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Original Research Article

Development of PH Triggered in-Situ Gel for Ophthalmic Delivery of Pilocarpine.

Article History:

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Abstract: Now days, natural polymers are being used as an alternative to synthetic polymers for the preparation of ophthalmic dosage forms. Such natural plant-based materials are very biocompatible, biodegradable with lack of side effects and economic. "The main focus of this research is to use Isabgol Husk Mucilage (IHM) as a novel pH triggered in situ gelling polymer in the formulation and development of pilocarpine in situ ocular gel." In current research in situ ocular gel of pilocarpine was prepared by using Isabgol Husk Mucilage (IHM) in the concentration 1-5 % W/V alone and in combination with Carbopol-940 in the concentration 0.3-0.8 % W.V. The in situ gel was subjected to various evaluation tests ocular gel including Organoleptic examination, In situ gelation, Viscosity, pH, In vitro drug release, Ex vivo drug permeation studies, Test of sterility, Osmolality or Isotonicity studies. The prepared in situ ocular gel fulfilled all the evaluation tests of in situ ocular gel. 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 best in situ ocular gel formulation batch I7 showed in situ gelation time 36 second, gel intact time 8.5 hours and osmolality 302.12±0.4 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. The best selected formulation I7 consists of the 3 % IHM powder in combination with 0.5 % Carbopol-940 and proven excellent gelation behavior. As per the dissolution studies and ex vivo permeability studies it can be concluded that the pH triggered in situ gel can increase the bioavailability of pilocarpine for 9 hours.

Keywords: Pilicarpinel; Isabgol Husk Mucilage; In situ gelation; Carbopol-940:

INTRODUCTION

Ophthalmic delivery of drug has always considered among the foremost demanding aspects for researchers worldwide as the organization of eye bears some of the physiological barriers that have to be targeted sequentially to accomplish a good rate of absorption and bioavailability. [1] Eye is considered to be a fascinating organ due to its uniqueness. In the advancement of an ocular drug delivery system, parameters of absorption, distribution, metabolism and elimination (ADME) have to be given emphasis by a pharmaceutical scientist so that a better ocular system could be developed and less problems are encountered. [2, 3]

Ocular delivery of drug has number hurdles such as ocular barriers, drainage of the drug due to tear flow which ultimately leads to poor bioavailability of drug, thus reducing the desired therapeutic effect of the drug. [4, 5] But ocular delivery has one of the advantage that drug enters the systemic circulation by passing the first-pass metabolism. [6, 7] Ocular formulations such as Ointments, suspensions and aqueous gels have certain drawbacks such as poor patient compliance, blurred vision, cumbersome for self-administration, ocular drainage of drug, instability of the formulation, poor retention and bioavailability of drug in ocular cavity etc. [8, 9]

To overcome the problem of poor retention and bioavailability of drug in ocular cavity pH triggered in situ gel can be a one of the good alternative approach. [10] This in-situ gelling system consists of pH-sensitive polymers which are polyelectrolytes contain an acidic (carboxylic or sulfonic) or a basic group (ammonium salts) that either accept or release protons in response to alteration in pH in the surrounding environment. [11, 12] At lower pH (pH 4.4), the formulation exists as a regular solution, however, it undergoes gel formation at pH 7.4, that is the pH of tear fluid. [13] The most commonly used pH-responsive polymers in ophthalmic preparation are Polyacrylic acid (PAA, Carbopol 940), polycarbophil, and cellulose acetate phthalate (CAP). [14]

pH triggered in situ gel offers several advantages such as ease of administration, ease of manufacturing, patient compliance, good sol to gel transformation by the tears, prolong ocular retention time of drug, increase bioavailability, sustained release of drug due to the gel network formation etc. [15]

MATERIAL AND METHOD

2.1 MATERIAL: The fresh Isabgol husk 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: Preformulation is the study of physicochemical properties of drug alone or in combination with excipients. It is the first step in the development of any project.

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

B. Determination of λmax: 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 λmax 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) and physical mixture of PLC and IHM 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) and physical mixture of PLC and IHM 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. Formulation of in situ ocular gel using IHM:

In situ gel formulations containing different concentrations of Isabgol Husk Mucilage 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 using IHM

Sr. No.	Ingredients	I1	I2	I3	I4	I5	I6	I7
1.	Pilocarpine	2	2	2	2	2	2	2
2.	IHM	1	3	5	-	-	-	3
3.	Carbopol-940	-	-	-	0.3	0.5	0.8	0.5
4.	HPMC-K4M	0.5	0.5	0.5	0.5	0.5	0.5	0.5
5.	NaCl	0.9	0.9	0.9	0.9	0.9	0.9	0.9
6.	Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02
7.	Distilled water	Q. S. to 30 mL (Applicable to all batches)						

