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Formulation, Optimization, and Evaluation of Metronidazole *In Situ* Gel for Local Treatment of Periodontitis

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ABSTRACT

The objective of this study was to prepare *In-situ* antibiotic gel for the local treatment of periodontitis, as it will reduce the frequency and increase its efficiency. Metronidazole monotherapy is used orally to reduce the bacterial infection but it has several side effects such as gastrointestinal discomfort, diarrhea, nausea, loss of appetite, metallic taste, urticaria, and discolored urine. These can be avoided by administration of metronidazole directly at periodontal pocket. In the present study, *In-situ* gel was prepared and optimized using 3^2 factorial design which optimized two formulation factors as concentration of Poloxamer 407 (12, 14, 16%) and concentration of Carbopol 934P (0.1, 0.25, 0.4%) to obtain desired gelation temperature, Adhesive force, and Drug release rate. A formulation containing 15.82% Poloxamer 407 and 0.39% Carbopol 934P was chosen as an anoptimized batch from DOE software.

INTRODUCTION

Periodontitis is a common oral bacterial infection that affects tooth-supporting tissues. Periodontitis is generally characterized by inflammation of the gum that damages soft tissues and bone that supports the tooth. It is the infection which affects the *Periodontium* (it is a tissue around the tooth and which supports the tooth)[1]. The disease progresses by affecting alveolar bone slowly and ultimately its complete destruction. The appearance of periodontal pocket is the first clinical manifestation of periodontal disease which offers a breeding ground for microbes which further worsens the condition leading to the deep pocket formation and ultimately tooth loss[2].

Narrow and broad-spectrum antibiotics are used to treat periodontal infections[3]. Antibiotics may be prescribed for patients with acute periodontal infections associated with systemic manifestations, for prophylaxis in medically compromised patients who do not positively respond to conventional mechanical thearapy[4].

Conventional therapy involves scaling and root planning which is painful and does not promise complete healing. Narrow as well as a broad-spectrum antibiotic can be used in periodontal therapy. Oral medication involves low bioavailability and higher dose frequency. This makes local applications attractive due to high bioavailability, low dose, and patient compliance[5].

Poloxamer 407 is a thermosensitive gelling agent and Carbopol 934P is a pH-sensitive gelling agent used in the preparation of *In-situ* gel. The present study describes optimization of Poloxamer and Carbopolconcentrations to obtain desired gelling temperature, drug release, and adhesive force.

MATERIALS AND METHODS:

MATERIAL:

Metronidazole was received as gift sample from Medley Pharmaceuticals, Mumbai. Poloxamer 407, Carbopol 934P and Propylene glycol were purchased from Loba Chemicals. All other chemicals and solvents used were of analytical grade.

METHOD:

Poloxamer was dissolved in a required quantity of water on ice bath and solution was allowed to stand in refrigerator overnight. Carbopol was added to this solution and dissolved with constant stirring using magnetic stirrer and the polymer solution was heated up to 70°C. In remaining quantity of water, Ethanol, Propylene glycol, and Metronidazole were dissolved at 70ºC. Both solutions were mixed with constant stirring. The formulations were stored in a refrigerator until further studies were performed.

Characterization of drug

Calibration of a drug by HPLC method

Calibration of Metronidazole in phosphate buffer pH 6.8 was performed by HPLC method. A stock solution of metronidazole (1000 µg/ml) was prepared in phosphate buffer pH 6.8and suitably diluted to prepare concentrations 2, 4, 6, 8, 10, 12, 14 µg/ml. The mobile phase used was Acetonitrile: Methanol: Water (6.5:2.5:1)[6] having the flow rate of 1ml/min and retention time of 3.4 minutes.

