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Research Article

STERCULIA FOETIDA GUM: PREPARATION AND EVALUATION OF OPHTHALMIC *IN-SITU* GEL OF STERCULIA FOETIDA GUM CONTAINING BRIMONIDINE TARTRATE

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

*[1] Department of Pharmaceutics. M.M.U College of Pharmacy, Ramanagara. Karnataka, INDIA.
[2] Department of Pharmaceutics. Acharya. BM Reddy College of Pharmacy. Soldevannahalli. Bangalore, INDIA.

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ABSTRACT

Objective: *The goal of this study was to develop and characterize an ion-activated in situ gel-forming Brimonidinetartrate (BT), solution eye drops containing sterculiafoetida gum as a mucoadhesive polymer.* ***Method:*** *sol-gel formulation was prepared by using gellan gum as an ion-activated gel-forming polymer, sterculiafoetida gum as mucoadhesive agent and hydroxy propyl methyl cellulose (HPMC E50LV) as release retardant polymer. Phenyl ethyl alcohol as preservatives in borate buffer. The 2^3 factorial design was employed to optimize the formulation considering the concentration of gelrite, sterculiafoetida gum and hydroxy propyl methyl cellulose as independent variables, gelation time, gel strength, mucoadhesive force (N). Viscosity (cP) and In-vitro percentage drug release were chosen as dependent variables. The formulation was characteristics for pH, clarity, isotonicity, sterility, rheological behavior, and in-vitro drug release, ocular irritation, and ocular visualization.* ***Result:*** *Based on desirability index of responses, the formulation containing a concentration of gelrite (0.24%), sterculiafoetida gum (0.13%) and hydroxy propyl methyl cellulose (HPMC E50 (0.4%) were found to be the optimized formulation concentration developed by 2^3 factorial design. The solution eye drops resulted in an in-situ phase change to gel-state when mixed with simulated tear fluid (STF). Drug release from the gel followed non-fickian mechanism with 94% of drug released in 10 h, thus increased the residence time of the drug.* ***Conclusion:*** *An in-situ gelling system is a valuable alternative to the conventional system with added benefits of sustained drug release which may ultimately result into improved patient compliance.*

KEYWORDS: *Sterculia Foetida gum, Hen's Egg Test – Chorioallantoic Membrane [HET-CAM] Test Brimonidinetartrate.*

INTRODUCTION

Eye diseases are treated by topical application of ophthalmic formulations; drugs dosed topically on eye penetrate the cornea and because of close proximity rapidly gain access to the diseased tissues. Several types of dosage forms like solutions, suspensions, powder for reconstitution and semisolid preparations like ointments and gels can be used to design delivery system for topical application of drugs to the eye. Among the various delivery systems, the most preferred dosage form is the eye drop solution. The eye drop dosage form is easy to instil bud suffer from the inherent drawbacks like tear drainage, passage via the naso-lacrimal duct into the GI tract, leading to side effects. Rapid elimination of the eye drop administered often results in a short duration of therapeutic effect making frequent dosing regimen necessary. Ocular therapy would be significantly improved if the precorneal residence time of drugs could be increased [1, 2]. Gels systems are better retained in the eye than conventional sys drops and better tolerated by patients than insets and ointments. Like ointments, gels are also difficult to administer for some patients. In this respect in-situ gels are interesting since these are conveniently dropped as a solution into the conjunctival sac, where they undergo a transition into a gel with its favourable residence [3]. A further approach to optimize the ocular dosage form is the incorporation of the mucoadhesive polymers. Interactions of suitable natural and synthetic polymers with the mucus layer of eye tissues increases the precorneal residence time of the preparation. The intimate contact may result in high drug concentration in the local area and hence high drug flux through the absorbing tissue [4-7].

The aim of this work is to formulate Brimonidinetartrate ocular *in situ* gelling system containing sterculiafoetida gum as a mucoadhesive polymer, and to evaluate the performance of the prepared *in situ* gelling system.

***Corresponding author:**
Vazir Ashfaq Ahmed
Department of Pharmaceutics,
M.M.U College of Pharmacy, Ramanagara,
Karnataka, INDIA.
** E-Mail: mail2vazir@gmail.com*

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MATERIALS & METHODS

The following materials are used for the study. Brimonidinetartrate (FDC Limited Mumbai), Gelrite (Applied biosciences (KELCO) Mumbai), sterculia foetida gum (YUCCA enterprise, Mumbai), Hydroxyl propyl methyl cellulose E50 LV (LOBA chemicals, Mumbai) Rhodamine B (Amrithal Chemuax Pvt Ltd. Mumbai). All other chemicals were of analytical grade.

Animals:

With the approval of Institute Animal Ethical Committee (IAEC/ABMRCP/PR/2012-2013/19), the study was performed and the protocol was approved as per CPCSEA guidelines. Albino rabbit (Newzeland white rabbit) were used as test species. The right eye was designated as control and left one as test eye. In the lower conjunctival cul-de-sac, two drops of the formulation were instilled and for few seconds after instillation, eyelids were held together, later normal blinking was allowed.

