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Development and optimization of *in situ* periodontal gel containing Levofloxacin for the treatment of periodontal diseases

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Abstract

Levofloxacin has a conventional dose of about 500 mg per day. Many drugs do not reach the site of action in the therapeutic concentrations intended. So, in present study, works has been done for administering the drug directly to the target site so that the efficacy of treatment can be improved. This site specific delivery of drug can thus overcome the problems faced during systemic administration of antimicrobials for periodontitis, where the drug get diluted many times before it reaches the site of action. This also reduces frequency of administration and dose size, and thereby improves patient compliance and minimizes systemic side effects. Levofloxacin periodontal gel was prepared by different concentrations of gellan gum, poloxamer 407. 32full factorial design was applied for optimization. Selected dependent variables are concentration of gellan gum (X1) and poloxamer 407 (X2). Selected independent variables are release at 1 hour (Y1), t90 % (Y2), gelation temperature (Y3) and viscosity (Y4). All the prepared formulations were evaluated for viscosity, pH, % drug content, syringeability, and effect of sterilization. By compatibility study drug was found to be compatible with formulation excipients. Gelation temperature and pH of all formulation found to be in the range of 40- 250C and 5.5-5.9 respectively. Viscosity of all prepared formulations was found in the range of 600-1500 centipoise. All the formulations except F3, F6 and F9 show satisfactory syringeability. Both the independent variable had the significant effect on the entire four response variable ($P< 0.05$). All the formulations were developed using combination of gellan gum and poloxamer 407. The developed formulations showed satisfactory results for in-vitro gelling capacity, rheology and other physical properties. Based on maximum desirability and cost effectiveness formulation containing 0.32%w/v of gellan gum and 14.2%w/v of poloxamer 407 was consider as an optimized batch.

Keywords: Periodontitis; Poloxamer 407; Gellan gum; Thermo-sensitive; Ion-sensitive

Introduction

Periodontal diseases are groups of infections and inflammatory conditions, including gingivitis and periodontitis that affect teeth supporting structures.[1] These diseases occur when bacteria from dental plaque invade surrounding tissues and from the accumulation of plaque at the gingival margin, which, in turn, induces an inflammatory response. The result is the formation of pockets between gingiva and tooth that causes gingival margin retraction and the development of an ideal environment for anaerobic bacteria growth responsible for the disease. The progression of this destructive process can cause tooth loss. Two particular problems common to many periodontal drug delivery systems are short retention time and difficult as well as time consuming application.[2]

Levofloxacin is L-isomer of fluoroquinolon antibiotic ofloxacin and it is found to be two fold more potent than ofloxacin in the treatment of periodontal diseases was chosen for present study. In the form of conventional dosage form such as tablets, parentrals and capsules Levofloxacin is available for the treatment of bacterial infection, but not available for treatment of infection locally. Hence it was a challenge to formulate in-situ periodontal gel containing Levofloxacin with rate controlling polymers which provides a longer duration of action and local antibacterial effect without loss of dosage.

Materials and methods

Materials

Gellan gum was purchased from ACS chemical (Mumbai), poloxamer 407 was purchased from S D fine chemical, (Mumbai), Levofloxacin was obtained as a gift sample from Moxy laboratories (Baroda), Methylparaben, Propylparaben, Sodium citrate, KH2PO4, Na2HPO4 were procured from S D fine chemical, (Mumbai), Nutrient agar media was purchased from Hi Media laboratories Pvt. Ltd (Mumbai).

Method

Preliminary study

Compatibility study

FT-IR study was carried out to identify the drug sample and to establish drug polymer compatibility.

Selection of poloxamer 407 concentration

Solution of different concentration ranging from 8-20 % of poloxamer 407 was prepared by cold process, as per literature review (3, 4). Required amount of polymer was accurately weighed and dispersed in distilled water with continuous mild stirring for 5 minutes. The beaker containing partially dissolved poloxamer 407 was sealed with aluminum foil and kept in refrigerator at 40C until the entire polymer was completely dissolved (about 48 hrs.). For optimization of poloxamer 407 concentrations, different solutions of various concentrations were prepared and optimization was done on the basis of gelation temperature and gelation time.

Selection of gellan gum concentration

Solution of different concentration ranging from 0.1-1.0 % of gellan gum was prepared by hot process, as per literature review (5, 6). Dry gellan gum powder was dispersed in distilled water maintained at 950C. The dispersion was stirred at 950C for 20 minutes using magnetic stirrer to facilitate the hydration of gellan gum. The solution was allowed cool at room temperature. For optimization of gellan gum concentrations, different solutions of various concentrations were prepared and optimization was done on the basis of gel strength in simulated saliva.

Preparation of in situ periodontal gel formulations

As described in table 1, dry gellan gum powder was dispersed in 25 ml of distilled water maintained at 950C(7). The dispersion was stirred at 950C for 2 minutes to facilitate the complete hydration of gellan gum. The required amount of preservatives (methyl paraben, propyl paraben) and sodium citrate were added to gellan gum solution with continuous stirring at 950C. The solution was allowed to cool to room temperature. The required amount of Levofloxacin was added to gellan gum solution with continuous stirring until the entire drug was dissolved. Then required amount of poloxamer 407 was added with continuous mild stirring for 5 minutes. The formulation containing partially dissolved poloxamer 407 were stored in the refrigerator until entire polymer gets completely dissolved. The prepared formulation then transferred to clean glass container and volume was make up to 30 ml with distilled water and store at cool place.

Table 1: Composition of in situ periodontal gel

Formulation code	Ingredients			
	Levofloxacin (mg)	Gellan gum (mg)	poloxamer 407 (gm.)	Distilled water
F1	150	60	3.6	In all the formulations final volume adjusted to 30 ml with distilled water.
F2	150	120	3.6	
F3	150	180	3.6	
F4	150	60	4.2	
F5	150	120	4.2	
F6	150	180	4.2	
F7	150	60	4.8	
F8	150	120	4.8	
F9	150	180	4.8	

All the formulations were added with 0.1% sodium citrate as a sequestering agent, 0.18% methyl paraben and 0.02% propyl paraben as a preservative.

Formulation optimization

To achieve the formulations with desired gelation temperature, viscosity, rheology, in-vitro drug release, and syringeability, the formulations prepared by using different concentrations of gellan gum and poloxamer 407 were evaluated using 32-full factorial design.

