

# Novel Long Retentive Posaconazole Ophthalmic Suspension

Jadhav Simta<sup>1,\*</sup>, Inamdar Kavita<sup>2</sup>, Bhitre Milind<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Chemistry, C. U. Shah College of Pharmacy, Mumbai, India

<sup>2</sup>R&D Department, Indoco Remedies Ltd, Mumbai, India

## Email address:

[simtjadhav@gmail.com](mailto:simtajadhav@gmail.com) (J. Simta)

\*Corresponding author

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**Abstract:** Ophthalmic formulations are not completely available for eliciting therapeutic action because of various reasons such as rapid tear drainage, blinking of eye, lower residence time and lower cul de sac volume. Ophthalmic formulation gets easily drained off due to eye blinking due to low viscosity. Posaconazole is known to have antifungal efficacy as oral route, however ophthalmic efficacy is not studied. The objective of present work was to develop long retentive posaconazole ophthalmic suspension based on polymer platform system of carbopol 974 P and xanthan gum. This synergistic polymer platform was pre-identified by means of experimental design study. Polymer based formulation helps to retain the drug at the site of action for longer duration and does not get washed away due to eye blinking phenomenon. Sterilization is key parameters for ophthalmic formulation. Different methods of sterilization were studied for posaconazole drug as well as polymer mixture and finished product. The developed formulations were characterized for homogeneity, pH, particle size, viscosity, osmolality, rheology study, mucoadhesive strength, contact angle, assay of posaconazole and benzalkonium chloride, degradation product, polymorphic form evaluation, eye irritation test and pharmacodynamic efficacy study. A stable long retentive posaconazole ophthalmic formulation was developed based on principles of quality by design. The developed formulation can be easily manufactured at bigger scale without need of any sophisticated equipment.

**Keywords:** Ocular Long Retentive, Posaconazole, Ophthalmic Suspension

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## 1. Introduction

Ophthalmic formulations have very less bioavailability as they may not be completely eliciting therapeutic action because of reasons such as rapid tear drainage, blinking of eye, lower residence time of ophthalmic formulation in eye and lower cul-de sac volume [1, 2]. Various attempts have been tried to improve the bioavailability by formulating different dosage forms however all these require specialized manufacturing equipment. Simplest way of increasing bioavailability is by formulating mucoadhesive formulation with help of polymers [3].

Formulation adhesiveness/retention in the eye is the function of viscosity being directly proportional; it plays a major role to extend the drug release by increasing the contact time in eye with help of muco-adhesive forces or by

polymer inter-penetrated network (IPN) [4]. Polymers were chosen based on viscosity. Significant synergies were considered for those combinations which have higher viscosities compared to their individual viscosities at lower concentrations. These systems would form more viscous solution which remains in the eye for a longer period of time and thus enhances the extended release.

The identified polymer system can be incorporated in different drugs to have better mucoadhesive properties. Synergistic polymer ratio is already been identified for polymer system with experimental design previously published in "Identification of Polymer Synergy with Help of DOE" in International Journal of Emerging Technologies in Engineering Research (IJETER) Volume 6, Issue 1, January (2018) [5]. The concentration of polymer system can be varied according to product requirement and drug property.

## 2. Materials and Methods

### 2.1. Materials

Posaconazole was sourced from MSN laboratories ltd. Monobasic sodium phosphate dihydrate was sourced from avantor performance materials, propylene glycol sourced from dow chemicals, polysorbate 80 from croda, dibasic sodium phosphate dihydrate, sodium hydroxide, hydrochloric acid were procured from Merck. Carbopol 974 P was sourced from Lubrizol. Xanthan gum (xantural 75) was procured from CP Kelco. Benzalkonium chloride was procured from novonordisk pharmatech.

### 2.2. Methods

Various trials were taken to finalized the formulation composition and final formulation composition is mentioned in table 1.

*Table 1. Formulation composition of posaconazole.*

S. no.	Ingredients	%w/v
1	Posaconazole	4.0
2	Benzalkonium chloride	0.025
3	Carbopol 974 P	0.125
4	Xanthan gum	0.750
5	Polysorbate 80	0.10
6	Monobasic sodium phosphate dihydrate	0.10
7	Dibasic sodium phosphate dihydrate	0.05
8	Propylene glycol	1.60
9	Sodium hydroxide	q. s.
10	Water for injection	q. s.

q. s. quantity sufficient.

In 25% of total batch quantity of water for injection carbopol 974 P was added slowly under stirring to form a homogenous dispersion. This dispersion was autoclaved at 121 degree centigrade for 15 minutes. In 30% of total batch quantity of water for injection ethylene oxide sterilized xanthan gum was added slowly under stirring to form a homogenous dispersion. Xanthan gum solution was added to carbopol 974PNF solution under stirring.

In 10% of total batch quantity of water for injection monobasic sodium phosphate & dibasic sodium phosphate were added. In 5% of total batch quantity of water for injection benzalkonium chloride and Polysorbate 80 was dissolved and added to buffer phase. Beaker was rinsed with water. Propylene glycol was added to buffer phase. Buffer phase solution was filtered using 0.22 micron polyethersulfone filter. Posaconazole (previously dry heat sterilized) was added slowly under stirring to form a homogenous dispersion. This dispersion was homogenized using overhead homogenizer for 15 minutes. Drug phase was added to polymer phase and mixed for 30 minutes. Finished product pH was adjusted to 6 to 7 which is more preferable for ophthalmic formulation with 0.22 micron polyethersulfone filtered 1 N sodium hydroxide solution

[6]. Final volume was made up using water for injection and suspension was stirred for 30 minutes.

