Indo Global Journal of Pharmaceutical Sciences, 2018; 8(3): 92-103

INDO GLOBAL JOURNAL OF PHARMACEUTICAL SCIENCES
ISSN 2249- 1023

Xyloglucan Based *In-Situ* Gel: Formulation Development and Evaluation of *In-Situ* Ophthalmic Gel of Brimonidine Tartarate

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*

Address for Correspondance
Vazir Ashfaq Ahmed,
mail2vazir@gmail.com

Received:
26.03.2018
Accepted:
21.05.2018

Keywords
Xyloglucan; Hen's Egg Test – Chorioallantoic Membrane (HET-CAM); Brimonidine Tartarate; Ocular irritation; Refractive index.

ABSTRACT: The poor bioavailability of ophthalmic solutions caused by dilution and drainage from the eye can be overcome by using in-situ gel forming ophthalmic drug delivery system prepared from a polymer that exhibits liquid–gel phase transition. The aim of the present study was to formulate and optimize Brimonidine tartarate in-situ gels for the management of glaucoma with the objectives of increasing contact time, achieving controlled release, reducing the frequency of administration and obtaining greater therapeutic efficacy of the drug. Brimonidine tartarate in-situ gel was prepared using various concentrations of polymers, such as gellan gum as an ion-activated gel-forming polymer, xyloglucan as mucoadhesive agent and hydroxy propyl methyl cellulose as release retardant. 23 factorial design was employed to in order to obtain optimized formulation considering the concentration of gelrite, xyloglucan and hydroxy propyl methyl cellulose as independent variables, gelation time, gel strength, mucoadhesive force (N).Viscosity (cP) and In-vitro percentage drug release as dependent variables. Based on desirability index of responses, the formulation containing concentration: gelrite (0.35%), xyloglucan (0.2%) and hydroxy propyl methyl cellulose (0.2%) were selected as the optimized. The formulation was characteristics for: pH, clarity, refractive index, isotonicity, sterility, rheological behavior, and in-vitro drug release, ocular irritation, and ocular visualization. Showed formulation has a pH (7.46), mucoadhesive force (49.53), refractive index (1.382), and gel strength (50.34). Drug release from the gel followed non-fickian mechanism with 90% of drug released in 10 h, thus increased the residence time of the drug. Sustained and prolonged release of the drug, biocompatibility characteristics make the in-situ gel of xyloglucan dosage forms very reliable.

Cite this article as: Ahmed, V.A.; Goli, D. Xyloglucan based *in-situ* gel: formulation development and evaluation of *in-situ* ophthalmic gel of brimonidine tartarate. Indo Global J. Pharm. Sci., 2018; 8(3): 92-103.

INTRODUCTION

Eye is most interesting organ due to its drug disposition characteristics. Generally, topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy. [1] Conventional ophthalmic formulations like solution, suspension, and ointment have many disadvantages, which result in to poor bioavailability of the drug in the ocular cavity. The specific aim of designing a therapeutic system is to achieve an optimal concentration of a drug at the active site for the appropriate duration. [2] The ophthalmic drug delivery based on *in-situ* gel can overcome these problems. As *in-situ* activated gel forming systems administered in drop form and create considerably fewer problems with vision and also

provide better-sustained properties than drops these *in-situ* gelling systems consist of polymers that exhibit sol-to-gel phase transitions due to change in specific physicochemical parameters. [3] xyloglucan having viscosity and mucoadhesive strength is a suitable candidate for increasing the residence time of drugs on the cornea. The increased drug absorption and the prolonged drug elimination phase obtained with the viscosified formulations indicate the usefulness of the tamarind seed polysaccharide as an ophthalmic delivery system for topical administration of drugs. Xyloglucan chains are hydrophilic and bind to water strongly. It is non-toxic, economical, and biodegradable [4-9]. Brimonidine is more lipophilic and alpha2 adrenergic agonist. In its ophthalmic form, it is used to treat open-angle glaucoma. Thus, the formulation of *in-situ* gel with controlled release patterns could provide a single dosing and ensure good patient compliance. Various proportions of the gelrite in combination with hydroxy propyl methyl cellulose and xyloglucan were used in order to prepare optimized *in-situ* gel formulation.

MATERIALS AND METHODS

The following materials are used for the study. Brimonidine Tartarate (FDC Limited Mumbai), Gelrite (Applied Biosciences (KELCO).Mumbai), Xyloglucan (Ultra fine gum, Mumbai).Hydroxyl propyl methyl cellulose E50 LV (LOBA chemicals, Mumbai) Rhodamine B (Amrithal Chemuax Pvt Ltd. Mumbai). Fluid Thioglycollate media (Microgen central drug house, New Delhi) Soybean-Casein Digest Media (Microgen central drug house, New Delhi). 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.

Analytical Methods

Scanning for drug absorption (λ_{Max}) using double beam spectrophotometer

Brimonidine Tartarate was *scanned* in 7.4 pH Phosphate Buffer (10 µg/ml) *by using double beam UV–Visible spectrophotometer (*UV- 1700 Pharma Spec/ Shimadzu Japan) in a wavelength range of 200-400 nm.

Development of UV Spectrophotometric method for analysis of Brimonidine Tartarate

A final concentration of 3, 6, 9, 12, 15, 18, and 21µg/ml respectively was prepared using 7.4 pH Phosphate Buffer. The absorbance of each concentration was measured at 248nm using UV spectrophotometer against blank. The standard curve was obtained by plotting absorbance V/s. concentration in µg/ml.

