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FORMULATION AND EVALUATION OF SUSTAINED RELEASE *IN-SITU* GEL OF AMIKACIN BY USING 3^2 FULL FACTORIAL DESIGN

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Keywords:

In-situ gelation, Amikacin, Poloxamer 407, Gelrite, Sustained-release, 3^2 full factorial designs

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ABSTRACT: Objectives: This work aimed to formulate and evaluate amikacin, fourth-generation fluoroquinolone antibiotic agent containing an *in-situ* gelling system based on sol-to-gel transition for ophthalmic delivery to overcome the problems of poor bioavailability and therapeutic response exhibited by conventional formulations based a sol-to-gel transition in the cul-de-sac upon instillation. **Methods:** In the present work, poloxamer 407 as a temperature-activated gelling agent and gelrite as an ion activated gelling agent were used in combination to prepared ion-sensitive and temperature-sensitive ophthalmic *in-situ* gel. The prepared formulations were evaluated for the parameters like pH, appearance, drug content, *in-vitro* gelation study, viscosity, *in-vitro* release study, sterility test, and stability studies. **Results:** In this study, the release profile depends on the concentration of gelrite and poloxamer 407. The selected formulation showed sustained release for a period of 10 h. Thus it showed increased residence and contact time with the eye. The draize test was performed with an optimized formulation for eye irritation test. It was found to be non-irritant to the rabbit eye. The *in-situ* gelling system showed favorable results in all studies. **Conclusion:** The results indicate that the formulation can be considered as a better option than conventional ophthalmic drops.

INTRODUCTION: Eye is a unique and vital organ [1]. It is considered as a window of the soul. A significant challenge to the formulator is to circumvent (bypass) the protective barriers of the eye without causing permanent tissue damage [2].

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So, many ophthalmic drug delivery systems are available as a remedial treatment. These are classified as conventional and newer drug delivery systems [3]. Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy [4].

Conventional ophthalmic formulations like a solution, suspension and ointment have many disadvantages which result in poor bioavailability of the drug in the ocular cavity. Conventional ophthalmic delivery systems often result in poor

bioavailability and therapeutic response, because high tear fluid turnover and dynamics cause rapid pre-corneal elimination of the drug [5]. A high frequency of eye drop instillation is associated with patient non-compliance [6]. Various ophthalmic vehicles such as inserts, ointments, suspensions, and aqueous gels have been developed in order to lengthen the residence time of instilled dose and enhance the ophthalmic bioavailability. These ocular drug delivery systems have not been used extensively because of some drawbacks such as blurred vision from ointments or low patient compliance from inserts [7].

The various approaches that have been attempted to increase bioavailability and the duration of the therapeutic action of ocular drugs can be divided into two categories [8]. The first one is based on the use of sustained drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs. The second involves maximizing corneal drug absorption and minimizing pre-corneal drug loss. Several *in-situ* gelling systems have been developed to prolong the pre-corneal residence time and improve ocular bioavailability.

These systems consist of polymers that exhibit sol to gel phase transitions due to change in specific physicochemical parameter (pH, temperature) in their environment, the cul-de sac in this case. Depending on the method employed to cause sol-to-gel phase transition on the eye surface the following three types of systems are recognized, pH triggered system, temperature dependent system and ion activated system. Using these three methods, *in-situ* gelling ophthalmic delivery system is developed [9]. Distinguishing from preformed hydrogels, *in-situ* forming gels are formulations, applied as a solution, which undergoes gelation after instillation due to physicochemical changes inherent to the biological fluids. In this way, the polymers which show sol-gel phase transition and thus trigger drug release in response to external stimuli are the most investigated. *In-situ* hydrogels are providing such "sensor" properties and can undergo reversible sol-gel phase transitions upon changes in the environmental condition. These "intelligent" or "smart" polymers play important role in drug delivery since they may dictate not only where a drug is delivered, but also when and with which interval it is released [10].

MATERIALS: Amikacin, poloxamer 407, gellan gum (gelrite) and carbopol 940 were obtained from was obtained from Indiana Ophthalmics Pvt. Ltd., Surendranagar and Gujarat. All the polymers received were of pharmaceutical grade. Other materials and solvents used were of analytical grade.

Drug Excipients Compatibility Study: Drug-excipients interaction plays a vital role in achieving the stability of the drug in a dosage form. Fourier transform infrared spectroscopy (FT-IR) was used to study the physical and chemical interactions between drugs and excipients. FT-IR spectra of amikacin, poloxamer 407, gelrite and their mixture (amikacin, poloxamer 407, gelrite) were recorded using KBr mixing method on FT-IR instrument. (FTIR-1700, Shimadzu, Kyoto, Japan) [11].

METHODS:

Preparation of *In-situ* Gelling Systems: Dissolve specified amount of polymer in a specified amount of solvent. If poloxamer 407 is used, dissolve it by cooling the solution. If gelrite is used, dissolve it by warming the solution. Dissolve a specified amount of other ingredients. Add the required amount of preservative and antioxidant. Dissolve a specified amount of drug into it and filter [12].

Preparation of Temperature-Dependent Gelling Systems: The *in-situ* gel was prepared by poloxamer 407. It was added to cold distilled water with continuous stirring and stirred until it completely mixed. The partially dissolved poloxamer 407 solutions were stored in a refrigerator and stirred periodically until clear homogenous solutions were obtained (approxi-mately few h.). Amikacin was dissolved in the required quantity of distilled water separately and then added to a polymer solution under constant stirring until a uniform solution was obtained.

Finally, sodium citrate, methylparaben, and propylparaben were added to the formulation under constant stirring until a uniform solution was obtained. The formulations, in their final pack, were subjected to sterilization by filtering the solution by using 0.2 μm membrane filter paper. Different concentrations of polymer were used to prepare *in-situ* gel, as shown in **Table 1**. The prepared formulations were evaluated [13].

TABLE 1: COMPOSITION OF TEMPERATURE DEPENDENT GELLING SYSTEMS

Ingredients	P1	P2	P3	P4	P5
Amikacin (% w/v)	0.3	0.3	0.3	0.3	0.3
Poloxamer (% w/v)	10	12	14	16	18
Methyl paraben (% w/v)	0.05	0.05	0.05	0.05	0.05
Propyl paraben (% w/v)	0.1	0.1	0.1	0.1	0.1
Disodium hydrogen phosphate (% w/v)	1.120	1.120	1.120	1.120	1.120
Distilled water	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml

Preparation of Ion Dependent Gelling Systems: The *in-situ* gel was prepared by gellan gum (gelrite). Gelrite was first added to distilled water with continuous stirring. Gelrite was dissolved by warming the solution at 80 °C for 15 min with continues stirring. Amikacin dissolved in the required quantity of distilled water separately and then it was added to polymer solution under constant stirring until a uniform solution was obtained. Finally, Sodium citrate, methylparaben, and propylparaben were added to the formulation under constant stirring until a uniform solution was obtained. The formulations, in their final pack, were subjected to sterilization by filtering the solution by using 0.2 µm membrane filter paper. Different concentrations of polymer were used to prepare *in-situ* gel, as shown in **Table 2**. The prepared formulations were evaluated [14].