To understand the impact of pH on degradation product extreme pH ranges samples were also manufactured and stability studies were conducted. To understand the impact of sterilization of container closure system optimum pH formulation was packed in gamma and ethylene oxide sterilized bottles.

Finished product formulation of pH (6.0, 6.5 and 7.0) was filled in low density polyethylene bottles with high density cap closure. Samples were kept at stability studies as per internal conference of harmonization (ICH) guideline for semipermeable container at  $40 \pm 2^\circ\text{C}/25 \pm 5\% \text{RH}$  and  $25 \pm 2^\circ\text{C}/40 \pm 5\%$  for 6 month.

## 3. Sterilization Method for Posaconazole

Sterility is a key issue in manufacture and use of ophthalmic products. Microbial content or bioburden of the raw materials, in-process intermediates, and drug substance or active product ingredient are potential sources of contamination and require incoming testing of ingredients. Major source of microbial load could be due to polymers & drug, being high in concentration in the formulation [7]. The most used methods of achieving a sterile product are moist heat sterilization, dry heat sterilization, gas sterilization, sterilization by ionizing radiation, sterilization by filtration, and aseptic processing [8].

Posaconazole was sterilized was steam, dry heat sterilization, gamma, ethylene oxide and steam sterilization.

## 4. Characterization of Posaconazole Ophthalmic Suspension

### 4.1. Differential Scanning Calorimetry Studies

Thermogram of the posaconazole powder and polymer mixture formulation were obtained from (TA instruments [differential scanning calorimetry] universal V4. 5A. DSC curves of pure samples were compared to that obtained from 4: 0.125: 0.375 mixture of the posaconazole: carbopol 974P: xanthan gum. Posaconazole and physical mixture along with polymer powder were sealed in an aluminum crucible and heated at the rate of  $10^\circ\text{C}/\text{minutes}$  up to  $400^\circ\text{C}$ . The exact peak temperature and melting point and heat of fusion were automatically calculated. It was assumed that the thermal properties (melting point, change in enthalpy, etc.) of blends were the sum of the individual components if the components are compatible with each other [9]. An absence, a significant shift in the melting of the components or appearance of a new exo/endothemic peak and/or variation in the corresponding enthalpies of reaction in the physical mixture indicates incompatibility. However, slight changes in peak shape height and width are expected due to possible differences in the mixture geometry.

#### 4.2. Physicochemical Characterization

Appearance of formulation was checked by visual observation under light for homogeneity. pH was checked using digital pH meter (Metler Toledo.) and viscosity was determined using Brookfield's viscometer (LVDV II<sup>+</sup> PRO model) in small volume adapter using S31 spindle at 5 rpm, Osmolality was measured on undiluted samples using an Osmometer- model 3250 of advanced Instruments, Inc. This instrument uses the principle of measuring osmolality precisely by measuring the difference in freezing point depression due to presence of solutes in the test product and in solvent alone.

#### 4.3. Polymorphic form Study

Posaconazole of Form-I was used for formulation development. To understand the impact of manufacturing process and stability studies on polymorphic form, XRD study was performed using Pan analytical instrument. Comparative evaluation of posaconazole API, Finished product sample at initial stage & stability sample of 25°C/40% RH study of 12 month time period were analyzed.

#### 4.4. Rheology Studies

To understand the formulated product structural bulk behavior under stress, rheology studies were evaluated using Anton Paar rheometer (Rheocompass model) with cone and plate geometry [10-12]. The samples of the formulations were carefully applied to the lower plate to minimize sample shearing and were allowed to equilibrate for 3 minutes prior to analysis. To simulate the formulation behavior with eye blinking rate viscosity with application of shear rate was done. Storage modulus  $G'$  which represents the cohesive property, longer or extended retentive formulation and Loss modulus  $G''$  which represents adhesive property with substrate in this case eye was studied. Amplitude sweep was studied to understand the deformation behavior of samples in the non-destructive deformation range and to determine the upper limit of this range in term of yield stress. Yield stress is measure of eye residence time. After the yield stress point with increasing deformation, the inner structure gets softer and starts to flow or breaks down in a brittle way. Viscoelastic region of the product was identified.

Frequency sweep was studied to understand the time-dependent product structural behavior of a sample in the non-destructive deformation range. The oscillation frequency was increased from 0 to 100 radian/sec while amplitude was kept constant.

#### 4.5. Mucoadhesive Strength

The mucoadhesive force between the sample probe and the formulation was assessed in a detachment test using a TA-XT plus texture analyzer (Stable Micro Systems, Surrey, UK). Ophthalmic suspension was kept into sample holder and the analytical probe was lowered to begin the test. The probe moved at a constant speed ( $0.1 \text{ mm}\cdot\text{s}^{-1}$ ) on the surface of the

formulation. The probe and the formulation were kept in contact for 60 seconds, and 5 g force was applied during this interval. After 60 seconds, the probe was drawn upward ( $0.1 \text{ mm}\cdot\text{s}^{-1}$ ) until the contact between the surfaces was broken. For comparison purpose the posaconazole ophthalmic formulation devoid of polymer system (immediate release formulation) was used. The Texture Exponent 32 software (Stable Micro Systems, Surrey, UK) was used to determine the force required for the detachment ( $F_{\text{adh}}$ ) and the work of adhesion ( $W_{\text{adh}}$ ) (the area under the force/distance curve). Triplicates reading were taken to understand the variability.