Purification of Sterculia foetida gum:

About 1% of Sterculiafoetida gum (SFG) powder was taken in distilled water. This solution was stirred at room temperature for 2

hours and it was hastened by heating the solution at 60 ^{0}C for about 1 hour. This solution was later centrifuged in order to remove water insoluble impurities, the supernatatened liquid was discarded and the residue was precipitated into ethanol. The obtained wet, precipitated SFG was dried. The dry, purified SFG was then milled to fine powder [8].

***In vitro* Gelation behavior studies of polymers with simulated tear fluid:** concentrations of gelrite, Sterculiafoetida gum alone and in combinations ranging from 0.1 to 1% were prepared and evaluated for *in- vitro* gelling studies.The gelling time of formulations of different batches was 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°C. The gel formation was visually observed and time for gelation was noted [9].

Procedure for preparation of *in-situ* gels:

Added required quantity of gelrite polymer to the borate buffer solution and heated to about 70 ^{0}C until it is completely dissolved. To prepared gelrite solution required quantity of Sterculiafoetida gum was added and stirred well on a magnetic stirrer with slight heating. To the above prepared gelrite/mucoadhesive solution, required quantity of drug (0.2% Brimonidinetartrate) for their respective batches was added with continuous stirring until it is thoroughly mixed. hydroxy propyl methyl cellulose E50 LV 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, hydroxy propyl methyl cellulose E50 LV, and xanthan gum. The independent variables levels were gelrite (0.2, 0.4), xanthan gum (0.2, 0.4), hydroxy propyl methyl cellulose E50 LV (0.2, 0.4) Levels were assigned after carrying out different trial studies on concentration ranging from 0.1 to 1% for the responses**.** Gelation time, gel strength, mucoadhesive force Viscosity in centipoise (cP) and *In-vitro* percentage drug release 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:

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

Where Y is the measured response associated with each factor level combination; b_0 is an intercept representing the arithmetic average of all quantitative outcomes of eight runs; $b_1$to b_{123} are regression coefficients computed from the observed experimental values of Y. X_1, X_2 and X_3 are the coded levels of independent variables. The terms X_1 X_2, X_2 X_3 and X_1 X_3 represent the interaction terms.

FTIR Study: Brimonidinetartrate and the 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

Measurement of Gel Strength:

A 50 gm of prepared gel (25 formulations:7 stimulated tear fluid maintained at 37°C ratio) was placed in a 100 ml graduated cylinder. A probe was placed on the gel and a weight of 15 gm was placed on the probe. The probe was allowed to penetrate a fixed distance of 5cm (30ml) and the time it took to travel the distance was recorded [10].

Mucoadhesive strength by modified balance method: The mucoadhesive strength was measured using a modified two-arm balance. 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 to 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 the 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 to establish adhesion bonding between gel and biological membrane. Preload was removed from the pan and another beaker was placed on to another 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 [11].

Rheological studies: Viscosity of the instilled ophthalmic solution is an important factor in determining residence time of the drug in the eye. 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 circulating water at 37^{o}C 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 an equal wait for each rpm. The viscosity measured at both the conditions was plotted (angular viscosity versus the angular velocity (rpm) [12].

***In-vitro* release studies:** The *in-vitro* drug release was studied by using a USP rotating paddle apparatus. Simulated tear fluid 7.4 maintained at 37^{o}C 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 UV spectrophotometer at their respective wavelength using simulated tear fluid as blank. The cumulative percentage drug release and release kinetics were evaluated [13].

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

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 the reference. The 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 [14].

Isotonicity Evaluation: Sheep blood was obtained from the slaughter house in a container containing 4% of tris-sodium citrate. Few drops of the formulation were taken china dish and added few drops of blood and gently shaken for mixing blood and formulation. The blood sample was drawn from the china dish into red blood cell (RBC) pipette up to 0.5 mark and further diluted with red blood cell (RBC) diluting fluid. On the hemocytometer, a drop of 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 red blood cell (RBC) for cremation or swelling and bursting [15].

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. 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 chorioallantoic membrane (CAM) was not injured. About 0.3 ml of formulation, positive control, and the negative control was applied directly onto the chorioallantoic membrane (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 chorioallantoic membrane (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: Haemorrhage (bleeding from the vessels), Vascular lysis (blood vessel disintegration) Coagulation (intra and extra-vascular protein denaturation) on chorioallantoic membrane (CAM) [16, 17].

Ocular visualization of *in-situ* gels with fluorophores (rhodamine B**):**

Two drops of the sterile formulation with rhodamine B (0.01%) were instilled into the rabbit eye. (One eye served as control and another eye as the test). The eyelids were held close for few second; the *in-situ* gel so formed was visualized [18].

RESULTS AND DISCUSSION

Compatibility studies of Drug(s) with the polymer(s) using FT-IR Spectrophotometer: FTIR spectra were measured using FTIR spectroscope (8400S/Shimadzu Japan) to determine the possible interactions between drug and polymers. The pure drug, polymers and drug-polymer physical mixture were scanned from 4,000-400cm^{-1} in Shimadzu FTIR 8400S spectrophotometer using KBr pellet method. The IR Spectrums of the physical mixture was compared with those of drug and polymers and matching was done to detect appearance or disappearance of peaks (Table No. 1).