Drug-polymer compatibility study[7]

The physicochemical interactions of MetronidazolewithPoloxamer 407, Carbopol 934P and propylene glycol were studied using Fourier transform infrared spectroscopy (FTIR). Drug and excipients (1:1) were stored in hermetically sealed glass vials at 40°C, 75% RH for one month. The infrared spectra were recorded in the FTIR (Shimadzu) instrument in the wavelength region between 4400 and 600cm-1 by KBr pellet method. The spectra obtained for drug, polymer and physical mixture of drug and polymer were compared and checked for any interaction.

Experimental design and statistical analysis

In this study, a 3^2 full factorial design was employed to optimize the formulation of *In-situ* gel (Table 1). In order to optimize formulations, the amount of Poloxamer 407 and Carbopol 934P were chosen as independent variables. Poloxamer is thermosensitive gelling polymer whereas Carbopol is mucoadhesive and release retardant polymer. Gellation temperature, % Drug release and Mucoadhesion were chosen as responses for the evaluation of formulation (Table 2).

Analysis of data

The data obtained by experimentation was evaluated using Design expert 11.0 software. 3D response surfaces curves were constructed to study the effect of two independent variables alone and in combination on Gelation temperature,%Drug release, and mucoadhesion. All the responses, observed were simultaneously fitted to quadratic models and were evaluated in terms of statistical parameters to get an optimized batch of the formulation. The experimental values of the responses were quantitatively compared with that of the predicted values by calculating residual and linear plots.

Table 1: Independent variable and their selection for a formulation of In-situ gel

Variables		Levels		
		-1	0	+1
A	Concentration of Poloxamer 407 (%)	12	16	18
B	Concentration of Carbopol 934P (%)	0.10	0.25	0.4
Responses		**Goals**		
Y1	Drug release	In range		
Y2	Gellation temperature	Formulation should form a gel at 37°C		
Y3	Mucoadhesive force	Maximum		

Table 2: The 3^2 factorial design of composition of *In-situ* gel containing Metronidazole

Batch code	Simvastatin (%)	Poloxamer 407 (%)	Carbopol 934P (%)	Propylene glycol (ml)
F1	2.5	400	100	0.19
F2	2.5	600	100	0.26
F3	2.5	800	100	0.34
F4	2.5	400	200	0.23
F5	2.5	600	200	0.30
F6	2.5	800	200	0.38
F7	2.5	400	300	0.26
F8	2.5	600	300	0.34
F9	2.5	800	300	0.42

EVALUATION OF *IN-SITU* GEL:

1. Determination of pH

A digital glass electrode pH meter was used for this purpose. pH was noted by bringing the electrode near the surface of the formulations and allowing it to equilibrate for 1 min.A test was performed in triplicate. The results were expressed as a mean of three determinations[8].

2. Syringeability

This test is used to check the ability of the formulation to pass from a needle. A syringeability study was carried out by using a 20 gauge needle. The formulation passes the test if it passes from 20 gauge needle. The test was performed at a temperature between 5-10°C[8].

3. Gelling temperature

Gelling temperature test was performed by two ways. Initially of the formulation without changing its pH and then by changing its pH to 6.8 (oral pH) to check the effect of temperature and effect of temperature and pH on gelling of a formulation. The test was carried out by Miller and Donavan technique[9], in this 2 ml of the aliquot of the formulation was taken in a test tube which was immersed in Cryobath (4ºC). The temperature was gradually increased by 1ºC and left to equilibrate for 5 min at each setting. The temperature at which formulation forms gel was reported as gelation temperature.A test was performed in triplicate and results were expressed as mean and standarddeviation[10].

4. Gelling capacity

The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of Phosphate buffer (pH 6.8) 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. Different grades were allotted as per the gel integrity with respect to time[9,11].

(+) Gels after few minutes dispersed rapidly

(++) Gelation immediate remains for few hours

(+++) Gelation immediate remains for an extended period

5. Viscosity

The viscosity was measured by Brookfield viscometer using spindle number 6 at 100 rpm. Viscosity was measured at two different points i.e. first, the viscosity of gel solution (at a cold temperature 5-10°C) was measured and then this solution was allowed to convert to gel by increasing the temperature of the solution with the help of water bath whose temperature was maintained at 37 ± 1∘C. The viscosity of this formed gel was measured. The test was performed in triplicate. The results were expressed as a mean and standard deviation.

