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FORMULATION AND EVALUATION OF OCULAR *IN-SITU* GELLING SYSTEMS OF VORICONAZOLE

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ABSTRACT

This research work aims in the formulation of an Ocular *in-situ* gelling systems of voriconazole to treat ocular keratitis. pH and Ion sensitive ocular *in-situ* gelling systems were formulated using the combination of polymers such as carbopol934, HPMC K 15 M and xanthan gum. Carbopol 934 and HPMC K 15 M were used to formulate pH-sensitive *in-situ* gels. The combination of pH and Ion-sensitive *in-situ* gels were formulated using carbopol934, HPMC K 15 M and xanthan gum. Ocular *in-situ* gelling systems are in the form of liquid/solution before administrating into the eye but once when they are administered, transition occurs from sol phase to gel phase due to the ocular environment. The prepared formulations were evaluated for various parameters such as clarity, gelling time, pH, drug content, spreadability, in-vitro drug release studies, anti-fungal studies, ex-vivo permeability studies, test for isotonicity, and drug-excipient compatibility studies. F13 consisting of carbopol 934 (0.4%w/v) and HPMC K 15 M (1%w/v), voriconazole (0.1gm) was selected as the best formulation as it exhibited the highest percentage and sustained drug release for 24 hours 97.5±0.5 %. The anti-fungal study of the best selected formulation was similar to that of marketed vozole® eye drops.

KEYWORDS: Voriconazole, Ocular *in-situ* gelling system, pH-sensitive, Ion-sensitive, Carbopol 934, HPMC K 15 M, Xanthan gum, and sustained drug release.

INTRODUCTION

Voriconazole is an Anti-fungal drug used to treat fungal infections caused by a variety of organisms including *Aspergillus spp*. And *Candida spp.*

Conventional dosage forms such as eye drops have many disadvantages like less residence time of drug, nasolacrimal drainage, rapid precorneal elimination, frequent administration of dosage form and non-patient compliance. These disadvantages can be overcome by *In-situ* gelling systems. These are formulated to release the drug in a sustained manner.

Ocular *In-situ* gels are less viscous solutions which undergo phase transition in cul-de-sac of the eye and forms viscoelastic gels due to ocular environment. This transition is generated by change in physicochemical parameters such as temperature, pH and presence of particular ions. The methods by which sol-gel phase transition occurred are temperature sensitive *in-situ* gelling systems, pH sensitive *in-situ* gelling systems and ion-sensitive *in-situ* gelling systems.

This research work aims to formulate and evaluate Ocular in-situ gelling systems of voriconazole to treat conjunctivitis, fungal keratitis and other fungal infections of the eye. pH sensitive ocular *in-situ* gelling systems and a combination of pH and ion-sensitive *in-situ* gelling systems were formulated using different concentrations of carbopol 934, HPMC K 15 M and xanthan gum. The formulated *in-situ* gels were evaluated for various parameters and compared to the marketed formulation of vozole® eye drops.

MATERIALS AND METHODS

Materials

Voriconazole was a kind gift sample from Chromo Laboratories INDIA Private limited. Carbopol 934, HPMC K 15 M and Xanthan gum, were procured from SDFCL (sd fINE chem limited) respectively.

Formulation of Ocular *in-situ* gel of Voriconazole

Preparation of simulated tear fluid (STF)

Preparation of simulated tear fluid was done by dissolving 0.67g of sodium chloride, 0.008g of calcium chloride, and 0.2g of sodium bicarbonate in 100 ml of double distilled water and the pH was adjusted to 7.4 at 37 °C using 0.1N Sodium Hydroxide.

METHODOLOGY

PREFORMULATION STUDIES

a. Determination of Melting point

Melting point of the sample was determined to test the purity of the sample by capillary tube method.

b. Solubility

Solubility is the important factor to be considered while preparing the formulation. The solubility of Voriconazole was tested in various solvents such as methanol, DMSO, ethanol, distilled water and in buffer solutions of varying pH such as acetate buffer I.P. (pH 4.6, 5.5, and 6.0), phosphate buffer (pH 6.0, 6.5 and 6.8) to select a suitable vehicle for the formulation. Solubility of the drug was observed visually.

c. Drug and excipient compatibility

Drug excipient compatibility studies were performed using FT-IR Spectroscopy. [Agilent Cary 630 FTIR]. The spectrum was recorded for pure drug Voriconazole and the physical mixture containing voriconazole, carbopol934 and HPMC K 15 M. Each sample was scanned at a 8cms resolution in the range between 4000 - 650 cm-1 wave number. The characteristic peaks were recorded for the samples. (Gladious Naguib EL-Hadidy1* H. K.-M., 2016)

ANALYTICAL METHOD DEVELOPMENT OF VORICONAZOLE

a. Determination of absorption maxima in methanol and pH 7.4 Phosphate buffer:

10µg/ml solution of voriconazole was scanned spectrophotometrically for absorbance using UV double beam spectrometer (TS60 UV-VIS Spectrophotometer) in the range of 200-400nm after calibration with corresponding blank.

b. Construction of calibration curve of voriconazole in methanol

Suitable dilutions of voriconazole were prepared in methanol and in pH 7.4 Phosphate buffer and the absorbance was measured using UV Spectrophotomer at the wave length of 257nm. The calibration curve was constructed with concentration on x-axis and absorbance on y-axis.

PREPARATION OF OCULAR IN-SITU GELLING SYSTEM OF VORICONAZOLE BY pH-SENSITIVE METHOD

Formulations were prepared using different concentrations of Carbopol934 and HPMC K15M. HPMC K15M was added to phosphate buffer pH 6.8 and stirred on magnetic stirrer to get a clear solution. Carbopol934 was sprinkled onto the clear polymer solution and allowed to hydrate overnight. The polymer solution was then stirred using overhead stirrer to form a clear solution. The pH of the above solution was adjusted to 6.8 using 0.1N Sodium Hydroxide.

PREPARATION OF OCULAR IN-SITU GELLING SYSTEM OF VORICONAZOLE ION-SENSITIVE METHOD

Formulations were prepared using different concentrations of Carbopol934, HPMC K15M and Xanthan gum. HPMC K15M was dispersed in phosphate buffer pH 6.8 and stirred on magnetic stirrer to get a clear solution, xanthan gum was then added under continuous stirring using a magnetic stirrer. Carbopol 934 was then sprinkled onto the clear polymer solution and allowed to hydrate overnight. The polymer solution was then stirred using overhead stirrer to form a clear solution. The pH of the above solution was adjusted to 6.8 using 0.1N Sodium Hydroxide. Different placebo formulations were prepared with same compositions. A drop of the solution was placed in a vial with 2 ml of freshly prepared simulated tear fluid (STF), the gelation was observed visually and the time taken for gelation was recorded. All the batches were assessed for pH, and gelation time.

FORMULATION OF pH-SENSITIVE AND ION-SENSITIVE OCULAR *IN-SITU* GEL OF VORICONAZOLE

Table 1: Formulation table of Ocular *in-situ* gelling systems of Voriconazole by pH-sensitive method.

