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Research Article

Design and Evaluation of Ion Activated *In-Situ* Ophthalmic Gel of Brimonidine Tartarate Using Kappa Carrageenan

Vazir Ashfaq Ahmed[1*], Divakar Goli[2]

[1]*M.M.U College of Pharmacy, Ramanagara. Karnataka*
[2]*Acharya & B.M Reddy College of pharmacy Soldevannahalli. Bangalore*

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ABSTRACT
The objective of the study was to develop optimized formulation of *In-situ* gel of Brimonidine tartarate (BT), anti-glaucoma agent using Ion activated polymer; Gelrite as gelling agent, kappa carrageenan as mucoadhesive agent and Hydroxy Propyl Methyl Cellulose (HPMC E50) as release retardant polymer. The 2^3 factorial design was employed to optimize the formulation considering concentration of Gelrite, kappa carrageenan and Hydroxy Propyl Methyl Cellulose as independent variables, mucoadhesive force (N). Viscosity (cP) and *In-vitro* percentage drug release as dependent variables. Based on mucoadhesive force (N), Viscosity(CPS) and *In-vitro* percentage drug release, formulation containing concentration Gelrite (0.39%), kappa carrageenan (0.21%) and HPMC E50 (0.4%) was found to be optimized formulation developed by 2^3 factorial design. Formulation was prepared successfully and assessed for gelling capacity, pH, rheological studies, refractive index, optical clarity, isotonicity and as ocular irriation by hen's egg chorioallantoic membrane (HET-CAM) Test. The overall results of this study revealed that the Brimonidine tartarate/kappa carrageenan *in-situ* system can be used to enhance ocular retention time.

Keywords: Kappa Carrageenan, HET-CAM TEST, Brimonidine Tartarate.

INTRODUCTION

Topical application of drugs to the eye is an established route of administration for the treatment of various eye diseases. The protective mechanisms of the eye, such as blinking, lachrymation and drainage, decreases the bioavailability of drug. Drops and ointments accounts to about 70% of the ophthalmic dosage forms in the market. But these preparations when instilled into eye, rapidly drained away from the ocular surface due to blinking tear flow and nasal drainage of the eye. Only a slight amount of drug is available for its therapeutic effect resulting in frequent dosing application to the eye. So to overcome to these problems, newer pharmaceutical ophthalmic formulation such as *in-situ* gel, nanoparticle, liposome, nanosuspension, microemulsion, iontophoresis and ocular inserts are developed to increase the bioavailability of the drug in a sustained and controlled manner with the aim of localizing the drug on site of action, improving the drug effectiveness, good stability, biocompatibility, easy of application. One such approach is *in-situ* gelling system; *In-situ* forming hydrogels is liquid upon instillation and form viscoelastic gels in response to environmental changes such as pH or temperature[1,2,3]. This *in-situ* gel system shows various advantages like: improved patient compliance, reduced dose, frequency, increased bioavailability, sustain and controlled delivery[4,5]. Mucoadhesive polymer are able to enhance retention time and drug penetration through the corneal barriers because of their bioadhesiveness[6,7]. Brimonidine tartarate is use as a drug to reduce the elevated intraocular pressure in the eye; Brimonidine is more lipophilic and alpha2 selective. The intraocular pressure lowering effect mediated via reductions in aqueous humor production and increase in uveoscleral outflow. The aim of this work is to formulate ocular *in situ* gelling systems using ion activated polymer containing Brimonidine tartarate, and to evaluate the performance of the prepared *in situ* gelling system.

MATERIALS AND METHODS

The following materials are used for the study. Brimonidine Tartarate (FDC Limited Mumbai), Gelrite (Applied biosciences (KELCO). Mumbai), Kappa Carragennan (Gurukrupa industries Ahmedabad) Hydroxyl propyl methyl cellulose E50 LV (LOBA chemicals, Mumbai) Rhodamine B (Amrithal Chemuax Pvt Ltd. Mumbai). All other chemicals were of analytical grade.

In vitro Gelation behavior studies of polymers with simulated tear fluid

The gelling capacity of polymer were determined by placing 1 or 2 drops of polymeric solution in a vial containing 2ml of freshly prepared simulated tear fluid (7.4 pH) equilibrated at 37^0C. The gel formation was visually observed. The stiffness of gel formed and the consistency of the polymeric solution was used as the basis for the evaluation of the gelling capacity[8].

Procedure for preparation of in-situ gels

*Author for Correspondence: mail2vazir@gmail.com

Table 1: Comparison of functional groups peaks (wave no ($cm^{-1)}$ of BT samples testing by FTIR spectroscopy.

S.no	Name of the samples	Characteristic Peak functional group wave length[wave length (cm^{-1}) of Brimonidine Tartarate		
		N-H stretching	-C=N- stretching	C-Br stretching
1	Brimonidine Tartarate (BT)	3400	1652	583
3	BT+Gelrite+kappa carrageenan+ HPMC	3427	1652	574

Added required quantity of gelrite polymer to the borate buffer solution and heated to about 70^0C until it is completely dissolved. To prepared gelrite solutions required quantity of kappa carrageenan, was added and stirred well on magnetic stirrer with slight heating. To the above prepared gelrite/mucoadhesive solution, required quantity of drug (0.2% Brimonidine tartarate) was added with continuous stirring until it is thoroughly mixed. HPMC E LV 50 and phenyl ethyl alcohol were added and stirred on magnetic stirrer. pH was checked and adjusted with the buffer.The prepared *in- situ* gel were filled in glass vials and closed with closures, capped with aluminum caps and sterilized by autoclaving

Design of experiments employing factorial design

Various batches of formulations were prepared by employing 2^3 factorial designs. The independent variables chosen were concentrations of Gelrite, Kappa carrageennan, HPMC E50, The independent variables levels were Gelrite (0.2, 0.4), Kappa carrageenan (0.2, 0.4), HPMC E50 (0.2, 0.4), Levels were assigned after carrying out *in-vito* gelation studies on different concentration ranging from 0.1 to 1% for the responses**.** Mucoadhesive Force Rheological studies (Viscosity before gelling at 50 rpm,) & *In-vitro* release studies (Drug release 10^{th} h) were taken as the response parameters and are categorized as dependent variables.

