ORIGINAL ARTICLE

Study of Intraocular Permeability Enhancement of Brimonidine Tartrate by Formulation in Its *In Situ* Thermoreversible Gel

Ashwini S. Mhetre, Ajay D. Shinde

Department of Pharmaceutics, SVPM's College of Pharmacy, Malegaon, Baramati, Pune, Maharashtra, India

Abstract

Aim: The present work describes the formulation and evaluation of thermoreversible *in situ* gel of brimonidine tartrate (BRT) by incorporating penetration enhancers such as Surfactant (Tween 20), Chelating agent (Disodium EDTA), Bile salt (Sodium tourocholate) in various percentage strength in combination with viscosifying agent to improve ocular bioavailability of drug, which will also improve corneal contact time and corneal permeation of drug. **Materials and Methods:** Thermoreversible ophthalmic gel were prepared by using Cold method by mixing Poloxamer 407 as a thermosensitive polymer, HPMC K4M as viscosifying agent, antiglaucomic drug BRT, Benzalkonium chloride as preservatives and one of the three penetration enhancers use alternatively. A 2_2 factorial design employed for optimization of BRT gels with HPMC K4M (% X1) and penetration enhancer (% X2) as the prime selected independent variable, which were varied at 2 different levels (low and high). **Result and Discussion:** Raising concentration of penetration enhancers at the certain limit increase *in vitro* permeation through goat cornea. At 0.5% w/v concentration of penetration enhancers increase percent drug content, improve apparent permeability coefficient of formulation, and gelation temperature, significantly ($P < 0.05$). The result revealed that optimized formulation containing Tween 20 as penetration enhancers show dominant increase in permeation of drug. **Conclusion:** The study reflects that the permeation of BRT influenced by various penetration enhancers concentration and also viscosifying agent, permeation was found to be concentration dependent.

Key words: Apparent permeability coefficient, brimonidine tartrate, factorial design, *in situ* gel, ocular penetration enhancers

INTRODUCTION

Ocular disposition and elimination of a therapeutic agent are dependent on physicochemical, microbiological, pharmaceutical properties, and ophthalmic irritancy properties of ocular dosage forms as well as the relevant ocular anatomy and physiology. To improve ocular drug contact time, bioavailability, residence time, to reduce the patient discomfort, frequency of dose as well as to slow down the elimination of the drug there are significant efforts concentrating towards newer drug delivery systems for ophthalmic administration.[1]

Glaucoma is a group of disease of the eye characterized by damage to the ganglion cells, and the optic nerve give to increased intraocular pressure remains the most important risk factor for the development of glaucoma.[2]

Successful delivery of drugs into the eye is extremely complicated because the eye is protected by a series of complex defense mechanisms which make it difficult to achieve an effective concentration of drug within the target area of the eye. Recently, the drug delivery systems use to treat glaucoma is eye drops, viscous solutions, suspensions, polymeric inserts, ointments were still acceptable, such dosage form are no longer sufficient to overcome the various ocular diseases such as glaucoma due to poor permeability.[3]

Incorporation of penetration enhancers with drug for transiently increasing the permeation of the cornea with

Address for correspondence:
Ashwini S. Mhetre, Monita Vishwa Apartment, Laxminager, Phaltan, Satara - 415 523, Maharashtra, India. E-mail: mhetre.ashwini777@gmail.com

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appropriate chemical substances is of wide application and probably holds the most promise from the standpoint of versatility and efficacy. The epithelium is the major barrier for most ocular drugs. The penetration enhancers are a class of vehicles that transiently change the permeability of the cornea.[4]

A significant challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. Thus, to increase the ocular bioavailability of the drug, we need to increase the ocular residence time of the drug. Several *in situ* gelling systems have been developed to prolong the precorneal residence time of a drug, improve patient compliance, and consequently enhance ocular bioavailability.[5]

Recently, ocular drug therapeutics research focused on thermoreversible gel. The *in situ* thermoreversible ophthalmic gels mostly prepared using cold method by mixing thermosensitive polymer pluronic F-127, viscosifying agent HPMC K4M and antiglaucomic drug.[6] Nowadays widely brimonidine tartrate (BRT) eye drops available to treat glaucoma also ocular implants, intravitreal injection for neuroprotection of RGC.

The current research topic is undertaken to formulate and evaluate thermoreversible *in situ* gel of BRT by incorporating penetration enhancers in various percentage strength to improve ocular bioavailability of drug, which will also improve corneal contact time and corneal permeation of drug.

MATERIALS AND METHODS

Materials

BRT (Indoco Remedies Pvt. Ltd., Navi Mumbai, India), Poloxamer 407 and HPMC K4M (BASF, Mumbai, India) Sodium chloride, Tween 20, Disodium EDTA, Sodium tourocholate (Loba Chemie Pvt Ltd., Mumbai, India) and Benzalkonium Chloride (Research Fine Chem Industries, Mumbai, India). All solvents used were of analytical grade, unless mentioned.

Methods

Preparation of thermoreversible poloxamer in situ gelling system

Thermoreversible *in situ* gel of BRT were prepared by cold method as desired by schmolka *et al.*[6] using mechanical stirrer. Accurately weighed quantities of poloxamer, bioadhesive polymers, penetration enhancer and Benzalkonium chloride were dissolved in distilled water. To these solution required amount of BRT and 0.9% w/v NaCl added under rapid stirring. The dispersions were cooled down to 4°C in refrigerator; the volume was adjusted with distilled water to reach final concentration of BRT of 0.2% w/v. The solution was mixed well and stored at 4°C for overnight which result in a clear solution. Composition of various prepared formulation is shown in Tables 1 and 2.

Differential scanning calorimetry (DSC) of BRT

The DSC study was carried out for BRT. The DSC pattern was recorded on a PerkinElmer 4000 DSC. Thermographs were obtained by heating 1 mg sample in crimped aluminium pans at heating rate of 10°C/min, from 30°C to 350°C and sample analysis were performed under nitrogen pumping (flow rate: 20 ml/min). Data were analyzed using PYRIS Version- 11.1.0.0488, 2009, PerkinElmer, Inc. software, for origin to obtain onset temperature (T onset); the peak temperature (T peak); and the end set temperature (T end set) of peak. DSC of BRT is shown in Figure 1.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of pure drug, Poloxamer 407, HPMC K 4M, Benzalkonium chloride, Tween 20, Disodium EDTA, Sodium taurocholate (bile salt) were recorded on Shimadzu FT-IR Spectrophotometer using KBr powder. The instrument was operated under dry air purge and the scans were collected at scanning speed 2 mm/s with resolution of 4/cm over the region 4000-400/cm. FT-IR of pure drug and polymers is shown in Figures 2-8.

EVALUATION OF FORMULATION

Visual appearance, clarity

The clarity and appearance of various developed formulations was determined by visual inspection under black and white background.[7]

pH, gelling capacity

The pH of each formulation was measured using Pocket pH meter which was calibrated using buffers of pH 4 and pH 7 before the measurements. Each recording was made in triplicates when they are in sol condition. The gelling capacity was determined by placing one drop of the formulation in a vial containing 2 ml of freshly prepared artificial tear

Table 1: Experimental design of ophthalmic *in situ* gel

Variable level	Low (–)	High (+)
X1=HPMC K4M concentration (% w/v)	0.10	0.50
X2=Tween 20 (% v/v) or	0.50	1
X2=Disodium EDTA (%w/v) or	0.25	0.50
X2=Sodium tourocholate (%w/v)	0.50	1

HPMS K4M: Hydroxyl propyl methyl cellulose K4M

Table 2: Composition of thermoreversible poloxamer gel

Ingredients	Formulation code											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Brimonidine tartrate (%w/v)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Poloxamer 407 (% w/v)	15	15	15	15	15	15	15	15	15	15	15	15
HPMC K4M (% w/v)	0.5	0.5	0.1	0.1	0.5	0.5	0.1	0.1	0.1	0.1	0.5	0.5
Tween 20 (% v/v)	1	0.5	1	0.5	-	-	-	-	-	-	-	-
Disodium EDTA (% w/v)	-	-	-	-	0.5	0.25	0.5	0.25	-	-	-	-
Sodium tourocholate (% w/v)	-	-	-	-	-	-	-	-	0.5	1	0.5	1
Benzalkonium chloride (% v/v)	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
NaCl (% w/v)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9

*All formulation contains quantity sufficient distilled water. HPMC K4M: Hydroxyl propyl methyl cellulose K4M

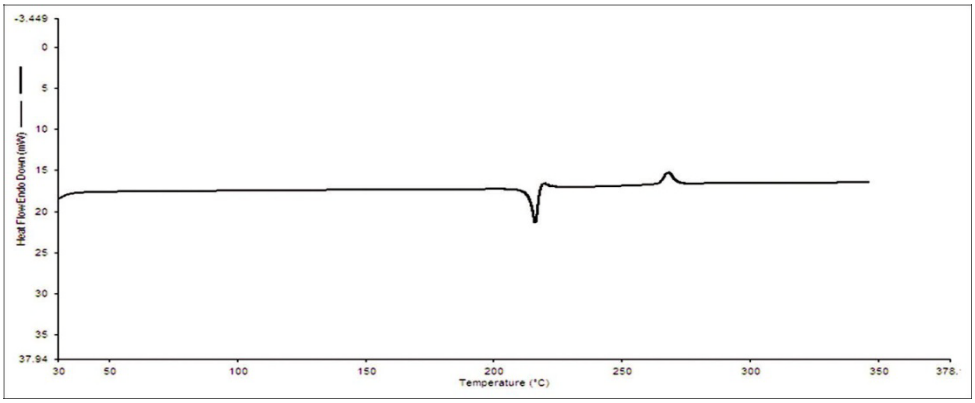

Figure 1: Differential scanning calorimetry thermogram of pure drug (brimonidine tartrate)

