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Design and Optimization of Timolol Maleate Ocular Insert by Statistical 3^2 Factorial Design

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Abstract

The purpose of this research was to design and optimize once a day ocular insert of Timolol maleate using hydrophilic carrier HPMC 15 CPS and lipophilic carriers Eudragit RLPO and Eudragit RSPO. Timolol maleate is a beta adrenoceptor antagonist widely used in the treatment of glaucoma available in the form of conventional eye drop dosage form. This conventional dosage form is facing certain drawbacks like poor bioavailability, tear turnover, lacrymal drainage and conjunctival absorption. Reservoir type once a day ocular insert would serve to replace this conventional dosage form. A 3^2 factorial design was applied for studying the effect of two independent variables (X1:Concentartion of HPMC 15 CPS and X2:Pay load of Eudragit RSPO) on drug release at the end of 12 h (DRL12), at the end of 18 h (DRL18) and time at which 50 % of drug released. FTIR and DSC study was performed for evaluation on compatibility of pure drug and excipients. All formulation was analyzed for their physicochemical parameters, *in vitro* release study and temperature sensitivity study. The in vitro release study data were fitted in to various kinetic models. Optimized formulation showed perfect zero order kinetic and drug release mechanism was case II transport. For optimized formulation DRL12, DRL18 and T50 % was found to be 51.50 %, 73.08 % and 11.67 h which were in close agreement with predicted responses. The result revealed that the two variables have significant effect on the selected responses.

Keywords: Timolol maleate, FTIR, DSC, factorial design, kinetic models.

Introduction

Glaucoma is the second cause of vision loss in the world. They are now an estimated 12 million people affected by glaucoma in India (Thomas 2011) and 60.5 million in the world and by 2020 this is expected to be 16 million in India and 79.6 million in the world (Quigley et al. 2006). Improved methods of screening and drug delivery systems are the urgent need to address this issue. Timolol maleate is an adrenoceptor antagonist widely used in treatment of

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glaucoma. Timolol maleate available in conventional eye drop. But this conventional dosage form is suffering from inherent drawbacks like patient has to take several times (Winfield et al. 1990), allergic reaction (Baudouin et al. 1990), limited bio availability (Barbu et al. 2005), tear dilution, solution drainage (Lang et al. 1995), tear turnover and conjuctival absorption (Robinson 1989). Eye drop contains the preservative having potential to cause adverse effect to ocular tissue (Hopes et al. 2010). Hence preservative free medication has potential to improve therapy of glaucoma. Despite these several limitations significant improvement in ocular drug delivery system have been made in last 10-20 years. In present study an attempt has been made to design and optimized once a day preservative free ocular insert of Timolol maleate with the prime objective of maintaining the drug in the biophase for an extended period of time. It is a challenge to the formulator to circumvent the protective barriers of the eye so that the drug reaches the biophase in sufficient concentration.

Materials and Methods

Timolol maleate was received as gift sample from INTAS pharmaceutical, Ahmedabad, Eudragit RSPO and Eudragit RLPO gifted by Degussa, Roehm pharma polymers. HPMC 15 CPS was gifted by IPCA laboratories. Dialysis membrane was procured from Himedia laboratories. Other chemicals and solvents used in this study were of analytical grade.

Preparation of Ocular Insert

The preparation of ocular insert involved three steps (Sultana et al. 2005)

I) Preparation of reservoir film: Reservoir film was prepared by solvent casting method. Polymer and drug were passed through sieve number 400. Weighed quantity of polymer HPMC 15 CPS was dissolved in 10 ml of doubly distilled water to prepare 1%, 3.5% and 6% polymeric solution under continues stirring. Timolol maleate equivalent to 0.450 mg per ocular insert was added to the polymeric solution. The medicated polymer solution was sonicated for 15 minutes to remove air bubbles. Then plasticizer PEG 400 (30 % w/w of dry weight of polymer) was added under continuous stirring. Resultant solution was stirred overnight to get uniform distribution. This casting solution was poured on Teflon coated petri plate (Diameter 5 cm and area 19.625 cm^2) and covered with inverted funnel. The solvent was allowed to evaporate by placing it inside an oven maintained at 40°C ± 2 °C for 24 h.

II) Preparation of rate controlling membrane: To prepare rate controlling membrane of Eudragit RLPO and Eudragit RSPO in the ratio of 1:1, 1:3.5 and 1:6 along with 15 % w/w of plasticizer Dibutyl phthalate were dissolved in 10 ml of acetone. The solutions were poured in Teflon coated petri plate (Diameter 6 cm and area 28.26 cm^2). The solvent was allowed evaporate at room temperature for 24 h.

III) Placing the rate controlling membrane around the reservoir and sealing them to obtain ocular insert: Circular shaped ocular insert (Diameter 6 mm) were cut out from medicated reservoir film with the help of cork borer. This reservoir film was placed on a rate controlling membrane (Diameter 8 mm) and another rate controlling membrane was kept over it. The two rate controlling membrane containing reservoir film between them were placed over a beaker saturated with methanol/acetone vapors (50:50) for 1-2 minutes. This procedure resulted in sealing the rate controlling membrane containing the medicated reservoir film between them. Nine formulations were prepared as per factorial design showed in Table 1.

