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A Novel *In Situ* Gelling System of Pefloxacin Mesylate for Ophthalmic Drug Delivery

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ABSTRACT

Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The anatomy, physiology and biochemistry of the eye render this organ highly impervious to foreign substances. The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid pre-corneal elimination of the drug may be overcome by the use of *in situ* gel forming systems that are instilled as drops into the eye and then undergo a sol-gel transition in the *cul-de-sac*. The purpose of the present work was to develop an ophthalmic drug delivery system of Pefloxacin mesylate for *in situ* gelling system byusing ion sensitive gelling agent (Gelrite) and viscofying agent (HPMCK4M). Optimization by 2^2 factorial design using Design expert software 8.0 version was applied to the formulations. Formulation with Gelrite (gellan gum) and HPMC K4M shows percent drug content of 99.83%, viscosity of 1864 cp at 20 rpm and *in-vitro* drug release of 88.32% which shows formulation is good. The developed formulation was found to be stable and provided sustained release of the drug over 8 hours.

Keywords: Pefloxacin mesylate, *In situ* gelling system, Ophthalmic drug delivery, Gelrite (gellan gum), HPMC K4M, Factorial design.

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INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging endeavours facing the pharmaceutical scientist. The anatomy, physiology and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage[1].

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of the drug may be overcome by the use of a gel system that are instilled as drops into the eye and undergo a sol-gel transition in the *cul-de-sac*[2].

An ideal ophthalmic drug delivery must be able to release the drug in a sustained manner and to remain in the area of front of the eye for prolonged period of time. As a result it is necessary to optimize ophthalmic drug delivery[3].

In situ activated gel-forming systems are those which are when exposed to physiological conditions will shift to a gel phase. Gelation occurs *via* the cross-linking of polymer chains that can be achieved by covalent bond formation (chemical cross-linking) or non-covalent bond formation (physical cross-linking).The progress that has been made in gel technology is in the development of droppable gel. The rate of *in situ* gel formation is important because between instillation in the eye and before a gel is formed; the solution or weak gel is produced by the fluid mechanism of the eye[4,5].

The fluoroquinolones represent an expanding class of broad-spectrum antibacterial which cover a host of Gram-negative and anaerobic species responsible for ocular infections. Fluoroquinolones were introduced as monotherapy for suspected bacterial keratitis owing to their broad spectrum of activity, low toxicity, good corneal penetration, and their efficacy at commercially available strength[6,7,8].

MATERIALS AND METHODS

Pefloxacin mesylate was obtained as a gift sample from Taj Pharmaceuticals, Gelrite and HPMCK4M were obtained as a gift sample from Colorcon Asia Pvt. Ltd. Respectively.

For the preparation of *in situ* gelling system, the polymer solution was prepared by dispersing the required amount in distilled water with continuous stirring. Drug was dissolved in dilute acetic acid and pH was adjusted to 6.5 using 0.1N NaOH. Benzalkonium chloride (0.02%v/v) solution was then added to the above solution. The drug solution was then added to polymer solution under constant stirring to obtain a uniform solution. Distilled water was then added to make the volume up to 100ml[9,10].

Table 1: Composition of pefloxacin mesylate *in situ* gel

Sr. No.	Ingredient	Quantity (gms)							
		F1	F2	F3	F4	F5	F6	F7	F8
1	Pefloxacin mesylate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
2	HPMC E50LV	0.75	0.75	-	-	-	-	-	-
3	Methyl cellulose	-	-	1.0	1.0	-	-	-	-
4	HPMC K4M	-	-	-	-	1.0	1.0	-	-
5	HPMC K15M	-	-	-	-	-	-	0.7	0.7
6	Sodium alginate	1.0	-	1.0	-	1.0	-	1.0	-
7	Gelrite	-	0.5	-	0.5	-	0.5	-	0.5
8	Benzalkonium chloride	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
9	Distilled water q.s	100ml	100ml	100ml	100ml	100ml	100ml	100ml	100ml

Differential Scanning Calorimetry (DSC) Characterization

Calorimetric characterization of the drug and polymers alone and their physical mixtures were carried out using a DSC 822^e (Mettler Toledo Star^e System, Switzer Land). Argon was used as purging gas at a rate of 80 ml min^{-1}. The calorimeter was calibrated for baseline using no pans, for cell constant and temperature using indium. All experiments were performed using non-hermetic aluminum pans, in which samples were accurately weighed, and then just covered with the lid. The samples were loaded on an autosampler tray. The samples for the DSC study were program-heated from 25ºC to 350ºC then cooled to 0ºC using liquid nitrogen, and finally heated to 350ºC again, always at the rate of 10ºC min^{-1}.