TABLE 2: COMPOSITION OF ION DEPENDENT GELLING SYSTEMS

Ingredients	G1	G2	G3	G4	G5
Amikacin (%w/v)	0.3	0.3	0.3	0.3	0.3
Gelrite (% w/v)	0.2	0.4	0.6	0.8	1.0
Methyl paraben (%w/v)	0.05	0.05	0.05	0.05	0.05
Propyl paraben (% w/v)	0.01	0.01	0.01	0.01	0.01
Disodium hydrogen phosphate (%w/v)	1.120	1.120	1.120	1.120	1.120
Distilled water	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml

Experimental Design of *In-situ* Gel of Amikacin Containing Poloxamer 407 and Gelrite: To achieve the formulation with desired *in-vitro* gelation strength and drug release, the formulation prepared by using different combination of poloxamer 407 (act as a temperature-dependent gelling agent) and gelrite (act as ion-dependent gelling agent) were optimized and evaluated using 3^2- full factorial design.

Full Factorial Design: This design is useful when a detailed analysis of higher-order interactions among the factors is needed. Runs are made at all possible combinations of factor levels. As the number of runs required increases rapidly as the number of factors increases, full factorials are usually used when a relatively small set of factors that are known to be important are available or when collecting a large number of observations is feasible. More information is obtained with less work, and effects are measured with maximum precision.

The number of experiments required for these studies is dependent on the number of independent variables selected. The response (Y) is measured for each trial.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2$$

In The 3^2- full factorial design 2 independent factors were evaluated, each at 3 levels and experimental trials were performed for all 9 possible combinations.

The design layout of 3^2- full factorial design as shown in **Table 3** and **Table 4**. Two independent variables were selected as below: X_1 = % w/v concentration of poloxamer 407 X_2 = % w/v concentration of gelrite.

TABLE 3: VARIABLES FOR EXPERIMENTAL DESIGN

Variables for 3^2- full factorial design					
Independent variables		Dependent variables			
X_1	X_2	Y_1	Y_2	Y_3	Y_4
Concentration of poloxamer 407	Concentration of gelrite	Viscosity	Gelling strength	Drug release after 4 h	Drug release after 10 h

TABLE 4: THREE LEVELS OF EACH VARIABLE

Level	X_1 (% w/v)	X_2 (% w/v)
Low (-1)	12	0.2
Medium (0)	14	0.6
High (+1)	16	1.0

Preparation of Ophthalmic *In-situ* Gelling System of Poloxamer 407 and Gelrite: The *in-situ* gelling system was prepared containing ion and temperature-dependent polymers. Firstly, poloxamer 407 was added to 50 ml distilled water with continuous stirring and stir until to completely mix. The partially dissolved poloxamer solutions were stored in a refrigerator and stirred periodically until clear homogenous solutions were obtained (approximately 24 h). Amikacin was dissolved in this solution with constant stirring that was marked as solution-I. gelrite was added to another 30 ml of distilled water with continuous stirring. Gelrite was dissolved by warming the solution at 80 °C for 15 min. with continuous stirring that was marked as solution-II. Both solutions were mixed and stirred it for 15 min. by using a magnetic stirrer. Disodium hydrogen phosphate was added to the formulation under constant stirring until a uniform solution was obtained. Finally, the volume was made up to 100 ml with distilled water. The formulations, in their final pack, were subjected to sterilization by filtering the solution by using 0.2 µm membrane filter paper. The formulations of *in-situ* gel were shown in **Table 5**.

TABLE 5: FORMULATION OF FULL FACTORIAL BATCHES

Ingredient (% w/v)	FORMULATION CODE (% w/v)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Amikacin	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Poloxamer 407	10	10	10	14	14	14	18	18	18
Gelrite	0.2	0.2	0.2	0.6	0.6	0.6	1.0	1.0	1.0
Propyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Methyl paraben	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Disodium hydrogen phosphate	1.120	1.120	1.120	1.120	1.120	1.120	1.120	1.120	1.120
Distilled water	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml

Drug Excipients Compatibility Study: Fourier transform infrared spectroscopy (FT-IR) was used to study the physical and chemical interactions between drugs and excipients. FT-IR spectra of amikacin, poloxamer 407, gelrite and their mixture of amikacin, poloxamer 407 and gelrite were recorded by using KBr mixing method on FT-IR instrument. The drug exhibited peaks due to the alcohol group, carbonyl group, amino group and C-C, C-N, and C-O stretching. It was observed that there were no or very minor changes in main drug peaks in the IR spectra of the mixture and pure drug. The FTIR study revealed no physical or chemical interaction of amikacin, poloxamer 407 and gelrite [15].