S.NO	Voriconazole (gm)	Carbopol 934 %w/v	HPMC K15M %w/v	Benzalkonium chloride (gm)	Phosphate buffer pH 6.8
F 1	0.1	0.3	0.5	0.01	Q.S
F 2	0.1	0.35	0.5	0.01	Q.S
F 3	0.1	0.4	0.5	0.01	Q.S
F 4	0.1	0.45	0.5	0.01	Q.S
F 5	0.1	0.5	0.5	0.01	Q.S
F 6	0.1	0.3	0.75	0.01	Q.S
F 7	0.1	0.35	0.75	0.01	Q.S
F 8	0.1	0.4	0.75	0.01	Q.S
F 9	0.1	0.45	0.75	0.01	Q.S
F 10	0.1	0.5	0.75	0.01	Q.S
F 11	0.1	0.3	1.0	0.01	Q.S
F 12	0.1	0.35	1.0	0.01	Q.S
F 13	0.1	0.4	1.0	0.01	Q.S
F 14	0.1	0.45	1.0	0.01	Q.S
F 15	0.1	0.5	1.0	0.01	Q.S

Table 2: Formulation table of Ocular in-situ gelling systems of Voriconazole by Combination of pH-sensitive and Ion-sensitive method.

S.No	Voriconazole (gm)	Carbopol 934 %w/v	HPMC K15M %w/v	Xanthan gum %w/v	Benzalkonium chloride (gm)	Phosphate buffer pH 6.8
FX 1	0.1	0.3	1.0	0.1	0.01	Q.S
FX 2	0.1	0.35	1.0	0.1	0.01	Q.S
FX 3	0.1	0.4	1.0	0.1	0.01	Q.S
FX 4	0.1	0.45	1.0	0.1	0.01	Q.S
FX 5	0.1	0.5	1.0	0.1	0.01	Q.S

CHARACTERIZATION OF OCULAR *IN-SITU* GELS OF VORICONAZOLE

a. Visual appearance and Clarity

Visual appearance and clarity was determined by observing the formulations by placing the formulations alternatively against white and black backgrounds for presence of any particulate matter. (Gladious Naguib EL-Hadidy1*, 2016)

b. Gelling time

The gelation time of all the test formulations were noted by placing a drop of test formulations in a vial containing 2ml of simulated tear fluid. (Sonjoy Mandal, 2012).

c. Determination of pH

The pH of the sol phase and gel phase were measured using digital pH meter. (Khan, 2019).

d. Drug content

Drug content was determined by dissolving accurately weighed quantity of selected in situ gels in methanol. After suitable dilution absorbance was recorded using UV spectrophotometer at 257nm. (Gladious Naguib EL-Hadidy1*, 2016).

e. Spreadability

A glass petri plate was taken and 0.1 g of the formulation was transferred onto the centre of the plate and this was compressed using another glass petri plate and a known weight was placed on the plate. After a minute, weight was removed and diameter of the spreaded area was measured. (Neslihan Üstündağ Okura, 2019).

f. In-vitro drug release

The *in vitro* drug release from the prepared formulations were studied using Franz diffusion cell through a dialysis membrane (24nm pore size, 10 to 12,000 D cut off of molecular weight). The receptor compartment consists of freshly prepared simulated tear fluid (pH 7.4) and the test formulation of 2ml was placed in the donor compartment. Dialysis membrane which was soaked overnight in the receptor medium (simulated tear fluid) was placed between the donor and receptor compartment. The entire assembly was placed onto the magnetic stirrer and was stirred at 100 rpm. The temperature was maintained at 37± 1°C. Aliquots, each of 3-ml volume, were withdrawn at regular time intervals for 24 hours and replaced with equal volume of the receptor medium and analysed by UV spectrophotometer at 257nm. *In-vitro* drug release of the Vozole® eye drops were performed in the similar manner. (Sonjoy Mandal, 2012).

g. Antifungal Studies

This was determined by the agar diffusion method. The nutrient agar medium was prepared by dissolving sabarouad dextrose in distilled water and heated. The medium was autoclaved at 121°C for 15 min. The developed formulations were poured into cups bored into sterile nutrient agar previously seeded (106 CFU/ ml) with the test organisms (*Candida albicans*). The plates were left for 30 minutes for diffusion and then incubated at 27°C for 48 hr. The diameters of zone of inhibition for *Candida albicans* were measured after 48 hr. The best selected formulations were compared with Vozole® eye drops. (Puranik K M, 2015).

h. Transcorneal permeability

Drug permeation studies were conducted using freshly excised goat cornea. Goat whole eyeballs were obtained from the slaughter house and were preserved in ice cold (4°C) normal saline to maintain viability while transporting to the laboratory within 1 hour of sacrificing the animal. The cornea was carefully excised along with surrounding sclera tissue and was washed with cold normal saline till the tissue was free from adhering proteins.

The receptor compartment in Franz diffusion cell was filled with fresh deaerated simulated tear fluid (pH 7.4). Freshly excised cornea was placed between the donor and the receptor compartments in such a way that its epithelial surface faces donor compartment. The two compartments were clamped together. 2 mL of the formulation was placed in the donar compartment on epithelial surface of the cornea. The temperature of the cell was maintained at 37°C. The whole assembly was placed on the magnetic stirrer. 2ml of the samples were withdrawn at regular intervals for 4 hours and the same volume of simulated tear fluid was replaced in the diffusion cell to maintain sink conditions. Withdrawn samples were analysed by UV-Vis spectrophotometer at 257nm. (Sakshi Malhotra A. K., 2014). Transcorneal permeability of Vozole® eye drops were performed in the similar manner.

i. Isotonicity

Test for isotonicity was conducted to ensure tissue safety. The test was conducted using Sodium chloride solutions of varying concentrations. 3% w/v solution was

hypertonic, 0.2% w/v solution was hypotonic, and 0.9% w/v solution was isotonic. 4 slides were prepared and named as Slide A, Slide B, Slide C and Slide D.

Slide A consists of small amount of blood along with EDTA solution and hypertonic solution. Slide B consists of small amount of blood along with EDTA solution and hypotonic solution. Slide C consists of small amount of blood along with EDTA solution and Isotonic solution. Slide D consists of small amount of blood along with EDTA solution and test solution (best formulation).

EDTA solution (1% w/v) was used to prevent blood coagulation. By using the edge of a cover slip, the contents were mixed and the slides were examined under a microscope to observe the red blood cell morphology. (Senthil Kumar Kannan*, 2017).

j. Sterility testing

Sterility testing was performed in accordance with procedure in Indian Pharmacopoeia for aerobic, anaerobic bacteria and fungi using fluid thioglycolate, alternate thioglycolate medium and soybean casein digest medium respectively. Direct inoculation method was used to test the sterility of the formulations. 5ml of best selected formulation F13 was sterilized in the final container at 121°C for 15 mins and directly inoculated in the 40 ml of these three sterilized media. Fluid thioglycolate media and alternative thioglycolate media were used for aerobic and anaerobic conditions respectively and were kept for incubation at 32°C to 35°C for 14 days. Soyabean caesin digest media was used for aerobic conditions at 20°C to 25°C for 14 days and observed for any microbial growth. The sterility test results were compared against the positive and negative controls. (Sudam Nagargoje, 2012).

RESULTS AND DISCUSSIONS

PREFORMULATION STUDIES

a. Determination of Melting point

The melting point of a substance can be defined as the temperature at which the substance changes from the solid state to the liquid state. It was determined by capillary tube method. Melting point of the voriconazole was found to be 132°C. Literature reported value of the melting point of voriconazole was 129-134°C.

b. Solubility

It was observed that voriconazole was soluble in methanol, ethanol and DMSO and was sparingly soluble in distilled water. The solution of voriconazole in pH 6.8 phosphate buffer was clear and hence was selected as the vehicle for the formulation.

c. Drug-excipient compatability studies

Drug excipient compatibility study was performed using FT-IR spectroscopy [Agilent Cary 630 FTIR]. FT-IR Spectrum of pure drug Voriconazole and physical mixture containing Voriconazole, Carbopol 934 and HPMC K 15 M are shown in the figure 1 and 2.