Table 1. Formulation of ocular insert

Formulation code	Drug reservior		Rate controlling membrane		Variables in coded form	
	Timolol maleate (mg)	Concentration of HPMC (%w/v)	Eudragit RLPO (mg)	Pay load of Eudragit RSPO (mg)	X1	X2
T_1	0.45	1	100	100	-1	-1
T_2	0.45	3.5	100	100	0	-1
T_3	0.45	6	100	100	1	-1
T_4	0.45	1	100	350	-1	0
T_5	0.45	3.5	100	350	0	0
T_6	0.45	6	100	350	1	0
T_7	0.45	1	100	600	-1	1
T_8	0.45	3.5	100	600	0	1
T_9	0.45	6	100	600	1	1
Casting solvent	Distilled water (10 ml)		Acetone (10 ml)			
Plasticizer	PEG 400 (30 % w/w of dry polymer)		Dibutyl phthalate (15 % w/w of dry polymer)			

Coded value	Actual values	
	X1(% w/v)	X2 (mg)
-1	1	100
0	3.5	350
1	6	600

Evaluation of Ocular Insert

The prepared ocular insert were subjected to physic chemical evaluations. Interaction study was performed by using FTIR spectrophotometer (Model IR Affinity 21 CE, Schimadzu, Japan) and Differential Scanning Calorimetry (DSC) analysis (David et al. 2008). Determination of average weight and weight variation was done by electronic weighing balance. Film thickness was determined by micrometer gauze. Surface pH (Harishankar et al. 2004) of the inserts was determined by digital pH meter. For determination of drug content formulated insert was transferred in to graduated glass stopper flask and dissolved with minimum quantity of methanol and rest of the dilution was carried out with simulated tear fluid (Gevaria et al. 2008). The content was stirred vigorously for 12 h on metabolic stirrer at 37°C. The solution was filtered and drug present in the filtrate was determined by UV spectrophotometer at 294 nm. The procedure was done in triplicate. The moisture absorption test (Ubaidulla et al. 2007) was done by using saturated solution of sodium chloride and moisture loss test (Mundada et al. 2006) was carried out by using calcium carbonate in desiccator.

In vitro Release Study

Semi permeable dialysis membrane number 60 procured from Himedia which has molecular weight cut off 12000 to 14000 with 2.4 nm pore size was used in the study. This dialysis membrane act as corneal epithelium. A simple cylindrical glass tube of 15mm internal diameter and 100 mm height was used (Mundada et al. 2007) (Figure 5). The dialysis membrane was tied to one end of open cylinder, which acted as a donor compartment. An ophthalmic insert was placed inside this compartment. The entire surface of the membrane was in contact with the receptor compartment comprising 25 ml of simulated tear fluid (pH 7.4) in a 100 ml beaker. The content of receptor compartment was stirred continuously using a magnetic stirrer and temperature was maintained at 37° ± 0.5°C. At specific intervals 1 ml aliquot of solution was withdrawn from the receptor compartment and replaced with fresh simulated tear fluid. The aliquot was analyzed for the drug content using UV-Vis spectrophotometer (Model 1800, Schimadzu, Japan) at 294 nm after appropriate dilutions against reference using simulated tear fluid.

Kinetic Analysis Study

In order to understand the mechanism and kinetics of drug release, the results of the *in vitro* drug release study were fitted in various kinetic models such as zero order, first order, Higuchi and Korsmeyer Peppas models (Costa et al. 2001). The correlation coefficient (r^2) was determined from all models. Release exponent n value was determined from Peppas equation $^{M_t}/_{M_\infty} = kt^n$ where M_t is the amount of drug released at time t is and M_∞ is the amount released at time infinitive. The $^{M_t}/_{M_\infty}$ is the fraction of drug released at time t, k is the kinetic constant and n is the diffusion exponent, a measure of the primary mechanism of drug release.

Temperature Sensitivity Study

The optimized insert was stored in amber colored glass bottles at 3 different temperatures 4°C, 25°C and 37 °C for a period of 3 months. The samples were withdrawn after 30, 60 and 90 days and analyzed for physical appearance, drug content and sterility (IP 2007).

Experimental Design

A 3^2 factorial design was applied for formulation of ocular insert of Timolol maleate to determine the effect of two independent variables (Concentration of HPMC (X1) and Pay load of Eudragit RSPO (X2) on drug release at the end of 12 h (DRL 12), drug release at the end of 18 h (DRL 18) and time required to release 50 % drug (T50 %). Each factor was tested at three levels designated as -1, 0 and +1. The values of the factors were transformed to allow easy calculation of co-efficient in polynomial equation. The reduced model was generated to identify the effect of significant variables. Interactive multiple regression analysis and F statistics was utilized in order to evaluate the response. The regression equation for the three responses were calculated using following equations

Response: Y1 (DRL 12) = b0+b1X1+ b2X2+ b3X12+ b4X22+ b5X1X2 (1)

Response: Y2 (DRL 18) = b0+b1X1+ b2X2+ b3X12+ b4X22+ b5X1X2 (2)

Response: Y3 (T 50 %) = b0+b1X1+ b2X2+ b3X12+ b4X22+ b5X1X2 (3)

Where, Y_i (Y1, Y2 and Y3) is the dependent variable, b0 is the arithmetic mean response of the nine runs and bi (b1, b2, b3, b4 and b5) is the estimated coefficient for the corresponding factor Xi (X1, X2, X12, X11, and X22), which represents the average results of changing one factor at a time from its low to high value. The interaction term (X1X2) depicts the changes in the response when two factors are simultaneously changed. The polynomial terms (X12 and X22) are included to investigate nonlinearity. The multiple regression was applied using Microsoft excel in order to deduce the factors having significant effect on the formulation properties. To identify the significant variables, the variables having p value > 0.05 in the full model were discarded and then the reduced model was generated for both the independent variables for each type of formulation.