In-vitro gelling capacity

To evaluate the formulations for its in-vitro gelling capacity by visual method, colored solution of prepared formulations (Formulation F1–F9) were prepared. The in-vitro gelling capacity of prepared formulations was measured by placing 2 ml of simulated saliva in a 15 ml borosilicate glass test tube and maintained at 37 ± 1°C temperature. One milliliter of coloured formulation solution was added with the help of a 1 ml pipette. The formulation was transferred in such a way that places the pipette at the surface of fluid in the test tube and formulation was slowly released from the pipette. As the formulation comes into contact with simulated saliva it was immediately converted into a stiff gel-like structure. The gelling capacity of formulation was evaluated on the basis of stiffness of formed gel and time period for which formed gel remains as such. Color was added to give a visual appearance to the formed gel. The in-vitro gelling capacity was graded in three categories on the basis of gelation time and time period for which formed gel remains (8).

pH measurements

The pH of all prepared formulations was measured directly with electronic pH meter.

Syringeability

All prepared formulations were transferred into an identical 5 ml plastic syringe placed with 20 gauge needle to a constant volume (1 ml). The solutions which were easily passed from syringe was termed as pass and difficult to pass were termed as fail.

Viscosity profile

The viscosity of all prepared formulations was measured using Digital Brookfield viscometer (DV-II+Pro, USA) (9). The measurements were carried out using spindle no.62 at the speed of a 10 rpm in the sample.

Rheological properties

The rheological properties of all prepared formulations were measured using a Brookfield viscometer DV-II+ Pro model viscometer using spindle no.62. The viscosity of the sample solutions was measured at different speeds at a temperature of 25 ± 1°C. A typical run involved changing the speed from 10 to 100 rpm (10).

Gelation temperature

Ten milliliters of the sample solution and a magnetic bead were put into a 30 ml transparent vial that was placed in a low temperature digital water bath. A thermometer was placed in the sample solution. The solution was heated at the rate of 1°C/min with the continuous stirring at lower rpm. The temperature was determined as gelation

temperature, at which the magnetic bead stopped moving due to gelation. Each sample was measured at least in triplicate.

Gelation time

For measurement of gelation time 2 ml of the formulation was placed in 15 ml borosilicate glass test tube. This test tube was placed in water-bath maintained at 37± 20C. Gelation time was noted when there was no flow when test tube was inverted.

Drug content uniformity

The container containing formulations were properly shaken for 2–3 min. One milliliter of the formulation was transferred into a 50 ml volumetric flask with a 1 ml calibrated graduated pipette. Twenty five milliliters of simulated saliva with pH 6.8 was added. The formed gel was completely crushed with the help of a glass rod followed by vigorous shaking until the formed gel was completely dispersed to give a clear solution. Final volume was adjusted to 50 ml with simulated saliva. Obtained solution was filtered through Whatman filter paper. One milliliters of this solution was transferred to a 10 ml volumetric flask and volume was adjusted with simulated saliva and the drug concentration was determined at 288 nm by using UV-Visible Spectrophotometer-1800 (Shimadzu, Japan) (9).

Effect of sterilization

The all prepared formulations was filled in 5 ml capacity glass vials, closed with rubber closures, and sealed with aluminum caps. The sealed vials were subjected to terminal sterilization by autoclaving at 121°C and 15 psi for 20 min. The sterilized formulations were evaluated for % transmittance, pH, and drug content.

In-vitro drug release Studies

In-vitro drug release study was performed by static dissolution method. Simulated saliva having pH 6.8 was used as a dissolution medium. Five ml of simulated saliva placed in test tube and maintained at 37± 10C. Then one ml of the prepared formulation was placed in test tube maintained at 37 ± 10C. Temperature was maintained to 37± 10C throughout the whole study. At pre-determined time interval one ml of the sample was taken and analyzed spectrophotometrically at 288 nm. The dissolution medium was replaced with fresh medium after sampling.

Microbiological studies of optimized formulation

Nutrient agar medium was prepared and sterilized by autoclaving under aseptic condition and transfer the medium to sterile petri plates. After solidification of nutrient agar medium, lawn was made with 0.1 ml microorganism i.e. S. aureus and E. coli in separate petri plates. Cups were made on the solidified agar layer with the help of sterile borer of 6 mm diameter. Appropriate amount of drug solution was poured into the cups and incubated for 48 hours at 370C. finally zone of inhibition was measured(11, 12).

Kinetic release study of optimized formulation

The in-vitro release data of optimized formulation were kinetically analyzed for establishing kinetic of drug release of drug. Model fitting was done using Microsoft Excel 2013. Zero order, First order, Higuchi, Hixoncrowel and Korsemeyerpeppas models were tested. The best fit model was selected on basis of relatively high co-relation coefficient value and least F value.

Results and discussion

Compatibility study

From the FT-IR spectra of levofloxacin and mixture it, can be seen that there no change in significant peak of levofloxacin in mixture indicating stability of drug in formulation mixture.

Results of selection of Poloxamer 407 concentration

Table 2: Gelation temperature and gelation time of poloxamer solutions

Poloxamer-407 concentration (%)	Gelation temperature (^{0}C)	Gelation time (min)
8	No gelation up to 50 ^{0}C	-
10		-
12	40	5
13	38	6
14	38	5
15	37	5
16	35	7
17	34	8
18	30	12
19	26	12
20	25	15

For the selection of poloxamer 407 concentration various solutions of different concentration ranging from 8-20% was prepared and finalization of concentration was done on the basis of gelation temperature and gelation time.

Gelation temperature of solution of 12 to 16% was observed in the range of desired gelation temperature (35-40 0C) so this range was selected for further study.

Results of selection of gellan gum concentration

Table 3: Gel strength of gellan gum solutions

Gellan gum concentration	Gel strength
0.1 %	+
0.2 %	+
0.3 %	+
0.4 %	++
0.5 %	++
0.6 %	++
0.7 %	+++
0.8 %	+++
0.9 %	+++
1.0 %	+++

(+), gels after few minutes, dispersed rapidly;
(++), gelation immediate, remains for few hours; and
(+++), gelation immediate, remains for an extended period.

For selection of gellan gum concentration various solutions of different concentration was prepared and finalization of concentration was done on the basis of gel strength. Here formulation having lower and intermediate gel strength was selected for preparation of formulations because when final formulation will prepared in combination, additive effect of individual gel strength of both polymers produced desired gel strength.

In-vitro gelling capacity of all prepared formulations

The main pre-requisite for in situ periodontal gels were viscosity and gelling capacity. The formulation should undergo rapid sol to gel transition in simulated saliva due to ionic interaction. To facilitate the sustained release of the drug to periodontal cavity, the formed gel should preserve its integrity without eroding or dissolving. Except the formulation F1 and F2 all the batches shows instantaneous gelation when come in contact with simulated saliva maintained at 37 ± 10C. However the nature of the gel formed depends upon the concentration of polymers. Formulation F1 and F2 shows weakest gelation after 8-10 minutes and dispersed rapidly on moderate shaking, which may be due to presence of very low concentration of gellan gum (0.2 and 0.4 %w/v) and poloxamer (12.0%). Formulation F3, F4 and F7 shows immediate gelation but the formed gels are less stiff and does not remains for extended period of time. Although the formulation F3 contains a relatively higher concentration of gellan gum (0.6%w-v) but it contains a lower concentration of poloxamer 407 (12%w/v) while formulation F4 and F7 contains lower concentration of gellan gum (0.2%w/v) but it contains higher concentration of poloxamer 407, 14.0 and 16.0%w/v respectively. So, formulation F3 and F7 shows immediate gelation. Formulation F5, F6, F8 and F9 shows immediate gelation and formed gel was stiff and remained for extended period of time, this is due to the presence of higher concentration of gellan gum and poloxamer 407.