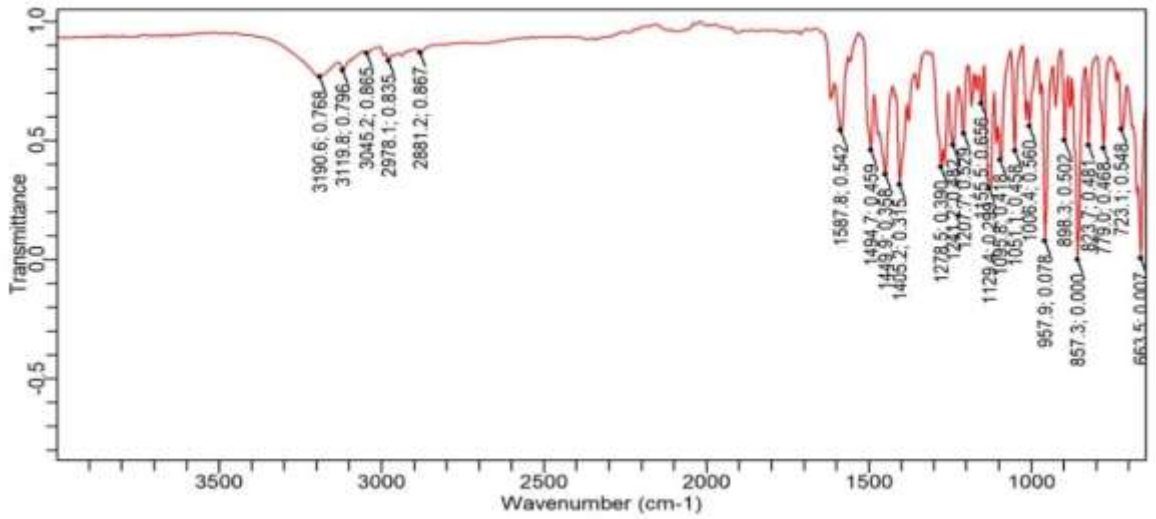

Figure 1: Representing the FT-IR Spectra of pure drug Voriconazole.

Table 3: Representing the data of wave number and groups of FT-IR Spectra of pure drug Voriconazole.

S.No	Wave number cm-1	Intensity	Group
1	663.466	0.00708	C-F group
2	823.74	0.480	C-H group
3	1006.4	0.559	C-N deformation
4	1129.4	0.298	C-O group
5	1587.8	0.542	C=N deformation
6	3190	0.767	O-H group.

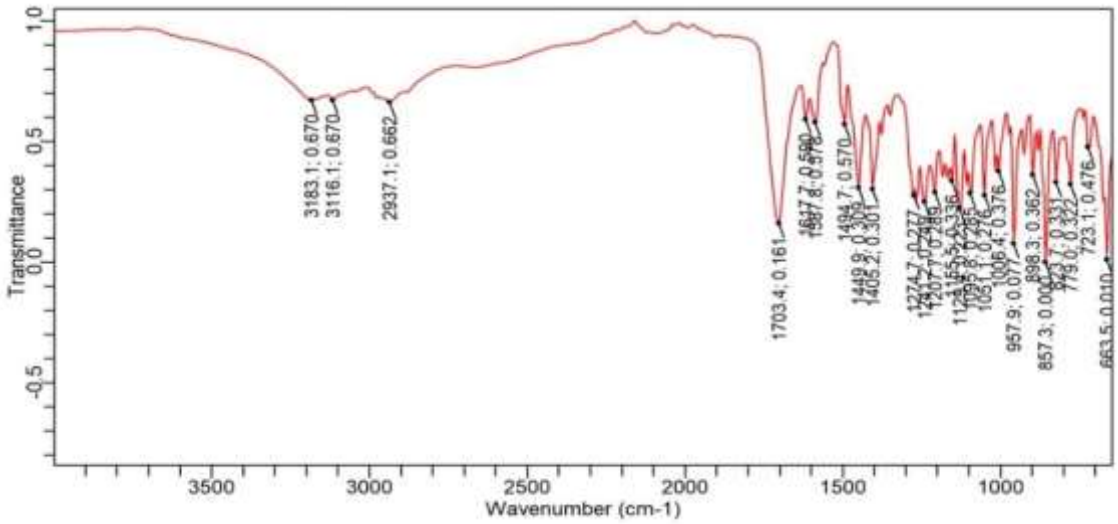

Figure 2: Representing the FT-IR Spectra of physical mixture.

Table 4: Representing the data of wave number and groups of FT-IR Spectra of physical mixture.

S.No	Wave number cm-1	Intensity	Group
1	663.466	0.00985	C-F group
2	823.741	0.33104	C-H group
3	1006.38	0.3759	C-N deformation
4	1129.383	0.222	C-O group
5	1587.8	0.577	C=N deformation
6	3183.14	0.6700	O-H group.

As seen in figures 1 and 2 results there are no considerable changes in the IR peaks of voriconazole when mixed with the polymers. Hence it is clear that there were no interactions between the pure drug and the polymers present in the formulation.

As seen in the tables 3 and 4 the peaks seemed to be retained at almost the same wave number with same intensity in the spectra of the physical mixture which indicates that the absence of any potential physical or chemical interaction between the pure drug Voriconazole and the polymers present in the physical mixture.

ANALYTICAL METHOD DEVELOPMENT OF VORICONAZOLE

a. Determination of Absorption maxima of Voriconazole in Methanol.

Voriconazole solution (10 µg/ml) in methanol was scanned in the UV-Visible Spectrophotometer from 200-400nm to determine the wavelength at which maximum absorbance is seen.

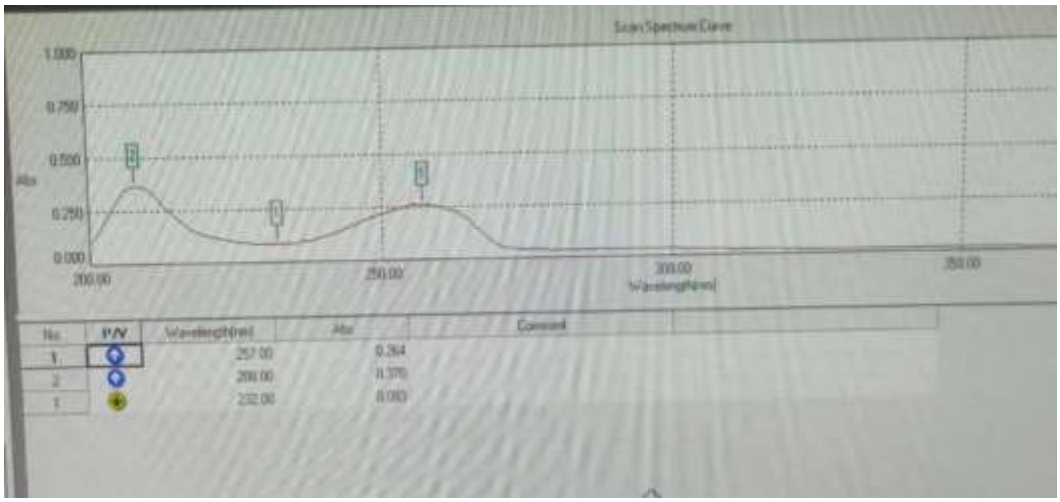

Figure 3: Absorption maxima of Voriconazole in Methanol As seen in fugure 3 drug exhibited maximum absorbance at 257nm.