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

Functional group	Frequency range (cm^{-1})	Observed Frequencies of pure drug: (Brimonidinetartrate)	Reported frequencies of drug in physical mixtures BGSTFH*
N-H stretching	3500-3300	3400	3400
Aromatic C-H stretching	3100-3000	3000	2978
N-H bending	1640-1550	1593	1597
C=O stretching	1740-1690	1729	1728
-C=N- stretching	1680-1620	1652	1651
C=C stretching	1600-1400	1486	1481
C-O stretching	1300-1000	1300	1300
OH bending	1085-1050	1072	1070
C-Br stretching	600-500	583	574

BGSTFH*= Brimonidine Tartarate with Gelrite, sterculia foetida gum & HPMC E50LV

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 & 3).

Polynomial equation coded factor:

Gelation time (sec) : As shown in the equation, the factors have a significant effect on the gelation time. The variables such as concentration of gelrite (A) sterculia foetida (B) and HPMC (C) have a negative effect on the gelation time. That means as increase in concentration of A, B and C will show decrease gelation time.Higher concentration level of gelrite gave low value of gelation time at all level. Sterculia foeitda showed less interaction effect compared to gelrite on gelation time (Table No. 4).

Gel Strength: As shown in the equation, the factors have a significant effect on the gel strength. The variables such as concentration of gelrite (A) sterculia foetida (STF) and HPMC (C) have a positive effect of gel strength. That means as increase in concentration of A, B and C will show increase in gel strength (Table No. 5).

Mucoadhesive force: As shown in the equation, the factors have a significant effect on the gel strength. The variables such as concentration of gelrite (A) and HPMC (C) have a positive effect where as sterculiafoetida (B) has negative effect on mucoadhesive force. Which means as increase in concentration of A and C will show increase in mucoadhesive force. And increase in concentration of B will decrease the mucoadhesive force (Table No. 6).

Viscosity before Gel: As shown in the equation, the factors have a significant effect on viscosity. The variables such as concentration of gelrite (A) sterculia foetida (B) and HPMC (C) have a positive effect on the viscosity. That means as increase in concentration of A, B and C will show increase viscosity (Table No. 7).

Cumulative Drug Release (%) 10th h :As shown in the equation; the factors have a significant effect on cumulative drug release. At 1st h the variables such as HPMC (C) have a negative effect on drug release. This means that C has drug release controlling capacity. Whereas gelrite (A) and sterculiafoetida (B) at 1$^{st\,h}$ is not able control the drug release. Hence A has shown positive effect. AB, AC, BC has shown negative effect. At 10thh, all polymers A, B, C and their combination AC has shown negative effect which indicates that increase in polymer concentration will reduce the % drug release. This is significant for drug release (Table No. 8).

Interaction studies of factors reveal that concentration of Sterculiafoetida gum, gelrite, and hydroxy propyl methyl cellulose E50 LV are critical factors. The concentration of xanthan gum should be carefully chosen in order to have proper mucoadhesive property. Desirability approach was utilized by setting a target in order to have a formulation which will have required properties of gelation time, gel strength, mucoadhesive property, viscosity and *in-vitro* drug release (Table No.9).

These were further evaluated for the optimization responses (gelation time, gel strength, mucoadhesive force (N). Viscosity (cP) and *In-vitro* percentage drug release) in order to confirm the validity of optimization process, Formulations exhibiting desirability like 0.906, close to 1 were selected as optimized formulation (Fig. 1).

Table No. 2: Experimental layout of factors

Batch code	Polymers (%)		
	Gelrite	sterculiafoetida gum	HPMC E50
F1	0.4	0.1	0.4
F2	0.4	0.1	0.2
F3	0.2	0.1	0.2
F4	0.2	0.2	0.2
F5	0.2	0.2	0.4
F6	0.4	0.2	0.4
F7	0.4	0.2	0.2
F8	0.2	0.1	0.4

Table No 3: Experimental layout of responses

Batch code	Gelation Time±S.D* (seconds)	Gel Strength ±S.D* (seconds)	Mucoadhesive Force (N)±S.D*	Viscosity (cP)At50 RPM±S.D*	Cumulative % drug release at 10thh±S.D*
F1	05±0.94	097±3.39	6.74±2.77	22±3.29	74.50±0.52
F2	07±0.47	028±3.37	5.52±0.69	27±2.86	84.66±0.69
F3	19±3.29	026±3.85	5.04±0.40	23±4.10	98.53±0.40
F4	15±0.47	015±2.05	3.83±0.77	20±4.49	65.23±0.77
F5	10±1.41	038±3.39	4.62±1.71	24±4.49	97.54±1.71
F6	04±0.47	150±3.38	6.94±0.96	27±2.44	61.36±0.96
F7	05±1.24	062±2.05	5.98±0.95	22±3.68	90.27±0.95
F8	10±0.47	032±1.65	6.12±0.79	17±4.49	98.74±0.79