Response Surface Plots

Graphical presentation of the data helped to show the relationship between the response and the independent variables. Surface plots and contour plot were drawn by using Design - Expert 8.0.5 trial version software. Graphs were shown in Figure 7 and 8.

Results and Discussion

Physicochemical parameters were presented in Table 2. Weight of all formulations ranged from 9.25 mg to 21.05 mg. The uniformity of weights of films indicates good distribution of drug, polymer and plasticizer. The thickness of the prepared formulations varied between 0.143 mm to 0.335 mm. The formulations did not produce irritation when placed in the cul de sac since they were not thick enough to produce irritation. Surface pH of all formulations also between 7.2 to 7.4 which further provides evidence of non irritancy of ocular insert as it

matches with the pH of tear fluid i.e. 7.4. The drug content of all formulations was found to be in the range of 99.15 to 100.05 %. The minimum intra batch variations revealed the suitability of the process used to prepare the ocular insert. Formulation T_7 showed least moisture absorption and formulation T_3 exhibited highest moisture absorption. This may be due to more concentration of HPMC 15 CPS and least hindrance provided by less amount of Eudragit RSPO. In formulation T_7 less concentration of HPMC 15 CPS and more proportion of Eudragit RSPO which provide more hindrance to entry of water molecule inside the formulation. Moisture loss for all formulation was in the range of 0.90 to 5.01 %. The result revealed that as concentration of HPMC increases and pay load of Eudragit RSPO decreases, moisture loss from formulation increases.

Table 2. Physicochemical evaluation of ocular insert

Formulation Code	Weight** (mg)	Thickness** (mm)	Drug content* (%)	Surface pH*	Moisture absorption*	Moisture loss*
T_1	9.25±0.45	0.143±0.01	99.57±0.25	7.2	1.58±0.10	1.8±0.11
T_2	11.85±0.50	0.146±0.05	99.40±0.28	7.4	3.49±0.15	3.5±0.12
T_3	12.25±0.46	0.172±0.03	99.15±0.35	7.2	5.75±0.17	6.05±0.14
T_4	13.45±0.35	0.227±0.05	99.89±0.40	7.2	1.15±0.12	1.09±0.09
T_5	15.75±0.25	0.256±0.02	100.0±0.26	7.4	2.84±0.15	3.1±0.15
T_6	18.05±0.51	0.29±0.01	99.55±0.30	7.4	4.91±0.13	5.01±0.10
T_7	17.59±0.40	0.26±0.03	100.05±0.45	7.4	0.75±0.16	0.9±0.08
T_8	19.54±0.54	0.253±0.01	99.67±0.34	7.4	1.85±0.11	2.15±0.14
T_9	21.05±0.25	0.335±0.01	100.03±0.48	7.2	4.05±0.10	3.9±0.15

Mean ± SD (**n=10, *n=3)

Effect of formulation variable on DRL 12 (Full and reduced model for DRL 12)

The quadratic model for DRL 12 was found to be significant (Table 4). F value for DRL 12 was found 316.71 which denotes that model is significant and there is only 0.01 % chance that a "model F value'' large could occur due to noise. The pred-R-square of 0.9748 is in reasonable agreement with adj-R-square of 0.9875. The variance inflation factor was 1 which showed good estimation of coefficient. Adequate precision ratio was found to be 46.30 indicates an adequate signal. This ratio greater than 4 is desirable hence it was concluded that this model can be used to navigate the design space. The polynomial equation for DRL 12 obtained was as follow.

$$DRL\ 12 = 51.658 - 5.53X1 - 13.77X2 - 0.593X11 - 1.028X22 + 1.067X12$$

The p value for variable X11, X22 and X12 was more than 0.05 hence they were omitted from above polynomial equation and equation was reduced to as follow (Table 4).

$$DRL\ 12 = 51.658 - 5.53X1 - 13.77X2$$

The above equation depict that both variable X1 and X2 exert significant effect on DRL 12. Negative sign for both the variable confirm that these two variable have decreasing effect on response Y1 i.e. DRL 12. Out of these two variables pay load of Eudragit RSPO exhibit significant effect on DRL 12. Drug released at 12 h from formulation T_3, T_6 and T_9 was found to be 56.81, 45.14 and 32.58 respectively (Table 3). Formulation T_9 consist highest proportion of Eudragit RSPO among these three formulations. This outcome may be attributed due to

low permeability nature of Eudragit RSPO which provides maximum hindrance to entry of water molecule inside the formulation.

Effect of formulation variable on DRL 18 (Full and reduced model for DRL 18)

The quadratic model for DRL 18 was found to be significant (Table 4). Model F value for DRL 18 was found 67.61 which denotes that model is significant and there is only 0.01 % chance that a "model F value'' large could occur due to noise. The Pred-R-square of 0.8936 is in reasonable agreement with Adj-R-square of 0.9434. The variance inflation factor was 1 which showed good estimation of coefficient. Adequate precision ratio was found to be 21.92 indicates an adequate signal. This ratio greater than 4 is desirable hence it was concluded that this model can be used to navigate the design space. The polynomial equation for DRL 18 obtained was as follow.

$$DRL\ 18 = 72.265 - 8.46X1 - 17.74X2 - 1.12X11 - 4.59X22 + 2.52X12$$

The p value for variable X11, X22 and X12 was more than 0.05 hence they were omitted from above polynomial equation and equation was reduced to as follow (Table 4).

