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Design And Characterization Of Latanoprost In situ Gel.

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Abstract

In recent years, the In Situ gelling system has emerged as a promising drug delivery system in the ophthalmic domain. The current study focuses on the development and evaluation of an in-situ gelling system for treating glaucoma by increasing ocular residence time and bioavailability. Temperature sensitive polymers such as Pluronic F-127 and viscosifying agents such as HPMC K4M in various concentrations were used to create in situ gelling formulations. The evaluation parameters such as appearance, pH, clarity, gelation studies, drug content, gelling capacity, gelation temperature, rheological studies, and in vitro drug release studies of the prepared In Situ Gels were all evaluated. Ex vivo permeation studies, microbial tests, in vivo ocular irritancy, and stability studies were performed on the optimised formulation. Following characterization, BT-4 demonstrated the most promising profile of drug release. This formulation appeared clear, and the sol to gel transition occurred quickly as the formulation approached body temperature. The formulation was determined not to cause irritation on the ocular surface and found to be stable after three months of stability testing. Latanoprost in the form of gel could prove to be having a good potential as a drug delivery system in the treatment of glaucoma by controlling intraocular pressure and increasing bioavailability.

Key Words: In Situ Gel Opthalmic System, Bioavailability, Enhanced Residential Time, Viscosity.

INTRODUCTION

Ophthalmic drug delivery has always been one of the most challenging aspects for researchers worldwide as the structure of eye bears some of the physiological barriers that have to be targeted in order to achieve a good rate of absorption and bioavailability. In the development of an ophthalmic drug delivery system, the parameters of Absorption, Distribution, Metabolism and Elimination (ADME) must be given emphasis by the scientist so that a better ocular system could be developed and less problems are encountered (Kumaran et al., 2010). Glaucoma is considered as a leading cause of initial blindness in the geriatric population as it primarily affects the optic nerve of eye and if it is not being treated at its detection, it can further lead to a severe form of blindness. Two forms of glaucoma have been found prominent which are the primary open angle and closed angle glaucoma. In open angle glaucoma, the trabecular meshwork which is situated near the cornea is associated with its fractional blockage which causes a less drainage of aqueous humor from eye resulting into an increased ocular pressure. When the problem deceit in the iris of eye due to its outward expansion resulting into the partial obstruction of the drainage angle present between the iris and cornea, due to which the flow of intraocular fluid into the trabecular meshwork is reduced and thus the intra ocular pressure is enhanced significantly. This is resulted into the formation of Closed angle Glaucoma

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(Schultz., 2011). Two forms of pathways are involved in the aqueous humor outflow from the eye which are the Trabecular Meshwork pathway and the other one is Uveoscleral pathway. The major portion of aqueous humor drainage (about 75%) is contributed by the trabecular meshwork pathway and the uveoscleral pathway accounts for a less percentage of its drainage from eye which further diminishes at a later age. The juxtacanalicular region of the trabecular meshwork is responsible for the major resistence in the outflow of aqueous humor as the extra cellular matrix of trabecular meshwork is deposited with glycosaminoglycan and some other proteins which further pose an obstruction in the exit of this fluid from the eye (Goel et al.,2010). Latanoprost is considered to be an effective medication in the treatment of glaucoma and it is a prostaglandin analogue which is believed to reduce the intraocular pressure by enhancing the outflow of aqueous humor via activating the trabecular meshwork pathway (pressure sensitive) or the uveoscleral pathway (pressure insensitive) (Lim et al.,2008). The recent advancements in Glaucoma therapy reveals the use of Latanoprost as an effective medication in the management of Primary Open Angle Glaucoma and further the approval of FDA for this drug supports robustly, the use of this drug in Glaucoma (Noecker and Earl,2004). Presently, Latanoprost is being used in the form of conventional eye drops for managing this disease in elderly patients but it bears some problems like a lesser ocular residence time, naso-lacrimal drainage, poor bioavailability, absence of sustained effect and repetitive administration by patients leading to poor patient compliance. To overcome all of these stated difficulties, attempt have been made in this study to develop and characterize an ophthalmic In Situ gelling drug delivery system which could prove to be much advantageous, stable and consistent in the management of Glaucoma (Jothi et al.,2012), (Pentyala et al.2023), (Narayan et al. 2022).

This type of In Situ gelling system is in the form of liquids but as they are instilled into the eye, they rapidly convert themselves into a viscous gel due to the presence of different concentration of polymers. This type of formulation is associated with physical and chemical changes once they reach into the cul de sac of the eye and a viscous gel is formed. This formation of gel attributes to some excellent properties like getting retained to the surface of cornea in an improved manner resulting into an increased time of contact and producing a sustained effect of drug for much higher time. (Srivastava et al.2023)

From the past literatures available on Ophthalmic In Situ gelling drug delivery systems, it has been established that usually three of the prime approaches are present for the preparation of such type of formulations. These can be formulated by a change in temperature, change in pH or by altering the ionic concentration (Ahmed and Goli, 2018). Thermosensitive or temperature activated In Situ gels are the formulations which are associated with an increase or alteration of temperature from room temperature (20-25°C) to the physiological temperature (35-37°C) and results into a transition from a liquid (sol) state to a gel form. This type of drug delivery systems contain polymers which are known as poloxamers containing some cross linked polypropylenes of Poly Ethylene Oxide (PEO) and Poly Propylene Oxide (PPO). These possess hydrophilic properties which are responsible for the formation of a translucent, stiff and viscous gel (Priyanka et al., 2016). Further, the formation of this gel into a dense gel is resulted due to the enhancement of the size and number of micelle formation inside the gel which make this delivery system as an ideal mode for the sustained release of drug (Gadad et al.,2016), (Daniya et al. 2022), (Narayan et al. 2017)

MATERIALS AND METHODS

Latanoprost pure drug of the pharmaceutical grade was purchased from Triveni Interchem Pvt Ltd, Vapi, Gujrat. Pluronic F-127 (Poloxamer 407) and HPMC K4M were received as a gift sample from Modi Mundi Pharma Pvt. Ltd., Modipuram , Uttar Pradesh. Other chemicals which were used in the present research work were of analytical grade.