**Standard Deviation (n=3)*

Table No. 4: ANOVA for response (Gelation Time)

ANOVA for selected factorial model:						
[Partial sum of squares - Type III]					Response: Gelation Time (sec)	
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	550.29	7	78.61	43.88	0.0001	Significant
A-Gelrite	376.04	1	376.04	209.88	0.0001	Significant
B-ST.Foetida	9.38	1	9.38	5.23	0.0361	Significant
C-HPMC E50	108.37	1	108.37	60.49	0.0001	Significant
AB	2.04	1	2.04	1.14	0.3016	Not Significant.
AC	40.04	1	40.04	22.35	0.0002	Significant
BC	9.38	1	9.38	5.23	0.0361	Significant
ABC	5.04	1	5.04	2.81	0.1129	Not Significant
Pure Error	28.67	16	1.79			
Cor Total	578.96	23				
Std. Dev.		1.34		**R-Squared**		0.9505
Mean		9.29		**Adj R-Squared**		0.9288
C.V. %		14.41		**Pred R-Squared**		0.8886
PRESS		64.50		**Adeq Precision**		18.547
Gelation Time =9.29-3.96*A-0.62*B-2.12*C+0.29*A*B+1.29*A*C+0.62*B*C-0.46*A*B*C						

Table No. 5: ANOVA for response (Gel Strength)

ANOVA for selected factorial model:						
[Partial sum of squares - Type III]					Response: Gelation Time (sec)	
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	44186.67	7	6312.38	477.91	0.0001	Significant
A-Gelrite	19040.67	1	19040.67	1441.56	0.0001	Significant
B-ST.Foetida	2646.00	1	2646.00	200.33	0.0001	Significant
C-HPMC E50	12696.00	1	12696.00	961.21	0.0001	Significant
AB	3174.00	1	3174.00	240.30	0.0001	Significant
AC	6144.00	1	6144.00	465.16	0.0001	Significant
BC	486.00	1	486.00	36.79	0.0001	Significant
ABC	0.000	1	0.000	0.000	1.0000	Not significant.
Pure Error	211.33	16	13.21			
Cor Total	44398.00	23				
Std. Dev.		3.63		**R-Squared**		0.9952
Mean		56.00		**Adj R-Squared**		0.9932
C.V. %		6.49		**Pred R-Squared**		0.9893
PRESS		475.50		**Adeq Precision**		64.021
Gel Strength= 56+28.17*A+10.50*B+23*C+11.50*A*B+16*A*C+4.50*B*C+0.00*A*B*C						

Table No. 6: ANOVA for response (Mucoadhesive Force)

ANOVA for selected factorial model:						
[Partial sum of squares - Type III]					Response: Mucoadhesive Force (N)	
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2465.57	7	352.22	43.99	0.0001	Significant
A-Gelrite	1208.70	1	1208.70	150.96	0.0001	Significant
B-ST.Foetida	163.07	1	163.07	20.37	0.0004	Significant
C-HPMC E50	637.16	1	637.16	79.58	0.0001	Significant
AB	440.84	1	440.84	55.06	0.0001	Significant.
AC	3.74	1	3.74	0.47	0.5038	Not significant
BC	12.04	1	12.04	1.50	0.2378	Not Significant
ABC	0.012	1	0.012	1.517E-003	0.9694	Not Significant
Pure Error	128.11	16	8.01			
Cor Total	2593.68	23				
Std. Dev.		2.83		R-Squared		0.9506
Mean		57.18		Adj R-Squared		0.9290
C.V. %		4.95		Pred R-Squared		0.8889
PRESS		288.25		Adeq Precision		19.375
Mucoadhesive Force =57.18+7.10*A-2.61*B+5.158C+4.29*A*B+0.39*A*C-0.71*B*C+0.023*A*B*C						

Table No.7: ANOVA for response (Viscosity before Gel at 50 RPM)

ANOVA for selected factorial model:						
[Partial sum of squares - Type III]					Response: Viscosity Before Gel at 50 RPM	
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.075E+002	7	32.33	1.48	<0.0420	Significant
A-Gelrite	882.01	1	776.04	3.54	<0.0284	Significant
B- S. Foetida	931.03	1	321.37	0.15	<0.0391	Significant
C-HPMC E50	7851.04	1	331.37	0.15	<0.0412	Significant
AB	885.07	1	500.04	0.23	<0.0630	Not Significant
AC	805.09	1	234.04	0.094	<0.0275	Significant
BC	1131.02 267.76	1	135.38	6.21	<0.0240	Significant
ABC	248.39	1	100.42	1.912E-003	<0.0.357	Significant
Pure Error	1.080E+002	16	212.79			
Cor Total		23				
Std. Dev.		04.67		R-Squared		0.9924
Mean		122.96		Adj R-Squared		0.9965
C.V. %		02.33		Pred R-Squared		0.9868
PRESS		784.50		Adeq Precision		1014.315
Viscosity Before Gel = 22.96+1.79*A+2.37*B+4.38*C+18.46*A*B+25.29*A*C+2.38*B*C+8.54*A*B*C						