Isolation of tamarind seed polysaccharide (TSP)

About 15g of tamarind kernel powder was taken in a 100 ml of cold distilled water to prepare a slurry. This was poured slowly into 400 ml of boiling distilled water and further boiled for 30 min under stirring conditions; the resulting mixture was kept overnight. Later it was centrifuged at 5000 rpm for 20 min. The supernatant liquid was separated and poured into twice the volume of absolute alcohol by continuous stirring to precipitate the polysaccharide. The precipitate was washed with 200 ml of absolute ethanol and then dried at 50^{o}C for 8h. The dried polymer was powdered and stored in desiccators. [10]

***In vitro* Gelation behavior studies of polymers with simulated tear fluid**

Concentrations of Gelrite, xyloglucan and in combinations with hydroxy propyl methyl cellulose E50 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. [11]

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 solutions required quantity of xyloglucan was added and stirred well on a magnetic stirrer with slight heating. To the above prepared gelrite/mucoadhesive solution, a required quantity of drug (0.2% Brimonidine) 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 a magnetic stirrer. Checked the pH and adjusted with the buffer. The prepared *in- situ* gel were filled in glass vials and closed with closures, capped with aluminium 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, and xyloglucan. The independent variables levels were gelrite (0.2, 0.4), xyloglucan (0.1, 0.2), hydroxy propyl methyl cellulose E50 (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 (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

Brimonidine Tartarate and a 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**.** [12]

Measurement of Gel Strength

A 50g 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 15g 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.[13]

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 1g of the preformed gel was put on the inverted beaker and was placed under the bottom of the stainless steel pan. A preload of 50g was placed on the pan for 5 min to establish adhesion bonding between the 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 g required to detach the pan from membrane, gave the measure of mucoadhesive strength. [14]

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). [15]

***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 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. [16]

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. [17]

Abbe's refractometer

Refractive index of the formulation was determined by Abbe refractometer. The light was turned 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, few drops of the formulation were placed. Hinged upper incident prism was locked with a 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.[18,19]

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. Blood sample was drawn from the china dish in to red blood cells (RBC) pipette up to 0.5 mark and further diluted with red blood cells (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 cells (RBC) for cremation or swelling and bursting. [20]

Test for Sterility

According to Pharmacopoeias, the sterility testing is intended for detecting the presence of viable forms of microorganisms in the pharmaceutical preparations

Growth Promoting Organism; *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231.[21,22] Incubation temperature; 35 °C &25 °C. Quantity of culture medium: 10ml. [23] Quantity of test sample: 1ml [24]

Method of Direct Transfer

Tests for sterility were performed for fungi, aerobic and anaerobic bacteria using soya bean casein digest media and fluid thioglycollate media. According to Indian Pharmacopoeia for ophthalmic preparation, if the number of items in a Batch is not more than 100 containers, a minimum number of items recommended to be tested are 2 containers. Two autoclaved glass vials each containing 10ml of the formulation (placebo) were used. This study was carried out to obtained sterile preparation, which can be instilled into rabbit eyes to understand the ocular behaviour and visualization of formulation when it comes in contact with the lachrymal fluid of the eye.[25]

Growth promotion (positive control) test

One culture tube containing 10ml of sterile media was inoculated with a sterile loop full of micro-organisms and incubated as per the specified conditions. It is labeled as a 'positive control'.

Sterility (negative control) test

Uninoculated sterile culture tube containing 10ml each for Fluid thioglycollate media and one for soya bean casein digest medium were taken. These were incubated as per the specified conditions. It is labeled as a 'negative control'.

Test for aerobic and anaerobic bacteria

Two culture tubes containing 10 ml each of sterile fluid thioglycollate media were labeled. 1 ml of the formulation was introduced to the depth of culture tube with help of sterile syringe aseptically and labelled as depth D* (for anaerobic). To another culture tube of sterile fluid thioglycollate media, 1 ml of the formulation was introduced on to the surface of the culture media with help of sterile syringe aseptically. The tube labeled as surface S *(for aerobic). The four tubes (positive, negative and two labeled test tubes) were incubated at 35ºC for 14 days.

Test for fungi

Three sterilized culture tubes containing 10 ml each of sterile soybean-casein digest media were taken. The tube labelled as positive control was inoculated with sterile loop full of viable microorganism, candida albicans aseptically. Uninculated culture tube was labelled as a negative control. 1ml of the formulation was added to the culture tube aseptically and labeled as a test. Three tubes were incubated at 25°C for 14 days.

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 inner chorioallantoic membrane was not injured. About 0.3 ml of formulation, positive control and a negative control was applied directly onto the chorioallantoic membrane 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 were observed for a period of 300s (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. [26, 27]

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. (One eye served to control and another eye as a test). The eyelids were held close for few second; the *in-situ* gel so formed was visualized. [28]

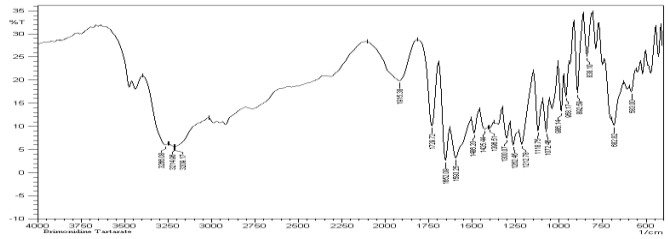


Fig No 1: IR Spectrum of Brimonidine Tartarate.