METHOD OF PREPARATION OF LATANOPROST LOADED IN SITU GELLING SYSTEM

Dose Selection of Latanoprost

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The previous literatures have depicted that the ophthalmic eye drops of Latanoprost which is used in the treatment of Glaucoma is 0.03%. This means that 1 milliliter of this In Situ Gel formulation contains 0.3 mg of drug. In the present study, six different formulations of 30 milliliter each were prepared in which the calculated drug for each formulation was 9mg.

Preparation Methodology for Ophthalmic In Situ Gelling System of Latanoprost

The cold method was used as a prime method to prepare all the different formulations. Pluronic F-127 was used in different concentrations to render the formulation with its thermosensitive property. To enhance the viscosity of the formulation, HPMC K4M was used in different concentrations which provided the formulation its required viscosity. To make the formulation isotonic, 0.9% of Sodium Chloride was employed which is a prerequisite for ophthalmic formulations and the required quantity 0.1 N Sodium Hydroxide solution was used to adjust the pH. The buffering agent which was employed in the study was Dibasic Sodium Phosphate and it was used in the quantity of 80mg. The formulations were further added with the required quantity of benzalkonium chloride which acts as a preservative.

To prepare the In Situ gelling formulation, the preliminary step was to disperse the required quantity of HPMC K4M in 20 milliliter of distilled water which is accompanied with constant stirring for a time period of 1 hour on a magnetic stirrer. When it is observed that HPMC K4M have been properly dissolved in distilled water after stirring, then the required concentration of Pluronic F-127 is being dispersed in this solution and further this solution is kept on a magnetic stirrer for a time period of 1 hour. This pluronic solution obtained was incompletely dissolved and thus placed in a refrigerator for a time period of 24 hours so that this polymer system can become entirely dissolved. The next step was to incorporate 9 mg of Latanoprost and 0.02% of benzalkonium chloride in a minute quantity of distilled water. Now, this solution of drug and preservative was further added to the above polymer solution by using a magnetic stirrer so that a consistent and a clear solution could be produced. At the final stage, the pH of the solution was adjusted by the incorporation of 0.1 N Sodium Hydroxide solution and further, distilled water was added to make up the volume of the formulation up to 30 milliliter. This solution was filtered through 0.2 mm filter paper and the prepared formulation was stored in sterile vials.

The formulation of six different In Situ Gelling formulations has been shown in the following Table 1:

Ingredients (gms)	**F_1**	**F_2**	**F_3**	**F_4**	**F_5**	**F_6**
Latanoprost (mg)	9	9	9	9	9	9
Pluronic F-127 (%)	13	14	15	16	17	18
HPMC K4M (g)	1	1.5	2	1	1.5	2
Benzalkonium Chloride (%)	0.02	0.02	0.02	0.02	0.02	0.02

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Dibasic Sodium Phosphate (mg)	80	80	80	80	80	80
Sodium Chloride (%)	0.9	0.9	0.9	0.9	0.9	0.9
0.1 N NaOH (ml)	qs to adjust pH	qs to adjust pH	qs to adjust pH	qs to adjust pH	qs to adjust pH	qs to adjust pH
DW (ml)	30	30	30	30	30	30

Table 1. Formulation of Ophthalmic In Situ Gelling System of Latanoprost

EVALUATION PARAMETERS OF LATANOPROST IN SITU GELLING SYSTEM

Incompatibility studies of Drug & Selected Polymers:

The compatibility between the drug and the selected polymers is of utmost concern in the design of a successful formulation and thus for this, FT-IR spectroscopy had been performed to check the compatibility between Latanoprost and the different polymers used. For this study, the FT-IR spectra of a mixture of Latanoprost along with the polymers were compared with the standard FT-IR spectrum of the pure Latanoprost.

Organoleptic Examination of the prepared formulations: A general visualization of prepared formulations was done to check out the changes in color, odor as well as the appearance and clarity. All the formulations were assessed visually on the first day of its preparation and afterwards the visual assessment was done on the third, seventh, fourteenth, twenty first and twenty eighth day of the preparation (Kurniawansyah et al.,2018).

pH: The pH of prepared formulations were evaluated with the help of a digital pH meter (Gupta et al.,2015).

Gelling Capacity: The important evaluation parameter with respect to In Situ gelling system is the gelling capacity and the method employed for this is by taking 5ml of test tube in which the thickness of the wall is minimal. A water bath was used in this and the prepared formulation (5 drops) was placed in the test tube. The test tubes were kept on different temperatures to assess the formation of gel. To check the formation of gel and to evaluate that the test tube was capable to hold the gel, the test tubes were inverted. If after inversion of the test tubes, the gel was able to remain in the test tube, then it means that the gel is being formed at that particular temperature and the gelling capacity of the prepared formulation is acceptable (Gupta and Samanta,2010).

