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FORMULATION AND EVALUATION OF THERMOREVERSIBLE *IN SITU* OCULAR GEL OF CLONIDINE HYDROCHLORIDE FOR GLAUCOMA

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ABSTRACT

The aim of this research is undertaken to formulate thermo sensitive drug delivery system of Clonidine Hydrochloride for glaucoma therapy in in-situ form to overcome the problem of rapid precorneal elimination, poor bioavailability and nasolachrymal drainage exhibited by conventional ocular formulation. Thermoreversible ophthalmic drop were prepared using cold method by mixing Poloxamer 407 as thermosensitive polymer, HPMC K15M as viscosity enhancing agent, antiglaucoma drug Clonidine Hydrochloride and Benzalkonium Chloride as preservative. The in-situ gels were evaluated for gelation temperature, drug content, bioadhesive strength, viscosity and in-vitro release. A 2² factorial design employed for optimization of Clonidine Hydrochloride gels with Poloxamer 407 amount (% X1) and polymer (% HPMC K15M, X2) as the prime selected independent variable, which were varied at 2 different levels (low and high). The effect of formulation variables on response variable were statistically evaluated by using trial version of Design Expert[®] SOFTWAR (version 9.0.4.1). The result revealed that as the increase of viscosifying polymer HPMC K 15 M concentration, decrease in gelation temperature. pH of all formulation were found to be within the range of 6 to 6.8. The increase in level of HPMC K15M, the mucoadhesive strength increases. This developed formulation had optimum viscosity. The optimized formulation shows the controlled drug release. This study further demonstrates that administration of Clonidine Hydrochloride in the form of ocular gel is a pleasant, safe and effective.

Keywords: Ocular drug delivery, Poloxamer 407, HPMC K15 M, Factorial design.

INTRODUCTION

Clonidine Hydrochloride is alpha 2 agonist specially acts on α_2 -receptor used in treatment of glaucoma and hypertension. Whenever drug act on α_2 receptor it responsible to mediate effect such as inhibition of adenylyl cyclase, stimulation of phospholipase D, stimulation of mitogenactivated protein kinase, stimulation of k⁺ current, inhibition of Ca⁺⁺current. Topically use to minimize the intra ocular pressure in treatment of open glaucoma, hemorrhagic glaucoma condition associated with hypertension and also treat secondary glaucoma. To lower desired intra ocular pressure by decreasing aqueous production and partially increase uveoscleral outflow. The conventional ocular dosage form available to treat

glaucoma but it shows very poor bioavailability of drug at posterior site of eye. There drug not available in sufficient amount to recovery of glaucoma because of drug loss via nasolachrymal drainage and most important barrier is corneal epithelium membrane and tear turnover (Nagaich U, Jain N. *at el.*, 2014). Various conventional ocular dosage forms such as suspension, ointments, insert, eye drop and aqueous gel have been used to increase bioavailability of drug by increasing residence time of instilled eye drop but some of them show drawbacks like blurred vision by use of ointment and insert are having very low patient compliance. To overcome drawbacks of this drug delivery system by using increasing

viscosity on eye drop formulation such as *In Situ* Gel formulation prepared by mixing water soluble polymer is in liquid state at room temperature but it whenever comes in contact with physiological fluid transit into a gel form. This increasing bioavailability of drug Clonidine Hydrochloride at site of action by enhancing the precorneal residence of thermoreversible formulation. Three types of ocular delivery system are recognized ion activated, pH triggered system, temperature dependent system (Darwhekar G, Jain P. *at el.*, 2011).

The objective of present research work to develop an temperature dependent in situ gelling system of Clonidine Hydrochloride alpha 2 agonist acts on α_2 -receptor used in treatment of glaucoma using Poloxamer 407 as temperature dependent polymer and HPMC K15 M as viscosity enhancing agent. The Poloxamer used as carrier for most routes of administration rectal, vaginal, ocular, intra nasal, topical, oral routes.