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

IV. Evaluation of in situ ocular gel containing IHM

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. Colored solutions were prepared by adding 1% amaranth dye solution in water and mixed with in situ ocular gel. The in vitro gelling capacities of prepared formulations were measured by placing 5 mL of the STF pH 7.4 in glass tube. To this 20 µl-50 µl of colored formulation solution was added with the help of pipette. As the solution comes in contact with STF fluid 7.4, it gets converted into stiff gel. The gelling capacity of solution was evaluated on the basis time required to form the gel. Colored dye was added in the formulation in order to visualize the appearance of in situ gel formation. 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 specific amount of gel was taken and diluted with STF pH 7.4. It was then stirred in order to dissolve the pilocarpine as well as formulation uniformly with the STF pH 7.4. The solution was further diluted with STF pH 7.4 in such a way that the resultant solution should have the concentration of pilocarpine 10 µg/mL.

This solution was then filtered and filtrate was subjected to UV visible spectrophotometric analysis at 213.3 nm and finally the absorbance was measured. [22]

It is possible to assess drug content by using the formula:

$$\text{Drug content (\%)} = \frac{\text{Practical concentration}}{\text{Theoretical concentration}} \times 100$$

F. In vitro drug release: In vitro release studies were carried out using franz diffusion cell. For this study the dialysis membrane was soaked overnight in STF fluid. The sample was applied on to the membrane and the membrane was placed in between donor and receptor compartment of the cell consisting of STF fluid pH 7.4. Samples were withdrawn at periodic intervals and replaced with fresh buffer to maintain sink condition. The drug release was analyzed using UV visible spectrophotometer at 213.3 nm using STF fluid as blank. [23, 24]

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 4° C in normal saline. The cornea was then carefully excised along with 2- 4 mm of surrounding scleral tissue and was washed with normal saline until the washings were free from proteins. The excised cornea was fixed between the clamped donor and receptor compartments of glass modified franz diffusion cell in such a way that its epithelial surface faced the donor compartment. The corneal area available for diffusion was 0.50 cm2. The receptor compartment was filled with 20 ml of freshly prepared simulated tear fluid pH 7.4 and all air bubbles were expelled from the compartment. Aliquot 1 ml of the prepared in situ gel was placed on the cornea and the opening of the donor cell was sealed in a glass cover slip. The receptor fluid was kept at 37 ºC with constant stirring using a teflon coated magnetic stirrer beads. 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: The formulations were kept in Fluid Thioglycolate medium (20 ml) to check the growth of bacteria and in Soyabean Casein digest medium (20 ml) to detect the growth of any fungi in the preparation. The method of direct inoculation was incorporated and 2 ml of the optimized formulation was transferred in to the respective mediums with the help of a sterile pipette or a syringe. The incubation of the inoculated media was done for not less than fourteen days at a temperature of 30-35 ◦C in Fluid Thioglycolate medium and at a temperature between 20-25 ◦C in Soyabean Casein digest medium. At the end, a visual inspection was done to check the growth of any undesirable microbial growth. [27, 28]

I. Osmolality or Isotonicity studies: The isotonicity was determined by using Digital Osmometer. The value in mOsm/kg is determined by the osmometer's measurement of freezing point depression, which is then converted into the osmolality reading. To obtain a reading, a sample is loaded into the instrument, which then cools it to its freezing point, measures the temperature at which freezing occurs, and displays the corresponding osmolality. [29]

J. Stability study: To assess the stability of the formulation, accelerated stability study was performed. 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 which can be referred to as the accelerated stability studies. For this study, the guidelines laid by the ICH were strictly adhered and the prepared optimized formulation was kept in a glass vial and the stability chamber was set at 40 ˚C ± 2 ˚C temperatures and 75 % ± 5 % relative humidity (RH) was also constantly maintained for the conduction of this study. [30]

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 (λmax): λmax of PLC of concentration 20 µg/mL in simulated tear fluid (STF) pH 7.4 was noted to be 213.3 nm.