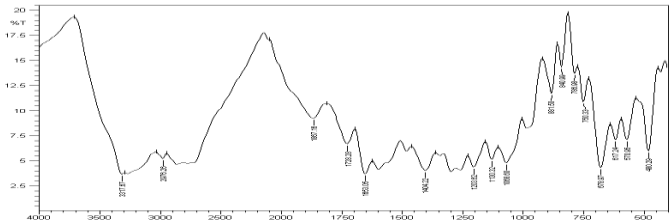


Fig No 2: IR Spectrum of Brimonidine Tartarate with the physical mixture.

Table No 1: Experimental layout of factors

Batch code	Polymers (%)		
	Gelrite	Xyloglucan	hydroxy propyl methyl cellulose 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

RESULTS AND DISCUSSION

Compatibility studies of Drug(s) with 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 (Fig. No.1, 2). Polymer combination having immediate gelation, the gel is stable, the vehicle is in the liquid form was chosen as the concentration for optimization study. 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. 1, 2). From the experiment carried out, the optimum ranges were assigned for the desirability approach. Gelation time to be between 5 to 10s, (below this range formulation was very viscous, higher than this was required, but the formulation should gel with a short period of time when it comes in contact with tear fluid). Gel strength between 50 to 100 s (below this range it was a weak gel and higher than this was required, but it forms a strong gel, taking into consideration about the drug release from the strong gel the range was limited to 100s) and mucoadhesive force to be between 4 to 6.5(N) (considering the gel strength and higher value will cause patient discomfort and lower will not have a good contact time with the mucin of the eye).

Polynomial equation coded factor:

Gelation time (s) =8.17-4.17*A+0.17*B-0.17***C**-0.67*A*B-0.67*A*C+0.17*B*C-0.17*A*B*C

As shown in the equation, the factors have a significant effect on the gelation time. The variables such as the concentration of gelrite (A) and hydroxy propyl methyl cellulose (C) have a negative effect on the gelation time. That means as increase in the concentration of A and C will show decrease gelation time. The variable such as the concentration of xyloglucan (B) showed a positive effect which means increase in concentration will an increase the gelation time. The higher the concentration level of gelrite gave the low value of gelation time at all level. The contour plot showed that lower conc. of gelrite and higher conc. of xyloglucan showed higher

gelation time, which explains the non interaction of xyloglucan with gelrite. The perturbation plot shows that factor gelrite has a more significant negative effect compared to hydroxy propyl methyl cellulose E50 and xyloglucan on gelation time.

Table No 2: Experimental layout of responses

Batch code	Gelation Time (seconds)	Gel Strength (seconds)	Mucoadhesive force (N)	Viscosity (cP) At 50 rpm	Cumulative % drug release at 1st h	Cumulative % drug release at 10th h
F1	04±0.942	124±2.624	6.28	55	24.23	72.60
F2	05±1.247	73±3.399	5.59	65	09.91	81.21
F3	11±0.942	52±3.027	4.96	68	24.19	91.64
F4	12±2.160	76±3.681	5.84	70	30.52	87.82
F5	14±1.414	70±2.885	6.11	43	04.20	78.53
F6	03±0.471	96±3.642	6.08	72	25.17	67.26
F7	04±0.942	44±2.494	4.80	68	25.54	94.99
F8	12±0.816	65±2.867	4.10	68	29.22	79.70

Table. No.3: Predicted and Experimental Observed Responses of the Optimized Formulation with % Prediction Error

Number	Gelrite	Xyloglucan	hydroxy propyl methyl cellulose E50	Gelation Time	Gel Strength	Mucoadhesive Force	Viscosity Before Gel at 50 RPM	Invitro release at 10h	Desirability
Predicted value									
1	0.35	0.2	0.20	6.47	52.22	51.84	68.53	93.07	**0.847**
Observed value									
2	0.35	0.2	0.20	6.53	50.34	49.52	71.20	90.68	
% Predicted error									
				0.918	03.73	04.68	03.75	2.63	

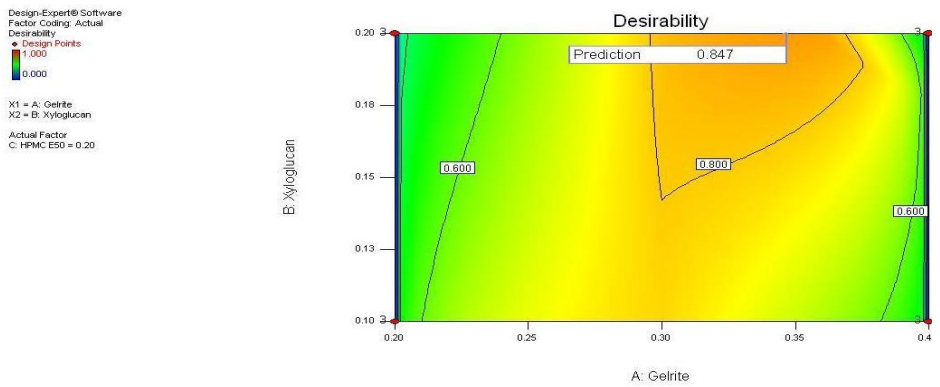


Fig. No. 3: Desirability plot related to the given data.

