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Design, Optimization and evaluation of peroral *in situ* gel containing Ranitidine HCl

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ABSTRACT

This research aimed to evaluate a new approach for preparation of *in situ* gel and to design innovative peroral delivery systems for Ranitidine Hydrochloride (RHCl) able to enhance the control release. The present study was carried out to optimize and evaluate an oral in-situ gel containing ranitidine HCl with Pluronic F-127 and hydrophilic HPMC E50 by the simple mixing method. The compatibility of the polymers was proved by FTIR. The prepared in-situ gel formulations were tested for their physicochemical characteristics such as clarity, gel strength, gelation temperature, drug content, sol-gel transition time and in vitro release studies of ranitidine HCl-loaded in-situ gel formulation in phosphate buffer (pH 1.2 and 7.4.) were performed using a modified diffusion cell across dialysis membrane. The prepared formulations were clear and Gel strength ranges from 31±1.6 to 33±1.2. The drug content for the prepared formulations was 97.23% to 99.02%.Then the Drug release at 12 h is 98.25 and thus shown controlled release.

Keywords: Oral in- situ gel, Ranitidine HCL; Pluronic F-127; HPMC E50.

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INTRODUCTION

The oral route is being increasingly used for the administration of drugs, which are mainly designed to release the drug over a long period of time in a controlled fashion[1].The peroral *in situ* gel forming polymeric drug delivery systems are designed with objective to controlled drug delivery system[2].

In situ gel forming polymeric drug delivery systems have shown some advantages such as ease of administration, reduced frequency of administration, improved patient compliance and comfort, high dose (over tablets) can be given for controlled release can be given. *In situ* gels are prepared to overcome the rapidly increasing cost and easy of manufacturing over than the other pharmaceutical dosage forms[3].

In smart polymeric systems represent promising means of delivering the drugs, these polymers undergoes sol-gel transition once administered. *In situ* gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation, solvent exchange and presence of ions, electrical stimuli etc[3].

Mucoadhesive polymers are essential in the development of oral muco-adhesive *in situ* gel. This polymer provides intimate contact between the dosage form and the absorbing tissue and increase the retention time. Increasing the retention time of the dosage form is essential in the development of this system & it has been reported that increase in retention time with an increase in the mucoadhesivity of the system. In the literature, bio-adhesive drug delivery systems for drugs like ciclopriox olamine[4] Metronidazole [5]Mebeverine hydrochloride [6] are reported.

Ulcers are popularly known as peptic ulcer disease. Peptic ulcers mainly form in the areas of duodenum which is the starting part of small intestine and in the linings of the stomach. Ulcer lesions forms in the size of 0.5 cm, in some cases it may be more than that.[7] RHCl is an important histamine H_2 receptor antagonist whose mechanism of action is to reduce the increased gastric acid level by acting on H^+K^+ATP' as an enzyme present in the gastric parietal cell. This drug is effective in treatment of gastric and duodenal ulcer, in treatment of heart burn disease and other symptoms associated with gastroesophagal reflux disease and its plasma elimination half-life is 2 to 3 hours. The idea behind a controlled in situ gel drug delivery system is to incorporate the drug within a polymeric carrier that controls the release rate of the drug.

The objective of present study is to develop *in situ* gel of RHCl with thermo sensitive polymer, pluronic F127and HPMCE50.This may give patient friendly, needle free controlled release dosage form.

MATERIAL AND METHODS

Materials:

RHCl was obtained as gift sample from Bal Pharmaceuticals (Bangalore, Karnataka). HPMCE50, Pluronic F127 and sorbitol were purchased from Yarrow chemicals and other chemicals used were analytical grade.

Experimental design:

2^2 factorial design was selected for the development of the formulation. The simplest factorial design is the two-factorial design where two factors are considered each at two levels, leads to four experiments, which are situated in 2- dimensional factor space at the corners of a rectangle. The number of experiments is given by 2^n, where 'n' is the number of factors[8].

The fitting of an empirical polynomial equation to the experimental result facilitates the optimization procedure. The general polynomial equation is as follows:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + \ldots + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + \ldots + B_{123}X_1X_3.$$

Effect of PluronicF127 (A) and effect of HPMC E50 (B) were selected as independent variables. PluronicF127 lower (-1) value is 900 mg and higher (+1) is 1500 mg were selected. Lower value (-1) of HPMC E50 is 50 mg and higher (+1) value is 100 mg were selected.

