WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 4, 1084-1101. Research Article ISSN 2277– 7105

MICONAZOLE LONG RETENTIVE OPHTHALMIC SUSPENSION DEVELOPED WITH SODIUM ALGINATE AND CARRAGEENAN POLYMER SYSTEM

Simta S. Jadhav[a,*], Kavita V. Inamdar[b] and Dr. Milind J. Bhitre[a]

[a]C.U. Shah College of Pharmacy, Department of Pharmaceutical Chemistry, Santacruz (West), Mumbai, Maharashtra, 400049, India.

[b]Indoco Remedies Ltd, R&D Department, Thane, Mumbai, Maharashtra, 400701, India.

Article Received on
26 January 2021,

Revised on 15 Feb. 2021,
Accepted on 07 March 2021

DOI: 10.20959/wjpr20214-19952

***Corresponding Author**
Simta S. Jadhav
C.U. Shah College of Pharmacy, Department of Pharmaceutical Chemistry, Santacruz (West), Mumbai, Maharashtra, 400049, India.

ABSTRACT

Due to poor bioavailability of ocular formulation due to various reasons such as rapid tear drainage, blinking of eye, lower residence time of ophthalmic formulation in eye and lower cul-de sac volume there is always need to improve the bioavailability. The objective of present work was to develop long retentive miconazole ophthalmic suspension using sodium alginate and carrageenan polymer system which was pre-identified by means of experimental design study. The developed formulations were characterized for homogeneity, pH, particle size, viscosity, osmolality, rheology study, mucoadhesive strength, contact angle, assay of miconazole and benzalkonium chloride, degradation product, in vitro drug release, eye irritation test and pharmacodynamic efficacy study. A stable long retentive miconazole ophthalmic formulation was developed based on principles of quality by design and as per industrial standards.

KEYWORDS: Polymer, ocular long retentive, miconazole, ophthalmic suspension, sodium alginate, carrageenan.

1. INTRODUCTION

Poor bioavailability of drugs from ophthalmic dosage forms is mainly due to tear production, transient residence time, non-productive absorption and impermeability to corneal epithelium.[1,2] Instilled ophthalmic formulations may not be completely available for eliciting therapeutic action because of various reasons such as rapid tear drainage, blinking of

eye, lower residence time of ophthalmic formulation in eye and lower cul-de sac volume.[3] There are different methodologies available to prolong the drug release in eye however these types of formulations require specialized manufacturing equipment and scalability has always remained a challenge. Hence prolonging the eye residence time with use of polymer becomes the most cost effective method.

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. Sodium alginate and carrageenan system was identified as best synergistic polymer system with various experimental studies.

The identified polymer system can be incorporated in different drugs to have better mucoadhesive properties. Syngerstic polymer ratio is already been identified for polymer system with experimental design previously published in "Identification of Polymer Synergy with Help of DOE" in European journal of pharmaceutical and medical research (ejpmr) 2018, 5(03), 343-348.[4] This polymer system can be incorporated with different drugs in identified synergistic ratio with different concentration to achieve the desired product quality attributes.

There is very less work done in area of antifungal ophthalmic segment, hence antifungal drug miconazole was selected for formulation development.[5-6]

2.0 MATERIALS AND METHODS

2.1 Materials

Miconazole was sourced from FDC limited. Monobasic sodium phosphate dihydrate, dibasic sodium phosphate dihydrate, sodium hydroxide, hydrochloric acid were procured from Merck. Propylene glycol was sourced from Dow chemicals. Sodium alginate and carrageenan were gifted by FMC bipolymer. Benzalkonium chloride was procured from novonordisk pharmatech.

2.2 Methods

Manufacturing composition detail is summarized in table 1.

Table 1: Formulation composition of miconazole.

S.no.	Ingredients	%w/v
1	Miconazole	1
2	Benzalkonium chloride	0.025
3	Sodium alginate	0.25
4	Carrageenan	0.75
5	Monobasic sodium phosphate dihydrate	0.1
6	Dibasic sodium phosphate dihydrate	0.05
7	Propylene glycol	1.92
8	Sodium hydroxide	q.s.
9	Water for injection	q.s.

q.s. quantity sufficient

In 50% of total batch quantity of water for injection sodium alginate followed by carrageenan was added slowly under stirring to form a homogenous dispersion. This dispersion was autoclaved at 121 degree centigrade for at least 15 minutes. In 10% of total batch quantity of water for injection monobasic sodium phosphate & dibasic sodium phosphate were added one after another. In 5% of total batch quantity of water for injection benzalkonium chloride 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. ETO sterilized Miconazole was added under stirring to sterile buffer solution phase. Suspension was stirred for 30 minutes to obtain a lump free solution and further it was homogenized using overhead homogenizer for 10 minutes.