Table No. 8: ANOVA for response (Cumulative Drug Release (%) 10th h

ANOVA for selected factorial model:						
[Partial sum of squares - Type III]					Response: Viscosity Before Gel at 50 RPM	
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	4456.38	7	636.63	710.51	< 0.0001	Significant
A-Gelrite	723.69	1	723.69	807.68	< 0.0001	Significant
B- S. Foetida	841.00	1	841.00	938.59	< 0.0001	Significant
C-HPMC E50	52.96	1	52.96	59.10	< 0.0001	Significant
AB	391.31	1	391.31	436.73	< 0.0001	Significant
AC	1646.56	1	1646.56	1837.65	< 0.0001	Significant
BC	24.18	1	24.18	26.99	< 0.0001	Significant
ABC	776.69	1	776.69	866.82	< 0.0001	Significant
Pure Error	14.34	16	0.90			
Cor Total	4470.72	23				
Std. Dev.		0.96		R-Squared		0.9968
Mean		83.19		Adj R-Squared		0.9954
C.V. %		01.14		Pred R-Squared		0.9928
PRESS		32.26		Adeq Precision		68.398
Cumulative Drug Release (%) = 83.19-5.49*A-5.92*B-1.49*C+4.04*A*B-8.28*A*C+1.00*B*C-5.69*A*B*C						

Table No. 9: Predicted and Experimental Observed Responses of the Optimized Formulation with % Prediction Error

S.No.	Gelrite	Sterculia Foetida	HPMC E50	Gelation Time	Gel Strength	Mucoadhesive Force	Viscosiy Before Gel at 50 RPM	Invitro release at 10h	Desirability
Predicted value									
1	0.24	0.13	0.40	08.63	49.99	60.79	20.00	91.37	0.906
Observed value									
2	0.24	0.13	0.40	08.51	47.89	58.43	18.89	94.12	
% Predicted error									
				01.41	04.38	04.03	04.87	02.92	

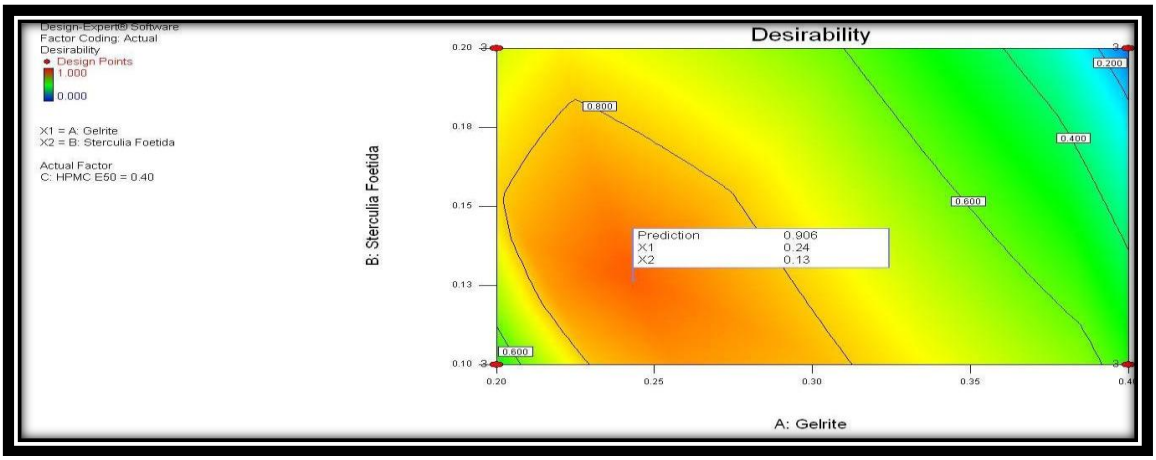

Fig. 1: Overlay graph of formulation optimization highlighting an area of operability

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. *In-vitro* release studies showed that hydroxy propyl methyl cellulose 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

The pH of formulations was within the range of comfort (6.8 to 7.8), Hence formulation will be tolerated by the eyes. 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 is dropped in the cul-de-sac where it forms a gel, the so formed gel will not spread over the eye (Table No.10).Rheological studies manifested that the shear stress and viscosity at 37°C with simulated tear fluid were higher than those at 25°C 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 cP. The ocular shear rate is about $0.03s^{-1}$ during inter-blinking periods and $4250\text{-}28500s^{-1}$ during blinking. The viscosity of the solution ranged from 27-351 cps before gelation and 300 to 675 cP 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.

The formulation incubated with media suitable for the growth and proliferation of aerobic/ anaerobic bacteria, fungi showed no growth at the end of 14 days at 35 °C and at 25 °C. No evidence of microbial growth/ turbidity was found in the test and negative samples when compared with positive control media. This indicated that formulations were free from micro-organisms; which also proved the effectiveness of moist heat sterilization. So the preparations being examined complies with the test for sterility (Table No. 10).Formulation showed no changes in size and shape of red blood cells (RBC) (neither hypertonic nor hypotonic). This qualitative study showed that formulations are isotonic with blood (Table No. 10).