Construction of calibration cure of Voriconazole in Methanol

The absorbance of different concentrations of voriconazole in methanol were measured in UV-Spectrophotometer at 257nm to plot the standard graph.

Table 5: The absorbance values of the concentrations in methanol.

S.No	Concentration (µg/ml)	Absorbance (nm)
1	6	0.158
2	12	0.304
3	18	0.482
4	24	0.618
5	30	0.778

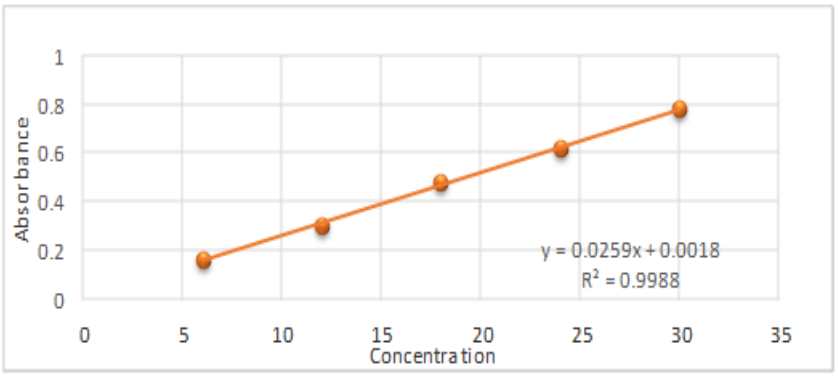

Figure 4: Representing Calibration cure of Voriconazole in Methanol.

Here, standard graph was plotted with concentration on x axis and absorbance on y axis.

The equation was found to be y=0.0259x+0.0018 with the coefficient of determination (R^2) = 0.9988. It represents that the graph 1 is linear in the range of 6µg/ml to 30 µg/ml.

Determination of Absorption maxima of Voriconazole in Phosphate buffer pH 7.4

Voriconazole solution (10 µg/ml) in pH 7.4 phosphate buffer was scanned in the UV-Visible Spectrophotometer from 200-400nm to determine the wavelength at which maximum absorbance is seen.

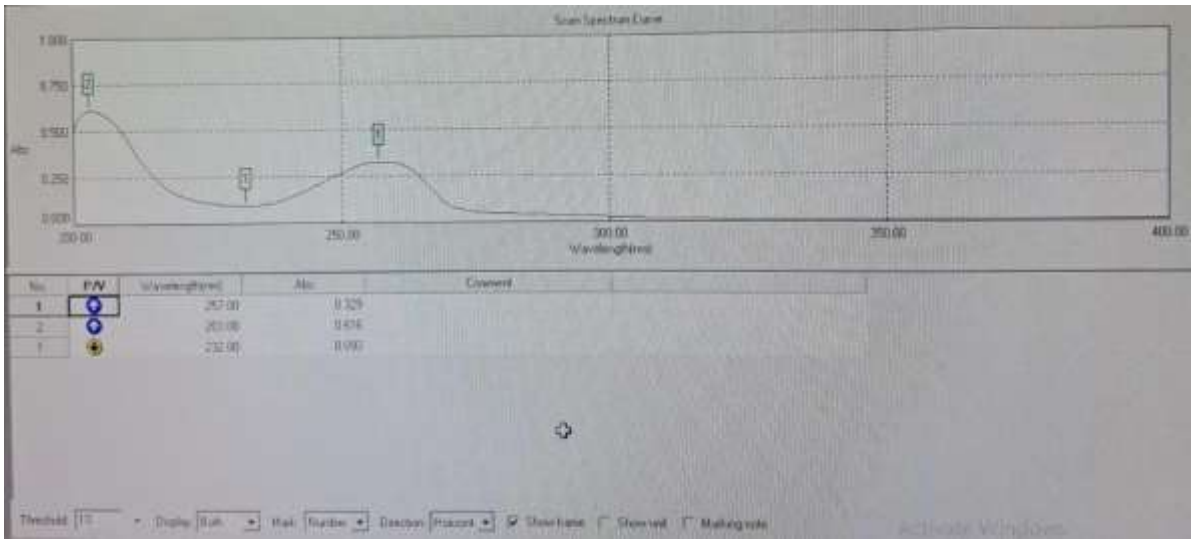

Figure 5: This figure describes that there was maximum absorption at the wavelength 257nm As seen in figure 5 drug exhibited maximum absorbance at 257nm.

Construction of calibration curve of Voriconazole in Phosphate buffer pH 7.4

The absorbance of different concentrations of voriconazole in pH 7.4 phosphate buffer were measured in UV-Spectrophotometer at 257nm to plot the standard graph.

Table 6: The absorbance values of the concentrations in Phosphate buffer pH 7.4.

S.no	Concentration (µg/ml)	Absorbance (nm)
1	4.0	0.168
2	8.0	0.290
3	12.0	0.453
4	16.0	0.583
5	20.0	0.708

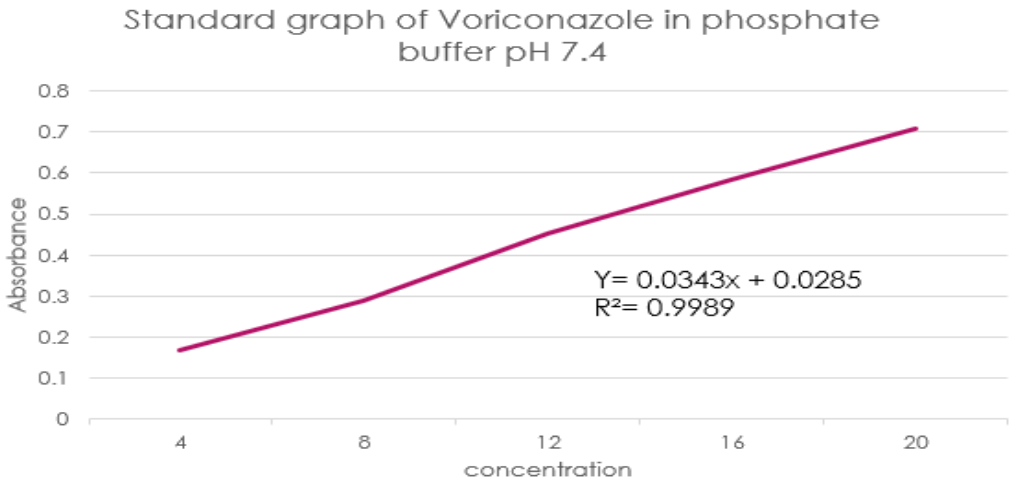

Figure 6: Representing the Standard graph of voriconazole in Phosphate buffer pH 7.4.

Here, standard graph was plotted with concentration on x axis and absorbance on y axis. The equation was found to be y=0.0343x+0.0285 with the coefficient of determination (R^2) = 0.9989 It represents that the graph 2 is linear in the range of 4µg/ml to 20 µg/ml.

Clarity, Texture and Visual appearance

Table 7: Describes the clarity of the sol phase before and after adjusting the pH, clarity of the gel at pH 7.4 and also the texture of the solution and gel.