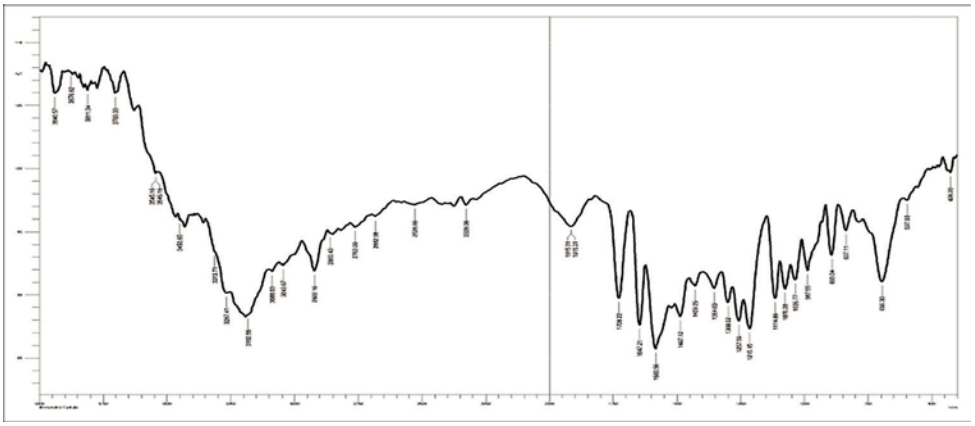

Figure 2: Fourier transform infrared spectrum of brimonidine tartrate

fluid and observing the time required to form gelation of formulation and also time taken for the gel redissolve; the composition of artificial tear fluid used was NaCl: 0.670 g, sodium bicarbonate: 0.200 g, calcium chloride: 0.008 g, in 100.00 g of purified water.[7]

Drug content

Tests for drug content were carried out for all the prepared gel formulations. The vials (n = 3) containing formulation were properly shaken for 2-3 min. 1 ml from each formulation

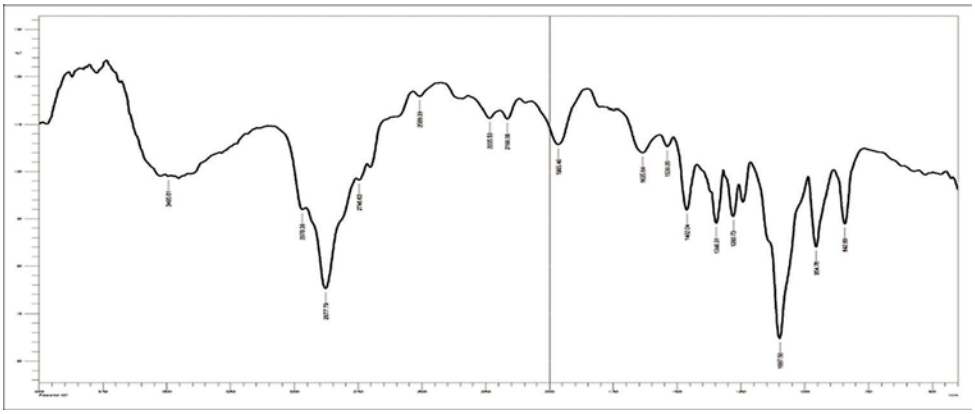

Figure 3: Fourier transform infrared spectrum of Poloxamer 407

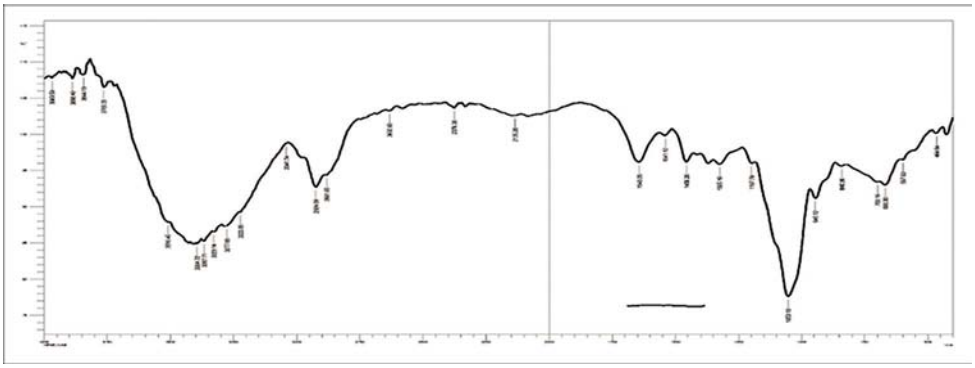

Figure 4: Fourier transform infrared spectrum of Hydroxyl propyl methyl cellulose K4M

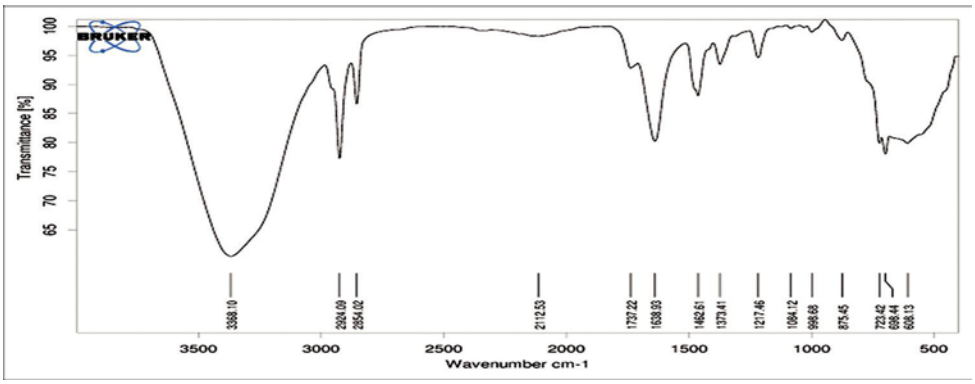

Figure 5: Fourier transform infrared spectrum of Benzalkonium chloride

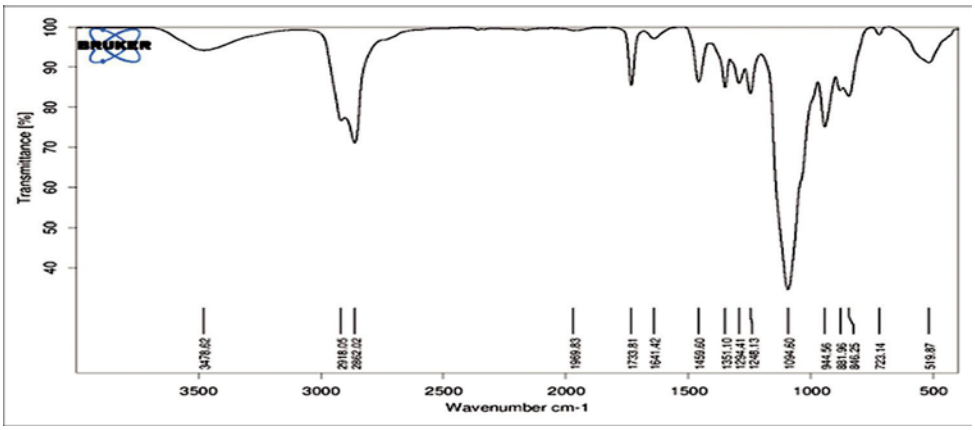

Figure 6: Fourier transform infrared spectrum of Tween 20

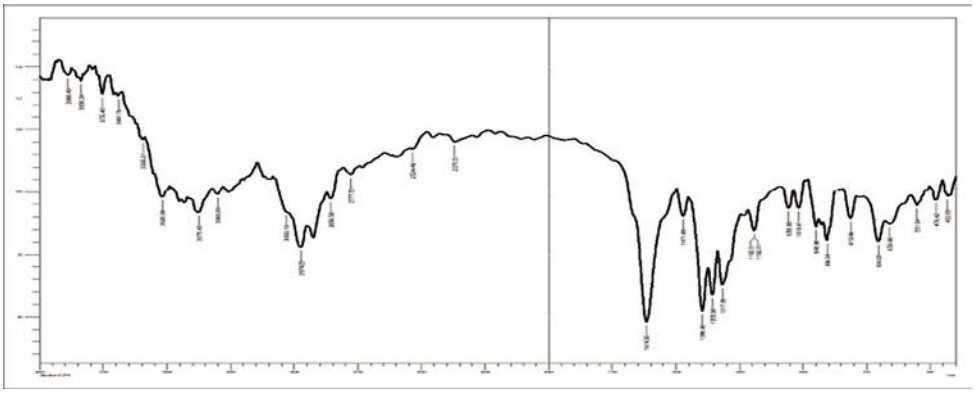

Figure 7: Fourier transform infrared spectrum of Disodium EDTA

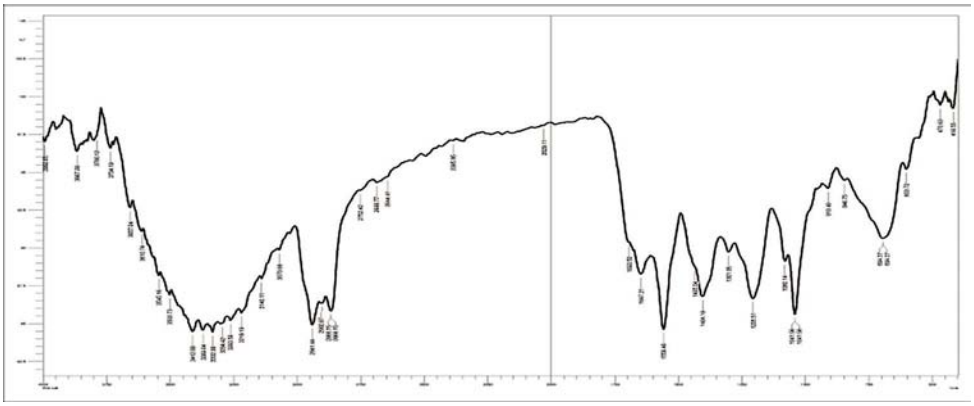

Figure 8: Fourier transform infrared spectrum of Sodium tourocholate

was taken in 50 ml volumetric flask, dissolved in phosphate buffer pH 7.4 with gentle stirring and final volume was adjusted to obtain concentration 25 µg/ml, respectively. The absorbance was measured at analytical wavelength 248 nm using phosphate buffer pH 7.4 as blank using shimadzu 1700 spectrophotometer.[8]