Composition of Simulated Tear Fluid

The simulated tear fluid (STF) was prepared using sodium chloride (0.670g), sodium bicarbonate (0.200g), calcium chloride dihydrate (0.08g) and de-ionized water (upto 100ml).

Optimization of ion sensitive *in situ* gel by the use of experimental design

A 2 level 2 factors factorial design (2^2) was employed to design ophthalmic *in situ* forming gel of pefloxacin mesylate. The design was employed for formulations containing gelling agent and viscofying agent respectively. The independent and dependent variables selected are common for the polymers and are as follows:

Independent Variables

a) Concentration of gelling agent- Gelrite (X_1)

b) Concentration of viscofying agent-HPMC K4M (X_2)

Dependent Variables

a) *In-vitro* release (Y_1)

b) Viscosity (Y_2)

Table 2: Experimental design of Pefloxacin mesylate *in situ* gel as per 2^2 factorial design

Sr.No.	Ingredients	Quantity (gms)			
		F9	F10	F11	F12
1	Pefloxacin Mesylate	0.3	0.3	0.3	0.3
2	HPMC K4M	0.75	0.75	1	1
3	Gelrite	1.2	1.5	1.2	1.5
4	Benzalkonium chloride	0.02	0.02	0.02	0.02
5	Distilled water q.s	100ml	100ml	100ml	100ml

Evaluation Parameters[11,12,13]

Appearance

All developed formulations were evaluated for clarity by visual observation against a black and white background.

pH

The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have pH range in between 5 to 7.4. The developed formulations were evaluated for pH by using Elico India Systronics digital pH meter.

Drug Content

Uniform distribution of active ingredient is important to achieve dose uniformity. The drug content was determined by diluting 1 ml of the formulation to 100 ml with STF solution (pH 7.4). Aliquot of 1ml was withdrawn and further diluted to 10 ml with STF. Pefloxacin mesylate concentration was then determined at 273 nm by using UV-Vis spectrophotometer.

***In-Vitro* Gelation Studies**

All prepared formulations were evaluated for gelling capacity and viscosity in order to identify the compositions suitable for use as *in situ* gelling systems. The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 37ºC and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve.

Rheological studies

Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye. The developed formulations were poured into the small sample adaptor of the Brookfield viscometer and the angular velocity increased gradually from 0.5 to 50 rpm. The hierarchy of the angular velocity was reversed. The average of the two readings was used to

calculate the viscosity.

***In-Vitro* Release Studies**

The *in-vitro* release of Pefloxacin mesylate from the formulations was studied by using modified USP apparatus II paddle method with STF (pH 7.4) as a dissolution medium. A glass cylinder of 2.5 cm in diameter open at both ends was designed for the purpose of the study. Dialysis membrane previously soaked in STF (pH 7.4) was taken, dried and tied on to one end of the glass cylinder and to this 1 ml of the formulation was accurately pipetted. The glass cylinder was attached to the shaft of USP apparatus II, in place of basket.The cylinder was then suspended in 50 mL of dissolution medium maintained at 37±1°C such that the membrane just touched the dissolution medium. The speed of the metallic device shaft was set at 50 rpm. Aliquots were withdrawn at hourly intervals and replaced by equal volumes of dissolution medium. Aliquots were suitably diluted with STF (pH 7.4) and analyzed by UV-Vis Spectrophotometer at 273 nm.

Sterility Test

All ophthalmic preparations should be sterile therefore the test for sterility is very important evaluation parameter. The sterility test was performed according to Indian Pharmacopoeia. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically transferred in soyabean-casein digest medium (20 ml). The liquid was mixed with the media. The inoculated media were incubated for not less than 14 days at 20^0C to25^0C.