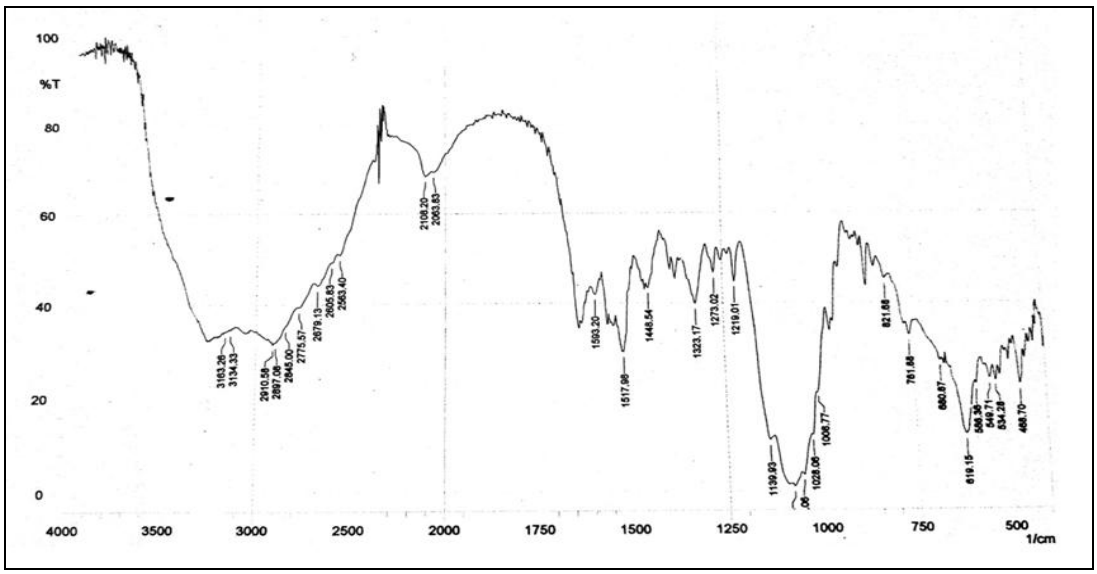

FIG. 1A: FT-IR SPECTRA OF AMIKACIN

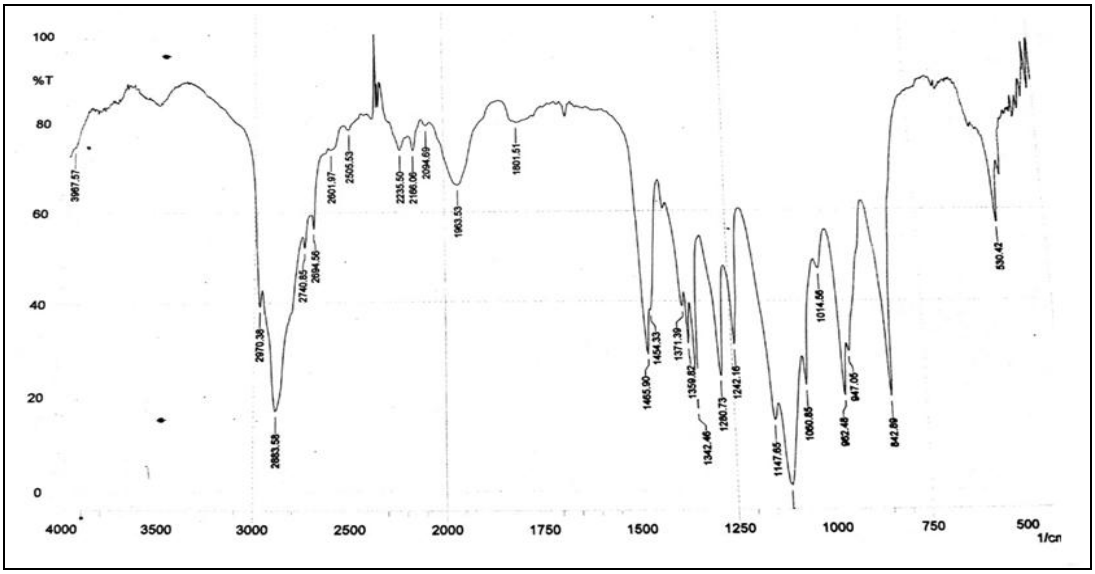

FIG. 1B: FT-IR SPECTRA OF POLOXAMER407

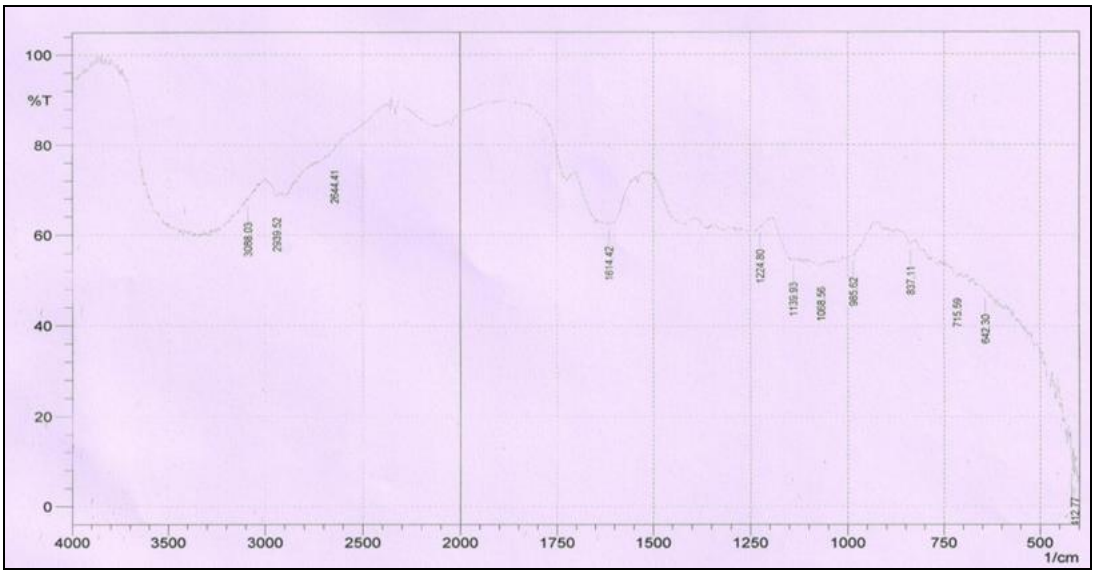

FIG. 1C: FT-IR SPECTRA OF GELRITE

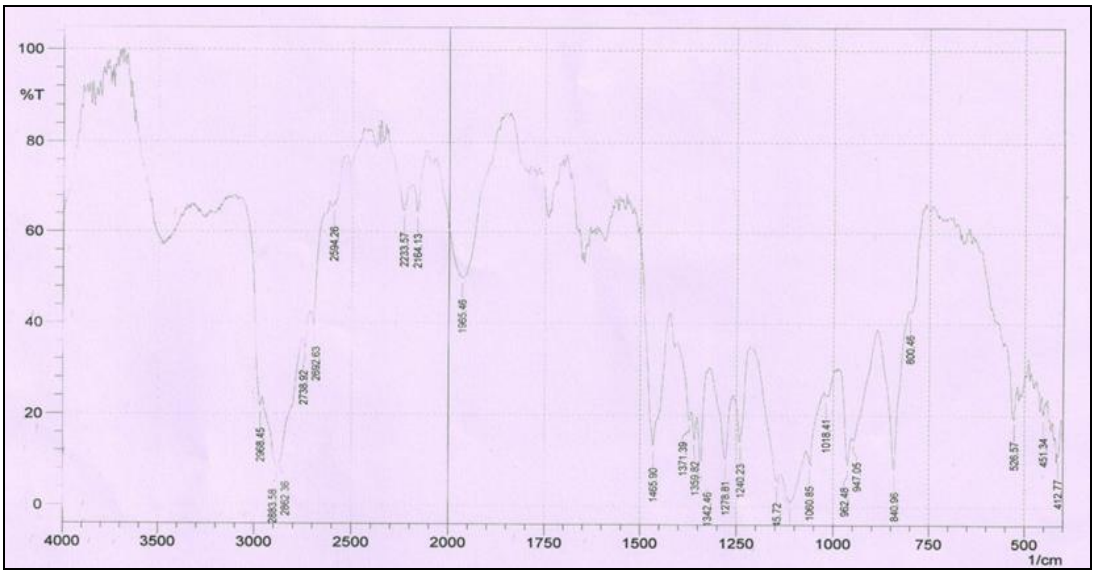

FIG. 1D: FT-IR SPECTRA OF MIXTURE OF AMIKACIN, POLOXAMER 407 AND GELRITE

Evaluation Parameter:

Appearance: Clarity is one of the most important characteristic features of ophthalmic preparations. Formulations containing poloxamer 407 were transparent than gelrite [16].

pH: pH is one of the most important parameters involved in the ophthalmic formulation. The pH of the formulations was in the range of 5.5-7.2, which was satisfactory. The pH of the gel was in the range of 6.3 – 7.35, which was satisfactory [17].

***In-vitro* Gelation Studies and Viscosity:** All formulations were evaluated for gelling capacity and viscosity in order to identify the compositions suitable for use as *in-situ* gelling systems. The viscosity of the formulations was found to be satisfactory [18].

Gellling Strength: The satisfactory results were found which is quite beneficial for gelation [19].