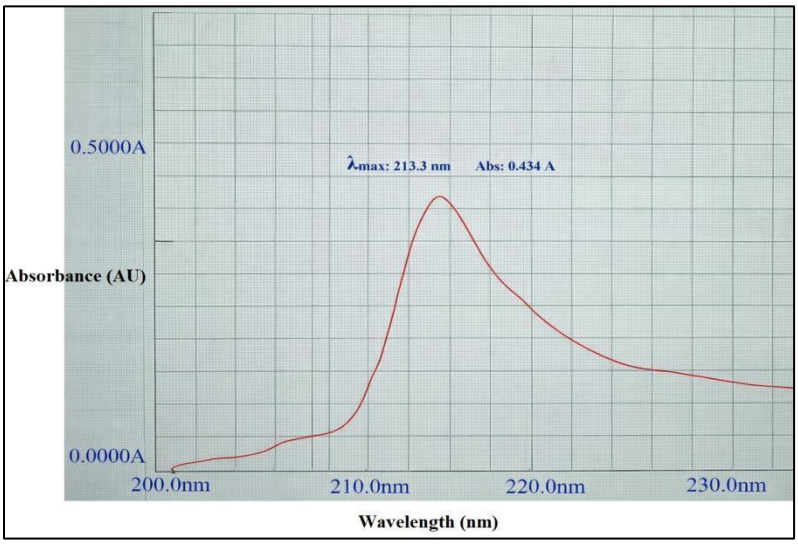

Figure 1: λmax of pilocarpine in STF pH 7.4

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

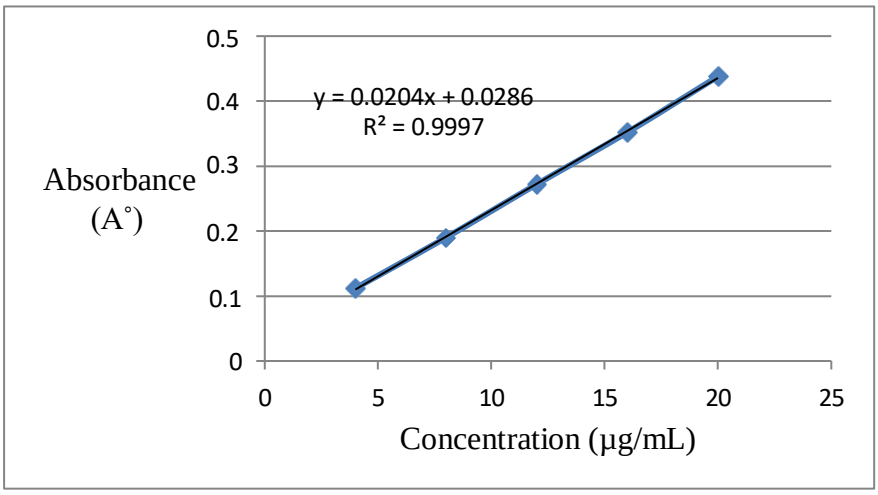

Figure 2: Calibration curve of pilocarpine in STF pH 7.4

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

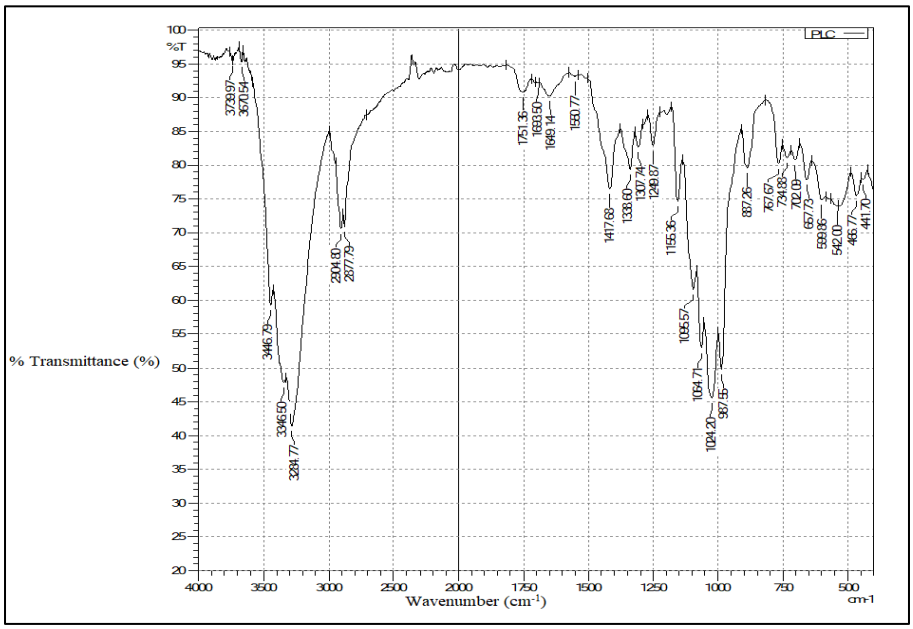

Figure 3: IR spectra of PLC

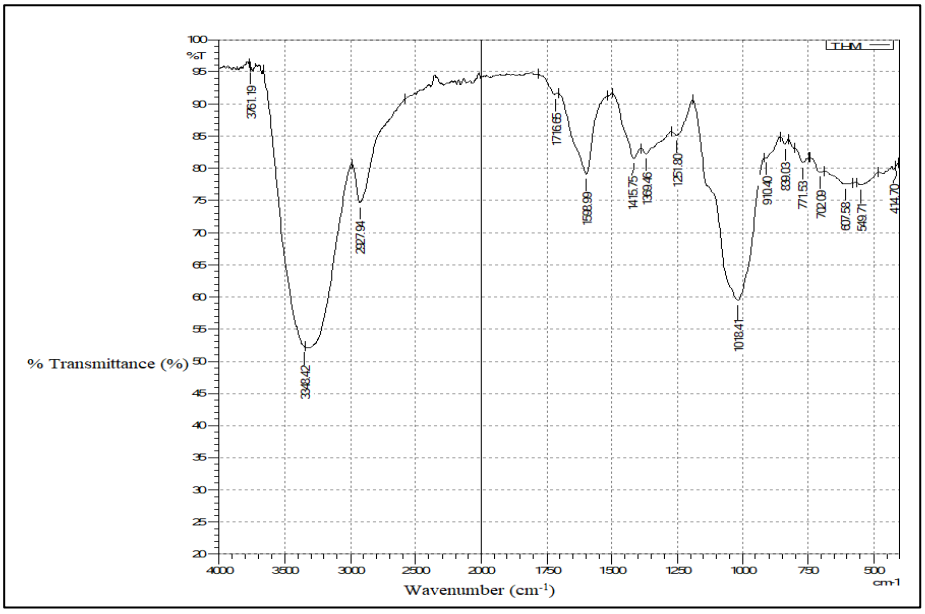