6. Estimation of drug content[12]

Solution (25 mg) was taken in 25 ml volumetric flask, dissolved in 0.1N HCl and volume was made up to 25 ml with 0.1N HCl. The solution was filtered and diluted appropriately with mobile phase [Acetonitrile: Methanol: water (6.5:2.5:1)] and analyzed by HPLC.The test was performed in triplicate and results were expressed as a mean and standard deviation.

7. Determination of gel strength

It was performed using Brookfield CT3 Texture Analyzer. The experiment was done by placing the gels in circular disc apparatus below the ebonite probe. In this probe is then immersed in the formulation (37°C). The formulation was analyzed by keeping 'gelling strength test' mode or compression mode with a test speed of 1.0 mm/s, hold time of 10.0 seconds with 5 g load and trigger value of 50 after which the probe was taken back at the speed of 1 mm/s. The Hardness was measured to know the force required to produce the deformation of gel [13,14].

8. Bioadhesion test

The Mucoadhesion force of the *In-situ* gel was measured using Texture Analyzer (CT3 Texture Analyzer, Make-Brookfield Engineering Labs, Inc., Model Texture Pro CT V1.4 Build 17) equipped with a 5 g load cell. The measurement of Mucoadhesive force was done on excised goat buccal mucosa procured from the slaughterhouse. Buccal mucosa was tied to the probe. The formulation was placed between two circular discs, the upper circular disc had a cavity of 12.7 mm diameter through which the mucosal membrane was exposed to the probe. The probe with the skin was lowered at a speed of 1.0 mm/s and skin was kept in contact with the formulation for 10.0 seconds with 5 g load and trigger value of 50 after

which the probe was taken back at the speed of 1 mm/s. Data collection and calculations were performed using Texture- Pro CT V1.3 Build 14 software. The adhesive force was used to evaluate the Mucoadhesive strength of *In-situ* gel[13,14].

9. *In vitro* drug release

Drug release studies were carried out by using Franz diffusion cell. Cellophane membrane stored in Methanolic: phosphate buffer pH 6.8 (3:7) for 24 h was mounted on a diffusion cell between the donor and receptor compartment. Insitu gel was applied evenly on cellophane membrane. Receptor compartment was filled with Methanolic: phosphate buffer pH 6.8 (3:7) maintained at 37±2°C and stirred continuously at 50±2rpm. Aliquots of 1ml were collected at predetermined 1 hr interval for 6 h and its volume was suitably diluted by mobile phase[Acetonitrile:Methanol: water (6.5:2.5:1)], filtered through a 0.45µm filter and analyzed by HPLC. Freshly prepared, pre-warmed Methanolic: Phosphate buffer pH 6.8 was added to maintain sink condition.

10. *In-vitro* permeation studies

Drug permeation studies were carried out by using Franz diffusion apparatus. Goat buccal (3 cm^2) mucosa was mounted on a diffusion cell between the donor and receptor compartment. Insitu gel was applied on the buccal mucosa. Receptor compartment was filled with Methanolic: Phosphate buffer pH 7.4 (3:7). The fluid was maintained at 37±2°C and stirred continuously at 50±2rpm. Aliquots of 1ml were collected at predetermined intervals for 6 hrs and its volume was made up to 10ml by mobile phase, filtered through a 0.45µm filter and analyzed by HPLC. Freshly prepared, pre-warmed Methanolic: Phosphate buffer was added to maintain sink condition.

RESULT AND DISCUSSION:

Characterization of drug

Calibration of Metronidazole by HPLC:

Calibration curve of Metronidazole in phosphate buffer pH 6.8 was established at 277 nm Beers law was obeyed in the range of 2-10 µg/ ml. A linear regression equation was obtained (y=714467x-363504) with a regression coefficient (R^2) of 0.9972 (fig. 1).