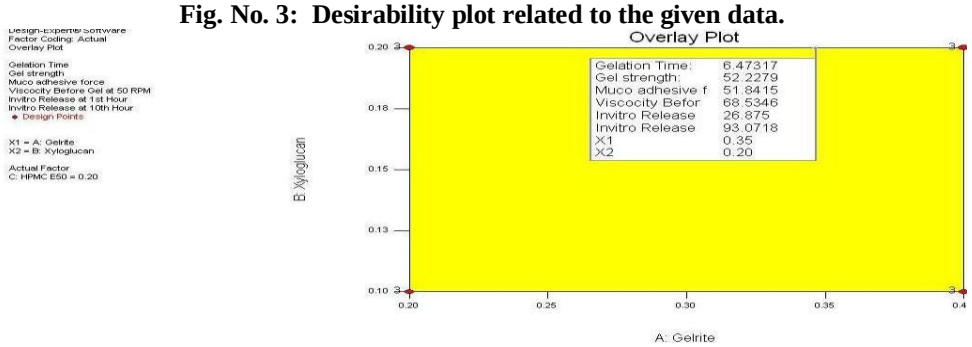


Fig. No. 4: Overlay graph of formulation optimization highlighting an area of operability

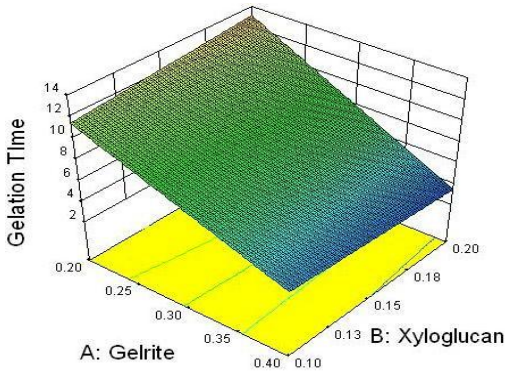


Fig.No.A: 3D Graph showing the effect of gelrite and xyloglucan on gelation time

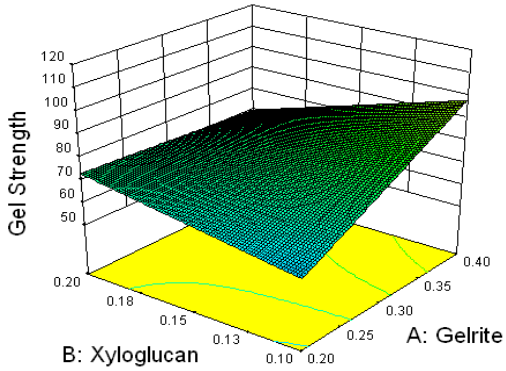


Fig.No.B 3D Graph showing the effect of gelrite and xyloglucan on gel strength

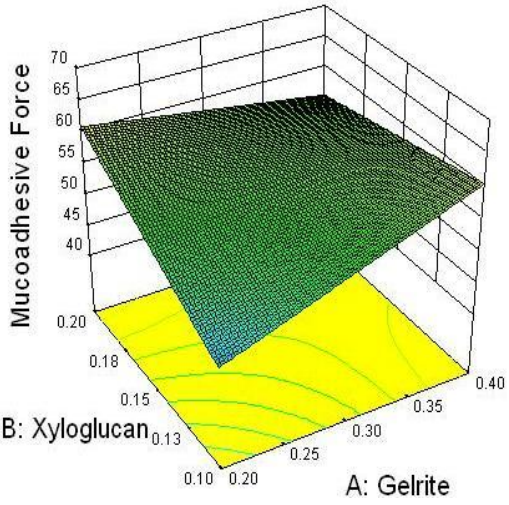


Fig.No.C, 3D Graph showing the effect of gelrite and xyloglucan on Mucoadhesive Force

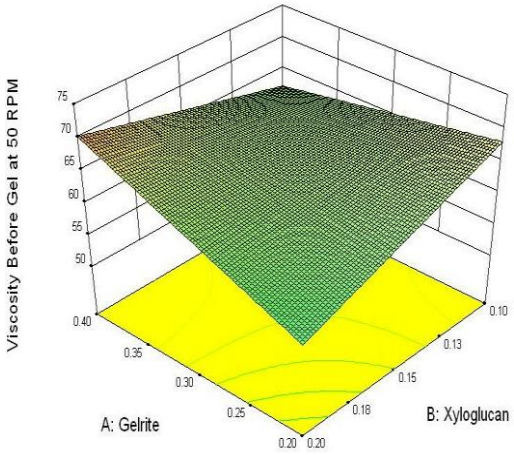


Fig.No.D. 3D Graph showing the effect of gelrite and xyloglucan on viscosity before gel

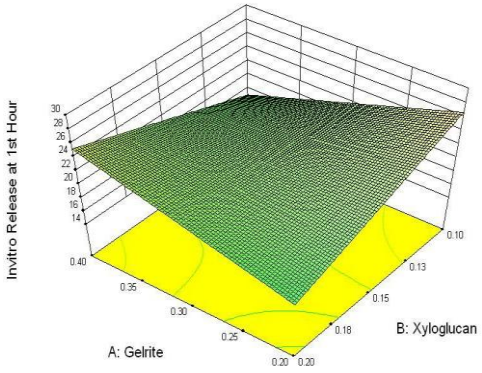


Fig.No.E.3D Graph showing the effect of gelrite and xyloglucan on cumulative drug release at 1st h