Optimization data analysis and model validation

ANOVA was used to establish the statistical validation of the polynomial equations generated by Design Expert® software (version 8.0, Stat Ease Inc., Minneapolis, MN). Fitting a multiple linear regression model to a 2^3 factorial design gave a predictor equation which was a first order polynomial, having the form:

$Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_{123}X_1X_2X_3$

Analytical Methods

FTIR Study

Brimonidine Tartarate and physical mixture containing pure drug and polymers were scanned (8400S/Shimadzu Japan) in the wave number region of 400-4000 cm-1 using KBr pellet method[9].

BT+G+K+H*= BT+Gelrite+kappa carrageenan+ HPMC,

Drug-polymer interaction study by the spectral shift method

Drug-polymer interactions between Brimonidine tartarate and polymers like Gelrite, kappa-carrageenan, HPMC E 50LV were studied by the spectral shift method[10]. The overlay spectra of pure drug along with polymers were taken in UV Visible Spectrophotometer (Shimadzu, Japan) by scanning from 200-400nm and the λ max was found out.

Mucoadhesive strength by modified balance method

The Mucoadhesive strength was measured using a modified two arm balance[11]. Fresh gastric mucosa of sheep was obtained from the laughter house and was preserved in normal saline. The biological membrane was fixed to the inverted bottom surface of a 100ml beaker; this was then placed in a larger beaker with membrane facing upward. Simulated tear fluid (7.4) was added into the larger beaker up to the upper surface of the gastric mucosa such that the media remains just moistened with the media. Accurately weighted 1gram of preformed gel was put on the inverted beaker and was placed under the bottom of stainless steel pan. A preload of 50g was placed on the pan for 5 min (preload time) to establish adhesion bonding between gel and biological membrane. The preload and preload time were kept constant for all the formulations. After completion of preload time, preload was removed from the pan and another beaker was placed on to other side of the pan. The addition of water was stopped when the other side of the pan got detached from the membrane. The mass, in grams required to detach the pan from membrane gave the measure of mucoadhesive strength.

Rheological studies

Viscosity of the instilled ophthalmic solution is an important factor in determining residence time of the drug

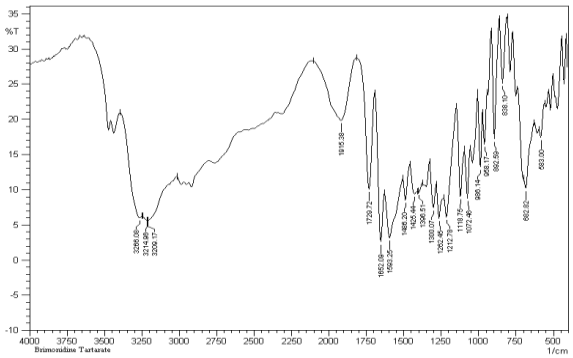

Figure 1: IR Spectrum of BT

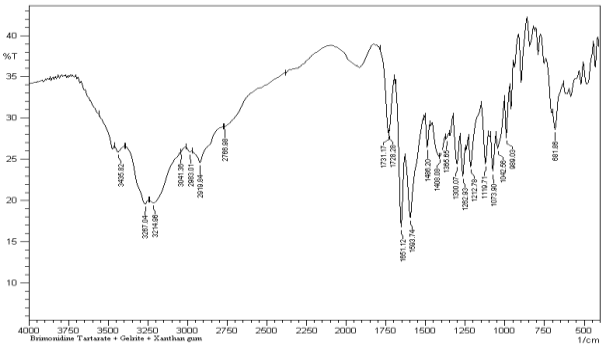

Figure 2: IR Spectrum of with BT with physical mixtue of polymers

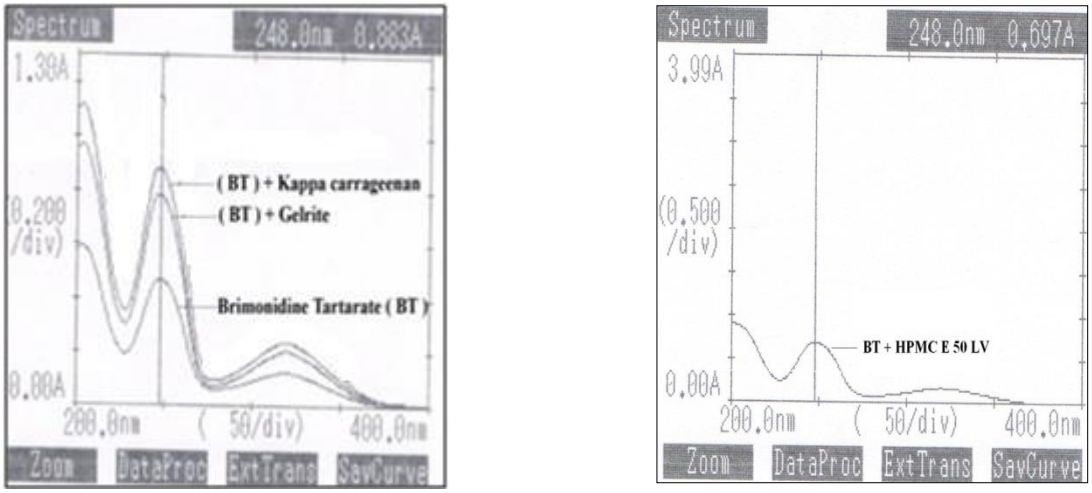

Figure 3: UV Overlay Spectra of Brimonidine Tartarate (BT) with polymers.

Table 2: Compositions of Different Polymer Concentrations.