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 seen after 5 minutes also. The scoring for 0.1N NaOH found to be 15.00/10.20. The study shows that the formulation was non irritant, as results obtained by Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) and those of the positive and negative controls (Fig. 2, 3 & 4).

Ocular visualization showed that *in-situ* gels were quickly formed when it comes in contact with the lachrymal fluid. Hence it is easy to instill in the eye (Fig No.5).

Table No. 10: Composite evaluation parameter of optimized Formulation

S. No.	Evaluation parameters	Optimised Formulation
1	pH	7.43±0.0169
2	Clarity(Before gel)	40
3	Clarity(After gel)	61
4	Mucoadhesive force	58.43±3.45
5	Viscosity before gel at 50 RPM	18.89±4.25
6	*In-vitro* drug release at 10^{th} h	94.12±4.12
7	Isotonicity	Isotonic
8	Ocular tolerance	Non-irritant
9	Sterility test	Sterile
10	Ocular visualization of in-situ gels	Easy to instill

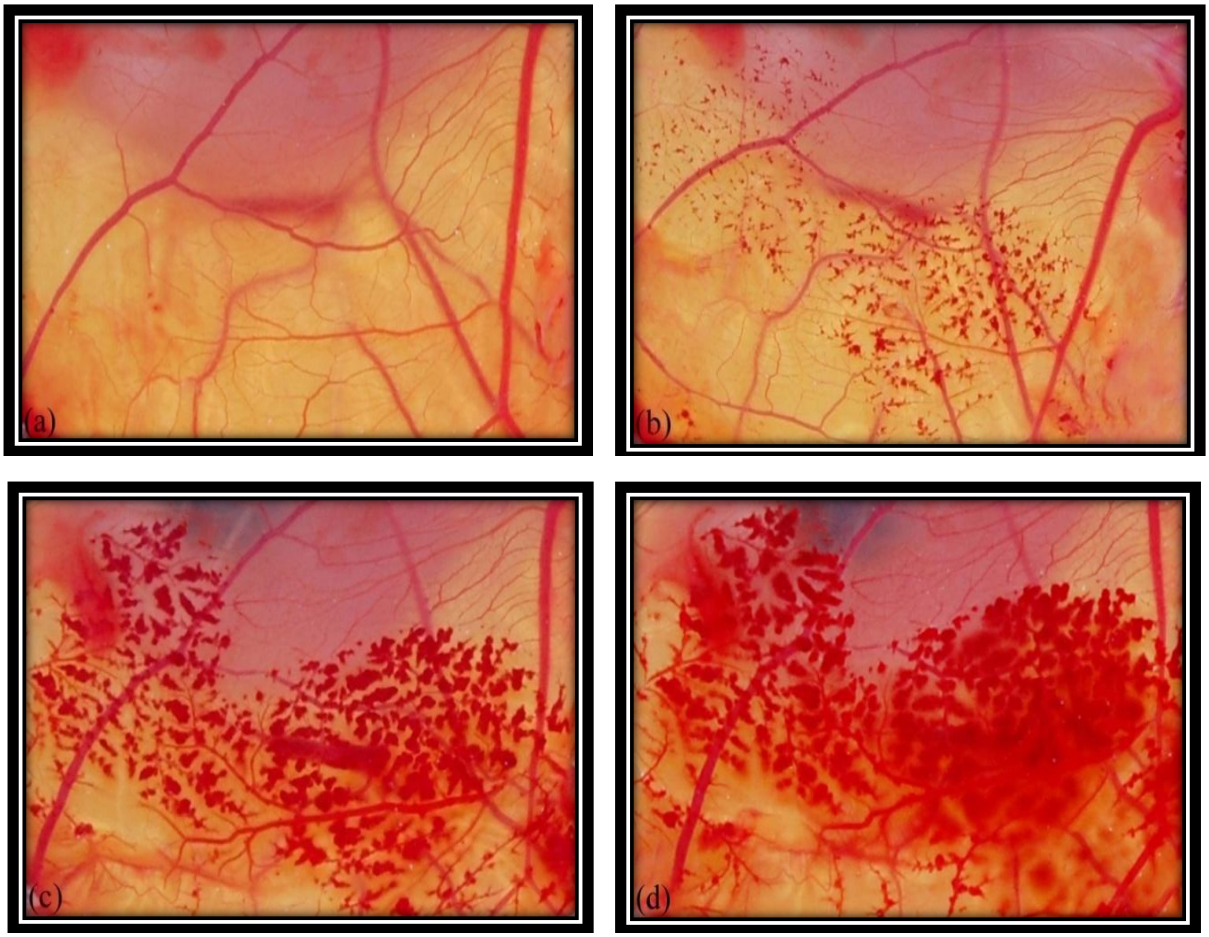

Fig. 2: (a) Membrane with 0.1% NaOH at 0 min, (b) NaOH at 0.5 min,(c) NaoH at 2 min,(d) NaOH at 5 min

