RESEARCH PAPER

Design and Development of pH Responsive In-Situ Ocular Gel of Pilocarpine Using Natural In-Situ Gelling Polymers

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ABSTRACT

Recently use of natural polymers in the designing of ocular dosage forms is increasing over synthetic polymers. This research emphasizes on the development and evaluation of in situ gelling system for management of glaucoma by using Isabgol Husk Mucilage (IHM) and Locust Bean Gum (LBG) to prolong the ocular retention time, and thereby increasing the bioavailability of pilocarpine. In current research in situ ocular gel of pilocarpine was prepared by the combined use of natural polymers such as Isabgol Husk Mucilage (IHM) in the concentration 2-4 % W/V and Locust Bean Gum (LBG) in the concentration 0.3-0.7 % W/V. The formulation batches were prepared as per the 3^2 full factorial design which provided 9 formulation batches. The in-situ gelation behavior and viscosity of the formulation found to be within range. Moreover, the dissolution pattern, ex vivo permeability osmolality was also found to be within range. The optimization study suggested that in situ ocular gel formulation batch R9 is optimized batch which showed in situ gelation time 16 second, gel intact time 12 hours and osmolality 293.45±0.5 mOsm/Kg. From this research, it is concluded that, there is decrease in in situ gelation time while increase in the gel intact time with corresponding increase in the concentration of IHM powder and LBG powder. The best sustained release 92.53% at 12 h was observed with the formulation R9 which contains the IHM powder and LBG powder in the concentration 4% and 0.7% respectively. On the other hand, PILOPINE HS, the marketed gel of pilocarpine showed drug release 83.85% only up to 10 h. The curve fitting results of drug release data indicated that release of pilocarpine from most of the batches of in situ gel formulation follows Higuchi model. The stability study indicated that the in situ ocular gel formulation batch R9 is stable under ambient condition. So ultimately the in situ ocular gel would increase the bioavailability of pilocarpine in the ocular cavity.

KEYWORDS: Pilocarpine; Isabgol Husk Mucilage; Locust Bean Gum; In situ gelation; Carbopol-940

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1. INTRODUCTION: Since the structure of the eye contains several physiological barriers that must be addressed one after the other in order to achieve a satisfactory rate of absorption and bioavailability, ocular drug delivery has long been regarded as one of the most challenging areas for researchers globally. [1] Because of its unique characteristics, the eye is regarded as an intriguing organ. Pharmaceutical scientists must prioritize the aspects of absorption, distribution, metabolism, and elimination (ADME) while developing an ocular drug delivery system in order to create a better system and encounter fewer issues. [2, 3]

There are several obstacles to ocular drug administration, including ocular barriers and drug drainage from tear flow, which eventually results in limited drug bioavailability and lowers the intended therapeutic benefit of the medication. [4, 5] However, one benefit of ocular administration is that the medication bypasses

first-pass metabolism and enters the systemic circulation. [6, 7] Ocular formulations such ointments, suspensions, and aqueous gels have a number of disadvantages, including poor patient compliance, obscured vision, difficulty administering the medication on one's own, ocular drug drainage, formulation instability, low drug retention and bioavailability in the ocular cavity, etc. [8, 9]

One potential new method for addressing the issue of inadequate medication retention and bioavailability in the ocular cavity is pH-triggered in situ gel. Polyelectrolytes with an acidic (carboxylic or sulfonic) or basic group (ammonium salts) that either accept or release protons in reaction to changes in pH in the surrounding environment make up this in-situ gelling system. [10, 11, 12] The formulation exists as a normal solution at lower pH values (pH 4.4); however, at pH 7.4, which is the pH of tear fluid, it gels. [13] In ophthalmic preparation, polyacrylic acid (PAA, Carbopol 940), polycarbophil, and cellulose acetate phthalate (CAP) are the most often utilized pH-responsive polymers. [14]

pH-triggered in situ gel has a number of benefits, including convenience of use, ease of production, patient compliance, good sol to gel transition by tears, extended drug retention duration in the eyes, increased bioavailability, and continuous drug release because of the gelling behavior. [15]

2. MATERIAL AND METHOD

2.1 MATERIAL: The fresh Isabgol husk, Locust bean and Pilocarpine was purchased from Surgical Home, Ambala Cantt. (Haryana). Labware Chemicals, Latur, Maharashtra, provided Carbopol-940, HPMC-K4M, NaCl, Benzalkonium chloride, Sodium bicarbonate and Calcium chloride dehydrate. During entire research distilled water was utilized.

2.2 METHOD

I. Preformulation studies on pilocarpine: It is the physicochemical analysis of drug alone or in combination with excipients.