Measurement of Viscosity: The contact time of the In Situ gel with the corneal surface depends upon the viscosity of the formulation and thus it becomes an important parameter. To measure the viscosity of the prepared formulations, Brookfield Viscometer was employed. The analysis was performed in all the formulations when they were in the solution state as well as they were analyzed after the sol-gel transition had took place. For this, spindle no

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61 of the instrument was used and then the angular velocity of the spindle was enhanced from five, ten, twenty, thirty, fifty and hundred. The measurement of viscosity were then measured (Sun and Zhou,2018).

Temperature of Gelation: This parameter was assessed by employing a 50ml beaker and then putting 10ml of the prepared formulation in the beaker. This beaker was kept on a magnetic stirrer which consisted of a thermo statistically controlled heater to maintain the temperature. The process was accompanied with an increase of 1 degree temperature of the magnetic stirrer and the measurement of temperature was done by using a thermometer. The rotation of the magnetic bead was visually assessed and the speed of the bead was found to become slow with passing time. The gelation temperature is the temperature at which the magnetic bead entirely stopped moving and this temperature was noted accordingly (T Avinash and Shinde,2015).

Assessment of Drug Content: To determine the drug content, a concentration of 1000µg/ml was prepared by taking 1ml of the formulation which is equal to 100 mg of the pure drug and it was further diluted with 100 ml of the simulated tear fluid. Further, the simulated tear fluid was used to make the final dilutions which were done according to the beer's range. The concentration of Latanoprost was determined at 294 nm by using a UV-Visible spectrophotometer (Bassi et al.,2015), (Narayan et al. 2023).

In Vitro Drug Release Studies of In Situ Gelling System: To perform the In Vitro release studies of the prepared formulation, a specialized fabricated apparatus was employed. This apparatus consisted of two separate chambers namely the donor compartment as well as the receiver compartment. 1 ml of the prepared formulation was kept in the donor compartment by using a cellophane membrane which was previously soaked in Simulated Tear Fluid for at least 24 hours. The immersion of donor compartment was done in the receiver compartment of the apparatus in which 50 ml of Simulated Tear Fluid was kept at 37±1°C by using a magnetic stirrer with the inclusion of continuous stirring. Further, 1 ml of the formulation was withdrawn from the receiver compartment at regular time intervals which was equally replaced by adding fresh Simulated Tear Fluid. At the end, the samples were analyzed for the amount of drug released by employing UV Visible Spectrophotometer at 294nm in which the Simulated Tear Fluid at pH 7.4 was used as a blank (Mahesh and Manjula,2012), (Sawhney et al. 2023), (Narayan et al. 2023).

Ex-Vivo Drug Permeation Studies: The evaluation of this parameter required the use of a Franz diffusion cell and the cornea from the eye of a goat was employed in this study. The procurement of a whole goat eyeball was done from a local slaughter house and the cold condition was maintained throughout the transportation of eye ball up to the laboratory which was done by using a normal saline at a temperature of 4°C. After the procurement of eye ball in the laboratory, the cornea was very carefully detached including a 5-6mm of the adjacent tissue of sclera which was further being washed with cold saline. This study required such an arrangement in which there was a continuous contact of the cornea with the prepared formulation in the donor compartment of the diffusion cell. This further requires 1 ml of the optimized formulation to be placed on the corneal surface which was present in the diffusion cell. The Simulated Tear Fluid was maintained at a pH of 7.4 with the temperature of 37±1°C. Continuous stirring of the receptor compartment was done with the help of a magnetic stirrer. To achieve the results of this study, 1 ml of the sample was taken out at predefined time intervals and further with the use of a UV Spectrophotometer, the absorbance was measured. Proper care was taken to refill an equal amount of Simulated Tear Fluid at the same time interval. A plot of the percent drug release and time was made finally to obtain the different dissolution curves (Gratieri et al.,2011), (Mall et al.,2023)

Test of Sterility: The ophthalmic preparation is of utmost concern with respect to the sterility factor. Such preparations must be absolutely sterile and must be free from any of the contaminant. Therefore this evaluation parameter is of very much importance in such preparations. This test was performed by using different types of mediums according to the guidelines of Indian Pharmacopoeia. The optimized formulation was kept in Fluid

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Thioglycolate medium (20ml) to check the growth of bacteria and in Soyabean Casein digest medium (20ml) to detect the growth of any fungi in the preparation. The method of direct inoculation was incorporated and 2ml 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 at least 14 days at 30-35°C in the case of Fluid Thioglycolate medium and at 20-25°C in the case of Soyabean Casein digest medium. At the end, a visual inspection was done to check the growth of any undesirable microbial growth (Maheshwara et al.,2011).

Isotonicity Studies: There should be no irritation in the eye when ophthalmic preparations are applied into the eye and further there must not be an accompanied damage to the tissue of eye. Thus isotonicity testing becomes an important parameter in case of ophthalmic formulations. To assess the isotonicity, the optimized formulation was mixed with one drop of blood and a laboratory microscope was used to assess the shape of the blood cells at 45x magnification and a comparison was done with the standard marketed Latanoprost eye drops to judge the nature of isotonicity of the prepared formulations (Dasankoppa et al.,2017).

Ocular Irritation Studies:

The protocol approved by the Institutional Animal Ethics Committee **(1204/PO/RC/S/08/CPCSEA/22/06)** for animal care at Swami Vivekanand Subharti University 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), Ministry of Fisheries, Animal Husbandry and Dairying, India. The guidelines which are based on the Draize technique have been followed extensively for undergoing the In Vivo ocular irritation study **[20].** The New Zealand Albino rabbits were taken for conduction of this study and the animals were divided into two groups which consisted of two albino rabbits each. The lower cul de sac of rabbit was instilled with two drops of prepared optimized formulation F5 in one animal of the first group and likewise in the other group 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 were carefully inspected visually (Nair et al.,2021).