Stability Studies

Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage and use (i.e. its shelf life), the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. All the three formulations were subjected to stability studies at ambient humidity conditions at 2^0C to 8^0C, ambient temperature and $40^0 1^0$C for a period of one month. The samples were withdrawn after 7, 15 and 30 days and were evaluated for following parameters.

1. Drug content
2. *In-vitro* drug release
3. Sterility study

RESULTS AND DISCUSSION

Differential Scanning Calorimetry (Dsc) Characterization

Figure 1, 2 and 3 compares the DSC thermogram of Pefloxacin mesylate, HPMC and physical mixture of pefloxacin with all excipients respectively. Pefloxacin mesylate showed a long and characteristic endothermic peak at 283ºC and HPMC showed small and characteristic endothermic peak at 105ºC whereas physical mixtures showed characteristic endothermic peaks corresponding to those of the individual components and there is no appearance of one or more new peak or disappearance of one or more peak corresponding to those of the individual components. From this result, it clears that there is no interaction in between Pefloxacin mesylate and excipients.

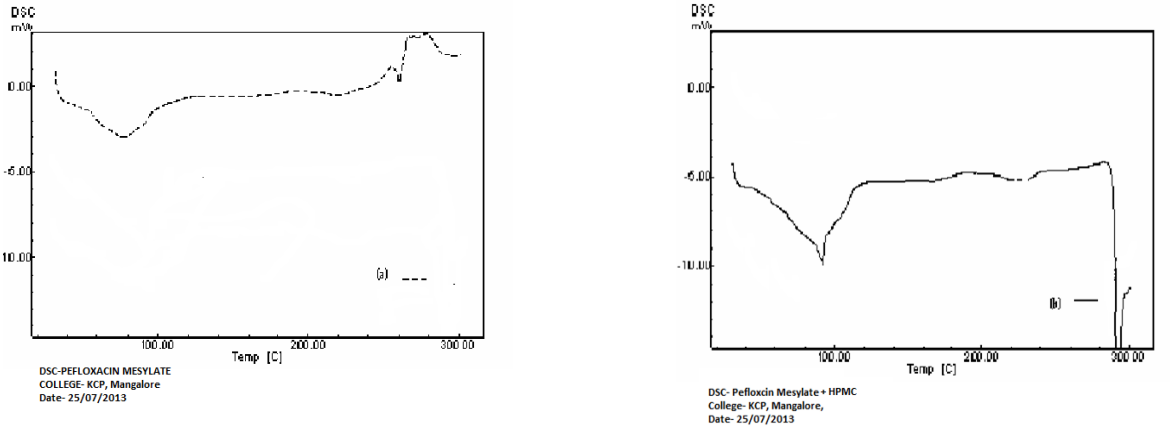


Figure 1 & 2: DSC thermogram of pefloxacin mesylate and pure drug with HPMC

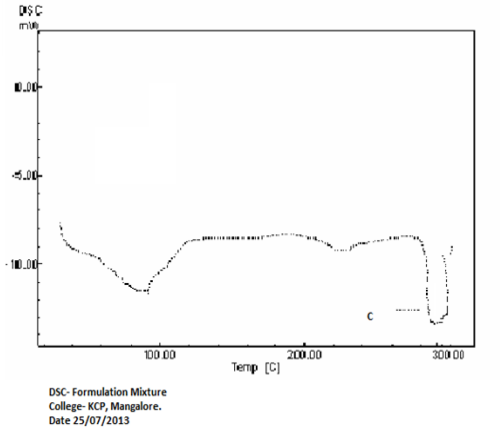


Figure 3: DSC thermogram of formulation mixture

Appearance, Clarity, pH, Drug Content

All the formulations were white in colour and were found to be clear. The pH of the formulations was within the acceptable range. Drug content was in the range of 92.34% to 99.83%. All these observations are listed in table 3.

***In-Vitro* Gelation Studies**

All the formulations gelled instantaneously (less than a minute) on contact with STF. The gelation may be due to ionic crosslinking of the alginate chains by the divalent cations. All these

observations were mentioned in the table 3.

