

Formulation and evaluation of periodontal *in situ* gel

Kevin Garala, Parth Joshi, Malay Shah¹, A Ramkishan², Jaydeep Patel

Department of Pharmaceutics, Atmiya Institute of Pharmacy, Kalawad Road, Rajkot, ¹Gujarat Forensic Sciences University, Institute of Research and Development, Sector 18A, Gandhinagar, Gujarat, ²Central Drugs Standard Control Organization, Government of India, New Delhi, India

Abstract

Background: The present study was aimed to develop and optimize *in situ* gel for the treatment of periodontal disease. **Materials and Methods:** Temperature-sensitive *in situ* gel containing 0.1% w/v Chlorhexidine hydrochloride was formulated by cold method using different polymers. Preliminary study was carried out to optimize different types and concentration of polymers such as Poloxamer 188, Poloxamer 407, Gellan gum, and Carbopol 934P. Central composite design was employed for optimization of the effect of independent variables such as Poloxamer 407 and Carbopol 934P on responses such as gelation temperature, spreadability, cumulative percentage release at 2 h, and time for 50% drug release (t_{50} %). Each formulations were evaluated for clarity, pH, gelation temperature, spreadability, drug content, *in vitro* drug release, t_{50} %, and cumulative percentage drug release at 2 h. **Results:** Results of evaluation parameters revealed that the drug release, gelation temperature was considerably decreased with increasing t_{50} % as the concentration of each polymer was increased. The desirability function was utilized to find out optimized formulation of the factorial design. Formulation F6 showed the highest overall desirability of 0.6283 and, therefore, this formulation was considered to be the optimized formulation. The % relative error was calculated, which showed that observed responses were in close agreement with the predicted values calculated from the generated regression equations. **Conclusion:** The clarity, pH, drug content of all formulations was found to be satisfactory. Further, all the formulations showed sustained drug release for a period of 6 h, which satisfied to treat periodontal disease.

Key words: Carbopol 934P, chlorhexidine hydrochloride, *in situ* gel, periodontal disease, poloxamer 407

INTRODUCTION

Periodontal disease is term used to ascribe to some pathological conditions characterized by degeneration and inflammation of gums, periodontal ligaments, alveolar bone, and dental cementum.^[1] It is a localized inflammatory reaction caused by bacterial infection of a periodontal pocket accompanying with sub-gingival plaque.^[2] Although bacteria are the principal cause of periodontal disease, the appearance of microbial pathogenic factors alone may not be enough to cause periodontitis. Periodontal pathogens generate destructive by-products and enzymes that break extracellular matrices as well as host cell membranes to

produce nutrients for their growth. In doing so, they start damage directly or indirectly by triggering host-mediated responses that lead to self-injury.^[1] In the early phase of the disease, inflammation is localized to the gingiva called gingivitis but extends to deeper tissues in periodontitis, leading to gingival swelling, bleeding, and bad breath. In the late phase of the disease, the supporting collagen of the periodontium is disintegrated, alveolar bone begins to resorb, and gingival epithelium migrates along the tooth surface forming a 'periodontal pocket.'^[2,3]

In situ gel forming formulations current a novel idea of deliver drugs to patients as a liquid dosage form, yet achieve sustained release of drug for the desired period.^[4] Different delivery systems based on polymers have been developed, which are able to increase the residence time of the formulation at absorption site of drugs. In recent years, there has been an increasing interest in water-soluble polymers that are able to form gels after application to delivery site. These so-called *in situ* gelling polymers are highly advantageous compared with other polymers because, in contrast to very strong gels, they can be easily applied in liquid form to the site of drug absorption. At the site of drug absorption, they swell to form a strong gel that is capable of prolonging the residence time of the active substance.^[5]

Chlorhexidine, a bisbiguanide compound, has been shown to possess a broad-spectrum of topical anti-microbial activity.^[6] It

Address for correspondence:

Mr. Kevin Garala,
Department of Pharmaceutics,
Atmiya Institute of Pharmacy, Yogidham Gurukul,
Kalawad Road, Rajkot - 360 005, Gujarat, India.
E-mail: kevincgarala@gmail.com

Access this article online

Quick Response Code: 	Website: www.jpionline.org
	DOI: 10.4103/2230-973X.108961

has been used by dental professionals for plaque control and for the treatment of gingival inflammation.^[7] Chlorhexidine was primarily used in mouth-rinses and was recommended in the hygiene phase of treatment as an adjunct to tooth-brushing. Most attention, however, has been focused on the use of chlorhexidine during the operative and immediate post-operative phases of non-surgical and surgical periodontal treatment.^[8]

The Poloxamer consist of more than 30 different non-ionic surface-active agents. These polymers are ABA-type triblock co-polymers composed of PEO (A) and PPO units (B).^[9] Pluronic are commercially available in a range of molecular weights, composition ratios, and forms, it would be useful to mention the nomenclature rules for these copolymers. The letter in the notation stands for liquid (L), paste (P), or flakes (F), whereas the first two numbers indicate the molecular weight of the PPO block and the last number the weight fraction of the PEO block. For example, the commonly used in biomedical applications F127 has a weight percentage of 70% PEO and a molecular weight of PPO around 4000.^[10]

Carbopol is a well-known pH-dependent polymer, which stays in solution form at acidic pH but forms a low viscosity gel at alkaline pH.^[11] Carbopols, which are very high molecular weight polymers of acrylic acid, have been used mainly in liquid or semi-solid pharmaceutical formulations, such as gels, suspensions and emulsions, as a thickening agent, in order to modify the flow characteristics.^[12]

MATERIALS AND METHODS

Materials

Chlorhexidine hydrochloride was obtained as a gift sample from Cadila Healthcare Pvt. Ltd. Ankleshwar, Gujarat. Poloxamer 188 was purchased from Hi media Laboratories Pvt. Ltd. Mumbai, India. Poloxamer 407 was purchased from Zeel pharmaceutical Pvt. Ltd. Mumbai, India.

Gellan gum was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. Carbopol 934P were obtained from Noveon Ltd. Mumbai, India. Triethanolamine were obtained from Burgoyne burbidges and co. Mumbai, India.

Optimization of types and concentration of polymers

Different grades of Poloxamer i.e., Poloxamer 188, Poloxamer 407, and Gellan gum was used for the preparation of *in situ* gel formulation. The periodontal *in situ* gel was prepared using different Poloxamer 188, Poloxamer 407 concentration by cold method. This method involved slow addition of polymer in cold water with continuous stir. The formed mixtures were stored overnight at 4°C and studied for their gelation temperature to select optimum concentration of Poloxamer grades for effective *in situ* gel formulation.