$$DRL\ 18 = 72.265 - 8.46X1 - 17.74X2$$

Above equation revealed that both variables X1 and X2 exhibit significant effect on DRL 18. It was evident from equation that both variables provide negative effect on response i.e DRL 18. Out of two variables second variable X2(pay load of Eudragit RSPO) play an effective role in decreasing of drug release from the formulations. A relationship was found between the polymer (type and concentration) and the rate of release. Formulation T_1, T2, T3 showed 99.76, 88.73, and 74.50 % of drug release (Table 3). In these pay load of Eudragit RSPO was same but concentration of HPMC 15 CPS was varied and it gives effect on cumulative % of drug release. Formulation T_7, T_8 and T9 showed 59.41, 52.89 and 44.23 % drug release (Table 3). Slow release from formulation T_9 may be due to high pay load of Eudragit RSPO and more concentration of HPMC 15 CPS.

Effect of formulation variable on T50 % (Full and reduced model for DRL T50 %)

The quadratic model for T50 % was found to be significant (Table 4). Model F value for T50 % was found 40.04 which denotes that model is significant and there is only 0.03 % chance that a "model F value'' large could occur due to noise. The Pred-R-square of 0.8198 is in reasonable agreement with Adj-R-square of 0.9071. The variance inflation factor was 1 which showed good estimation of coefficient. Adequate precision ratio was found to be 16.88 indicates an adequate signal. This ratio greater than 4 is desirable hence it was concluded that this model can be used to navigate the design space. The polynomial equation for T50 %obtained was as follow.

$$T50\ \% = 11.59 - 2.10X1 - 4.40X2 - 0.161X11 - 1.57X22 + 1.00X12$$

The p value for variable X11, X22 and X12 was more than 0.05 hence they were omitted from above polynomial equation and equation was reduced to as follow (Table 4).

$$T50\ \% = 11.59 + 2.10X1 + 4.40X2$$

From above equation it was evident that both variables X1 and X2 exhibit significant effect on T50 %. It was revealed from equation that both variables provide positive effect on response i.e T50 %. As concentration of HPMC 15 CPS and pay load of Eudragit RSPO increases there was increase in time taken for 50 % drug released from formulations. Pay load of Eudragit RSPO play a vital role in increasing the value of T50 %. Formulation T_7, T_8 and

T9 showed 14.63,17.01 and 21.39 h (Table 3). This may be due to higher proportion of Eudragit RSPO in the formulation.

In vitro Release Study

The in vitro dissolution data were presented in Figure 6. The cumulative release of drug from formulation T1 99.76 % in 18 h, Formulation T8 showed 100 % at the end of 22 h while formulation T6 showed 95.24 % at the end of 24 h. Hence observation was carried out that as concentration of HPMC and pay load of Eudragit RSPO increases, there was decrease in rate of drug release. Formulations T_7, T_5 and T_3 showed 72.81 %, 69.25 % and 56.89 % at the end of 24 h. This may be attributed due to low permeability of Eudragit RSPO and at higher concentration HPMC may form viscous gel layer from this it will not allow to diffuse drug at a faster rate.

Release Kinetic

In vitro release data were fitted in to various kinetic models such as zero order, first order, Higuchi and Korsmeyer Peppas model. The value of correlation coefficient showed in Table 5 confirms that correlation was observed in Zero order, Higuchi and Korsmeyer Peppas model than first order model. Peppas model is very useful model to determine mechanism of drug released from the formulation. The value of n=0.5 indicates Fickian diffusion mechanism, if $0.5< n <1$, indicates anomaleous (non-fickian) mechanism and n=1 implies case II (relaxation controlled) transport. In present study the value for n was found in the range of 0.84 to 1.05 which confirms that release mechanism followed anomalous (non-fickian) and case II transport (relaxation controlled).

Optimization

Optimization was done by Design - Expert 8.0.5 trial version software by setting target for dependent responses. The data for the in vitro release study were fitted in to Design Expert software. It provides ideal composition of independent variable. As per this concentration of HPMC 15 CPS (X1) should be 3.49 % w/v and pay load of Eudragit RSPO should be 342.73 mg (Table 7) which may showed 0.978 desirability (Figure 9). FTIR spectra (Figure 1 and 2) and DSC spectra (Figure 3 and 4) of pure drug and medicated film ruled out possible interaction between drug and excipients. Result from FTIR study observed that all the important peaks of Timolol maleate remain unaltered in medicated film. DSC spectra of Timolol maleate showed melting endotherm 205.75 which is equal to the melting point of Timolol maleate shows good purity of drug used in the formulation and melting endotherm remain unchanged in medicated film confirms the compatibility of excipients used in the formulation process. The optimized formulation (T10) was prepared as per composition suggested by the software and it was checked for its feasibility. The physicochemical parameters of optimized formulation were optimum and data were presented in Table 7. The in vitro release data were also fitted in various kinetic models. Results based on value of correlation coefficient (r^2) it follow Zero order, Higuchi and Korsmeyer Peppas models (Table 5). Drug release mechanism from formulation was case II transport. The optimized formulation provides perfect zero order release and passes the temperature sensitivity parameters shown in Table 9. Experimental and predicted values of responses were (Table 8) match in close agreement for optimized formulation (Figure 7 and 8).