Rheological Profile: The rheological property of the formulation will measure using Brookfield's viscometer LV DV II+ pro model using appropriate spindle (S02) selected on the basis of the viscosity of the formulation. The formulation of batches F1 to F9 indicated that all formulation showed pseudoplastic rheology, as shown by shear thinning and a decrease in viscosity [20].

Drug Content: The drug content was found to be in the acceptable range for all the formulations indicating uniform drug content [21].

Sterilization: Sterilization of the formulation was done by the filtration sterilization method. In this method, the formulation was passed through 0.2 µm membrane filter paper in aseptic condition [22].

***In-vitro* Release Studies:** *In-vitro* release profile of the formulation F1 to formulation F9 was shown in **Table 6** for amikacin. The *in-vitro* release profile represented that the *in-situ* gelling formulation could provide sustained and controlled release of the drug. All the formulations showed an initial burst release. The prolonged-release in the later stage can be attributed to the slow diffusion of the drug through the polymer matrix.

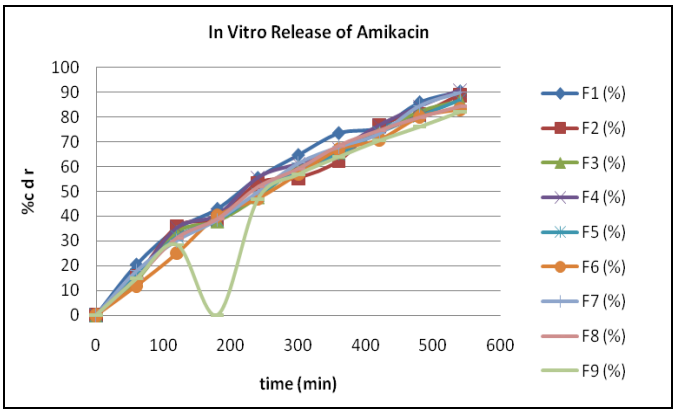

FIG. 2: *IN-VITRO* DRUG RELEASE OF BATCH F1 TO F9 OF AMIKACIN *IN-SITU* GEL

The initial burst release of the drug can be explained by the fact that the *in-situ* gelling system is formulated in water and hence the polymer was completely hydrated. When they come in contact with STF, gelation occurs and a prehydrated matrix is formed in which hydration and water penetration no longer limit drug release, leading to an apparent diffusion-controlled release. However, the results clearly show that the gels have the ability to retain the drug for a prolonged period of time and that premature drug release will not occur. In the cul-de-sac, the gels will probably undergo faster dissolution due to the shearing action of the eyelid and eyeball movement. No discernible relationship between the extent of swelling and gel composition could be established. Also, no apparent changes or disruptions in the integrity of the gels were noticed during the course of experiment [23].

TABLE 6: RESULT OF *IN-VITRO* DRUG RELEASE PROFILE DATA OF BATCH F1 TO F9 OF AMIKACIN

Time (min)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)	F9 (%)
0	0	0	0	0	0	0	0	0	0
60	20.46	15.61	14.11	15.61	16.22	11.95	17.91	15.01	14.70
120	34.81	35.81	33.61	35.28	31.78	25.03	30.18	31.32	28.38
180	42.90	38.78	38.02	40.44	38.51	40.01	38.56	38.98	3s5.91
240	55.38	53.20	47.71	55.51	48.73	47.09	49.81	51.84	46.80
300	64.60	55.42	59.80	61.08	58.91	57.01	61.38	59.16	56.81
360	73.41	62.20	65.78	67.01	65.20	66.89	68.01	68.51	63.81
420	75.68	76.63	74.08	76.56	73.81	70.80	73.34	74.63	70.41
480	85.81	81.05	82.31	84.18	80.43	79.93	84.61	79.98	75.98
540	90.20	88.91	86.60	90.80	87.01	83.17	89.98	84.38	82.01

Sterility Testing: The sterility test was performed. The formulation F4 passed the sterility test as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for a period of 7 days at 30-35 °C in case of fluid thioglycolate medium and at 20-25 °C in the case of soya bean casein digest medium [24].

***Ex-vivo* Ocular Diffusion Study:** The *ex-vivo* study for an optimized F4 batch was performed [25].

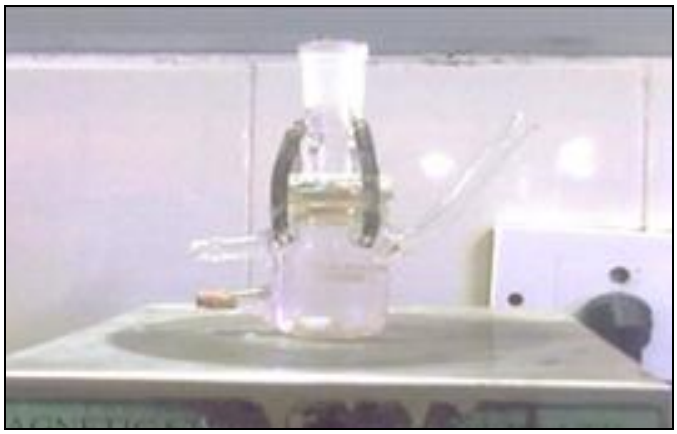

FIG. 3: FRANZ DIFFUSION CELL

TABLE 7: RESULTS OF *EX-VIVO* STUDY OF OPTIMIZED BATCH F4

Time (min)	% CDR of amikacin
0	0
60	12.88
120	25.06
180	35.93
240	44.56
300	39.98
360	62.93
420	71.82
480	78.11
540	82.09
600	85.58

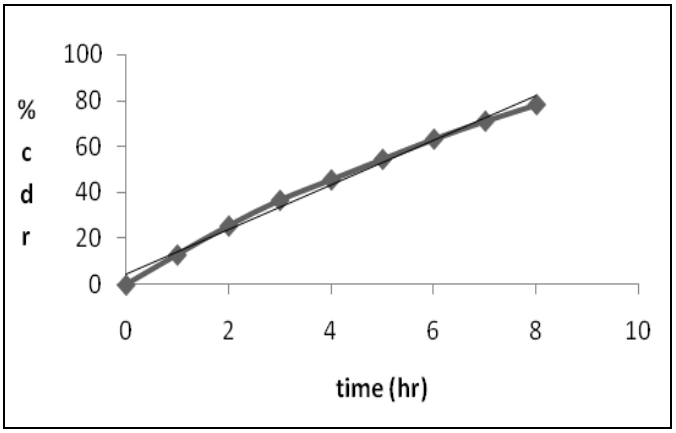

FIG. 4: *EX-VIVO* RELEASE PROFILE OF AMIKACIN FROM FACTORIAL BATCH F4 THROUGH GOAT CORNEA

Prediction of Release Mechanism: The dissolution profile of selected batches were fitted to kinetic models such as zero order, first order, Higuchi, Korsmeyer, and peppas, to ascertain the kinetic of drug release. The diffusion exponent n is indicative of the mechanism of drug release from the formulation. The n value is used to characterize different release mechanisms, concluding for values for a slab, of $n < 0.5$ for fickian diffusion mechanism, $0.5 < n < 1.0$ to non-fiction transport, values of $n = 1$ Case-II transport, and $n > 1.0$ to super case II transport [26].