Gelation temperature

The sol-gel phase transition temperature (gelation temperature) were determined for all prepared formulations by taking 2 ml of refrigerated sample to a test tube sealed with a parafilm. Then these test tubes were placed on the water bath to heat. The temperature was increased in steps of 1°C/min. The gelation temperature was measured the gel not follow down when test tube in invert position due to gelation. The temperature was allowed to increase with constant rate until the gel again comes in liquid form to measure sol temperature. Measurement was carried in triplicate for each formulation.[9]

Determination of bioadhesive strength

The bioadhesive strength of all the prepared formulation were determined using the mucoadhesive force measuring device, which is modified balance that was developed in our laboratory according to previously reported methods. The mucoadhesive force of the formulation under study was determined by measuring the force required to detach the formulation from biological membrane using the measuring device.[10]

Rheological evaluation

Viscosity is an expression of resistance of a fluid flow, higher the viscosity greater the resistance. The viscosity of various prepared formulation determined using cone and plate viscometer (Brookfield viscometer Model cap 2000+2). Few drops of formulation were applied to lower plate viscometer using glass rod. The apparent viscosity was measured as a function of the temperature (°C) . The viscosity of formulation measured at temperature of 25°C and 35°C.[11,12].

Isotonicity evaluation

The tonicity of thermoreversible ophthalmic *in situ* gel was checked by formulation were mixed with few drop of blood and observe under microscope at 45× and observe the effect of formulation on red blood cells (RBCs) such as, swelling, bursting, and cremation. Finally, compare the shape of formulation mixed blood cell with RBC alone.[13]

FT-IR spectroscopy

FT-IR spectroscopy of optimized formulations was obtained using Shimadzu FT-IR spectrophotometer using KBr powder. The instrument was operated under dry air purge and the scans were collected at scanning speed 2 mm/s with resolution of 4/cm over the region 4000-400/cm. The scans were evaluated for the presence of principle peaks of drug, shifting and masking of drug peaks and appearance of new peaks due to polymer interaction.

DSC

The DSC study was carried out for optimized formulations. The DSC pattern was recorded on The DSC pattern was recorded on a PerkinElmer 4000 DSC. Thermographs were obtained by heating 1 mg sample in crimped aluminum pans at heating rate of 10°C/min, from 30°C to 350°C and sample analysis were performed under nitrogen pumping (flow rate: 20 ml/min). Data were analyzed using PYRIS Version-11.1.0.0488, 2009, PerkinElmer, Inc. Software, for origin to obtain onset temperature (T onset); the peak temperature (T peak); and the end set temperature (T end set) of peak, as shown in Figures 9-11.

In vitro transcorneal permeation study

In vitro transcorneal permeation study was performed using modified Franz diffusion chamber. Simulated tear fluid was used as the diffusion medium. Fresh goat corneal membrane was separated, soaked in simulated tear fluid, and mounted on by sandwiching between the clamped donor and receptor compartment. Before the application of formulations, the membrane was allowed to equilibrate for 30 min, as shown in Figure 12. One milliliter of sample was withdrawn and replaced with fresh simulated tear fluid in order to maintain sink conditions. The samples were appropriately diluted and the absorbance was measured at 248 nm using a Shimadzu ultraviolet Visible Spectrophotometer. The results were the means of three runs.[14] Percent cumulative drug release of optimized formulations (F2, F5, and F11) is shown in Figure 13.

Apparent permeability coefficient (*Papp*)

Apparent permeability coefficient was calculated using the following equation:

$$\text{Papp} = \frac{Q}{t} 1/(ACo..60)$$

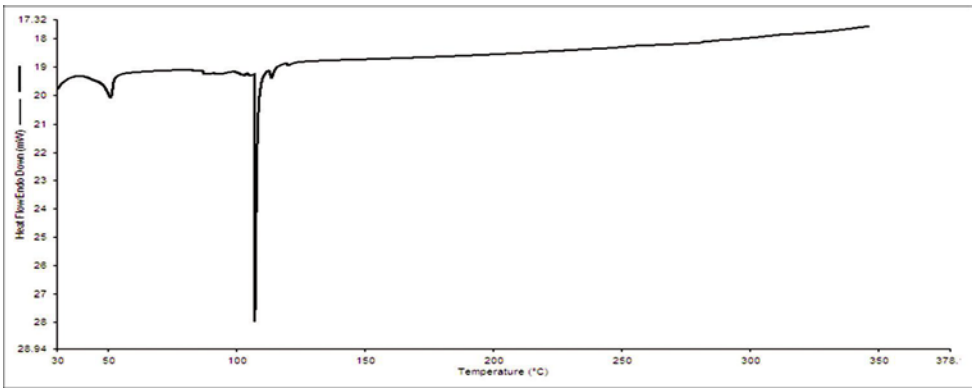

Figure 9: Differential scanning calorimetry thermogram of F2 optimized formulation (Hydroxyl propyl methyl cellulose K4M: Tween 20 [0.5% w/v: 0.5%v/v])

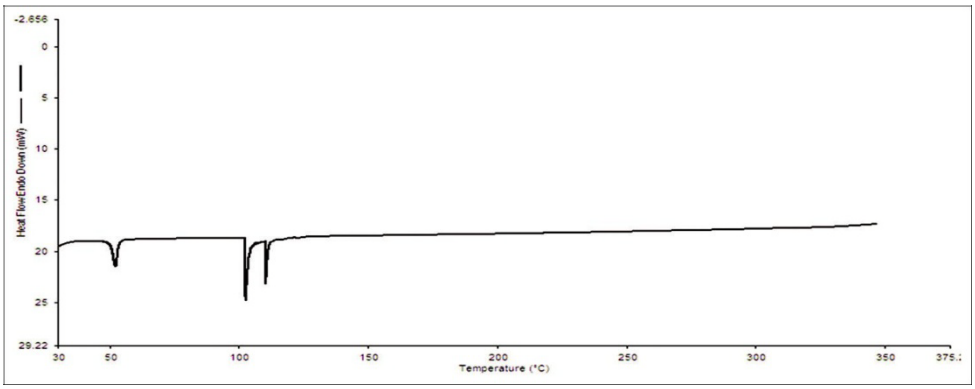

Figure 10: Differential scanning calorimetry thermogram of F5 optimized formulation (Hydroxyl propyl methyl cellulose K4M: Disodium EDTA [0.5% w/v: 0.5% w/v])

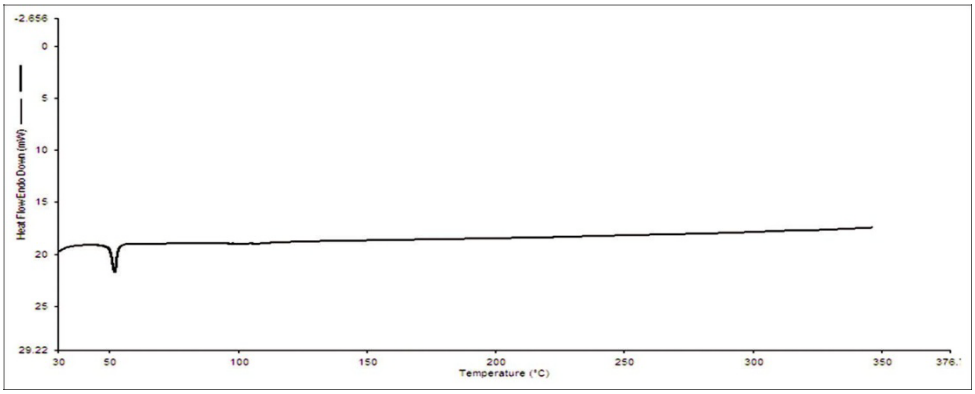

Figure 11: Differential scanning calorimetry thermogram of F11 optimized formulation (Hydroxyl propyl methyl cellulose K4M: Sodium tourocholate [0.5% w/v: 0.5% w/v])

Figure 12: Diffusion cell Assembly used in experimental work for drug release study

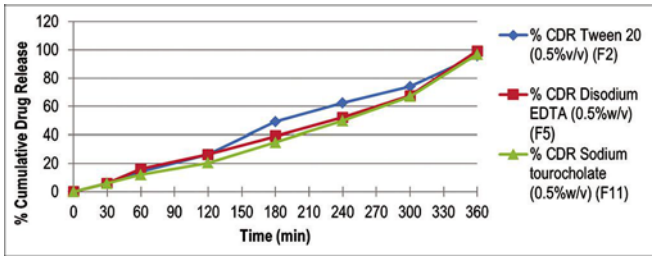

Figure 13: Percent cumulative drug release of optimized formulations (F2, F5, and F11)

Where, ΔQ/Δt (µg/min) is the flux across the corneal tissue. A is the area of diffusion (cm_2), Co (µg/cm_3) is the initial concentration of drug in donor compartment, and 60 is taken as the factor to convert minute into second. The flux across the cornea was obtained from the slope of the regression line obtained from the linear part of the curve between the amount permeated (Q) Vs time (t) plot.[15] Apparent Permeability Coefficient of formulations is shown in Table 3.