#### 4.6. Contact Angle

Contact angle is measurement of spreading and wetting ability of the formulation [13]. Formulation is non-wetting and non-spreading if the contact angle is greater than  $90^\circ$ , and formulation will be clinically ineffective in that case. For comparison purpose posaconazole ophthalmic suspension along with only placebo formulation comprising of polymer platform was evaluated using goniometer.

#### 4.7. Zeta Potential

Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion/attraction between particles and is one of the fundamental parameters known to affect stability [14]. Its measurement brings detailed insight into the causes of dispersion, aggregation or flocculation. Zeta potential was evaluated using Zetasizer Ver. 7.12.

#### 4.8. Posaconazole Assay and Degradation Product

Weigh accurately 1 g of Sample (equivalent to 40 mg of Posaconazole), into a 50 mL volumetric flask, add 30 mL of diluent (water: acetonitrile) in 40: 60 was added. Flask was sonicated to dissolve. Sample was cooled and then diluted with diluent to volume of 50 mL and mixed. Filter through a glass microfibre filter or  $0.45 \mu\text{m}$  PVDF filter, discard first 3.5 mL filtrate then transfer into HPLC vial.

Mobile phase, stationary phase and chromatographic conditions were selected based on drug product profile and available literature information. Further stability indicating HPLC method was developed.

*Chromatographic conditions:*

Mode: HPLC

Column: Hypersil ODS C18, 100 mm $\times$ 4.6 mm, 5 $\mu\text{m}$  (Part No: M05CSB15)

Detector: UV 260 nm

Column Temperature: 30°C

Sampler Temperature: 25°C

Injection size: 10  $\mu\text{L}$

Flow Rate: 1.0 mL/minute

Run time: 15 minutes.

Retention time: About 4.0 minutes

For degradation product same chromatographic conditions as of assay were used however further composition of mobile phase and run time extended to ensure adequate separation of peak of interest.

#### 4.9. Benzalkonium Chloride (BKC) Assay and Antimicrobial Efficacy Study

Standard was prepared by weighing about 80.87 mg of BKC in to 100 ml volumetric flask. 70 ml purified water was added and mixed, sonicated to dissolve. Volume make up was done with purified water. Pipette out 1.6 ml of this solution and transfer to 100 ml volumetric flask. Dilute it with diluent (water: acetonitrile) in 10: 90 ratio. Solution was filtered through 0.45 micron nylon syringe filter. Initially 2 to 3 mL filtrate was discarded before keeping in HPLC vials. Sample was prepared as of similar standard concentration. Mobile phase, stationary phase and chromatographic conditions were selected based on available literature information. Further stability indicating HPLC method was developed with UV detector.

Antimicrobial preservative testing at lower concentration of preservative i.e. 90% of label claim of benzalkonium chloride was tested to account for worst case study.

## 5. Animal Study Design

### 5.1. Ocular Irritation Studies

Ocular irritation study was performed as per protocol number MET. IOP. IAEC. 2017-18. PR-08 at MET institute of Nashik. New Zealand white rabbits (three), each weighing about 2 to 3 kg were used for study. A dose of one drop of the test formulation was instilled in to right eye of each rabbit. The left eye served as control. The eyes of the rabbits were carefully examined, observed at 1 hour, 24 hour, 48 hour and 72 hour post application and the observations extended to determine the reversibility or irreversibility till the end of the observation period of 7 days. Score methodology was used for evaluation of cornea opacity, iris, conjunctivae redness, chemosis for eye lids and/or nictating membranes [15].

### 5.2. Pharmacodynamic Studies (In-vivo Antifungal Efficacy Studies)

Antifungal efficacy study was performed in as per protocol number MVC/IAEC/ 10 /2019 at Bombay veterinary college, Mumbai. Wistar rat (six) of both genders, each weighing

about 150-250 g was used for study. The animals were housed in individual cages, and the experiments were conducted in a sanitized room at a temperature maintained around 25°C. Immunosuppression in all test groups animals were induced by cyclophosphamide marketed preparation. The optimized dose of the drug used was 8 mg/kg bodyweight for 15 consequent days through oral route. The suppressed animals showed the signs of decrease body weight dullness and other motor responses. Fungal infection was induced by inoculating live culture of candida albicans species of  $10^{-8}$  cfu/ml concentration. The initial marginal injury was done on eye lid membrane to hasten the infection. Further inflammation and all markers like mucous membranes, opacity of lens etc. were taken in to consideration before instillation of posaconazole ophthalmic formulation. Another group of fungi induced infected animals (six) was kept as positive control. A dose of two drops of the test formulation was instilled in to eyes of each rat twice a day. The eyes of the rats were carefully examined, observed everyday post application and the observations extended till complete recovery of fungal infection had happened. Score methodology was used for evaluation of chemosis, eyelid membranes (hyperaemia), corneal membrane opacity, corneal reflex, blindness or vision impairment. A score of 0 to 5 was used for all physiological observations except for corneal reflex scale of 5-0 were used, which indicates 5 scale is normal reflex action. At the end of study corneal tear fluid sample was collected for fungal count.

## 6. Results and Discussion

### 6.1. Sterilization Method for Posaconazole

For sterilization of posaconazole degradation product was found to be higher in case of gamma sterilization. Impurities were found to be similar for steam, dry heat & ethylene oxide sterilization. Steam sterilization resulted in hard cake formation due to high solid content. Hence dry heat sterilization was selected for further formulation development. Comparative impurities profile for various sterilization techniques is summarized in table 2.