Preparation of Oral In situ gel:

The *in situ* gel formulations containing RHCl were prepared by simple mixing method by cooling in refrigerator. Pluronic F-127 and HPMC E50 were used as polymers in the preparation of in-situ gel formulation. Cold water used as a solvent. Weighed required quantity of polymers (Pluronic F-127, HPMC E-50) and drug were dissolved separately in 5 ml of cold water and mixed it well, vortexed till bubbles have gone. The Entire preparation was done in dark room condition[4].

Evaluation:

Fourier Transform Infrared Spectroscopy (FT-IR α - e ATR Module Bruker):

IR spectroscopy was carried out for the following A) Pure drug, RHCl B) RHCl + HPMCE50C) RHCl + Pluronic F127 using FT-IR α - e ATR Model Bruker.

Determination of pH:

EUTECH pH meter is used to determine the gel pH which is a calibrated pH meter. The readings were taken for average of 3 samples[9].

Gelation time:

Gelation time of the gel was determined by taking the solution and added pH 6 phosphate buffer solution. The time taken for the solution to convert into gel is noted[10].

Viscosity studies:

Brookfield programmable LV DVIII Model pr (UCP) having T-bar spindle is used to determine the rheological studies. The viscosity of in situ gel and the solution (250 ml) were determined at different angular velocities (0, 10, 20, 30, 40….to 50 rpm) average of two reading were used to calculate the viscosity. Evaluation was conducted in triplicate[11].

Measurement of gel strength:

50 gm of gel sample was taken in a graduated cylinder of capacity 100 ml. Then the gel sample weighing 20gms is allowed for penetration and time recorded by weight to sink to 5 cm down. The gel strength was determined by time in seconds at temperature (37.5^0 c) [12].

***In vitro* drug release studies:**

Drug release was monitored by the USP paddle method USP. Phosphate buffer of pH 6 at 35^0C±0.5^0Cwere used and maintaining the speed of rotation at 50 rpm. The instrument was covered with a black sheet to protect the drug from light as it is photosensitive. 5 mL of in situ gel is taken in a small vial and immersed in the dissolution medium maintaining the speed at 50 rpm. Aliquots, each of 5 mL, were withdrawn from the release medium at 1 hour time intervals for12 h and each aliquot was replaced by 5 mL of fresh buffer. The concentration of drug was determined from a previously constructed calibration curve. The release experiments were run in triplicates using phosphate buffer and the results were averaged[13].

Drug content by HPLC

The HPLC system was fitted with an autosampler with a 5 µL loop, a detector set at 228 nm, and a data station. A 3 µm spherisorbcyano-bonded phase column was used at ambient temperature with a mobile phase flow rate of 1.0 mL/min. Other equipment used was as follows: centrifuge, UV-VIS spectrophotometer connected to a HP 85 computer with plotter and disk drive, auto titrator equipped with a 5 mL burette and a glass calomel electrode, and a wrist action shaker. The mobile phase consists of ammonium phosphate buffer and it was prepared by adding 0.025 M ammonium hydroxide to 0.025 M ammonium dihydrogen phosphate to obtain a final solution of pH 5. A 300 mL aliquot of acetonitrile was placed in a suitable vessel and 0.025 M ammonium phosphate buffer (pH 5.0) was added to obtain a final volume of 1 L. The mixture was well stirred, filtered, and de-aerated under reduced pressure[14].

Regression analysis:

The response parameters were statistically analyzed by applying one way ANOVA at 0.05 levels using commercially available software Design-Expert software (Stat-Ease Inc, Minneapolis, USA). The individual parameters were evaluated using the F test and Linear, 2FI; Quadratic models were generated for each response parameter using the multiple linear regression analysis (MLRA) equation:

$$R = b_0 + b_1 A + b_2 B + b_3 AB + b_4 A^2 + b_5 B^2 + b_6 AB^2 + b_7 A^2 B \quad (1)$$

Where, R is the level of measured response, b_0 is the intercept of the arithmetic mean response of the 13 runs, A and B are the coded level of the independent variables. The AB is the interaction term, show how response changes when two factors are simultaneously used. A^2, B^2 are quadratic terms of the independent variables to evaluate the nonlinearity.

Stability Studies:

An accelerated stability study was carried out according to ICH guide lines. The optimized formulation was kept in 2 ml of glass vial and closed. The vials were kept at 40 ± 2^0C / 75 RH ± 5% RH for six months after end of every month drug content[15].