Table 3. Different batches with their experimental coded level of variables and response

Formulation code	Variable level in coded form		*DRL 12 ± SD	*DRL 18± SD	*T 50 % ± SD
	X1	X2			
T_1	-1	-1	69.51±0.89	99.76±1.01	7.3±0.11
T_2	0	-1	65.71±1.89	88.73±2.01	9.25±0.08
T_3	1	-1	56.81±3.06	74.50±3.41	10.05±0.12
T_4	-1	0	57.23±1.78	79.45±1.56	10.15±0.15
T_5	0	0	51.42±1.16	74.99±1.23	11.66±0.09
T_6	1	0	45.14±2.05	69.10±3.25	13.29±0.11
T_7	-1	1	41.01±1.45	59.41±1.56	14.63±0.16
T_8	0	1	35.79±3.25	52.89±2.56	17.01±0.14
T_9	1	1	32.58±2.59	44.23±3.09	21.39±0.14

Concentration of HPMC (%w/v), Pay load of Eudragit RSPO (mg) Mean ± SD (*n=3)

Table 4. Response of full model and reduced model.

	DRL 12				DRL 18				T50 %			
	Full model		Reduced model		Full model		Reduced model		Full model		Reduced model	
Response	X Coeff	P value	X Coeff	P value	X Coeff	P value	X Coeff	P value	X Coeff	P value	X Coeff	P value
X1	-5.536	0.0019	-5.536	0.0019	-8.465	0.0081	-8.465	0.0081	2.108	0.006	2.108	0.006
X2	-13.775	0.00012	-13.775	0.00012	-17.743	0.0009	-17.743	0.0009	4.405	0.0007	4.405	0.0007
X11	-0.593	0.566	-	-	-1.128	0.662	-	-	0.161	0.781	-	-
X22	-1.028	0.346	-	-	-4.593	0.143	-	-	1.571	0.060	-	-
X12	1.067	0.200	-	-	2.52	0.224	-	-	1.002	0.076	-	-
Intercept	51.658	1.47E	51.658	-	75.265	7.69E	75.265	-	11.592	0.0002	-	-
Model F value	316.71				67.61				40.04			
Level of significance	Significant				Significant				Significant			
VIF	1				1				1			

Regression statistics				
	Multiple R	R Square	Adjusted R Square	Adequate Precision
DRL 12	0.9980	0.9961	0.9897	46.36
DRL 18	0.9932	0.9864	0.9639	21.925
T50 %	0.9944	0.9889	0.9704	16.881

Table 5. Kinetic analysis of ocular insert

Formulation code	Zero order	First order	Higuchi	Korsmeyer Peppas		Type of release mechanism
	r^2	r^2	r^2	r^2	n	
T_1	0.996	0.863	0.979	0.997	0.90	Anomaleous
T_2	0.998	0.847	0.975	0.997	1.001	Case II
T_3	0.990	0.803	0.987	0.995	1.03	Case II
T_4	0.997	0.835	0.975	0.995	1.05	Case II
T_5	0.994	0.850	0.957	0.995	1.007	Case II
T_6	0.987	0.812	0.991	0.995	0.921	Anomaleous
T_7	0.995	0.823	0.983	0.995	0.959	Anomaleous
T_8	0.987	0.814	0.991	0.996	0.922	Anomaleous
T_9	0.997	0.860	0.961	0.993	1.004	Case II
T10	0.998	0.847	0.972	0.999	1.01	Case II

T10 was optimized formulation, n-release exponent, r^2 – correlation coefficient

Table 6. Goals for optimization of formulation

Name	Goal	Lower limit	Upper limit
Concentraion of HPMC 15 CPS X1 (% w/v)	In range	1	6
Pay load of Eudragit RSPO X2 (mg)	In range	100	600
DRL 12 (Y1) %	51	50	55
DRL 18 (Y2) %	72	70	75
T50 % (Y3) h	12	11	13

Table 7. Suggested formulation and physicochemical parameters of optimized formulation

Formulation		Weight** (mg)	Thickness** (mm)	Drug content* (%)	Surface pH*	Moisture absorption* (%)	Moisture loss* (%)
X1	X2						
3.49	347.07	15.70±0.15	0.251±0.01	100.01	7.4	2.78±0.10	2.89±0.13

X1- % of HPMC 15 CPS, X2- Pay load of Eudragit RSPO, mean ±SD(**n=10, *n=3)

Table 8. Observed value and predicted value for optimized formulation

Responses	*Predicted	*Observed
DRL 12 (Y1) %	51.00	51.50±0.65
DRL 18 (Y2) %	72.001	73.08±0.58
T50 % (Y3) h	12.61	11.67±0.04

Mean ± SD (*n=3)

Table 9. Temperature sensitivity study

Time (Days)	4°C			25°C			37°C		
	PA	% *DC	SRT	PA	%* DC	SRT	PA	%* DC	SRT
0	+	99.50±0.12	√	+	99.48±0.13	√	+	99.80±0.07	√
30	+	99.12±0.11	√	+	99.59±0.09	√	+	99.67±0.10	√
60	+	99.15±0.08	√	+	99.35±0.10	√	+	99.17±0.11	√
90	+	99.25±0.10	√	+	99.30±0.07	√	+	99.05±0.15	√

PA- Physical Appearance (+ indicates good), DC- Drug Content, SRT- Sterility Test (√ indicates passes the test)