Table 2. Degradation data of different sterilization technique of Posaconazole.

S. no	Impurity	Limits (%)	Initial before sterilization	ETO sterilization	DHS 160°C/2 hrs	Gamma sterilization (2.5 KG)	Steam sterilization (121 degree for 15 minutes)
1	Tosylated compound	NMT 0.12	0.12	0.12	0.12	0.12	0.07
2	Hydroxyl Triazole	NMT 0.12	ND	ND	ND	ND	ND
3	Deshydroxy posaconazole	NMT 0.12	0.05	0.05	0.05	0.05	0.03
4	Bezylated posaconazole	NMT 0.12	0.01	0.01	0.01	0.01	0.01
5	Single max. unspecified impurity	NMT 0.10 of each	0.03	0.03	0.03	0.19	0.02
6	Total impurities	1.0	0.32	0.35	0.33	0.67	0.2

NMT: Not more than ND: Not detected.

For sterilization of posaconazole degradation product was found to be higher in case of gamma sterilization. Impurities

were found to be similar for steam, dry heat & ethylene oxide sterilization. Steam sterilization resulted in hard cake

formation due to high solid content. Hence dry heat sterilization was selected for further formulation development.

## 6.2. Differential Scanning Calorimetry Studies

Posaconazole was found to be compatible with identified polymer system. Figure 1 and Figure 2 shows DSC scan of posaconazole and DSC scan of posaconazole and polymer mixture.

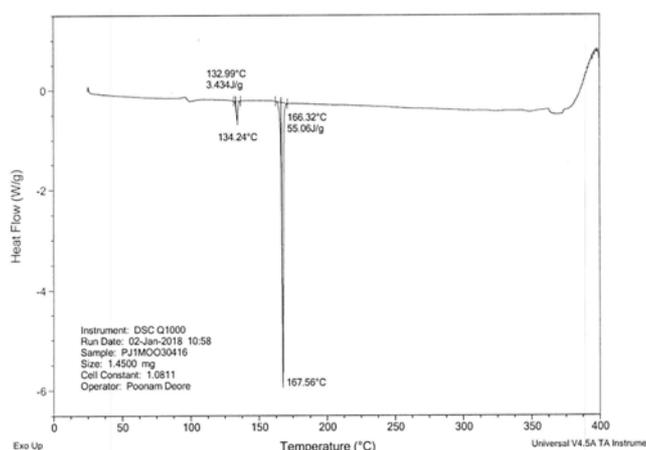


Figure 1. DSC scan of posaconazole.

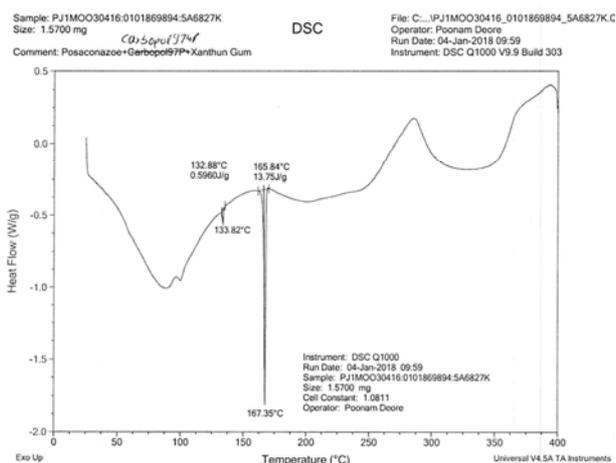


Figure 2. DSC scan of posaconazole & polymer mixture.

## 6.3. Physicochemical Characterization

White to off white homogenous suspension was formed. pH range of 6 to 7 was also studied in stability & there was no pH drop observed in stability. Also there was no significant drop in viscosity & osmolality observed in stability. There was no impact of container closure sterilization ethylene oxide (ETO) and gamma on physical parameters such as pH, viscosity and osmolality was observed. Figure 3 shows viscosity data of pH range and container closure sterilization.

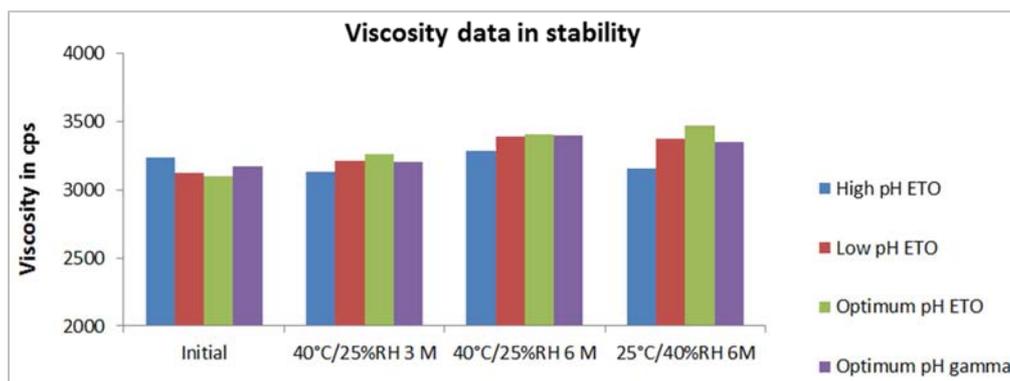


Figure 3. Viscosity data of pH range & container closure sterilization.