Photo stability studies:

Photo stability studies are done according to ICH Q1B guidelines. These studies are carried out in Neutronic photo stability chamber. The sample are kept under U.V light and the tube light for 1.2 million lux hours and integrated under UV energy of not less than 200 watt per hour per square meter to allow the compound. The results of drug substance and the drug product were compared.

RESULTS AND DISCUSSION

FTIR studies

The peaks of drug, binary mixture of drug and eudragit RS 100, drug and eudragit RL 100 are shown in the table 1. In the drug-excipient interaction study, it was found that RHCl was compatible with all the excipients used in the formulation. Fourier-transform infrared (FTIR) spectroscopy was performed on each of the samples to identify the presence of specific functional groups within a sample. Furthermore, drug-polymer interactions were examined using the resulting spectra. In the drug-excipient interaction study, it was found that RHCl was compatible with all the excipient used in the formulation .As there are no extra peaks and no shifting of peaks of the functional groups of RHCl (all peaks are within the ± 5 cm^{-1}) in the spectra of binary mixtures of drug and excipients.The IR spectrum of RHCl reveals characteristic shoulders in the RHCl IR spectrum that occur at 1132.57cm^{-1} for the C=N, 3181 cm^{-1} for the N-

H, 1218.55 cm^{-1} for the C-O-C, 689.18 cm^{-1} for the of the C-S respectively. These related bands were also observed for the physical mixture of polymers along with ranitidine HCl with the same absorbance. From these results, it can be confirmed that there is no interaction between ranitidine HCl and polymers (Pluronic F-127 and HPMC E-50) in the physical mixture. This can be known from table 1.

Table 1: FT-IR data of Ranitidine alone and with excipients

Bond	Wave no. cm^{-1} (Pure drug)	Wave no. cm^{-1} (pluronicF127 + drug)	Wave no. cm^{-1} (HPMCE50 + drug)
NH_2	3181.24	3481.24	3380.36
C-N	1132.57	1145.68	1132.57
C=C	1621.28	1627.37	1617.54
C-O-C	1218.55	1241.01	1219.09
NO2	1568.36	1627.37	1571.17
C-S	689.18	840.92	756.36

In vitro Release

Drug release studies are carried out using USP paddle dissolution apparatus *in vitro* dissolution studies of RHCl *in situ* gels were carried out in dissolution apparatus in buffer, PBS pH 6 solution and their graphical representation given in figure 1-5. *In vitro* drug release was shown in table 2

Table 2: Design and Summary of Responses Data

Run	Formulation code	Pluronic F127 (gm)	HPMCE50 (mg)	SORBITOL (mg)	In-vitro release 1hr	In-vitro release 8hr	In-vitro release 12hr
1	F1	0.9	100	0	20.93	62.03	96.15
2	F2	1.6	100	25	15.56	56.76	85.98
3	F3	1.6	50	25	16.32	55.67	86.09
4	F4	1.6	100	0	14.56	52.99	83.25
5	F5	0.9	50	25	22.78	67.95	98.25
6	F6	0.9	100	25	21	66.56	97.36
7	F7	0.9	50	0	22.65	62.65	97.25
8	F8	1.6	50	0	15	53.05	85.23