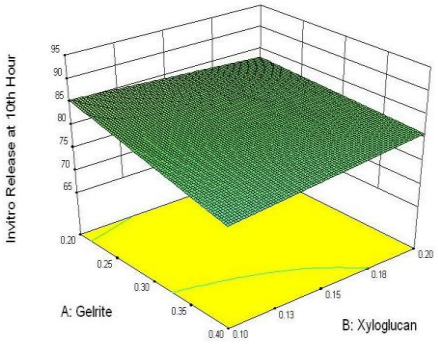
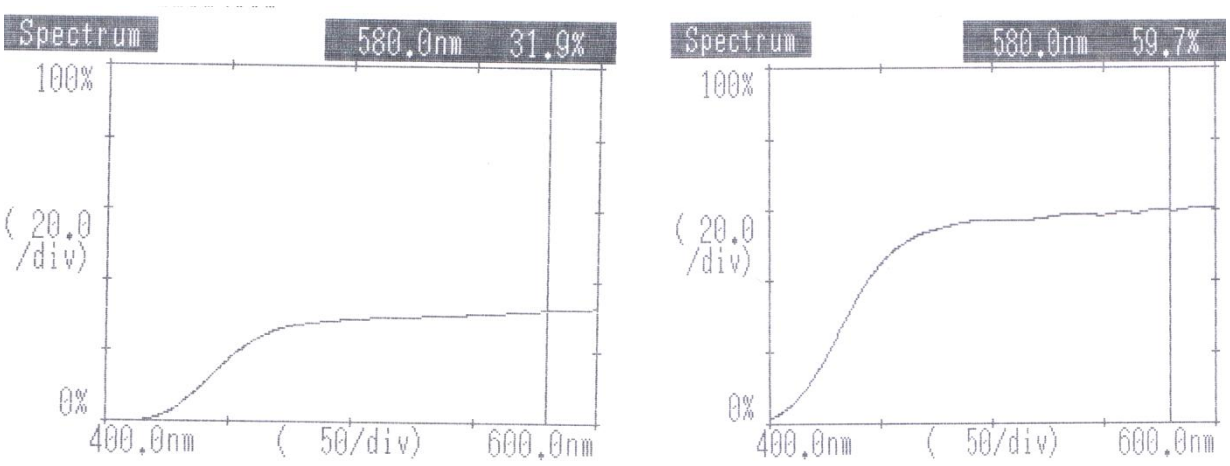


Fig.No. F.3D Graph showing the effect of gelrite and xyloglucan on cumulative drug ' release at 10th h

Fig. No. 5 3D Graph showing the effect of xyloglucan on various responses



Relative % transmittance of XY* before gel **Relative % transmittance of XY* after gel**

Fig. No. 6: Percentage transmittance of xyloglucan (XY*)