(+), gels after few minutes, dispersed rapidly;
(++), gelation immediate, remains for few hours; and
(+++), gelation immediate, remains for an extended period

pH of all prepared formulations

It was reported that the apparent viscosity of gellan gum solution can be markedly influence by the pH(13). Therefore, the pH of the formulation was adjusted and maintained between 5–6 with the help of a non-ionic alkalinizing agent like Triethanolamine if necessary. The pH of all prepared formulations was observed in the range of 5.5–5.9. Therefore, there was no need for pH adjustment by any external alkalinizing agent.

Syringeability of all prepared formulations

The syringeability of each formulation is represented in table 6. As the concentration of gellan gum and poloxamer

407 increases, the viscosity of formulations were increased and increase the force required to expel each formulation from the syringe equipped with 20 gauge needle. Formulation F3, F6, F8 and F9 fail the syringeability test because they contain higher amount of polymer concentration.

Viscosity of all prepared formulations

One important pre-requisite for a periodontal gel was viscosity of the formulation. As indicated, a formulation suitable for application to the periodontal pocket should ideally have a low viscosity when applied, and after administration should have a high viscosity in order to stay at the application site. All the formulations of batches F1–F9 showed a polymer concentration-dependent rise in viscosity. The order of viscosity of all formulations with gellan gum and poloxamer 407 was F9 > F6 > F3 > F8 > F5 > F2 > F7 > F4 > F1. Batches F3, F6, and F9 containing 16% w/v of Poloxamer 407 with varying concentrations of gellan gum had significant higher viscosity, which may be a disadvantage in formulation development of injectable periodontal gel. Therefore, it was not possible to formulate an in situ periodontal gel with poloxamer 407 using concentrations higher than 16% w/v of the formulation in combination with gellan gum.

Rheological properties of all prepared formulations

The flow curve (viscosity against speed) of all prepared formulations indicates that, at the examined polymer concentration pseudoplastic systems were obtained. The prepared formulations tend to thin when being exposed to shearing force. Figure 1 compares the shear dependent viscosity of prepared formulations containing gellan gum and poloxamer 407.

Table 3: Rheological properties of prepared formulations

RPM	Viscosity (centipoise)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
10	610.3	857.3	1380	751.6	962.3	1465.5	820.6	1180	1530.2
20	503.5	710.8	1048	605.2	749.5	1098.5	656.5	910.3	1168.9
30	425.6	605.9	813.8	512.2	642.8	845.6	562.8	737.8	897.2
50	342.1	478.1	563.4	405.6	503.5	668.4	432.7	557.3	725.6
70	310.2	441.4	510.5	358.9	465.8	595.7	395.3	490.2	652
100	253.4	327.4	460.8	289.3	365.2	512.3	306.3	402.6	605.4

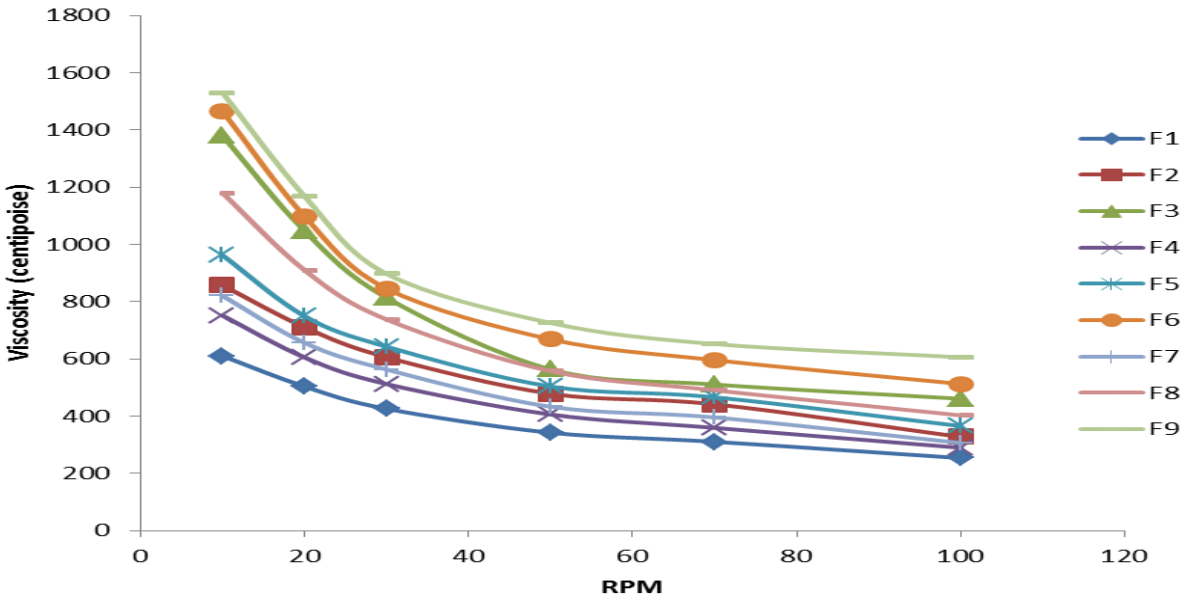

Figure 1: Rheological properties of prepared formulations

Effect of sterilization of all prepared formulations

Table 4: Effects of sterilization of prepared formulations

Formulation code	% Transmittance		% Drug content		pH	
	Before autoclave	After autoclave	Before autoclave	After autoclave	Before autoclave	After autoclave
F1	98.3	98.2	99.43	99.47	5.5	5.5
F2	98.1	98.2	96.33	94.76	5.6	5.5
F3	95.8	95.6	99.13	101.68	5.8	5.8
F4	98.2	98	96.97	94.03	5.5	5.6
F5	97	96.9	94.07	91.82	5.7	5.7
F6	96.4	96.5	93.53	92.85	5.7	5.7
F7	96.8	96.8	99.23	98.25	5.6	5.5
F8	96.5	96.4	100.01	99.43	5.7	5.7
F9	96	96.1	99.28	99.47	5.9	5.8

The results of effects of sterilization indicate insignificant change in % Transmittance, % Drug content and pH indicating no effect of sterilization on prepared formulations.