Rheological Studies

Table 3 shows the viscosity values obtained for all the formulations using Brookfield DV-111+ rheometer. The viscosity was directly dependent on the polymeric content of the formulation. The viscosity increased with increasing concentration of Gellan gum (Gelrite) and HPMC.

Table 3: pH, Drug content, Gelling capacity and viscosity of Pefloxacin mesylate *in situ* gel

Formulations	pH	Drug content (%)	Gelling capacity	Viscosity (cp)
F1	6.7	94.07	++	1096
F2	6.8	93.21	++	1241
F3	6.8	94.98	++	1296
F4	6.8	92.65	++	970
F5	6.7	94.33	++	1077
F6	6.8	95.12	++	1335
F7	6.7	92.34	++	1149
F8	6.8	96.55	++	1278
F9	6.8	97.32	+++	1398
F10	6.8	96.87	+++	1522
F11	6.7	99.83	+++	1864
F12	6.8	97.45	+++	2012

- , No gelation; + Gels after few minute, dissolves rapidly; ++, Gelation immediate, remains for few hours; +++, Gelation immediate, remains for extended period.

***In-Vitro* Release Studies**

The *in-vitro* release studies were carried out for all formulations using STF as the dissolution medium. The data of these studies are presented in Table No. 4. And the comparative plot is shown in figure.4 and 5.

Table 4: *In-vitro* release profile of pefloxacin mesylate *in situ* gel formulations F1-F8

Time (T) (hrs)	*In-vitro* drug release (%)							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	20.56	18.67	19.76	22.63	21.72	19.66	18.43	21.29
2	38.87	27.29	29.04	40.98	37.03	29.18	31.67	29.84
3	52.90	39.73	43.81	54.68	49.11	40.45	43.21	35.23
4	64.23	48.90	53.64	61.29	58.75	52.09	58.39	47.10
5	78.09	57.31	62.72	76.28	78.56	61.48	76.31	54.26
6	-----	66.47	79.71	------	----	74.67	-----	62.34
7	-----	76.08	-----	-----	-----	80.72	----	78.33

It was found that formulations F1, F4, F5 and F7 showed drug release 78.09%, 76.28%, 78.56% and 76.31% in 5 hours respectively. F3 shows drug release 79.71% in 6 hours respectively. F2, F6 and F8 showed drug release 76.08%, 80.72% and 78.33% in 7 hours respectively. These

values indicated that F6 showed better sustaining effect than the other trial formulations. So further study was planned to carry out by applying 2^2 factorial design by using design expert software.

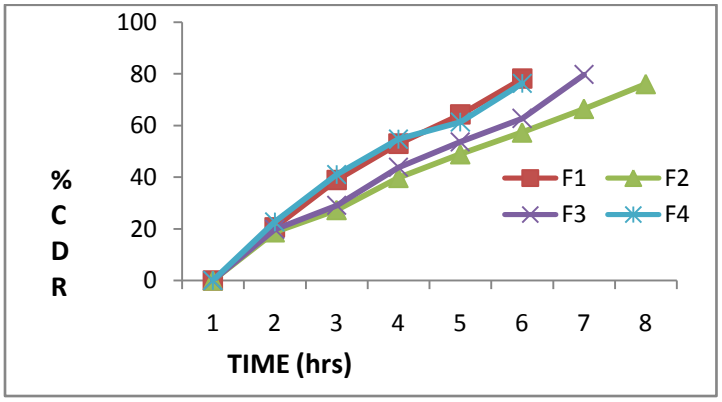


Figure 4: *In-vitro* drug release profile of pefloxacin mesylate *in situ* gel formulations F1- F4

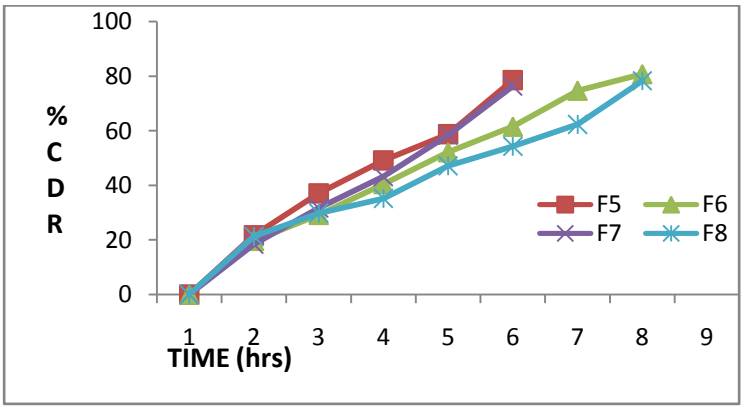


Figure 5: *In-vitro* drug release profile of pefloxacin mesylate *in situ* gel formulations F5- F8