Method of preparation of *in situ* gel

The method of preparation of *in situ* gel involved slow addition of polymers (i.e., Poloxamer 188, Poloxamer 407), and methyl

paraben were solubilized in required quantity of cold deionized water. An appropriate amount of (0.1% w/v) Chlorhexidine HCl was solubilized in polymer solution with continuous stirring until uniform drug solution obtained. 0.1% w/v Chlorhexidine thermosensitive hydrogel is a strong candidate as a local drug delivery system for periodontal treatment.^[13] Required quantities of Carbopol 934P were added into the solution with continuous stirring by mechanical stirrer) until uniform solution obtained. The final solution was kept overnight in refrigerator at 5°C to completely dissolve polymers in solution, and then addition of small amount of triethanolamine (TEA) was added to adjust the pH 7.^[14]

Experimental design

Central composite design (CCD) was employed for systemic study of joint influence of the effect of independent variables [Poloxamer 407 (X_1) and Carbopol 934P (X_2)] on responses such as gelation temperature (°C), spreadability (gm·cm/sec), cumulative percentage release (CPR) at 2 h and time for 50% drug release ($t_{50\%}$). Based on preliminary trials, two factors were determined as follows: Poloxamer 407 (X_1): 15–19% w/v and Carbopol 934P (X_2): 0.20–0.40% w/v. In this design, two factors with five levels were probed to investigate the main effects and interaction of the two factors on two responses [Table 1]. The design consists of nine runs (4 factorial points, 4 star points, and 1 center point) and four replicated runs (center points) yielding 13 experiments in total. The main purpose of the replication runs was to increase the precision and to minimize experimental error. The data obtained for the four responses in each trial were fitted to classical second order polynomial model. The mathematical model was expressed as follow, second order polynomial model:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2 \quad \dots(1)$$

Where y is the measured response, b_0 is an intercept, and b_1 - b_5 is the regression co-efficients, X_1 , X_2 represents the main effect, the quadratic effect, and $X_1 X_2$ is the interaction effect. The effects were evaluated statistically at 0.1 level ($\alpha = 1.414$). Data were

Table 1: Layout of Experimental design batches

Formulations	X1 (% w/v)	X2 (% w/v)			
F1	- α	0			
F2	-1	-1			
F3	-1	+1			
F4	0	- α			
F5	0	+ α			
F6	+1	-1			
F7	+1	+1			
F8	+ α	0			
F9-F13	0	0			
Factor - α	Level				
	- α	-1	0	+1	+ α
X1:	14.17	15	17	19	19.83
Poloxamer 407 (% w/v)					
X2: Carbopol 934P (% w/v)	0.16	0.2	0.3	0.4	0.44

($\alpha = 1.414$)

further analyzed by Microsoft Excel® 2007 for regression analysis. Analysis of variance (ANOVA) was implemented to assure that there was no significant difference between the developed full model and the reduced model. Response surface plots were plotted to study response variations against two independent variables using Design Expert® Version 8 software.^[15,16]

Desirability function

The desirability value is a satisfaction index ranging between 0 and 1, characterizing the level of a response considering a particular objective. For the optimization of a process involving multiple responses, the individual responses have to be combined to define a product with the desired characteristics. The desirability value^[17,18] is used to classify experiments according to the degree of satisfaction. For each response (Yi), the desirability function D (Yi) assigns values from 0 and 1 to the possible values of Yi; D (Yi) =0 corresponding to a completely undesirable value of Yi, and D (Yi) =1 corresponding to a completely desirable or ideal response value. Two desirability functions D (Yi) were used depending on whether the response Yi had to be maximized or minimized up to a target value (T).

Evaluation of trans-dermal films clarity

The *in situ* gel solutions was prepared and evaluated visually for clarity against white and black backgrounds in light.^[19]

pH measurement

The two areas of critical importance are the effect of pH on solubility and stability. The pH of *in situ* gel formulation should be such that the formulation was be stable at that pH and at the same time, there would be no irritation to the patient upon administration of the formulation. *In situ* gel formulations should have pH range in between 6.2 and 7.4. The developed *in situ* gel formulations were evaluated for pH by using calibrated digital pH meter. The pH meter was calibrated before each use with standard pH 4, 7, and 9.2 buffer solutions. The formulation temperature was maintained at 25°C.^[20,21]

Gelation temperature

Gelation temperature was assessed using a modified Miller and Donovan technique.^[22] Two mL aliquots of the gel were transferred to test tubes sealed with parafilm and immersed in a water bath at 4°C. The temperature of the bath was increased in increments of 1°C and left to equilibrate for 15 min at each new setting. The samples were examined for gelation, which was deemed to have occurred when the meniscus would no longer move upon tilting through 90°C. All measurements were performed in triplicate (n = 3).^[23]

Drug content

The prepared *in situ* gel formulations were analyzed for drug content by transferring 1 mL of formulation in 100 mL volumetric flask. In this volumetric flask, 50 mL of phosphate buffer with pH 6.8 was added, followed by continuous shaking until the gel was totally dispersed to give a clear solution. Final volume was

adjusted to 100 ml with the help of phosphate buffer pH 6.8 and filtered the solution. Drug concentration in filtrated solution was determined spectrophotometrically at 257 nm using UV-Visible spectrophotometer (Shimadzu 1700, Japan).^[20]

Instrumental analysis

Fourier transform infrared spectroscopy

Pre-formulation studies regarding the drug-polymer interaction are, therefore, very critical in selecting appropriate polymers. The FTIR spectra of the samples were recorded using KBr pellets method. The samples were then placed in the sample holder of the instrument. These were analyzed by FTIR to study the interference of polymer for drug analysis. The integrity and compatibility of the pure drug and polymer were evaluated with the help of IR spectra of the pure drug, and polymer was carried out using FTIR spectrophotometer.^[24]

Differential scanning calorimetry

DSC study was carried out using DSC-60 instrument (Shimadzu corporation, Japan) to check the formation as well as the compatibility of ingredients. Thermogram of pure drug (Chlorhexidine hydrochloride) and optimized formulation was scanned for DSC. Accurately weighed samples were placed on aluminum plates sealed with aluminum seals and heated at constant temperature of 10°C/min over a temperature range of 0-300°C.^[25,26]

Viscosity

The viscosity determination of the prepared optimized *in situ* gel formulation was determined using Brookfield Digital Viscometer (LVDV III U, Brookfield Engineering Labs. USA). The optimized *in situ* gel formulation was taken in a beaker and maintained at room temperature. The measurements were carried out using spindle no. 62 at the speed of 50 rpm in the sample, and the viscosity was measured at 10 min after the rotation of the spindle.^[20,26]

Spreadability test

Spreadability was measured by apparatus, which was suitably modified in the laboratory and used for the study. It consists of a wooden block, which was provided by a pulley at one end. A ground glass slide was fixed on this block. An excess of gel (about 1 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. One kg weight was placed on the top of the two slides for five minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gms. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability. The time required to separate the two slides and spreadability measured by using following equation^[27,28]

$$S = M \times \frac{L}{T} \quad \dots(2)$$

Where,

M = weight tide to upper slide

L = length moved on the glass slide

T = time taken to separate two slides.

In vitro drug release study

The *in vitro* drug release study of Chlorhexidine HCl from *in situ* gel was determined using a Franz-diffusion cell. The cellophane membrane was fixed on the receptor cell.^[29] The donor cell was filled with 1 g of *in situ* gel formulation. The receptor compartment was filled with phosphate buffer pH 6.8 and constantly stirred with a small magnetic bar at a speed of 200-250 rpm during the experiments to confirm homogeneity, and temperature was maintained at $37 \pm 1^\circ\text{C}$ by circulating hot water through the jacket of Franz-diffusion cell. The 0.5 mL samples were withdrawn at scheduled time intervals (0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 min) and were replaced with same volume of pH 6.8 phosphate buffer to maintain the sink condition. Samples were analyzed at 257 nm on UV-visible spectrophotometer.^[20]

Stability study

Drug decomposition or degradation occurs during stability, because of chemical alteration of the active ingredients or due to product instability, lowering the concentration of the drug in the dosage form. The stability of pharmaceutical preparation should be evaluated by accelerated stability studies. Stability studies of periodontal *in situ* gel were carried out to determine the effect of contents on the stability of the drug. The accelerated stability studies were carried out according to ICH guidelines by storing the samples at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH for 1 month using stability chamber (Remi, India). The optimized formulation were evaluated for clarity, pH measurement, drug content, gelation temperature, spreadability, CPR at 6 h, CPR at 2 h, and $t_{50\%}$.^[30]