MATERIALS AND METHODS

Clonidine Hydrochloride sample was gifted from neon laboratory ltd. Mumbai. Poloxamer 407 and HPMC K15 M were gifted from BASF Mumbai, sodium chloride purchased from HiMedia laboratory pvt. Ltd and Benzalkonium Chloride was purchased from research fine chem industries. Mumbai

Preparation of Thermoreversible Poloxamer In Situ Gel

Thermoreversible in situ gels of Clonidine Hydrochloride were prepared by cold method using mechanical stirrer. Accurately weighed of poloxamer and bioadhesive quantities polymers and Benzalkonium chloride were dissolved into distilled water. To these solutions, the required amount of Clonidine hydrochloride and 0.9 % NaCl added under rapid stirring. The dispersions were cooled down to 4°C in refrigerator; the volume was adjusted with distilled water to reach a final concentration of Clonidine Hydrochloride of 0.125% (w/v). The solution was mixed well and stored at 4°C for overnight which result into a clear solution. All final formulations were evaluated for their clarity, pH, content uniformity, gelation temperature, mucoadhesive strength, viscosity study and diffusion study. Compositions of various prepared formulations are given in Table no.1

Evaluation of Prepared In Situ Gel *Visual Appearance and Clarity*

The clarity and appearance of various developed formulations was determined by visual inspection under black and white background.

pН

The pH of each formulation was measured by using pocket pH meter which was calibrated using buffers of pH 4 and pH 7 before the measurements. Each recording was made in triplicates when they are in sol condition.

Gelling Capacity

The gelling capacity was determined by placing one drop of the formulation in a vial containing 2 ml of freshly prepared artificial tear fluid and observing the time required to form gelation of formulation and also time taken for the gel redissolve; the composition of artificial tear fluid used was NaCl: 0.670 gm, sodium bicarbonate: 0.200 gm, calcium chloride: 2H₂O 0.008 gm, in 100.00 gm of purified water.

Observations recorded in following format:

I. + Gelation slowly and dissolve.

II. ++ Gelation immediate and remains for few hours

III. +++ Gelation immediate and remain for extended period of time.

Drug Content

Tests for drug content were carried out for all the prepared gel formulations. The vials (n=3) containing formulation were properly shaken for 2-3 min. 1 ml from each formulation was taken in 50 ml volumetric flask, dissolved in phosphate buffer pH 7.4 with gentle stirring and final volume was adjusted to obtain concentration 25 μ g/ ml respectively. The absorbance was measured at analytical wavelength 271 nm using phosphate buffer pH 7.4 as blank using shimadzu 1700 spectrophotometer.

Gelation Temperature

The sol-gel phase transition temperature (gelation temperature) were determined for all prepared

formulations by taking 2 ml of refrigerated sample to a test tube sealed with a parafilm. Then these test tubes were placed on the water bath to heat. The temperature was increased in steps of 1^{0} C/minute. The gelation temperature was measured the gel not follow down when test tube in invert position due to gelation. The temperature was allowed to increase with constant rate until the gel again comes in liquid form to measure sol temperature. Measurement was carried in triplicate for each formulation.

Determination of Bioadhesive Strength

The bioadhesive strength of all the prepared determined formulation were using the mucoadhesive force measuring device which is modified balance that was developed in our laboratory according to previously reported methods. The mucoadhesive force of the formulation under study was determined by measuring the force required to detach the formulation from biological membrane using the measuring device. The biological membrane was fixed onto the glass slide using rubber band and it attach to outer surface of the bottom of the 50 ml beaker then placed in a 500 ml beaker. Phosphate buffer ph 7.4 was added into beaker up to the upper surface of gastric mucosa such that the media remains just above the mucosa. Accurately 1 ml gel was measured were placed on the lower slide with biological membrane. A preload of 50 gm was placed on to glass slide for 5 min (preload time) to establish adhesion bonding between gel and biological membrane. The preload time and force were kept constant for all the tested formulation. The biological membrane was detached from the tested sample and the weight required detaching the tested sample from the biological membrane calculated by difference. The results were the mean of three runs.