Drug phase was added to polymer phase and mixed for 30 minutes. Finished product pH was adjusted to 6.5 with 0.22 micron polyethersulfone filtered 1 N sodium hydroxide solution. 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 low density polyethylene bottles with high density cap closure and stability studies were conducted.

3.0 Characterization of miconazole ophthalmic suspension

3.1 Differential scanning calorimetry studies

Thermogram of the miconazole 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 1:0.0.250:0.750 mixture of the miconazole :

sodium alginate : carrageenan. Miconazole and physical mixture along with polymer powder were sealed in an aluminum crucible and heated at the rate of 10°C/minutes up to 400°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. An absence, a significant shift in the melting of the components or appearance of a new exo/endothermic 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.

3.2 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^+ PRO model) in small volume adapter using S34 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.

3.3 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. 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.

3.4 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 $mm \cdot s^{-1}$) 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 mm·s−1) until the contact between the surfaces was broken. For comparison purpose the miconazole 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_{adh}) and the work of adhesion (W_{adh}) (the area under the force/distance curve). Triplicates reading were taken to understand the variability.

3.5 Contact angle

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

3.6 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. Its measurement brings detailed insight into the causes of dispersion, aggregation or flocculation. Zeta potential was evaluated using Zetasizer Ver. 7.12.

3.7 Particle size analysis

Particle size is important criteria for ophthalmic formulation. Generally acceptance criteria for ophthalmic suspension formulation to have particle size below 15 micron. Formulation particle size was analysed by motic microscopy.

3.8 Miconazole assay

Standard solution of Miconazole API was prepared by dissolving an accurately weighed amount of Miconazole 50 mg in 100 mL of solvent mixture of acetonitrile and methanol having 1:1 ratio to obtain a standard solution of Miconazole concentration 500µg/mL. The prepared solution was filtered through glass microfiber filter or 0.45 µm PVDF filter. Initial 3-4 mL filtrate was discarded and then transferred into HPLC vial. Finished product sample solution was prepared accurately weighing sample equivalent to 25 mg of Miconazole (2.5 g of Sample), in a 50 mL volumetric flask and 70 mL of solvent mixture of acetonitrile and methanol having 1:1 ratio, was added and sonicated for 2 minutes. The volume was made up with same solvent mixture to obtain Miconazole concentration of 500µg/mL.

The mobile phase was prepared by dissolving 6.0 g of Ammonium acetate in 1000 mL water. From this preparation, 350 ml solution was mixed with solvent mixture of acetonitrile and methanol having 1:1 ratio. Prior to use the mobile phase was filtered through 0.45 µm membrane filters and degassed by sonication for 10 min. Analysis was carried using HPLC with analytical column C4, 5 µm, 150 × 4.0 mm with a detection wavelength of 235 nm. The operating temperature of the column was set at 30°C. The injection volume was 10 µL and the flow rate was maintained at 1.2 mL/min. The run time was 20 minutes.

3.9 Benzalkonium chloride (BKC) assay

A standard solution of Benzalkonium chloride was prepared by dissolving an accurately weighed amount of Benzalkonium chloride (50 mg) in 100 mL of acetonitrile and further diluted to obtain standard solution of Benzalkonium chloride (25µg/mL), then transferred into HPLC vial. A sample solution was prepared by accurately weighing sample equivalent to 50 mg of Benzalkonium chloride (2 g of Sample), was transferred completely to 20 mL volumetric flask, and diluted with acetonitrile to obtain a solution of Benzalkonium chloride 25µg/ml.

The mobile phase was prepared by dissolving 6.0 g of Ammonium acetate in 1000mL water. From this preparation, 350 ml was mixed with solvent mixture of acetonitrile and methanol having ratio (1:1).

Buffer solution for mobile phase was prepared by dissolving 4.0 mL of orthophosphoric acid, 8.0 mL of Triethylamine in 2000mL of water. The buffer solution was filtered through 0.45 µm membrane filters. The mobile phase A was prepared by mixing buffer solution with

acetonitrile having ratio (1100:900). Mobile phase B was prepared by mixing buffer solution with acetonitrile having ratio (900:1100). Both the mobile phase A and mobile phase B were degassed by sonication for 10 minutes.