Particle size data of pH range formulation and formulation packed in gamma and ETO sterilized container closure was below 10 micron. Table 3 shows particle size data in stability.

Table 3. Particle size (d 90) data in stability.

Particle size data (d 90) in micron				
Condition	High pH ETO	Low pH ETO	Optimum pH ETO	Optimum pH gamma
Initial	6.82	5.02	6.8	6.8
40°C/25%RH 1 M	6.52	4.82	6.62	6.58
40°C/25%RH 3 M	5.89	5.68	6.64	6.55
40°C/25%RH 6 M	6.29	5.78	6.29	6.68
25°C/40%RH 6 M	6.51	5.8	6.39	6.94

## 6.4. Polymorphic Evaluation Study

There was no impact of manufacturing process and stability of the formulation was observed on polymorphic form. From the

XRD spectra it was observed that two theta values of all XRD spectra are identical and it is comparable with form-I of Posaconazole. Figure 4 shows the XRD overlay of posaconazole API, Finished product sample at initial stage & stability sample

of 25°C/40% RH -12 month time period.

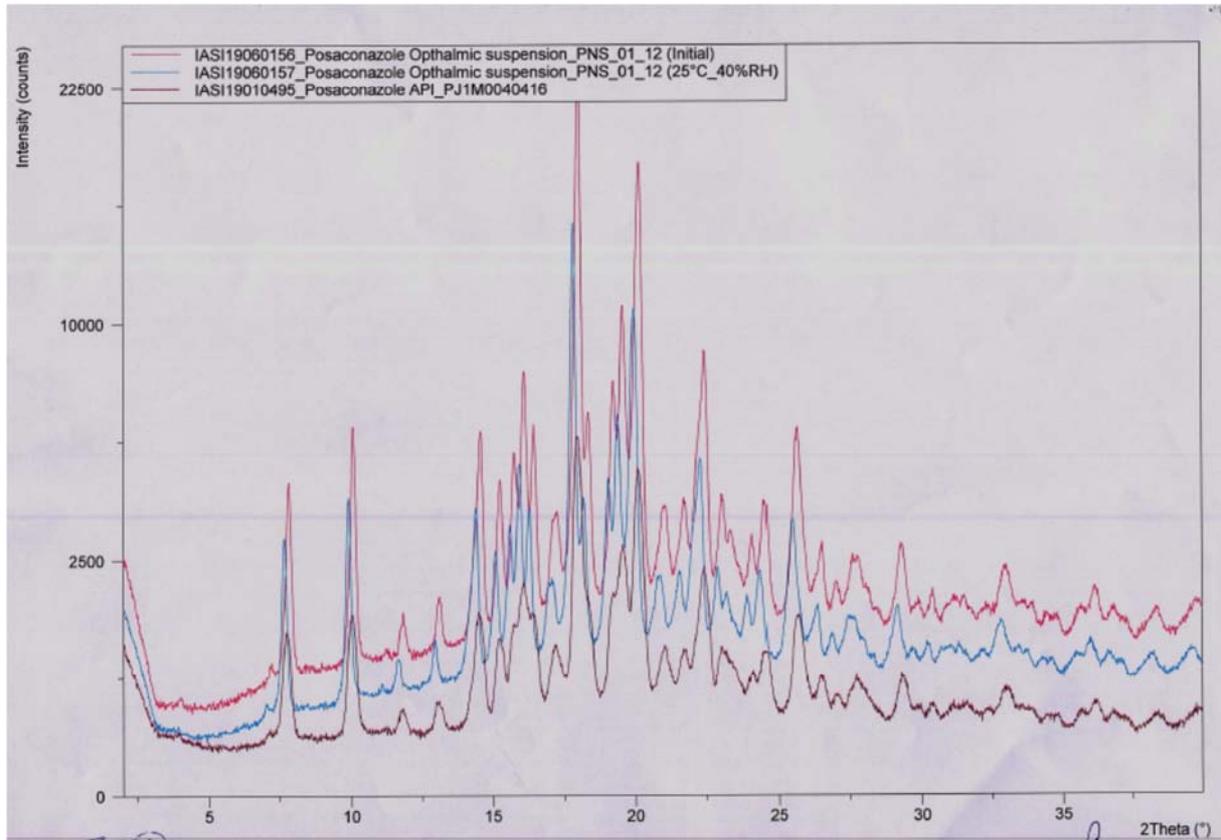


Figure 4. XRD overlay of Posaconazole API, Finished product initial & stability sample.

### 6.5. Rheology Study

1. Viscosity of formulation decreases with application of shear rate of  $1000 \text{ sec}^{-1}$  which indicates formulation showed pseudoplastic behavior.
2. Storage modulus  $G'$  represents the stored deformation energy, higher extended release, elastic portion or solid state of viscoelastic behavior and loss modulus  $G''$  characterizes the deformation energy lost (dissipated) through internal friction when flowing. Viscoelastic solids with  $G' > G''$  have a higher storage modulus than loss modulus. This is due to links inside the material, for example chemical bonds or physical-chemical interactions. Storage modulus  $G'$  which represents the elastic or cohesive property was found to be about 14.701 for formulation. Higher  $G'$  modulus gives longer retention or extended release as well as good flow.
3.  $G''$  loss modulus which represents the adhesive property with any other substrate in this case it would be eye cornea.  $G''$  was 6.66 for the formulation. An amplitude sweep test was performed to define the fluid linear viscoelastic region (LVER), the results showed that this region was at 100% shear strain for the formulation which indicates formulation has good structural behavior. Angular frequency of 0 to 100 rad/sec (radian/sec measurement of rotational speed) was applied to understand the product structural behavior.