Batch code	Gelrite (%)	Kappa Carrageenan (%)	HPMC E50(%)	Mucoadhesive force* (N)	Viscosity(CPS *)At 50 RPM*	CDR*10thh (%)
F1	0.4	0.4	0.2	4.41	179	62.13
F2	0.2	0.2	0.2	4.50	60	63.44
F3	0.4	0.4	0.4	7.21	328	41.47
F4	0.2	0.2	0.4	6.49	70	73.40
F5	0.2	0.4	0.4	4.41	104	76.88
F6	0.4	0.2	0.2	6.84	241	80.82
F7	0.4	0.2	0.4	4.09	195	90.81
F8	0.2	0.4	0.2	5.12	93	95.40

*Standard Deviation (n=3)

in the eye[12]. Rheological behaviors of different ratio of *in-situ* gelling polymeric solutions were evaluated on a Brook Field's DV-I+ model. Based on the viscosity range and torque the spindles were selected. The temperature was maintained by circulatig water at 37ºC across the sampler. For gelation, the sample solution was mixed with simulated tear fluid in 25 µl: 7µl ratio. The angular viscosity was increased gradually from 10 to 100 rpm with equal wait for each rpm. The viscosity measured at both the conditions was plotted (angular viscosity versus the angular velocity (RPM).

In-vitro release studies

The *in-vitro* drug release was studied by using a USP rotating paddle apparatus[13]. Simulated tear fluid 7.4 maintained at 37ºC was used as the medium. The paddle speed was set to 50 rpm. 3ml of the formulation was placed in a dialysis tube with cellophane membrane covered cells and it was placed such that it just touches the diffusion medium. The drug samples were withdrawn at the interval of one hour for a period of ten hours from the medium and were analyzed by U.V spectrophotometer at their respective wavelength using simulated tear fluid as blank. The cumulative percentage drug release and release kinetics were evaluated.

pH

The pH of the prepared *in-situ* gelling system was measured using pH meter[14].

Refractive index

Refractive index of the formulation was determined by Abbe refractometer[15]. Light was turn on. Opened incident prism and the prism face were carefully cleaned with acetone and after drying it was bolted. On polished surface of the lower refracting prism, drops of formulation were place. Hinged upper incident prism was locked with knob, so that the liquid on the face of refracting prism gets evenly distributed. Dispersion correction knob was used to align the X-Mark in the eye piece with the shadow boundary separating the dark and bright area.Centered the boundary in the crosshairs of the telescope using the lower large adjustment knob and read the refractive index on the scale.

Optical Clarity studies

Optical clarity of solutions/gels was carried out by using UV Visible Spectrophotometer (Shimadzu, 1700 Japan) against simulated tear fluid (7.4) as reference[16].

Formulation was placed in a glass cuvette containing simulated tear fluid, care was taken to avoid air bubbles and the cuvette was inverted up and down to confirm gel formation. Transmission of light was measured at 580nm and it was kept constant for all batches.

Isotonicity Evaluation

Sheep blood was obtained from the slaughter house in a container containing 4% of tris-sodium citrate[17]. Few drops of the formulation was taken china dish and added few drops of blood and gently shaken for mixing blood and formulation. Blood sample was drawn from the china dish in to RBC pipette up to 0.5 mark and further diluted with RBC diluting fluid. On the haemocytometer, a drop of

Table 3: Constraints for various responses by 2^3 factorial design.

	Constraints			
Name	Goal	Lower limit	Upper limit	Importance
Gelrite	is in range	0.2	0.4	3
Kappa Carrageenan	is in range	0.2	0.4	3
HPMC E50	is in range	0.2	0.4	3
Mucoadhesive Force	is target = 45	34.54	86.23	2
Viscosity Before Gel at 50 RPM	is target = 200	58	332	4
Invitro release at 10h	is target = 95	40.38	95.4	5

Table 4: Predicted and Experimental Observed Responses of the Optimized Formulation with % Prediction Error.

Number	Gelrite (%)	Kappa carragennan (%)	HPMC E50 (%)	Muco-adhesive Force (gr)	Viscosity Before Gel at 50 RPM	Invitro release at 10h (%)	Desir-ability
Predicted value							
1	0.39	0.21	0.40	45.00	200	86.21	**0.973**
Observed value							
2	0.39	0.21	0.40	46.50	192	88.69	
% predicted error							
				3.22	04.16	02.79	

sample was placed and covered with a cover slip on the counting chamber. By placing the counting chamber on the mechanical stage of the microscope the cells were observed. The tonicity of the formulation was checked under the microscope (45x) for the effect on RBC for cremation or swelling and bursting.

Ocular Irritation Test (HET-CAM Test)

Procedure

In this test, 9th day incubated White Leghorn chicken eggs weighing between 50 and 60 g was selected. Marked air cell of the egg and placed it on the egg cup holder[18]. With help of a dentist blade, a window (2 × 2 cm) was made on the egg air cell, pared off the outer shell. With the forceps, the outer membrane was removed and care was taken to ensure that the inner membrane CAM (chorioallantoic membrane) was not injured. About 0.3 ml of formulation, positive control and negative control was applied directly onto the CAM surface and left in contact for 5 minutes. Monitored and recorded the time for the appearance of each of the noted endpoints in minutes. Positive Control: 0.3 mL of 0.1N NaOH to provide a baseline for the assay endpoints Negative Control: 0.3 ml of 0.9% NaCl solution to provide a baseline for the assay endpoints. Treatment: 0.3 mL of formulation on the chorioallantoic membrane of the 9th day egg. Observed the reactions on the CAM were observed for a period of 300 seconds (0.5 min, 2 min and 5 min). Monitored and recorded the time for the appearance of each of the noted endpoints, in minutes.

End points

Observed endpoints are: Hemorrhage (bleeding from the vessels), Vascular lysis (blood vessel disintegration) Coagulation (intra and extra-vascular protein denaturation) on CAM.

Irritation Scores

Ocular visualization of in-situ gels with flurophores (rhodamine B)

Two drops of the sterile formulation with rhodamine B (0.01%) were instilled into the rabbit eye[19]. (One eye

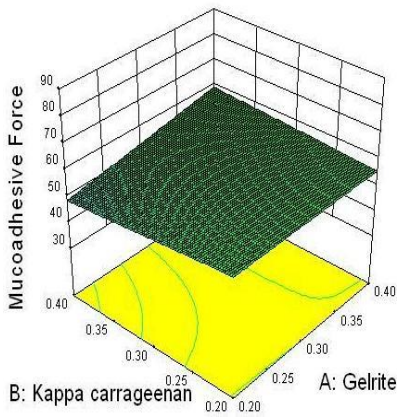

Figure 4: 3D Graph showing the effect of gelrite and kappa carrageenan on mucoadhesive force.