RESULTS AND DISCUSSION

Fourier transform infrared spectroscopy

FTIR study was performed to evaluate compactability between drug and polymer utilized in study. IR spectrum of Chlorhexidine HCl is characterized by principal absorption peaks at 2542 , 2956.67 cm^{-1} (N-H stretch, salt of secondary amine), 1519.80 cm^{-1} (N-H bending, secondary aromatic amine), 1259.43 cm^{-1} (C-N stretch, secondary aromatic amine), 1022.20 , 1155.28 cm^{-1} (C-N stretch) shown in Figure 1. IR spectrum of poloxamer 188 is characterized by principal absorption peaks at 2883.38 cm^{-1} (C-H stretch aliphatic), 1348.15 cm^{-1} (in-plane O-H bend), and 1107.06 cm^{-1} (C-O stretch) shown in Figure 2. IR spectrum of poloxamer 407 is characterized by principal absorption peaks at 2893.02 cm^{-1} (C-H stretch aliphatic), 1355.86 cm^{-1} (in-plane O-H bend), and 1124.42 cm^{-1} (C-O stretch) shown in Figure 3. IR spectrum of Carbopol 934P is characterized by principal absorption peaks at 3089.75 cm^{-1} (O-H stretching), 1706.88 cm^{-1} (carboxyl group) shown in Figure 4. The IR spectrum of physical mixture of drug and polymers is characterized by principal absorption peaks at 2935.45 cm^{-1} (N-H

stretch, salt of secondary amine), 1529.45 cm^{-1} (N-H bending, secondary aromatic amine), 1155.28 cm^{-1} (C-N stretch).

The interaction between the drug and the polymers often leads to identifiable changes in the FTIR profile of solid systems. FTIR spectra for pure drug, polymers, and physical mixture of drug and polymers have been depicted in Figures 1-5. The spectrum of physical mixture of drug and polymers was equivalent to the addition spectrum of pure drug, indicating no interaction occurring in the simple physical mixture of drug and polymer as shown in Figure 5.

Selection of polymers

Different grades of Poloxamer i.e. Poloxamer 188, Poloxamer 407, and Gellan gum were used for the preparation of *in situ* gel formulation. From all polymers, Poloxamer 188 and Poloxamer 407 were formed viscous clear solution and formed gel at elevated temperature. Gellan gum was not used to prepare formulation because of its gel formation during formulation shown in Table 2. So, Poloxamer 188 and Poloxamer 407 were selected as polymer for *in situ* gel formulation on bases of visual examination and gelation temperature.

Selection of polymers concentration

The gelation temperatures have been considered to be suitable if they are in the range of body temperature. As the temperature of the periodontal cavity is nearest 37°C , this study aimed at preparing the liquid formulations that may gel below 37°C . The gelation of Poloxamer vehicles was known to result from the change in micellar number with temperature. With increasing temperature, the number of micelles formed increases as a consequence of the negative co-efficient of solubility of block copolymer micelles. Eventually, the micelles become so tightly packed that the solution becomes immobile, and gel is formed. Conformational changes in the orientation of the methyl groups in the side chains of poly (oxypropylene) polymer chains, constituting the core of the micelle, with expulsion of the hydrating water from the micelles will contribute to the gelation phenomenon. Gelation temperatures for Poloxamer 188 and Poloxamer 407 gels were observed for the different concentration range of polymer, and it was found that the gelation temperature of formulation decreased with increasing concentration of polymer.

As the concentration of polymer increases, the gel structure becomes more closely packed with the arrangement in the lattice pattern.^[23] Result revealed that the formulation containing Poloxamer 188 have found to be higher gelation temperature compared to body temperature. So, gelation temperature of Poloxamer 188 *in situ* gel was not fulfilling the requirement of *in situ* gel formulation. Only 15% w/v and 20% w/v concentration

Table 2: Selection of polymer

Polymers	Observation
Poloxamer 188	Clear solution formed
Poloxamer 407	Clear solution formed
Gellan gum	Gel formed