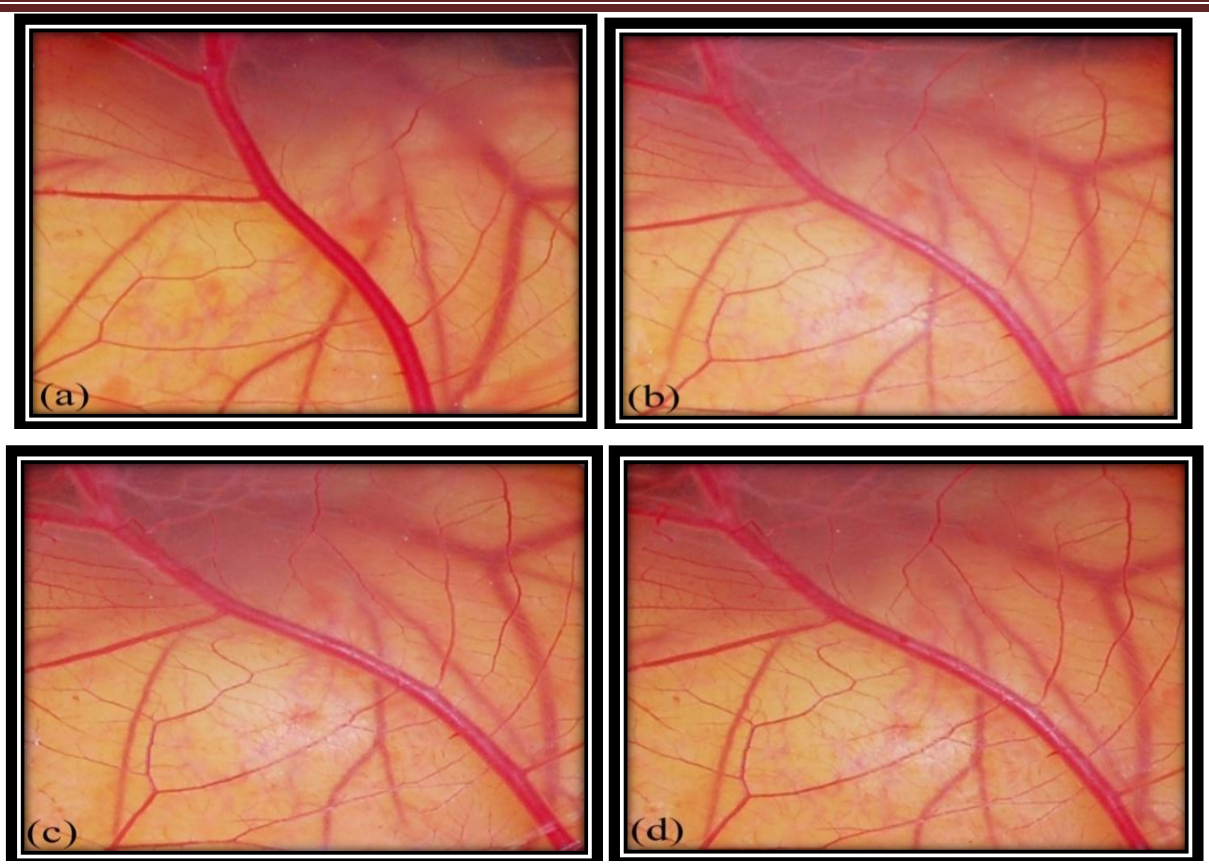

Fig. 3: (a) Membrane with 0.9% NaCl at 0 min, (b) NaCl at 0.5 min, (c) NaCl at 2 min, (d) NaCl at 5 min