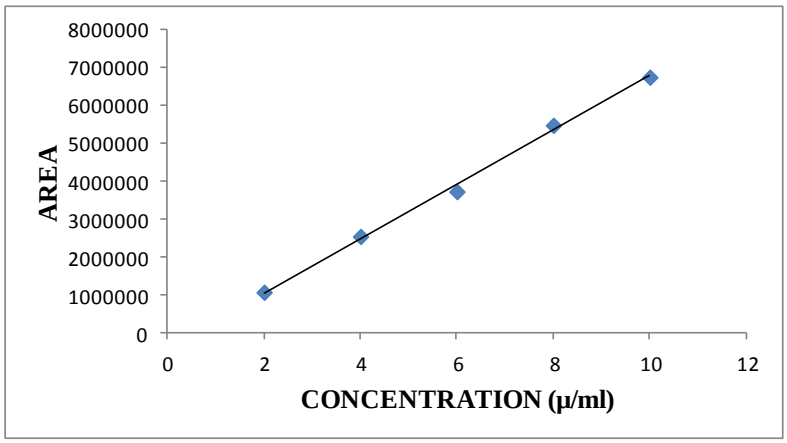

Figure 1: Calibration of Metronidazole in Phosphate buffer pH 6.8

Drug-Polymer Compatability

The FTIR spectral analysis of Metronidazole showed the principal peaks at wave numbers 3530.10, 3213.41, 1456.26, 1263.37 and 1725 cm-1 which are characteristic. In the FTIR spectra of the physical mixture of Metronidazole, Poloxamer, Carbopol, Propylene glycol all peaks of Metronidazole were observed at wave numbers 3245.28, 1454.38, 1251.84 and 1710.99 cm-1 (Figure 2). Since all the peaks were retained in the physical mixture and no new peaks were observed it can be interpreted as there is no interaction between drug and other excipients.

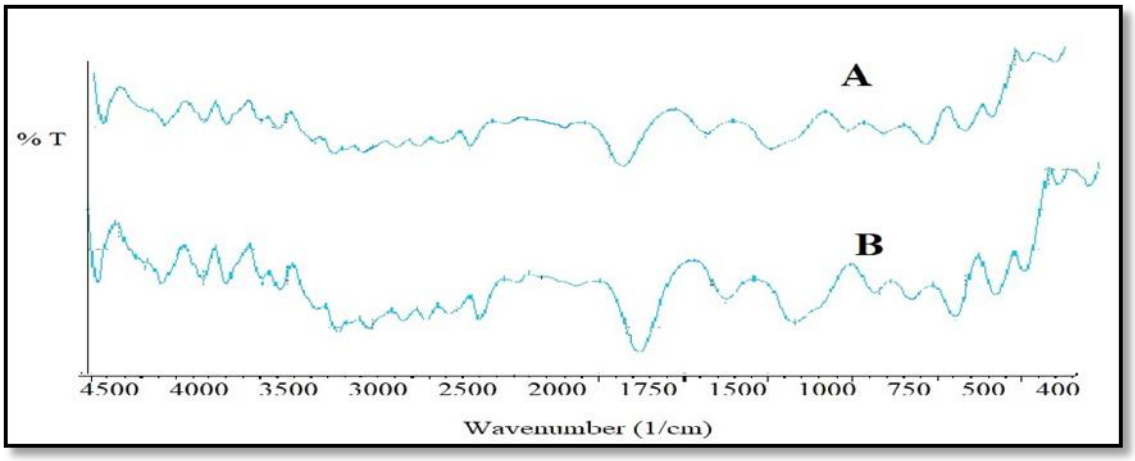

Figure 2: FTIR spectra A) Metronidazole and B) Physical mixture

EVALUATION OF *IN-SITU* GEL (Table 4)

1. Determination of pH

The values (5.73±0.16 to 6.32±0.47) were found to be within the range of oral pH indicating no irritation will be caused by formulation. Since Carbopolcontains carboxylic acid (COOH) between 56-68% which is calculated on the dry basis, thus it is an acidic polymer and has a tendency to decrease formulation pH. From the determined values, it was found that as the concentration of Carbopol increases there is a decrease in formulation pH.