3.10 Degradation product

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

3.11 In-vitro drug release

Enhancer cell with 2 g sample size was used for dissolution. Sample was placed in enhancer cell. It was covered with 40 mesh filter (425 micron). Based on solubility trials data, simulated tear fluid with 0.5% sodium lauryl sulphate, 200 mL, 70 rpm was used as dissolution media. Assay method was adopted for drug release. Concentration of standard and samples were modified accordingly.

3.12 Antimicrobial efficacy studies

Antimicrobial preservative testing at lower concentration of preservative i.e. 90% of label claim is tested, as there is a drop in levels of benzalkonium chloride to around 90% of the label claim at the end of 3 months accelerated stability at 40ºC/25%RH, hence worst case study was performed.

3.13 Accelerated stability studies

Finished product formulation of pH (6.0, 6.5 and 7.0) was filled in low density polyethylene bottles with high density cap closure. Optimum pH formulation was packed in gamma and ethylene oxide sterilized low density polyethylene bottles with high density cap closure to understand the impact on sterilization of container closure system. Samples were kept at stability studies as per internal conference of harmonization (ICH) guideline[7] for semipermeable container at 40 ± 2°C and 25 ± 5% RH for 3 month and 25 ± 2°C and 40 ± 5% RH for 6 month.

3.14 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 hr and 24 hours, 48 hours and 72 hours 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.[8]

3.15 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. Immunosupression 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 miconazole 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 every 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.

4.0 RESULTS AND DISCUSSION

4.1 Differential scanning calorimetry studies

Miconazole was found to be compatible with identified polymer system. Figure 1 and figure 2 shows DSC scan of miconazole and DSC scan of miconazole and polymer mixture.

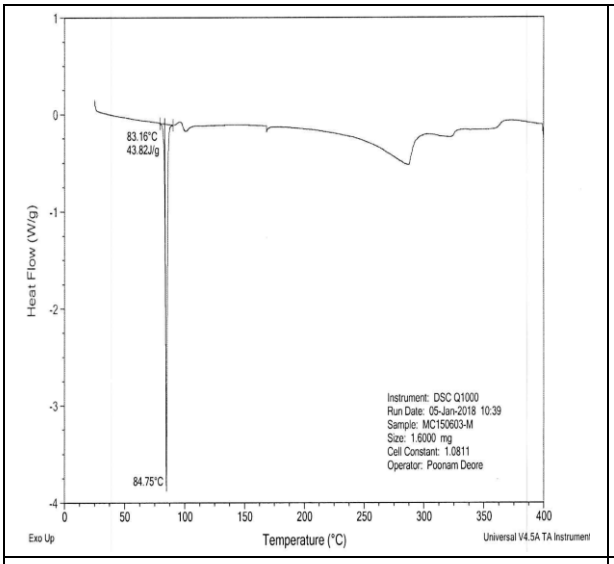

Fig 1: DSC scan of miconazole.

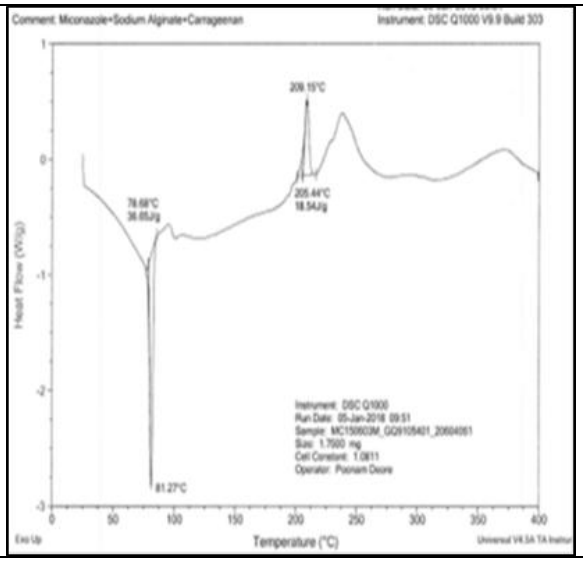

Fig 2: DSC scan of miconazole & polymer mixture.

4.2 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.