Accelerated Stability Study: The stability study indicated that the formulation F4 was physically and chemically stable with no significant changes in any of the evaluated parameters when stored at the 60 °C ± 2 °C and at 75% RH conditions. From stability studies, it was concluded that the *in-situ* gelling system of amikacin was stable [27].

TABLE 8: MODEL FITTING FOR RELEASE PROFILE OF BATCH F1 TO F9

Batch code	Zero-order	First-order	Higuchi matrix	Korsmeyer and peppas	
	R^2 M	R^2 M	R^2 M	R^2 M	Release exponent
F4	0.9653	0.8642	0.9666	0.9363	0.8767

TABLE 9: RESULT OF SHORT TERM STABILITY STUDY OF OPTIMIZED F4 BATCH

Evaluation parameters	Before the stability period	After the stability period
Appearance	Transparent	Transparent
pH	6.79	7.39
Viscosity	272	276
Drug content of amikacin	38.98	37.36
Drug release after 4 h of amikacin	39.98	38.20
Drug release after 10 h of amikacin	85.58	83.62

Mucoadhesion Study: The mucoadhesive force was measured as the minimum weight required for detaching two beakers. The mucoadhesive force of the optimized F4batch was found to be 0.05136N, which indicates that the formed gel has good mucoadhesion force [28].

Isotonicity Study: Istonicity testing of optimized batch F4 formulation exhibited no change in the shape of blood cells (bulging or shrinkage), which revealed the isotonic nature of the formulation and compared with that of standard marketed ophthalmic eye drop of amikacin. By this study, it was concluded that the optimized formulation could not damage tissue because the is tonicity of formulation was to be maintained to prevent tissue damage or irritation of eye [29].

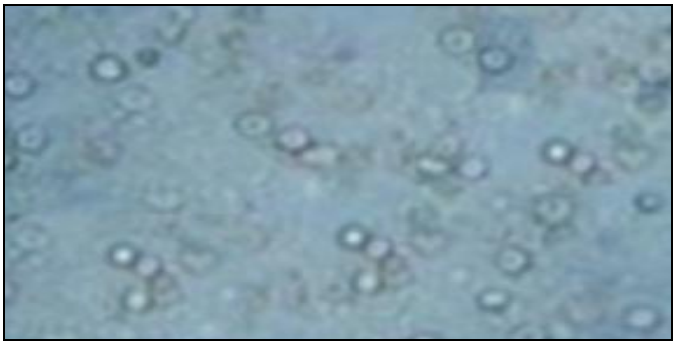

FIG. 5A: BLOOD CELLS WITHOUT MAINTAINED ISOTONICITY OF FORMULATION

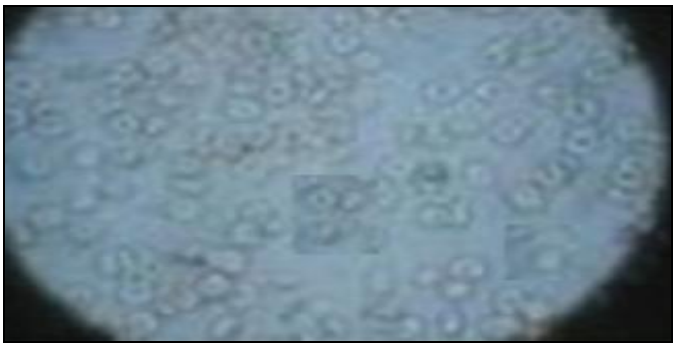

FIG. 5B: BLOOD CELL WITH STANDARD EYE DROPS

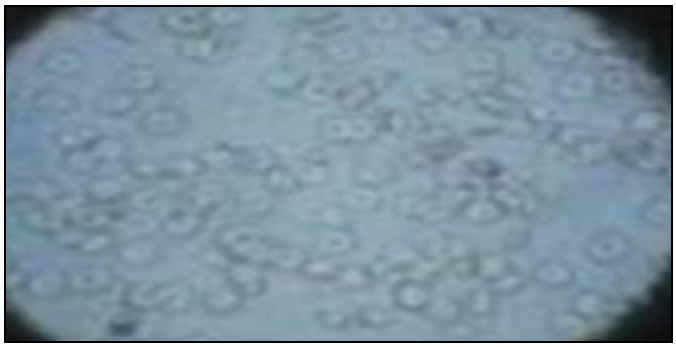

FIG. 5C: BLOOD CELLS WITH OPTIMIZED BATCH

Eye Irritation Study: The results of the ocular irritation study for optimized batch F4 indicated that the formulation was non-irritant. There was no irritation to sensitive ocular tissues by the formulation, and no ocular damage or abnormal clinical signs like redness, swelling, itching or watery eye was observed during the study period. Hence, the formulation was safe to use in ocular treatment [30].

Statistical Analysis: The statistical analysis of the factorial design batches was performed by multiple linear regression analysis. The viscosity (Y_1), gelling strength (Y_2) % drug release after 4 h of amikacin (Y_3), % drug release after 10 h of amikacin (Y_4), was selected as dependent variables. **Table 10** shows a list of variables. The polynomial equation for 3^2 factorial designs is described as follows

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \ldots\ldots\ldots (1)$$

Where Y is the dependent variable, β^0 arithmetic means the response of nine batches and β^1 estimated coefficient for factor X_1. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction term "$X_1 X_2$" shows how the response changes when the two factors change simultaneously. The polynomial terms (X_1^2 and X_2^2) are included to investigate nonlinearity [31, 32]. The fitted equations (full model) relating the responses that are, viscosity (cps), gelling strength, Percentage drug release at 4 h and Percentage drug release at 10 h to the transformed factor are shown in **Table 10**. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (*i.e.*, positive or negative). Data were analyzed using the design of expert version [9]. R^2 values for viscosity (CPS), gelling strength, drug release after 4 h (%) of amikacin, drug release after 10 h (%) of amikacin were 0.9993, 0.9824, 0.5706, 0.9987 and 0.9554 respectively indicating good correlation between dependent and independent variables. There was no need to develop reduced models because the response variable was

significant *i.e.*, P<0.05. The terms with P<0.05 were considered statistically significant and retained in the full model.

The results of ANOVA suggested that *F* values calculated for viscosity, gelling strength, drug release after 4 h of amikacin, drug release after 10 h of amikacin were 852.50, 33.46, 18.80 and12.87, respectively **Table 12**. Calculated *F* values were greater than tabulated for all dependent variables therefore factors selected have shown significant effects. From the results of multiple regression analysis, it was found that both factors had a statistically significant influence on all dependent variables as p<0.05 **Table 11**.