Effect of Independent variables On Dependent variables By 2^2 Full Factorial Design Of *in Situ* Pefloxacin Mesylate Ophthalmic Gel

The factorial batches were prepared by using independent variables like concentration of Gelrite (X_1) and HPMC K4M (X_2). To check its effect on dependent variables like *in-vitro* drug release and viscosity which are tabulated in Table 5.

Table. 5:Effect of Independent variable on dependent variable by 2^2 full factorial design of *in situ* pefloxacin mesylate ophthalmic gel.

Std	Run	Factor 1 A:Conc of Gelrite (X_1)	Factor 2 B:Conc of HPMC K4M(X_2)	Response 1 *In-vitro* release(Y_1)	Response 2 Viscosity (Y_2)
		gm	gm	%	cps
1	2	1.2	0.75	84.39	1398
2	4	1.5	0.75	81.85	1522
3	3	1.2	1	88.32	1864
4	1	1.5	1	85.94	2012

Factorial batches of *in situ* pefloxacin mesylate ophthalmic gel release were evaluated for the *in-vitro* drug release and viscosity by its regression analysis. The results obtained in the *in-vitro* drug release and viscositystudyaretabulated in Table.5. Thecumulativepercentageof *in situ* pefloxacin mesylate ophthalmic gel released for all the formulations(F9toF12) are shown in Figure 16.The Concentration of Gelrite(X_1), Concentration of HPMC K4M (X_2).The result of regression analysis showed that all the co-efficient bear a different sign, which indicate that both the Independent variables shows significant effect on dependent variables.

In-vitro drug release gives correlation co-efficient 0.99971.The P value for variable X_1 and X_2 were 0.0207 and 0.0127 respectively ($P<0.05$), it indicate that both variables shows significant effect on drug release. Result of ANOVA for selected factorial model Analysis of variance table are shown in table.6 and result of ANOVA for selected factorial model Classical sum of squares - Type II are shown in table. 7. Combination co-efficient was positive the P value was not less than 0.05, which indicates that combination of independent variable does show significant effect. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 93.38 indicates an adequate signal. This model can be used to navigate the design space. The "Pred R-Squared" of 0.9954 is in reasonable agreement with the "Adj R-Squared" of 0.99913. The Model F-value of 1729.04 implies the model is significant. It shows that there is only a 1.70% chance that a "Model F-Value" this large could occur due to noise.

Final equation in terms of coded factors

In-vitro release = 285.125-1.23+16.04.

Table 6: ANOVA for selected factorial model (Response *In-vitro* release)

Source	Squares	df	Square	Value	Prob> F	
Model	22.1317	2	11.06585	1729.039062	0.0170	Significant
A-Conc of Gelrite	6.0516	1	6.0516	945.5625	0.0207	
B-Conc of HPMC K4M	16.0801	1	16.0801	2512.515625	0.0127	
Residual	0.0064	1	0.0064			
Cor Total	22.1381	3				

Table. 7: Analysis of variance table [Partial sum of squares - Type III]

Column1	Column2	Column3	Column4	Column5
Std. Dev.	0.08		R-Squared	0.999710906
Mean	85.125		Adj R-Squared	0.999132717
C.V. %	0.093979442		Pred R-Squared	0.99537449
PRESS	0.1024		Adeq Precision	93.38640604

The response data was analyzed by using stat ease design expert software. The software gives statistical analysis of data. The interaction effect of these formulation factors on the drug release

can be studied using the results of statistical analysis. Responses are given below as shown in figure. 6 to 10:

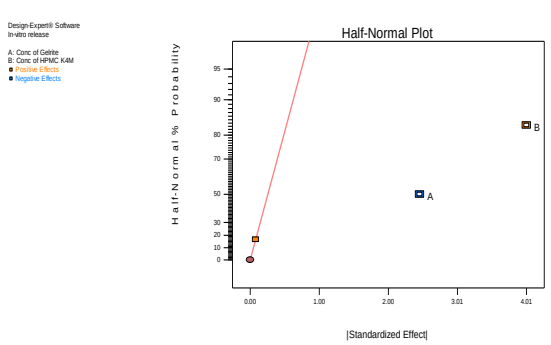


Figure 6: Half normal plot for *in-vitro* drug release

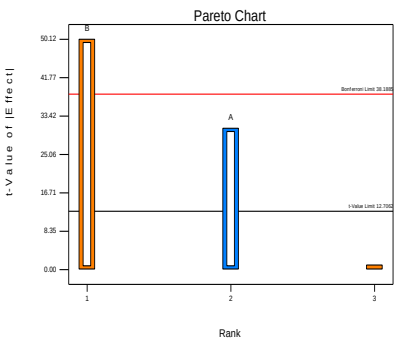


Figure. 7: Pareto chart of *in-vitro* drug release

Table. 8: Predicted response shown by software

Response	Prediction	Std Dev	SE Mean	95% CI low	95% CI high
In-vitro release	85.93125	0.08	0.05414795	85.24323512	86.61926488
Viscosity	1601.95	12	8.12219182	1498.747768	1705.152232

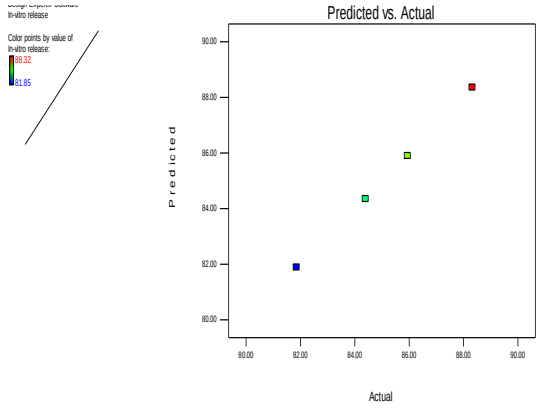


Figure. 8: Predicted Vs Actual result of *in-vitro* release

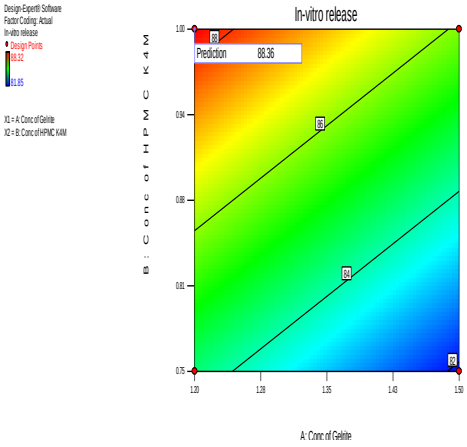


Figure 9: Counter plot of *in-vitro* release

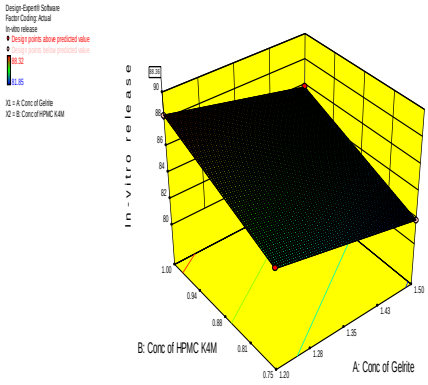


Figure10: 3-D Response surface plot for *in-vitro* release

Viscosity of formulation gives correlation co-efficient 0.9994.The P value for variable X_1 and X_2were 0.0560 and 0.0160 respectively($P<0.05$), it indicate that both variables shows significant effect on drug release. Result of ANOVA for selected factorial model Analysis of variance table are shown in table No.10 and result of ANOVA for selected factorial model Classical sum of squares - Type II are shown in table No. 11. Combination co-efficient was positive the P value wasnotlessthan0.05, which indicates that combination of independent variable does show significant effect. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 59.082 indicates an adequate signal. This model can be used to navigate the design space. The "Pred R-Squared" of 0.9906 is in reasonable agreement with the "Adj R-Squared" of 0.998. The Model F-value of 857.569 implies the model is significant. It shows that there is only a 2.41% chance that a "Model F-Value" this large could occur due to noise.