4.3 Rheology study

- Viscosity of formulation decreases with application of shear rate of 1000 sec-1 which indicates formulation showed pseudoplastic or shear thining behavior.
- 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 33.808 for formulation. Higher G’ modulus gives longer retention or extended release as well as good flow.
- G” loss modulus which represents the adhesive property with any other substrate in this case it would be eye cornea. G” was 7.97 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 δ was less than 1 till an angular frequency of 0.0185 rad/sec which shows gel kind nature & further it increased more than 1 which shows fluid like 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 0.766 Pa for the formulation. Figure 3 and Figure 4 shows frequency sweep study data. Figure 5 is Shear strain vs. storage modulus and loss modulus. Figure 6 is viscosity vs. shear rate graph.

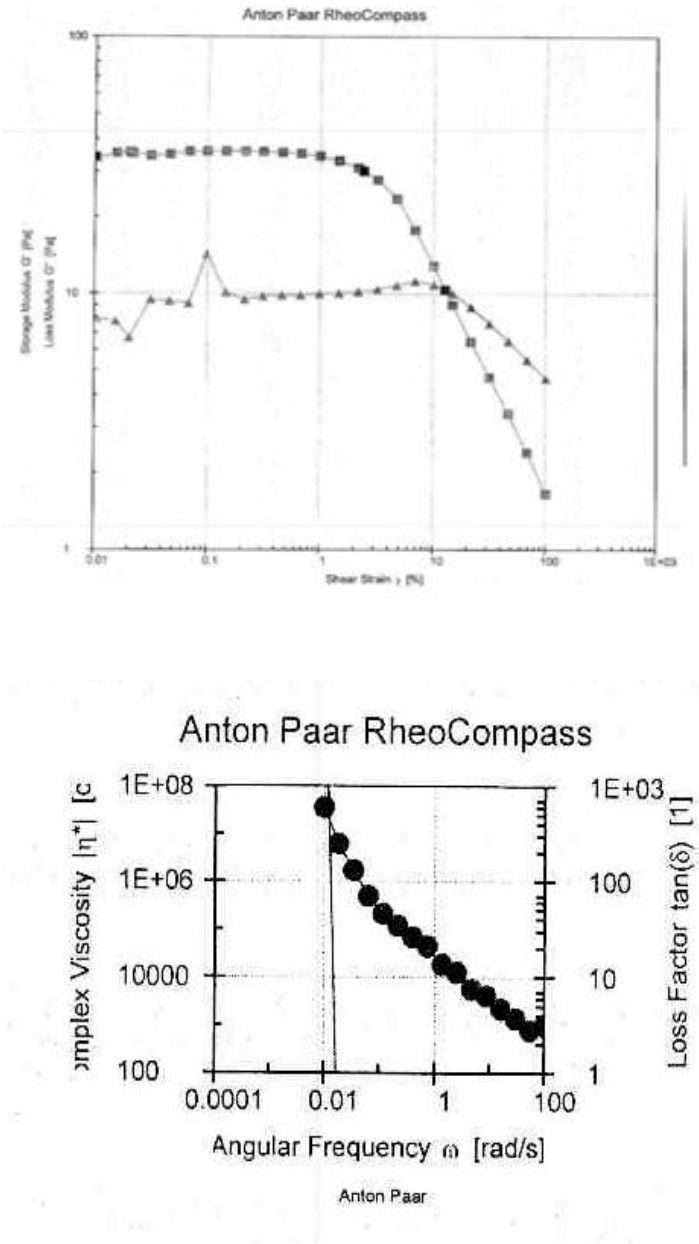

Fig 3 & 4: Frequency sweep study data.

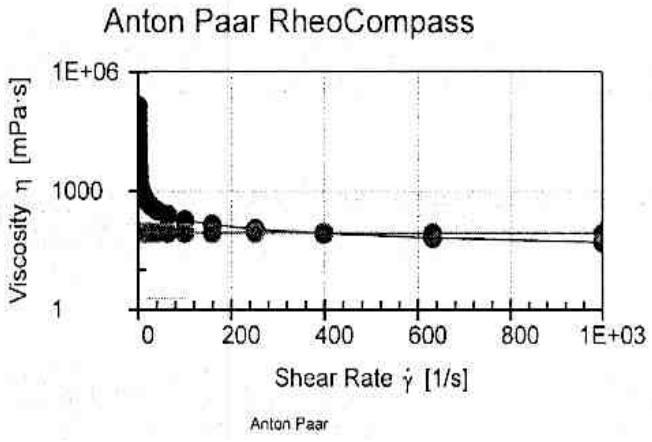

Fig 5: Shear strain vs. storage modulus and loss modulus.

