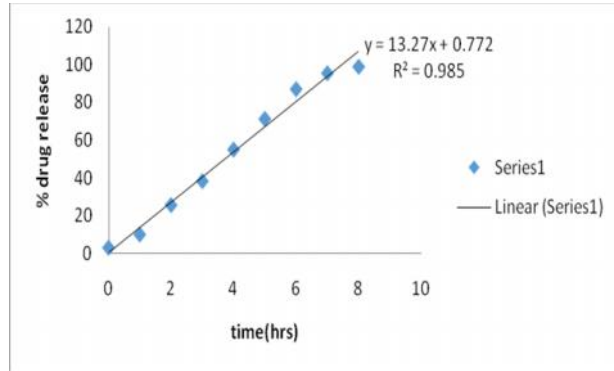


Fig 5: Plot showing zero- order release kinetics of formulation F-3

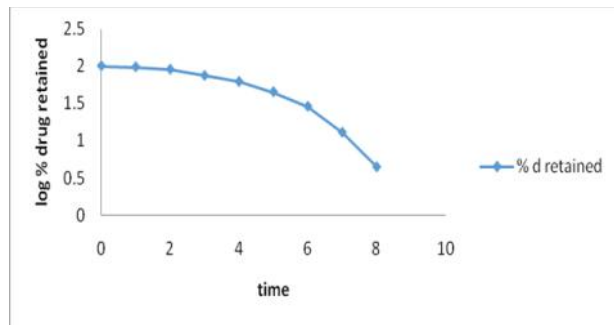


Fig 6: Plot showing first - order release kinetics of formulation F-3

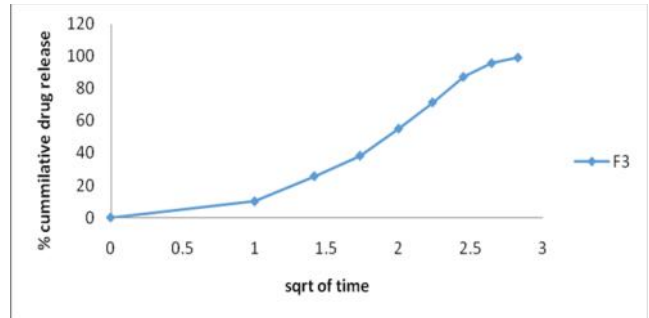


Fig 7: Plot showing Higuchi model of formulation F-3

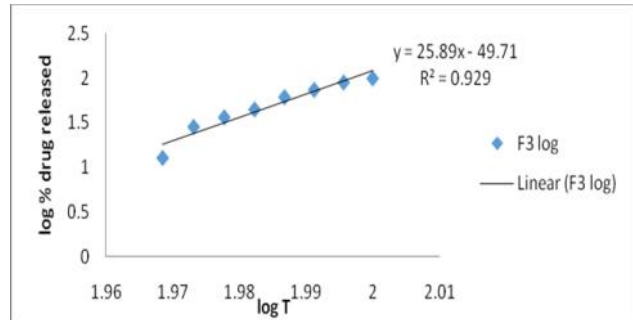


Fig 8: Plot showing korsmeyer- peppas model of formulation F-3

Sterility test:

In sterility studies, there was no appearance of turbidity or any odd growth of microorganism. Hence no evidence of microbial growth in formulation.

Accelerated Stability Studies:

For the stability studies different tests were done. In Table 7 the results of accelerated stability studies are shown. The appearance of the gels remained clear and no significant variation in pH was observed after subjecting the formulations to stability stress for 2 months. Also there was no significant change in drug content was observed after 2 months period.

Table 7: Data showing stability studies of Formulation at 40 ± 2°C & 75 ± 5°C

| Time (days) | Physical Appearance | pH | %Drug content |
|-------------|---------------------|-----|---------------|
| 0 | Clear | 6.9 | 99.76 |
| 15 | Clear | 6.9 | 99.62 |
| 30 | Clear | 6.9 | 99.38 |
| 45 | Clear | 6.8 | 99.06 |
| 60 | Clear | 6.8 | 98.20 |

Table 8: Comparison of drug release data

| Time | % cumulative drug release | |
|------|---------------------------|---------------|
| | Before storage | After storage |
| 0 | 0 | 0 |
| 1 | 10 | 9 |
| 2 | 25.6 | 23.74 |
| 3 | 38.30 | 35.16 |
| 4 | 55.06 | 52.50 |
| 5 | 71.33 | 69.12 |
| 6 | 87.10 | 84.67 |
| 7 | 95.57 | 92.34 |
| 8 | 98.97 | 95.85 |

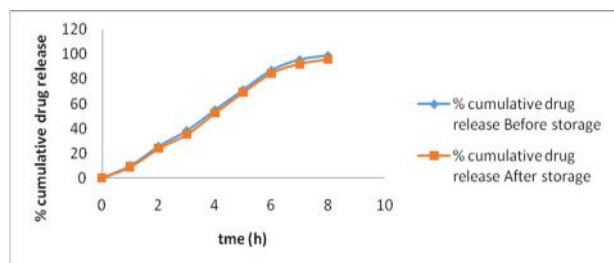


Fig 9 : Comparison of drug release before and after storage

4. CONCLUSION

The aim was to prepared in situ gel of gatifloxacin using pH triggered system to increase the retention time of gel into the eye and increase the effectiveness of the drug. In situ gel produced the prolonged drug release and decrease the nasolacrimal drainage. The mechanism of the gatifloxacin was inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, and recombination. The three formulation showed sustain release for 8 hours. HPMC, HPMCK15M, Carbopol 934 use as polymers to increase the viscosity of the formulation. Final it can be concluded that in-situ ophthalmic gel is alternative to conventional eye drops.

5. REFERENCES

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