Final equation in terms of coded factors for Viscosity

In-vitro release = 1699+68+239.

Table 10: Analysis of variance table [Partial sum of squares - Type III]

Column1	Sum of	Column2	Mean	F	p-value	Column3
Source	Squares	Df	Square	Value	Prob> F	
Model	246980	2	123490	857.569444	0.0241	significant
A-Conc of Gelrite	18496	1	18496	128.444444	0.0560	
B-Conc of HPMC K4M	228484	1	228484	1586.69444	0.0160	
Residual	144	1	144			
Cor Total	247124	3				

Table. 11: Analysis of variance table [Partial sum of squares - Type III]

Column1	Column2	Column3	Column4	Column5
Std. Dev.	12		R-Squared	0.9994173
Mean	1699		Adj R-Squared	0.99825189
C.V. %	0.70629782		Pred R-Squared	0.99067675
PRESS	2304		Adeq Precision	59.0821775

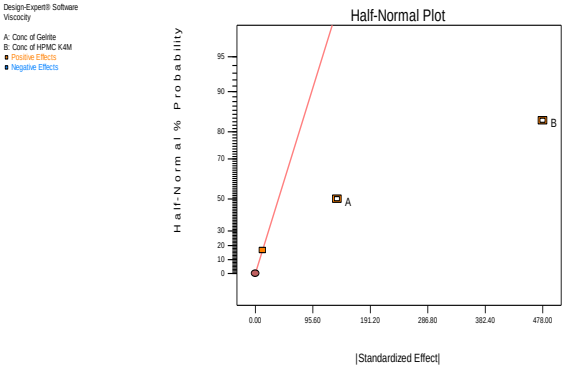


Figure 11: half normal plot for viscosity

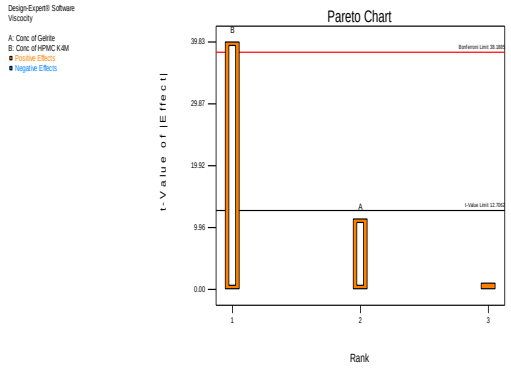
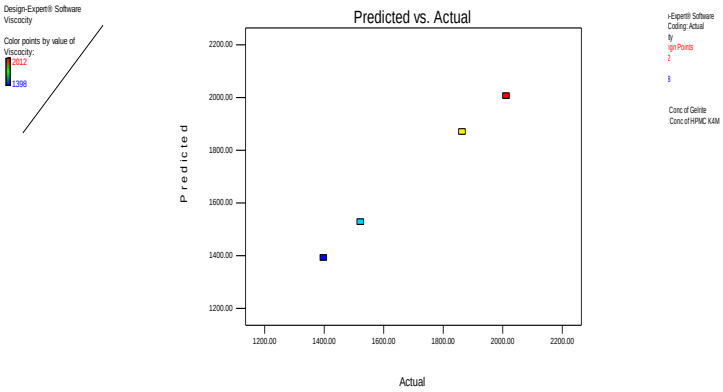


Figure 12: Pareto chart of viscosity

The response data was analyzed by using stat ease design expert software. The software gives statistical analysis of data. The interaction effect of these formulation factors on the viscosity can be studied using the results of statistical analysis. Responses are given below as shown in figure 11 to 15:



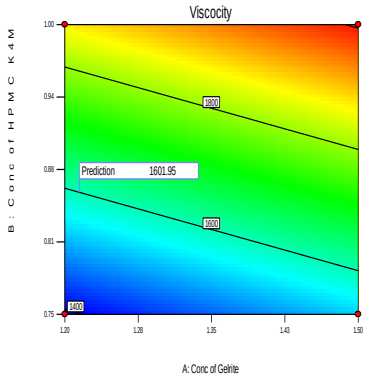


Figure. 13: Predicted Vs Actual result of viscosity Figure. 14:Counter plot of viscosity