2_2 Factorial design and regression analysis

Batches were prepared using a 2_2 factorial design. The advantages of a factorial design include greater precision. Using a factorial design allows examination of the effect of one variable when other factors are changed, something which is not possible using traditional methods of investigation. A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses. The effect of formulation variables on the response variables were statistically evaluated by applying one-way ANOVA at $P < 0.05$ level using a commercial available software package Design-Expert® version 9.0.6.2 (Stat-Ease Inc.). To describe the response surface curvature, the design was evaluated by design model, which bears the form of equation:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_1X_2 + b_4X_1^2 + b_5 X_2^2$$

Where, Y is Response variable, b_0 the constant, b_1, b_2,…, b_5 the regression coefficient, X_1 and X_2 stand for main effect, X_1X_2 are interaction terms, show how response changes when two factors are simultaneously changed.[16]

Data analysis of release kinetics

Different kinetic models such as zero order (cumulative amount of drug released vs. time), first order (log cumulative percentage of drug remaining vs. time), Higuchi model (cumulative percentage of drug released vs. square root of time), Korsmeyer-Peppas model and Hixson-Crowell model were applied to interpret the drug release kinetics from the formulations. Based on the highest regression values for correlation coefficients for formulations, the best-fit model was decided.[17]

Stability study

The 30 days stability studies were carried out for optimized formulation according to International Conference on Harmonization guidelines. Selected sterile formulations were subjected to stability testing. Sterile gel forming ophthalmic solution were filled in glass vials, closed with gray butyl rubber closures and sealed with aluminum cap. The formulations vial kept in stability chamber maintained at 40°C ± 2°C temperature and relative humidity 75±5% for 30 days.[18] Sample were withdrawn at 0, 7, 15, 30, day's interval and evaluated for drug content, pH, visual

appearance, clarity, stability study of optimized formulations reported in Table 4.

RESULT AND DISCUSSION

Clarity and appearance

Formulation of BRT in the form of thermoreversible *in situ* ocular gel was found to be very clear and transparent at room temperature in liquid phase and also in gel formulation was found to be clear and transparent represented in Table 5.

pH

pH of all formulation BRT in the form of thermoreversible ocular gel were found to be within the range of 6.0-7.0 as shown in Table 5.

Table 3: Apparent permeability coefficient (*Papp*)

Penetration enhancer	formulation code	HPMC K4M:Penetration enhancer (%w/v)	Permeability coefficient (*Papp*) (cm/s×10^{-6})
Tween 20	F1	0.5:1	2.5310±0.43
	F2	0.5:0.5	3.0079±0.14
	F3	0.1:1	2.9500±0.5
	F4	0.1:0.5	2.6273±0.25
Disodium EDTA	F5	0.5:0.5	3.3818±1.02
	F6	0.5:0.25	2.2540±0.74
	F7	0.1:0.5	2.3673±0.62
	F8	0.1:0.25	1.8968±0.34
Sodium tourocholate	F9	0.1:0.5	1.467±0.58
	F10	0.1:1	2.065±0.69
	F11	0.5:0.5	2.5939±1.13
	F12	0.5:1	2.1417±0.37

HPMC K4M: Hydroxyl propyl methyl cellulose K4M

Table 4: Stability study of optimized batch F2, F5, F11

Penetration enhancers	Evaluation parameters	Time (days)			
		0	7	15	30
F2 Tween 20 (0.5% w/v)	Drug content (%w/v)	99.43±1.25	98.36±1.02	97.48±1.05	98.51±1.11
	pH	6.4±1.22	6.6±1.0	6.8±1.03	6.7±0.4
	Viscosity at 37°C	580±0.89	600±0.69	620±1.04	630±1.21
	Appearance	Transparent	Transparent	Transparent	Transparent
	Clarity	Clear	Clear	Clear	Clear
F5 Disodium EDTA (0.5% w/v)	Drug content (%w/v)	99.31±1.25	98.65±0.36	97.2±0.58	97.83±0.98
	pH	6.8±0.32	6.9±0.56	6.5±0.24	6.3±0.11
	Viscosity at 37°C	810±1.85	820±1.57	860±0.25	830±0.45
	Appearance	Transparent	Transparent	Transparent	Transparent
	Clarity	Clear	Clear	Clear	Clear
F11 Sodium tourocholate (0.5% w/v)	Drug content (%w/v)	99.31±1.58	98.65±0.74	97.2±0.26	97.83±0.35
	pH	6.8±0.45	6.9±0.57	6.5±0.14	6.3±0.33
	Viscosity at 37°C	660±0.87	700±0.49	710±0.41	730±0.31
	Appearance	Transparent	Transparent	Transparent	Transparent
	Clarity	Clear	Clear	Clear	Clear

Drug content

The percentage drug content of all formulation was found to be in range 96-99% w/v, as shown in Table 6.

Formulation containing Tween 20 as penetration enhancer (F1-F4)

In case of % drug content, (P = 0.0001) value of $P > F < 0.05$ indicated model terms are significant. The statistical model comprising incorporation interactive and polynomial terms was utilized to evaluate response. The polynomial equations for percentage drug content in terms of coded factors were found as follows:

$$y = +97.98 + 1.05A - 0.31B - 0.17AB \quad R_2 = 0.9937$$

From the equation the drug content, positive sign of A (HPMC K4M) and negative sign of B (Tween 20) indicate increase in A and B, the decrease percentage drug content and interaction terms A-B indicate, their combined increase in value increases percentage drug content. The 3D surface

Table 5: Visual appearance, clarity, pH, and gelling capacity of all formulations

Formulation code	Penetration enhancer	HPMC K4M:Penetration enhancer (%w/v)	Clarity	Appearance of gel	pH (*n*=3)	Gelling capacity
F1	Tween 20	0.5:1	Clear	Transparent	6.2±0.43	+++
F2		0.5:0.5	Clear	Transparent	6.4±0.84	+++
F3		0.1:1	Clear	Transparent	6.9±0.23	++
F4		0.1:0.5	Clear	Transparent	6.7±0.14	+
F5	Disodium EDTA	0.5:0.5	Clear	Transparent	6.8±0.61	+++
F6		0.5:0.25	Clear	Transparent	6.3±0.11	++
F7		0.1:0.5	Clear	Transparent	6.6±0.56	++
F8		0.1:0.25	Clear	Transparent	6.5±0.98	++
F9	Sodium tourocholate	0.1:0.5	Clear	Transparent	6.4±0.55	+
F10		0.1:1	Clear	Transparent	6.6±0.65	++
F11		0.5:0.5	Clear	Transparent	6.8±0.41	+++
F12		0.5:1	Clear	Transparent	6.7±0.63	++

+: Gelation slowly and dissolve, ++: Gelation immediate and remains for few hours, +++: Gelation immediate and remain for extended period of time. HPMC K4M: Hydroxyl propyl methyl cellulose K4M

Table 6: Evaluation parameters of F1 to F12

Formulation code	Mean±SD			
	Drug content (%w/v)	Gelation temperature (°C)	Viscosity at 25°C (cPoise)	Detachment Force (dyne/cm$_2$)
F1	97.15±1.25	35±1.0	580±6.5	17662±3.65
F2	99.43±2.32	36±1.33	340±6.2	19263±3.98
F3	96.70±2.65	33±1.25	510±5.3	14462±3.74
F4	98.63±3.21	38±1.24	290±4.6	19263±2.33
F5	99.31±1.25	37±1.62	670±5.6	14451±5.21
F6	96.93±1.48	37±2.30	450±2.1	17661±2.56
F7	98.75±1.78	35±2.01	630±3.4	20866±3.21
F8	97.04±1.95	34±1.96	410±3.5	17666±1.25
F9	96.5±0.89	36±1.85	480±1.89	16055±1.65
F10	97.38±1.02	36±1.87	770±1.57	12833±1.00
F11	99.2±1.58	35±0.65	530±2.89	17652±1.89
F12	95.23±2.52	34±0.85	790±1.47	14447±3.04

Value expressed as mean±SD, *n*=3. SD: Standard deviation

response plot was constructed using Design Expert Software as shown in Figure 14.

Formulation containing Disodium EDTA as penetration enhancer (F5-F8)

In case of percentage drug content ($P = 0.0001$) value of $P > F < 0.05$ indicated model terms are significant. The polynomial equations for percentage drug content in terms of coded factors were found as follows:

y = +98.01+ 0.11 A + 1.02B +

0.34AB R_2 = 0.9742

From the equation the drug content, positive sign of A (HPMC K4M) and B (Disodium EDTA) indicate increase in A and B, the increase percentage drug content and interaction terms A-B indicate, their combined increase in value increases percentage drug content. The 3D surface response plot was constructed using Design Expert Software as shown in Figure 15.