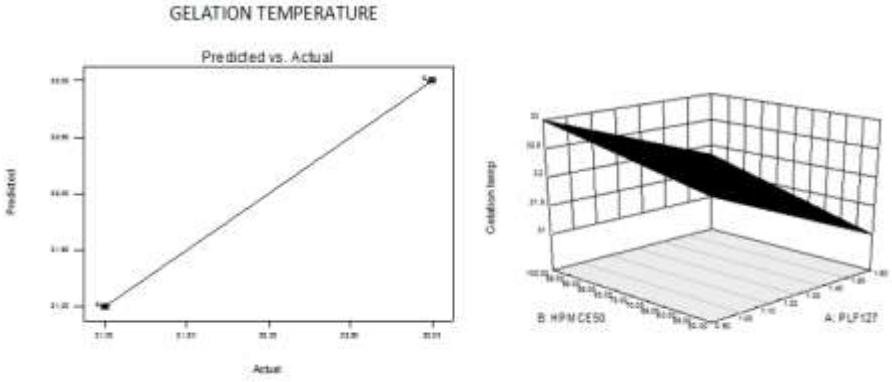

Figure 1: Effect of formulation variables on Gelation temperature

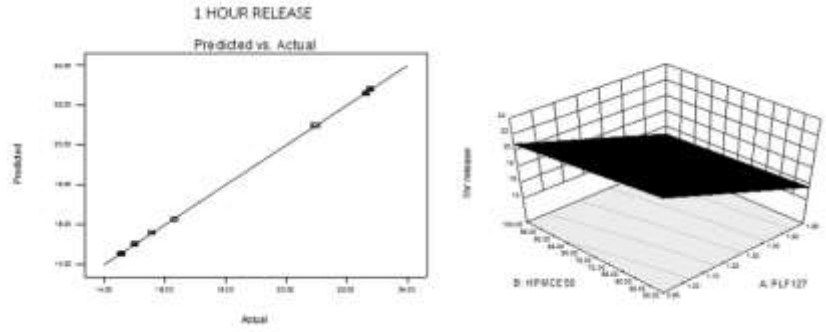

Figure 2: Effect of formulation factors on in vitro drug release after 1st h

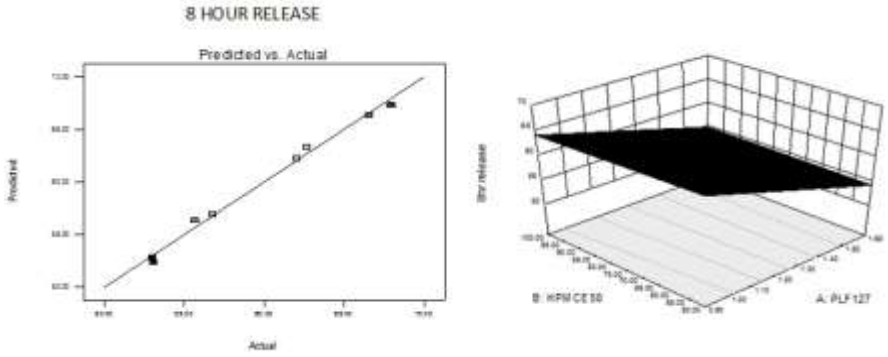

Figure 3: Effect of formulation factors on in vitro drug release after 8th h

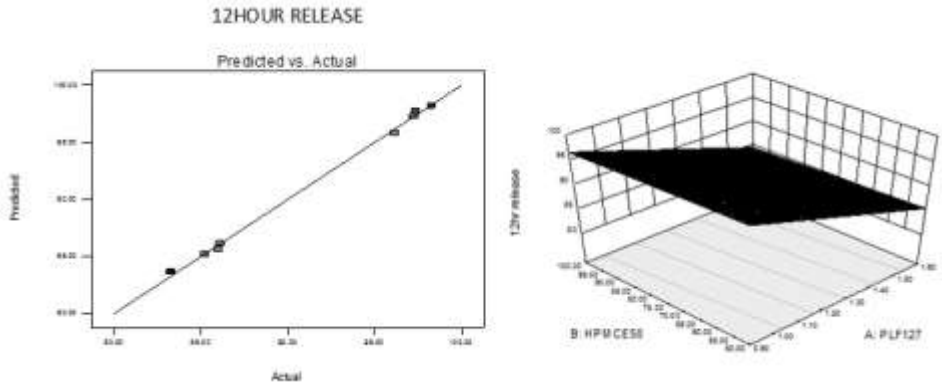

Figure 4: Effect of formulation factors on in vitro drug release after 12th h

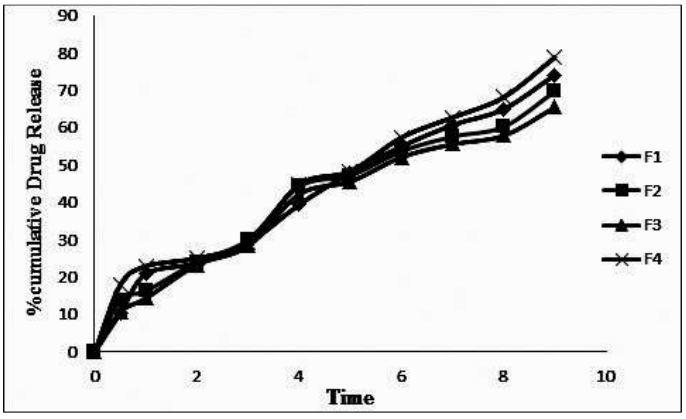

Figure 5: In vitro drug release of RHCL *in situ* gel formulations

Effect of formulation variables on Gelation temperature

Gelation temperature of the developed formulations varied from 31 ± 1.6 to 33 ± 1.24^{0}C (table 3). The Gelation temperature increased with decrease in Pluronic F-127 concentration. As increases the temperature, copolymer molecules aggregate into micelles, this micellization is due to the dehydration of hydrophobic PO blocks, which represents the very first step in the gelling process. These micelles are spherical with dehydrated poly PO core with an outer shell of hydrated swollen PEO chains. This micelle was followed by gelation for sufficient concentrated samples