A. Melting point determination of pilocarpine: Pilocarpine melting point was determined by Thiele tube. [16]

B. Determination of λ_{max}: The stock solution of PLC having concentration 20 µg/mL was prepared by using phosphate buffer pH 7.4 i.e, simulated tear fluid (STF) and then analyzed with UV visible spectrophotometer by scanning between 200-400 nm to identify the wavelength (λmax) at which maximum absorbance is obtained. [17]

C. Standard calibration curve: In the similar way, for λ_{max} determination, pilocarpine standard solution series was prepared with concentrations of 4, 8, 12, 16, and 20 µg/mL by using simulated tear fluid (STF). The resultant solutions were then analyzed with UV visible spectrophotometer at 213.3 nm. [17]

D. Compatibility between drug and excipients

a. IR (Infra-red) study: IR spectra of PCL, Isabgol Husk Mucilage (IHM), Locust Bean Gum (LBG) and physical mixture of PLC, IHM and LBG were performed on Fourier Transform Infrared Spectrophotometer (MIRacle 10). Small quantity of sample was taken and directly put on IR platform. Then the spectrum was studied in the 4000 to 400 cm^{-1} wavelength region. [18]

b. DSC (Differential Scanning Calorimetry) study: DSC thermogram of PCL, Isabgol Husk Mucilage (IHM), Locust Bean Gum (LBG) and physical mixture of PLC, IHM and LBG were performed on a Shimadzu DSC 60. This instrument is calibrated for temperature and enthalpy by using pure indium. 3-5 mg of sample was placed on non-fermetic Aluminum pans and crimped and finally covered with lid. It was then scanned at 50-300 ºC. Meanwhile the heating rate is maintained at 10 ºC/min under a continuous nitrogen gas purging (rate of flow 20 mL/min). The instrument uses a refrigerated cooling system. [19]

II. Preparation of Isabgol Husk Mucilage (IHM): The Plantago ovata husk were soaked in distilled water for 48 hrs. Then boiled for 20 minutes. The collected material was squeezed through muslin cloth to separate them. Then, an equal volume of acetone was added to the filtrate for precipitation of the mucilage. The separated mucilage was dried at 40 ºC in a tray dryer. The dried mucilage was powdered and sieved in sieve no # 80. The resultant powder was stored in a desiccator and used for the present study.

III. Preparation of Locust Bean Gum (LBG): The extraction and purification of the gum began by boiling the seed for several hours in order to dehusk it to obtain the endosperm. The endosperms were separated from the husk and ground gently using a blending machine. It was then dispersed in hot water and the shaft was sieved out using a clean muslin cloth. The filtrate (which contains the gum content) was collected. Ethanol (Analytical grade) was added onto the filtrate in order to extract the gum. This was left to stand for more than twelve hours and the supernatant was decanted, leaving the slurry. The slurry was centrifuged for ten minutes at 4000 RPM, and dried at 60 °C for 24 h by cutting into pieces and properly spread in a clean brown paper to hasten drying. The dry gum was then pulverized using porcelain mortar and pestle. The weight was taken using a weighing balance.

IV. Formulation of in situ ocular gel: In situ gel formulations containing different concentrations of Isabgol Husk Mucilage, Locust Bean Gum and

Carbopol-940 in combination with HPMC-K4M were prepared by dispersion method. Briefly, about 20 mL distilled water was preheated to 70 °C to dissolve Benzalkonium chloride and then sodium chloride (NaCl), HPMC and Carbopol-940 were incorporated into the solution. The mixture was left at room temperature overnight to allow the polymer to hydrate. Pilocarpine was dissolved in 5 mL distilled water separately. It was added into above polymeric solution and stirred until a uniform solution was obtained. The final product was filled into sterile amber colour bottles and sterilized in autoclave at 121 °C for 15 min. The prepared formulations were stored in refrigerator at 4 °C until further use. [20]

Table 1: Formula for in situ ocular gel

Sr. No.	Ingredients	R1	R2	R3	R4	R5	R6	R7	R8	R9
	Pilocarpine	2	2	2	2	2	2	2	2	2
	IHM	2	2	2	3	3	3	4	4	4
	LBG	0.3	0.5	0.7	0.3	0.5	0.7	0.3	0.5	0.7
	Carbopol-940	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	HPMC-K4M	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	NaCl	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
	Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	Distilled water	Q. S. to 30 mL (Applicable to all batches)								

**All values in the table are in % W/V.*

V. Evaluation of in situ ocular gel

A. Organoleptic examination: A general visualization of prepared formulations was done to check out the changes in color, odor as well as the appearance and clarity on the first third, seventh, fourteenth, twenty first and twenty eighth day of the preparation. [20]

B. In situ gelation: The determination of in vitro gelling capacity was done by visual method. It was performed as per the previously established method. The in vitro gelling capacity was graded in two categories on the basis of gelation time and time period for which the formed gel remains as such i.e, gel intact time. [21]

C. Rheological study: Viscosity of the formulation was checked by using Brookfield viscometer (Cole Parmer), before and after gelation using spindle number. L3. The angular velocity of the spindle was increased 10 to 100, and the viscosity of the formulation was measured before and after gelation. [21]

D. Determination of pH: The developed formulations were evaluated for pH by using digital pH meter. [22]

E. Drug content: The drug content of in situ gel was determined as per the previously established method. [22]

F. In vitro drug release: In vitro release studies were carried out using franz diffusion cell by using STF fluid pH 7.4. It was performed as per the previously established method. The drug release was analyzed using UV visible spectrophotometer at 213.3 nm using STF fluid as blank. [23, 24] In dissolution studies 2 parameters were studied. It includes determination of *f1* and *f2* by comparing the optimized batch R9 with PILOPINE HS and release kinetic studies.