Formulation containing Sodium tourocholate as penetration enhancer (F9-F12)

In case of % drug content, ($P = 0.0001$) value of $P > F < 0.05$ indicated model terms are significant. The polynomial equations for % drug content in terms of coded factors were found as follows:

y = +97.10 + 1.19A − 0.80B −

0.22AB R_2 = 0.9939

From the equation the drug content, positive sign of A (HPMC K4M) and negative sign of B (Sodium tourocholate) indicate increase in A and B, the decrease percentage drug content

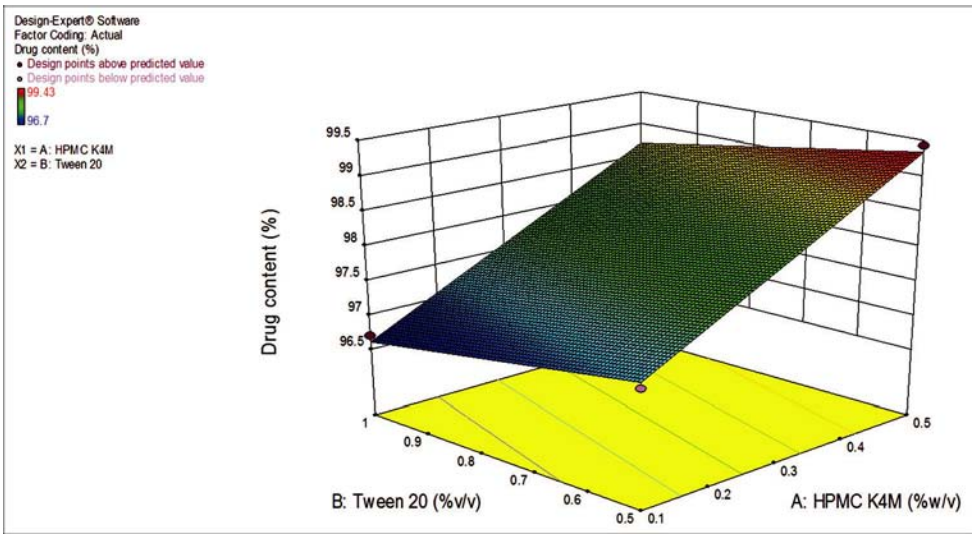

Figure 14: Effect of main factor on %drug content presented by 3D surface plot of Hydroxyl propyl methyl cellulose K4M: Tween 20

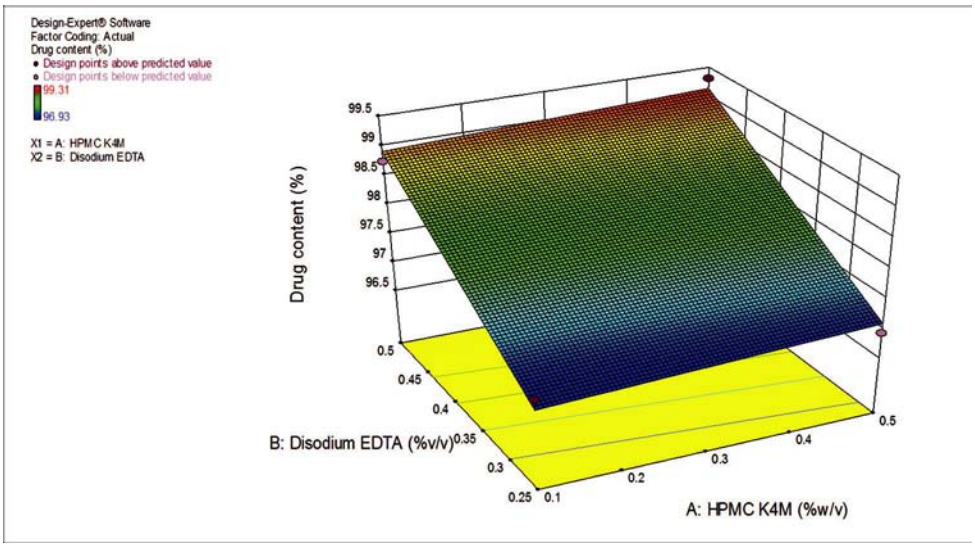

Figure 15: Effect of main factor on %drug content presented by 3D surface plot of Hydroxyl propyl methyl cellulose K4M: Disodium EDTA

and interaction terms A-B indicate, their combined increase in value decreases percentage drug content. The 3D surface response plot was constructed using Design Expert Software as shown in Figure 16.

Gelation temperature

The gelation temperature (T_g°C) of formulation was found within the range of 33-37°C. as shown in Table 6.

Formulation containing Tween 20 as penetration enhancer (F1-F4)

In case of gelation temperature ($P = 0.0001$) value of $P > F < 0.05$ indicated model terms are significant. The polynomial equations for percentage drug content in terms of coded factors were found as follows:

$y = +35.75 - 0.75A - 1.25B + 0.25$

AB $R_2 = 0.9714$

From the equation the gelation temperature, negative sign of A (HPMC K4M) and B (Tween 20) indicate increase in A and B, the decrease gelation temperature and interaction terms A-B indicate, their combined increase in value increases gelation temperature, as shown in Figure 17.

Formulation containing Disodium EDTA as penetration enhancer (F5-F8)

In case of percentage drug content, ($P = 0.0003$) value of $P > F < 0.05$ indicated model terms are significant. The polynomial equations for gelation temperature in terms of coded factors were found as follows:

$y = +35.75 + 1.25\ A + 0.25B - 0.25$

AB $R_2 = 0.9670$

From the equation the gelation temperature, positive sign of A (HPMC K4M) and B (Disodium EDTA) indicate decrease in A and B, the increase gelation temperature and interaction

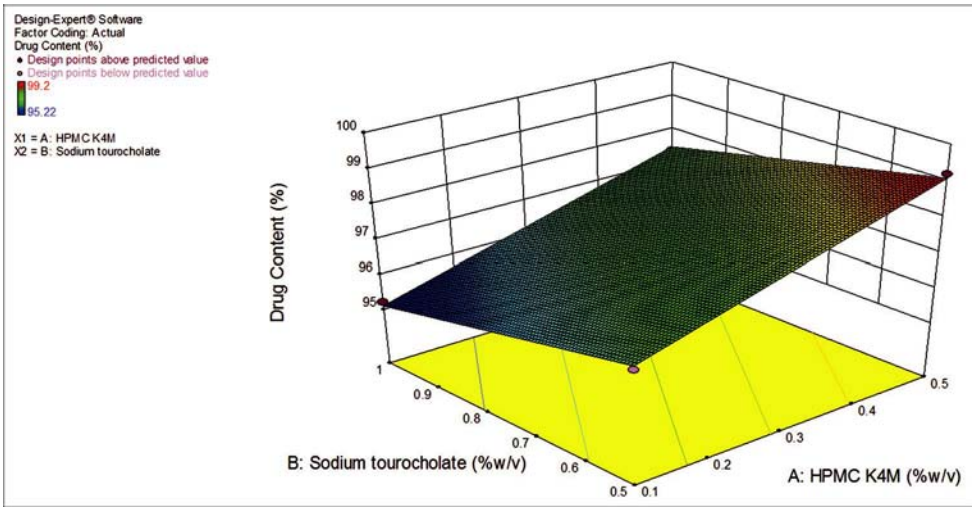

Figure 16: Effect of main factor on %drug content presented by 3D surface plot of Hydroxyl propyl methyl cellulose K4M: Sodium tourocholate

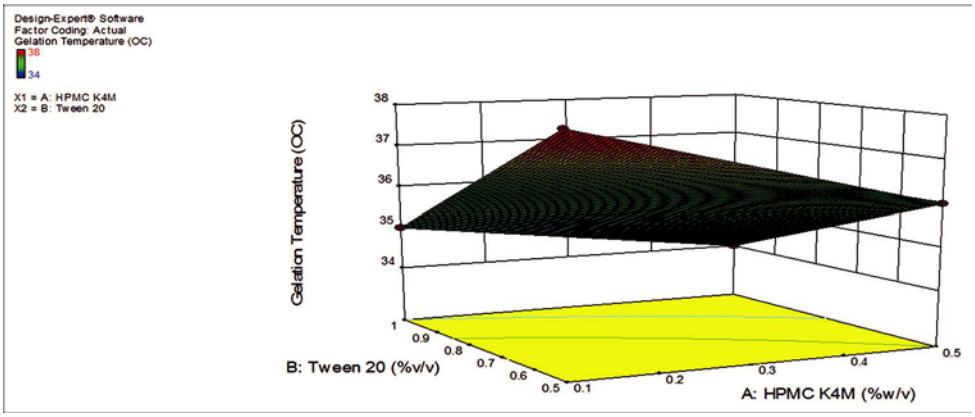

Figure 17: Effect of main factor on Gelation temperature presented by 3D surface plot of Hydroxyl propyl methyl cellulose K4M: Tween 20

terms A-B indicate, their combined increase in value decreases gelation temperature, as shown in Figure 18.

Formulation containing Sodium tourocholate as penetration enhancer (F9-F12)

In case of gelation temperature ($P = 0.0025$) value of $P > F < 0.05$ indicated model terms are significant. The polynomial equations for gelation temperature in terms of coded factors were found as follows:

$y = +35.25 - 0.75A - 0.25B -$

$0.25AB \; R_2 = 0.9091$

From the equation the gelation temperature, negative sign of A (HPMC K4M) and negative sign of B (Sodium tourocholate) indicate increase in A and B, the decrease gelation temperature and interaction terms A-B indicate, their combined decrease in value increases gelation temperature, as shown in Figure 19.

Bioadhesive strength

The batches code F2, F4, F5, F7, and F11 showed better mucoadhesive strength, it was found that all mucoadhesive polymers under study show increase in mucoadhesion as compared to control Poloxamer 407 solutions, as shown in Table 6.