Stability Studies for the Optimized formulation: The stability of the formulation becomes a crucial factor in determining its overall efficacy and the result it will show in a long term. To assess the stability of the formulation, a time period of 3 months was decided for this study. The different evaluation parameters such as the appearance of formulation, pH, clarity as well as the content of drug were assessed for the first, second and third month which can be referred to as the short term stability studies of total three months. 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 room temperature conditions of 25 ± 2°C and 60 ± 5 % relative humidity (RH). Further, an accelerated temperature range of 40 ± 2°C and 65 ± 5 % Relative Humidity (RH) was also constantly maintained for the conduction of this study (FDA,2003).

RESULTS & DISCUSSION

FTIR Spectroscopy

The compatibility of the pure drug Latanoprost was checked with the polymers employed in the formulation with the help of FTIR studies. After the conduction of studies, it was realized that the pure drug was compatible with the polymers and it was recognized that there was no additional peak visualized in the spectra, thus confirming that the drug and polymer had no chemical interaction and thus accepted to be used in the formulation of ocular In Situ gel. The following Figure No:1 depicts the FTIR spectra of the pure drug Latanoprost and the physical mixture of Latanoprost with Poloxamer (Pluronic F-127):

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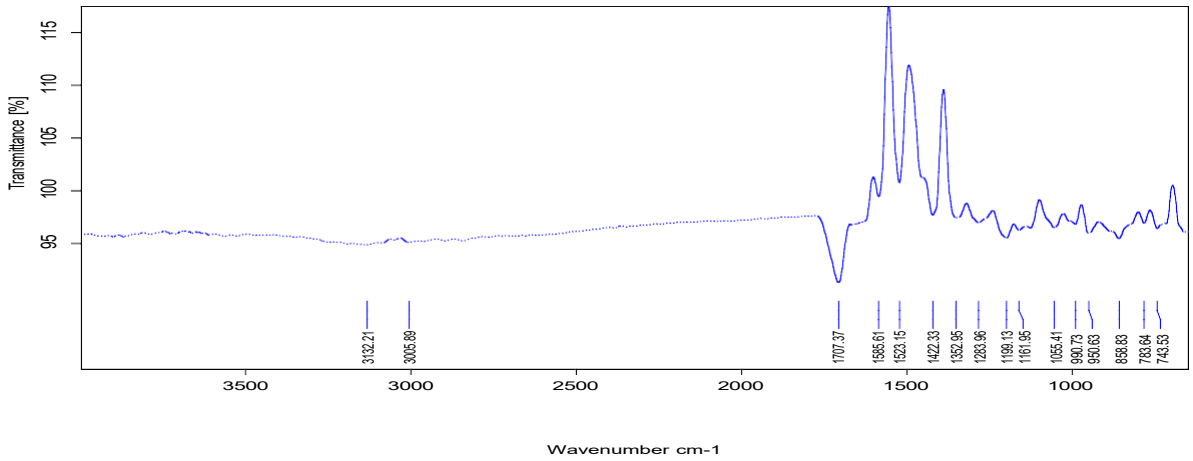
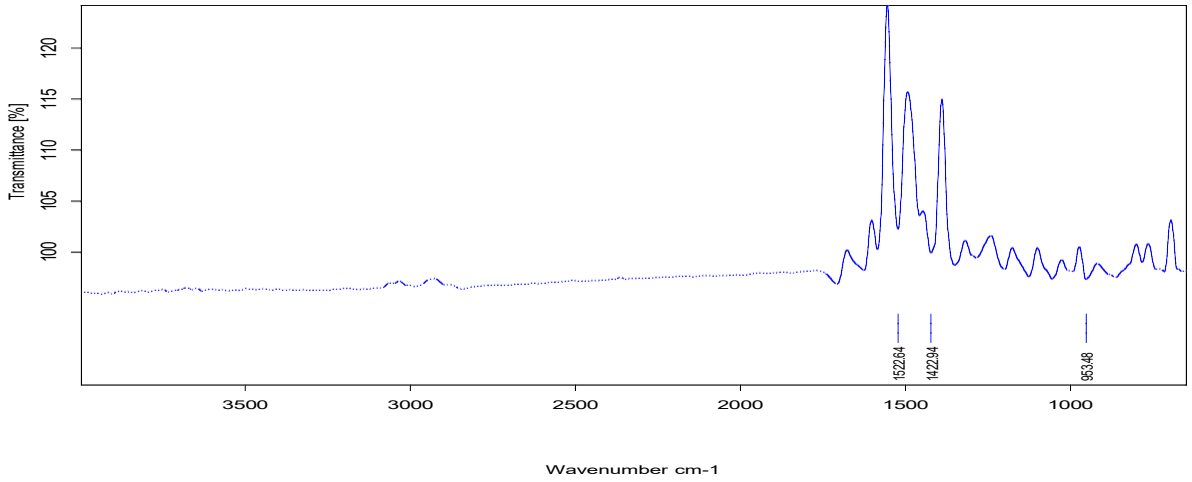
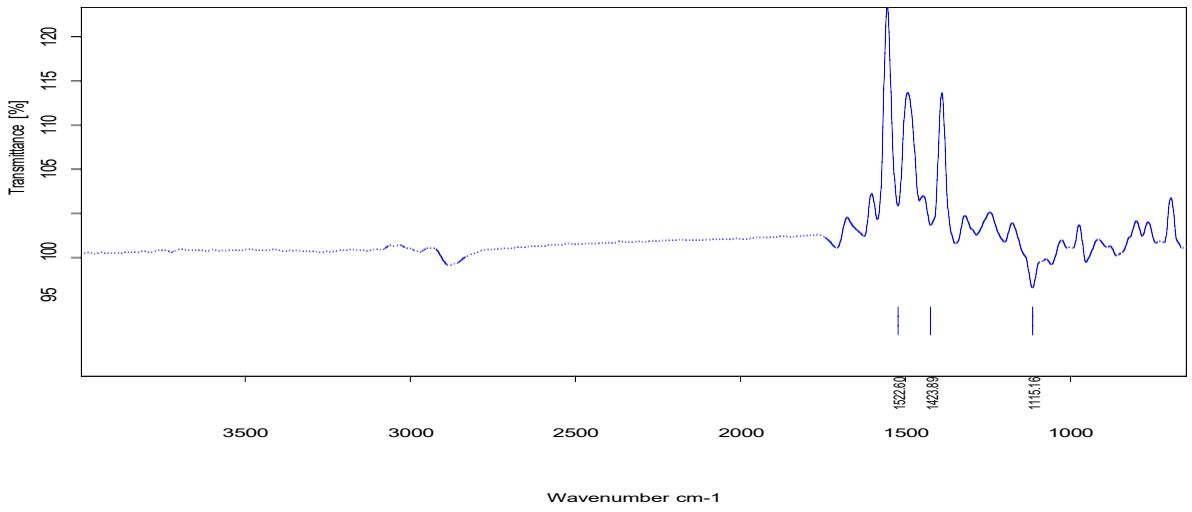