TABLE 10: EXPERIMENTAL RUNS AND MEASURED RESPONSES

Batch	X_1 (conc. of poloxamer 407)	X_2 (conc. of gelrite)	Y_1 viscosity (cps)	Y_2 gelling Strength	Y_3 (% drug release after 4 h)	Y_4 (% drug release after 10 h)
F1	-1	-1	206	8	33.48	90.63
F2	0	-1	244	10	41.42	82.17
F3	+1	-1	284	11	42.06	80.23
F4	-1	0	272	10	39.98	85.58
F5	0	0	314	11	40.42	81.92
F6	+1	0	346	11.5	37.42	78.96
F7	-1	+1	313	10	43.40	85.23
F8	0	+1	355	10.5	35.42	77.18
F9	+1	+1	386	11	41.09	74.28

TABLE 11: SUMMARY OF RESULTS OF REGRESSION ANALYSIS

Viscosity (CPS)							
Response (Y_1)	**β_0**	**β_1**	**β_2**	**β_{12}**	**β_{11}**	**B_{22}**	**R^2 value**
Coefficient	+313.62	+53.33	+37.50	-1.25	-13.17	-3.67	0.9987
P Value	<0.0001	<0.0001	<0.0001	0.1831	<0.0001	0.0087	
Gelling strength							
Response (Y_2)	**β_0**	**β_1**	**β_2**	**β_{12}**	**β_{11}**	**B_{22}**	**R^2 value**
Coefficient	+10.56	+2.67	+0.17	1.00	-1.33	-0.83	0.9824
P Value	0.0078	0.0012	0.5015	0.4195	0.0389	0.1152	
Drug release after 4 h (%) of amikacin							
Response (Y_3)	**β_0**	**β_1**	**β_2**	**β_{12}**	**β_{11}**	**B_{22}**	**R^2 value**
Coefficient	+40.35	+0.49	-0.60	+4.14	+0.41	-1.93	0.8956
P Value	0.0004	0.2113	0.1371	<0.0001	0.4628	0.0081	
Drug release after 10 h (%) of amikacin							
Response (Y_4)	**β_0**	**β_1**	**β_2**	**β_{12}**	**β_{11}**	**B_{22}**	**R^2 value**
Coefficient	+81.57	-2.72	-4.66	-0.14	-1.01	+1.59	0.9094
P Value	0.0002	0.0009	<0.0001	0.8262	0.2085	0.0647	

TABLE 12: RESULTS OF THE ANOVA FOR DEPENDENT VARIABLES

Source of Variation	DF	SS	MS	F	P
Viscosity (CPS)					
Regression	5	26233.64	5246.73	1834.52	0.0001
Residual	3	20.05	2.86		
Total	8	26253.69			
Gelling Strength					
Regression	5	48.03	9.61	33.13	0.0078
Residual	3	0.86	0.29		
Total	8	48.89			
Drug release after 4 h (%) of amikacin					
Regression	5	82.75	16.55	21.49	0.0004
Residual	3	5.36	0.77		
Total	8	88.11			
Drug release after 10 h (%) of amikacin					
Regression	5	182.46	36.49	25.16	0.0002
Residual	3	10.18	1.45		
Total	8	192.64			

Full and Reduced Model for Viscosity of Amikacin: For Viscosity as seen from the **Fig. 6A** and **6B** of counter plot and response surface plot revealed that a corresponding increase in the viscosity of *in-situ* gel was observed with an increase in the concentration of poloxamer 407. It may lead to increase more retention time of drugs to remain in the precorneal area for a longer period

of time and it may increase the bioavailability of the drug in the eye. The coefficient of X_2 was higher than the X_1 which indicated that the effect of concentration of poloxamer 407 was more significant than the effect of concentration of gelrite. It was observed that only X_1, X_2, and X_2^2 were significant model terms that affect the viscosity. Interaction and nonlinearity were not observed. For Viscosity, the significant levels of the coefficients β_{12} were found to have a P-value of 0.1831. So, it was omitted from the full model to generate a reduced model. The coefficients β_0, β_1, β_2, β_{11}, and β_{22} were found to be significant at $P<0.05$. Hence, they were retained in the reduced model. The reduced model for Viscosity was:

$$\text{Viscosity} = + 313.62 + 53.33 * X_1 + 37.50 * X_2 - 13.17 * X_1^2 - 3.67 X_2^2$$

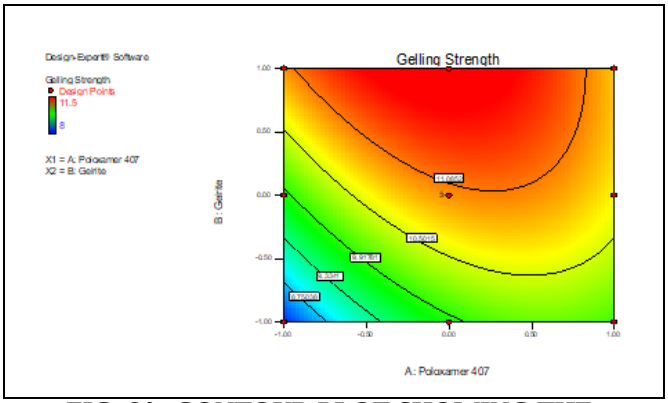

FIG. 6A: CONTOUR PLOT SHOWING THE EFFECT OF CONC. OF POLOXAMER 407 (X_1) AND GELRITE (X_2) ON VISCOSITY (Y_1)

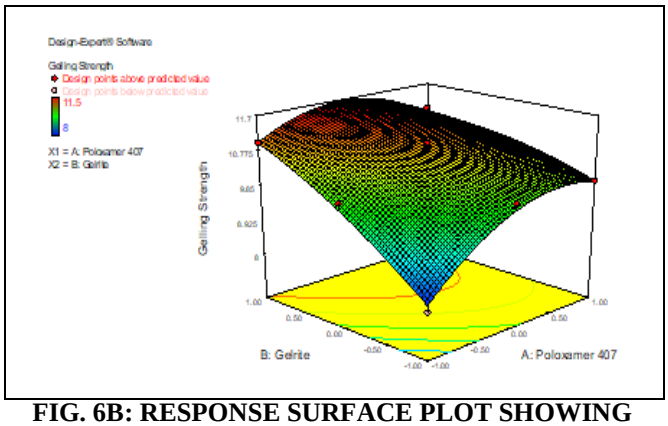

FIG. 6B: RESPONSE SURFACE PLOT SHOWING THE EFFECT OF POLOXAMER 407 (X_1) AND GELRITE (X_2) ON VISCOSITY (Y_1)