In-vitro drug release study of all prepared formulations

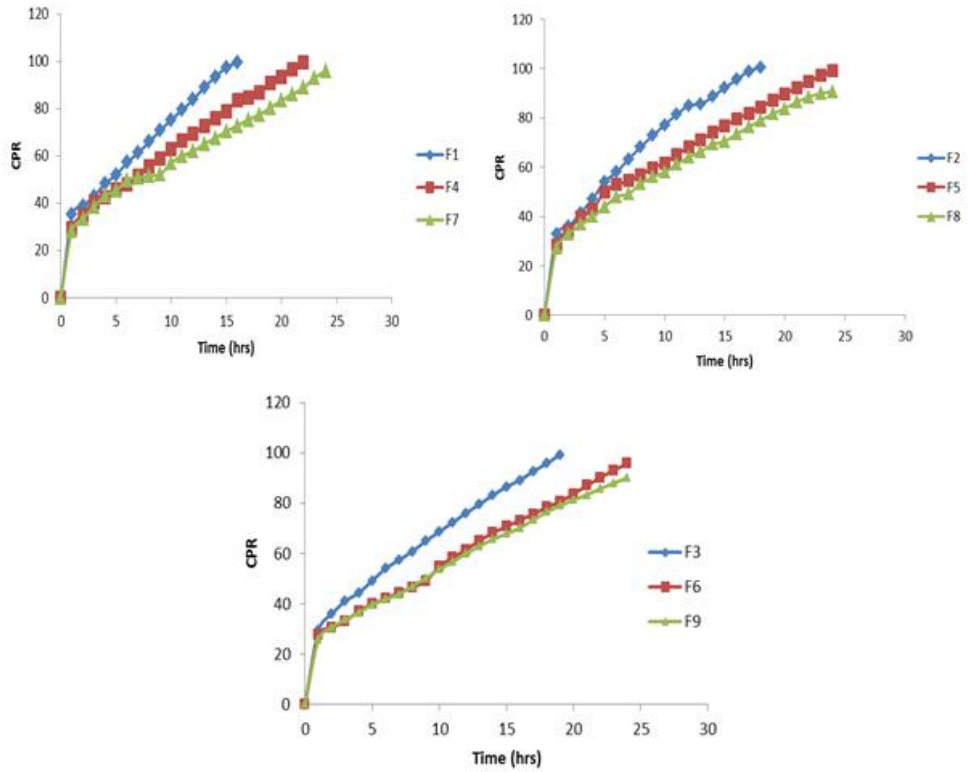

Figure 2 : Comparative dissolution profile of formulation F1 to F9

The cumulative amount of levofloxacin released vs. time profile for the selected formulations is shown in Figure 2. First sampling was done 1 h after the formulation placed in test tube. One hour time for first sampling was selected in order to evaluate the effect of increasing polymer concentration on the cumulative amount of drug released. The results showed that the amount of drug released in the first hour decreased with increasing polymer concentration, and the trend continued for the entire duration of the study. The initial burst release of the drug from the prepared formulations could be

explained by the fact that these systems were formulated in an aqueous vehicle. The matrix formed on gelation was already hydrated and hence hydration and water permeation could no longer limit the drug release. The release of drug decreased significantly as the concentration of polymer increased. The release from various formulations can be ranked as follows at each time point: F1 > F2 > F3 > F4 > F5 > F6> F7 > F8 > F9. This indicates that the structure of gel becomes more closely packed and functioned as an increasing resistant barrier to drug release as the concentration of polymer increased. In general there was a reduction in drug release as the concentration of polymers increases. Slowest drug release was observed from formulation containing 0.6%w/v of gellan gum and 16%w/v of poloxamer 407, and relatively faster drug release was observed from formulation containing a different concentrations of gellan gum (0.2 and 0.4%w/v) and poloxamer 407 (12.0 and 14.0%w/v). Initial burst release of drug also compared with viscosity of the formulation and found that as the viscosity of the formulations increase the percentage drug release at first hour decreases.

Table 5: Gel strength, Gelation temperature, Gelation time, pH, Syringeability, Viscosity and % Drug content of prepared formulations.

Formulation	Gel strength	Gelation temperature (^{0}C)	Gelation time	pH	Syringeability	Viscosity (Centipoise)	% Drug content
F1	+	40-41	15	5.5	Pass	610.3	99.42%
F2	+	38-39	11	5.6	Pass	857.3	96.33%
F3	++	37-38	9	5.8	Fail	1380	99.13%
F4	++	36-37	12	5.5	Pass	751.6	96.97%
F5	+++	34-35	10	5.7	Pass	962.3	94.07%
F6	+++	32-33	9	5.7	Fail	1465.5	93.53%
F7	++	31-32	9	5.6	Pass	820.6	99.22%
F8	+++	28-29	8	5.7	Fail	1180	100.01%
F9	+++	24-25	6	5.9	Fail	1530.2	99.27%

Statistical optimization of *in situ* periodontal gel formulation

Desirable values of dependent variables

The criteria for selection of suitable feasible region were as shown in table 6.

Table 6: Desirable values of dependent variable for optimization

Response	Desirable value
Release at 1 hour	30%
Time required releasing 90 % ($t_{90\%}$)	20 hour
Gelation temperature	37^{0}C
Viscosity	Less than 1000 centipoise

Table 7: Values of response variables

Formulation code	Y_1	Y_2	Y_3	Y_4
F1	36.26	13.4	40	610.3
F2	29.52	14.13	38	857.3
F3	28.22	16	37	1380
F4	33.9	18.62	36	751.6
F5	28.43	19.93	34	962.3
F6	27.04	21.85	32	1465.5
F7	29.49	22.25	31	820.6
F8	27.54	23	28	1180
F9	25.81	24	24	1530.2

Optimization of polymer concentration for release drug release at 1 hour (Y_1)

Following equation shows full polynominal equation for drug release at 1 hour

$Y1 = 28.707 - 3.096\ X_1 - 1.86\ X_2 + 1.623\ X_1^2 - 0.316\ X_2^2 + 1.09\ X_1X_2$ …… (Equation 1)

The data clearly indicated that values of release at 1 hour are strongly dependent on the selected independent variables. The fitted equation (for full model) relating the response (release at 1 hour) to the transformed factor is shown in equation 1. The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The coefficients b_1 and b_2 were found to be significant at $p<0.05$ to the prediction of release at 1 hour.

The results of multiple linear regression analysis showed that coefficient b_1 (-3.096) and coefficient b_2 (-1.86) bear a negative sign. Therefore, increasing the values of X_1 and X_2 expected to decreases the values of release at 1 hour of the formulation. The coefficient value of X_1 is more than that of X_2 indicating that X_1 is more effective in relation to release at 1 hour than X_2. As, p values of coefficient X_1^2, $X_2^2$and X_1X_2 were more than 0.05. Hence reduce model was generated by excluding these insignificant variables. Polynominal equation for reduce model is as follows.