a) Pure Latanoprost



b) Poloxamer (Pluronic F-127)



c) Latanoprost + Poloxamer (Pluronic F-127)

Figure 1. IR Spectrum of (a) Pure Latanoprost , (b) Poloxamer (Pluronic F-127) &

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(c) Latanoprost + Poloxamer (Pluronic F-127)
Organoleptic Examination of Prepared In Situ Formulation

Appearance:

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After the general visualization of the all the prepared six formulations, it was seen that all the prepared batches of In Situ gel formulations were found to be clear, transparent and their character was also found to be as free flowing liquids before gelation. Table 2 shows the result of this study.

Clarity:

It was observed that no particulate matter, contaminants or particles were seen in all the prepared formulations and a clear formulation was resulted in every batch. The result is depicted in Table 2.

pH:

The prepared In Situ Gel ophthalmic formulations after assessing with a digital meter were found to be in the range of 6.5-7.4 and confirmed that all the six formulations were inside the physiological range of human eye. The result is shown in Table 2:

Table 2. Evaluation study of Appearance, Clarity, pH, Gelling capacity and Temperature of Gelation of Ocular In Situ Gel formulations of Latanoprost

Code of Formulation	Appearance	Clarity	pH	Gelling Capacity	Gelation Temperature
F1	Free Flowing Liquid	Clear with no particulate matter	6.7 ± 0.11	++	35.4
F2	Free Flowing Liquid	Clear with no particulate matter	7.4 ± 0.03	++	37.2
F3	Free Flowing Liquid	Clear with no particulate matter	6.8 ± 0.38	++	36.1
F4	Free Flowing Liquid	Clear with no particulate matter	7.3 ± 0.14	+++	35.6
F5	Free Flowing Liquid	Clear with no particulate matter	7.4 ± 0.33	+++	37.4
F6	Free Flowing Liquid	Clear with no particulate matter	7.1 ± 0.05	+++	36.8

Here, ++ designates the batch of formulations in which the gel form was maintained upto **8 hours**
+++ designates the batch of formulations in which the gel form was maintained upto **10 hours**

Gelling Capacity:

An immediate gelation was observed in all the prepared batches of formulations and the same consistency of gelation was seen till an extended period of time. To facilitate the sustained release of drug to the ocular tissue, the prepared In Situ gelling system must maintain its integrity without getting dissolved or getting eroded for a extended period of time. The formulation which had a higher concentration of Pluronic F-127, showed higher gelling capacity as

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compared to the formulations containing a lesser amount of this polymer due to the development of micelles due to the inclusion of polypropylene polymers in the structure of Pluronic F-127.The formulations F1-F3 which consisted of 13%,14% and 15% of the Pluronic F-127 showed an immediate gelation and the gel form was retained upto a time period of 8 hours. The formulations F4-F6 which had a polymeric concentration of 16%,17% and 18% respectively also showed an immediate gelation but the gel form in this case was retained upto an extended period of 10 hours. The results have been depicted in Table 2 and is denoted by ++ which indicates the batch of formulations in which the gel form was maintained upto 8 hours and +++ shows the formulations in which the gel form was maintained upto 10 hours.

Measurement of Viscosity:

The test was performed with the employment of Brookfield Viscometer at different angular velocities. The following Table 3 depicts the results of different viscosity values obtained for all of the formulations i.e. F_1, F_2, F_3 ,F_4, F_5 and F_6 before gelation and Table 4 represents the results of viscosity values of all the formulations after gelation at physiological pH. From the tests conducted, an inference was made that as the rpm was diminished, the viscosity parameter of the different formulations was enhanced, thus depicting the Non Newtonian flow character of the liquids which could be appreciated in the rheological graphs. The improvisation of the ocular residence time of the drug present in the In Situ gelling system depends upon its Non Newtonian nature and also to get the drug properly distributed on the corneal surface of eye. The concentration of the polymer used in the study was a dependent factor for the viscosity of the formulations and it was observed that as the polymeric concentration was increased, the viscosity was also increased. As the concentration of Pluronic F-127 and HPMC K4M was enhanced, it was seen that the viscosity was also increased.