Formulation	Clarity before adjusting the pH (Sol)	Clarity after adjusting the pH (Sol)	After gelation at pH 7.4	Texture of solution (visually)	Texture of gel (visually)
F1	Clear	Clear	Clear	Smooth	Soft gel
F2	Clear	Clear	Clear	Smooth	Soft gel
F3	Clear	Clear	Clear	Smooth	Soft gel
F4	Clear	Clear	Clear	Smooth	Soft gel
F5	Clear	Clear	Clear	Smooth	viscous gel
F6	Clear	Clear	Clear	Smooth	Soft gel
F7	Clear	Clear	Clear	Smooth	Soft gel
F8	Clear	Clear	Clear	Smooth	Soft gel
F9	Clear	Clear	Clear	Smooth	Soft gel
F10	Clear	Clear	Clear	Smooth	viscous gel
F11	Clear	Clear	Clear	Smooth	Soft gel
F12	Clear	Clear	Clear	Smooth	Soft gel
F13	Clear	Clear	Clear	Smooth	Soft gel
F14	Translucent	Clear	Clear	Smooth	Soft gel
F15	Translucent	Clear	Clear	Smooth	viscous gel
FX1	Clear	Clear	Clear	Smooth	Soft gel
FX2	Clear	Clear	Clear	Smooth	Soft gel
FX3	Translucent	Clear	Clear	Smooth	Soft gel
FX4	Translucent	Clear	Clear	Smooth	Soft gel
FX5	Translucent	Clear	Clear	Smooth	viscous gel

As seen in the table 7 all the formulations were determined by visual inspection against the black and white background. F14, F15, FX3, FX4 and FX5 formulations were found to be translucent and the other formulation were found to be clear and free from foreign particles. Upon gelation, all the formulations formed clear gels with no precipitation or phase separation. The formulations were observed for clarity before and after adjusting the pH of the sol and after gelation by placing in 2ml of simulated tear fluid.

pH: pH of all test formulations were measured using digital pH meter.

Table 8: Describes the pH values before and after adjusting the pH and the values of pH after gelation

Formulation	Before adjusting the pH (Sol)	After adjusting the pH (Sol)	After gelation
F1	5.8 ±0.02	6.8 ±0.02	7.3 ±0.03
F2	5.6 ±0.01	6.7 ±0.03	7.4 ±0.01
F3	5.5 ±0.02	6.6 ±0.01	7.4 ±0.02
F4	5.4 ±0.03	6.6 ±0.04	7.3 ±0.02
F5	5.2 ±0.03	6.4 ±0.04	7.4 ±0.03
F6	5.8 ±0.04	6.8 ±0.02	7.3 ±0.02
F7	5.6 ±0.01	6.7 ±0.02	7.4 ±0.04
F8	5.5 ±0.02	6.6 ±0.04	7.4 ±0.02
F9	5.4 ±0.02	6.6 ±0.04	7.3 ±0.03
F10	5.2 ±0.03	6.4 ±0.05	7.4 ±0.02
F11	5.9 ±0.01	6.7 ±0.01	7.3 ±0.02
F12	5.7 ±0.03	6.6 ±0.02	7.4 ±0.01
F13	5.7 ±0.02	6.6 ±0.03	7.4 ±0.02
F14	5.6 ±0.04	6.6 ±0.03	7.3 ±0.02
F15	5.2 ±0.04	6.5 ±0.05	7.4 ±0.03
FX1	5.8 ±0.02	6.8 ±0.01	7.3 ±0.02
FX2	5.6 ±0.03	6.8 ±0.02	7.4 ±0.01
FX3	5.5 ±0.02	6.7 ±0.03	7.4 ±0.02
FX4	5.4 ±0.04	6.7 ±0.02	7.3 ±0.03
FX5	5.2 ±0.03	6.5 ±0.02	7.4 ±0.04

As seen in the table 8 before adjusting the pH of sol, all the formulations were within the range of 5.2 to 5.9. The pH was adjusted using 0.1N NaOH. After adjusting the pH of sol all the formulations were within the range of 6.4 to 6.8. After gelation the pH was found to be within the range of 7.3 to 7.4. This is non-irritating to the eye.

GELATION TIME: A drop of test formulation was taken in a vial containing 2ml of simulated tear fluid (STF) and gelation time was noted.

Table 9: Describing the values of Gelation time.

FORMULATION	GELATION TIME
F1	45 ± 0.5secs
F2	35 ± 0.3secs
F3	30 ±1 secs
F4	25 ±2 secs
F5	20 ± 0.2secs
F6	48 ±2 secs
F7	40 ± 0.7secs
F8	30 ±0.4 secs
F9	25 ±0.5 secs
F10	20 ±0.5 secs
F11	45 ±0.3 secs
F12	37 ±0.5 secs
F13	25 ±0.3 secs
F14	15 ±0.6 secs
F15	15 ±0.5 secs
FX1	30 ±0.5 secs
FX2	25 ±1 secs
FX3	25 ±1 secs

FX4	20 ±0.5 secs
FX5	15 ±1 secs

The gelation time for all the formulations are shown in table 9. All the formulations gelled between 15 to 50 seconds. As per literature review gelation time of more than 40 seconds causes nasolacrimal drainage. The formulations with gelation time less than 30 seconds were selected for further studies.

Drug content and Spreadability

The drug content of all the formulations was determined by UV-VIS Spectrophotometer at 257nm. The values for drug content and spreadability are given in table 10.

Table 10: Describing the values of drug content and spreadability.

Formulation	Drug Content	Spreadability (cm2/gm)
F1	84 ±0.02 %**%**	0.622 ± 0.12
F2	85.02 ±0.02 %	0.565 ±0.20
F3	76.2 ± 0.02 %	0.509 ±0.25
F4	78.6 ±0.02 %	0.474 ±0.07
F5	77 ±0.02 %	0.440 ±0.05
F6	83.74 ±0.02 %	0.565 ±0.02
F7	84.95 ±0.02 %	0.492 ±0.08
F8	79.5 ±0.02 %	0.457 ±0.27
F9	81.06 ±0.02 %	0.424 ±0.12
F10	74.87 ±0.02 %	0.392 ±0.02
F11	87.8 ±0.02 %	0.509 ±0.02
F12	85.9 ±0.02 %	0.492 ±0.34
F13	102.6 ±0.02 %	0.474 ±0.23
F14	93.9 ±0.02 %	0.424 ±0.12
F15	76.2 ±0.02 %	0.376 ±0.39
FX1	74.5 ±0.02 %**5**	0.474 ±0.22
FX2	85 ± 0.02 %	0.440 ±0.16
FX3	97 ± 0.02 %	0.408 ±0.25
FX4	99 ± 0.02 %	0.376 ±0.10
FX5	87 ± 0.02 %	0.361 ±0.38

As seen in table 10 drug content values were found in between 74.5 and 102.6 ±0.02 %.

Formulation F13 showed highest drug content value of 102.6 ±0.02 %.

Spreadability values were found in between 0.361 ±0.38 and 0.622 ± 0.12 (cm2/gm).

In-vitro drug release: The formulations (F3, F4, F5, F9, F10, F13, F14, F15, FX3, FX4 and FX5) were selected based on desired gelling time and pH and drug release was performed using dialysis membrane.

Table 11: In-vitro drug release of selected formulations and marketed formulation Vozole ® eye drops.

