Research Article

QUALITY BY DESIGN APPROACH IN FORMULATION DEVELOPMENT AND EVALUATION OF IN SITU OPHTHALMIC GEL OF OLOPATADINE HCl

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ABSTRACT

Objective: The aim of present study was to apply Quality by Design (QbD) for development of an optimized ion sensitive in situ ophthalmic gel Olopatadine HCl to increase drug residence time, ocular bioavailability and decreased dosing frequency of drug administration. **Method:** To develop the optimized formulation, 3² full factorial design method was adopted. The material attributes and critical quality attributes of the in situ gel were identified. Materials attributes were amount of Sodium Alginate and HPC and CQA's identified were % drug release and viscosity. The formulations were assessed for appearance, gelling ability, sterility, pH, drug content, viscosity, drug release through cellophane membrane, drug release through corneal membrane of goat, and stability study as per ICH guidelines. Formulations F3 and F8 were selected and were again analysed by grid analysis. **Result:** The optimized batches exhibited all results in acceptable limits. F8 was found to be the best formulation from the nine formulations developed by 3² full factorial design. The optimized formulation showed better drug release when compared with marketed formulation. **Conclusion:** The study revealed that the in situ gelling system of Olopatadine HCl sustained the effect of drug up to 10 hours. The formulation F8 extended the release of drug up to 10 hours as compared to marketed preparation of Olopatadine HCl, Zyopta (solution), which gives 98.39% drug release within 6 hours.

KEYWORDS: Olopatadine HCl, Sodium alginate, HPC, Quality by design, 3² full factorial design.

INTRODUCTION

Eye is a special sensory organ which provides sensation of vision¹. It is one of the challenging organ due to its complex make up and functionalities. It is well supplied by nerve and blood to carry its sensory functions. Thus, it is an extremely important organ. Therefore, ophthalmic dosage forms need to be formulated with utmost precautions. Drug delivery to the eye has been one of the most challenging tasks to pharmaceutical scientists. The unique anatomy and physiology of the eye renders it a highly protected organ, and the unique structure restricts drug entry at the target site of action. The usual ophthalmic dosage forms account for 90% of currently accessible ophthalmic formulations. The major disadvantage is quick pre corneal drug drainage. Many drug delivery systems for administration of drug using ophthalmic route are being studied. Tear fluid turnover is high and also the ocular fluid dynamics result in quick elimination of drug from ophthalmic route administered using conventional dosage forms and also drug delivery techniques. To subjugate these problems, ophthalmic in situ gel can be developed.^{2,3}

Definition of Ophthalmic Dosage Forms

USP defines ophthalmic dosage forms as, "Sterile dosage forms, essentially free from foreign particles, suitably compounded and packed for instillation into the eye."

Eye has got a complex anatomy, physiology and also drug administration is difficult due to high tear turnover and blinking of the eyes. Two major absorption routes are present; corneal (cornea-aqueous humour-intraocular tissues) and non-corneal (conjunctiva-sclera-choroid/retinal pigment epithelium (RPE))⁴

Topical administration to eyes is very useful and much easier than systemic administration through the eye. The drug given by ocular route has to overcome many barriers of the eye. In case of topical administration drug has to cross the pre corneal barriers. Our body has its own defence system which exhibits response when any medication is administered. In case of ophthalmic drug delivery, blinking of eyes and tear production are stimulated when a medication is instilled into the eyes. These physiological mechanisms cause drug loss by rapid clearance. Rapid drug clearance is a result of lachrymation, conjunctival and naso lachrymal absorption. Therefore, rapid drug clearance in addition to low residence volume of drug effect bioavailability of ocular drug delivery system.⁵

Following characteristics are required to optimize ocular drug delivery system

- Good corneal penetration
- Prolong contact time with corneal tissue
- Simplicity of instillation for the patient
- Non-irritant and comfortable form (viscous solution should not provoke lachrymal secretion and reflex blinking)
- Appropriate rheological properties