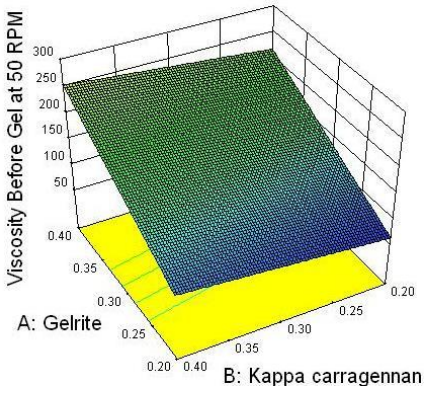

Figure 5: 3D Graph showing the effect of gelrite and kappa carrageenan on Viscosity Before Gel at 50 RPM.

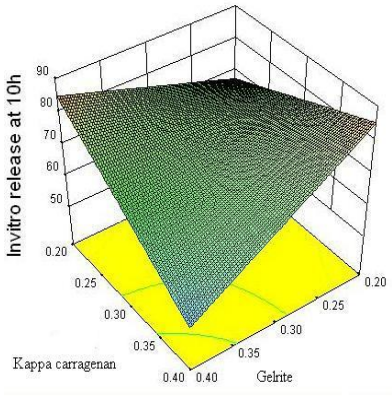

Figure 6: 3D Graph showing the effect of gelrite and kappa carrageenan on cumulative drug release at 10th hour.

Table 5: Compositions of Optimized formulation by 2^3 factorial design.

Formulation code	Ingredients %					
	Brimonidine Tartarate	Gelrite	Kappa carrageenan	HPMC E 50LV	Phenyl ethyl alcohol	Borate buffer q.s
Optimized formulation	0.2%	0.39%	0.21%	0.4%	0.5%	100 ml

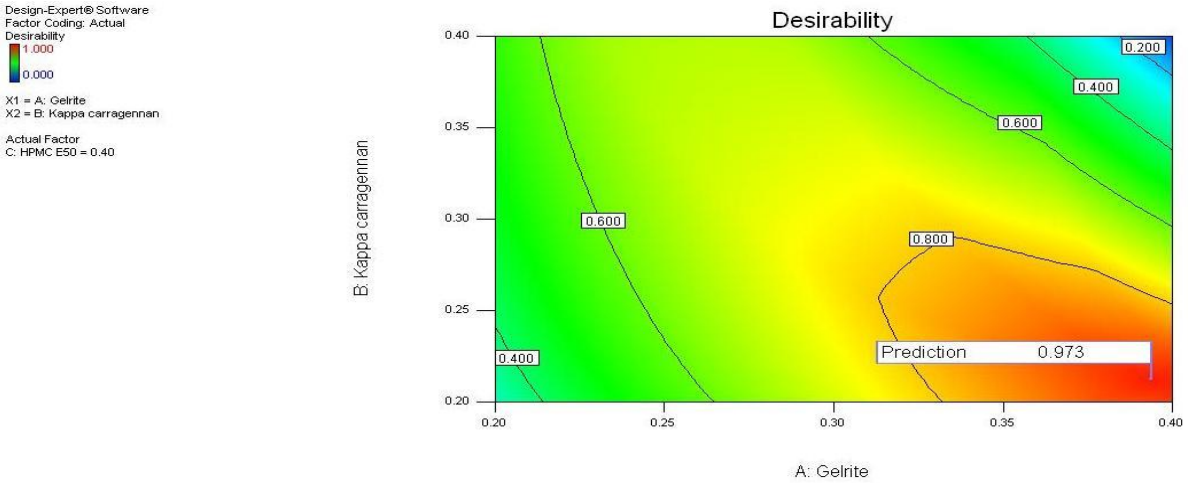

Figure 7: Desirability plot related to the given data.

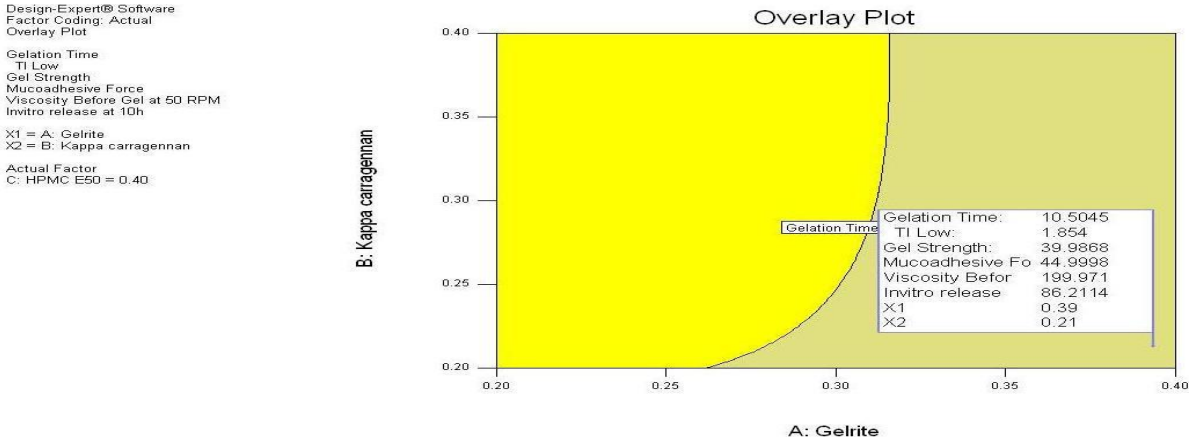

Figure 8: Overlay graph of formulation optimization highlighting an area of operability.

served control and other eye as test). The eyelids were held close for few second; the *in-situ* gel so formed was visualized.