Time in hours	F3	F4	F5	F9	F10	F13	F14	F15	FX3	FX4	FX5	Standard (Vozole ® eye drops)
1	10.5±2.5	7.75±3.5	11.25±0.2	5.05±0.5	4.55±1.5	16.75±0.2	15±0.71	12±0.5	23.5±1.5	20.55±0.2	18.4±0.5	28±3.5
2	16±1.5	13±2	13.95±0.5	8.65±0.5	7.35±0.5	24±0.5	21.3±1.5	14±0.5	30.5±2.5	29.3±0.5	25.9±1.5	43.65±2
3	25±2	17.75±1.5	18±2.5	13.8±1.5	10.5±2	32±1	28.3±3.5	25±1.25	38.1±0.5	36.55±1	35.3±0.5	64.1±1
4	32±0.5	20±0.5	20±1.5	18.4±2.5	14.3±1.5	38±2.5	37.2±2.5	32±3.5	46.8±0.7	44.4±1.5	41.5±0.45	83.8±2.5
5	37±0.8	26.2±2.1	25±0.5	22.6±0.5	20.5±2.5	46.5±1.5	47.6±1.7	39±0.1	55.4±1,7	55.35±2.5	47.5±1.5	100.9±2
6	42±1.2	30±3.5	28±1	30.2±2.5	23.7±1.5	53.2±0.57	55±1.5	47±0.57	64.1±2	64.1±0.25	55.25±2	-
7	47±3.2	34± 0.5	32±0.5	45.4±1.5	27.15±1	64.45±0.95	68.8±1	57±0.63	73.4±2	73.2±3.5	64.6±0.2	-
8	52±2.5	38.15±1.5	37±0.7	51.6±2.5	30±1.5	87.5±1.5	79.6±2.3	65±0.59	82.4±0.2	82.6±0.57	73±3.5	-
24	59.7±4.5	55.45±2	52.5±2.4	84.75±0.5	71.65±0.2	97.5±0.5	95.9±0.2	92±0.5	97.15±0.2	95.7±0.3	91.2±1.5	-

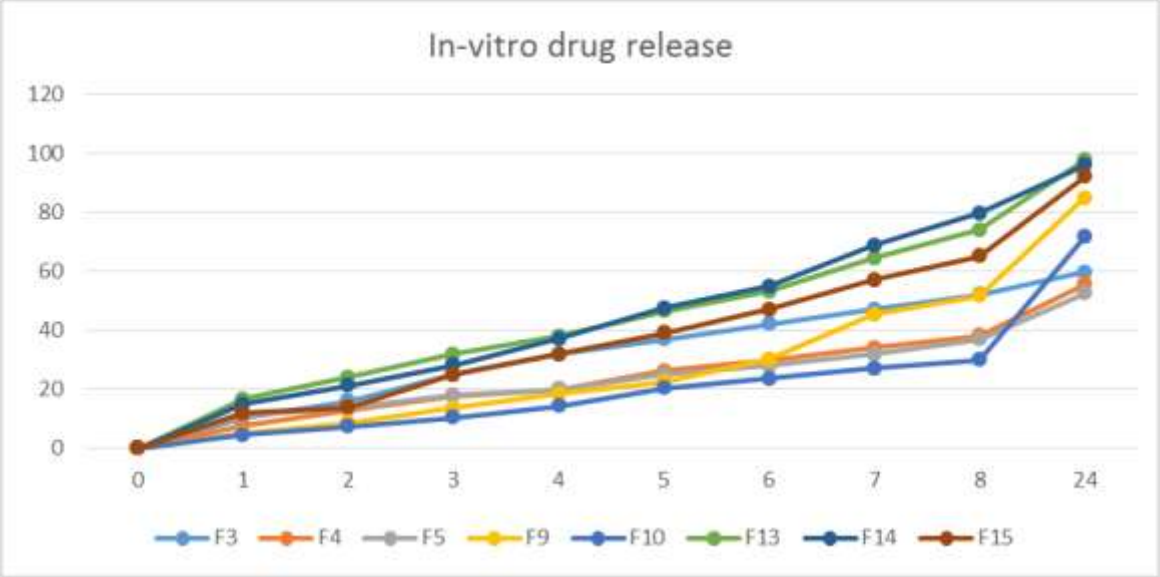

Figure 8: Representing the percentage drug release of F3, F4, F5, F9, F10, F13, F14 and F15.

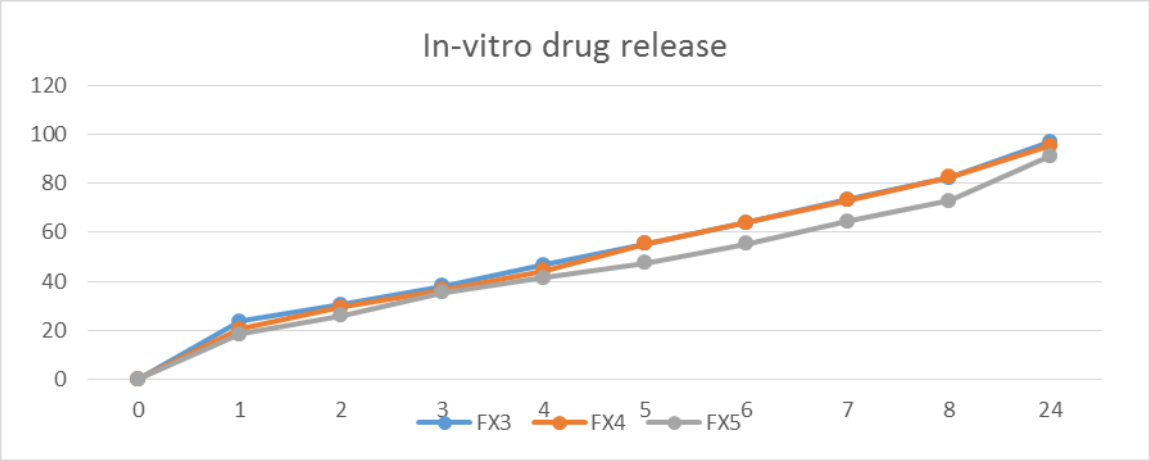

Figure 9: Representing the percentage drug release of the formulations FX3, FX4 and FX5.

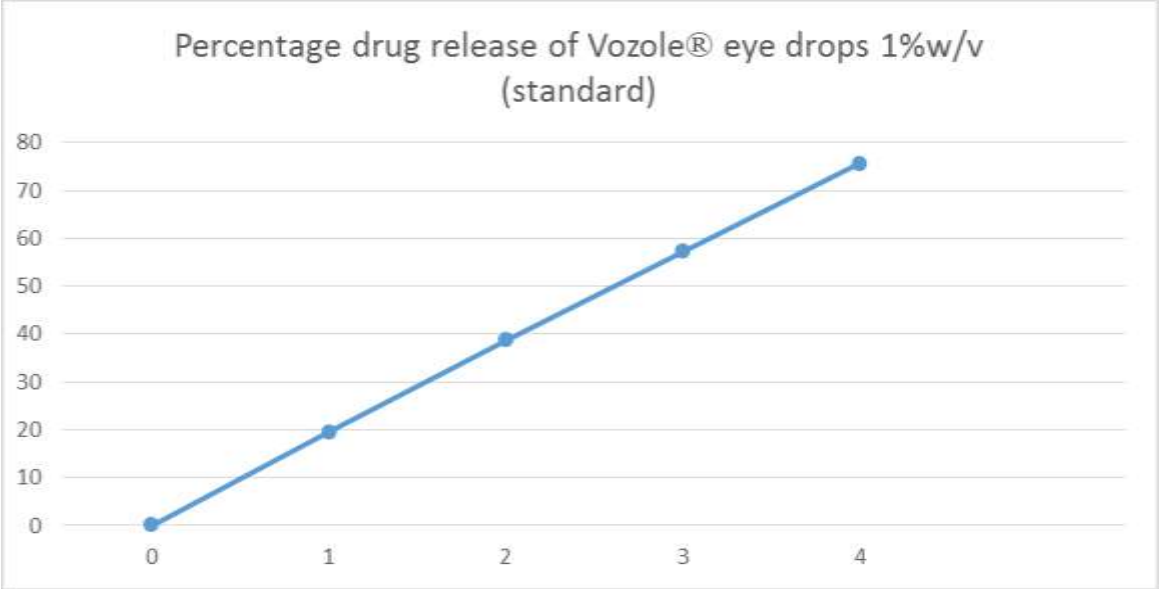

Figure 10: Representing the percentage drug release of Vozole® eye drops (standard) marketed formulation Vozole ® eye drops.