$Y_1 = 29.57889 - 3.09667\ X_1 - 1.86\ X_2$ ……..…….... (Equation 2)

Table 8: Statistically predicted values of release at 1 hour

	X_1 (Gellan gum)											
X_2 (poloxamer 407)		**-1**	**-0.8**	**-0.6**	**-0.4**	**-0.2**	**0**	**0.2**	**0.4**	**0.6**	**0.8**	**1**
	-1	34.50	33.88	33.26	32.64	32.02	31.40	30.78	30.16	29.54	28.92	28.30
	-0.8	34.12	33.51	32.89	32.27	31.65	31.03	30.41	29.79	29.17	28.55	27.93
	-0.6	33.75	33.13	32.51	31.89	31.28	30.66	30.04	29.42	28.80	28.18	27.56
	-0.4	33.38	32.76	32.14	31.52	30.90	30.28	29.66	29.05	28.43	27.81	27.19
	-0.2	33.01	32.39	31.77	31.15	30.53	29.91	29.29	28.67	28.05	27.43	26.82
	0	32.64	32.02	31.40	**30.78**	**30.16**	29.54	28.92	28.30	27.68	27.06	26.44
	0.2	32.26	31.65	31.03	**30.41**	**29.79**	29.17	28.55	27.93	27.31	26.69	26.07
	0.4	32.26	31.27	30.65	30.03	29.42	28.80	28.18	27.56	26.94	26.32	25.70
	0.6	31.52	30.90	30.28	29.66	29.04	28.42	27.80	27.19	26.57	25.95	25.33
	0.8	31.15	30.53	29.91	29.29	28.67	28.05	27.43	26.81	26.19	25.57	24.96
	1	30.78	30.16	29.54	28.92	28.30	27.68	27.06	26.44	25.82	25.20	24.58

The statistically predicted values of the release at 1 hour generated by the reduced polynomial regression equation are given in table 9.

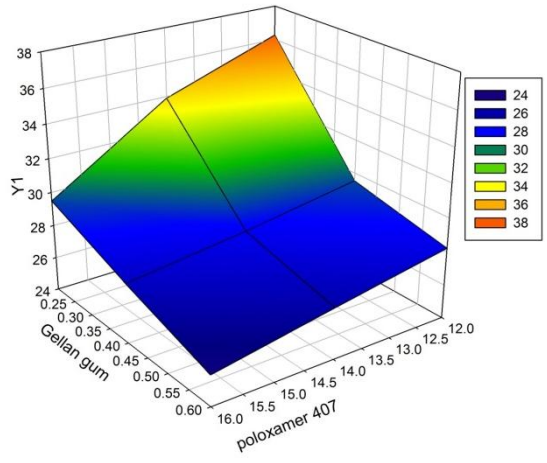

Figure 3: 3D surface plot of release at 1 hour

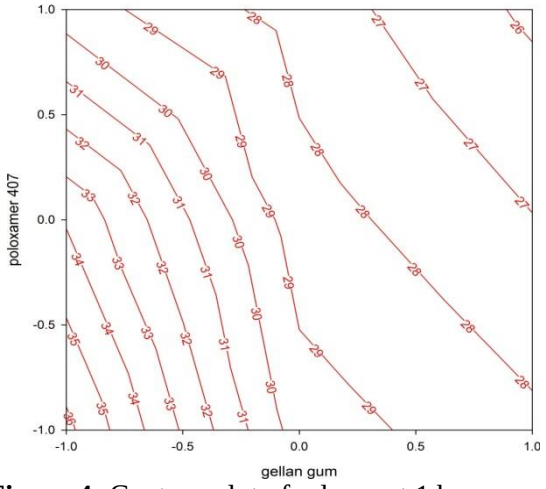

Figure 4: Contour plot of release at 1 hour

Figure 4 & 5 shows the 3D surface plot and contour plot of release at 1 hour verses amount of gellan gum (X_1) and amount of poloxamer 407 (X_2). The plots were drawn using Sigma plot 11.0. Response surface analysis revealed that percentage release of drug at 1 hour decreases by increasing an amount of gellan gum and poloxamer 407.

Optimization of polymer concentration for time required to release 90 % of drug ($t_{90\%}$, Y_2)

Full polynominal equation for $t_{90\%}$ is as follows.

$$\mathbf{Y_2 = 19.911+1.26\ X_1+4.28\ X_2+0.333\ X_1^2-1.336\ X_2^2-0.122X_1X_2} \text{.....} \mathbf{\text{(Equation 3)}}$$

The data clearly indicated that $t_{90\%}$ (Time required for 90% release of drug) values are strongly dependent on the selected independent variables. The fitted equation (for full model) relating the response ($t_{90\%}$) to the transformed factor is shown in equation 3. The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The coefficients b_1 and b_2 were found to be significant at $p<0.05$ to the prediction of $t_{90\%}$.

The results of multiple linear regression analysis showed that coefficient b_1 (1.2633) and coefficient b_2 (4.2866) bear a positive sign. Therefore, increasing the values of X_1 and X_2 expected to increases the time required for release of 90% of drug from the formulation. The coefficient value of X_2 is more than that of X_1 indicating that X_2 is more effective in relation to $t_{90\%}$ than X_1. As, p values of coefficient $X_1^2$and X_1X_2 were more than 0.05. Hence reduce model was generated by excluding these insignificant variables. Reduce polynominal equation for $t_{90\%}$ is described below.

$$\mathbf{Y_2 = 20.133+1.26\ X_1+4.28\ X_2\ -1.336\ X_2^2} \mathbf{\text{ ……...… (Equation 4)}}$$

Table 9 : Statistically predicted values for t_{90}% (Y_2)

	X1 (Gellan gum)										
X2 (poloxamer 407)	**-1**	**-0.8**	**-0.6**	**-0.4**	**-0.2**	**0**	**0.2**	**0.4**	**0.6**	**0.8**	**1**
-1	13.25	13.50	13.75	14.00	14.26	14.51	14.76	15.02	15.27	15.52	15.77
-0.8	14.59	14.84	15.09	15.34	15.60	15.85	16.10	16.35	16.61	16.86	17.11
-0.6	15.82	16.07	16.32	16.57	16.83	17.08	17.33	17.59	17.84	18.09	18.34
-0.4	16.94	17.19	17.45	17.70	17.95	18.20	18.46	18.71	18.96	19.22	19.47
-0.2	17.96	18.21	18.46	18.72	18.97	19.22	19.48	19.73	19.98	20.23	20.49
0	18.87	19.12	19.38	**19.63**	**19.88**	20.13	20.39	20.64	20.89	21.14	21.40
0.2	19.67	19.93	20.18	**20.43**	**20.68**	20.94	21.19	21.44	21.70	21.95	22.20
0.4	20.37	20.62	20.88	21.13	21.38	21.63	21.89	22.14	22.39	22.64	22.90
0.6	20.96	21.21	21.47	21.72	21.97	22.22	22.48	22.73	22.98	23.23	23.49
0.8	21.44	21.70	21.95	22.20	22.45	22.71	22.96	23.21	23.47	23.72	23.97
1	21.82	22.07	22.33	22.58	22.83	23.08	23.34	23.59	23.84	24.09	24.35

The statistically predicted values of the release at 1 hour generated by the reduced polynomial regression equation are given in table 10.