Angular Velocity(rpm)	Viscosity (cps)					
	F_1	F_2	F_3	F_4	F_5	F_6
10	40.13	50.65	63.82	77.13	89.24	98.65
20	27.45	41.24	45.25	52.24	63.51	72.73
30	23.62	34.31	39.83	44.81	52.78	61.68
40	17.84	27.82	30.21	33.66	41.82	35.29
50	12.71	17.36	21.36	26.21	31.56	33.54
60	10.37	13.52	18.94	22.37	27.53	29.17
70	8.14	12.28	14.42	18.27	24.91	27.12
80	7.62	8.43	10.17	13.18	18.37	23.73
90	6.88	7.16	9.34	11.66	14.28	17.26

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100	5.47	6.85	8.58	10.25	12.72	13.84

Table 3. Rheological properties of In Situ gelling formulations of Latanoprost at different rpm before gelation

Angular Velocity(rpm)	Viscosity (cps)					
	F_1	F_2	F_3	F_4	F_5	F_6
10	240.52	512.31	910.13	1002.54	1244.76	1411.52
20	224.31	485.47	820.56	910.26	1127.12	1289.43
30	198.17	388.62	710.91	835.73	1007.44	1165.37
40	184.53	321.51	620.54	750.41	910.83	1022.15
50	168.72	288.37	510.72	625.33	812.27	925.84
60	152.48	225.78	412.37	519.18	718.13	832.51
70	139.27	192.13	362.83	425.63	600.32	740.28
80	124.82	167.81	290.51	375.82	510.53	650.84
90	118.64	147.26	210.26	290.25	422.48	510.32
100	110.25	135.65	185.65	224.41	315.71	402.67

Table 4. Rheological properties of In Situ gelling formulations of Latanoprost at different rpm after gelation

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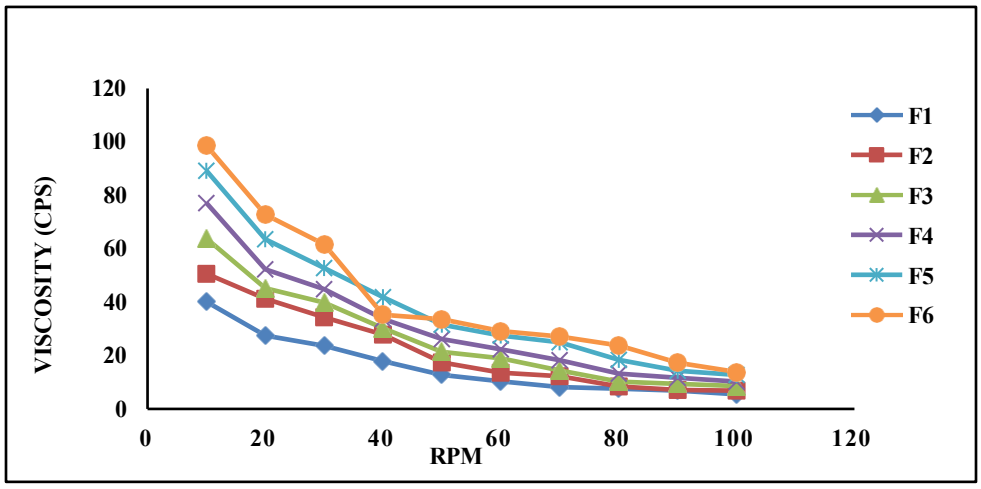

Fig 2. Rheological graph of Latanoprost In Situ gelling formulations before gelation (F1-F6)

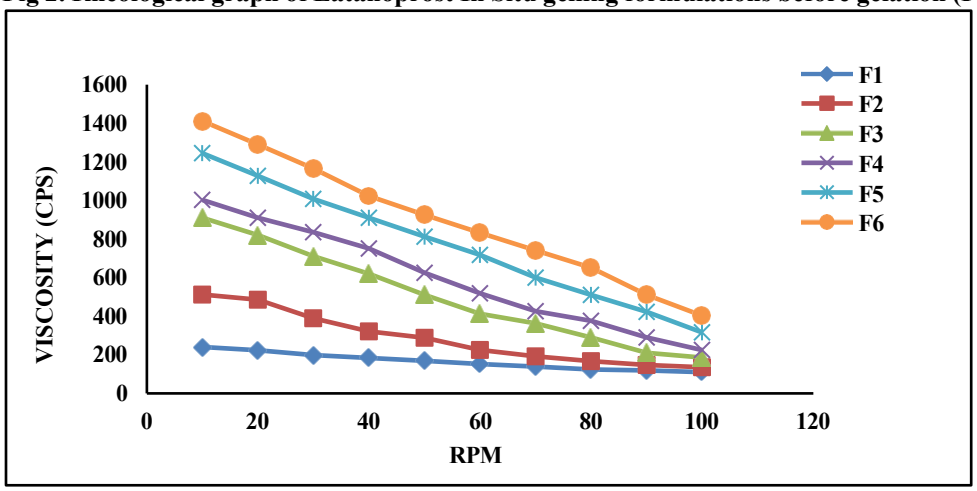

Fig 3. Rheological graph of Latanoprost In Situ gelling formulations after gelation (F1-F6)

Temperature of Gelation:

The temperature at which the gelation occurred in all of the prepared six formulations was in the range of 35.4- 37.4°C. From the performed studies, it was concluded that the formulation no F1 and F3 showed a gelation temperature of 35.4 and 36.1°C respectively but the prepared formulation no F2 shown a higher gelation temperature of 37.2°C. The reason behind this could be made that because in this batch of formulation, HPMC K4M was used in the optimum amount which was 1.5 grams and due to this attribute, this prepared formulation was not that much viscous which could have resulted into micelle formation to effect the gelling capacity.