Therefore, the ophthalmic drug delivery needs to be optimized for better bioavailability and overcome the disadvantages of conventional ophthalmic delivery system. One such approach is formulation of the ophthalmic solutions as in situ gels. In situ forming gels are formulations, applied as solutions, sols, or suspensions, that undergo gelation (phase transition) after instillation due to physicochemical changes (thermosensitive, pH triggered, ion activated). In case of ion activated gelling systems, gelling of the instilled solution is triggered by change in the ionic

strength or due to presence of ions. Gelation is triggered by the presence of cations (Na^+ , Mg^{++} , Ca^{++}) in the tear fluid. Various polymer grades can be used depending on the requirement of gel to be formulated.⁶

Olopatadine HCl⁷ is a selective histamine H_1 antagonist that binds to the histamine H_1 receptor. This blocks the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms brought on by histamine. Olopatadine HCl inhibits the release of histamine from mast cells. It is used to treat ocular itching associated with allergic conjunctivitis. Olopatadine HCl eye drops (0.1% and 0.2%) are available for the treatment of allergic conjunctivitis.⁸

Quality by Design approach encompasses designing and developing formulations, in which manufacturing processes ensure pre-defined product specifications. The important part of this approach is to understand how process and formulation parameters affect the product quality and subsequent optimization parameters with respect to final specifications. QbD uses multivariate experiments to understand product and process and to establish a design space through design of experiments (DoE). DoE is an organized method to determine the relationship between the inputs and outputs of a process.⁹⁻¹²

The present research work has been carried out keeping in mind the safety and importance of ophthalmic dosage form and therefore use of "Quality by Design" for formulation development of ophthalmic in situ gel has been attempted. In present work in situ gelling system of Olopatadine HCl using ion sensitive Sodium Alginate as gelling agent and viscosity modifier HPC for sustained drug delivery to enhance the drug residence time in eye has been developed.

MATERIALS AND METHOD

Olopatadine HCl and Sodium Alginate were obtained as a gift sample from Cipla R&D Vikhroli, Mumbai, India. HPC was a gift sample from Wockhardt Pharmaceuticals Ltd, Aurangabad. All chemicals used were of analytical grade.

EXPERIMENTAL METHOD

Identification of Material Attributes of Excipients Used in Preparation of In Situ Gel

CQA's as defined by ICH Q8 (R2),¹³ is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. CQA's are generally associated with raw materials & process parameter. This parameter directly affects the quality of final product. Preliminary trials for the selection of polymers was carried out based on literature survey. The polymers were studied for drug content, drug release, viscosity and pH. These data were used to optimize concentration of polymers for the preparation of ophthalmic in situ gel.

UV Method Development

The method development was done by determining λ_{max} , precision, recovery, LOD, LOQ, linearity and by preparing calibration curve using Shimadzu UV 1700 spectrophotometer.¹⁴

Preliminary Study^{15,16}

The preliminary compatibility study for Olopatadine HCl and Sodium alginate was done using infrared spectroscopy (Shimadzu, Prestige-21).

Preliminary batches of 0.1% Olopatadine HCl were formulated using Sodium Alginate, HPC, Dibasic sodium phosphate, Benzalkonium Chloride and mannitol. The batches were evaluated for in vitro in situ gelation in artificial tear fluid (ATF) to optimize the concentration of Sodium Alginate and HPC for final formulation.

Preparation of In Situ Gels with Selected Variables Using 3² Factorial Design

Design of Experiments (DoE)

A two factor three level full factorial response surface methodology was used to optimize and evaluate the effects of material attributes on CQA's of ophthalmic in situ gel. The design consisted of nine experimental points. A design used is suitable for exploring quadratic response surfaces and constructing second-order polynomial models with Design Expert software (Version 11, Stat-Ease Inc.)