Figure 4: IR spectra of IHM

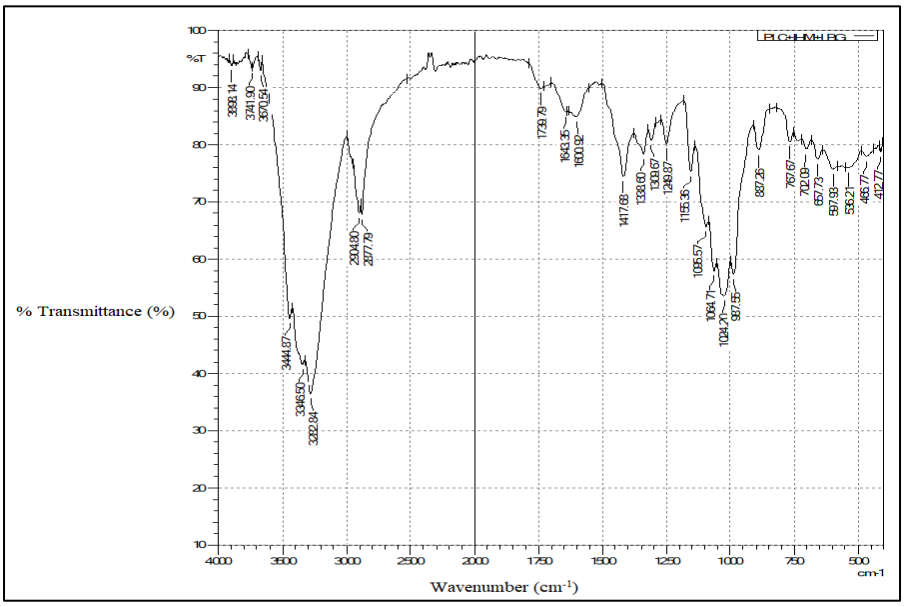

Figure 5: IR spectra of physical mixture of PLC and IHM

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

b. DSC (Differential Scanning Calorimetry) study: DSC spectra of PLC, IHM and physical mixtures of PLC and IHM as shown in fig. 6, 7 and 8 respectively were recorded to see the thermal behavior of drug and also to check drug-excipients compatibility.