G. Ex vivo drug permeation studies: Ex-vivo trans corneal permeation study was carried out on freshly excised goat cornea. The fresh, whole eye balls of the goat were obtained from local slaughter shop and transported to the laboratory in cold condition at 4º C in normal saline. It was performed as per the previously established method. The permeation study was carried out and the samples were withdrawn from the receptor and analyzed for drug concentration by measuring absorbance at 213.3 nm in a UV-Visible spectrophotometer. [25, 26]

H. Test of sterility: Sterility test was performed as per the previously established method by using Fluid Thioglycolate medium and Soyabean Casein digest medium for testing of bacteria fungi in the preparation respectively. [27, 28]

I. Osmolality or Isotonicity studies: The isotonicity was determined by using Digital Osmometer. [29]

J. Optimization study for prepared in situ gel

The optimization study was performed by using response surface plot (3 dimensional plot) and contour plot (2 dimensional plot) to determine the effect independent variables on dependent variables. [30]

K. Ocular irritation studies

The protocol approved by the Institutional Animal Ethics Committee for animal care (SPJCOPR/IAEC/M-3/2025-26/02) was followed during the conduction of experiment which was entirely based on the guidelines of 'The Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA). Six adult male Wistar albino rats, weighing 180–200 g were used in this study. Each rat was housed freely in a controlled setting which was maintained at 21 °C ±1 °C and 55 % ±1 % relative humidity. The animals remained in 12 hours light as well as 12 hours dark cycle conditions. The animals were fed and watered as needed. All rats were given intramuscular injections of 5.0 mg/kg xylazine

hydrochloride and 50 mg/kg ketamine hydrochloride to induce anesthesia. The rats were instilled with two drops of prepared optimized formulation R9 in one eye once in a day for a time period of 21 days. The other eye which remained untreated was considered as a control. The parameter of irritation as well as various adverse signs like swelling, redness, discharge of fluid from the eye, hemorrhage, cloudiness as well as blindness was carefully inspected visually. [31]

L. Stability study:

Optimized formulation batch R9 was subjected to accelerated stability study by using previously established method. The different evaluation parameters such as the clarity, gel intact time, viscosity after gelation, pH and drug content were assessed at the initial time, after 3 months and after 6 months. For this study, the guidelines laid by the ICH were strictly followed. [32]

3. RESULT AND DISCUSSION

I. Preformulation studies on pilocarpine

A. Melting point determination of pilocarpine: The melting point of Pilocarpine by using melting point apparatus was noted to be 204-205 °C.

B. Determination of maximum wavelength (λ_{max}): λ_{max} of PLC of concentration 20 µg/mL in simulated tear fluid (STF) pH 7.4 was noted to be 213.3 nm.

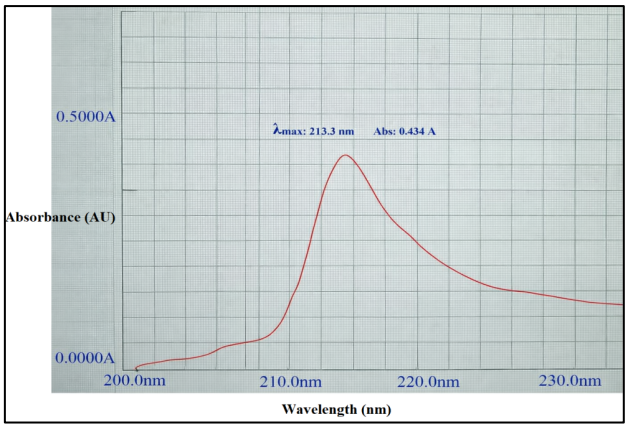

Figure 1: λmax of pilocarpine in STF pH 7.4

C. Standard calibration curve: The standard calibration curve of prepared solutions of known concentration of PLC is mentioned in figure 2.

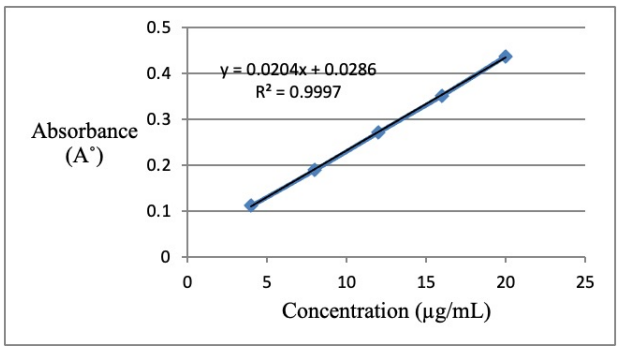

Figure 2: Calibration curve of pilocarpine in STF pH 7.4

D. Compatibility between drug and excipients

a. IR (Infra-red) study: Various prominent peaks with their corresponding functional groups are given in table 2.