The linear & quadratic models for predicting the optimal point were expressed using the following equation:¹⁷

$$\text{Linear model:} \quad Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 \quad (1)$$

$$\text{Quadratic model:} \quad Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_3 X_1^2 + \beta_4 X_2^2 + \beta_5 X_1 X_2^2 + \beta_6 X_1^2 X_2 \quad (2)$$

Where, Y is the measured response associated with each factor level combination, β_0 is intercept, β_1 to β_6 are regression coefficients computed from the observed experimental values of Y, and X_1 , X_2 , and X_3 are the coded levels

of independent variables. The terms $X_1 X_2$ and X_i^2 ($i=1, 2$, or 3) represent the interaction and quadratic terms, respectively. The validity of the model was determined by comparing the experimental and predicted values. Statistical validity of the polynomials was established on the basis of ANOVA provision in the design expert software. Subsequently, the feasibility and grid searches were performed to locate the composition of optimum formulations. Also 3-D response surface graphs were constructed using the design expert software. Materials attributes and CQAs (process output) selected with the results are shown in table 3. Materials attributes studied were Sodium Alginate conc. (%) (X_1) and HPC conc. (%) (X_2). The CQAs were cumulative percentage drug release (Y_1) and viscosity (Y_2). The optimum formulation of this study is based on the constraints for CQAs given in table 3.

Formulation and Development of in Situ gel

The 0.1% w/v solutions of Olopatadine HCl were prepared using different concentrations of Sodium Alginate as per figure 1. The two independent variables selected were Sodium Alginate (X_1) and HPC (X_2), and the dependent variables were % Drug release (Y_1) and viscosity (Y_2). The factorial designed batches are shown in table 4.

EVALUATION OF IN SITU GELLING FORMULATION¹⁷

Clarity, pH, Gelling ability and Drug content

The ophthalmic formulations were evaluated for various physical and performance characteristics i.e. for appearance/ clarity, pH, gelling ability and drug content.

The clarity of solutions was further assessed by observing them against a dark and white background as described in the USP.

The pH was measured using pH meter (Equiptronics model no. 614)

Artificial tear fluid (ATF) was the medium used for testing gelling ability. The formulation which is a solution, forms a viscous gel which was observed visually and scores were designated based on time required for gel formation and the time required for the gel to break down upon shaking.

The drug concentration was examined spectrophotometrically using Shimadzu UV 1700 spectrophotometer. Olopatadine HCl ophthalmic solution must contain NLT 90.0% and NMT 110.0% of labelled amount.

Viscosity

The viscosity of gel was determined using rheometer (Brookfield R/S-CPS+Rheometer). Gelation was induced in formulation by adding Artificial tear fluid (ATF).

In Vitro Drug Release Study

In vitro release was performed through cellophane membrane (pore size 0.45 μ m) using modified dissolution testing apparatus,¹⁸ figure 2. The basket attached to the shaft of USP apparatus I (Basket type) was replaced with a glass cylinder. 50ml of ATF maintained at 37 $\pm$ 0.5 $^{\circ}$ C was used as the dissolution medium. 1 ml of sample solution was withdrawn after every hour i.e. 0,1,2, up to 12 hrs. To maintain sink conditions, the sample solutions were replaced simultaneously with same volume of ATF. The concentration in sample solutions were determined at 300.6 nm using UV spectrophotometer.

Kinetics of Drug Release

The dissolution profile of all the formulations were fitted to zero order kinetics, first order kinetics, Higuchi, Hixson-Crowell and Korsmeyer-Peppas to ascertain the kinetic modelling of drug release the model with the higher correlation coefficient was considered to be the best fit model.

Ex Vivo Drug Release Study using Corneal Membrane

For ex vivo study the biological membrane used was freshly cut goat cornea. Same test conditions prevalent in in-vitro study were followed with respect to temperature and stirring rate. In place of cellophane membrane goat corneal membrane was used in ex vivo study. The formulations F3 and F8 were subjected to ex vivo study.

Isolation of Goat Corneal Membrane: Goat eyeballs from nearby slaughter house were obtained. These were stored in cold saline (0-2 $^{\circ}$ C) media in an airtight container. The cornea was isolated keeping 5-6 mm scleral layer for attaching to the glass cylinder of dissolution apparatus. The isolated cornea was washed using cold saline and preserved in fresh ATF (kept at 0-2 $^{\circ}$ C).