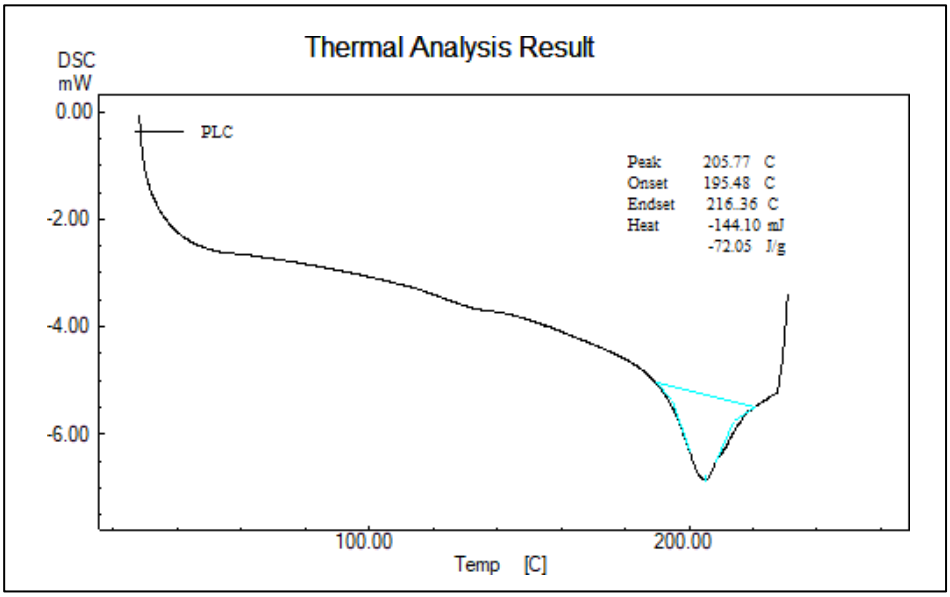

Figure 6: DSC spectrum of PLC

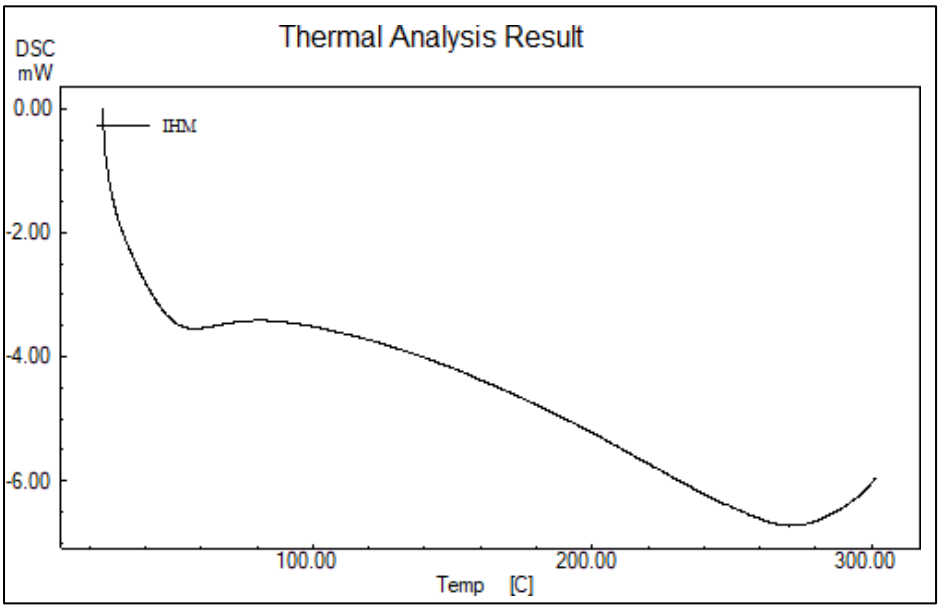

Figure 7: DSC spectrum of IHM

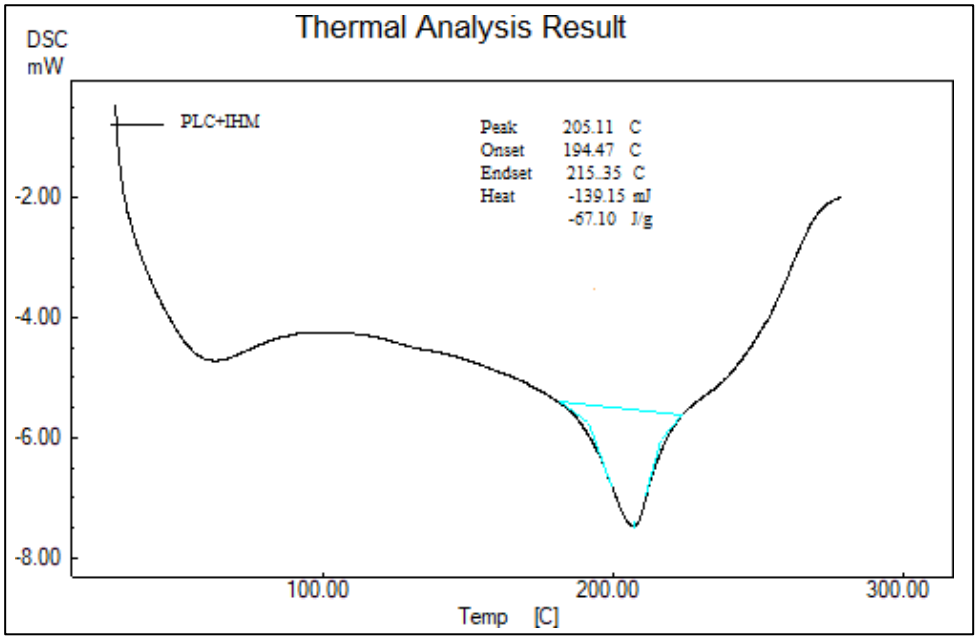

Figure 8: DSC spectrum of physical mixture of PLC and IHM

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	PLC + IHM	205.11	

Figure 6 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 does not show sharp endothermic peak as these are natural compounds. When DSC spectrum of PLC alone was compared with physical mixture of PLC and IHM, no shifting of the endothermic peak of drug was found. Thus it can be revealed that, there is no interaction among the PLC and IHM.