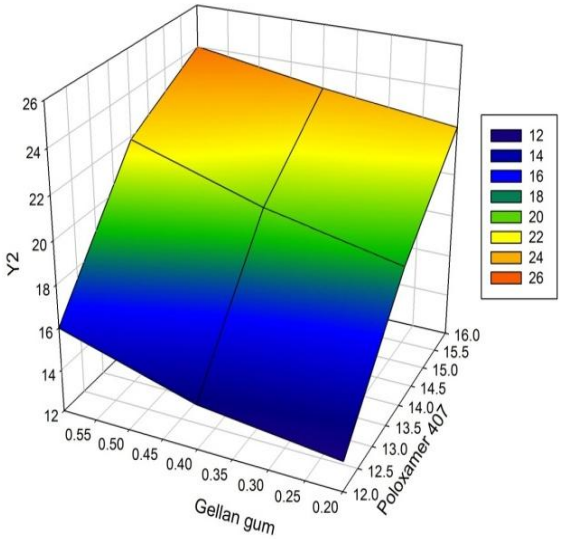

Figure 5: 3D surface plot of t_{90}%

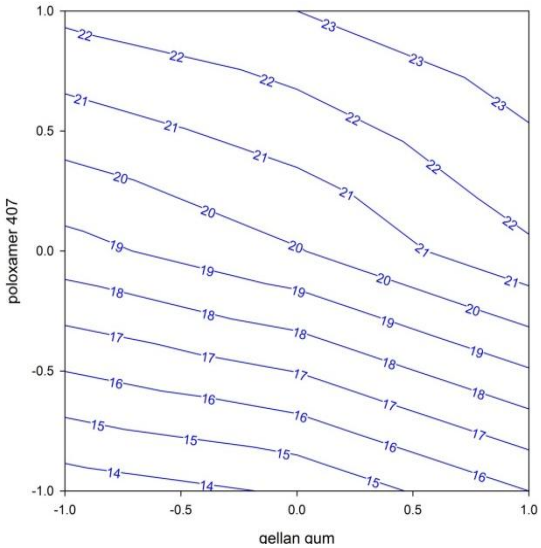

Figure 6: Contour plot of t_{90}%

Figure 6 & 7 shows the 3D surface plot and contour plot of time required for 90% release of drug verses amount of gellan gum (X_1) and amount of poloxamer 407 (X_2). The plots were drawn using Sigma plot 11.0. Response surface analysis revels that time required for 90% release of drug increases by increasing an amount of gellan gum and poloxamer 407.

Optimization of polymer concentration for gelation temperature (Y_3)

Full polynominal equation for gelation temperature is as follows**.**

$$Y_3 = 34 - 2.33 X_1 - 5.33 X_2 + 2.9 (10^{-16}) X_1^2 - 1 X_2^2 - 1 X_1X_2 \quad \text{(Equation 5)}$$

The data clearly indicated that values of gelation temperature are strongly dependent on the selected independent variables. The fitted equation (for full model) relating the response (gelation temperature) to the transformed factor is shown in equation 5. The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative

The results of multiple linear regression analysis showed that coefficient b_1 (-2.3333) and coefficient b_2 (-5.333) bear a negative sign. Therefore, increasing the values of X_1 and X_2 expected to decreases the values of gelation temperature of the formulation. The coefficient value of X_2 is more than that of X_1 indicating that X_2 is more effective in relation to gelation temperature than X_2. As p values of coefficient X_1^2, X_2^2 were more than 0.05. Hence reduce model was generated by excluding these insignificant variables. Reduced polynominal equation is as follows.

$$Y_3 = 33.3333 - 2.3333 X_1 - 5.3333 X_2 \quad \text{(Equation 6)}$$

Table 10: Statistically predicted values of gelation temperature

	X1 (Gellan gum)											
X2 (poloxamer 407)		-1	-0.8	-0.6	-0.4	-0.2	0	0.2	0.4	0.6	0.8	1
	-1	39.83	39.80	39.77	39.73	39.70	39.67	39.63	39.60	39.57	39.53	39.50
	-0.8	40.06	39.95	39.83	39.72	39.61	39.49	39.38	39.27	39.15	39.04	38.93
	-0.6	40.07	39.88	39.69	39.49	39.30	39.11	38.91	38.72	38.53	38.33	38.14
	-0.4	39.87	39.60	39.33	39.05	38.78	38.51	38.23	37.96	37.69	37.41	37.14
	-0.2	39.46	39.11	38.75	38.40	38.05	37.69	37.34	36.99	36.63	36.28	35.93
	0	38.83	38.40	37.97	**37.53**	**37.10**	36.67	36.23	35.80	35.37	34.93	34.50
	0.2	37.99	37.48	36.97	**36.45**	**35.94**	35.43	34.91	34.40	33.89	33.37	32.86
	0.4	36.94	36.35	35.76	35.16	34.57	33.98	33.38	32.79	32.20	31.60	31.01
	0.6	35.68	35.01	34.33	33.66	32.99	32.31	31.64	30.97	30.29	29.62	28.95
	0.8	34.20	33.45	32.70	31.94	31.19	30.44	29.68	28.93	28.18	27.42	26.67
	1	32.51	31.68	30.85	30.01	29.18	28.35	27.51	26.68	25.85	25.01	24.18