Sterility Study

The test for sterility for optimized formulations was confirmed by method B described in USP. The media (Sterile Fluid Thioglycolate) was then allowed to incubate for 14 days at 30-35 $^{\circ}$ C. The end point was judged visually noting the presence of turbidity in the inoculated media. Both positive and negative controls were also maintained simultaneously. The method of detection was visual inspection of turbidity.¹⁹

Stability Study

Stability studies as per ICH guidelines for optimized formulations were done. Appearance, gelling ability, drug content and pH of the formulations, viscosity and sterility study was performed.

RESULTS AND DISCUSSIONS

Identification of Material Attributes of Excipients used in Preparation of In Situ Gel

According to Quality by Design, pharmaceutical development includes identifying potential CQAs of the drug product, determining material attributes of excipients, selecting an appropriate manufacturing process (CPPs) and defining a control strategy. Preliminary trials were carried out for the selection of polymers, concentration and combination ratio. The role of Polymers used in ophthalmic formulations is to increase the residence time on the ocular surface and thereby to increase permeability and bioavailability. In particular, an ophthalmic ion sensitive in situ gel, possesses liquid characteristic and forms gel when comes into contact with a certain ions present in tears. temperature defined as a sol-gel transition temperature. Sodium Alginate is an economic and easy to use as a gelling agent and has a good gelling ability at physiological conditions hence was selected to be used in formulation. HPC was used to retard drug release and further enhance the viscosity of formulation when it comes in contact with physiological conditions of the eye. The final Screening was based on gelling ability of prepared formulations. Numerical scores were assigned for gelling ability. optimization was carried out via 3² full factorial experimental design. The potential CQAs for development of ophthalmic in situ gel were identified to be % Cumulative release and viscosity of preformed gels.

UV Method Development

The drug, Olopatadine HCl was characterized by observing its UV spectrum. The λ_{max} of drug in ATF was found to be 300.6 nm and the regression coefficient for calibration curve was found to be 0.99859 (figure 4). The UV spectrum obtained is given in figure 3.

Identification of Olopatadine HCl Using FT-IR

The FTIR spectrum of Olopatadine HCl was recorded using Prestige-21 (SHIMADZU) with IR resolution software. The drug sample was placed in FTIR sample holder and scanned over the range of 400 to 4000 cm^{-1} . The spectrum obtained is shown in figure 5.

Preliminary Study

Compatibility Study Using FT-IR

The spectrum obtained after compatibility studies are shown in figure 6 and figure 7.

Preliminary Batches

The preliminary batches indicated that:

1. HPC alone does not possess any in situ gelling properties (P1 to P5).
2. Sodium alginate above the concentration of 1% forms in situ gel in artificial tear fluid (P8 to P12).
3. Sodium alginate above 1% concentration produced highly viscous liquid that is not suitable for instillation into the eye (P13 to P15).

At 3% concentration of sodium alginate direct gelling is induced of the formulation (P16), therefore level of sodium alginate is decided based on in situ gelling capacity.

Characterization

The formulations were examined carefully. Colour, pH, drug content, gelling ability and suspended particulate matter if any as physical parameters were assessed. Clarity test was performed by

visual inspection as per USP against a dark and white background. The ophthalmic formulations were found to contain clear solutions free from particulate matter. The pH observed was in the range of 7.01-7.21. The drug content was in the range of 97.60 to 102.21% of labelled amount in ophthalmic formulations. Gelling ability was carried to study drug release from in situ gel in ATF. The designated scores for gelling ability varied as the concentration of sodium alginate differed. The transformation of solution to viscous gel depended on concentration of gelling agent i.e. Sodium Alginate as given in table 6. More the concentration of Alginate lesser was the time taken for gelling.