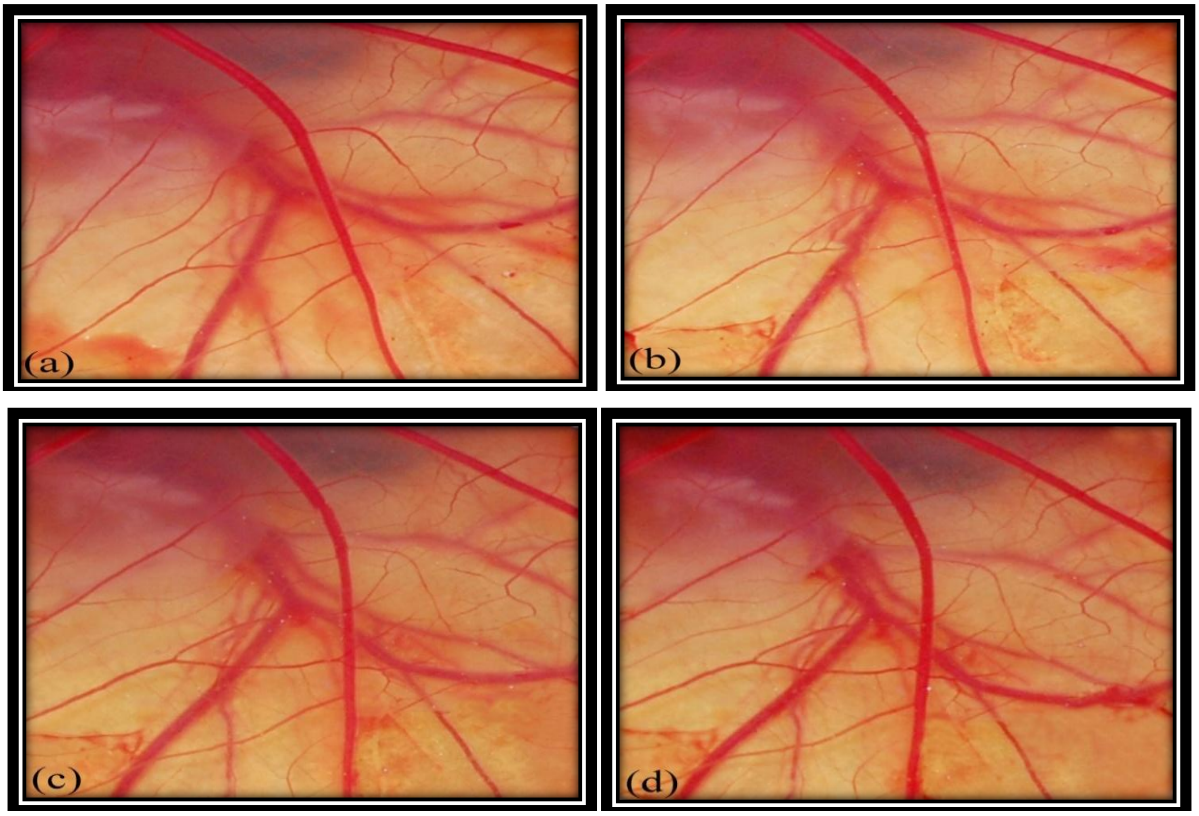

Fig. 4: (a) Membrane with optimised formulation at 0 min, (b) 0.5 min, (c) 2 min, (d) 5 min.

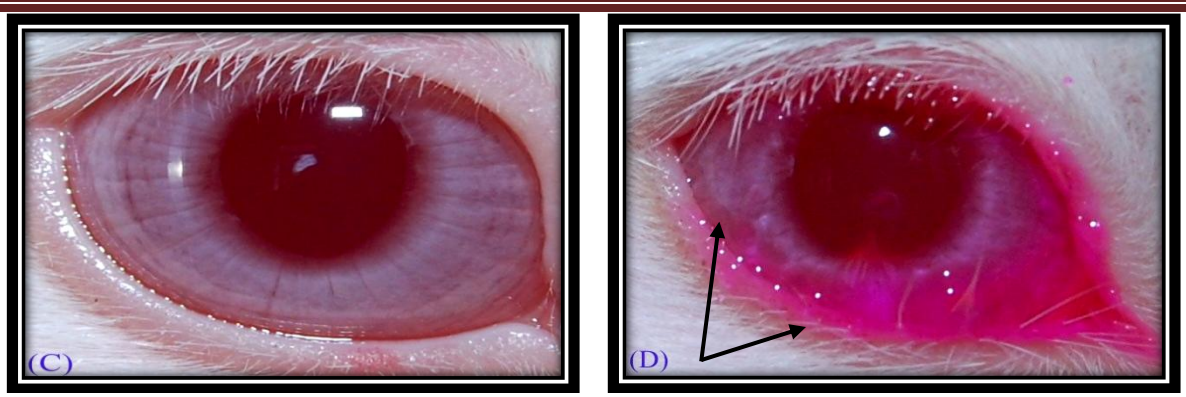

Fig. 5: (C) Normal rabbit left eye. (LE) (D): Formulation F (coloured gel formation) with Rhodamine B dye. (LE)

CONCLUSION

An *in-situ* gel-forming Brimonidinetartrate / sterculiafoetida gum eye drop using gellan gum as an ion-activated polymer was developed. The application of experimental design methodology helped to prepare the optimized formulation, which showed appropriate mucoadhesive force and In-vitro percentage drug release. From the factorial design, the optimum concentrations of Gelrite, HPMC E50 and sterculia foetida gum as mucoadhesive for *in-situ* ocular drug delivery system were 0.24%, 0.4% and 0.13% (w/v), respectively. FTIR spectroscopy study reveals no significant interaction between drug and polymers. So it is concludes that the drug to be compatible with polymers, Ocular visualization showed optimized formulation showed evidence of phase transition and in situ gel structure formation upon contact with cations of the simulated tear fluid. The *in-situ* gel-formed was viscoelastic in nature and sustained the drug release for 10 hours. The drug release from the *in-situ* gel formed was by diffusion from the gel matrix. Formulation was sterile. 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 showed optimized formulation showed evidence of phase transition and *in-situ* gel structure formation upon contact with cations of the simulated tear fluid. Use of biodegradable and water soluble polymers for the *in-situ* gel formulations can make them more acceptable and excellent drug delivery systems. 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 also promises to reduce the frequency of drug administration, thus improving patient compliance. Use of biodegradable and water soluble polymers for the in situ gel formulations can make them more acceptable and excellent drug delivery systems.

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