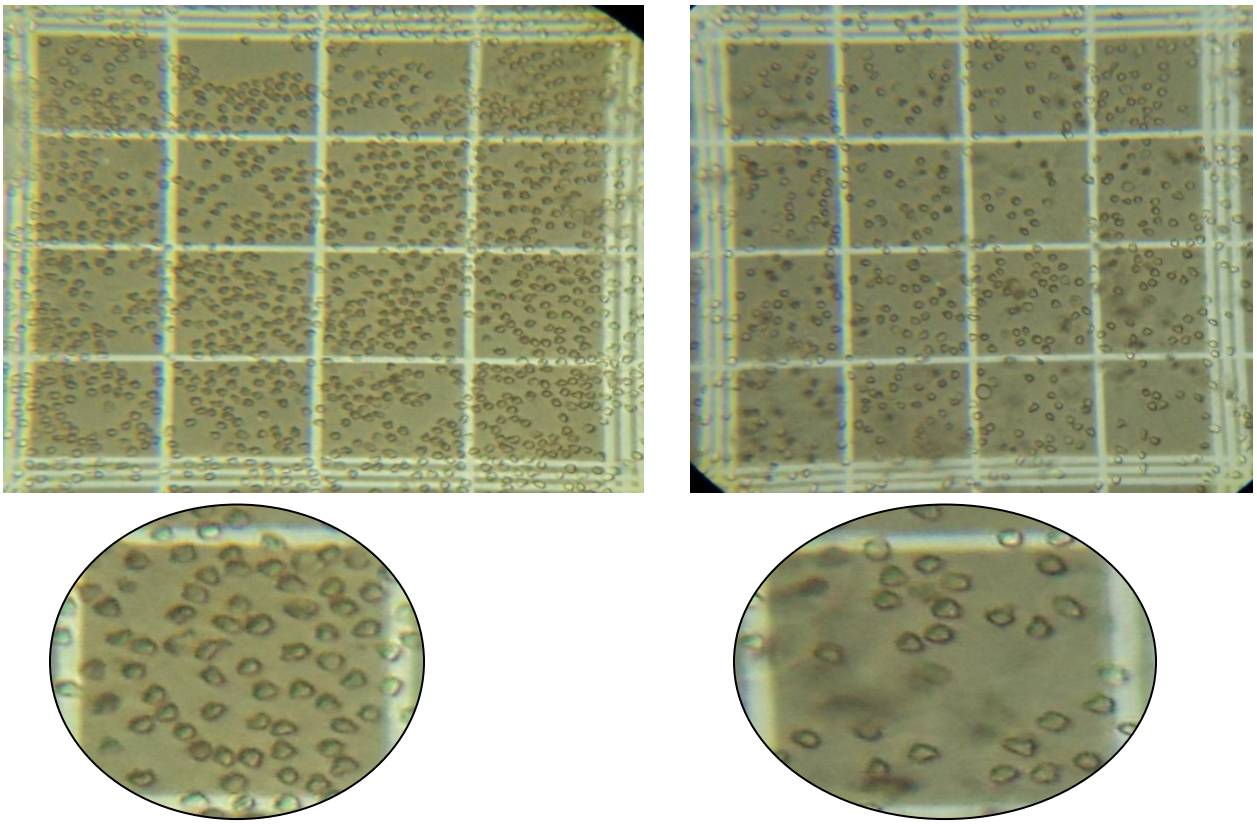


Fig. No.7: RBC'S without formulation and RBC'S with optimized formulation

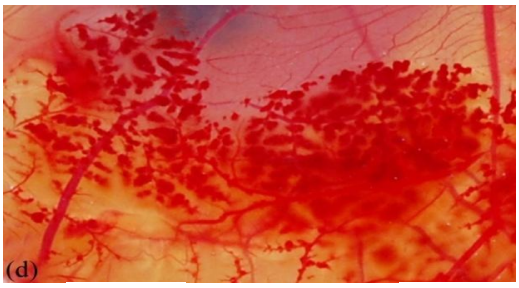


Fig. No.8: **(d) NaOH at 5 min**

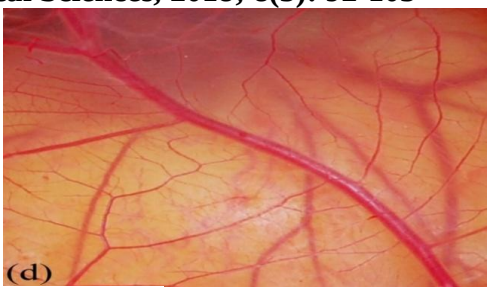


Fig. No.9 **(d) NaCl at 5 min**

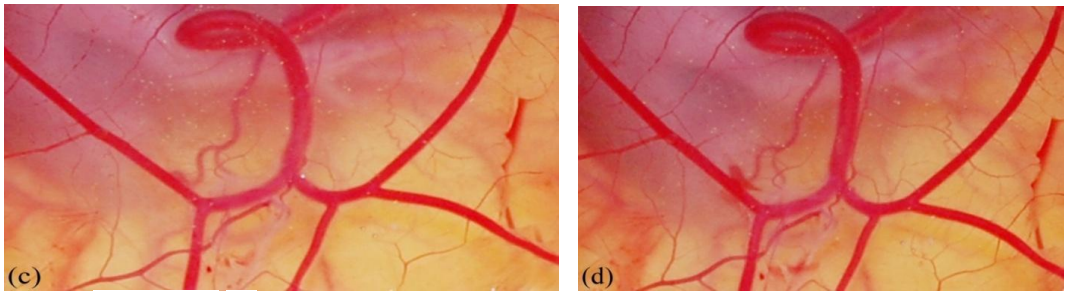


Fig. No.10: (a) Membrane with optimized formulation at (c) 2 min, (d) 5 min.

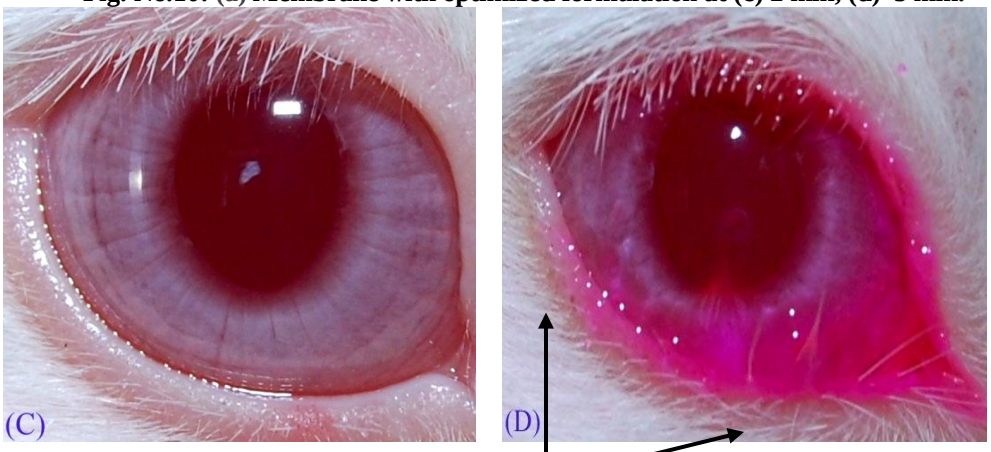


Fig. No.11 (C): Normal rabbit left eye. (LE)(D): optimized formulation (colored gel formation) with Rhodamine B dye. (LE)

Table No 4: Composite evaluation parameter of optimized formulation

Sl.no	Evaluation parameters	Optimised Formulation
1	pH	7.46±0.0326
2	Clarity(Before gel)	31%
3	Clarity(After gel)	59%
4	Gelation Time	06.53±3.85
5	Gel Strength	50.34±3.85
6	Mucoadhesive force	49.52±3.89
7	Viscosity before gel at 50 RPM	71.20±3.69
8	*In-vitro* drug release at 10^{th} h	90.68±2.45
9	Refractive index	1.382±0.0020
10	Isotonicity	Isotonic
11	Ocular tolerance	Non irritant
12	Sterility test	Sterile
13	Ocular visualization of in-situ gels	Easy to instill

*Standard Deviation (n=3)

Gel Strength (s) =74.88+9.21*A-3.29*B+13.96*C-10.79*A*B+11.96*A*C-2.04*B*C+2.46*A*B*C

As shown in the equation, the factors have a significant effect on the gel strength. The variables such as the concentration of gelrite (A) and hydroxy propyl methyl cellulose (C) have a positive effect where as xyloglucan (B) have negative effect on gel strength. That means an increase in the concentration of A, and C will show increase in gel strength and an increase in the concentration of B will decrease the gel strength.

The Perturbation plot shows that factor gelrite, hydroxy propyl methyl cellulose E50 has a significant positive effect on gel strength with factors showing elevation at the positive side. The xyloglucan shows the elevation on the negative side which shows it negative effect. Surface response plot shows that positive effect of hydroxy propyl methyl cellulose E50 and gelrite is more compared to xyloglucan.