RESULTS AND DISCUSSION

FTIR studies, the characteristic absorption bands of important functional group of pure drug were identified which confirm the structure of the drug. Similar peaks were identified in the spectrums of physical mixture with minor differences in frequencies. Hence the drug had no interaction with polymers. (Fig No.1, 2, Table No.1) Drug-polymer interaction*:* spectral shift method was employed to study the interaction between Brimonidine tartarate and polymers like Gelrite, kappa- carrageenan, and HPMC E 50 LV. There was no shift in the λ max of brimonidine tartarate (248nm) in the presence of polymers as shown in overlay spectrums. This study revealed non existence of interaction between drug and polymer. (Fig No.3). 2^3 factorial design was employed to under the factors that are critical for the response. The main effect study and interaction study of factors reveals that concentration of the polymer plays as important role in viscosity, mucoadhesive study and % drug release in the development of formulation. (Table No.2)

Polynomial equation coded factor

Mucoadhesive force=54.98+2.59*A-1.00*B+1.71*C+2.72*A*B-1.56*A*C+3.64*B*C+10.53*A*B*C

From the ANOVA test the models of mucoadhesive force was found to be significant (P<0.05).As shown in the equation, the factors have a significant effect on the mucoadhesive force. The variables such as concentration of gelrite (A) and HPMC (C) have a positive effect where as kappa carrageenan (B) has negative effect on mucoadhesive force. And increase in concentration of B will decrease the mucoadhesive force. Increasing the

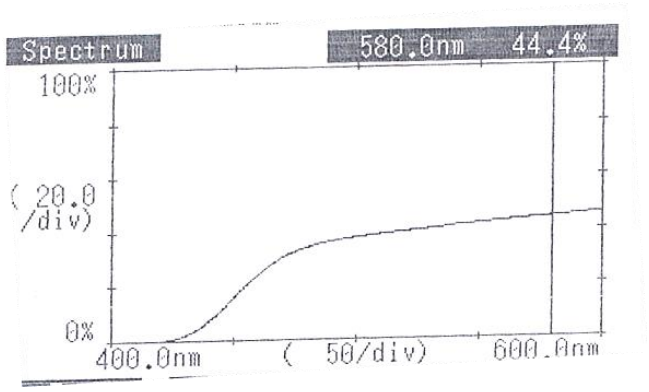

Figure 9: Relative % transmittance of BT Optimized formulation before gel

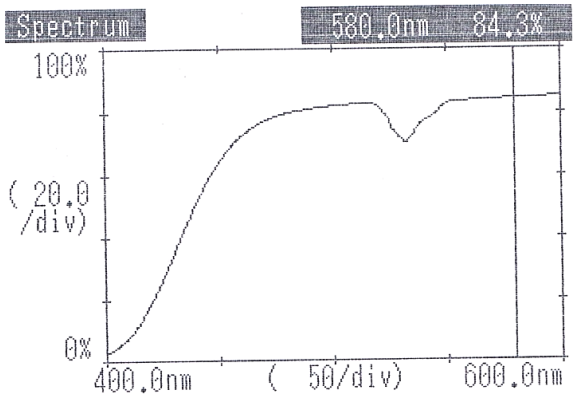

Figure 10: Relative % transmittance of BT*optimized formulation after gel

Table 6: Composite evaluation parameter of optimized Formulation

S. No	Evaluation parameters	Optimised Formulation
1	pH	7.46±0.094
2	Refractive index	1.350±0.016
3	Clarity(Before gel)	44
4	Clarity(After gel)	84
5	Mucoadhesive force	46.50±3.48
6	Viscosity before gel at 50 RPM	192±04.89
7	Invitro drug release at 10^{th} h	88.69±3.21
8	Isotonicity	Isotonic
9	Ocular tolerance	Non irritant
10	Sterility test	sterile
11	Ocular visualization of in-situ gels	Easy to instill

*Standard Deviation (n=3)

Table 7: Kinetic model for response (Cumulative Drug Release (%) of optimized formulation

Order	Batch Code	Optimized Formulation
Zero order	R2	0.88
	K0	7.47
	SSR	663.61
First order	R2	0.97
	K1	-0.0841
	SSR	0.018
Higuchi equation	R2	0.987
	KH	26.71
	SSR	66.59
Koresmeyer peppas equation	R2	0.979
	Km	1.53
	SSR	0.0023
	n	0.481

K0= zero-order rate constant, SSR= Sum of Squares due to Regression, K1= first order constant, KH= Higuchi dissolution constant, Km= kinetic constant, n= Release exponent

concentration of factors doesn't have much effect on mucoadhesive force. So selection of appropriate concentration of mucoadhesive is important. The surface response plot shows the response of gelrite and kappa carrageenan. The results are shown in Fig No 4.

Viscosity before Gel =158.71+77.21*A+17.38*B+15.54*C+0.54*A*B+10.21 *A*C+24.54*B*C+24.21*A*B*C

From the ANOVA test the models of viscosity before gel at 50 rpm obtained for different batches was found to be significant (P<0.05). As shown in the equation, the factors have a significant effect on viscosity before gel. The variables such as concentration of gelrite (A) kappa carrageenan (B) and HPMC (C) have a positive effect on the viscosity. This means as increase in concentration of A, B and C will show increase viscosity. Higher concentration level of gelrite and kappa carrageenan gave higher viscosity the factors can be observed in 3D response surface plot. The results are shown in Fig No 5

Cumulative Drug Release (%1^{st}h=19.88-1.55*A+0.41*B-2.46*C-3.63*A*B-1.93*A*C-7.00*B*C- 1.13*A*B*C

Cumulative Drug Release (%) 10^{th}h=72.20-4.22*A-3.95*B-2.40*C-12.89*A*B-0.13*A*C-7.52*B*C-0.14*A*B*C

From the ANOVA test, the models of *In-vitro* release was found to be significant (P<0.05). As shown in the equation, the factors have a significant effect on cumulative drug release. At 1^{st} h the variables such as gelrite (A) and HPMC (C) have a negative effect on drug release. Which means that A and C has drug release controlling capacity.