Detachment force $(dyne/cm^2) = mg/A$ Where,

m is the weight of water in grams;

g is acceleration due to gravity taken as 980 cm/sec^2 ,

A is the area of the biological membrane (area of contact) and is equal to πr^2 (r is the radius of the mucosa)

Rheological Evaluation

The viscosity of various prepared formulation determined by using cone and plate viscometer (Brookfield Viscometer Model Cap 2000+2). Few drops of formulation were applied to lower plate of the viscometer using glass rod. The temperature was increased from 25° C to 37° C. The apparent viscosity was measured as a function of the temperature ($^{\circ}$ C).

The results obtained were given in the following observation tables.

Isotonicity Evaluation

The tonicity of thermoreversible ophthalmic insitu gel was checked by formulation were mixed with few drop of blood and observe under microscope at 45X magnification and observe the effect of formulation on red blood cells like, swelling bursting and cremation. Finally compare the shape of formulation mixed blood cell with RBC alone.

Infrared Spectroscopy

IR Spectroscopy of each formulation was obtained using Shimadzu FT-IR by spectrophotometer. The spectra were scanned over wavelength range of 4000 to 400 cm⁻¹ at resolution 4 cm⁻¹. The KBr pellet techniques were used for the infra-red absorption. The procedure consists of dispersing samples in KBr and compressing into discs by applying a pressure of 5 tons for 5 minutes in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained and reported in graph no.1-4.

Differential Scanning Calorimetry (DSC)

The DSC study was carried out for selected formulation. The DSC pattern was recorded on a Perkinelmer 4000 DSC. Thermographs were obtained by heating 1-5 mg sample in crimped aluminium pans at heating rate of 10 °C min, from 30 to 400 °C, and were sample analysis performed under nitrogen pumping (flow rate: 20 ml/min). Data was analyzed using PYRIS Version -11.1.0.0488, 2009, PerkinElmer, Inc. software, for origin to obtain onset temperature (T onset); the peak temperature (T peak); and the endset temperature (T endset) of peak. To

evaluate the internal structure modification after drug incorporation into the formulation, analysis was performed on selected formulations (F3) and compared with pure drug reported in graph no.5.

Ex-Vivo Diffusion Study

Ex-vivo permeation study was carried out by using modified Franz diffusion chamber (nasal diffusion cell) and goat corneal membrane used to separate donor and receptor compartment. The whole eyeball of goat were procured from a slaughter house and carried to laboratory in cold condition in normal saline maintained at 4^oC.The corneal membrane were carefully removed and washed with cold normal saline. The washed corneal membranes were kept in cold freshly prepared solution of phosphate buffer of pH 7.4 and then it mounted on by sandwiching between the clamped donor and receptor compartment. application of formulations, Prior to the membrane was allowed to equilibrate for 30 minutes. Accurately weighed 1 ml of gel spread uniformly on corneal membrane which was in contact with receptor medium. The receptor compartment was filled with phosphate buffer pH 7.4 at 37 ± 0.5 °C and stirred it continuously at 20

Observations for Evaluation of Formulations

rpm to simulate blinking action of eyelids. Whole assembly adjusted on magnetic stirrer. At predetermined interval (30 min, 1 hr, 2 hr, 3hr up to 6 hr) 1 ml sample was withdrawn from receptor compartment, replacing the sampled volume with phosphate buffer solution pH 7.4 after each sampling, for a period of 6 hours. The samples withdrawn were analyzed spectrophotometrically. The amount of permeated drug was determined using a UV Vis. Spectrophotometer at 271 nm (Linearity range= $10 \ 1 \ \mu g/ml$ to $100 \ 1 \ \mu g/ml$, R² = 0.999).