II. Formulation of in situ ocular gel using IHM: The in situ ocular gel was formulated using IHM powder in the batch of 30 mL. Here the ability of IHM 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 containing IHM

Organoleptic examination: After the general visualization of the all the prepared seven 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.

In situ gelation: The determination of in vitro gelling capacity was done by visual method. The gelling capacity of solution was evaluated on the basis time required to form the gel. Actually the in vitro gelling capacity was performed by two ways on the basis of gelation time and time period for which the formed gel remains as such. 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 of IHM powder

Batch	In situ gelation time (s)	Gel intact time (h)
I1	156±0.2	3±0.1
I2	140±0.6	3.5±0.2
I3	130±0.7	4±0.4
I4	118±0.3	4±0.7
I5	98±0.2	5±0.2
I6	72±0.7	6±0.4
I7	36±0.2	8.5±0.1

*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 36 to 156 sec. An ideal time of in situ gelation for an ocular gel is up to 2 min. It is very acceptable range for the in situ ocular gel. Because the formulation should be transformed from sol to gel immediately when instilled in to ocular cavity in order to avoid the loss of formulation through ocular drainage. On the other hand all batches showed the gel intact time in the range 3 to 8.5 h. Here formulations I1, I2 and I3 contain only IHM powder in the concentration 1, 3 and 5 %W/V respectively. In situ gel containing IHM showed the increase in in situ gelation time as well as gel intact time with increase in the concentration of IHM. On the other hand the formulations I4, I5 and I6 contain only Carbopol-940 in the concentration 0.3, 0.5 and 0.8 %W/V respectively. In situ gel containing Carbopol-940 also showed the increase in in situ gelation time as well as gel intact time with increase in the concentration of Carbopol-940. The last batch, I7 contains the combination of both IHM and Carbopol-940 in the concentration 3 and 0.5 %W/V respectively. Here batch I7 showed better results of the in situ gelation time as well as gel intact time i.e, 36 sec. and 8.5 h. This indicate that when IHM and Carbopol-940 are used in combination shows better results than the formulations containing IHM alone or Carbopol-940 alone.

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 of IHM powder

Batch	Viscosity of solution before gelation (cps)	Viscosity of solution after gelation (cps)
I1	68±0.7	1022±0.2
I2	76±0.3	1270±0.4
I3	88±0.2	1450±0.1
I4	106±0.2	2248±0.7
I5	118±0.2	2790±0.1
I6	127±0.7	3245±0.2
I7	137±0.6	3520±0.4

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

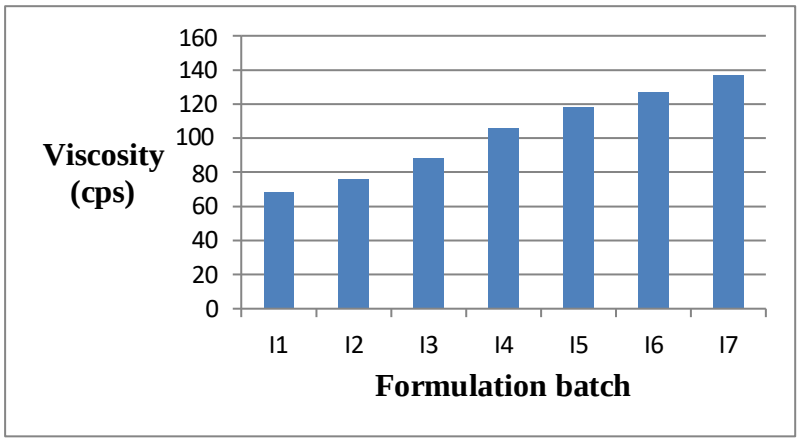

Figure 9: Comparison of viscosity before gelation of I1 to I7

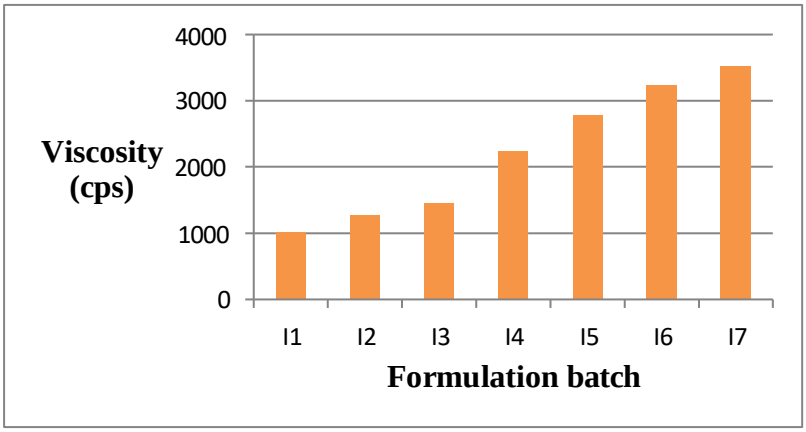

Figure 10: Comparison of viscosity after gelation of I1 to I7

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 9 and 10 it could be said that the viscosity of in situ ocular gel before as well as after the gelation increases for both IHM powder and Carbopol-940 as their corresponding concentration increases. But the better viscosity was obtained when both IHM and Carbopol-940 were used in the combination. Determination of pH: pH values of the in situ ocular gel from batch I1 to I7 are mentioned in table 6. pH values of all the formulations were found in the range 5.7 to 6.5 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 of IHM powder