Table 2: Interpretation of pilocarpine by IR

Sr. No.	Observed peak (cm^{-1})	Functional group
1.	3446.79	O-H Stretching
2.	3284.77	N-H stretching
3.	1751.36	C=O Stretching
4.	1649.14	C=N stretching
5.	1580.77	N-H Bending

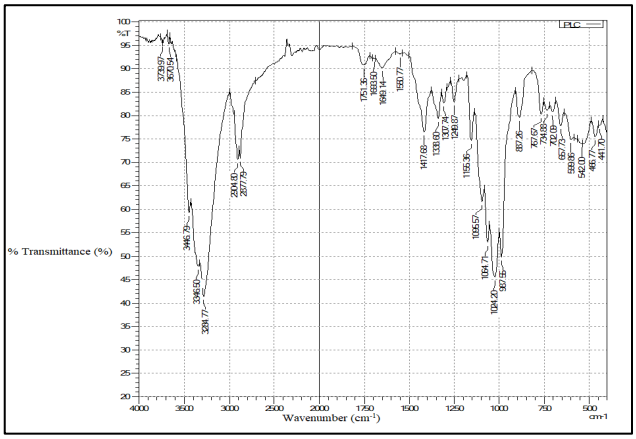

Figure 3: IR spectra of PLC

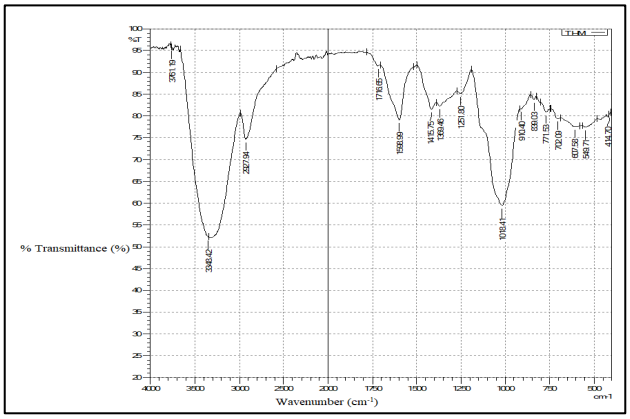

Figure 4: IR spectra of IHM

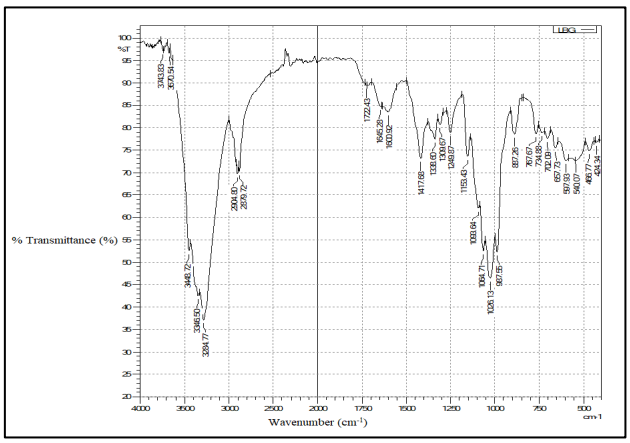

Figure 5: IR spectra of LBG

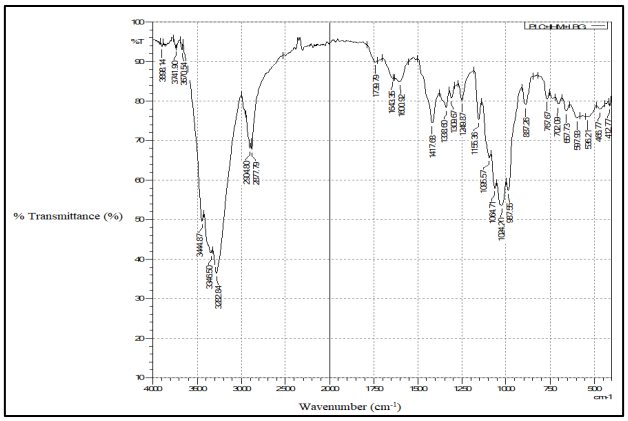

Figure 6: IR spectra of physical mixture of PLC, IHM and LBG

By cross checking the prominent peaks of some characteristic functional groups in PCL, IHM, LBG and physical mixture of PLC, IHM and LBG, it can be revealed that there is no significant shifting of peaks observed. As a result, there appears to be no chemical interaction of IHM and LBG with PLC.

b. DSC (Differential Scanning Calorimetry) study: DSC spectra of PLC, IHM, LBG and physical mixtures of PLC, IHM and LBG as shown in fig. 7, 8, 9 and 10 respectively were recorded to see the thermal behavior of drug and also to check drug-excipients compatibility.