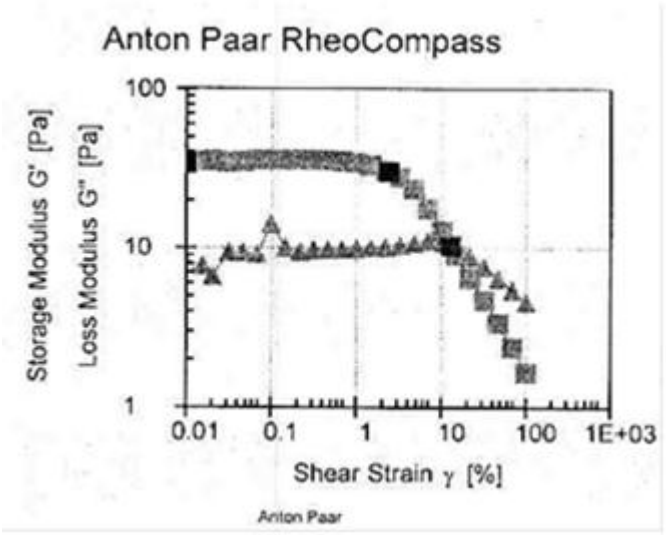

Fig 6: Viscosity vs. shear rate graph.

4.4 Mucoadhesive strength

Mucoadhesive force i.e. force of adhesiveness (F_{adh}) and work of adhesion (W_{adh}) of miconazole 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 miconazole ophthalmic suspension which would remain in eye for longer time. The F_{adh} value was 0.041 N and 0.011 N respectively for long retentive and immediate release formulation. The W_{adh} value was 0.543 N.sec and 0.196 N.sec respectively for long retentive and immediate release formulation. Figure 7 and figure 8 shows mucoadhesive force for long retentive and immediate release formulation.

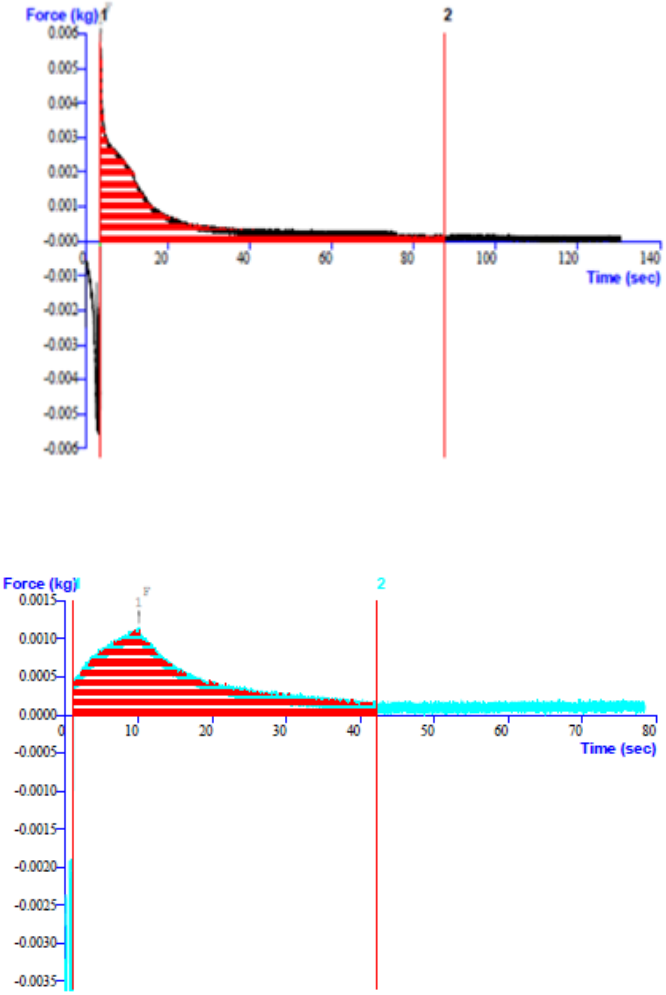

Fig 7 and 8: Mucoadhesive force for long retentive and immediate release formulation.