Stability Study

The 30 days stability studies were carried out for optimized formulation according to international conference on harmonization (ICH) guidelines. Sterile gel forming ophthalmic solution were filled in glass vials, closed with gray butyl rubber closures and sealed with aluminium caps. The formulations vials kept in stability chamber maintained at $40 \pm 2^{\circ}$ C temperature and relative humidity $75\pm 5\%$ for one month. Sample were withdrawn at 0, 7, 15, 30, day's interval and evaluated for drug content, pH, visual appearance, clarity.

Sr. No.	Formulation Code	Clarity of Solution	Clarity of Gel	Appearance of Solution	Appearance of Gel
1	F1	Clear	Clear	Transparent	Transparent
2	F2	Clear	Clear	Transparent	Transparent
3	F3	Clear	Clear	Transparent	Transparent
4	F4	Clear	Clear	Transparent	Transparent

Table 1: Clarity and Appearance

Table 2: pH	and Gelling	Capacity
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Sr. No.	Formulation Code	рН n=3	Gelling Capacity
1	F1	6.6	+
2	F2	6.7	++
3	F3	6.8	+++
4	F4	6.9	+++

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Table 3: Drug Content and Gelation Temperature

Sr. No.	Formulation Code	Drug Content (% w/v) n=3	Gelation Temperature
1	F1	98.46	37
2	F2	97.48	35
3	F3	98.20	34
4	F4	99.23	31

Table 4: Bioadhesive strength

Sr. No.	Formulation Code	$F=(dyne/cm^2) n=3$
1	F1	1820.58
2	F2	2273.29
3	F3	2955.39
4	F4	3411.57

Table 5: Rheological Study

Sr. No.	Formulation Code	Viscosity at 25 ^o C (cPoise)	Viscosity at 37 ^o C (cPoise)
1	F1	270	850
2	F2	390	930
3	F3	510	1060
4	F4	670	1170

Isotonicity Test

The isotonicity testing of thermoreversible in situ Clonidine Hydrochloride ocular gel was performed. It found that there no change in the shape of blood cell (bulging or shrinkage). Which reveals the isotonic nature of the optimized formulation (F3) as showed in figure 1 and 2.



Figure 1: Red Blood Cell alone

Figure 2: RBC with optimized formulation (F3) of Clonidine Hydrochloride ocular gel

Infrared Spectroscopy I. IR Spectrum for F1 Formulation



Graph 1: IR Spectrums for F1 Formulation

II. IR Spectrum of F2 Formulation



Graph 2: IR Spectrum of F2 Formulation

III. IR Spectrum of F3 Formulation

Graph 3: IR Spectrum of F3 Formulation

IV. IR Spectrum of F4 Formulation

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Graph 4: IR Spectrum of F4 Formulation

Differential Scanning Calorimetry of Optimized Formulation

Graph 5: DSC of Optimized Formulation

Times (hr)	F1 (%)	F2 (%)	F3 (%)	F4 (%)
0	0	0	0	0
0.5	10	6	6	6
1	18.2	16.2	14.12	18.24
2	32.56	26.52	28.40	28.6
3	53.20	39.04	40.96	41.16
4	72.24	51.80	63.76	53.96
5	81.64	78.80	83	75
6	97.20	98.32	96.6	98.44

Table 10: Ex-vivo Diffusion Study

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Graph 6: % Cumulative Drug Release of All formulation

Table11: Data Analysis of Release Kinetic

Formulation	Zero order	First order	Higuchi matrix	Peppas plot	Hixson Crowell	Best Fit Model
F1	0.9911	0.9910	0.9676	0.9370	0.9910	Zero order
F2	0.9929	0.9928	0.9337	0.9370	0.9928	Zero order
F3	0.9981	0.9980	0.9399	0.9290	0.9980	Zero order
F4	0.9928	0.9928	0.9481	0.9430	0.9929	Hixson Crowell

Table 12: Stability Study of Optimized Batch at $40 \pm 0.5^{\circ}$ C; 75%, 5% RH condition