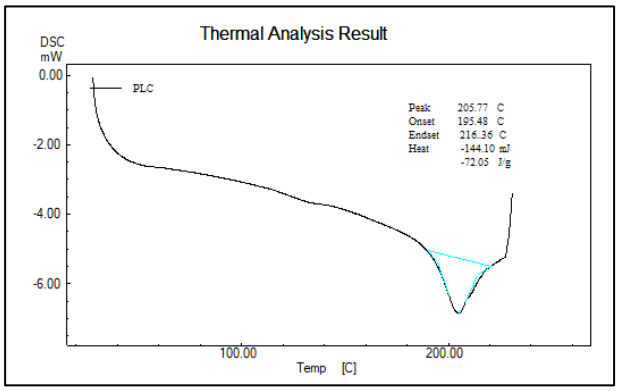

Figure 7: DSC spectrum of PLC

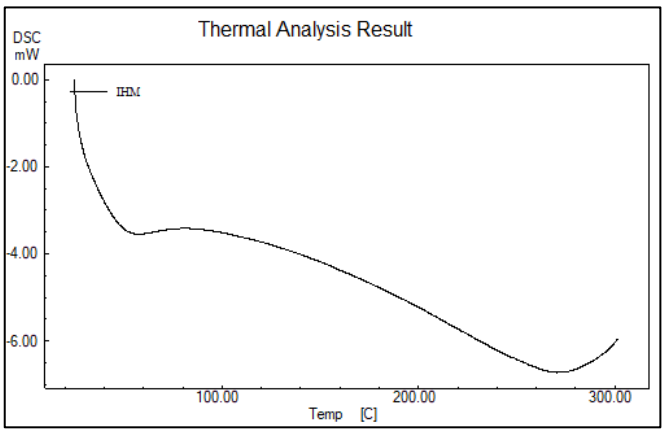

Figure 8: DSC spectrum of IHM

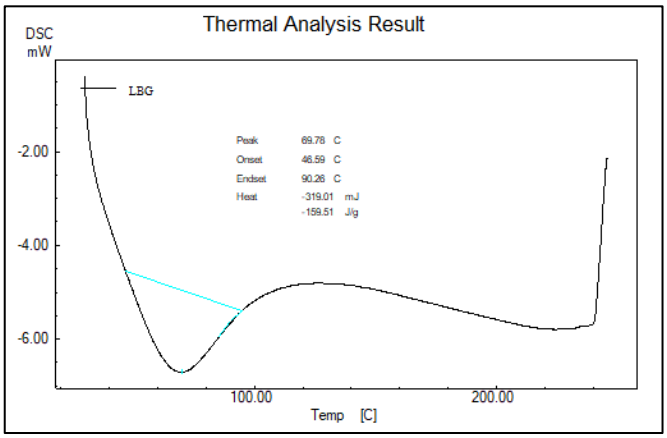

Figure 9: DSC spectrum of LBG

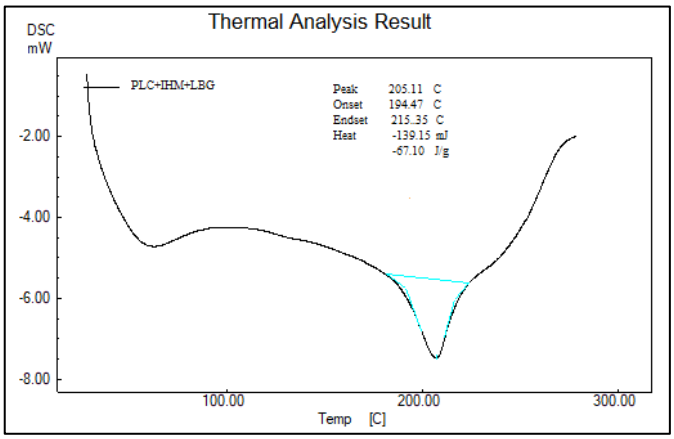

Figure 10: DSC spectrum of physical mixture of PLC, IHM and LBG

Table 3: Interpretation of Pilocarpine by DSC

Sr. No.	Name of sample	Sharp endothermic peak (°C)	Inference
1	PLC	205.77	No significant shifting of sharp endothermic peak observed in the physical mixture.
2	IHM	No peak	
3	LBG	No peak	
4	PLC + IHM + LBG	205.11	

Figure 7 illustrates the DSC thermogram of pilocarpine indicated a characteristic endothermic peak at 205.77 °C

which suggests the melting point of pilocarpine. DSC thermogram of IHM and LBG do not show sharp endothermic peak as these are natural compounds. When DSC spectrum of PLC alone was compared with physical mixture of PLC, IHM and LBG, no shifting of the endothermic peak of drug was found. Thus, it can be revealed that, there is no interaction of IHM and LBG with PLC.

II. Formulation of in situ ocular gel: The in situ ocular gel was formulated using IHM powder and LBG powder in the batch of 30 mL. Here the ability of IHM and LBG to transform *sol to gel* by pH triggered approach was highlighted for the development of in situ ocular gel.

III. Evaluation of in situ ocular gel

A. Organoleptic examination: After the general visualization of the all the prepared 9 formulations, it was observed that all the prepared batches of in situ gel formulations were found to be clear, transparent and free from particulate matter. No changes in color, odor, appearance and clarity was noted on the third, seventh, fourteenth, twenty first and twenty eighth day of the preparation.

B. In situ gelation: The results of the in-situ gelation time and time of gel remain as such i.e, gel intact time are mentioned in table 4.

Table 4: Gelation behavior of in situ ocular gel

Batch	In situ gelation time (s)	Gel intact time (h)
R1	54±0.4	5±0.5
R2	44±0.8	6.5±0.6
R3	36±0.7	8±0.8
R4	40±0.3	7±0.1
R5	32±0.4	8.5±0.6
R6	26±0.9	10±0.4
R7	28±0.2	9±0.1
R8	20±0.7	10.5±0.5
R9	16±0.4	12±0.7

***n=3; values are expressed as mean ± SD**

From table 4 it could be said that the in-situ gelation time of all batches lies within 16 to 54 sec. An acceptable and ideal time of in situ gelation for an ocular gel is up to min. The formulation should be transformed from sol to gel immediately when instilled in to ocular cavity. On the other hand, all batches showed the gel intact time in the range 5 to 12 h. The results of the in-situ gelation suggested that, as concentration of IHM powder and LBG powder increases, the in-situ gelation time decreases while as gel intact time increases. Formulation R9 containing IHM powder at 4 % while as LBG powder at 0.7 %, showed highest gel intact time i.e, 12 h among all the formulations. So formulation batch R9 could be considered the best among all. This suggests that the combined use of IHM powder, LBG powder and carbopol-940 in in situ ocular gel formulation helps to reduce the in-situ gelation time and prolong the gel intact time, which could be attributed to in situ gelation property of both novel polymers used in the research.