Viscosity

Viscosity of the ophthalmic formulations depended on both the polymers Sodium Alginate and HPC. As the concentration of polymers Sodium alginate and HPC increased from 1 to 2% and 0.5 to 0.9% respectively viscosity also increased. Increased viscosity of formulation as an in situ gel was required to prolong and thereby sustain drug release from formulation upon instillation into the eye. Enhanced viscosity was responsible for increased pre corneal residence time. Increased optimum viscosity was achieved due to Sodium alginate in formulation which gels in presence of calcium or sodium ions of tear fluid. Viscosity of all formulations were found to be in the range of 362 – 1478 cps.

In Vitro Drug Release

HPC plays an important role in drug release from the formulations. HPC contributes to the formation of gel structure which effect drug release from cellophane membrane. As concentration of HPC is increased there is reduction in number and dimensions of channels present in gel due to swelling of polymer. In vitro drug release from cellophane membrane decreases on increasing the polymer concentration. Sodium Alginate further assists in retarding drug release upon increasing conc. from 1% to 2% as shown in figure 8 (batches F4, F5, F6 and F7, F8, F9).

Drug Release Kinetics

The kinetics of drug release mechanism revealed Higuchi model kinetics. Thus, the increase in viscosity of gel formed by phase transition of Sodium Alginate and swelling of HPC chains due to accommodation of ATF within matrix together contributed for decrease in release of Olopatadine HCl. As a result, a reduction in drug release rate is observed. The drug release from HPC formulation is controlled by polymer swelling and diffusion.

Optimization of CQA's using Experimental Design and Data Analysis

The 3^2 full factorial design was selected to study the effect of independent variables Sodium Alginate (X_1) and HPC (X_2) on dependent variables % drug release and viscosity. The independent variables X_1 and X_2 affect % drug release and viscosity. The equation obtained gives the effect of these variables on responses. High regression coefficient (r^2) was obtained which shows acceptable fitting of model used for analysis. Polynomial equations with negative and positive coefficients were given by design expert. These equations are used to study the nature and effect of variables on responses.

Variable X_2 i.e. HPC in case of response showed negative coefficient indicating that as HPC concentration was increased, release value decreased. Viscosity coefficient for X_2 had positive sign showing increased HPC concentration increases viscosity of the in situ gel.

In case of variable X_1 i.e. sodium alginate, it showed negative coefficient for drug release and positive coefficient for viscosity.

Final equation in terms of actual factor (release)

Drug Release = $+120.73750 - 9.79333 \text{ sod alginate} - 23.32500 \text{ HPC}$

Final equation in terms of coded factor (release)

Drug Release = $+120.73750 - 9.79333 X_1 - 23.32500 X_2$

Final equation in terms of actual factor (viscosity)

viscosity = $886.56 + 386.00 \text{ sod alginate} + 138.17 \text{ HPC} + 39.00 \text{ sod alginate HPC} - 4.33 \text{ sod alginate}^2 - 28.83 \text{ HPC}^2$

Final equation in terms of coded factor (viscosity)

viscosity = $886.56 + 386.00 X_1 + 138.17 X_2 + 39.00 X_1 X_2 - 4.33 X_1^2 - 28.83 X_2^2$

p-values are important in evaluation and explanation of research results. They serve as an important analytical tool for establishment of research findings. The coefficients of independent variables X_1 and X_2 were found to be significant with $p < 0.05$, therefore it showed significant effect of both variables X_1 and X_2 on the responses drug release and viscosity. ANOVA for the dependent variables Y_1 and Y_2 i.e. % drug release and viscosity respectively is given in table 7. ANOVA and Multiple regression analysis was carried out by Stat-Ease Design Expert software version 11.