The constant and regression coefficient for R_2 are as follows:

Gelation temp =+32.00-1.00 * A(coded values)

=+35.57-2.85* A-4.88*B(actual values)

It was found that linear model was significant for the gelation temperature. The Model F-value of 6.366 and value of p is less than 0.05 indicate the model is significant. The factor A has negtive effect which indicates that Gelation time decrease as the factor increases. The effect of A and B can be further elucidated with help of response surface plot. At high level of factor A gave low value of gelation temp at all level of factor B which indicates factor A has significant negative effect.

Table 3: Gelation temperature, Drug Content of Ranitidine *In situ* gel

Formulation code	Gelation temperature (sec)	Drug content (%)
F1	35 ± 1.6	98.19 ± 0.84
F2	31 ± 0.47	97.23 ± 0.58
F3	29 ± 1.6	98.78 ± 0.67
F4	36 ± 1.24	99.02 ± 0.88
Optimized formula	32. 97	98.219 + 0.26

Effect of formulation factors on in vitro drug release after 1st h

Total amount of ranitidine released from all formulations ranged from 14.56% to 22.78 % in 1 h (Table 2) Amount of ranitidine HCl released from all formulations at 1hr. ranges from 14.56 % to 22.78 %. Decreased rate of drug release was observed with increased concentration of polymers. Polypropylene oxide forms central hydrophobic core wherein methyl groups interact by vanderwals forces with substances undergo solubilization. However water solubility is believed to be due to the polyethylene oxide (PEO) block by hydrogen bonding interactions of ether oxygen with water molecules. Due to these interactions pluronic are readily soluble in polar solvents and non- organic polar solvents. When the gels contact with water could retard the release of the drug since the structure of the gel functioned as barrier to the drug release. In this

case, effect of both polymers can be explained by mathematical equation in terms of actual factors:

Drug release at 1hr =+18.60-3.24 * A-0.59*B (Coded Factors)

=+35.50-12.4*A-0.06*B(Actual Factors)

The linear model is selected for this response with Model F-value 1751.02 and p value is less than 0.05 indicate the model is significant. Both the factors A, Pluronic and B, HPMC E-50 decreases drug release from the gels. The factor A has negative effect which indicates that drug release decrease as the factor increases. The effect of A and B can be further elucidated with help of response surface plot. At high level of factor A gave lower value of drug release at all level of factor B which indicates factor A has significant negative effect.

Effect of formulation factors on in vitro drug release after 8thh

Amount of RHCLreleasedfromallformulationsat8thhrangedfrom 52.99%to 67.95%(Table 2).

Amount of ranitidine HCl released from all formulations at 8th hr. ranges from 52.99% to 67.95 %. Decreased rate of drug release was observed with increased concentration of polymers. When the gels contact with water occurs which acts as rate controlling matrix for the release of drug molecules. In this case, effect of both polymers can be explained by mathematical equation in terms of actual factors:

Drug release at 8hr = + 59.71 - 5.09 * A - 0.12 * B(coaded factors)

= +80.4-17.8*A+0.06 * B(actual factors)

The linear model is selected for this response with Model F-value 49.78and p value is less than 0.05 indicate the model is significant. Both the factors A, Pluronic and B, HPMC E-50 decreases drug release from the gels. The factor A has negative effect which indicates that drug release decrease as factor increases. The effect of A and B can be further elucidated with help of response surface plot. At high level of factor A gave lower value of drug release at all level of factor B which indicates factor A has significant nagetive effect

Effect of formulation factors on in vitro drugreleaseat12th h

Amount of RHCL released from all formulations at12th h ranged from 83.25% to 98.25 % (Table 2). Amount of ranitidine HCl released from all formulations at 8th hr. ranges from 83.25% to 98.25 %. Decreased rate of drug release was observed with increased concentration of polymers. When the gels contact with water occurs which acts as rate controlling matrix for the release of drug molecules. In this case, effect of both polymers can be explained by mathematical equation in terms of actual factors:

Drug release at 12hr =+91.20 -6.06 * A-0.51 * B (coaded factors)

=+114.28-17.2 *A-0.02 *B (Actual factors)

The linear model is selected for this response with Model F-value 206.27 and p value is more than 0.05 indicate the model is not significant. Both the factors A, Pluronic and B, HPMC E-50 decreases drug release from the gels. The factor A has nagetive effect which indicates that drug release decrease as factor increases. The effect of A and B can be further elucidated with help of response surface plot. At high level of factor A gave lower value of drug release at all level of factor B which indicates factor A has significant nagetive effect.