Batch	I1	I2	I3	I4	I5	I6	I7
pH	6±0.5	5.7±0.4	6.5±0.3	6.3±0.2	6.3±0.7	6.5±0.4	6.3±0.4

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

Drug content: Drug content values of all the batches of in situ ocular gel were found within acceptable range i.e, 96.25 to 103.45 %.

In vitro drug release: Drug release pattern of in situ ocular gel containing IHM is shown in table 7 and figure 11. In situ gel containing IHM such as I1, I2 and I3 showed the decrease in drug release with increase in the concentration of IHM. It showed the drug release up to 5 h. On the other hand the in situ gel containing Carbopol-940 also showed the decrease in drug release with increase in the concentration of Carbopol-940. It showed the drug release up to 7 h. The last batch, I7 contains the combination of both IHM and Carbopol-940 in the concentration 3 and 0.5 %W/V respectively. Here batch I7 showed better sustained release profile up to 9 h. This indicate that when IHM and Carbopol-940 are used in combination shows better sustained release profile than the formulations containing IHM alone or Carbopol-940 alone.

So it can be said that when in situ gel remain in the ocular cavity for prolong period of time i.e, 9 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.

Table 7: Drug release of in situ ocular gel of IHM powder

Time (h)	% Cumulative drug release (%)						
Batch	I1	I2	I3	I4	I5	I6	I7
0	0	0	0	0	0	0	0
0.5	22.7±0.5	18.7±0.6	20.8±0.5	21.5±0.3	17.0±0.7	16.2±0.9	12.6±0.3
1	54.6±0.4	34.5±0.4	26.9±0.6	42.4±0.2	28.1±0.8	24.3±0.6	22.6±0.9
1.5	61.7±0.9	57.8±0.7	45.4±0.9	57.5±0.7	41.2±0.1	36.4±0.3	30.6±0.5
2	75.6±0.7	75.6±0.7	66.2±0.4	73.4±0.5	57.0±0.2	47.2±0.5	42.8±0.1
3	85.1±0.7	85.4±0.2	78.6±0.4	82.5±0.9	72.4±0.2	58.6±0.4	48.9±0.8
4	-	91.6±0.5	87.1±0.2	87.4±0.7	83.9±0.6	69.1±0.8	60.7±0.4
5	-	-	89.4±0.9	-	90.2±0.1	77.4±0.3	68.1±0.9
6	-	-	-	-	-	82.7±0.5	76.6±0.3
7	-	-	-	-	-	89.6±0.5	82.4±0.3
8	-	-	-	-	-	-	87.7±0.5
9	-	-	-	-	-	-	91.1±0.2

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

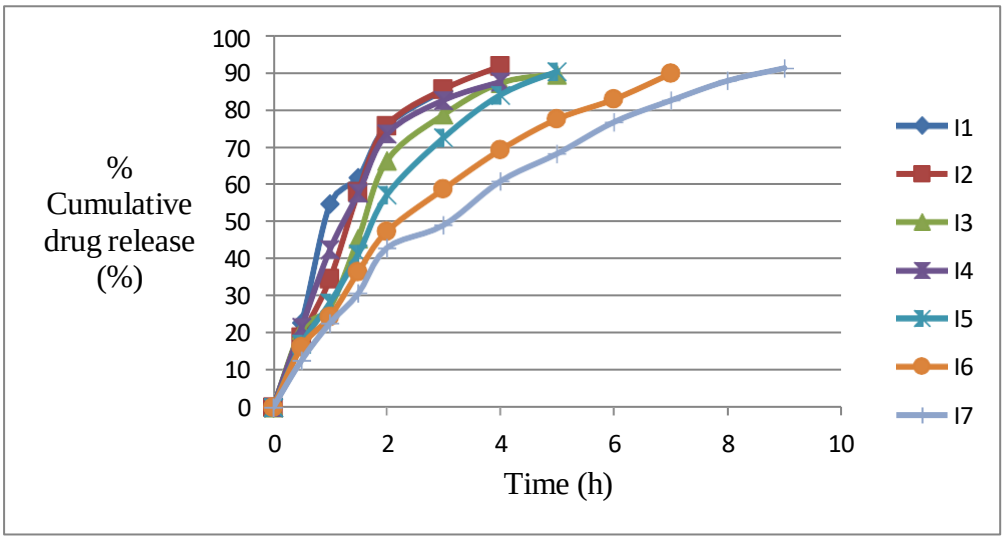

Figure 11: Drug release of in situ ocular gel of IHM powder

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 table 8 and figure 12.