The Variance Inflation Factor (VIF) is calculated for release and viscosity and was near to one. This shows that the model has good estimation of the coefficient values. Also, the model terms A, B, AB, A^2 and B^2 were found to be significant based on values of Probe > F which were less than 0.05 in all cases. "Pred R-squared" values were in reasonable agreement with the "Adj R-squared" values with difference less than 0.2. Adel-Precision measures the signal to noise ratio. A ratio > 4 is acceptable. The Adeq-Precision values were 9.6309 and 11.8000 for the responses drug release and viscosity respectively indicating an adequate signal. Hence, both the variables X_1 and X_2 i.e. sodium alginate and HPC can be used to prepare sustained release in situ gel of Olopatadine HCl. Selection of optimized level for drug release and viscosity respectively was done by grid analysis. Most acceptable results for these responses were observed at middle value of Sodium Alginate and lower value of HPC. Thus, the formulation F₈ was selected as the best optimized formulation.

The release profile of the F₃ and F₈ were also compared with that of marketed preparation (Zyopta, 0.1% w/v Ophthalmic solution, Zydus Occucare), as shown in figure 9 and the developed formulations showed better release profiles than the marketed formulation (Zyopta).

Response Surface Analysis

The response surface plot is used to explain combination of independent variables. It also takes into consideration the factors associated with dosage form. It is used to study a specific requirement, in this case, sustained drug release up to 10 hrs. Figure 10, depicts independent variables X_1 and X_2 and explains the effect of these variables as a combination on drug release.

Table 1: Calibration curve of olopatadine HCl

Sr.no	Conc (µg/ml)	Absorbance
1	2.00	0.05
2	4.00	0.07
3	6.00	0.09
4	8.00	0.11
5	10.00	0.13
6	12.00	0.15

Table 2: Formulation of preliminary batches and their evaluation

Sr.no	Batch code	Sodium alginate (% Conc)	HPC (% Conc)	Gelling capacity*
1	P1	-	0.2	No gelation
2	P2	-	0.4	No gelation
3	P3	-	0.6	No gelation
4	P4	-	0.8	No gelation
5	P5	-	1.0	No gelation
6	P6	0.5	0.3	-
7	P7	0.75	0.4	-
8	P8	1.00	0.5	+
9	P9	1.25	0.6	++
10	P10	1.50	0.7	++
11	P11	1.75	0.8	++
12	P12	2.00	0.9	+++
13	P13	2.25	1.0	Highly viscous liquid
14	P14	2.50	1.1	Highly viscous liquid
15	P15	2.75	1.2	Highly viscous liquid
16	P16	3.00	1.3	Direct gelling

*(-): Solutions did not undergo phase transition at all, (+): Solutions which showed phase transition after 60 sec. but the in situ gels formed collapsed within 2 hrs. (++) : The solutions showed gels after 60 secs, but the gels were not stable for more than 3-4 hrs. (+++) : The solutions showed phase transition within 60 sec. and the gels were stable for more than 8 hrs.

Table 3: Material attributes and CQA'S of in situ ophthalmic gel in 3² factorial design with results and their constraints

Material attributes and CQA's of in situ gel in 3 ² factorial design				
Variables		Level of variables		
Independent (material attributes)		-1	0	+1
X ₁ = conc of sod Alginate (%)		1	1.5	2
X ₂ = conc of HPC (%)		0.5	0.7	0.9
Dependent (CQA's)		Criteria for selection		
Y ₁ = % drug release		Min of 85% drug release in 10 hrs		
Y ₂ = viscosity		Not less than 700 cps		
No.	Coded factor (actual factor)		Response	
Batch	X ₁ (conc of Sod Alginate)%	X ₂ (conc of HPC) %	Y ₁ (drug release)	Y ₂ (viscosity)
F1	-1 (1)	-1 (0.5)	96.27	362
F2	0 (1.5)	-1 (0.5)	97.90	684
F3	+1 (2)	-1 (0.5)	88.50	1104
F4	-1 (1)	0 (0.7)	94.81	489
F5	0 (1.5)	0 (0.7)	95.25	997
F6	+1 (2)	0 (0.7)	81.50	1165
F7	-1 (1)	+1 (0.9)	87.27	580
F8	0 (1.5)	+1 (0.9)	85.63	921
F9	+1 (2)	+1 (0.9)	80.39	1478