C. Rheological study: In rheological study the viscosity of the formulation was measured before and after gelation. The results of the rheological study are mentioned in table 5.

Table 5: Rheological study of in situ ocular gel

Batch	Viscosity of solution before gelation (cps)	Viscosity of solution after gelation (cps)
R1	106±0.5	2246±0.4
R2	116±0.3	2470±0.6
R3	125±0.9	2896±0.1
R4	122±0.7	2674±0.9
R5	131±0.3	3013±0.3
R6	144±0.2	3446±0.2
R7	138±0.2	3220±0.6
R8	147±0.2	3763±0.8
R9	156±0.3	4236±0.4

***n=3; values are expressed as mean ± SD**

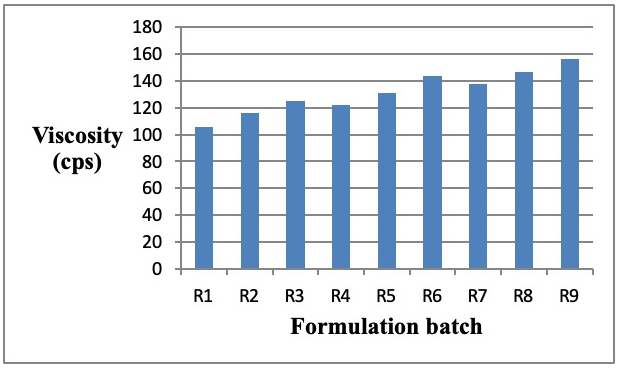

Figure 11: Comparison of viscosity before gelation of R1 to R9

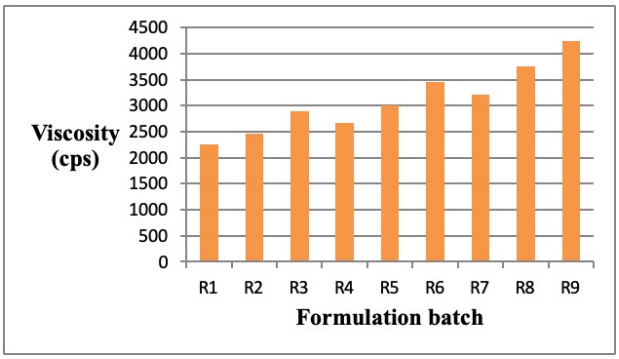

Figure 12: Comparison of viscosity after gelation of R1 to R9

An ideal viscosity of the in situ ocular gel before gelation and after gelation should be in the range 50 to 160 cps and 500 to 6000 cps respectively. From figure 11 and 12 it could be said that the viscosity of in situ ocular gel before as well as after the gelation increases as the

concentration of both IHM powder and LBG powder increases. Formulation batch R9 showed the highest viscosity. So, the in-situ gel containing the combination of IHM powder, LBG powder and Carbopol-940 gives better results of viscosity than used alone.

D. Determination of pH: pH values of all the formulations were found in the range 5.5 to 6.3 which could be considered as ideal and non-irritating for the ocular cavity and stable under storage conditions.

Table 6: pH of in situ ocular gel

Batch	R1	R2	R3	R4	R5	R6	R7	R8	R9
pH	6.3 ±0.2	6.3 ±0.7	5.7 ±0.4	6.3 ±0.4	6± 0.5	5.5 ±0.4	5.8 ±0.3	6.2 ±0.3	5.9 ±0.3

***n=3; values are expressed as mean ± SD**

E. Drug content: Drug content values of all the batches of in situ ocular gel were found within acceptable range i.e, 95 to 105 %. So drug content satisfied the criteria of Pilocarpine.

Formulation	R1	R2	R3	R4	R5	R6	R7	R8	R9
f1	12.5±0.5	8.3 ±0.4	6.5 ±0.8	6.6 ±0.4	9.9±0.2	5.0 ±0.1	9.0±0.5	4.9 ±0.6	5.8 ±0.2
f2	66.6±0.6	67.6 ±0.4	81.6±0.3	71.4 ±0.7	62.3±0.3	66.5±0.9	67.6±0.3	68.7±0.2	65.2±0.6

*** n=3; values are expressed as mean ± SD**

F. In vitro drug release: Drug release pattern of in situ ocular gel is shown in figure 13. HPMC-K4M used in the in-situ gel showed the sustained release effect in all batches of in situ gel. The novel polymers IHM powder and LBG powder offer the sustained release effect to very lesser extent. The best sustained release 92.53 % at 12 h. was observed with the formulation R9 which contains the IHM powder and LBG powder in the concentration 4% and 0.7 % respectively. On the other hand, PILOPINE HS, the marketed gel of pilocarpine showed drug release 83.85 % only up to 10 h. On the basis of drug release study, it could be said that, as the concentration of IHM powder and LBG powder increases, the drug release decreases; which actually is not related to these polymers but due to the in-situ gel formation which helps for the sustained release of pilocarpine from the gel.