Time in	Drug Content	pН	Viscosity at 36 ⁰ C	Visual	Clarity
days	$(40 \pm 0.5^{\circ}\text{C}; 75 \pm 5 \% \text{RH})$	$(40 \pm 0.5^{\circ}\text{C}; 75 \pm 5\% \text{RH})$	$(40 \pm 0.5^{\circ}\text{C}; 75 \pm 5 \% \text{RH})$	appearance	
0	98.36%	6.6	1010	Transparent	Clear
7	97.48%	6.8	990	Transparent	Clear
15	98.51%	6.7	975	Transparent	Clear
30	97.48%	6.6	1005	Transparent	Clear

RESULT

Clarity and Appearance

Formulation of Clonidine Hydrochloride in the form of thermoreversible ocular gel was found to be very clear and transparent at room temperature in liquid phase and also in gel formulation was found to be clear and transparent.

рН

pH of all formulation Clonidine Hydrochloride in the form of thermoreversible ocular gel were found to be within the range of 6.0-7.0.

% Drug Content

The percentage drug content of all formulation was found to be in the range of 96-99 % w/v. the percentage drug content of all formulations from the same batch was found to be uniform as shown in observation table no.3. The polynomial equations for % drug content in term of coded factors were found as follows: y= + 99.332 - 0.003A - 1.5145B + 0.0145 AB

$R^2 = 0.998$

From the equation the % drug content, negative sign of A (Poloxamer 407) and B (HPMC K15M) indicate increase in A and B the decrease % drug content and interaction term A-B indicate, their combined increase in value increases % drug content. The fitted equation relates the responses, showed good correlation coefficient of 0.998. The 3D surface response plot were constructed using Design Expert Software (figure no 3). These plots were used to estimate the effect of independent factors on response.

Figure 3: Effect of main factor on % Drug Content presented by 3D surface plot of Poloxamer 407: HPMC K15 M.

Gelation Temperature

The gelation temperatures $(T_g^0 C)$ of formulation were found within the range of 31-37^oC. The polynomial equations for Gelation Temperature in term of coded factors were found as follows: y= + 60.440 - 1.4681A - 1.0909 B - 0.1818 AB

$R^2 = 0.998$

From the equation the Gelation Temperature, negative sign of A (Poloxamer 407) and B (HPMC K15M) and interaction term A-B indicate, their combined increase in value decrease Gelation Temperature also increase in A and B the decrease Gelation Temperature. The fitted equation relates the responses, showed good correlation coefficient of 0.998. The 3D surface response plot were constructed using Design Expert Software (figure no 4). These plots were used to estimate the effect of independent factors on response.

Figure 4: Effect of main factor on Gelation Temperature presented by 3D surface plot of Poloxamer 407: HPMC K15 M.

Bioadhesive Strength

The batches code F3 and F4 showed better mucoadhesive strength. it was found that all mucoadhesive polymers under study show increase in mucoadhesion as compared to control Poloxamer 407 solution. The polynomial equations for Bioadhesive Strength in term of coded factors were found as follows: y= -5596.6 + 486.90 A + 8.1818 B + 39.090 AB $R^2 = 0.996$

From the equation the Bioadhesive Strength, positive sign of A (Poloxamer 407), B (HPMC K15M) and interaction term A-B indicate, their combined increase in value decrease Gelation Temperature also increase in A and B the increase Bioadhesive Strength. The fitted equation relates the responses, showed good correlation coefficient of 0.998. The 3D surface response plot was constructed using Design Expert Software (figure 5). These plots were used to estimate the effect of independent factors on response.

Figure 5: Effect of main factor on Bioadhesive Strength presented by 3D surface plot of Poloxamer 407: HPMC K15 M

Rheological Study

From the observation table in was observed that increase in polymer concentration result in decrease in gelation temperature and increase in viscosity. All formulation shows decrease in gelation temperature as increasing polymer concentration. The order of increasing viscosity was found to be F1 < F2 < F3 < F4.