Table 4: Formulation composition of olopatadine HCl in situ ophthalmic gel

Sr.no	Formulation code	Olopatadine HCl (% w/v)	Sodium Alginate % w/v	HPC % w/v	BKC % w/v	Dibasic sodium phosphate % w/v	Mannitol % w/v
1	F1	0.1	1	0.5	0.01	0.05	5
2	F2	0.1	1.5	0.5	0.01	0.05	5
3	F3	0.1	2	0.5	0.01	0.05	5
4	F4	0.1	1	0.7	0.01	0.05	5
5	F5	0.1	1.5	0.7	0.01	0.05	5
6	F6	0.1	2	0.7	0.01	0.05	5
7	F7	0.1	1	0.9	0.01	0.05	5
8	F8	0.1	1.5	0.9	0.01	0.05	5
9	F9	0.1	2	0.9	0.01	0.05	5

Table 5: Validation parameters for UV method development of olopatadine HCl

Parameters		Results		
Wavelength (nm)		300.6 nm		
Linearity range		2-12 µg/ml		
Regression equation		Y (Absorbance) = 0.011x + 0.0227		
Correlation coefficient (r ²)		0.99859		
Slope		0.0110		
Intercept		0.227		
Precision		% RSD		
Intraday and interday		< 2%		
Recovery study	Level of addition	80%	100%	120%
	% RSD	0.3453	0.8357	0.3459
LOD		1.2712 µg/ml		
LOQ		3.8521 µg/ml		

Table 6: Numerical scores for gelling ability of formulations

Sr.no	Formulation code	Sodium Alginate % w/v	HPC % w/v	Gelling ability*
1	F1	1	0.5	+
2	F2	1.5	0.5	+
3	F3	2	0.5	++
4	F4	1	0.7	+
5	F5	1.5	0.7	++
6	F6	2	0.7	++
7	F7	1	0.9	++
8	F8	1.5	0.9	+++
9	F9	2	0.9	+++

Table 7: Analysis of variance for CQA'S from 3² full factorial design

Source	df	Sum of squares	Mean square	F-value	p-value
% drug release					
X ₁	1	143.86	143.86	12.16	0.0130
X ₂	1	130.57	130.57	11.04	0.0160
Viscosity (cps)					
X ₁	1	8.940E	8.940E	75.51	0.0032
X ₂	1	1.145E	1.145E	9.67	0.0529
X ₁ X ₂	1	6084	6084	0.5139	0.5252
X ₁ ²	1	37.56	37.56	0.0032	0.9586
X ₂ ²	1	1662.72	1662.72	0.1404	0.7328

Cleaning of glasswares and container
<ul style="list-style-type: none"> All glasswares were washed with distilled water and then sterilized by drying at 160-165°C for 1 hr in hot air oven. the closures were sterilized with preservative (BKC 0.02% w/v) by autoclaving at 115-116°C for 30 min
Preparation of solutions
<ul style="list-style-type: none"> Solution A: Accurately weighed quantity of dibasic sodium phosphate was dissolved in part of purified water. Separately polymer solution of HPC and alginate was prepared by dispersing HPC in small quantity of purified water with continuous stirring. Sodium alginate in fixed concentration was then added to HPC solution until dissolved and this polymer solution was added to buffer solution of dibasic sodium phosphate and autoclaved at 121°C for 20 min Solution B: In a small quantity of purified water Olopatadine HCl was dissolved under slow stirring and to this BKC and mannitol were added
Compounding of ophthalmic solution
<ul style="list-style-type: none"> solution B was mixed to solution A with continuous stirring using a mechanical stirrer to produce clear transparent. The pH of the formulation was checked and adjusted with 0.1 N NaOH and volume made up with distilled water to 100 ml
Sterilization of ophthalmic solution
<ul style="list-style-type: none"> The final formulation was sterilized by membrane filtration technique. The solution was filtered through 0.22 micron cellulose nitrate membrane filter
Aseptic filling into container
<ul style="list-style-type: none"> The formulation was aseptically transferred to previously sterilized glass container