Whereas B at $1^{st\ h}$ is not able control the drug release. Hence B has shown positive effect. AB, AC, BC has shown negative effect. At 10^{th}h, all polymers A, B, C and their combination AB, AC, BC has shown negative effect which indicates that increase in polymer concentration will reduce the % drug release. Which is significant for drug release? The surface response plot shows that negative effect is more by gelrite and HPMC E50 at 1^{st} h. and at10^{th} h all polymers have prominent negative effect. The results are shown in Fig No 6. Interaction studies of factors reveal that concentration of kappa carrageenan, gelrite, and HPMC E 50 are critical factors. Concentration of kappa carrageenan should be carefully chosen in order to have proper mucoadhesive property. Desirability approach was utilised by setting a target in order to have a formulation which will have required properties of mucoadhesive

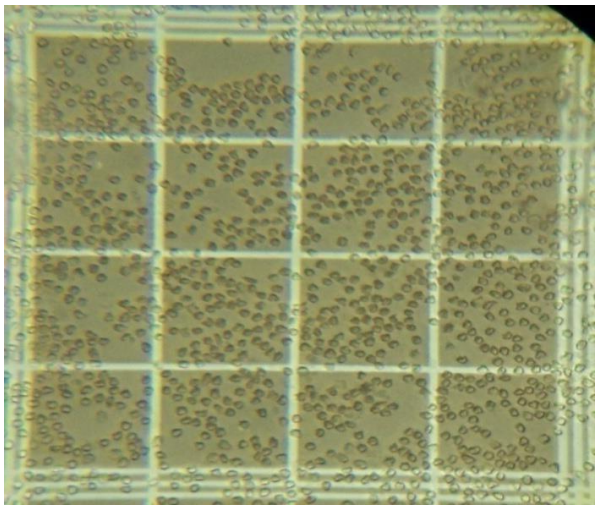

Figure 11: RBC'S without Formulation

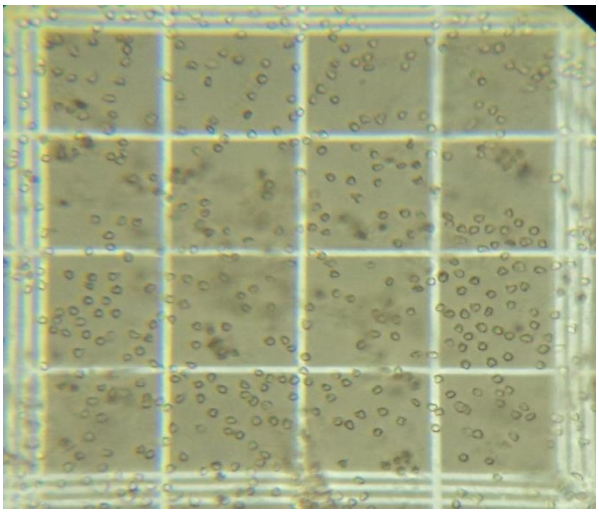

Figure 12: RBC'S with optimized Formulation

Table 8: Summary of HET-CAM Score Ranges Used in Irritancy Classification

HET-CAM Score Range	Irritation Category
0-0.9	Non-irritant or Practically None
1-4.9	Weak or Slight Irritation
5-8.9 or 5-9.9	Moderate Irritation
9-21 or 10-21	Strong or Severe Irritation

Table 9: HET-CAM Scoring System

Endpoint	Score at Different Observation Times		
	0.5 min	2 min	5 min
Hyperemia	5	3	1
Haemorrhage	7	5	3
Coagulation	9	7	5

property, viscosity and *in-vitro* drug release (Table No.3). These were further evaluated for the optimization responses (mucoadhesive property, viscosity and *in-vitro* drug release) in order to confirm the validity of optimization process, Formulations exhibiting desirability like 0.962, close to 1 were selected as optimized formulation. The statistically optimized formulation fulfilled all the physicochemical criteria. The observed values were in close agreement with the model predictions. The relative errors (%) between the predicted and experimental values for each response were calculated, and the values found to be within 5%. The experimental values were in agreement with the predicted values, confirming the predictability and validity of the optimization process (Table.No.4, 5 Fig No 7, 8). *In-vitro* release studies showed that HPMC E50 LV act as release retardant. From the kinetic study it was found the drug release from the optimized formulation followed first-order kinetics, since a straight line was obtained. From higuchi plots, the plots were found to be linear which indicates the drug release from the *in-situ* gel was by diffusion. The 'n' values obtained from the peppas equation were less than 0.5, which indicates the drug release by fickian diffusion mechanism (Table No.7). The pH of formulations was within the range of comfort (6.8 to 7.8), Hence formulation will be tolerated by the eyes. (Table No.6). Solutions showed less % transmittance bcoz of the presence of polymers. Formed gels (mixing with simulated tear fluid (pH 7.4) showed greater % transmittance compared to solutions. Gels with optical transmission ≥90% are termed as transparent, ≤ 90% but ≥10% as translucent, and ≤ 10% as opaque. The study reveals that in-*situ* gels were translucent. The sol-gel are dropped in the cul-de-sac where it form a gel, the so formed gel will not spread over eye. (Figure No.9, 10) Rheological studies manifested that the shear stress and viscosity at 37ºC with simulated tear fluid were higher than those at 25ºC without simulated tear fluid. It was noted from the various literature that the solution before gelling should have a viscosity of 5 to 1000cps and after gelling in the eye a viscosity from about 50-50,000 cps. The ocular shear rate is about $0.03s^{-1}$ during inter-blinking periods and $4250\text{-}28500s^{-1}$ during blinking. Viscosity of the solution ranged from 27-351 cps before gelation and 300 to 675 cps after gelation. Viscoelastic fluids having high viscosity under low shear rates and low viscosity under high shear rates, i.e. Pseudo plastic fluid is often preferred. This may favour the sustained release of drug in the conjunctival sac of the eye and also without much blinking difficulty for shear thinning. Refractive index of tear fluid is 1.340 to 1.360. Formulation showed refractive index 1.350. Hence it does not cause impairment the vision or discomfort to the patient (Table No.6). Formulation showed no changes in size and shape of RBC's cells (neither hypertonic nor hypotonic). This qualitative study showed that formulations are isotonic with blood. (Figure No.11, 12) Formulations scoring were compared with those obtained using normal saline, 0.1N NaoH as controls. A means score of 0 was obtained for normal saline as well as for *In-situ* gel based formulation up to 5 minutes and no change was see after 5 minutes also. The scoring for 0.1N NaoH found to be 15.00/10.20. Study shows that the formulation was non irritant, as results obtained by HET-CAM and those of the positive and negative controls (Fig No.13, 14, 15). Ocular visualization showed that *in-situ* gels were quickly formed when it