Isotonicity Test

The isotonicity testing of thermoreversible in situ Clonidine Hydrochloride ocular gel was performed. It found that there no change in the shape of blood cell (bulging or shrinkage). Which reveals the isotonic nature of the formulation as showed in figure 1 and 2.

Infrared Spectroscopy

IR spectra of prepared formulation F1 to F4 represented in graph no.1 to 4. Te observed spectrum represent drug to polymer superimposed pattern with their significant functional group at specific wave length indicated in its pure form. The spectrum represents significant functional groups OH, C=O, C-O-C, C-N 1^0 amine. From drug to polymer, no significant peak formation was observed indicated that there was no any chemical interaction between drug and polymers only the physical interaction was take place in term of hydrogen bonding.

Differential Scanning Calorimetry (DSC) of Optimized Formulation

The Differential Scanning Calorimetry (DSC) for pure drug Clonidine Hydrochloride and selected formulation batch F3 were shown in graph no.5. The DSC study of formulation small blunt endothermic at 80°C graph indicate the formulation containing drug and its polymeric complex get melt. Further broad exothermic peak at 100°C represent the polymorphs remain present in more % in its amorphous form further the

narrow endothermic peak represent the drug polymorphs represent in its less crystalline form. Absence of any additional endotherm indicated that there was no any chemical interaction between drug and excipient.

Ex-Vivo Diffusion Study

Ex-vivo diffusion studies for all formulations carry out by using goat corneal membrane. Results obtained were represented in observation table no. 6 to 9. Percent cumulative drug release obtained from various formulations was given in observation table no.10

Data Analysis of Release Kinetic

Best release kinetic fitting model to all formulation shown in table no. 11 were the formulations F1, F2, F3 shows Zero order dissolution mathematical model while formulation F4 fitted into Hixson Crowell mathematical model. From all above evaluation parameter formulation F3 was optimized formulation. Which was further studied for 30 days stability study.

Stability Study

Accelerated stability study was selected for 30 days; sample was kept to 40 ± 2 ⁰C, $75\pm5\%$ relative humidity and room temperature represented stability study of optimized (F3) formulation exhibited the following observations also it is represented in observation table no.12. Observation of stability study:

Visual appearance, Clarity and pH

Formulation kept for stability studies were examined. There was no any change in visual appearance, clarity and pH in optimized formulations F3 over period of 30 days in accelerated and room temperature exaggerated conditions.

Viscosity

Viscosity was observed for optimized formulation F3 for both condition show small change in viscosity studied by viscometer. At optimized formulation batch shows 990, 975, 1005 cp viscosity after 7, 15, 30 days sample withdrawing respectively and room temperature viscosity was found to be 840 cp.

% w/v Drug content

Drug content was determined after every specified interval of time in days for 7, 15 and 30 days. At the end of 30 days the drug content for stability chamber condition was found to be 97.48% w/v and there was no significant change in % drug content as compared with initial values and at room temperature it shows 98.36% w/v. This indicates that Clonidine hydrochloride was stable in presence of excipients used at accelerated temperature.

CONCLUSION

This study evaluated suitability and feasibility of thermoreversible mucoadhesive gel for ocular drug delivery of Clonidine hydrochloride with a view of enhancing bioavailability and, Based upon the experimental finding. Formulation F3 containing drug to polymer ratio in 17% P407 with 0.45% HPMC K1 M in thermoreversible in situ gel formulation of Clonidine Hydrochloride shows better results, Formulation F3 was optimized formulation On the basis of various evaluation parameters.

The IR spectrum of optimized formulation revealed that there was no chemical interaction between polymers and drug and the DSC graph of optimized formulation revealed that endothermic and exothermic graph of polymer and drug respectively. This is significant for formulation and development. The pH of all the formulation was found to be between 6-7 and percentage drug content of all formulation was found to be in the range of 97.48- 99.23% w/v and Bioadhesive strength for formulations increase with increasing mucoadhesive polymer concentration.

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