$\tan \delta$  was less than 1 across frequency of 100 rad/sec which shows gel kind nature. Yield stress value studied over amplitude sweep which is measure of residence time of the formulation with other substrate in this it would be eye cornea was observed to 1.702 Pa for the formulation. Figure 5 shows frequency sweep study data. Figure 6 is Shear strain vs. storage modulus and loss modulus. Figure 7 is viscosity vs. shear rate graph.

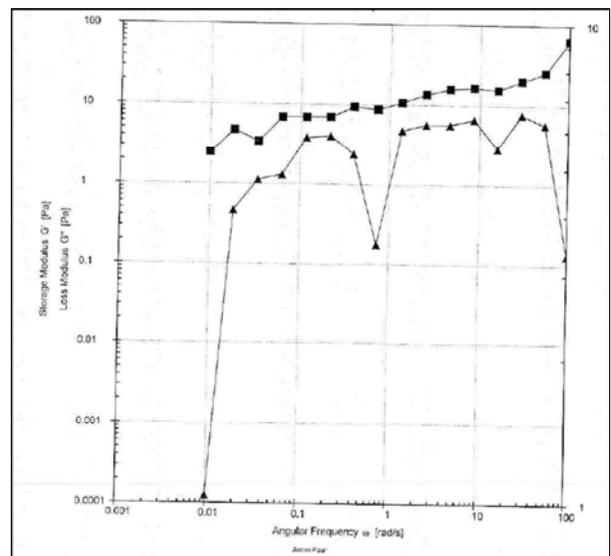


Figure 5. Frequency sweep study data.

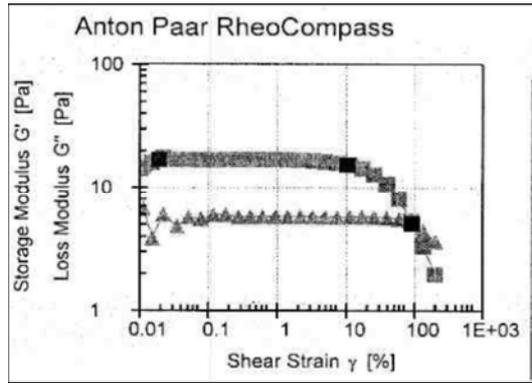


Figure 6. Shear strain vs. storage modulus and loss modulus.

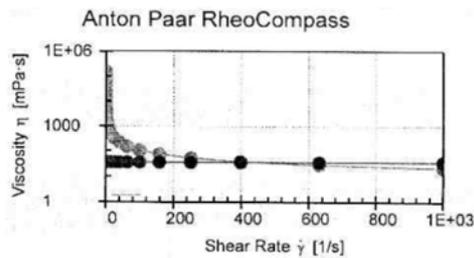


Figure 7. Viscosity vs. shear rate graph.

### 6.6. Mucoadhesive Strength

Mucoadhesive force i.e. force of adhesiveness ( $F_{adh}$ ) and

work of adhesion ( $W_{adh}$ ) of posaconazole long retentive formulation was found to be higher than immediate release formulation devoid of any polymers. This concludes that polymer system increased the mucoadhesive strength of developed posaconazole ophthalmic suspension which would remain in eye for longer time. The  $F_{adh}$  value was 0.059 N and 0.011 N respectively for long retentive and immediate release formulation. The  $W_{adh}$  value was 0.396 N.sec and 0.240 N.sec respectively for long retentive and immediate release formulation. Figure 8 and figure 9 show mucoadhesive force for long retentive and immediate release formulation.

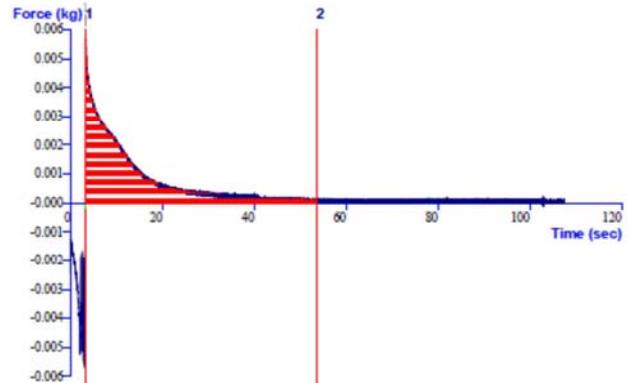


Figure 8. Mucoadhesive force for long retentive formulation.

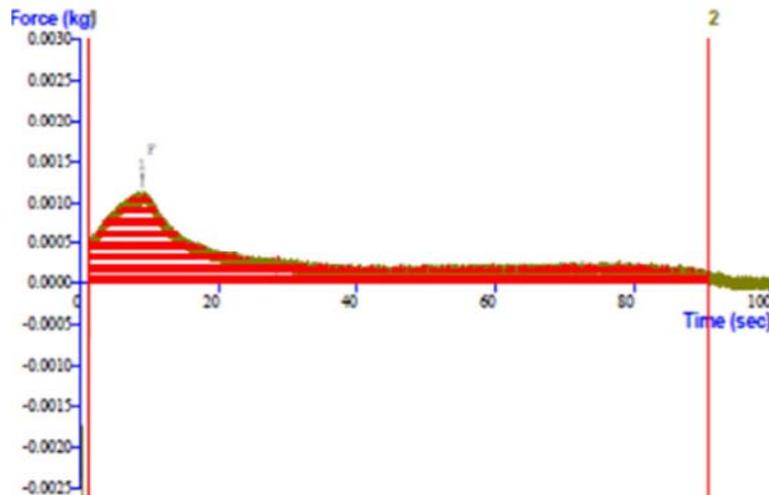


Figure 9. Mucoadhesive force for immediate release formulation.