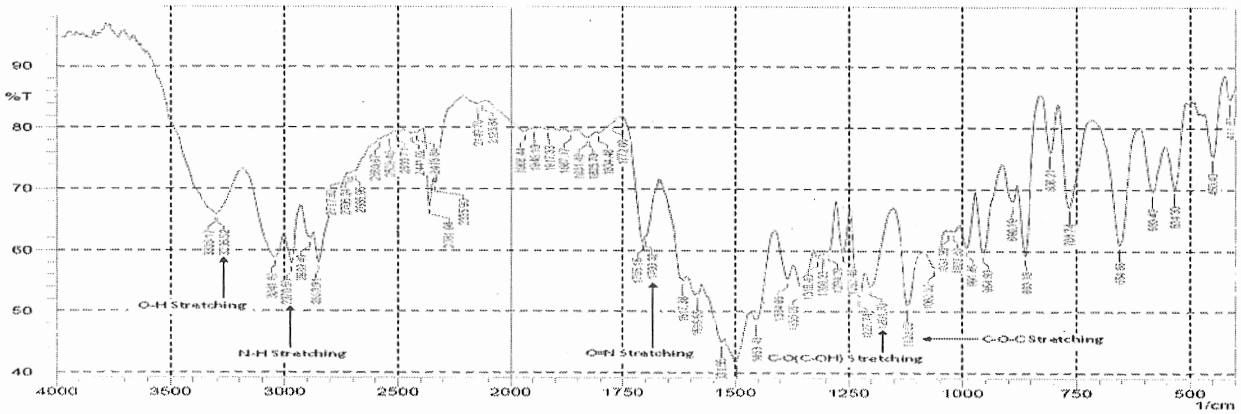

Figure 1. FTIR spectra of Timolol maleate

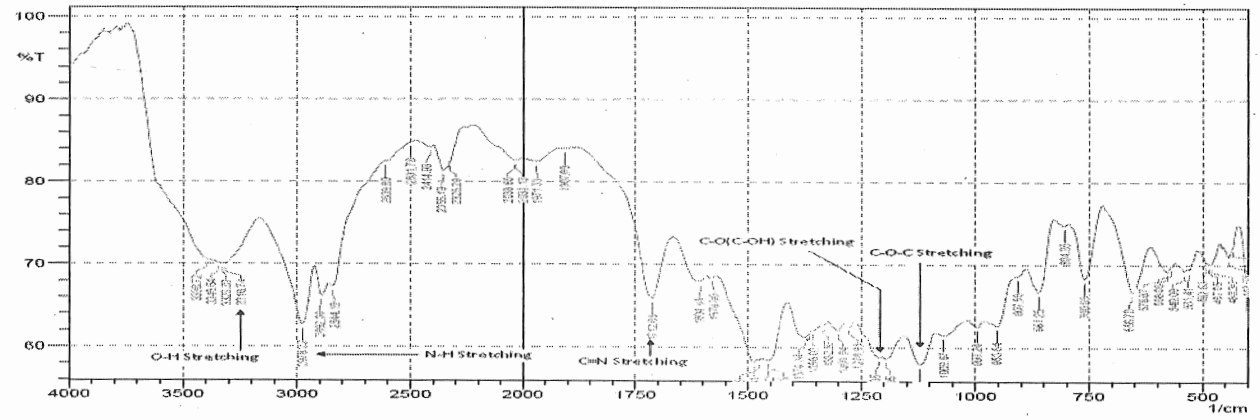

Figure 2. FTIR spectra of optimized ocular insert of Timolol maleate.