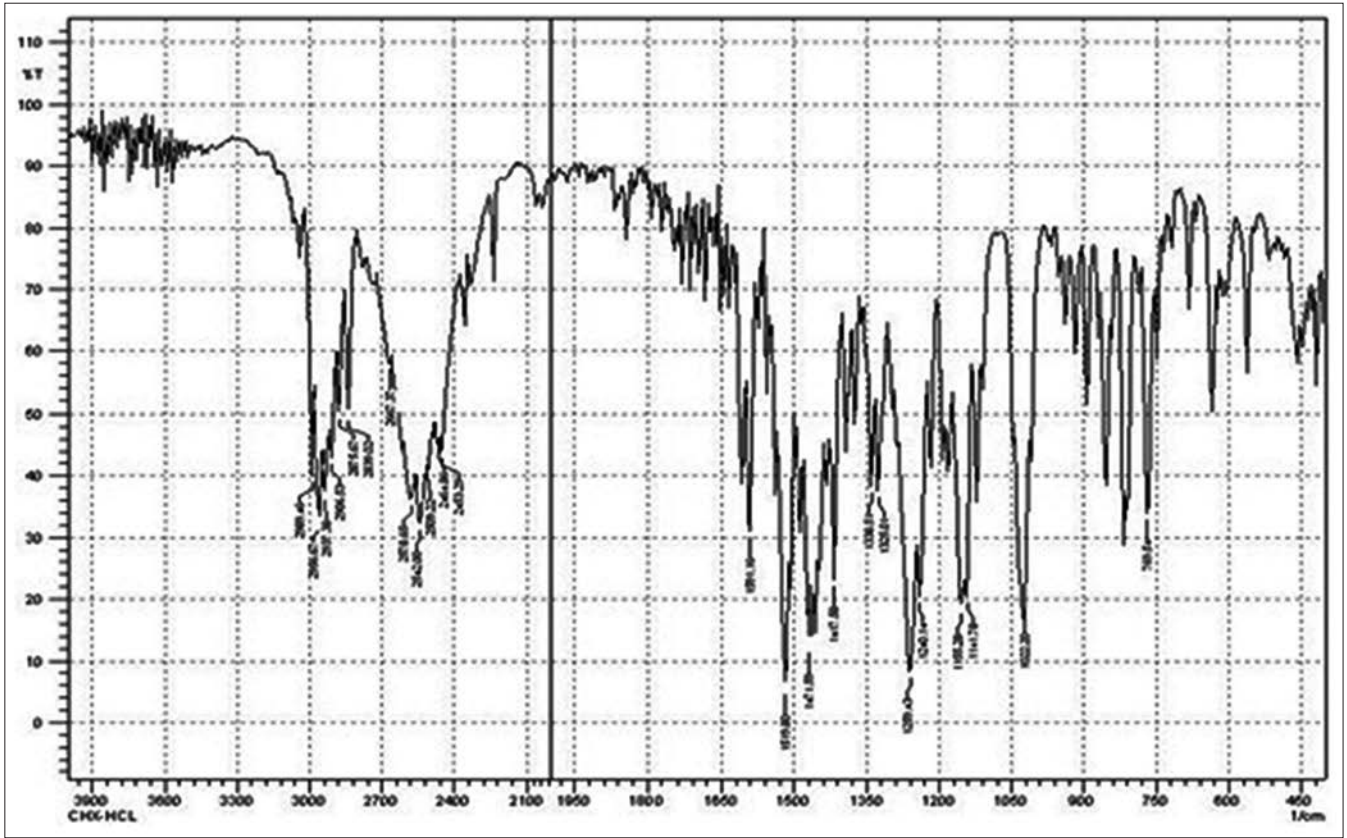


Figure 1: FTIR spectrum of chlorhexidine HCl

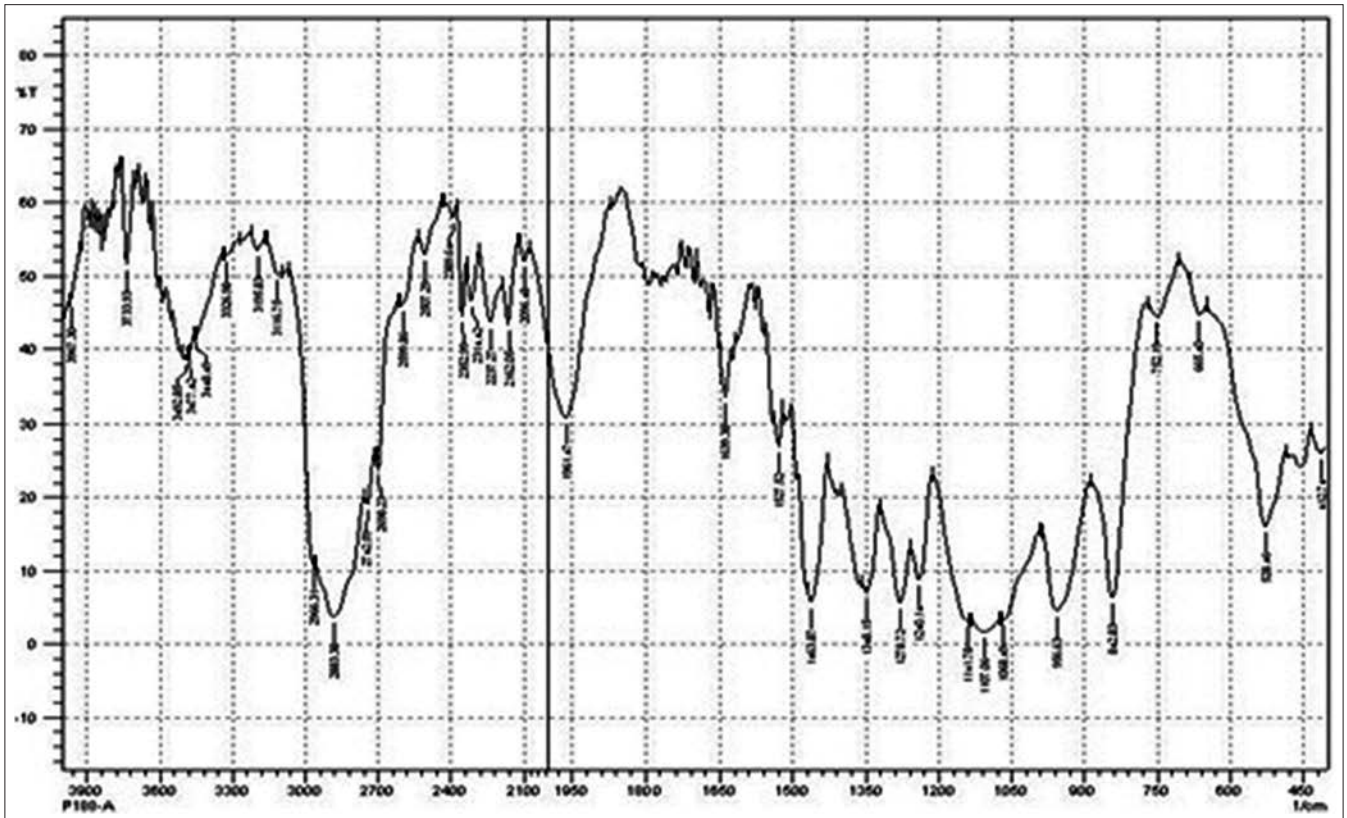


Figure 2: FTIR spectrum of poloxamer 188

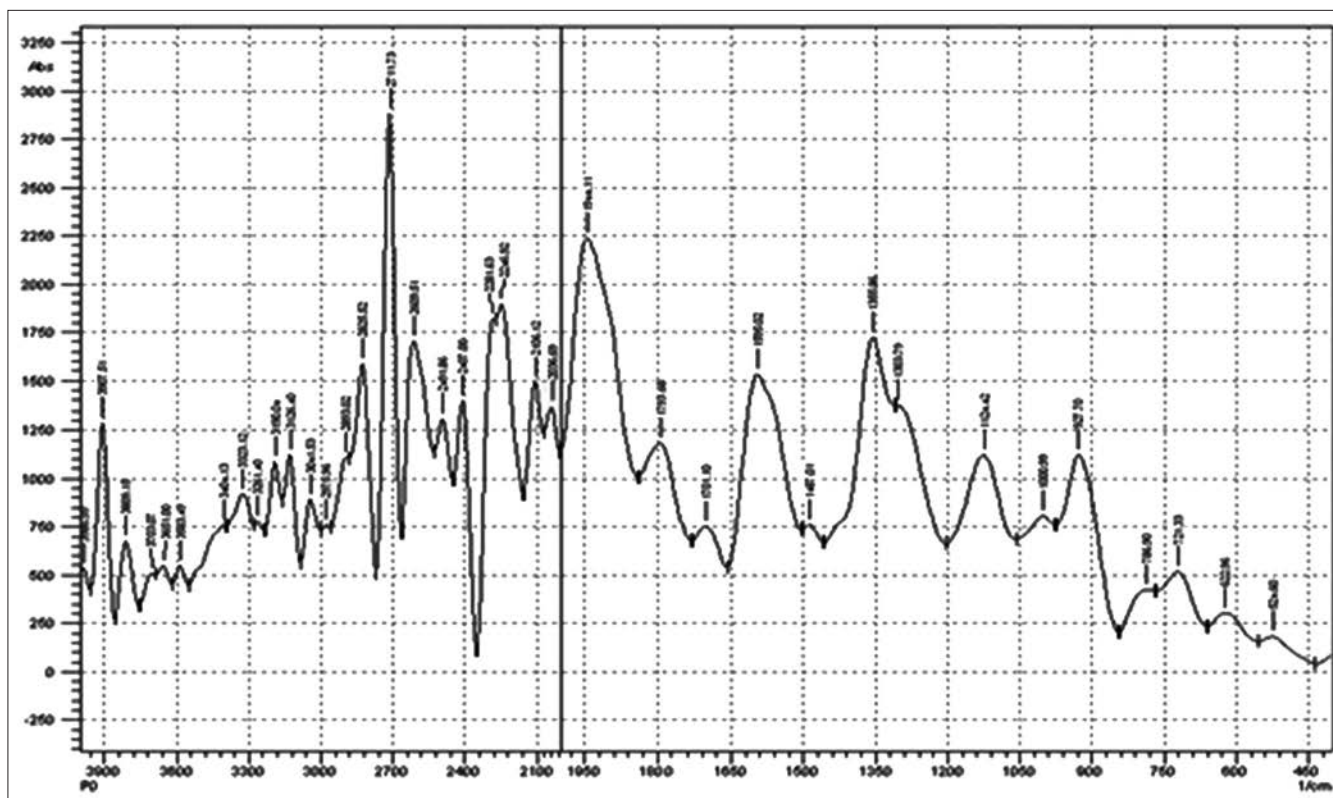


Figure 3: FTIR spectrum of poloxamer 407

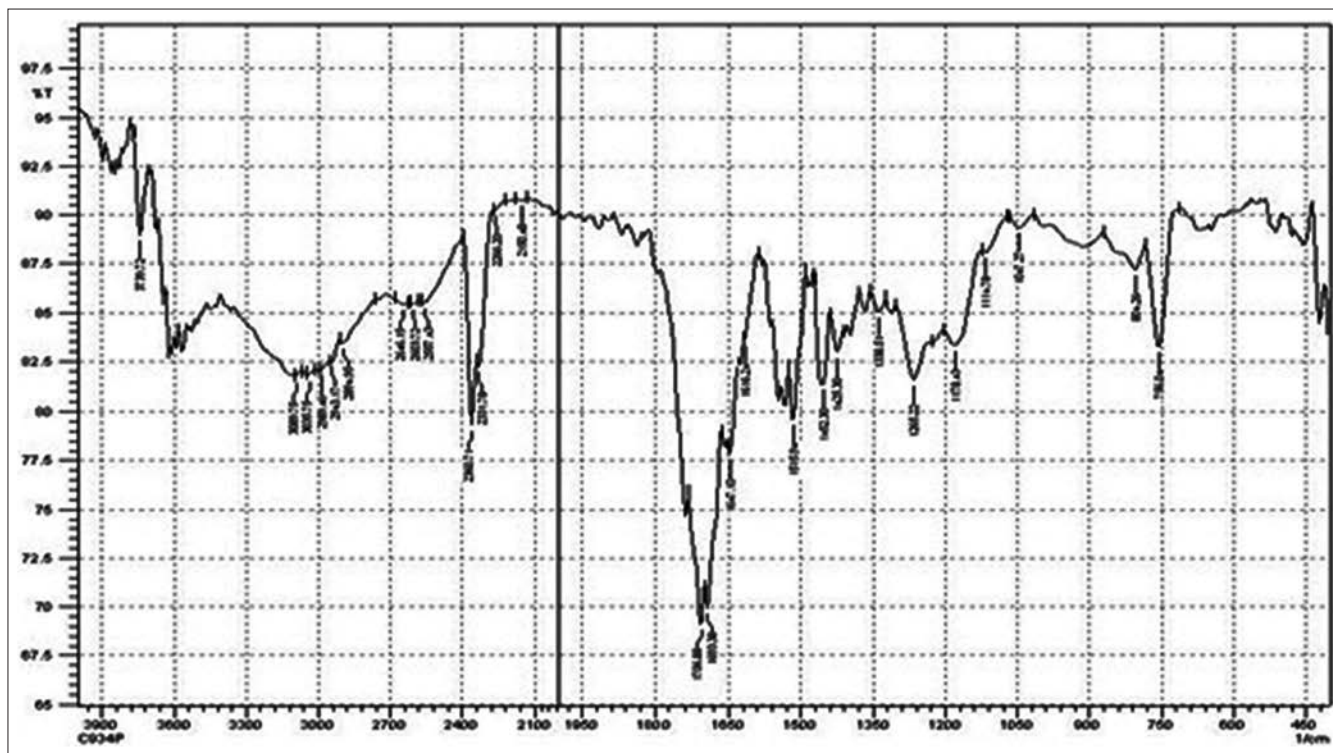


Figure 4: FTIR spectrum of carbopol 934P

of Poloxamer 407 *in situ* gel showed ability to form gel in the range of body temperature shown in Table 3. So, 15-20% w/v concentration of Poloxamer 407 was used for further studies.

Potential drawbacks of Poloxamer gels include their weak mechanical strength, rapid erosion, and the non-biodegradability of PEO-PPO-PEO, which prevented the use of high molecular

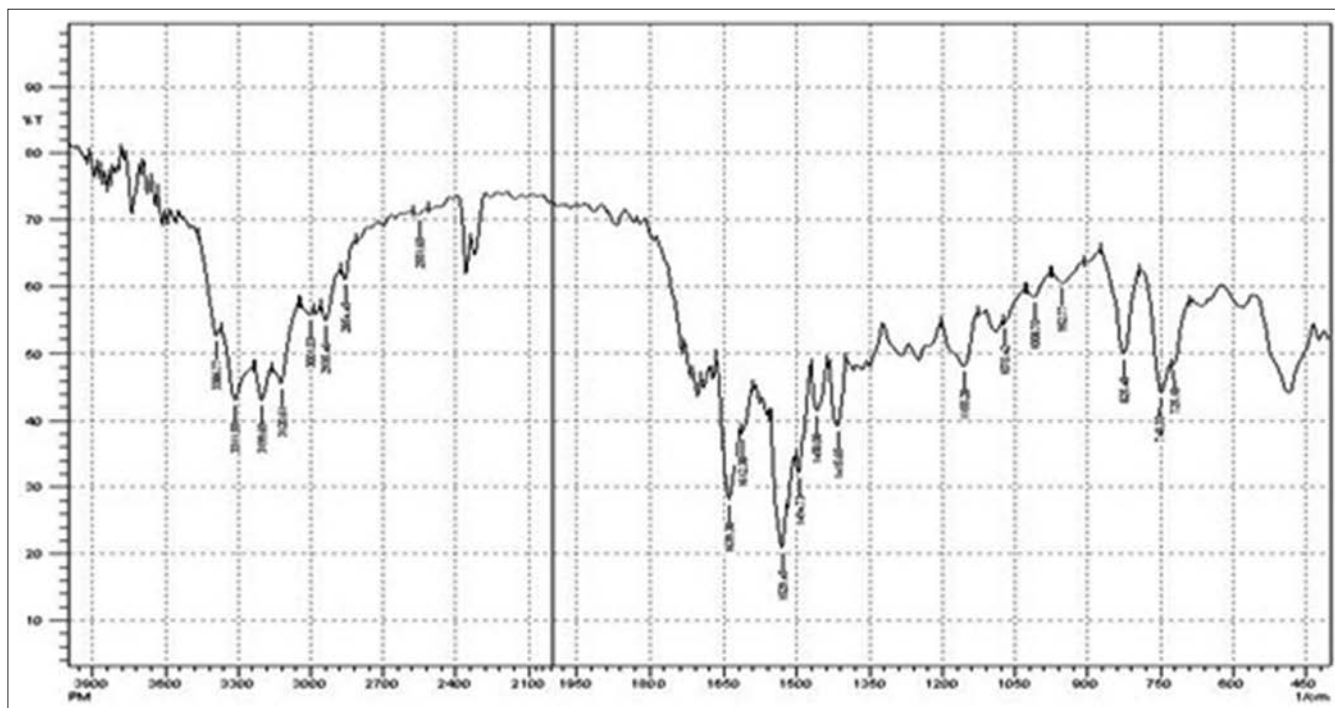


Figure 5: FTIR spectrum of physical mixture

Table 3: Optimization of different polymer concentration

Polymers	Concentration (% w/v)	Gelation temperature (°C)
Poloxamer 188	5	70
	10	65
	15	59
	20	55
	25	49
Poloxamer 407	5	50
	10	44
	15	39
	20	28

weight polymers. Thus, it was necessary to formulate them with other bioadhesive polymers, including cellulose ethers polymers such as carboxy methylcellulose (CMC), HPMC, hydroxyl ethyl cellulose (HEC), hydroxyl propyl cellulose (HPC), acrylic polymers.^[31]

Experimental design

Preliminary investigations of the process parameters exposed that factors such as Poloxamer 407 (X_1) and Carbopol 934P (X_2) exhibited significant influence on gelation temperature, spreadability, CPR at 2h, $t_{50\%}$; hence, they were utilized for further systematic studies. All selected dependent variables for all 13 batches showed a wide variation of data [Table 4]. The polynomial equations can be used to draw conclusions after considering the magnitude of co-efficients and the mathematical sign carried: Positive or negative.^[16]

Effect of formulation variable on gelation temperature (Y_1)