Formulation containing Tween 20 as penetration enhancer (F1-F4)

In case of mucoadhesive strength ($P = 0.031$) value of $P > F < 0.05$ indicated model terms are significant. The

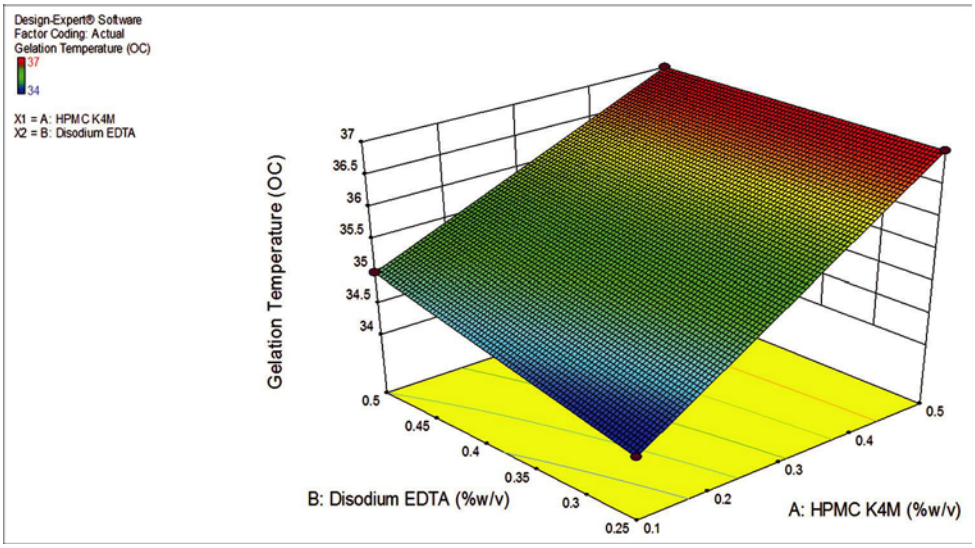

Figure 18: Effect of main factor on Gelation temperature presented by 3D surface plot of Hydroxyl propyl methyl cellulose K4M: Disodium EDTA

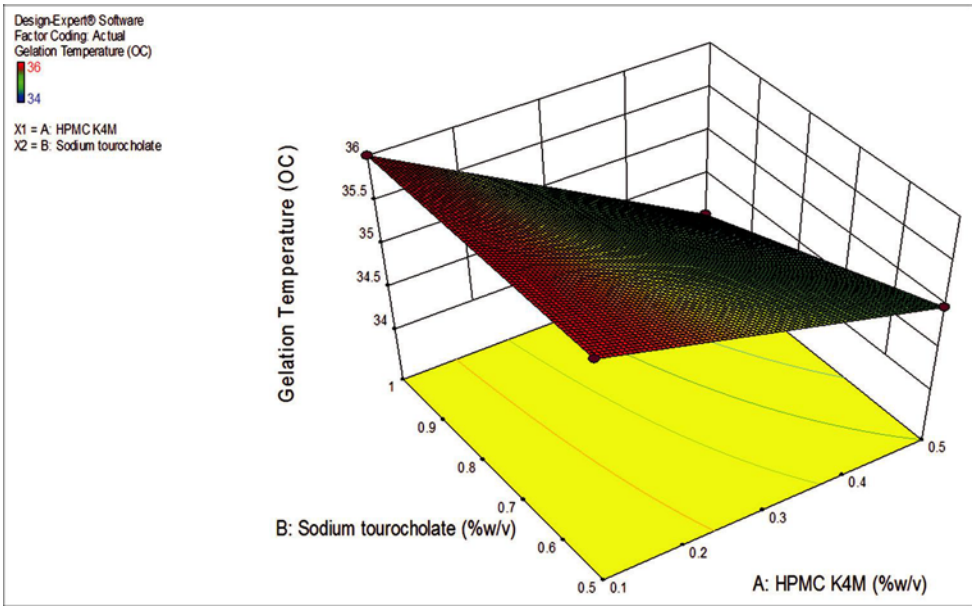

Figure 19: Effect of main factor on gelation temperature presented by 3D surface plot of Hydroxyl propyl methyl cellulose K4M: Sodium tourocholate

polynomial equations for mucoadhesive strength in terms of coded factors were found as follows:

$y = +16806.75 - 1655.75A - 744.75B +$

$55.75AB \; R_2 = 0.9991$

From the equation the mucoadhesive strength, negative sign of A (HPMC K4M) and negative sign of B (Tween 20) indicate increase in A and B, respectively, the decrease mucoadhesive strength and interaction terms A-B indicate, their combined increase in value increase mucoadhesive strength, as shown in Figure 20.

Formulation containing Disodium EDTA as penetration enhancer (F5-F8)

In case of mucoadhesive strength, ($P = 0.0003$) value of $P > F < 0.05$ indicated model terms are significant. The polynomial equations for mucoadhesive strength in terms of coded factors were found as follows:

$y = +17661 + 1605 A + 2.50B -$

$1620.50AB \; R_2 = 0.9089$

From the equation the mucoadhesive strength, positive sign of A (HPMC K4M) and B (Disodium EDTA) indicate decrease in A and B, the increase mucoadhesive strength and interaction terms A-B indicate, their combined increase in value increases mucoadhesive strength, as shown in Figure 21.

Formulation containing Sodium tourocholate as penetration enhancer (F9-F12)

In case of mucoadhesive strength (P= 0.0025) value of Prob > F less than 0.05 indicated model terms are significant. The polynomial equations for mucoadhesive strength in terms of coded factors were found as follows:

$y = +15246.75 + 802.75A - 1606.75B$

$+4.25AB \; R_2 = 0.9998$

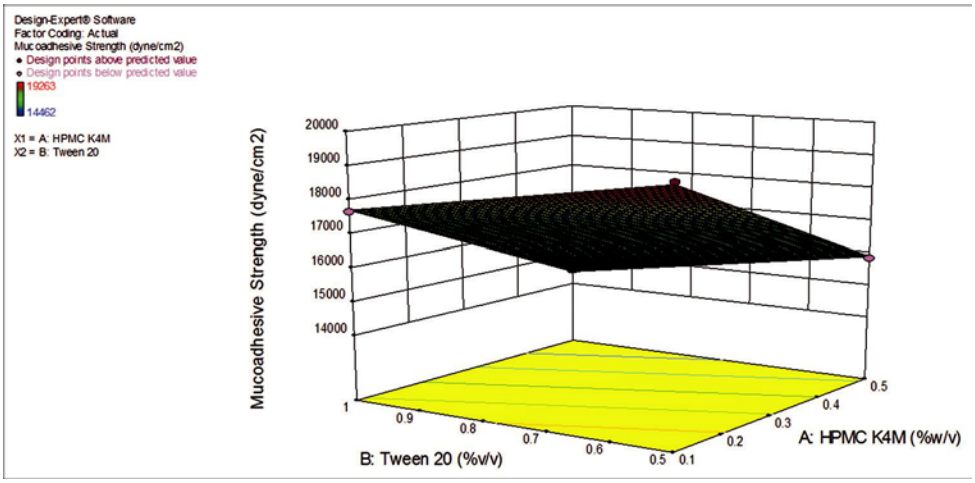

Figure 20: Effect of main factor on Mucoadhesive strength presented by 3D surface plot of Hydroxyl propyl methyl cellulose K4M: Tween 20

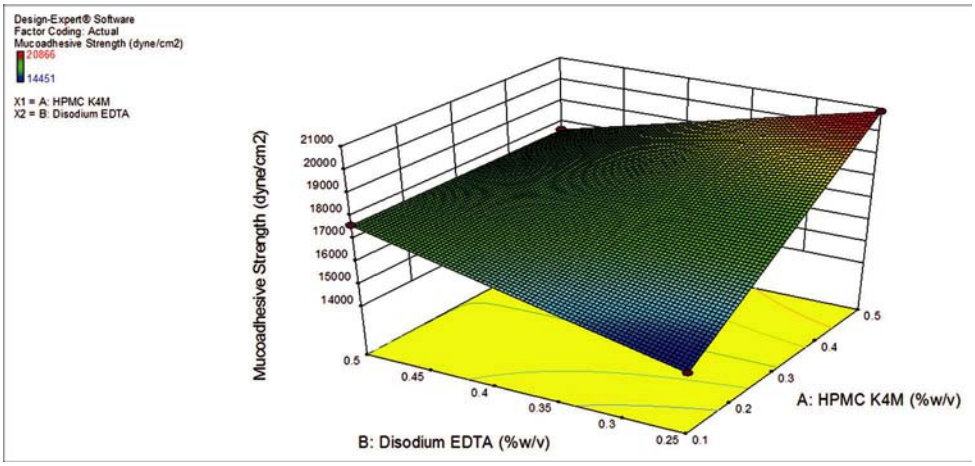

Figure 21: Effect of main factor on Mucoadhesive strength presented by 3D surface plot of Hydroxyl propyl methyl cellulose K4M: Disodium EDTA

From the equation the mucoadhesive strength, positive sign of A (HPMC K4M) and negative sign of B (Sodium tourocholate) indicate decrease and increase in A and B, respectively, the increase mucoadhesive strength and interaction terms A-B indicate, their combined increase in value increases mucoadhesive strength, as shown in Figure 22.

Apparent permeability coefficient

Papp of Optimized formulations F2, F5, F11 is found to be $3.0 \times 10_{-6}$, $3.38 \times 10_{-6}$, and $2.59 \times 10_{-6}$ respectively, as shown in Table 3.

Formulation containing Tween 20 as penetration enhancer (F1-F4)

In case of apparent permeability coefficient ($P = 0.0053$) value of $P > F < 0.05$ indicated model terms are significant. The polynomial equations for apparent permeability coefficient in terms of coded factors were found as follows:

$y = +3.28 - 0.010A - 0.54B -$

$0.20AB \; R_2 = 0.8774$

From the equation the apparent permeability coefficient, negative sign of A (HPMC K4M) and negative sign of B (Tween 20) indicate increase in A and B respectively, the decrease Apparent permeability coefficient and interaction terms A-B indicate, their combined decrease in value increase Apparent permeability coefficient, as shown in Figure 23.

Formulation containing Disodium EDTA as penetration enhancer (F5-F8)

In case of apparent permeability coefficient, ($P = 0.0024$) value of $P > F < 0.05$ indicated model terms are significant.