4.5 Contact angle: Contact angle of miconazole ophthalmic suspension was found to be 57.52 whereas for only placebo polymer it was 49.43. 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.

4.6 Zeta potential: Zeta potential of miconazole ophthalmic suspension was – 51.3 mv shows the developed suspension is electrically stabilized and has good stability behavior against coagulation/flocculation.

4.7 Particle size analysis: Particle size data of pH range formulation and formulation packed in gamma and ETO sterilized container closure was below 10 micron. Table 2 shows particle size data in stability.

Table 2: Particle size data in stability.

Particle size data (d90) in micron					
Condition	**Months**	**High pH ETO**	**Low pH ETO**	**Optimum pH ETO**	**Optimum pH gamma**
Initial	0	9.87	8.84	7.9	7.65
40°C/25%RH	3	9.46	8.67	7.32	7.91
25°C/40%RH	3	9.74	8.26	7.48	7.29
25°C/40%RH	6	9.90	8.54	7.49	7.49

4.8 Miconazole assay and impact of pH & sterilization of container closure system on assay

Across the pH range 6.0, 6.5 and 7.0 (low, optimum and high) the miconazole 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 of container closure has more deleterious effect on stability; miconazole content was beyond the acceptance limit as compared to that of ethylene oxide sterilization (ETO). ETO sterilization is better choice for container closure. Graphical representation of Miconazole assay and Benzalkonium chloride assay across pH range and impact of container closure sterilization is shown in below Figure 9 and 10.

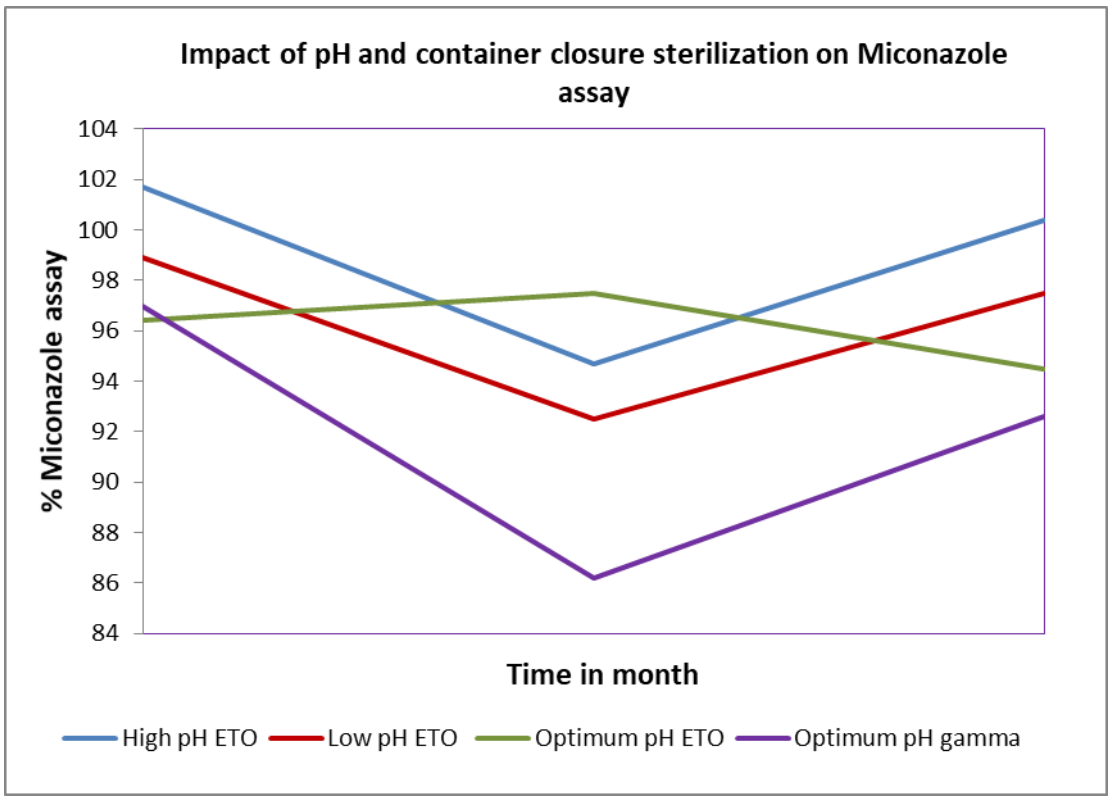

Fig 9: Miconazole assay in stability.