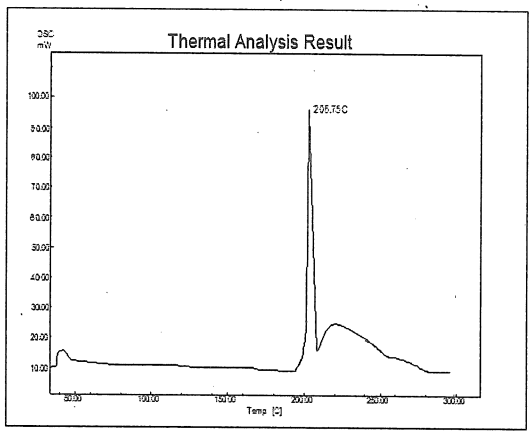

Figure 3. DSC spectra of Timolol maleate

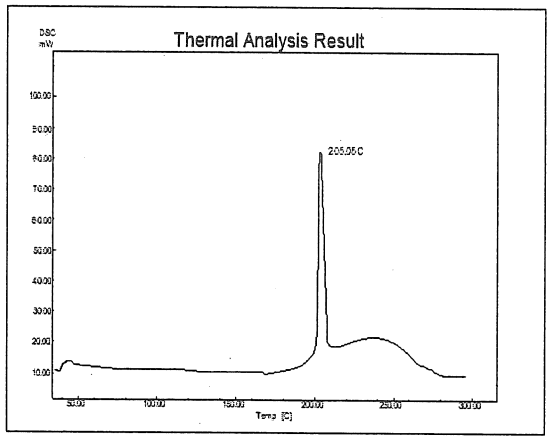

Figure 4. DSC spectra of optimized ocular insert

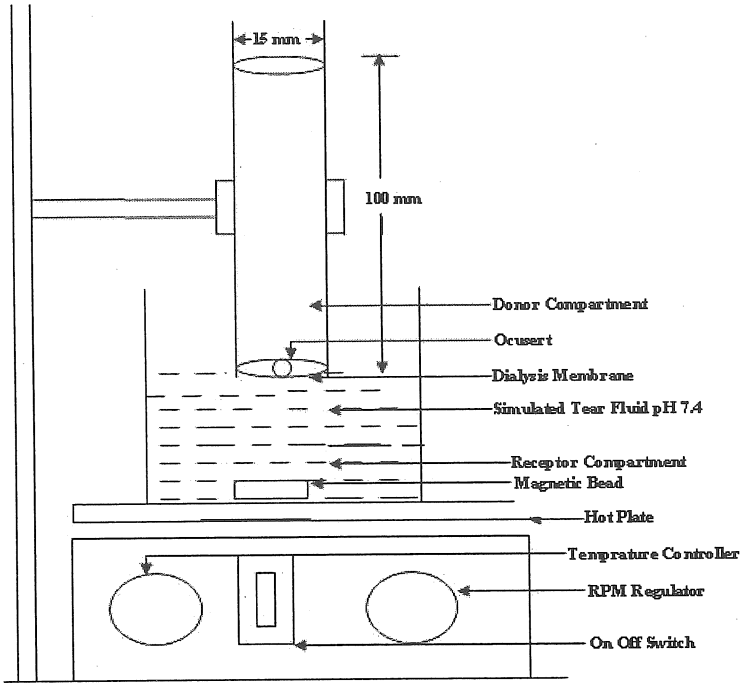

Figure 5. In vitro dissolution assembly

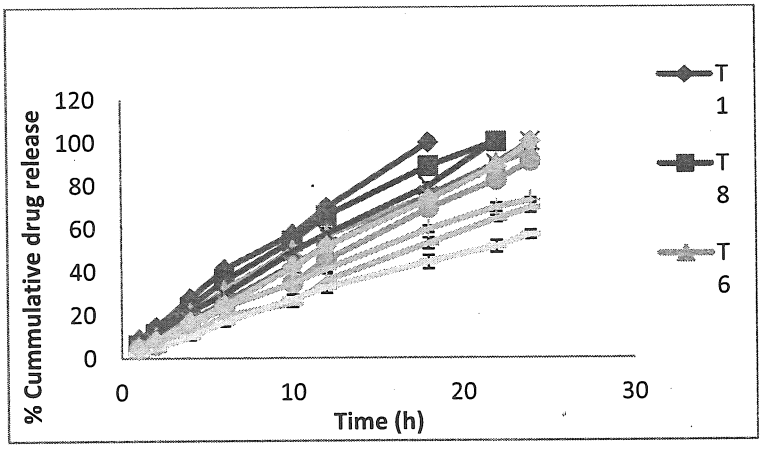

Figure 6. In vitro release study of prepared ocular insert and optimized formulation.