Concerning Y_1 , the results of multiple linear regression analysis showed that both the co-efficients b_1 and b_2 bear a negative sign [Table 5]. The negative of both X_1 and X_2 co-efficient indicates that as the concentration of X_1 (Poloxamer 407) and X_2 (Carbopol 934P) increases, there is decrease in the gelation temperature. The fitted equation relating the response Y_1 to the transformed factor is shown in following equation,

$$Y_1 = 33.33 - 3.769X_1 - 0.755X_2 - 0.590X_1^2 + 0.6699X_2^2 + 0.5825X_1X_2 \quad \dots(3)$$

The Y_1 for all batches F1 to F13 shows good correlation co-efficient of 0.910009. Variable X_1 has P value 0.0000916 ($P < 0.05$), and variable X_2 has P value 0.038169 ($P < 0.05$). Variables, which have P value less than 0.05, significantly affect the gelation temperature. As the concentration of Poloxamer 407 increases, there is micelle formation, followed by micellar aggregation. The gel phase can only occur when the concentration is above the micellar concentration. When the material is in cold water, hydrogen bonding between POP chains and water keeps the hydrophobic portions of the pluronic separate. When the temperature is increased, the hydrogen bonding is disrupted, and hydrophobic interactions cause a gel to be formed. It is to be noted that the addition of increasing concentrations of Carbopol 934P from 0.2% to 0.4% further lowered the gelation temperature.^[32] As the concentration of mucoadhesive polymers (Carbopol 934P) increased, the gelation temperature of gel decreased. This ability of mucoadhesive polymers to lower

gelation temperature may be due to increased viscosity after dissolution of polymers. The ability of mucoadhesive polymers to lower gelation temperature could be explained by their ability to bind to the polyoxyethylene chains present in the Poloxamer 407 molecules. This would promote dehydration, causing an increase in entanglement of adjacent molecules and extensively increasing intermolecular hydrogen bonding, thus leading to gelation at lower temperature.^[33] Combination of bioadhesive agents and Poloxamer 407 on gelation temperature that the packing and entanglements of micelles were promoted by adding Carbopol 934P, which accordingly lead to decrease of gelation temperature.

The relationship between formulation variables (X_1 and X_2) and Y_1 was further elucidated using response surface and contour plot. The effects of X_1 and X_2 on Y_1 are shown in Figures 6a and b. Table 4 showed that with the increase of amount of Poloxamer 407 and Carbopol 934P, gelation temperature was reduced.

Effect of formulation variable on spreadability (Y_2)

Concerning Y_2 , the results of multiple linear regression analysis showed that co-efficient b_1 bear positive sign and co-efficient b_2 bear a negative sign [Table 5]. The positive X_1 co-efficient indicates that as the concentration of X_1 (Poloxamer 407) increases, there is increase in the spreadability of *in situ* gel. The negative X_2 co-efficient indicates that as the concentration of X_2 (Carbopol 934P) increases, spreadability of *in situ* gel

decreases. The fitted equation relating the response Y_2 to the transformed factor is shown in following equation,

$$Y_2 = 21.573 + 0.9412X_1 - 2.677X_2 - 0.842X_1^2 - 0.681X_2^2 - 1.68X_1X_2 \quad \dots(4)$$

The Y_2 for all batches F1 to F13 shows good correlation co-efficient of 0.749198. Variable X_1 has P value 0.003824 ($P < 0.05$), and variable X_2 has P value 0.006702 ($P < 0.05$). Variables, which have P value less than 0.05, significantly affect the spreadability.

The relationship between formulation variables (X_1 and X_2) and Y_2 was further elucidated using response surface and contour plot. The effects of X_1 and X_2 on Y_2 are shown in Figures 7a and b. As the concentration of X_1 (Poloxamer 407) increases, there is increase in the spreadability of *in situ* gel, and as the effect of concentration of X_2 (Carbopol 934P) increases, spreadability of *in situ* gel decreases.

Results of the spreadability testing are shown in Table 4. All the prepared *in situ* gels using different polymers concentrations were spreadable. Data in Table 4 revealed that increasing the concentration of any of the gelling agents was always associated with a decrease in the spreadability.^[34] One of the criteria for a gel to meet the ideal qualities is that it should possess good spreadability. It is the term expressed to denote the extent of area,

Table 4: Results of experimental design batches of variables

Formulation	X1 (%)	X2 (%)	Y1* (°C)	Y2* (gm-cm/sec)	Y3* (%)	Y4* (min)
F1	14.17	0.3	39.33±0.58	21.73±1.22	52.48±0.36	153.54±0.69
F2	15	0.2	37.33±1.53	17.33±0.77	47.57±0.35	175.54±0.75
F3	15	0.4	34.33±0.58	15.80±0.42	47.60±0.43	180.79±0.37
F4	17	0.16	35.67±1.15	25.42±1.68	50.19±0.51	178.19±0.49
F5	17	0.44	34.00±1.00	17.21±1.35	37.53±0.35	224.19±0.90
F6	19	0.2	31.00±1.00	25.48±2.16	37.44±0.55	238.96±0.84
F7	19	0.4	30.33±1.53	17.23±1.48	36.31±0.56	258.23±0.57
F8	19.83	0.3	25.33±0.58	20.26±1.06	39.32±0.27	211.09±0.38
F9	17	0.3	33.33±0.58	21.20±0.88	46.47±0.30	186.59±0.51
F10	17	0.3	32.67±0.58	22.24±0.83	48.10±0.60	175.21±0.40
F11	17	0.3	33.67±1.15	21.73±1.22	47.66±0.24	179.25±0.59
F12	17	0.3	33.33±0.58	21.22±1.13	47.42±0.32	180.77±0.53
F13	17	0.3	33.67±0.58	21.45±0.77	46.63±0.36	188.28±0.41

*All the values are in mean±S.D. (n=3), X1: Poloxamer 407 (% w/v), X2: Carbopol 934P (%w/v), Y1: Gelation temperature (°C), Y2: Spreadability (gm-cm/sec), Y3: CPR at 2 h, Y4: t₅₀% (min)

Table 5: Summary of regression analysis

Co-efficients	b0	b1	b2	b3*	b4*	b5*
Gelation temperature						
FM	33.3348	-3.76997	-0.75533	-0.59003	0.669967	0.5825
RM	33.38385	-3.76997	-0.75533	-	-	-
Spreadability						
FM	21.57322	0.941213	-2.67748	-0.84223	-0.68128	-1.68
RM	20.63846	0.941213	-2.67748	-	-	-
CPR at 2h						
FM	47.26385	-4.84002	-2.40705	-1.32433	-2.32277	-0.45
RM	45.02615	-4.84002	-2.40705	-	-	-
t ₅₀ %						
FM	181.9037	27.9006	11.43165	5.652703	15.20705	3.6725
RM	194.7023	27.9006	11.43165	-	14.48642	-

FM: Full model, RM: Reduced model, t₅₀% time required for 50% drug release, *Response is insignificant at $P \geq 0.05$

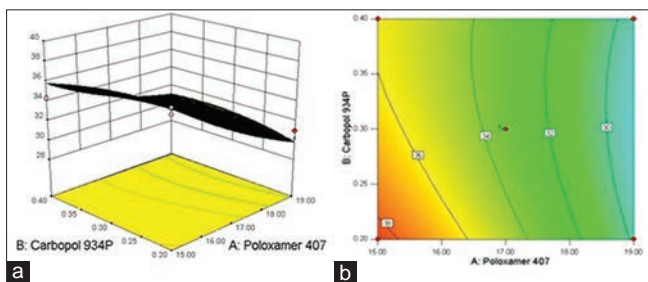


Figure 6: (a) Response surface plot showing effect of gelation temperature of variables [Poloxamer 407 (X_1) and Carbopol 934P (X_2)] (b) the corresponding Contour plot