The highest percentage drug release was found for the formulations F13 was found to be **97.5±0.5 %**, FX3 was found to be **97.15±0.2 %** and Fx4 was found to be **95.7±0.3 %** respectively at 24th hour and this was compared to marketed formulation Vozole ® eye drops which was found to be 100.9±2% at 4th hour, it is the conventional dosage form.

Hence formulations F13, FX3 and FX4 were selected as they could sustain the drug release for 24 hours and increase the residence time in the eye. The objective of the study was met. Further studies were performed using these formulations.

Anti-fungal studies

Anti-fungal studies were performed by agar cup diffusion method using the organism *Candida albicans.* Test formulations which showed higher drug release were selected.

Table 12: Representing the values of zone of inhibition of test formulations, control and standard (Vozole® eye drops).

Anti-fungal studies			
Candida albicans			
S.No	**Test**	**Control (Placebo)**	**Standard (vozole® eye drops)**
F13	27mm	0	28mm
FX3	23mm	0	28mm
FX4	22mm	0	28mm

Figure 11: 11a 11b 11c

11a. representing the zone of inhibition of standard (vozole® eye drops), 11b.zone of inhibition of formulation F13 and11c. control with placebo.

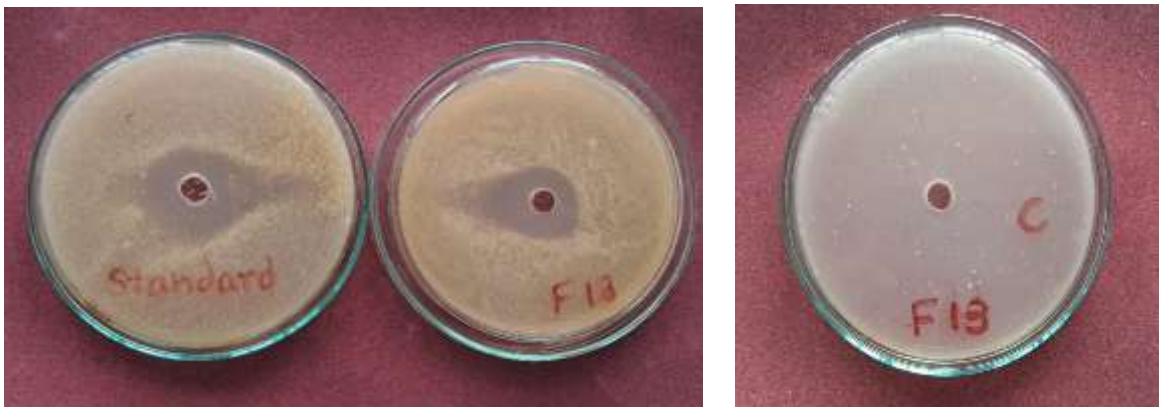

Figure 12: 12a 12b 12c

12a. representing the zone of inhibition of standard (vozole® eye drops), 12b.zone of inhibition of formulation FX3 and 12c. control with placebo.

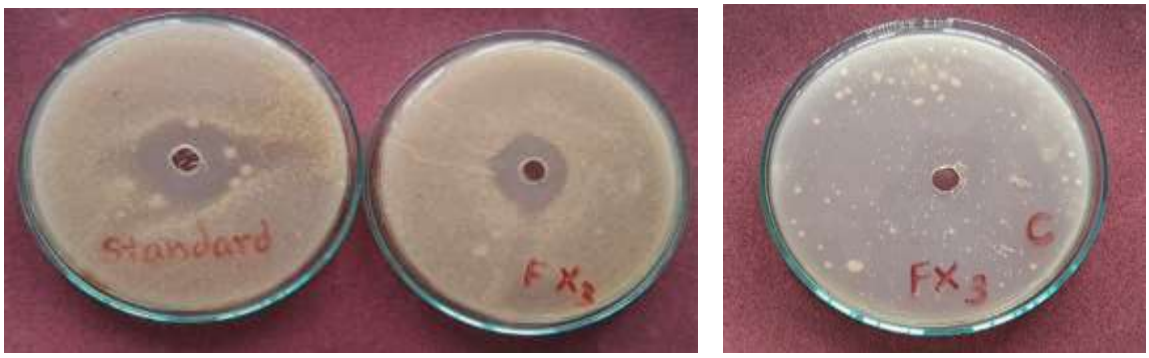

Figure 13: 13a 13b 13c

13a. representing the zone of inhibition of standard (vozole® eye drops), 13b. zone of inhibition of formulation FX4 and 13c. control with placebo.

As seen in the table 11 the zone of inhibition of formulations F13 was 27mm, FX3 was 23mm and FX4 was 22mm respectively. No zone was observed in the control which contains placebo in it. The values of zone of inhibition of formulations were compared against the Vozole® eye drops. It was found that the value of formulation F13 was comparable with the zone of inhibition of marketed formulation Vozole® eye drops which was 28mm. Hence it was selected as best formulation.

Transcorneal permeability

The goat eye balls were collected from local slaughter house. The cornea was removed from the eye balls. As the excised goat cornea was viable only for 4 hours, drug permeation study was performed for 4 hours. The percentage drug release of best selected formulation F13 was compared with that of Vozole® eye drops.

Table: 13: Comparision of percentage drug release of Formulation F13 and Vozole® eye with dialysis membrane and goat cornea.

Time in hours	% drug release of F13 with dialysis membrane	% drug release of F13 with goat cornea	% drug release of Standard (Vozole® eye drops) with dialysis membrane	% drug release of Standard (Vozole® eye drops) with goat cornea
1 hour	16.75	3.45	28	19.5
2 hours	24	14.9	43	38.75
3 hours	32	28.3	64.1	57.24
4 hours	38	36.05	83.8	75.6

Figure 14.

Figure 15.

Figure 14: Franz diffusion cell with goat cornea.
Figure 15: Separation of cornea from goat eye ball.