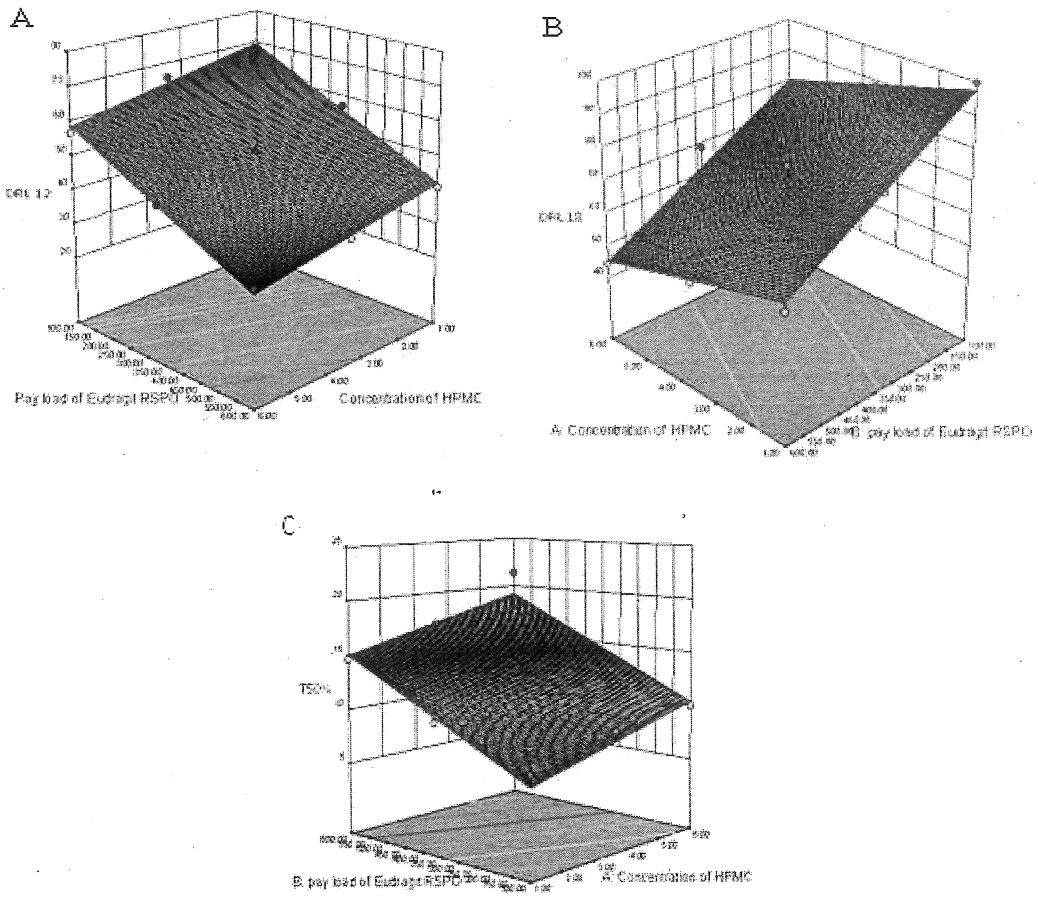

Figure 7. Response surface plot of A) DRL 12 B) DRL 18 and C) T50 %

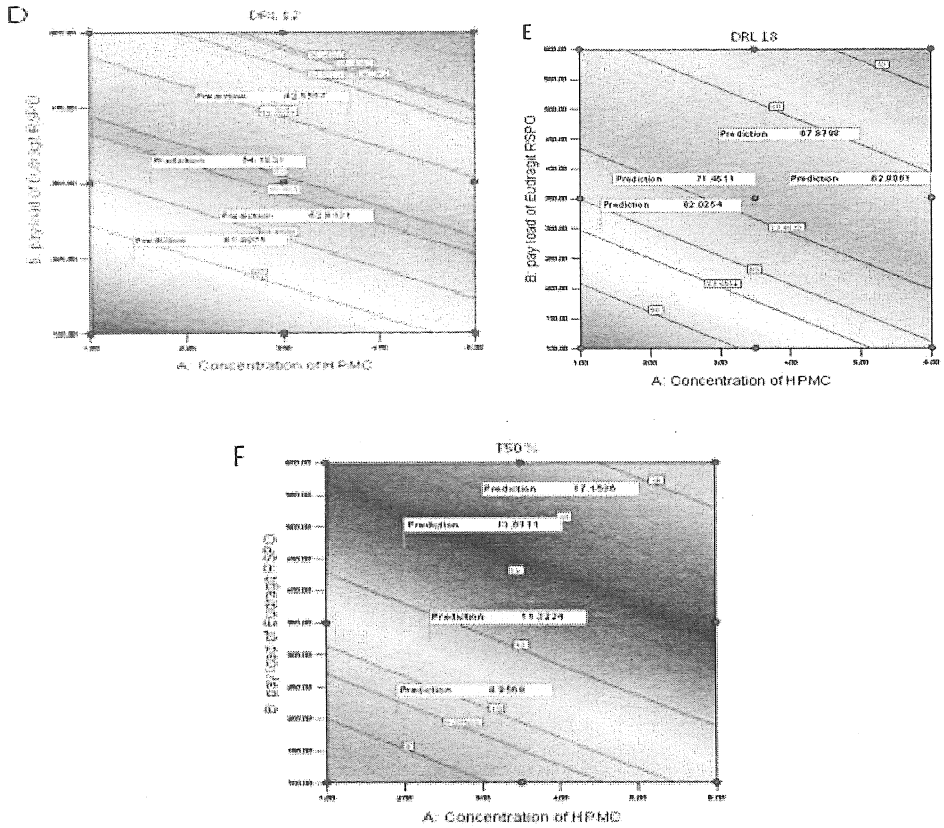

Figure 8. Contour plot of D) DRL 12 E) DRL 18 and F) T50 %

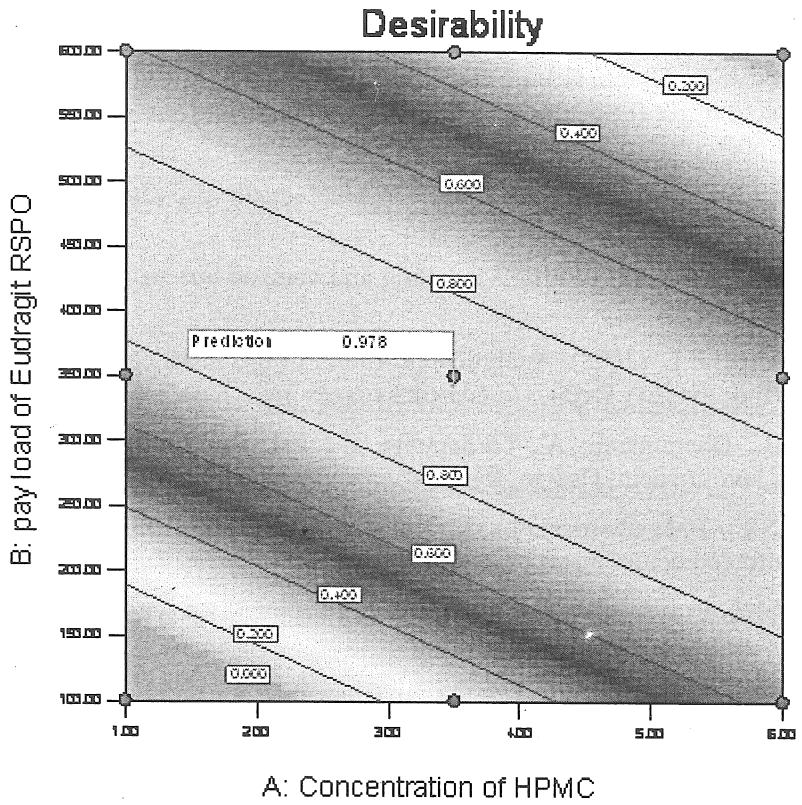

Figure 9. Desirability graph for optimized formulation

Conclusion

The application of factorial design gave statistically systemic approach for formulation of ocular insert with desired in vitro drug release. Concentration of HPMC 15 CPS and pay load of Eudragit RSPO significantly influence on selected response. Hydrophilic carrier like HPMC 15 CPS and lipophilic carriers like Eudragit RSPO, Eudragit RLPO were useful in formulation of reservoir type of ocular insert. Further studies are needed to confirm its performance in vivo.

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