Regression analysis:

The result of ANOVA demostrate that the model was singnificant for all dependent variables. Regression analysis was carried out to determine the regression coefficients. All the independent variables (Factors) were found to be significant for R1, R2, and R4,response variables. The linear model was found to be significant for R1, R2, and R4 responses.

ANOVA

The results of ANOVA demonstrate that the model was significant. The summary of ANOVA was given in the table 4,5.The result of ANOVA demostrate that the model was singnificant for all dependent variables. Regression analysis was carried out to determine the regression coefficients. All the independent variables (Factors) were found to be significant for all R1, R2, R3, R4, R_5 and R_6 response variables. The linear model was found to be significant for all responses. So,above result indicate that both the factors play an important role in the formulation of in-situ gels containing ranitidine HCl. The data of pure error and lack of fit, which can provide a mean response and an estimate of pure exprimental uncertainty.

Optimization

The predicted values of drug release of optimized formulation at 1 h, 8 h, 12 h and gelation temperature were found to be 16.67%, 55.94%, 87.68%, and 31 and actual values were 17.35%, 59.95%, 88.89% and 32 respectively.

Table 4: Summary of ANOVA table for dependable variables

Source	d.f.	Sum of squares	Mean square	F value	Probability
Drug release at 1 hour					
A	1	83.98	83.98	9938.56	0.0064*
B	1	2.76	2.76	326.78	0.0352
C	1	0.79	0.79	93.94	0.0655
AB	1	0.66	0.66	78.25	0.0717
AC	1	0.56	0.56	66.49	0.0777
BC	1	0.018	0.018	2.14	0.3820
Drug release at 8 hour					

A	1	207.26	207.26	213.68	0.0046*
B	1	0.12	0.12	0.12	0.7586
C	1	32.89	32.89	33.90	0.0283
AB	1	1.16	1.16	1.19	0.3891
BC	1	0.018	0.018	0.019	0.9040
Drug release at 12 hour					
A	1	293.55	293.55	1007.88	0.0010*
B	1	2.08	2.08	7.14	0.1161
C	1	4.20	4.20	14.44	0.0628
AB	1	1.250	1.250	4.29	0.9537
BC	1	0.54	0.54	1.86	0.3061
Gelation temperature					
A	1	8.00	8.00	6.366	0.0001*
Tmax					
A	1	6.12	6.12	49.00	0.0060*
B	1	0.12	0.12	1.00	0.3910
C	1	0.12	0.12	1.00	0.3910
AB	1	0.12	0.12	1.00	0.3910

Table 5: Summary of ANOVA results model, residual and corrected total

Source	Sum of squares	d.f.	Mean square	F value	Probability > F
Drug release at 1 hr					
Model	88.78	6	14.80	1751.02	0.0183*
Residual	8.450	1	8.450	-	-
Corrected total	88.79	1	-	-	-
Drug release at 8 hr					
Model	241.44	5	48.29	49.78	0.0198*
Residual	1.94	2	0.97	-	-
Corrected total	243.38	7	-	-	-
Drug release at 12hr					
Model	300.37	5	60.07	206.27	0.0048*
Residual	0.58	2	0.29	-	-
Corrected total	300.96	7	-	-	-
Tmax					
Model	6.00	6	1.00	6.366	0.0001*
Residual	0	1	0	-	-
Corrected total	6.00	7	-	-	-
Gelation temperature					
Model	8.00	6	1.33	6.366	0.0001*
Residual	0	1	0	-	-
Corrected total	8.00	7	-	-	-

pH determination:

The pH for all the four formulations are nearly same value.

Drug content:

The drug content for four formulations can be known from table 3.

Stability Studies:

Stability studies were carried out on optimized formulation as per ICH Guidelines Q1C. The physicochemical parameters and the dissolution studies for the formulation after stability studies are same as before.

Table 6: Photo stability studies

S No	Experiment Sample	Light exposed	Light protective
1	Drug substance	94.02%	98.89%
2	Drug product	93.27%	98.48%

CONCLUSIONS

Thus oral controlled *in situ* gel of RHCL was prepared successfully. The factorial design was used to find out the effect of independent variables on the dependent variables.These results show the feasibility for oral controlled delivery system. Among the studied polymers, pluronicF127 has influenced the controlled release of the drug.

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