4.9 Benzalkonium chloride assay and impact of pH & sterilization of container closure system on assay

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 10 shows benzalkonium chloride assay in stability.

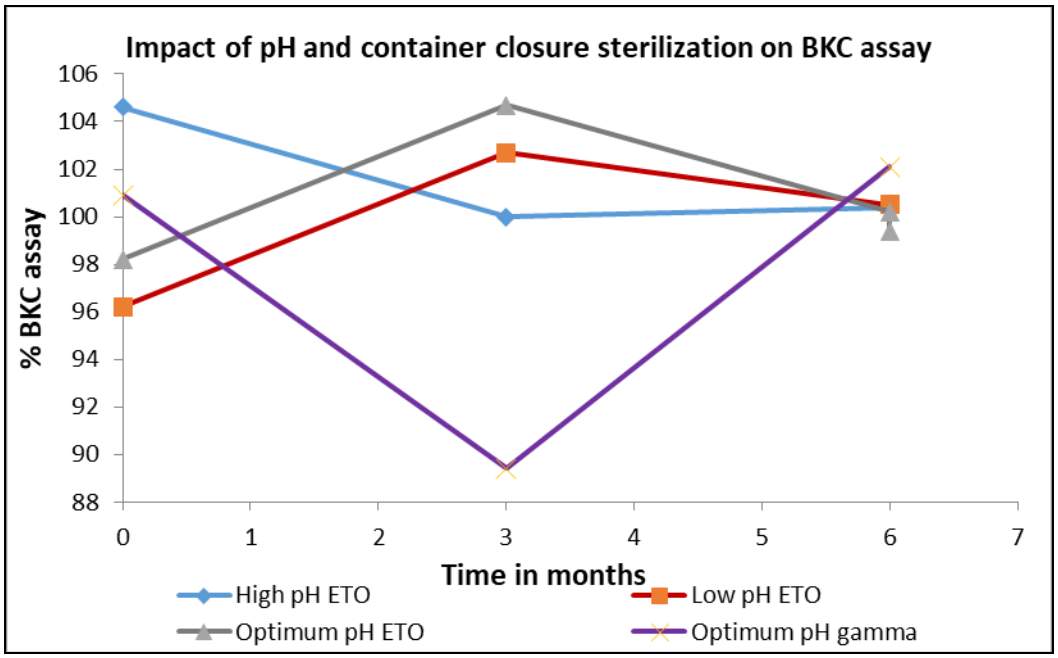

Fig 10: Benzalkonium chloride assay in stability.

4.10 Degradation product

Gamma sterilization container closure system showed higher degradation product hence ethylene oxide sterilization is better choice. Degradation products were found to be similar across the pH range.

4.11 In vitro drug release study

To understand the impact of Polymer in controlling the drug release formulation developed with polymer system and without polymer system was studied for dissolution. Table 3 shows comparative drug release for extended release and immediate release formulation.

Table No 3: Comparative dissolution profile of long retentive Miconazole formulation and immediate release formulation in simulated gastric fluid with 0.5% sodium lauryl sulphate, 200 ml, 70 rpm paddle, 425 micron in enhancer cell.

Time in min	Miconazole long retentive ophthalmic formulation (E5)	Miconazole Immediate release ophthalmic formulation (E8)
0	0	0
1 hr	25	74
2 hr	43	91
4 hr	67	96
6 hr	82	101
8 hr	98	-

4.12 Antimicrobial efficacy studies

Table 4 shows summary results of Antimicrobial effectiveness test at lower concentration of benzalkonium chloride (90% of label claim).

Table 4: 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	4.38	5.63	No increase	Complies
Pseudomonas aeruginosa ATCC 9027	4.15	6.42	No increase	Complies
Staphylococcus aureus ATCC 6538	4.12	4.18	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 compliance**
Limit	**No increase form '0'hr**	**No increase form '0'hr**	**No increase form '0'hr**	
Candida albicans ATCC 10231	No increase	No increase	No increase	Complies
Aspergillus brasiliensis ATCC 16404	No increase	No increase	No increase	Complies

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.

4.13 Ocular irritation studies

Ocular irritation study data proved that developed formulation is non-irritant to rabbit eyes.

4.14 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 5.