2. Syringeability study

This evaluation parameter indicates the ease with which formulation can be injected into the periodontal cavity. All the developed formulations were syringeable through 20 gauge needle. Results revealed that all the formulations were syringeable at a temperature range of (5-15°C).

3. Gelling temperature

Since Carbopol is pH sensitive polymer this test was carried out for formulation alone and for the formulation whose pH was adjusted by an alkalizing agent. From the results, it revealed that gelling temperature for pH adjusted formulation was less as compared to the formulations whose pH was not adjusted before the test (Table 4).

$$\text{Gelation temperature} = 37.75 - 2.43\,A - 2.77\,B + 0.9000\,AB + 0.2875\,A^2 + 1.59\,B^2$$

…..(1)

Equation (1) shows the quantitative effect of independent variables. The value of correlation R^2 was found to be 0.9255.

As per equation and figure 3, it can be observed that Poloxamer and Carbopolhavea negative effect on gelation temperature i.e. as the concentration of both the polymers increases there is a decrease in gelation temperature.

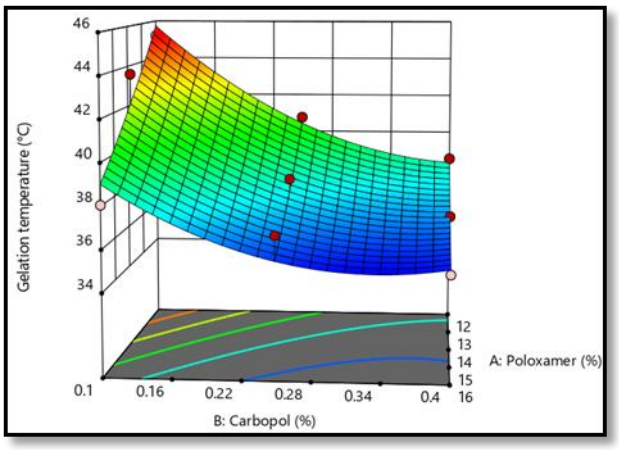

Figure 3: Response surface plots showing the effect of Poloxamer and Carbopol on Gellation temperature of In-situ gel

4. Gelling capacity

Gelling capacity and gelling temperature both play an important role for *In-situ* gel. It was determined to check the ability of the formulation to form a gel due to interaction with different environmental conditions like temperature and pH. The formulation should undergo rapid sol to gel transition at the site of application due to change in pH and temperature and maintain its integrity without eroding or dissolving. Results revealed that as the concentration of polymers increased, the integrity of formed gel also increased and it remained in gel form for a longer period of time ensuring proper drug release from the formed gel (Table 4).

5. Viscosity

Viscosity was found to increase with the concentration of the polymers. Viscosity also affects drug release i.e. as the concentration of the polymers increases there is a decrease in drug release in the formulation. The viscosities of all the formulations at cold and at 37°C were in the range between 980 to 1839 and 6032 to 8907 centipoise respectively (Table 5).

6. Estimation of drug content

The percent drug content for all formulations was determined (Table 5) and was found to be in the range of 96.22-104.05%.

7. Determination of gel strength

Hardness measures the force required to produce the deformation of gel[14]. A formulation having lower hardness value will be easy to remove from the container and ease of administration. From the observations, it can be observed that hardness of formulation increases with the increase in the concentration of polymers (Table 5).

8. Bioadhesion test

Adhesiveness for gel is required to maintain the intimate contact with the mucosa. Both polymer Poloxamer and Carbopol being mucoadhesive in nature show good mucoadhesive property (Table 5).

$$\text{Adhesive force} = 12.96 + 2.13\ A + 1.88\ B + 0.05\ AB + 0.4250\ A^2 - 0.0250\ B^2 \ldots..(2)$$

Above equation (2) indicates that both polymers have a significant effect on the Adhesive force. The value of co-relation R^2 was found to be 0.8689.