So, it can be said that when in situ gel remain in the ocular cavity for prolong period of time i.e, 12 h, it will provide sustained release of pilocarpine for the same time period. So ultimately the in situ ocular gel would increase the bioavailability of pilocarpine in the ocular cavity.

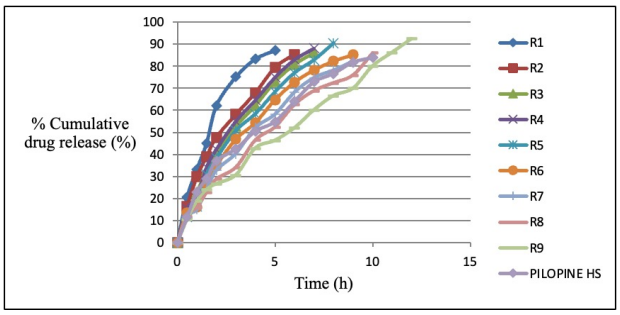

Figure 13: Drug release of in situ ocular gel

Estimation of difference factor (*f1*) and similarity factor (*f2*): In this research, all prepared batches of in situ gel are compared with marketed formulation PILOPINE HS. Table 7 showed *f1*and *f2* values. The *f1* values of all formulation are less than 15 whiles as *f2* values are more than 50, which fulfills the criteria of similarity and dissimilarity factor.

Table 7: *f1* and *f2* values of in situ ocular gel

Release kinetic studies: Dissolution study data was fitted to various release kinetic models to know the drug release mechanism. The curve fitting results of drug release data indicated that release of pilocarpine from most of the batches of in situ gel formulation follows Higuchi model. Some formulation also follows Korsmeyer Peppas model due to presence of HPMC-K4M rate controlling polymer matrix. The values of the release exponent (n) primarily show that the drug release mechanism is anomalous (non-fickian) diffusion.

Table 8: Curve fitting of drug release of in situ ocular gel

Formulation	R^2				Release exponent (n)
	Zero order	First order	Higuchi	Korsmeyer Peppas	
R1	0.896±0.6	0.771±0.2	0.972±0.5	0.776±0.5	0.469±0.6
R2	0.893±0.7	0.733±0.7	0.973±0.9	0.982±0.4	0.438±0.8
R3	0.875±0.7	0.863±0.8	0.986±0.8	0.993±0.7	0.522±0.7
R4	0.872±0.8	0.741±0.1	0.982±0.6	0.991±0.2	0.491±0.8
R5	0.944±0.2	0.750±0.2	0.991±0.2	0.990±0.9	0.419±0.3
R6	0.822±0.4	0.833±0.2	0.991±0.2	0.994±0.6	0.482±0.5

R7	0.852± 0.2	0.637± 0.5	0.997± 0.4	0.934± 0.3	0.595± 0.7
R8	0.918± 0.9	0.762± 0.2	0.997± 0.1	0.939± 0.2	0.543± 0.9
R9	0.930± 0.2	0.857± 0.1	0.953± 0.2	0.944± 0.9	0.606± 0.4
PILOPINE HS	0.966± 0.2	0.860± 0.8	0.976± 0.6	0.945± 0.7	0.600± 0.9

*** n=3; values are expressed as mean ± SD**

G. Ex vivo drug permeation studies: The prepared in situ gel formulations were subjected to ex vivo drug permeation studies to understand the diffusion pattern of the drug through the goat cornea. The rate of drug permeation of all the formulation was observed to be slow because the pilocarpine is highly hydrophilic in nature. On the other hand the corneal epithelium is highly lipophilic. Hence the corneal epithelium is expected to be the rate-limiting barrier for ocular absorption of pilocarpine. It results into a slower diffusion rate of drug through cornea. The results of the ex vivo drug permeation studies are mentioned in figure 14.

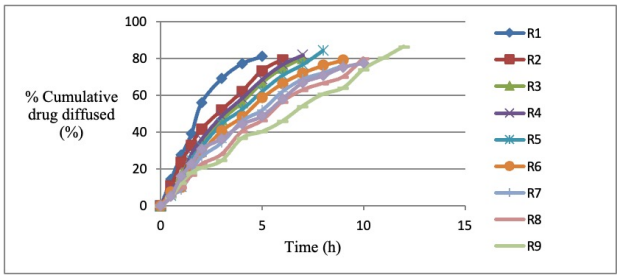

Figure 14: Ex vivo drug diffusion of in situ ocular gel

H. Test of sterility: After the incubation time period of 14 days at a temperature of 30-35 °C in Fluid Thioglycolate medium and 20-25 °C in Soyabean Casein digest medium, no turbidity or growth of microorganisms observed in any of the 9 in situ ocular gel formulations. So, it could be said that the prepared in situ ocular gel passed the test of sterility.

I. Osmolality or Isotonicity studies: An ideal osmolality for ophthalmic formulations is generally considered to be isotonic with human tears, falling within the range of 290 to 310 mOsm/kg (or mOsm/L). If it is altered drastically, it may cause cell swelling or cell shrinkage. Ophthalmic gels should be isotonic with the human tear to minimize irritation and promote a longer ocular residence time.

The results of the osmolarity are mentioned in table 9 which suggest that the osmolarity of all the formulation batches of in situ ocular gel are in the acceptable range.