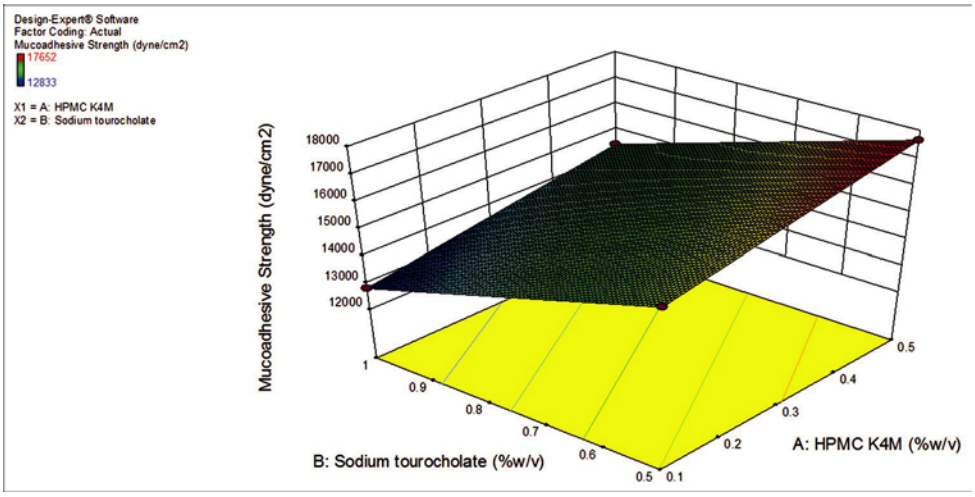

Figure 22: Effect of main factor on Mucoadhesive strength presented by 3D surface plot of Hydroxyl propyl methyl cellulose K4M: Sodium tourocholate

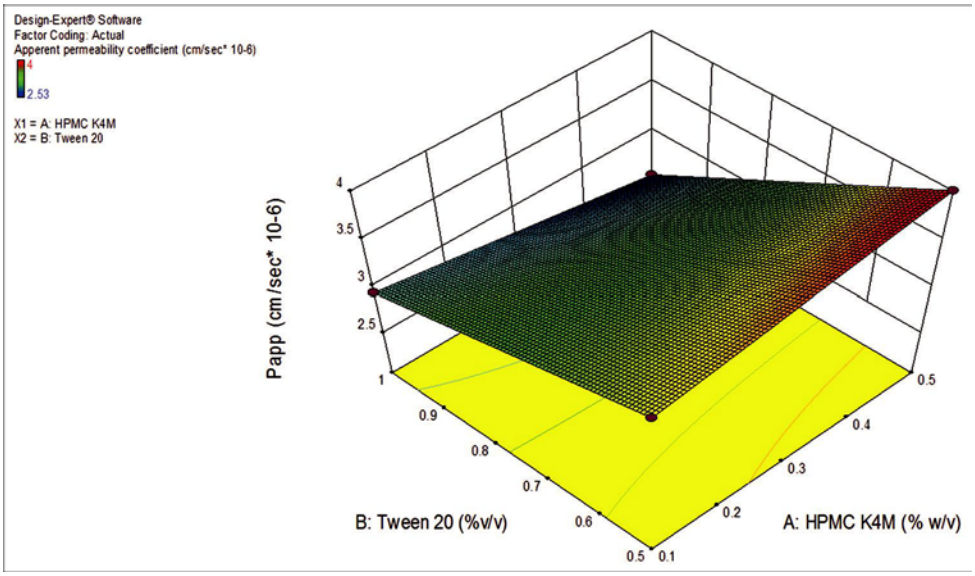

Figure 23: Effect of main factor on apparent permeability coefficient presented by 3D surface plot of Hydroxyl propyl methyl cellulose K4M: Tween 20

The polynomial equations for apparent permeability coefficient, (in terms of coded factors were found as follows:

$y = +2.47+ 0.35 A + 0.40B +$

$0.16AB R_2 = 0.9111$

From the equation the apparent permeability coefficient, positive sign of A (HPMC K4M) and B (Disodium EDTA) indicate increase in A and B, respectively, the increase apparent permeability coefficient, and interaction terms A-B indicate, their combined increase in value increases apparent permeability coefficient, as shown in Figure 24.

Formulation containing Sodium tourocholate as penetration enhancer (F9-F12)

In case of apparent permeability coefficient ($P = 0.0015$) value of $P > F < 0.05$ indicated model terms are significant. The polynomial equations for apparent permeability coefficient in terms of coded factors were found as follows:

$y = +2.31 + 0.55A - 0.51B -$

$0.21AB R_2 = 0.9263$

From the equation the apparent permeability coefficient, positive sign of A (HPMC K4M) and negative sign of B (Sodium tourocholate) indicate increase and decrease in A and B, respectively, the increase apparent permeability coefficient and interaction terms A-B indicate, their combined increase in value increases apparent permeability coefficient, as shown in Figure 25.

FT-IR study of optimized formulations

The observed spectrum of drug to polymer represents superimposed pattern with their significant functional group at specific wavelength indicated that there was no any chemical interaction between drug and polymers only the physical interaction was takes place in terms of hydrogen bonding. FT-IR spectra of optimized formulations are shown in Figures 26-28.

DSC

In DSC study of formulation F2, a small blunt endothermic peak at 52.62°C, graph indicates the formulation containing drug and its polymeric combination get melt. Further sharp endothermic peak at 108°C indicates that the drug presents in more percentage in its amorphous polymorphic form. The consequent next exothermic peak at 115°C indicates that the drug present in less percentage in its crystalline form, as shown in Figure 9. In DSC study of formulation F5, a small blunt endothermic peak at 53.61°C, graph indicates the formulation containing drug and its polymeric combination get melt. Further consequently two sharp endothermic peak at 103°C and 110°C indicates that the drug presents in more percentage in its amorphous polymorphic form, as shown in Figure 10. In DSC study of formulation F11, a small sharp endothermic peak at 53.49°C, graph indicates the formulation containing drug and its polymeric combination get melt, as shown in Figure 11.

Rheological study

From the observation Table 6, it was observed that increase in polymer concentration result in decrease in gelation

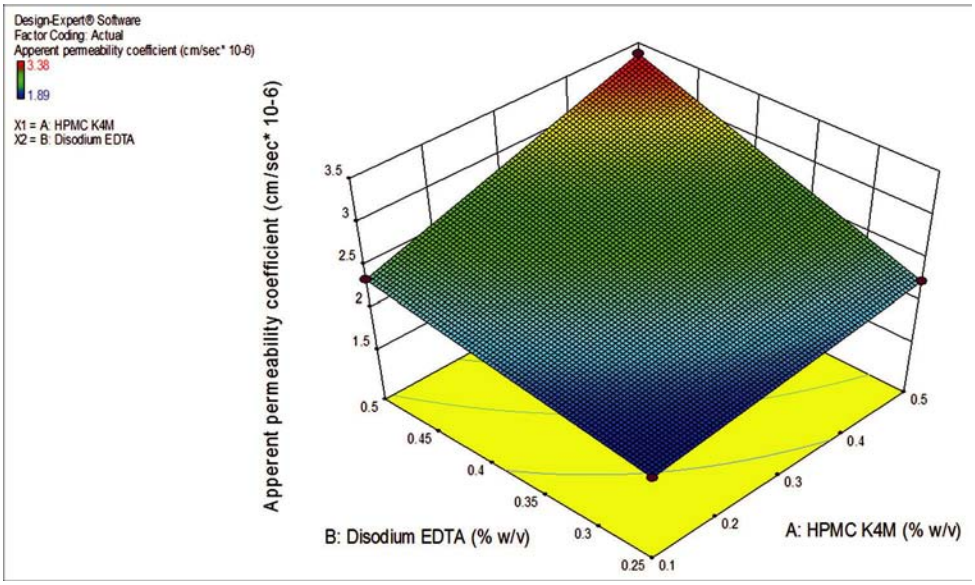

Figure 24: Effect of main factor on apparent permeability coefficient presented by 3D surface plot of Hydroxyl propyl methyl cellulose K4M: Disodium EDTA

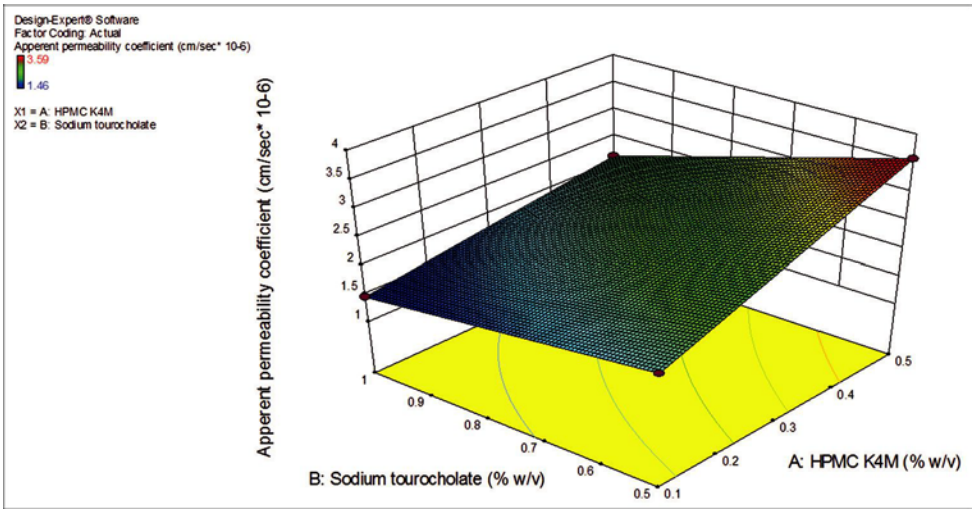

Figure 25: Effect of main factor on apparent permeability coefficient presented by 3D surface plot of Hydroxyl propyl methyl cellulose K4M: Sodium tourocholate