Figure 1: Method for preparation of Ophthalmic in situ gel of Olopatadine HCl

Figure 2: Modified dissolution testing apparatus

Figure 3: UV spectrum of Olopatadine HCl

Figure 4: Calibration curve of Olopatadine HCl at 300.6 nm

Figure 5: IR spectra Olopatadine HCl

Figure 6: Infrared Spectrum of drug- polymer blend

Figure 7: Infrared Spectrum of drug-excipient blend

Figure 8: In vitro drug release from formulations F1-F9

Figure 9: Comparison of release profile with marketed formulation

Figure 10: Response Surface Plots for (a) drug release and (b) viscosity

Figure 11: Design space overlay plot

Figure 12: Drug release through corneal membrane

Optimization of Formula and Validation of Response Surface Methodology

After generating the polynomial equations relating the dependent and independent variables, the in situ formulations were optimized for the responses R_1 and R_2 . The optimum values of the variables were obtained by graphical and numerical analyses using the Design-Expert software which are based on criterion of desirability. The optimized formula was achieved with 1.65 % of Sodium Alginate and 0.52 % of HPC. Therefore, to verify the evolved models, the optimum formulation was prepared according to the above values of the factors and subjected to the analysis of responses. It was demonstrating that the observed value was quite closer to predicted value indicating validity of the model.

Design Space

“Design space” is a space within which desired quality of product can be built. Regulatory point of view changes within design space are not considered, but changes outside design space would normally initiate regulatory post approval process. Concentration of sodium alginate and HPC was found to be critical on responses %drug release and viscosity. Based on the requirement of product quality the criteria considered for responses was minimum of 85% drug release in 10 hrs and viscosity not less than 700 cps. When, critical variables operated within the established design space compliance to CQAs would be assured. Design space (Overlay plot) shown in figure 11 had shaded region with yellow colour indicates that region of successful operating ranges.

Ex Vivo Drug Release Study using Corneal Membrane

The corneal goat membrane showed slow release of drug Olopatadine HCl as compared to drug release from cellophane membrane. Therefore, the in situ gel formulation of Olopatadine

HCl containing Sodium Alginate sustained the release of drug up to 10 hours as depicted in figure 12.

Sterility Testing

After incubation period of 14 days no turbidity was detected in the samples while positive control showed dense turbidity under same test conditions. This indicates the absence of any microbial contamination.

Stability Study

There were no any significant changes during the duration of study under the conditions prescribed for accelerated stability study.

CONCLUSION

In the present research work, Quality by Design (QbD) was applied for pharmaceutical development of Olopatadine HCl Ophthalmic in situ gel containing Sodium Alginate as in situ gelling polymer in the combination with HPC which acts as a viscosity modifier to increase pre corneal residence time and decrease frequency of administration. A 3^2 full factorial response surface methodology for 2 factors at 3 levels each was employed to optimize formulation. Polynomial mathematical models were generated for various response variables using multiple linear regression analysis and were found to be statistically significant ($p < 0.050$). Batches containing combination of above polymers gives drug release up to 10 hr. As per Quality based design, full factorial response surface methodology, it is concluded that batch F8 [1.5% Sod alginate, 0.9% HPC] as an optimized formulation. Stability studies revealed that there was no decomposition in physicochemical properties of in situ gel. Therefore, it can be concluded that a stable formulation of ion sensitive in situ

ophthalmic gel has been successfully developed using 3² factorial design.

ABBREVIATIONS

HCl	Hydrochloride
HPC	Hydroxyl propyl cellulose
Sod	Sodium
Conc	Concentration
ATF	Artificial Tear Fluid
BKC	Benzalkonium Chloride
CQA	Critical Quality Attributes
DoE	Design of Experiments
ICH	International Conference of Harmonization
LOD	Limit of Detection
LOQ	Limit of Quantitation
NLT	Not less than
NMT	Not more than
RSD	Relative Standard Deviation
cps	Centipoise
FT-IR	Fourier Transform Infrared

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