### 6.7. Contact Angle

Contact angle of posaconazole ophthalmic suspension was found to be 45.74 whereas for only placebo polymer it was 42.68. Contact angle data proves that polymer platform as such also has good wetting and spreading properties & incorporation of hydrophobic drugs also doesn't alter much these properties.

### 6.8. Zeta Potential

Zeta potential of posaconazole ophthalmic suspension was  $-47.3$  mv shows the developed suspension is electrically

stabilized and has good stability behavior against coagulation/flocculation.

### 6.9. Posaconazole Assay and Degradation product

Across the pH range 6.0, 6.5 and 7.0 (low, optimum and high) the posaconazole content was well within specification limit of 90.0 to 110.0% which indicates formulation remains stable across pH range of 6.0 to 7.0. Gamma sterilization and ethylene oxide sterilization (ETO) of container closure did not show much difference on impact on assay. Figure 10 shows posaconazole assay in stability.

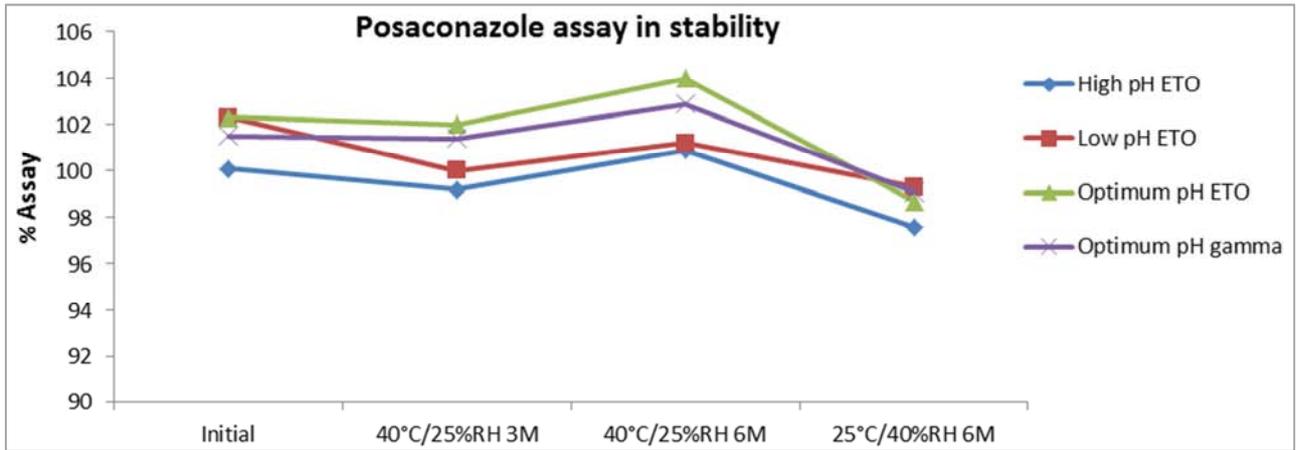


Figure 10. Posaconazole assay in stability.

There was no significant difference observed in degradation product due to change in container closure sterilization, gamma sterilization and ethylene oxide sterilization resulted in similar degradation products. Degradation products were found to be similar across the pH range.

Table 4. Degradation product in stability.

Impurity	Limit	High pH (7.0) ETO		Low pH (6.0) ETO		Optimum pH (6.5) ETO		Optimum pH (6.5) ETO	
		Initial	40°C/25% RH 6 M	Initial	40°C/25% RH 6 M	Initial	40°C/25% RH 6 M	Initial	40°C/25% RH 6 M
Single max unknown	NMT 0.2%	0.06	0.06	0.06	0.05	0.06	0.06	0.06	0.06
Total impurity	NMT 1%	0.24	0.21	0.24	0.20	0.32	0.23	0.33	0.23

6.10. Benzalkonium Chloride (BKC) Assay and Antimicrobial Efficacy Study

Across the pH range 6.0, 6.5 and 7.0 (low, optimum and high) the benzalkonium chloride content was well within specification limit of 90.0 to 110.0% which indicates formulation remains stable across pH range of 6.0 to 7.0. There was no impact of container closure sterilization on benzalkonium chloride assay was observed. Figure 11 shows benzalkonium chloride assay in stability.