As the concentration of polymer increases, there is an increase in viscosity of gel imparting more adhesiveness property to the formulation. From the equation, it can be inferred that both the polymers have a positive effect on adhesive force i.e. as the concentration of both the polymers increases there is an increase in the adhesive force of the formulation.

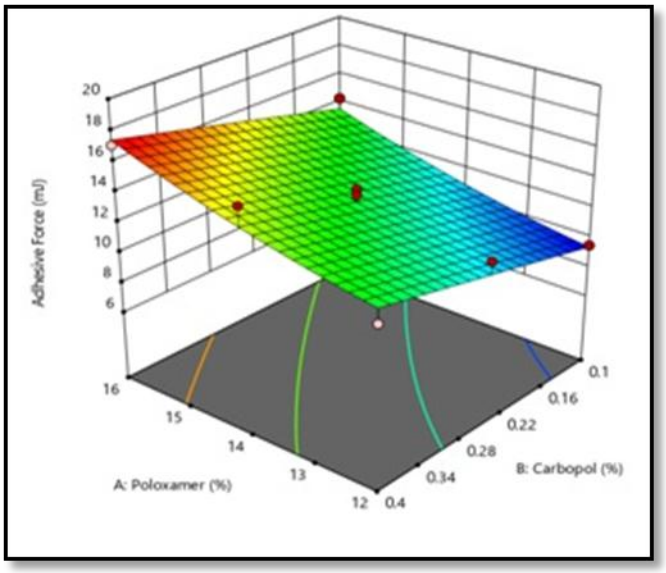

Figure 4: Response surface plots showing an effect of Poloxamer and Carbopol on the Adhesive force of In-situ gel

9. *In vitro* drug release

Studies were carried out for six hours and samples were analyzed by HPLC, the plot of drug release rate vs time is shown in figure 4, formulation F9 showed the prolonged release of metronidazole (6.95% per hour) from the formulation. The prolongation of release is due to increase in polymer concentration leading to high viscosity.

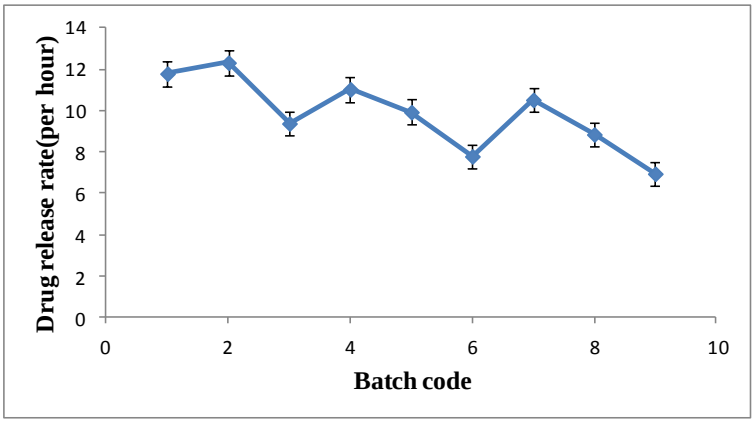

Figure 5: Drug release rate of formulations F1-F9

$$\text{Drug release rate} = 10.02 - 1.54\,A - 1.20\,B - 0.2912\,AB - 0.7369\,A^2 + 0.4381\,B^2$$

…..(3)

The above equation (3) shows the quantitative effect on independent variables. The value of correlation R^2 was found to be 0.9341.

As per the equation, it can be concluded that th0at both the polymers i.e. Poloxamer and Carbopol has a negative effect on drug release leading to the conclusion that as the concentration of polymer increases there is an increase in viscosity of In-situ gel at oral temperature leading to slow release of drug from the formulation. F9 showed the prolonged release of drug from formulation (Table 4).