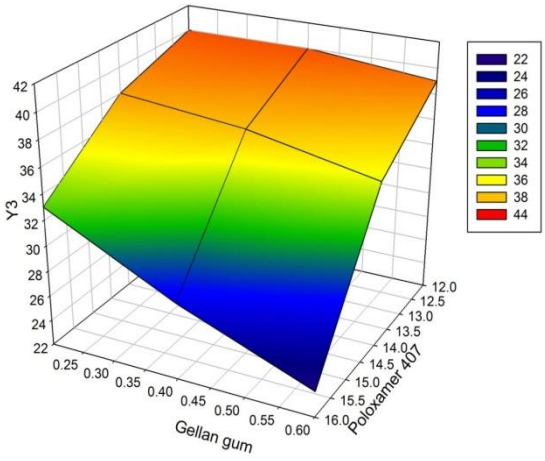

Figure 7: 3D surface plot of gelation temperature

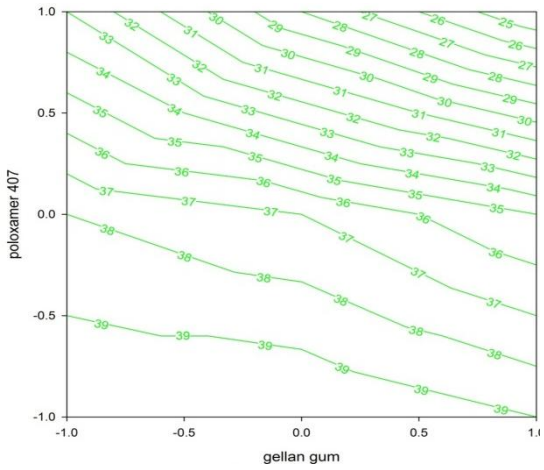

Figure 8: Contour plot of gelation

Figure 8 & 9 shows the 3D surface plot and contour plot of gelation temperature verses amount of gellan gum (X_1) and amount of poloxamer 407 (X_2). The plots were drawn using Sigma plot 11.0. Response surface analysis revels that gelation temperature decreases by increasing an amount of gellan gum and poloxamer 407.

Optimization of polymer concentration for viscosity (Y_4)

$$Y_4=997.68+365.53\ X_1+113.866\ X_2+93.166\ X_1^2+3.266\ X_2^2-15.02\ X_1X_2 \quad \text{.....} \quad \textbf{(Equation 7)}$$

The data clearly indicated that values of viscosity are strongly dependent on the selected independent variables. The fitted equation (for full model) relating the response (viscosity) to the transformed factor is shown in equation 7. The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The coefficients b_1 and b_2 were found to be significant at $p<0.05$ to the prediction of viscosity.

The results of multiple linear regression analysis showed that coefficient b_1 (997.6886) and coefficient b_2 (365.53) bear a positive sign. Therefore, increasing the values of X_1 and X_2 expected to increases the values of viscosity of the formulation. The coefficient value of X_1 is more than that of X_2 indicating that X_1 is more effective in relation to gelation temperature than X_1. As, p values of coefficient X_1^2, X_2^2 and X_1X_2 were more than 0.05. Hence reduce model was generated by excluding these insignificant variables. Reducepolynominal equation for viscosity is described below.

$$Y_4 = 1061.978+365.53\ X_1+113.866\ X_2 \quad \textbf{……..……..……. (Equation 8)}$$

Table 11: Statistically predicted values of viscosity

	X_1 (Gellan gum)											
X_2 (poloxamer 407)		**-1**	**-0.8**	**-0.6**	**-0.4**	**-0.2**	**0**	**0.2**	**0.4**	**0.6**	**0.8**	**1**
	-1	582.6	655.7	728.8	801.9	875.0	948.1	1021.2	1094.3	1167.4	1240.5	1313.6
	-0.8	605.4	678.5	751.6	824.7	897.8	970.9	1044.0	1117.1	1190.2	1263.3	1336.4
	-0.6	628.1	701.2	774.3	847.4	920.6	993.7	1066.8	1139.9	1213.0	1286.1	1359.2
	-0.4	650.9	724.0	797.1	870.2	943.3	1016.4	1089.5	1162.6	1235.7	1308.9	1382.0
	-0.2	673.7	746.8	819.9	893.0	966.1	1039.2	1112.3	1185.4	1258.5	1331.6	1404.7
	0	696.4	769.6	842.7	**915.8**	**988.9**	1062.0	1135.1	1208.2	1281.3	1354.4	1427.5
	0.2	719.2	792.3	865.4	**938.5**	**1011.6**	1084.8	1157.9	1231.0	1304.1	1377.2	1450.3
	0.4	742.0	815.1	888.2	961.3	1034.4	1107.5	1180.6	1253.7	1326.8	1399.9	1473.1
	0.6	764.8	837.9	911.0	984.1	1057.2	1130.3	1203.4	1276.5	1349.6	1422.7	1495.8
	0.8	787.5	860.6	933.8	1006.9	1080.0	1153.1	1226.2	1299.3	1372.4	1445.5	1518.6
	1	810.3	883.4	956.5	1029.6	1102.7	1175.8	1249.0	1322.1	1395.2	1468.3	1541.4

The statistically predicted values of the viscosity generated by the reduced polynomial regression equation are given in table 12.

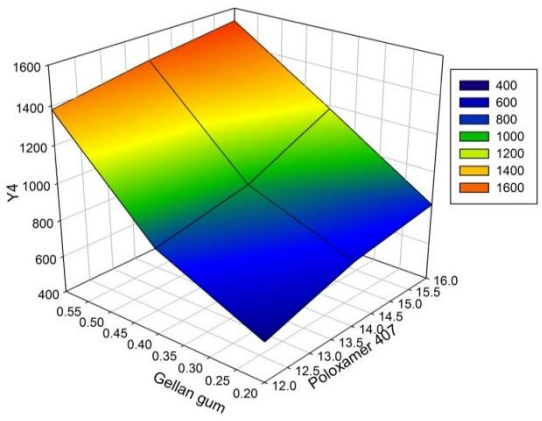

Figure 9: 3D surface plot of viscosity

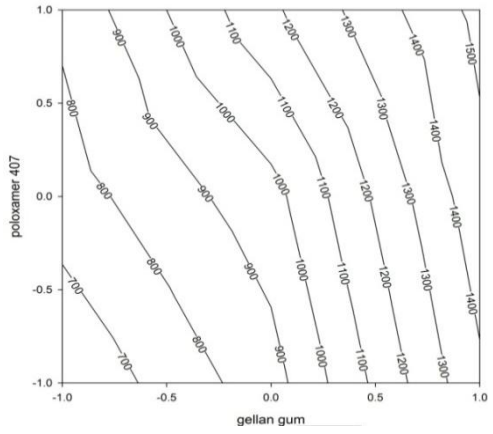

Figure 10: Contour plot of viscosity

Figure 10 & 11 shows the 3D surface plot and contour plot of viscosity verses amount of gellan gum (X_1) and amount of poloxamer 407 (X_2). The plots were drawn using Sigma plot 11.0. Response surface analysis revels that viscosity of formulations increases by increasing an amount of gellan gum and poloxamer 407.