Table 5: Comparative pharmacodynamics efficacy score study data of Miconazole ophthalmic suspension developed with sodium alginate and carrageenan vs. positive control.

Parameters	Animal	Positive control (infected eye)	Miconazole ophthalmic suspension 1% (Test product)
Chemosis	1	5	3
	2	4	2
	3	5	1
	4	5	2
	5	2	3
	6	5	4
Average ± SD		**4.33 ±1.21**	**2.50± 1.05**
Eyelid membranes (hyperaemia)	1	5	2
	2	4	3
	3	5	2
	4	5	3
	5	5	3
	6	4	2
Average ± SD		**4.67 ± 0.52**	**2.50 ± 0.55**
Corneal membrane opacity	1	5	2
	2	5	3
	3	4	1
	4	4	2
	5	5	2
	6	5	3
Average ± SD		**4.67 ± 0.52**	**2.17 ± 0.75**

Corneal reflex	1	2	3
	2	1	3
	3	2	2
	4	1	3
	5	2	4
	6	2	3
Average ± SD		**1.67 ± 0.52**	**3.0 ± 0.63**
Blindness vision impaired/not impaired	1	VM	NI
	2	VM	NI
	3	VM	NI
	4	VM	NI
	5	VM	NI
	6	VM	NI

For stastical evaluation one way anova and turkey's multiple comparison test was applied and data is summarized in Table No. 6.

Table No. 6: Anova data of score study data of Miconazole ophthalmic suspension developed with sodium alginate and carrageenan.

Parameters	Mean difference	p value as per Anova	95 % CI of difference
Chemosis	1.83	< 0.0001	0.4344 to 3.232
Hyperaemia	2.17	< 0.0001	0.9996 to 3.334
Corneal membrane opacity	2.5	< 0.0001	1.333 to 3.667
Corneal reflex	-1.33	< 0.0001	-2.484 to -0.1828

After completion of study tear fluid sample was collected with help of syringe and fungal count was done after 7 days by inoculating in to agar media. No fungal colony was observed. In-vivo antifungal pharmacodynamics study proved that developed Miconazole ophthalmic formulation using sodium alginate and carrageenan is efficacious against fungal infection. Also dose given was two drops twice daily which proved long retention property of developed formulation. Candida albicans is the most virulent strain of fungi and Miconazole was found effective against this strain. Hence other fungal strains also can be treated with developed formulation.

5.0 CONCLUSION

A stable long retentive miconazole 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. Pharmacodynamic in-vivo antifungal efficacy and ocular irritation study proved that the developed formulation is non-irritant to eyes and efficacious against most pathogenic fungi candida albicans species. Twice a day administration coupled with its ability to provide sustained release could probably result in less frequent administration, thus enhancing patient compliance.

6.0 Disclosures

The author reports no conflict of interest in this work. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

7.0 ACKNOWLEDGEMENT

Simta S. Jadhav acknowledges the help provided by Indoco Remedies Ltd. for the laboratory facilities in carrying out the research work.

8.0 REFERENCES

1. Kaur IP, Kanwar M: Ocular preparations: the formulation approach. Drug development and industrial pharmacy, 2002 may; 28(5): 473-93.
2. Geroski DH, Edelhauser HF. Transscleral drug delivery for posterior segment disease. Advance Drug Delivery Rev., 2001; 52: 37–48.
3. Cholkar K, Dasari SR, Pal D, Mitra AK. Eye: anatomy, physiology and barriers to drug delivery. In: Mitra AK (ed.). Ocular transporters and receptors: their role in drug delivery. Woodhead Publishing Series in Biomedicine, 2012: 1–36.
4. Geroski DH, Edelhauser HF. Transscleral drug delivery for posterior segment disease. Advance Drug Delivery Rev., 2001; 52: 37–48.
5. Sandle, Tim. Sterile Ophthalmic Preparations and Contamination Control. Journal of GXP Compliance, 2014; 18: 1-5.
6. Jadhav S., Bhitre M., Inamdar K., Identification of Polymer Synergy with Help of DOE, European journal of pharmaceutical and medical research (ejpmr), 2018; 5(03): 343-348.
7. Stability testing of new drug substances and products, ICH Q1A(R2), 2003.
8. Draize J, Woodward G, Calvery O, Methods for the study of toxicity and irritation of substance applied locally to the skin surface and mucous membrane, Journal of Pharmacology and Experimental Therapeutics, 1944; 82(3): 377-390.