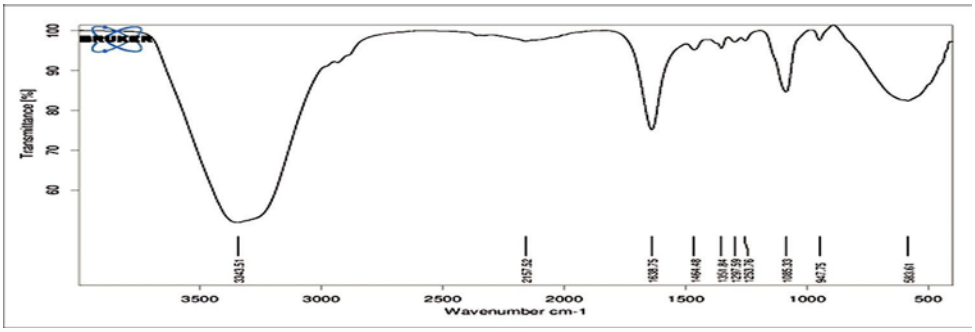

Figure 26: Fourier transform infrared spectrum of optimized formulation (F2)

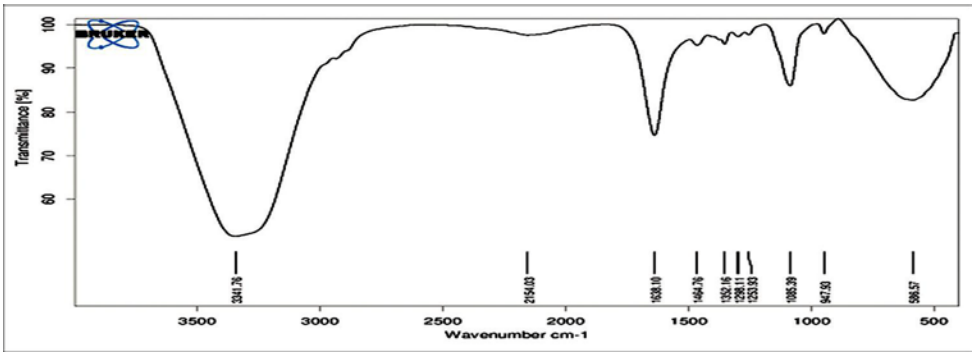

Figure 27: Fourier transform infrared spectrum of optimized formulation (F5)

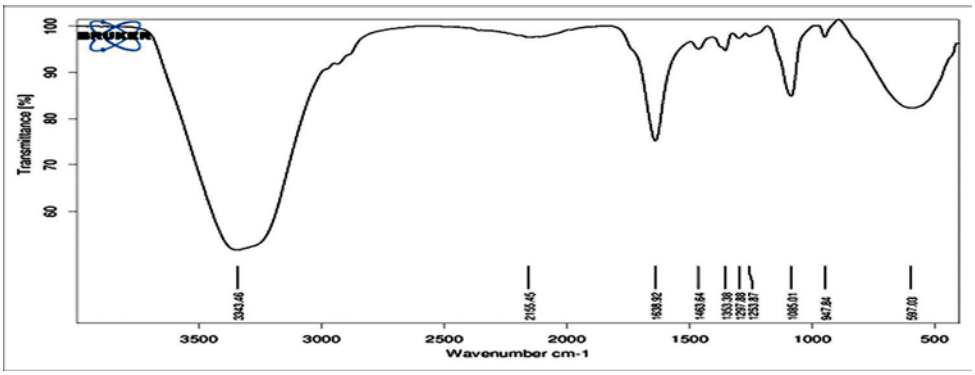

Figure 28: Fourier transform infrared spectrum of optimized formulation (F11)

temperature and increase in viscosity. All formulation shows decrease in gelation temperature as increasing polymer concentration. The order of increasing viscosity was found to be F3<F8=F12<F7=F11=F1<F2=F9=F10=F5=F6<F4.

Isotonicity test

The isotonicity testing of thermoreversible *in situ* BRT ocular gel was performed. It found that there no change in the shape of blood cell (bulging or shrinkage). Which reveals the isotonic nature of the optimized formulation (F2, F5, and F11) as showed in Figures 29-32.

Data analysis of release kinetics

Best release kinetic fitting model to optimized formulation as shown in Figure 33. All formulations F1 to F12 shows Zero order dissolution mathematical model.

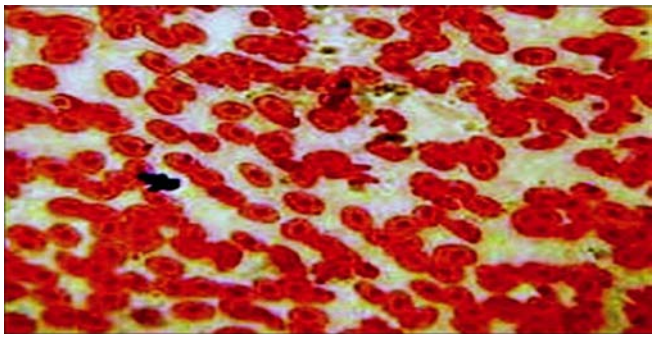

Figure 29: Red blood cell alone

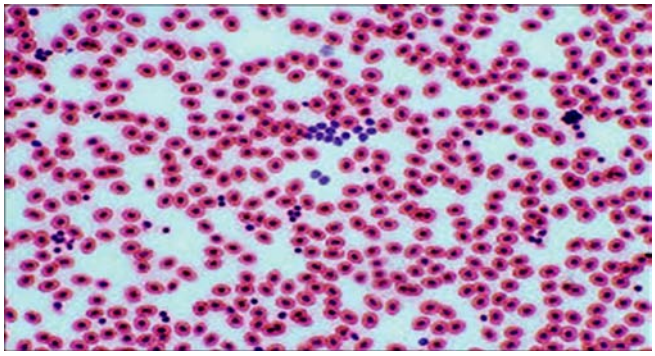

Figure 30: Red blood cells with optimized formulation (F2)

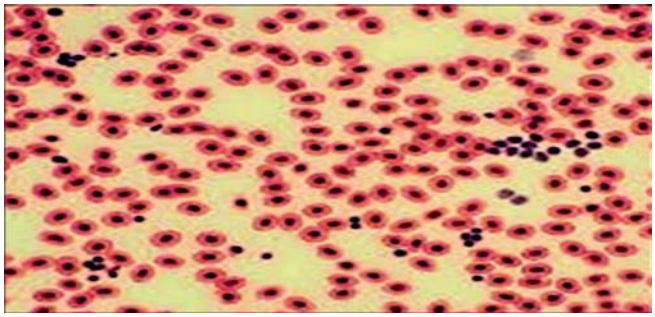

Figure 31: Red blood cells with optimized formulation (F5)

Stability study

Accelerated stability study was selected for 30 days; sample was kept to 40°C ± 20°C, 75% ± 5% relative humidity and room temperature represented stability study of optimized formulations (F2, F5, F11), it is represented in observation Table 4.

CONCLUSIONS

This study evaluated suitability and feasibility of penetration enhancers through thermoreversible *in situ* gel for BRT with a view of enhancing permeability, bioavailability. The result of *ex vivo* diffusion studies indicate that Tween 20 (Surfactant), Disodium EDTA (Chelating agent), Sodium tourocholate (Bile salt) at conc. 0.5% v/v, 0.5% w/v, 0.5% w/v respectively, improve *in vitro* permeation. FT-IR spectrum of optimized formulations revealed that there was no chemical interaction between polymers and drug (BRT); hence, they were found compatible. DSC graphs of optimized formulation revealed that endothermic and exothermic graph of polymer and drug, respectively. This is significant for formulation and development. The addition of increasing concentration of HPMC K4M from 0.1% to 0.5% w/v with increasing penetration enhancers concentrations, Tween 20 (0.5-1% w/v), Disodium EDTA (0.25-0.5% w/v), Sodium tourocholate(0.5-1% w/v) lowered the gelation temperature from 37°C to 31°C regardless of the concentration of viscosifying enhancing polymer, all the formulations gelled at the temperature ranging from 31°C to 37°C. From overall study, the formulations (F2, F5, F11) *in situ* gel seems to be promising formulation for the safe and effective ocular

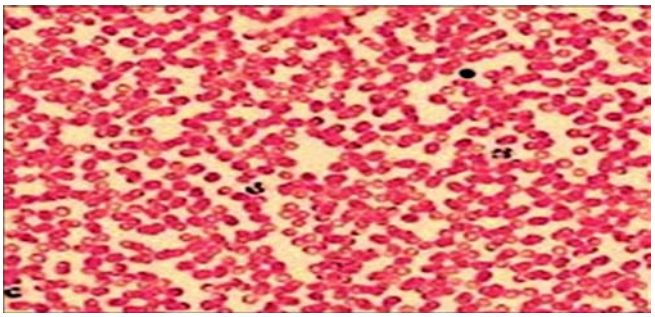

Figure 32: Red blood cells with optimized formulation (F11)

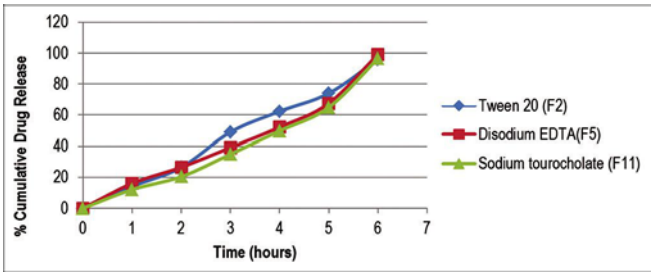

Figure 33: Zero order model of optimized formulations (F2, F5, F11) of thermoreversible *in situ* gel

delivery of BRT for Glaucoma. By considering all evaluation parameters Formulation F2 containing 0.5% v/v Tween 20 as penetration enhancer with 0.5% w/v HPMC K4M as viscosifying agent shows promising improvement in *ex vivo* diffusion, apparent permeability coefficient. The study reflects that the permeation of BRT influenced by various penetration enhancers concentration and also viscosifying agent, permeation was found to be concentration dependent.

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