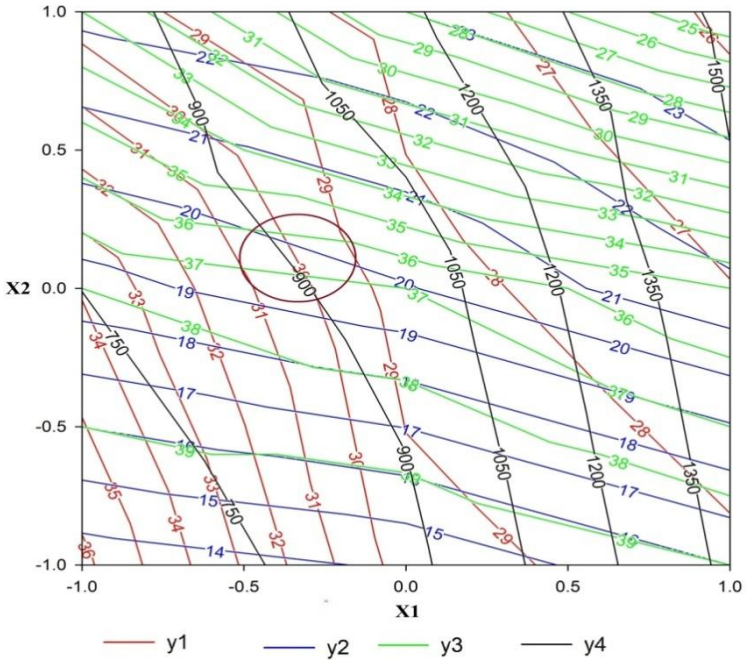

Figure 11: Overlay contour plot of Y_1, Y_2, Y_3 and Y_4

Figure 12 shows overlay of contour plot of Y_1, Y_2, Y_3 and Y_4. In above figure area marked with red circle shows probable area where optimized polymer concentration may obtain.

Table 12: Possible optimum formulation

Formulation no	X_1	X_2	Y_1	Y_2	Y_3	Y_4
1	-0.2	0.2	29.78733	20.6845	35.94163	1011.645
2	-0.2	0.1	29.97333	20.29593	36.54743	1000.259
3	-0.2	0	30.15933	19.88064	37.10003	988.872
4	-0.3	0.2	30.097	20.55817	36.1983	975.0923
5	-0.3	0.1	30.283	20.1696	36.7841	963.7057
6	-0.3	0	30.469	19.75431	37.3167	952.319
7	-0.4	0.2	30.40667	20.43184	36.45497	938.5393
8	-0.4	0.1	**30.59267**	**20.04327**	**37.02077**	**927.1527**
9	-0.4	0	**30.77867**	**19.62798**	**37.53337**	**915.766**

Table no 10, 11 and 12 polymer concentration -0.2 and -0.4 for gellan gum and 0 and 0.2 for poloxamer 407 was selected and further extended to obtain optimized batch having closer desirable values. Table 12 shows possible optimum formulation. Formulation no.1 and 2 have viscosity beyond the desirability. Based on maximum desirability and less utilization of polymers, formulation no 8 and 9 was short listed. Among these two formulations, the values of response variables of formulation no 8 was found closer to desirable value as compared to formulation no 9. Hence formulation no 8 was considered as an optimized formulation.

Table 13: Decoded values of optimized formulation

Independent variable	Coded value	Decoded value
X_1 (Gellan gum)	-0.4	0.32 %
X_2 (Poloxamer 407)	0.1	14.2 %

Table 14: Composition of optimized formulation

Ingredients	Quantity
Levofloxacin	150 mg
Gellan gum	90 mg
Poloxamer 407	4.26 gm.
Distilled water	up to 30 ml

Formulations were added with 0.1% sodium citrate as a sequestering agent, 0.18% methyl paraben and 0.02% propyl paraben as a preservative.

Evaluation of optimized formulation

Table 15: Evaluation of optimized formulation

	Predicted value	Experimental value
Y_1	30.59	29.54
Y_2	20.04	19.84
Y_3	37.02	36.8
Y_4	927.15	935.3

Microbial study of optimized formulation

Zone of inhibition in mm was observed after 24 hours. The data obtained was shown in table no.17.

Table 16: Zone of inhibition

	Zone of inhibition (mm)	
Microorganism	***E.coli***	***S.aureus***
Formulation	**38**	**36**
Drug solution (1mg/ml)	**39**	**37**

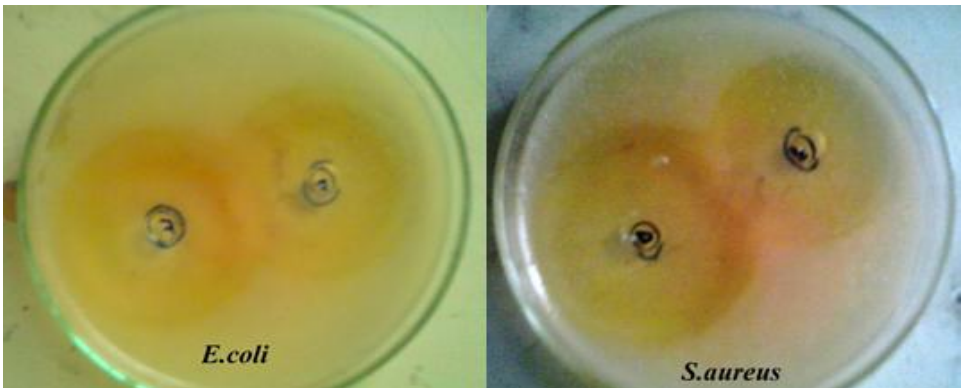

Figure 12: Zone of inhibition (mm)

Figure 15 illustrates the results of antimicrobial studies against *E.coli* and *Staphylococcus aureus* after 24 hours. The formulation and pure drug solution shows identical zone of inhibition in both *E.coli* and *S.aureus.* Results revealed that prepared formulations were found to be effective against *E.coli* and *S.aureus* when compared to pure drug solution.

Kinetic release study of optimized formulation

Table 17: Kinetic release study of optimized formulation

	Zero order	First order	Higuchi	Hixoncrowel	Korsmeyerpeppas
R^2	0.9891	0.7892	0.9928	0.9367	0.9847
F value	1.09	1.26	1.007	1.067	1.0154
					n - value 0.3375

It is evident from above data that Higuchi model was the best fit model for optimized batch. The value of diffusion exponent (n) of the optimized formulation was 0.3375. The obtained n-value for the optimized formulation was 0.3375, indicating that the optimized formulation follow purely Fickian diffusion mechanism for drug release.

Conclusion

In present research work *in situ* periodontal gel containing Levofloxacin was developed with combination of gellan gum and poloxamer 407 using ion sensitive and temperature sensitive approaches. By doing compatibility study, drug was found to be compatible with formulation excipients, it is concluded that the selected polymers are likely to be suitable for preparation of *in situ* periodontal gel formulation. The developed formulations shows satisfactory results for gelation time, gelation temperature, syringeability and other physical properties. Based on maximum desirability and cost effectiveness formulation containing 0.32%w/v of gellan gum and 14.2%w/v of poloxamer 407 was consider as an optimized formulation.

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