The other three batches of formulations also gave similar results and it was concluded that in formulation no F5, a higher gelation temperature of 37.4°C was seen in comparison to the remaining two formulations, F4 and F6 which showed a lesser temperature of 35.6 and 36.8°C respectively. The performed study gave an inference that with the inclusion of HPMC K4M in an optimum concentration, the gelling temperature of the gelling system could be achieved near to the physiological temperature. The results for the gelation temperature of the formulations are shown Table 2.

Assessment of Drug Content:

The drug content of all the formulations was assessed with the use of UV Spectrophotometer and the absorbance was measured at 294nm against blank. For the formulation F1-F3, the drug content was found to be within the range of

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93.58 ± 3.28% to 97.82 ± 4.17% and in the formulations F4-F6, the drug content came to be within a range of 94.48 ± 3.27% to 98.62± 2.50%. The results of drug content uniformity analysis of Latanoprost In-Situ gelling system were established to be in an acceptable range.

In Vitro Drug Release Studies of In Situ Gelling System:

The formulations F1, F2 and F3 in which the concentration of Pluronic F-127 was 13%, 14% and 15% respectively exhibited a drug release of 76.27-83.47% up to the time period of 8 hours and the drug release was found to be better with the use of an increased concentration of Pluronic-F127 and the gelling capacity of these formulations has been found to be retained for 8 hours. Further in the formulations F4, F5 and F6, the concentration of Pluronic F-127 used was 16%, 17% and 18% respectively and exhibited a release of drug in the range of 82.38-87.42% up to an extended time period of 10 hours.

The concentration of the polymer HPMC K4M also manifested a change in drug release. It was observed that the polymer HPMC K4M, when used in the concentration of 1 gram and 1.5 gram showed an enhanced drug release rate but when HPMC K4M was used in the concentration of 2%, it resulted into a diminished rate of drug release due to a rise in the viscosity of the formulation which further showed a diminished rate of permeation through the corneal membrane and thus the ending release of drug was also found to be lesser. After the conduction of these studies, a confirmation was obtained that amongst all of the prepared six formulations, the formulation number F5 have exhibited the maximum rate of drug release of 87.42% up to a time period of 10 hours and was considered as the optimum formulation based on these studies.

The reason of this optimum result exhibited by the formulation F5 could be due to the use of an enhanced concentration of Pluronic F-127 which was 17% and the concentration of HPMC K4M used was 1.5 grams which was also found to be optimum in the terms of viscosifying agent.

From the performed studies, it was inferred that the formulation F5 has been selected as the optimized formulation. The release of the drug release studies has been depicted in the following Figure 4.

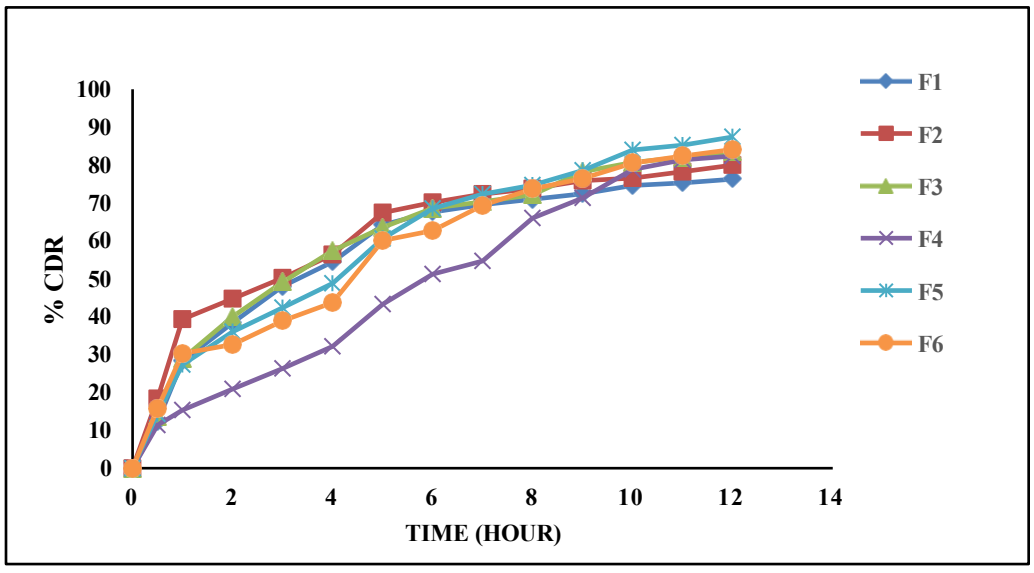

Figure 4. Latanoprost In Situ Gel Drug release study profile

Ex-Vivo Drug Permeation Studies:

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The optimized formulation of prepared In Situ gelling formulation F5 was employed in this study and a study of 12 hours was conducted. The rate of drug permeation of formulation F5 was observed to be 62.37% from the performed studies. The reason behind this slow drug permeation rate was based on the fact that the cornea present in eye is composed of different type of layers in which the stroma, epithelium and endothelium possess a very high lipophilic nature which results into a poor diffusion rate of drug through these layers when this is compared with a dialysis membrane which is not lipophillic . A representation of the comparison between the ex vivo rate of drug permeation and in vitro release rate of the optimized formulation F5 is made in the following figure.