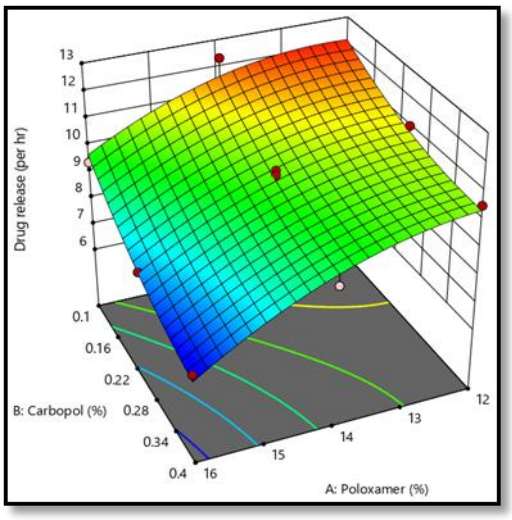

Figure 6: Response surface plots showing the effect of Poloxamer and Carbopol on Drug release of In-situ gel

10. *In vitro* permeation studies

Considering all the responses optimized batch was derived with the help of DOE design software. The optimized batch was found to be with a concentration of Poloxamer 407 and Carbopol 934 P as 15.82% and 0.39% respectively. *In vitro* permeation studies were carried out for an optimized batch using goat buccal mucosa. Methanolic: Phosphate buffer pH 7.4(3:7) was used as diffusion medium and samples were removed, diluted appropriately by mobile phase [Acetonitrile:Methanol: water (6.5:2.5:1)] and analyzed by HPLC. A flux of optimized batch was found to be 1.98 mg/hr/cm^2.

Table 4: pH, Syringibility, Gelation temperature, Gelling capacity, Drug release rate

Batch code	pH	Syringibility	Gelation temperature (°C)		Gelling capacity	Drug release rate at 6th h (%)
			treatment without an alkalizing agent	treatment with an alkalizing agent		
F1	6.25±0.12	Test passes	47.6	45.2	++	11.79
F2	6.2±0.35	Test passes	46.1	43.6	++	12.32
F3	6.32±0.47	Test passes	44.3	38.1	++	9.38
F4	6.14±0.14	Test passes	42.7	40.8	+++	11.03
F5	6.1±0.34	Test passes	40.3	38.4	+++	9.93
F6	5.98±0.1	Test passes	37.1	35.8	+++	7.79
F7	5.73±0.16	Test passes	36.4	34.6	+++	10.52
F8	5.67±0.21	Test passes	34.2	32.6	+++	8.85
F9	5.45±0.13	Test passes	33.4	31.1	+++	6.95

Table 5: Viscosity, Drug content, Hardness, Adhesive force

Batch code	Viscosity		Drug content (%)	Hardness (g)	Adhesive force (g)
	Viscosity at 4°C	Viscosity at 37°C			
F1	980	6032	97.2±0.38	38.1	9.5
F2	1037	6439	102.23±0.11	39.2	10.2
F3	1179	6772	96.22±0.59	43.7	14.3
F4	1246	6976	98.79±0.07	48.59	12.1
F5	1369	7357	104.05±0.18	49.6	13.8
F6	1448	7619	99.75±0.33	50.5	15.1
F7	1566	8024	102.57±0.04	51.8	12.1
F8	1689	8497	96.32±0.12	52.5	16.1
F9	1839	8907	98.68±0.1	53.97	17.1

CONCLUSION:

DOE was used to establish design space for the development of the formulation with desired attributes using 3^2 factorial design. Combination of pH-sensitive polymer Poloxamer 407 and pH-sensitive polymer Carbopol 934P was used to develop a formulation for the local treatment of Periodontitis. An optimized batch containing 15.82% Poloxamer 407 and 0.39% Carbopol 934P was chosen from DOE software which showed prolonged drug release from the formulation.

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