Full and Reduced Model for Gelling Strength of Amikacin: For gelling strength, as seen from the **Fig.7A** and **7B** of counter plot and response surface plot revealed that a corresponding increase in the viscosity of *in-situ* gel was observed with increase in the concentration of poloxamer 407. It may lead to increase more retention time of drugs to remain in the precorneal area for a longer period of time and it may increase the bioavailability of the drug in the eye. The coefficient of X2 was higher than the X1 which indicated that the effect of concentration of poloxamer 407 was more significant than the effect of concentration of gelrite. It was observed that only X1, X2, and X22 were significant model terms that affect the viscosity. Interaction and nonlinearity was not observed. For gelling strength, the significant levels of the coefficients β12 and β22 were found to be P-value of 0.419 and 0.389, respectively, so they were omitted from the full model to generate a reduced model. The coefficients β0, β1, and β11 were found to be significant at $P < 0.05$. Hence, they were retained in the reduced model. The reduced model for gelling strength was:

$$\text{Gelling strength} = + 10.56 + 2.67 * X_1 + 0.17 * X_2 - 1.33 * X_{12}$$

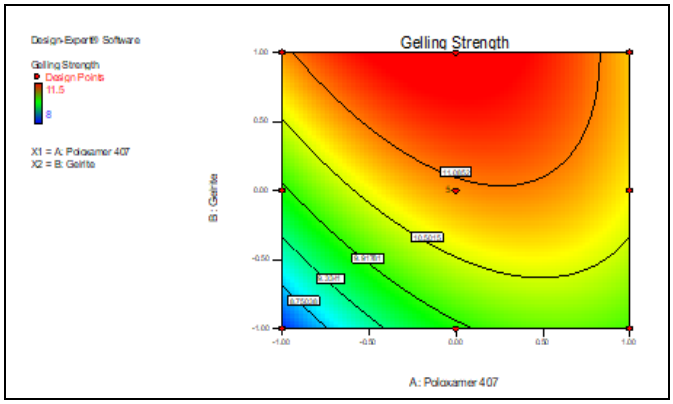

FIG. 7A: CONTOUR PLOT SHOWING THE EFFECT OF CONC. OF POLOXAMER 407 (X_1) AND GELRITE (X_2) ON GELLING STRENGTH (Y_2)

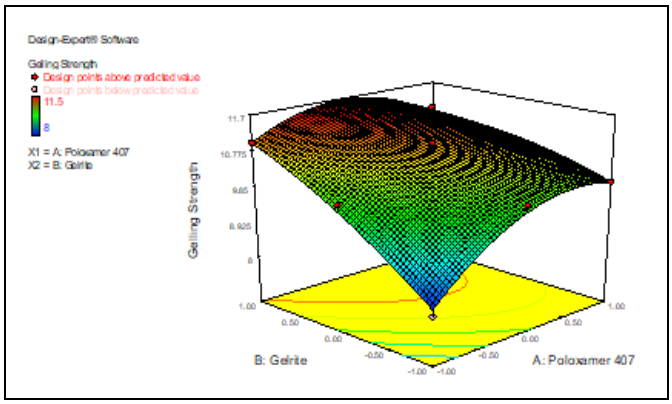

FIG. 7B: RESPONSE SURFACE PLOT SHOWING THE EFFECT OF POLOXAMER 407 (X_1) AND GELRITE (X_2) ON GELLING STRENGTH (Y_2)

Full and Reduced Model for Drug Release After 4 h of Amikacin: For Drug release after 4 h of amikacin, as seen from **Fig. 8A** and **8B** of the contour plot and response surface plot revealed that the drug release after 4 h was slower in case of a lower concentration of gelrite.

The lower drug release after 4 h could be explained by the higher concentration of poloxamer 407 as compared to gelrite. The contour plot and response surface plot showed that the concentration of poloxamer 407 and gelrite showed negative effects on drug release after 4 h. An increase in their concentration would decrease the drug release and concentration of poloxamer 407 was more significant than the concentration of gelrite. For Drug release after 4 h of amikacin, the significant levels of the coefficients β11 were found to have a P-value of 0.4628. So, it was omitted from the full model to generate a reduced model. The coefficients β0, β1, β2, β12, and β22 were found to be significant at $P < 0.05$. Hence, they were retained in the reduced model.

Drug release after 4 h = + 40.35 + 0.49 * X_1 – 0.60 * X_2 + 0.41 * X_{12}

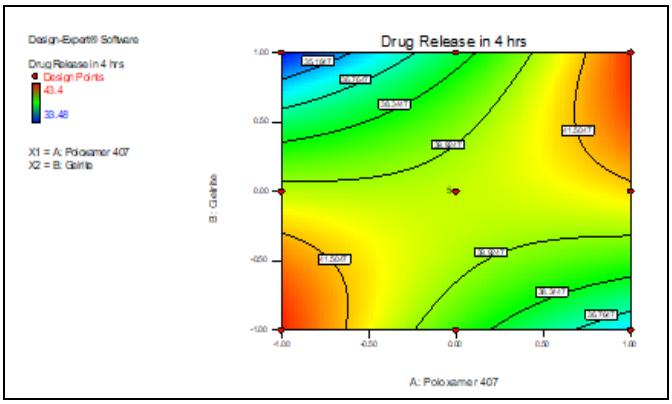

FIG. 8A: CONTOUR PLOT SHOWING THE EFFECT OF POLOXAMER 407 (X_1) AND GELRITE (X_2) ON DRUG RELEASE AFTER 4 H (Y_2)

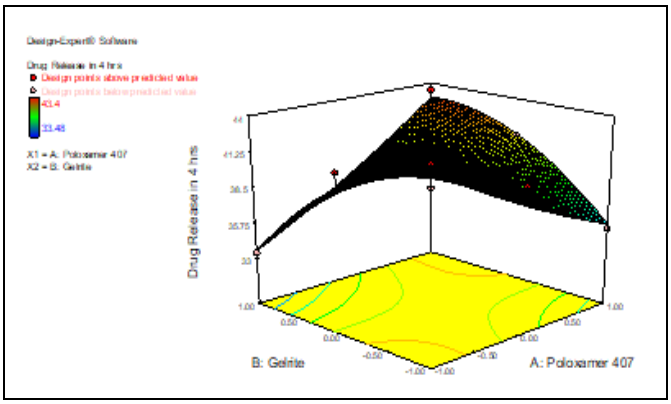

FIG. 8B: RESPONSE SURFACE PLOT SHOWING THE EFFECT OF POLOXAMER 407 (X_1) AND GELRITE (X_2) ON DRUG RELEASE AFTER 4 H (Y_2)

Full and Reduced Model for Drug Release After 10 h of Amikacin: For drug release after 10 h, as seen from **Fig. 9A** and **9B** of contour plot and Response surface plot revealed that the drug release appeared to decrease with an increase Concentration of sodium alginate and poloxamer 407. The contour plot and response surface plot showed that the concentration of sodium alginate and poloxamer 407 showed negative effects on Drug release after 10 h. An increase in their concentration would decrease the drug release, and concentration of sodium alginate was more significant than the concentration of poloxamer 407. For drug release after 10 h of amikacin, the significant levels of the coefficients β12, β11, and β22 were found to have a P-value of 0.8262, 0.2085, and 0.0647 respectively, so they were omitted from the full model to generate a reduced model.