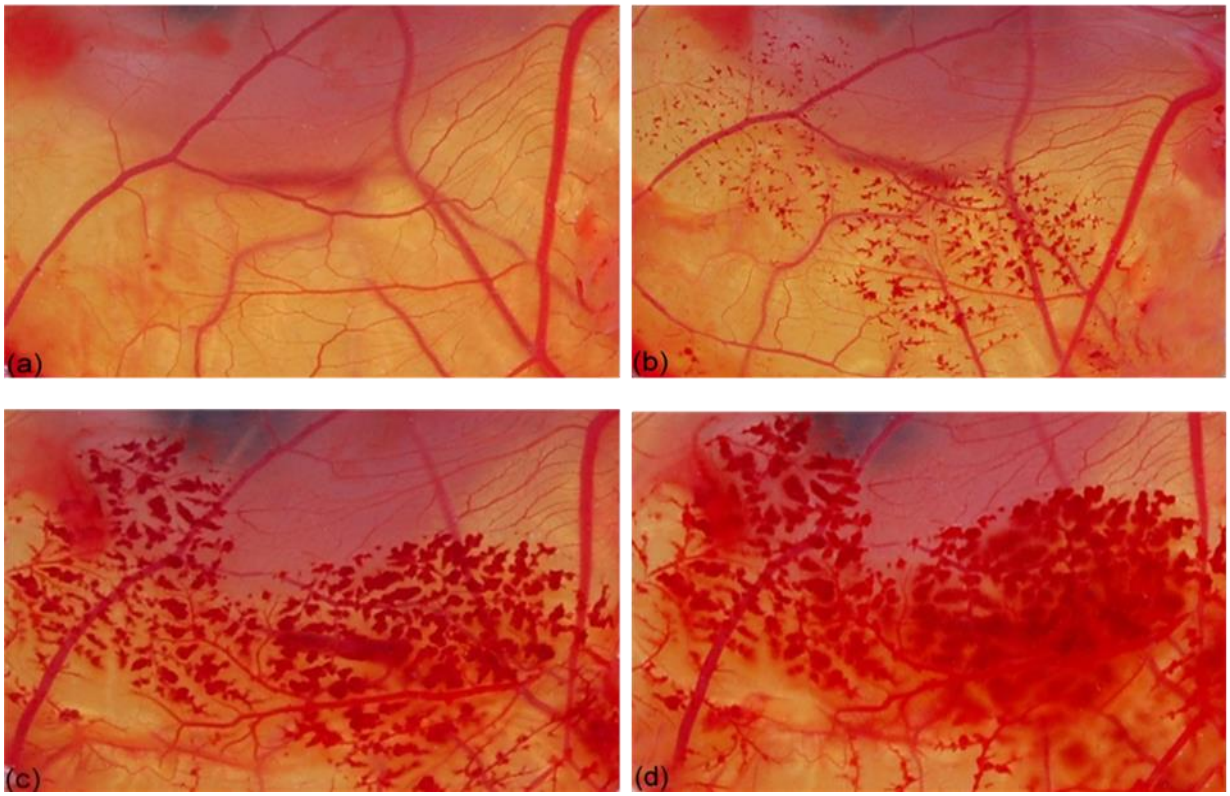

Figure 13: CAM Membrane with 0.1N NaoH at 0, 0.5, 2 and 5 min

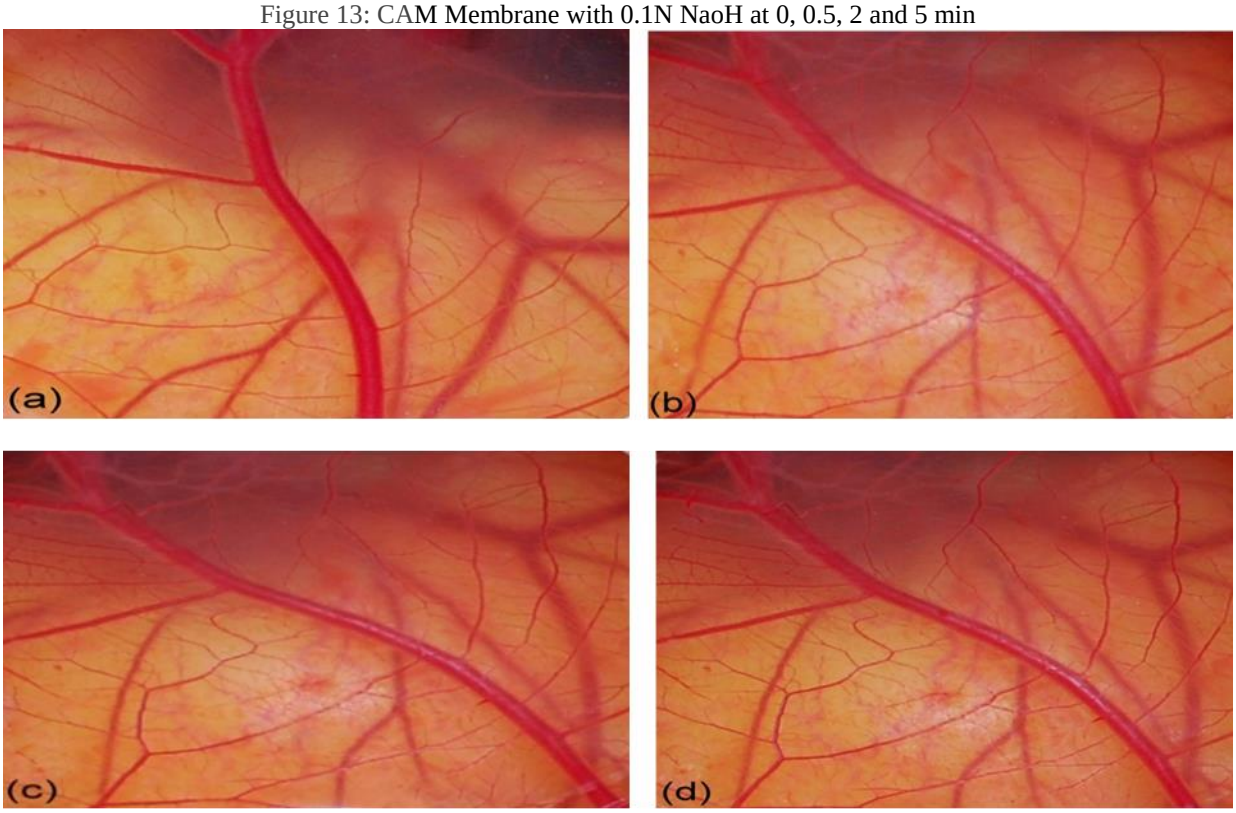

Figure 14: CAM Membrane with 0.9% Nacl at 0, 0.5, 2 and 5 min

comes in contact with lachrymal fluid. Hence it is easy to instil in the eye (Fig No.16).

CONCLUSION

The present studies, therefore, shows the successful formulation of ion activated *In-situ* gels of Brimonidine Tartarate using Gelrite, kappa carrageenan and HPMC E50 as suitable polymers. The application of experimental design methodology helped to prepare the optimized formulation, which showed appropriate mucoadhesive

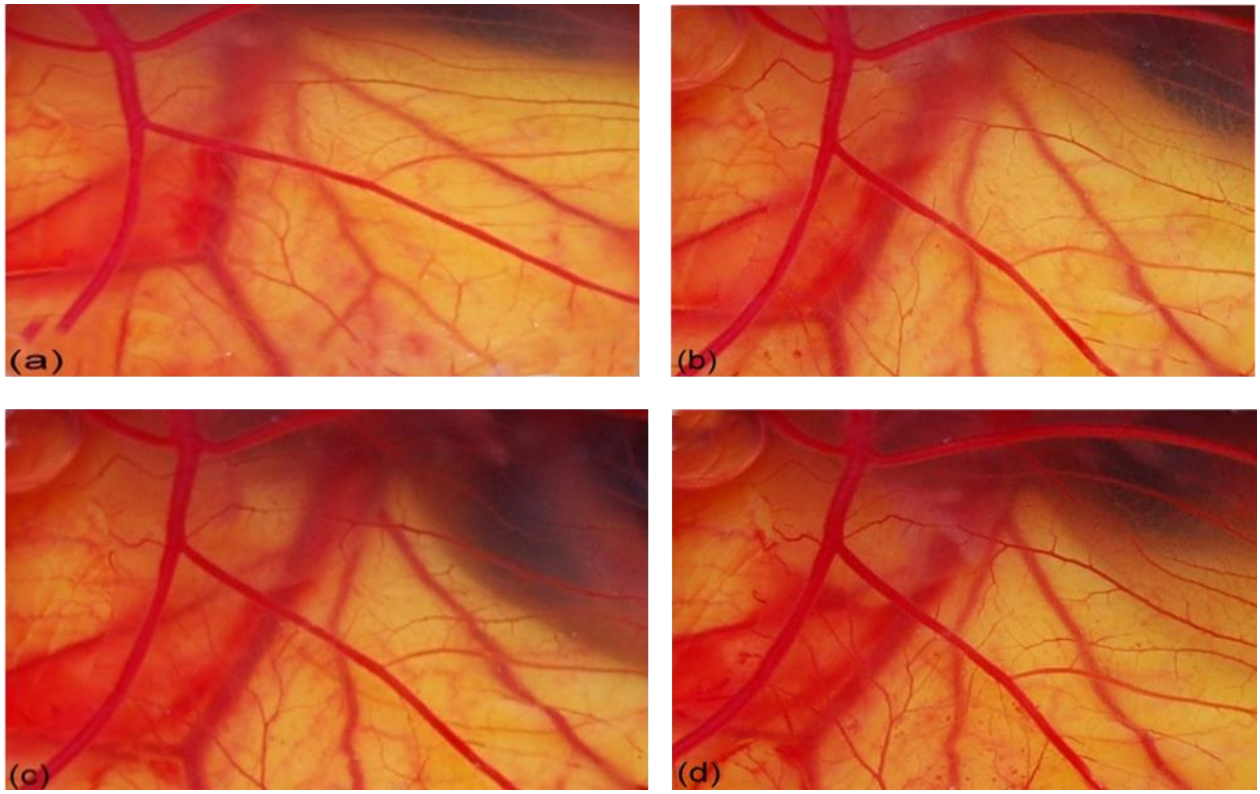

Figure 15: CAM Membrane optimized Formulation at 0, 0.5, 2 and 5 min

Figure 16 **(A):** Normal rabbit right eye.(RE) **(D):** optimized Formulation (coloured gel formation) with Rhodamine B dye.(RE)

force and *In-vitro* percentage drug release. From the factorial design, the optimum concentrations of Gelrite, HPMC E50 and kappa carrageenan as mucoadhesive for *in-situ* ocular drug delivery system were 0.39%, 0.4% and 0.21% (w/v), respectively. The drug release to be diffusion controlled and followed Higuchi kinetics for the formulation. FTIR spectroscopy study reveals no significant interaction between drug and polymers. So it is concludes that the drug to be compatible with polymers,

Ocular irritation studies showed absence of Hyperemia, Haemorrhage and Coagulation. We can conclude that an optimized formulation was non irritant, as results obtained by HET-CAM and with those of the positive and negative controls. Ocular visualization shows that sol- gel transition takes place quickly when it comes in contact with lachrymal fluid. The effect of combining a mucoadhesive polymer to gelrite showed its ability to enhance bioavailability through its greater mucoadhesive strength which indicates longer precorneal residence time and its ability to sustain the release of the drug. Therefore, the Gelrite/ kappa carrageenan *in-situ* gel be use as ophthalmic drug delivery system.

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