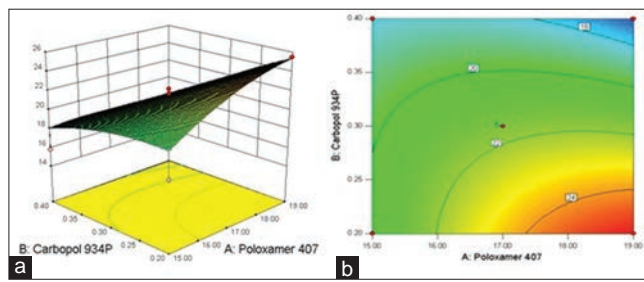


Figure 7: (a) Response surface plot showing effect of spreadability of variables [Poloxamer 407 (X_1) and Carbopol 934P (X_2)] (b) the corresponding Contour plot

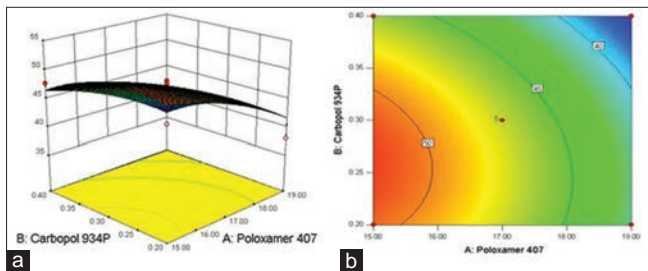


Figure 8: (a) Response surface plot showing effect of CPR at 2 hr of variables [Poloxamer 407 (X_1) and Carbopol 934P (X_2)] (b) the corresponding Contour plot

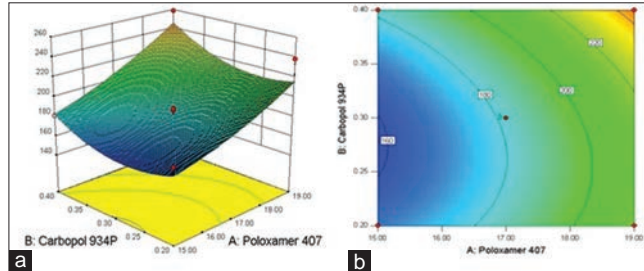


Figure 9: (a) Response surface plot showing effect of $t_{50\%}$ of variables [Poloxamer 407 (X_1) and Carbopol 934P (X_2)] (b) the corresponding Contour plot

to which gel readily spreads on application site. Lesser the time taken for separation of two slides, better the spreadability.^[35] The spreadability of all prepared *in situ* gel formulations were found be in range of 17.21-25.48 gm·cm/sec.

Effect of formulation variable on CPR at 2 hr (Y_3)

Concerning Y_3 , the results of multiple linear regression analysis showed that both the co-efficients b_1 and b_2 bear a negative sign [Table 5]. The negative of both X_1 and X_2 co-efficient indicates that as the concentration of X_1 (Poloxamer 407) and X_2 (Carbopol 934P) increases; there is decrease in the cumulative percentage drug release. The fitted equation relating the response Y_3 to the transformed factor is shown in following equation,

$$Y_3 = 47.263 - 4.840 X_1 - 2.407 X_2 - 1.324 X_1^2 - 2.322 X_2^2 - 0.45 X_1 X_2 \quad (5)$$

The Y_3 for all batches F1 to F13 shows good correlation co-efficient of 0.852097. Variable X_1 has P value 0.001251 ($P < 0.05$), and variable X_2 has P value 0.036108 ($P < 0.05$). Variables, which have P value less than 0.05, significantly affect the release profile.

The relationship between formulation variables (X_1 and X_2) and Y_3 was further elucidated using response surface and contour plot. The effects of X_1 and X_2 on Y_3 are shown in Figures 8a and b. This finding was probably due to the increased strength of the formed gel structure. It is worthwhile that the drug diffusion is controlled by the penetration of liquid through the gel structure and retard release of drug into the medium.^[36]

Effect of formulation variable on $t_{50\%}$ (Y_4)

Concerning Y_4 , the results of multiple linear regression analysis showed that both the co-efficients b_1 and b_2 bear a positive sign [Table 5]. The positive of both X_1 and X_2 co-efficient indicates that as the concentration of X_1 (Poloxamer 407) and X_2 (Carbopol 934P) increases, there is increase in the time for the drug release from formulation. The fitted equation relating the response Y_4 to the transformed factor is shown in following equation,

$$Y_4 = 181.90 + 27.90 X_1 + 11.431 X_2 + 5.6527 X_1^2 + 15.207 X_2^2 + 3.672 X_1 X_2 \quad \dots(6)$$

The Y_4 for all batches F1 to F13 shows good correlation co-efficient of 0.848547. Variable X_1 has P value 0.00125 ($P < 0.05$), and variable X_2 has P value 0.042157 ($P < 0.05$). Variables, which have P value less than 0.05, significantly affect the release profile. The relationship between formulation variables (X_1 and X_2) and Y_4 was further elucidated using response surface and contour plot. The effects of X_1 and X_2 on Y_4 are shown in Figures 9a and b. A strong influence of both independence variables (Poloxamer 407 and Carbopol 934P) was observed on *in vitro* drug release ($t_{50\%}$). The highest $t_{50\%}$ value (258.23 ± 0.57) was observed with Batch 7 [Poloxamer 407 (19% w/v) and Carbopol 934P (0.4% w/v)]. It is possible that at higher polymers concentration, drug is trapped in smaller polymer cells, and it is structured by its close proximity to the polymer molecules. So, increasing the amount of the polymer in the formulations increased the time for the drug to leave the formulation and delayed release of drug into the medium.^[36]

Analysis of variance

The R^2 value for gelation temperature (Y_1), spreadability (Y_2), CPR at 2 h (Y_3), and $t_{50\%}$ (Y_4) are 0.910009, 0.749198, 0.852097, and 0.848547, respectively, indicating good correlation between dependent and independent variables [Table 6]. The reduced models were developed for response variables by omitting the insignificant terms with $P > 0.05$. For all selected dependent variables, co-efficients b_3 , b_4 , b_5 were found to be insignificant, as P values were more than 0.05 and hence removed from the full model.

Table 6 shows the results of analysis of variance (ANOVA) performed to justify the removal of insignificant factors. The high values of correlation co-efficients for gelation temperature, spreadability, CPR at 2 h, and $t_{50\%}$ indicate a good fit. The calculated values of F for gelation temperature, spreadability, CPR at 2 h, and $t_{50\%}$ was found to be 1.43, 1.55, 2.19, and 1.91, respectively, which were less than F critical value (4.35). These observations suggest no significant difference between the full and reduced model.

Clarity and pH

The clarity of first three formulations (F1-F3) was slightly hazy, and all other formulations (F4-F13) were found to be clear. The pH value of all prepared *in situ* gel formulations (F1-F13) were found in range 6.72 to 7.10 [Table 7]. The pH values of all prepared formulations were within the limit of neutral pH; this indicates formulations can be used without any irritation in the oral cavity.