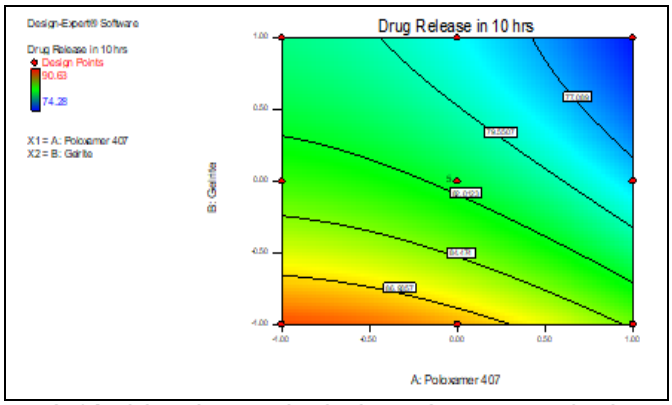

FIG. 9A: CONTOUR PLOT SHOWING THE EFFECT OF POLOXAMER 407 (X1) AND GELRITE (X_2) ON DRUG RELEASE AFTER 10 (Y_2)

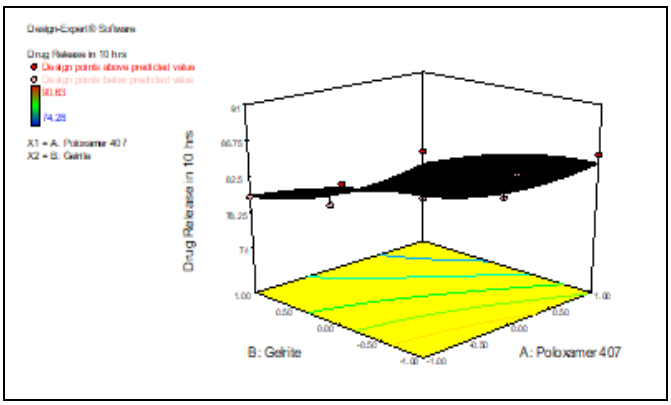

FIG. 9B: RESPONSE SURFACE PLOT SHOWING THE EFFECT OF POLOXAMER 407 (X_1) AND GELRITE (X_2) ON DRUG RELEASE AFTER 10 H (Y_2)

The coefficients β_0, β_1, β_2 were found to be significant at $P < 0.05$. Hence, they were retained in the reduced model

Drug release after 10 h = + 81.57-2.72 * X_1 – 4.66 * X_2

Validation by Check Point Batch: To confirm the validity of response surface plot and equation generated by multiple regression analysis, a checkpoint batch was prepared shown in **Table 13**. An overlay plot was obtained by adding a desired range of evaluation parameters from design expert [9]. The overlay plot is shown in **Fig. 10** a yellow color area in overlay plot showed optimum concentration range for the desired result. A batch was prepared by taking a concentration of poloxamer 407 (X_1) and concentration of gelrite (X_2) observed in the overlay plot and the actual responses were evaluated from the prepared checkpoint batch. The overlay plot indicated that optimum concentration which showed the best result. The practically obtained values were closer to the predicted values as shown in **Table 14**. Thus, it justified the validation of the design.

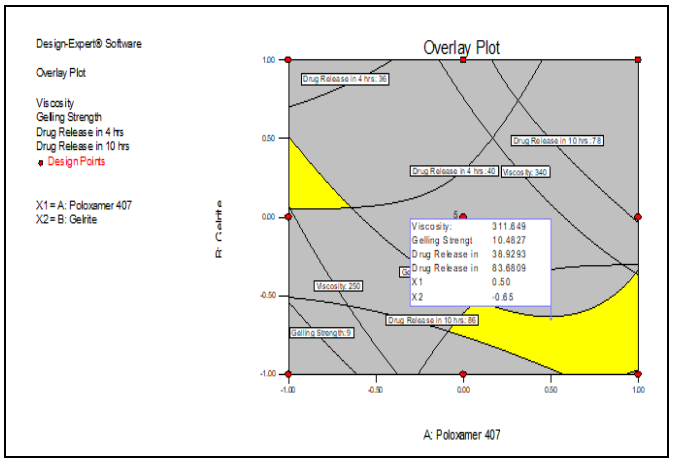

FIG. 10: CHECKPOINT BATCH OVERLAY PLOT

TABLE 13: FORMULATION OF CHECKPOINT BATCH

Batch Code	Coded value		Actual value	
CP1	X_1	X_2	X_1 (mg)	X_2 (mg)
	0.5	-0.65	15	0.48

TABLE 14: RESULTS OF CHECKPOINT BATCH METHOD

Response	Predicted value	Experimental value
Viscosity (cps)	311.649	313
Gelling strength	10.482	10.282
Drug release after 4 h of amikacin	38.929	40.821
Drug release after 10 h of amikacin	83.680	84.246

RESULTS: The most commonly available ophthalmic preparation is eye drops and ointments. But eye drops when instilled into the cul-de sac were rapidly drained away from the ocular cavity due to the tear flow and nasolacrimal drainage. This problem can be overcome by an *in-situ gel*-forming system which is a viscous liquid that shifts to a gel phase upon exposure to physiological condition. Preliminary screening was performed for the selection of polymer and its concentration by using different polymers like pH-dependent carbopol, temperature-dependent poloxamer 407 and ion-activated gelrite. Among this ion activated gelrite and poloxamer 407 shows good results. Preformulation studies were carried out in order to establish the compatibility between the drug and polymers by infrared spectroscopy.

The studies revealed that drug and polymers were satisfactorily compatible. The *in-situ* gel was developed by using 3^2 full factorial designs by employing gelling agent poloxamer 407 and gelrite to increase residence time. From the results obtained from the preliminary screening, two factors were selected *i.e.*, concentration of poloxamer (X_1) and concentration of gelrite (X_2) as independent variables. Dependent variables selected were viscosity (cps), gelling strength, drug release after 4 h (%), and drug release after 10 h (%). The prepared formulations were evaluated for different parameters like pH, appearance, drug content, in vitro gelation study, viscosity, *in-vitro* release study, sterility test, stability studies, mucoadhesion study, and *ex-vivo* ocular diffusion study.

DISCUSSION: Based on the desirability approach, a formulation containing Poloxamer 407 and gelrite in a concentration of 14.0% w/v and 0.6 % w/v batch was selected as an optimized batch. The eye irritation test and is tonicity study revealed that formulation was non-irritant. The release profile of the formulation follows zero-order models and the release mechanism was non-Fickian diffusion. From the *in-vitro* study, it was found that the developed formulation was provided sustained release of the drug over 10 h by formulating in the form of ophthalmic amikacin *in-situ* gel.

CONCLUSION: Amikacin, a fourth-generation fluoroquinolone agent a non steroidal anti-

inflammatory agent used in the treatment of antibiotic and anti-inflammation to the eye infection was successfully formulated *in-situ* gel-forming eye drops using gelrite (ion activated gelling agent) and poloxamer 407 (temperature-activated gelling agent). The formulations were liquid before instillation and underwent rapid gelation upon instillation into eye. The 3^2 full factorial designs were used to select the optimized batch. The gel formed *in-situ* afforded sustained drug release over a 10 h period. Eye irritation test was carried out in rabbits and it was found that formulations were non-irritant.

The optimized formulation passed is tonicity study. Thus, would not damage the tissue of the eye. Stability data recorded over a one month period under accelerated temperature condition indicated that the formulation to be stable. The developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustained drug release. It was concluded that the method attempted to formulate the *in-situ* gel of amikacin is being simple and acceptable. The developed formulation was therapeutically efficacious, stable, and provided sustained release of the drug over an extended period of time.

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