Table 9: Osmolality of in situ ocular gel

Batch	Osmolarity (mOsm/Kg)
R1	289.89±0.7
R2	304.77±0.4
R3	300.12±0.4
R4	293.45±0.5
R5	284.52±0.7
R6	305.56±0.3
R7	286.58±0.2
R8	300.12±0.4
R9	293.45±0.5

***n=3; values are expressed as mean ± SD**

J. Optimization study for prepared in situ gel: Optimization study suggested that, as concentration of IHM powder increases, gel intact time and gelling viscosity increases. On the other hand, concentration of LBG powder also has similar effect on gel intact time and gelling viscosity but to the lesser extent as compared to IHM powder. Concentration of IHM powder and LBG powder has very less effect on t_{75} of % CDR. It can be seen that among all the batches of in situ gel formulation, R9 is the most efficient and could be considered as optimized formulation. Hence used novel polymers IHM powder and LBG powder could be used as good pH triggered in situ gelling polymers in the designing of in situ ocular gel.

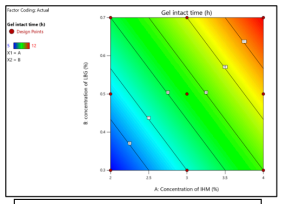

Figure 15: Contour plot of gel intact time

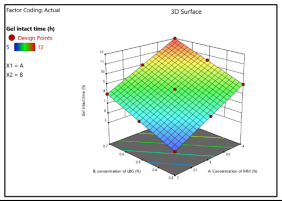

Figure 16: Response surface plot of gel intact time

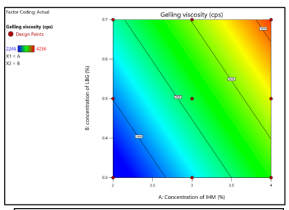

Figure 17: Contour plot of gelling viscosity

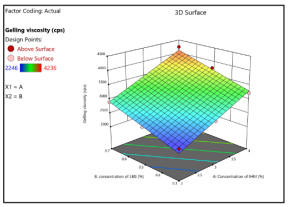

Figure 18: Response surface plot of gelling viscosity

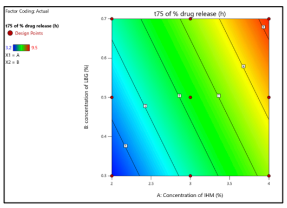

Figure 19: Contour plot of t_{75}

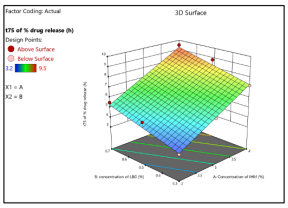

Figure 20: Response surface plot of t_{75}

K. Ocular irritation studies: It was investigated visually that, after instilling the optimized formulation R9, there was no occurrence of swelling, redness or unwanted lachrymation in the eye. No ocular damage or any other clinical symptom was observed in various parts of the eye like cornea, iris or conjunctiva which further proved that the optimized formulation of in situ ocular gel used in the study was entirely harmless and non-irritant for the administration in the eye.

K. Stability study: The results of the stability study of optimized formulation R9 are mentioned in table 10.

Table 10: Accelerated stability study

Duration of stability study	Clarity	Gel intact time (h)	Viscosity after gelation (cps)	pH	Drug content (%)
Initial	Clear	11.5±0.8	4305±0.6	6.8±0.6	101.33±0.5
After 3 months	Clear	12.5±0.7	4156±0.2	6.5±0.9	100.22±0.6
After 6 months	Clear	12±0.3	4258±0.8	6.3±0.5	98.55±0.9

*** n=3; values are expressed as mean ± SD**

The accelerated stability study was carried out for the optimized in situ ocular gel formulation i.e, batch R9. The stability study results suggested that clarity, gel intact time, viscosity after gelation, pH and drug content are within acceptable limits. No considerable changes in the results observed after 3rd and 6th month of stability study. Thus, the formulation R9 can be said to be stable.

4. CONCLUSION: On the basis of result and discussion in this research, formulation R9 of in situ ocular gel containing IHM powder and LBG powder at 4 % and 0.7 % concentration respectively showed pH triggered in situ gelation behavior i.e, in situ gelation time 16 sec. and gel intact time 12 h. On the other hand, in situ gel showed expected sustained release profile for 12 h with the use of HPMC-K4M. The results suggested that the in-situ gel of pilocarpine (R9) showed better sustained release profile than that of PILOPINE HS. Optimization study suggested that concentration of IHM powder and LBG powder has a predominant effect on in situ gelation behavior when used in combination. As the concentration of IHM powder increases the gel intact time as well as viscosity after gelation increases. In the similar way as the concentration of LBG powder increases the gel intact time as well as viscosity after gelation increases. But the effect of IHM powder is more predominant than that of the LBG powder. These both novel polymers have very less sustained release effect. The sustained release effect of in situ ocular gel is mainly attributed to HPMC-K4M a release retardant polymer used in the formulation. On the basis of optimization studies, it is very clear that IHM powder and LBG powder could be used as effective pH triggered in situ gelling novel natural polymers with desired gelation behavior and viscosity after gelation to prepare in situ ocular gel of pilocarpine. An accelerated stability studies showed that, no considerable changes in the results observed after 3rd and 6th month of stability study. Thus, the formulation R9 can be said to be stable.

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