Mucoadhesive Force (N) =55.80+2.21*A+2.45*B+1.75*C-4.95*A*B+3.25*A*C+2.19*B*C-0.68*A*B*C

As shown in the equation, the factors have a significant effect on the mucoadhesive force. The variables such an concentration of gelrite (A) xyloglucan (B) and hydroxy propyl methyl cellulose (C) have a positive effect on mucoadhesive force. That means an increase in the concentration of A, B and C will show an increase in mucoadhesive force.

The Perturbation plot shows that factor gelrite, xyloglucan. hydroxy propyl methyl cellulose E50 has a significant positive effect on gel strength with factors showing elevation at the positive side. The surface response shows that positive effect of xyloglucan. As the concentration of xyloglucan increase mucoadhesive force also increase.

Viscosity before Gel =64.29+2.04*A-0.88*B-3.63*C+4.88*A*B+3.13*A*C-1.96*B*C+4.79*A*B*C

As shown in the equation, the factors have a significant effect on the viscosity. The variables such as concentration of gelrite (A) xyloglucan (B) and hydroxy propyl methyl cellulose (C) have a negative effect were as gelrite (A) has positive effect on the viscosity. That means as increase in concentration of B and C will show decrease in viscosity. The variable such as the concentration of gelrite (A) showed a positive effect which means an increase in the concentration will increase the viscosity.

The Contour plot showed that higher conc. of gelrite and lower conc. of xyloglucan showed higher viscosity, which explains the non interaction of xyloglucan with gelrite. The Perturbation plot shows that factor gelrite has a more significant positive effect compared to hydroxy propyl methyl cellulose E50 and xyloglucan on viscosity.

Cumulative Drug Release (%) 1^{st} h = 21.62-0.41*A-0.26*B-0.92*C+4.41*A*B+4.40*A*C-5.75*B*C+2.08*A*B*C

Cumulative Drug Release (%) 10^{th} h = 81.72-2.71*A+0.43*B-7.20*C+1.68*A*B-1.89*A*C-2.06*B*C-2.72*A*B*C

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) xyloglucan (B) and hydroxy propyl methyl cellulose (C) have a negative effect on drug release. Which means that A, B and C have drug release controlling capacity. At 10^{th} h, all polymers A, B, C and their combination AC, BC has shown a negative effect which indicates that increase in polymer concentration will reduce the % drug release. Which is significant for drug release?

The Perturbation plot shows that factor gelrite (A) xyloglucan (B) and hydroxy propyl methyl cellulose E50 (C) has a significant negative effect on drug release with factors showing elevation at the negative side. Surface response plot shows that negative effect is more by hydroxy propyl methyl cellulose E50 at 1^{st} h. and at10^{th} h all polymers have a prominent negative effect. (Fig. No. 5).

Interaction studies of factors reveal that concentration of xyloglucan, gelrite, and hydroxy propyl methyl cellulose E50 are critical factors. The concentration of xyloglucan 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. These were further evaluated for the optimization responses (gelation time, gel strength, mucoadhesive force (N). viscosity (CPS) and *In-vitro* percentage drug release) in order to confirm the validity of optimization process, Formulations exhibiting desirability like 0.847, 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.3 & Fig No 3, 4, 5). *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 the fickian diffusion mechanism.

The composition of the optimized formulation is shown in Table. No 4.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 (Fig No.6).Rheological studies manifested that the shear stress and viscosity at 37^{o}C with simulated tear fluid were higher than those at 25^{o}C without simulated tear fluid. It was noted from the various literature that the solution before gelling should have a viscosity of 5 to 1000cP 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 4250-28500s^{-1} during blinking. The viscosity of the solution ranged from 27-351 cP 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 favor 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 comply with the test for sterility (Table No.4).

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 (Fig No.7, 8).Formulations scoring was 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 min and no change was seen after 5 min 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 HET-CAM and those of the positive and negative controls (Fig No.8, 9, 10). 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.11).

CONCLUSION

An *in-situ* gel-forming Brimonidine Tartarate/ xyloglucan 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, hydroxy propyl methyl cellulose E50 and xyloglucan as mucoadhesive for the *in-situ* ocular drug delivery system were 0.4%, 0.21% and 0.24% (w/v), respectively. FTIR spectroscopy study reveals no significant interaction between drug and polymers. So it is concluded 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 to be sterile. Ocular irritation studies showed the absence of Hyperemia, Haemorrhage and Coagulation. We can conclude that an optimized formulation was non irritant, as results obtained by Hen's Egg Test – Chorioallantoic Membrane (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. 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.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