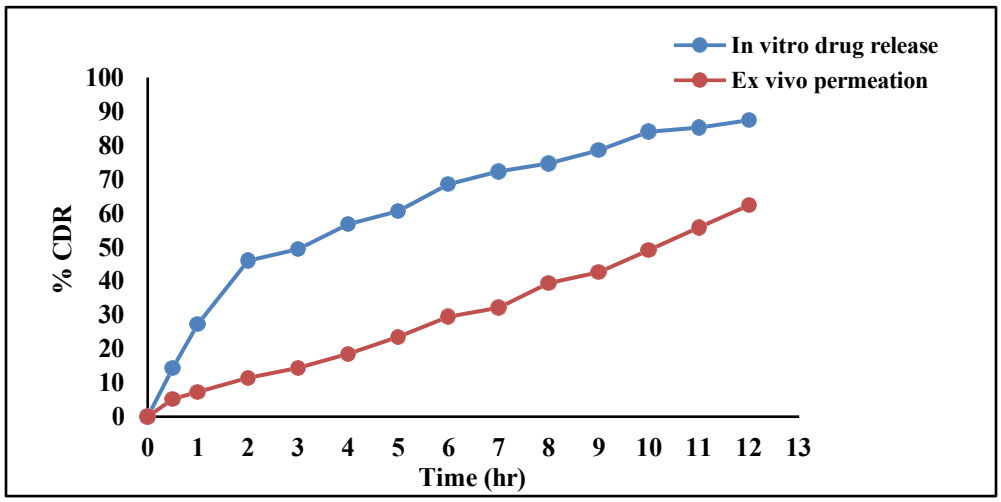

Figure 5. Representation of ex vivo drug permeation and In Vitro drug release comparison of optimized formulation F5

Test of Sterility:

When an observation was done in the positive control, it was seen that a growth of microorganisms has been initiated in case of Fluid Thioglycolate and Soyabean Casein digest agar medium after the incubation of a time period of 7 days which was confirmed by the appearance of turbidity. In the case of negative control, no growth of microbes was observed as well as in the case of the optimized formulation F5, no microbial growth was seen which proved the formulation to be entirely sterile.

Isotonicity Studies:

The isotonicity studies were conducted and the shape of the RBC's were observed by using a microscope and an absence of shrinking as well as bulging was observed in the case of optimized formulation (F5) on comparing with the marketed Latanoprost eye drops. A conclusion was made that the prepared formulation was isotonic with the Red Blood Cells.

Ocular Irritation Studies:

It was investigated visually that after instilling the optimized formulation F5, 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 used in the study was entirely harmless and non irritant for the administration in the eye.

Stability Studies for the Optimized formulation:

A short term study of three months was performed by taking the optimized formulation F5 and different parameters like appearance, clarity, pH and the content of drug was assessed to check the stability of prepared formulation. After

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the conduction of this study, it was concluded that there was absence of any significant change in the parameters that were being evaluated and the prepared formulation was quite stable up to a time period of three months. The following Table 5 represents the results of performed stability studies:

Assessment of stability studies in optimized formulation F5 at 25 ± 2°C/60 ± 5% RH				
Months	Appearance	Clarity	pH	Drug Content
1	Free flowing	Clear	7.2±0.16	96.23±1.42
2	Free flowing	Clear	7.4±0.37	95.64±0.21
3	Free flowing	Clear	7.3±0.24	96.38±0.84

Assessment of stability studies in optimized formulation F5 at 40 ± 2°C/65 ± 5% RH				
Months	Appearance	Clarity	pH	Drug Content
1	Free flowing	Clear	7.1±0.31	96.01±0.28
2	Free flowing	Clear	7.2±0.22	96.31±0.33
3	Free flowing	Clear	7.3±0.41	95.63±1.41

Table 5. Stability studies including assessment of appearance, clarity, pH and drug content of F5 at: 25 ± 2°C/60±5% RH and at 40 ± 2°C/65±5% RH

CONCLUSION

The management of Glaucoma was attempted through this research work by developing In Situ Gelling System of Latanoprost. Six different formulations were developed by employing the cold method. Pluronic F-127 was used in different concentrations to render the formulation with its thermosensitive property. HPMC K4M was used in the present study to enhance the viscosity of the formulations, which was used in different concentrations. The different evaluation parameters were conducted to assess the practicability of the developed formulations and how these could help in dealing with Glaucoma. From the performed studies, it could be inferred that all the different formulations were found to be clear, transparent and free flowing. The prepared formulations were found to be in the range of 6.5-7.4 confirming to be inside the physiological range of human eye. The formulation which had a higher concentration of Pluronic F-127, showed higher gelling capacity as compared to the formulations containing a lesser amount of this polymer and an immediate gelation was observed in all formulations. An optimum viscosity was observed in all the developed formulations with a pseudoplastic flow nature, leading to an enhanced ocular residence time and proper distribution of drug onto the corneal membrane of eye. The In Vitro drug release studies confirmed that amongst all of the prepared six formulations, the formulation number F5 have exhibited the maximum rate of drug release of 87.42% up to a time period of 10 hours and was considered as the optimum formulation based on these studies. All the developed In Situ Latanoprost gel formulations were found to be sterile with no visibility of microbial growth and these were also found to be isotonic with red blood cells. The conduction of In Vivo studies was done in all the six formulations and it was investigated visually that after instilling the required dose of the optimized formulation F5,

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there was no occurrence of swelling, redness or unwanted lachrymation in the eye. No ocular damage or any other clinical symptom was observed in the different parts of the eye of albino rabbits and the prepared formulation proved to be harmless and non irritant. At the end of this present research work, it could be comprehended that the management of Glaucoma could be effectively achieved by employing a better approach of In Situ Gelling System of Latanoprost and could prove to be a promising approach in future.

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