Table 8: Ex vivo drug diffusion of in situ ocular gel of IHM powder

Time (h)	% Cumulative drug diffused (%)						
Batch	I1	I2	I3	I4	I5	I6	I7
0	0	0	0	0	0	0	0
0.5	17.7±0.7	13.7±0.9	15.8±0.3	17.5±0.3	12.0±0.7	13.2±0.9	10.6±0.1
1	49.6±0.4	29.5±0.6	21.9±0.6	36.4±0.4	22.1±0.8	20.3±0.4	18.6±0.9
1.5	55.7±0.1	50.8±0.7	45.4±0.1	52.5±0.7	36.2±0.3	30.4±0.3	25.6±0.2
2	68.6±0.7	68.6±0.7	60.2±0.4	67.4±0.7	52.0±0.2	42.2±0.5	36.8±0.3
3	82.1±0.7	78.4±0.1	70.6±0.2	78.5±0.9	66.4±0.4	52.6±0.4	42.9±0.2
4	-	85.6±0.5	82.1±0.2	83.4±0.9	78.9±0.6	62.1±0.4	54.7±0.4

5	-	-	85.4±0.9	-	85.2±0.3	71.4±0.3	62.1±0.9
6	-	-	-	-	-	76.7±0.5	72.6±0.7
7	-	-	-	-	-	84.6±0.4	76.4±0.5
8	-	-	-	-	-	-	82.7±0.5
9	-	-	-	-	-	-	87.1±0.2

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

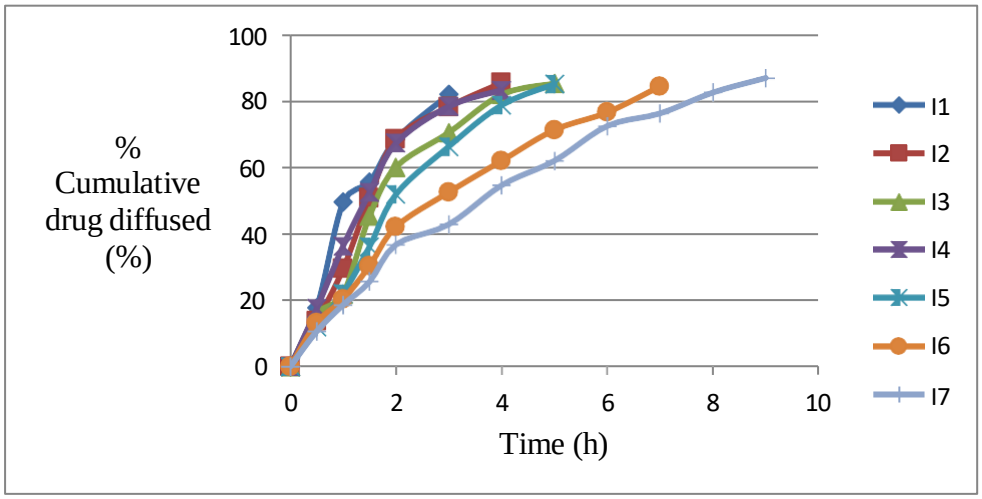

Figure 12: Ex vivo drug diffusion of in situ ocular gel of IHM powder

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 7 in situ ocular gel formulations. So it could be said that the prepared in situ ocular gel passed the test of sterility.

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 of IHM powder

Batch	Osmolarity (mOsm/Kg)
I1	295.45±0.5
I2	286.52±0.7
I3	307.56±0.3
I4	288.58±0.2
I5	291.89±0.7
I6	306.77±0.4
I7	302.12±0.4

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

Stability study: The results of the stability study of optimized formulation I7 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	8.5±0.4	3540±0.4	6.9±0.5	96.55±0.1
After 3 months	Clear	8±0.5	3557±0.6	6.4±0.7	99.44±0.2
After 6 months	Clear	9±0.1	3505±0.4	6.6±0.9	103.46±0.7

* 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 I7. 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 I7 can be said to be stable.

CONCLUSION:

On the basis of result and discussion in this research, formulation I7 of in situ ocular gel containing IHM powder and Carbopol-940 at 3 % and 0.5 % concentration respectively showed pH triggered in situ gelation behavior i.e, in situ gelation time 40 sec. and gel intact time 8.5 h. On the other hand, in situ gel showed expected sustained release profile for 9 h with the use of HPMC-K4M. The results suggested that as the concentration of IHM powder increases the gel intact time as well as viscosity after gelation increases. The novel polymers IHM has 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. The research says that IHM could be used as effective pH triggered in situ gelling novel natural polymer with good 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 I7 can be said to be stable.

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