REFERENCES

1. Sasaki H, Yamamura K, Nishida K, Nakamurat J, Ichikawa M. Delivery of drugs to the eye by topical application. Progress in Retinal and Eye Research, 15(2), 1996, 553-620. 2
2. Macha S, Mitra AK. Ophthalmic drug delivery systems; 2nd ed. revised and expanded. Chapter 1 Overview of Ocular Drug Delivery, 2008, 1-3.
3. Sechoy O, Tissie G, Sebastian C, Maurin F, Driot JY, Trinquand C, A new long acting ophthalmic formulation of Carteolol containing alginic acid. International Journal of Pharmaceutics, 207(1-2), 2007, 109–116
4. Durai RD, Joseph J, Rajalakshmi G. Tamarind seed polysaccharide: A promising natural excipient for pharmaceuticals. Int J Green Pharmac. 2012; 12:270-78
5. Uccello-Barrettaa G, Balzanoa F, Vannia L, Sansòb M. Mucoadhesive properties of tamarind-seed polysaccharide/hyaluronic acid mixtures: A nuclear magnetic resonance spectroscopy investigation. Carbohydrate Polymers. 2013; 91: 568– 572
6. Sahoo S, Sahoo R, Nayak PL. Tamarind Seed Polysachharide: A versatile biopolymer for mucoadhesive applications. J Pharma Bio Sci. 2011; 8(8):1-12
7. Rolando M, Valente C. Establishing the tolerability and performance of tamarind seed polysaccharide (TSP) in treating dry eye syndrome: results of a clinical study. BMC Ophthalmo. 2007; 7(5):1-8.
8. Ghelardi E, Davini P, Salvetti S, Parisio E, Campa M. A mucoadhesive polymer extracted from tamarind seed improves the intraocular penetration and efficacy of rufloxacin in topical treatment of experimental bacterial keratitis. Antimicrob Agents Chemother. 2004; 48(9):3396-401.
9. Tvantia A, Celandronia F. Effect of a novel mucoadhesive polysaccharide obtained from tamarind seeds on the intraocular penetration of gentamicin and ofloxacin in rabbits. J Antimicro Chemo.2000; 46: 831–34
10. Srinivasan B, Ganta A, Veerabhadraiah BB, Varadharajan M. Evaluation of tamarind seed polysaccharide as a drug release retardant. Int J Pharma Sci Rev Res 2011;9(2):27-31
11. Nagargoje S, Phatak A, Bhingare C, Chaudhari S. Formulation and evaluation of ophthalmic delivery of fluconazole from ion activated *in situ* gelling system. Der Pharmacia Lettre 2012; 4 (4):1228-35
12. Dyer JR. Application of absorption spectroscopy of organic compounds. 8th edition. Prentice Hall Learning, 1991 (fitr)
13. Kumar JR, Muralidharan S. Development of Microparticle Loaded Gel (MPLGs) for Prolong Ocular Drug Delivery Containing Ketorolac Tromethamine. J Pharm Sci Res 2014 6(3):148-52
14. Kanoujia J, Sonker K, Pandey M, Koshy M, Shubhini AK, Saraf. Formulation and characterization of a novel pH-triggered *in-situ* gelling ocular system containing Gatifloxacin. Int Cur Pharma J 2012; 1(3): 43-9
15. Dasankoppa FS et al.; Formulation and evaluation of a novel *in situ* gum base ophthalmic drug delivery system of linezolid. Sci Pharm.2008; 76: 515–32
16. Farheen T, Sadhana RS, Shaikh AM, Zudbuke N, Ali SA . Formulation development and evaluation of *in-situ* ophthalmic gel of sodium cromoglycate. Der Pharmacia Sinica, 2013; 4(2):109-118
17. Esser MH et al Antiperspirant compositions US.PATENT 2000061082. 2000 Oct 19
18. Koohyar F. Refractive index and its applications. J Thermo dyn Catal 4:e117. doi:10.4172/7544.1000e1177544.1000e117
19. Kumar PK, Padma Jeeth A. Refractive index of liquids using abbe's refractometer .Experiments18. [Internet] cited June 2015. Available from www. web.mst.edu/~tbone/Subjects/TBone/chem224/riproc.pdf
20. Dave V, Paliwal S, Yadav S, Sharma S. Effect of *in vitro* transcorneal approach of aceclofenac eye drops through excised goat, sheep, and buffalo corneas. Sci World J 2015;1-7
21. Kumar K, Mishra I.N, Sandhya J. Study of storage periods of culture suspension of *Escherichia coli*. Int J Pharma Sci and Drug Res 2013; 5(1): 36-7
22. Smith R, Mark von tress, Tubb C, Vanhaecke E. Evaluation of the scanRDI_ as a rapid alternative to the pharmacopoeial sterility test method: comparison of the limits of detection. J Pharm Sci and Tech 2010; 64: 356-63
23. Sterility testing : pharmaceutical products : [Internet].cited February 2016 Available from http://www.newagepublishers.com/sample chapter.pdf
24. General requirements for the sterility of biological substance : [Internet].cited February 2016Availablefromhttp://www.who.int/biologicals/publications/sterility/WHO_TRS_530_A4.pdf
25. Pharmaceutical microbiology and biotechnology [Internet].cited February 2016 Available from http://nsdl.niscair.res.in/sterilization methods and Principles.pdf
26. Current Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants:Hen's Egg Test - Chorioallantoic Membrane Test Method. [Internet].cited august 2015. Available fromhttp://iccvam.niehs.nih.gov/methods/ocudocs/ocu_brd.htm#hetcam
27. Day 9 of incubation : the story inside the chicken egg [Internet].cited august 2015 Available from http://www.raising-happy-chickens.com/incubation-day-9.html)
28. Guss R, Johnson R, Maurice D. Rhodamine B as a test molecule in intraocular dynamics. Invest Ophthalmol Vis Sci. 1984 Jun;25(6):758-62.

Indo Global Journal of Pharmaceutical Sciences(ISSN 2249 1023 ; UGC Journal No.: 44477; CODEN- IGJPAI; NLM ID: 101610675) indexed and abstracted in EMBASE(Elsevier), UGC Journal List, National Library of Medicine (NLM) Catalog, Elsevier(EMBASE), ResearchGate, Publons, CAS (ACS), Index Copernicus, Google Scholar and many more. For further details, visit http://iglobaljournal.com