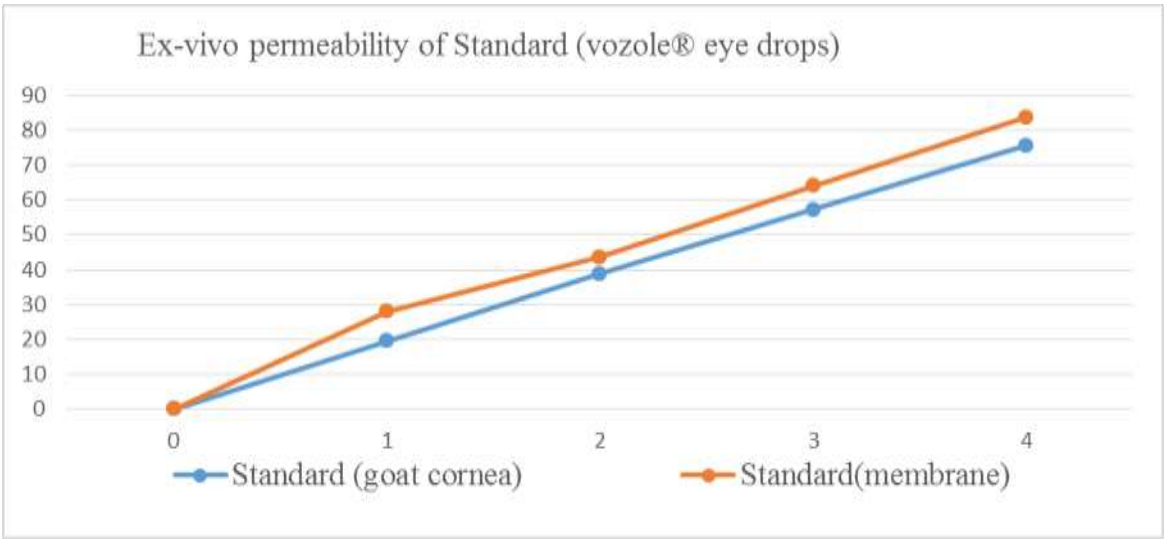

Figure16: Comparision of the percentage drug release of Vozole® eye drops with goat cornea and with semipermeable membrane.

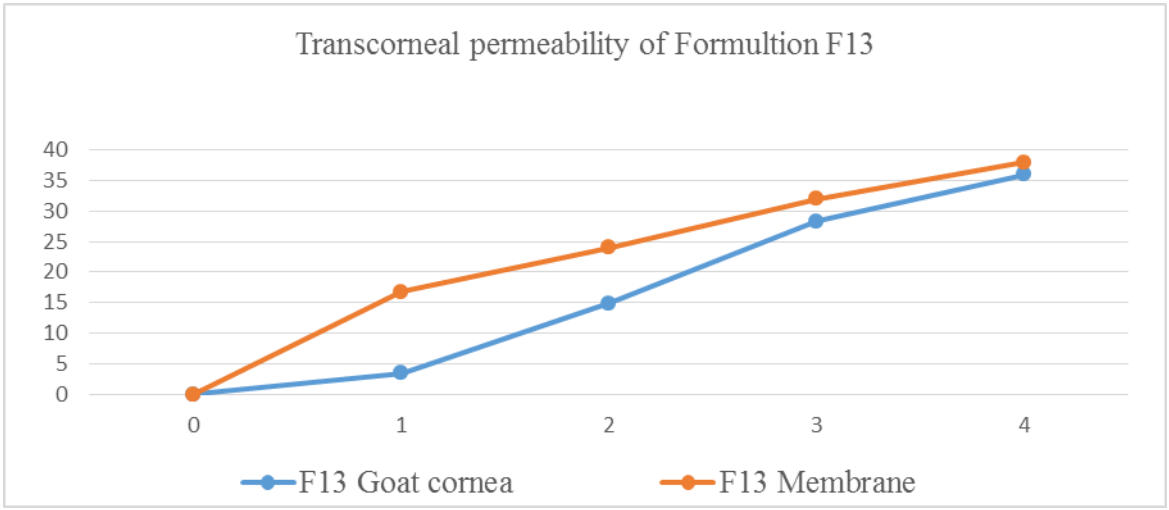

Figure17: Comparision of the percentage drug release of formulation F13 with goat cornea and with semipermeable membrane.

As seen in the table 13 the percentage drug release of F13 and Vozole® eye drops with goat cornea was found to be 36.05% and 75.6% at 4th hour respectively. It was found that there was similar percentage drug release of formulation F13 and Vozole® eye drops with dialysis membrane and goat cornea.

Isotonicity Studies

The isotonicity studies were performed for the best selected formulation F13. As shown in the figures, Slide A consists of small amount of blood along with EDTA solution and hypertonic solution. Slide B consists of small amount of blood along with EDTA solution and hypotonic solution. Slide C consists of small amount of blood along with EDTA solution and Isotonic solution. Slide D consists of small amount of blood along with EDTA solution and test solution (best formulation).

After observing Slide A, Slide B, Slide C and Slide D under the microscope, it was found that the Slide C and Slide D were similar and there was no change in the morphology of RBC's. This showed that the formulation F13 which was on the Slide D was similar to isotonic control solution on Slide C. The objective of this study was met and therefore it can be said that the best selected formulation is isotonic.

Figure 18: Slide A.

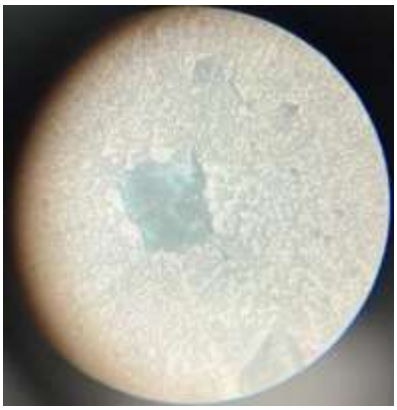

Figure 19: Slide B

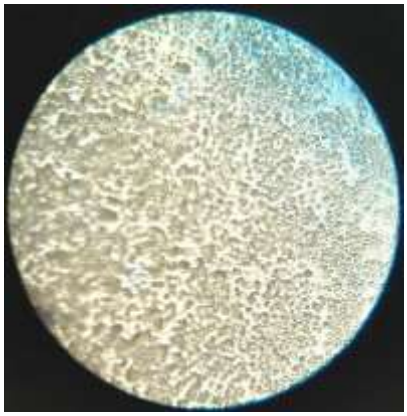

Figure 20: Slide C.

Figure 21: Slide D.

Sterility Studies

For Fluid thioglycolate medium and Alternative thioglycolate medium the organism used was *Bacillus subtilis*. The organism placed in the positive control for Soya bean caesin digestive medium was *Candida albicans*.

There was no microbial growth when incubated for more than 14 days. The media in which the best formulation F13 placed, was clear and has no microbial growth was observed when compared with positive and negative control.

Positive control consists of the organism and Negative control consists of only media without any organism or formulation.

CONCLUSION

Voriconazole is an anti-fungal agent used to treat ocular fungal infections such as Conjunctivitis, fungal keratitis and keratomycosis etc. Ocular *in-situ* gelling systems of voriconazole were formulated by two mechanisms. pH-sensitive *in-situ* gelling systems were formulated using the polymer carbopol 934 and a combination of pH and ion sensitive *in-situ* gelling systems using the polymers carbopol 934 and xanthan gum. HPMC K15M was used to in the formulation to increase viscosity. Benzalkonium chloride was used as preservative.

F13 containing voriconzole (0.1gm), carbopol 934 (0.4%w/v) and HPMC K15M (1%w/v) was selected as the best formulation as it could sustain the drug release for 24 hours with highest percentage drug release **97.5±0.5 %**. Anti-fungal studies of F13 were similar to that of marketed vozole® eye drops. Transcorneal permeability of formulation F13 and vozole® eye drops were performed with goat cornea and were compared which shown comparable results. Test for isotonicity of best selected formulation was found to be isotonic. The results of sterility testing shown that the F13 formulation was free from microbial growth. The ocular in-situ gelling systems of voriconazole were formulated to release the drug in a sustained manner, to reduce the frequent administration of dosage form, to improve patient compliance, to increase the precorneal residence time. Hence, objective of the study was met.

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