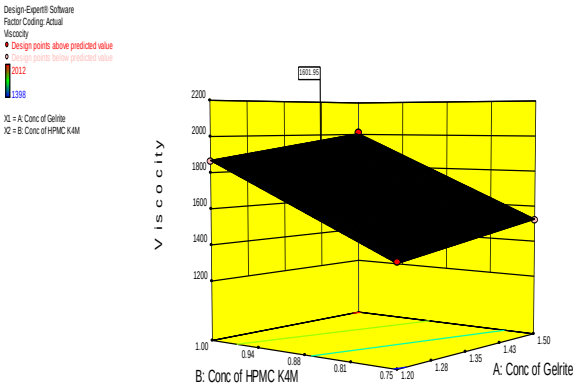


Figure. 15: 3-D Response surface plot of viscosity

Table 12: *In-vitro* release profile of pefloxacin mesylate *in situ* gel formulationsF9-F12

Time (T) (hrs)	*In-vitro* drug release (%)			
	F9	F10	F11	F12
0	0	0	0	0
1	20.44	19.59	17.93	16.22
2	29.67	27.62	36.74	25.43
3	37.10	31.41	49.21	36.76
4	43.74	44.86	58.11	42.31
5	56.09	52.09	67.53	52.02
6	68.56	66.31	73.38	68.14
7	76.02	74.59	78.74	77.20
8	84.39	81.85	88.32	85.94

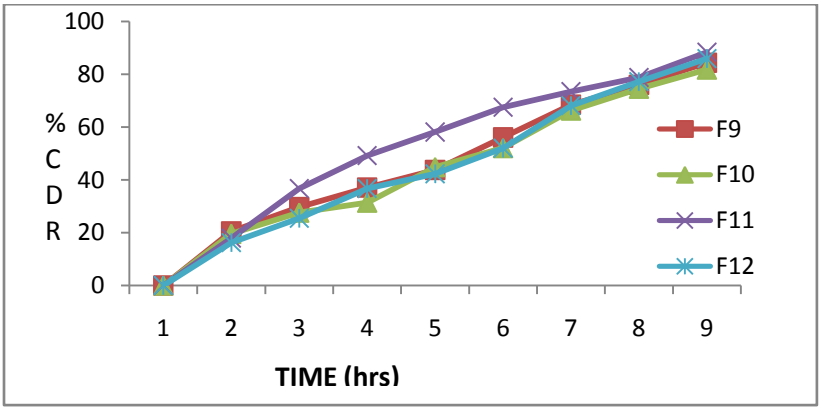


Table. 16: *In-vitro* release profile of pefloxacin mesylate *in situ* gel formulations F9-F12

Stability Studies

From stability studies it was observed that there was no significant change in any of the parameters evaluated as all the results obtained were within acceptable range. The stability data revealed that the formulation was found to be stable.

sterility studies

All the formulations were found to be sterile when subjected to sterility study by direct inoculation method and no growth of any forms of microorganisms were observed in the formulations.

CONCLUSION

Ophthalmic *in situ* gelling system of Pefloxacin mesylate was successfully formulated by using Ion sensitive method and were developed to a satisfactory level, in terms of pH, gelling capacity, viscosity, drug content and *in-vitro* drug release. The clarity of the prepared formulations was found to be satisfactory.Appropriatefactorialdesignandoptimizationtechniquecanbesuccessfully used in the development of ophthalmic *in situ* gelling system of pefloxacin mesylate. Optimization of enabled formulation of *in situ* gelling system of Pefloxacin mesylate. The optimized formulations how release profiles and responses which werecloseto predicted responses.From the optimization results, we observed that Gelrite and HPMC K4M is an excellent gelling agent and viscofying agent in combination for the preparation of pefloxacin mesylate *in situ* ophthalmic gel.Results of sterility test confirmed that all the formulations were sterile. Accelerated stabilitystudies, proved that the formulation is quitestable.

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