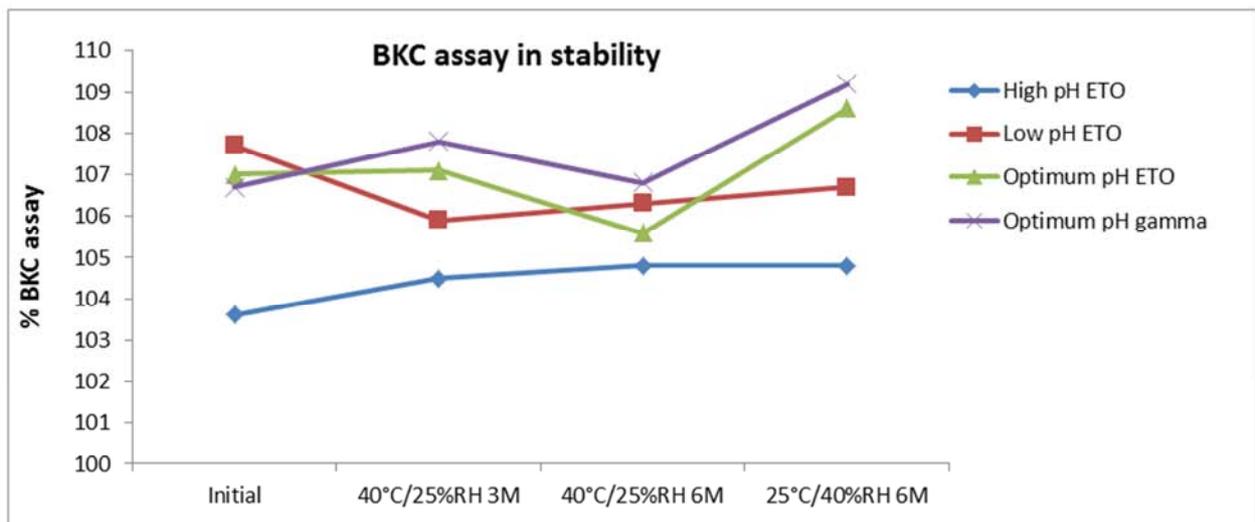


Figure 11. Benzalkonium chloride assay in stability.

The results of AET test 90% benzalkonium chloride concentration is summarized below: Table 5 shows summary results of Antimicrobial effectiveness test at lower concentration of benzalkonium chloride (90% of label claim).

Preservative efficacy data was well within the USP acceptance criteria for all the specified bacteria and yeasts and fungi. Thus benzalkonium chloride in the formulation acts effectively as a preservative.

**Table 5.** Summary results of Antimicrobial effectiveness test at lower concentration of benzalkonium chloride (90% of label claim).

Name of microbial culture Bacteria	Log reduction in viable count from initial calculated viable count at '0' hour		Log of viable count at 28 days	USP compliance
	After 7 days (Limit: NLT 1)	After 14 days (Limit: NLT 3)	Limit: No increase from 14 days	
Escherichia coli ATCC 8739	3.83	4.68	No increase	Complies
Pseudomonas aeruginosa ATCC 9027	3.21	5.21	No increase	Complies
Staphylococcus aureus ATCC 6538	2.98	6.56	No increase	Complies
Yeasts and Molds	Log of viable count at 7 days	Log of viable count at 14 days	Log of viable count at 28 days	USP
Limit	No increase form '0'hr	No increase form '0'hr	No increase form '0'hr	compliance
Candida albicans ATCC 10231	No increase	No increase	No increase	Complies
Aspergillus brasiliensis ATCC 16404	No increase	No increase	No increase	Complies

## 7. Animal Study Results

### 7.1. Ocular Irritation Studies

Score study data observation till 7 days showed no sign of irritation. Developed formulation is non-irritant to rabbit eyes.

### 7.2. In-vivo Antifungal Efficacy Studies

The test formulations were administered in to the infected eye twice a day of animals for 15 consecutive days. 80% animals showed recovery in one week time in test formulations and rest of the animals were treated for complete 15 days for healing the

remnants of infections. Improvements in the clinical parameters post instillation suggesting the propensity of the prepared systems to sustain drug release with a minimal loss due to drainage. Gross examination of the ocular tissues showed that the formulations caused no undue irritation and no leakage of the developed polymer based formulation was observed from any part of the eye.

Score data for positive control & test formulation is presented in table number 6. Stastical analysis for positive control and test formulation was done using t test for all physiological parameters and differences were found to be statistically significantly at  $p < 0.05$ . Fungal count performed after completion of study was nil, which concludes antifungal efficacy of developed formulation.

**Table 6.** Score study data.

Parameters	Chemosis	Eyelid membranes (hyperaemia)	Corneal membrane opacity	Corneal reflex	Blindness vison impaired/not impaired
Animal number					
1	5	5	5	2	VM
2	4	4	5	1	VM
3	5	5	4	2	VM
4	5	5	4	1	VM
5	2	5	5	2	VM
6	5	4	5	2	VM
Average	4.33	4.67	4.67	1.67	-
1	2	2	2	5	VM
2	1	3	2	4	NI
3	2	2	2	4	NI
4	1	1	3	4	NI
5	2	2	1	3	NI
6	2	1	1	4	VM
Average	1.67	1.83	1.83	4.00	-
p value	Less than 0.05				-

VM: vision impaired.

NI: Vision not impaired.

Figures 12 and 13 showed images of eye for untreated positive control and treated eye of test product after study completion.

**Figure 12.** Positive untreated eye.**Figure 13.** Treated eye after study completion.

## 8. Conclusion

A stable long retentive posaconazole ophthalmic suspension was developed using polymer system identified by principles of quality by design which will reduce the adverse effects associated with frequent dosing. Unlike other long acting ophthalmic formulation this formulation developed by simple manufacturing process without use of any sophisticated equipment. Developed formulation can be directly scale up for bigger scale. Being ophthalmic formulation sterilization method is key parameters and suitable sterilization method for formulation, drug and container closure system was identified to mitigate risks. The product is stable up to 6 months at accelerated conditions in low density polyethylene bottles. Thus, a proposed shelf life of 24 months can be assigned to the product without the need for any special storage conditions.

## Disclosures

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