Table 6: Calculation of testing the model in portions

Model	DF	SS	MS	R2
Gelation temperature				
Regression				
FM	5	125.5653	25.11307	0.910009
RM	2	117.9135	58.95677	0.854554
Error				
FM	7	12.41718	1.773883	
RM	10	20.06898	2.006898	
Spreadability				
Regression				
FM	5	82.738	16.5476	0.749198
RM	2	64.24645	32.1232	0.581756
Error				
FM	7	27.69737	3.956768	
RM	10	46.18892	4.618892	
CPR at 2 h				
Regression				
FM	5	278.4977	55.69953	0.852097
RM	2	233.0623	116.5311	0.713082
Error				
FM	7	48.34025	6.90575	
RM	10	93.77561	9.377561	
$t_{50\%}$				
Regression				
FM	5	8997.068	1799.414	0.848547
RM	2	7251.536	3625.686	0.683904
Error				
FM	7	1605.841	229.4058	
RM	10	3351.536	335.1536	

DF: Degree of freedom, SS: Sum of squares, MS: Mean of squares, R_2 : Regression co-efficient

Drug content

Drug content was one of a significant requirement for any type of dosage form. Amount of the drug present in the formulation should not deviate beyond certain specified limits from the labeled amount. All formulations were found to having drug content in the range of 96.89-100.32%, representing homogenous drug distribution throughout gel shown in Table 7.

Viscosity

One essential necessity for a periodontal *in situ* gel was viscosity of the formulation. As indicated, a formulation suitable for application to the periodontal pocket should ideally have a low viscosity when apply and after instillation, should have a high viscosity in order to stay at the application site. The viscosity of the optimized *in situ* gel formulation measured at 37°C was found to be 30596.33 ± 1.52 cps.

Differential scanning calorimetry study

Drug excipients compatibility study was carried out by Shimadzu DSC-60 in dry N_2 atmosphere (flow rate 20 mL/min), and temperature scanning rate was 10°C/min up to 300°C. About 2 mg of each sample was weighed using closed aluminum pans. Thermogram of pure drug (Chlorhexidine HCl) is shown in Figure 10. Melting transition of Chlorhexidine HCl was observed from 266.63°C (Onset) to 275.46°C (Endset). Sharp melting transition of Chlorhexidine HCl was observed at 272.08°C. In the optimized formulation drug and excipients had two melting endotherm was observed first from 36.72°C (Onset) to 181.09°C (Endset), and second was observed at 265.69°C (Onset) to 276.4209°C (Endset). Sharp melting transition of Chlorhexidine HCl in optimized formulation was observed at 271.06°C, which is shown in Figure 10. There was no much difference in the melting point of the drug in all the thermogram, it was concluded that the drug is in the same state, even in the optimized formulation without interacting with polymers. Co-operation of thermogram of pure drug and optimized formulation is shown in Figure 10.

In vitro drug release

The effect of polymer concentration on *in vitro* drug release from *in situ* gels was shown in Figure 11. The cumulative percent of Chlorhexidine HCl released as a function of time is shown in Figure 11. The combination of Carbopol 934P with

Table 7: Result of clarity and pH

Formulations	Clarity	pH*	Drug content*
F1	Slightly hazy	6.72±0.02	96.892±0.159
F2	Slightly hazy	6.84±0.03	97.551±0.159
F3	Slightly hazy	6.79±0.03	98.730±0.375
F4	Clear	6.92±0.02	97.794±0.312
F5	Clear	6.95±0.02	97.412±0.159
F6	Clear	6.94±0.01	98.835±0.275
F7	Clear	7.00±0.02	100.326±0.217
F8	Clear	7.10±0.02	98.037±0.217
F9	Clear	6.83±0.02	99.320±0.262
F10	Clear	6.87±0.03	99.147±0.375
F11	Clear	6.90±0.03	99.112±0.159
F12	Clear	6.82±0.03	99.043±0.375
F13	Clear	6.81±0.03	99.320±0.217

*All the values are in mean±S.D (n=3)

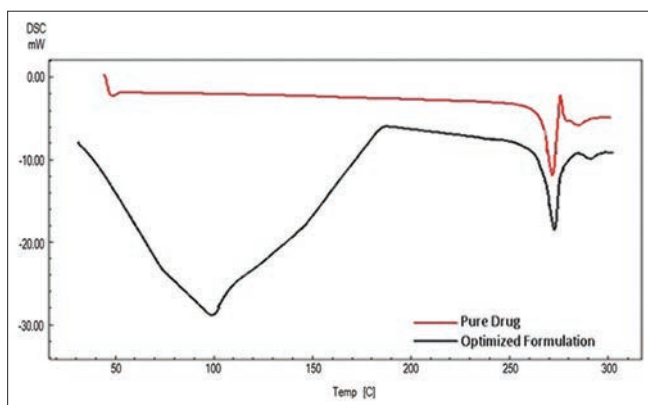


Figure 10: DSC thermogram of pure drug and optimized formulation

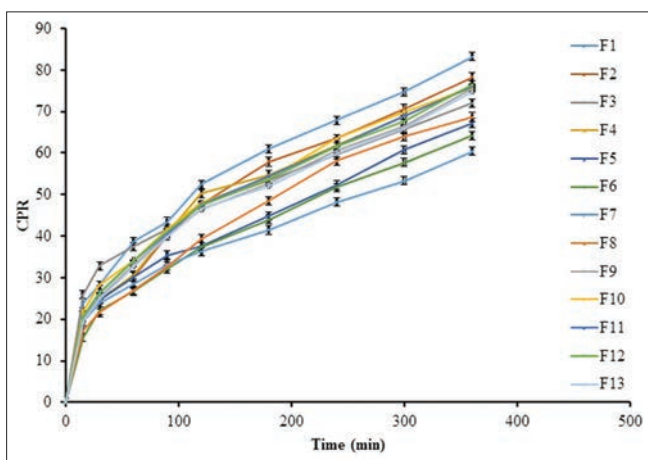


Figure 11: *In vitro* drug release profile of Chlorhexidine HCl

Poloxamer 407 decreased the release rate for Chlorhexidine HCl from gel. The results indicated that combination of bioadhesive agents with Poloxamer 407 could prolong drug action time in periodontal cavity due to the increase of viscosity with addition of Carbopol 934P. The mechanism for resistant barrier of single Poloxamer 407 gels to drug release may be due to reduction in the number and dimension of water channels and the increase in the number and size of micelles within the gel structure. The shorter intermicellar distance leads to greater numbers of cross-links between neighboring micelles, which result in higher viscosity and lower rate of drug release.^[37]

Optimization of the formulation using the desirability function

The desirability function was used for optimization of the formulation. During optimization of formulations, the responses have to be combined in order to produce a product of desired characteristics. The application of the desirability function combines all the responses in one measurement and gives the possibility of predicting optimum levels for the independent variables.^[18]

The combination of the responses in one desirability function requires the calculation of the individual functions. A suitable

in situ gel should have an optimum gelation temperature, maximum spreadability, minimum % drug release, and maximum time require to 50% of drug release ($t_{50\%}$). The individual desirability for each response was calculated using the following methods.^[18,38] In this particular study, there were no specific requirements for gelation temperature of the optimum formulation. Therefore, the range of values of the produced formulations was selected. As moderate gelation temperature was desired, the formulations that have its value within the range of 25-37°C have a desirability of 1, while the formulations that have values outside this range have a desirability of 0. These can be described by the following equations:

$$d_1 = 0 \text{ for } Y_i < Y_{\min} \\ d_1 = 1 \text{ for } Y_{\min} < Y_i < Y_{\max} \\ d_1 = 0 \text{ for } Y_i > Y_{\max} \quad \dots(7)$$

Where d_1 = the individual desirability of the gelation temperature.

The spreadability values were maximized in the optimization procedure, as suitable *in situ* gel should have high spreadability. The desirability functions of these responses were calculated using the following equation:

$$d_2 = \frac{Y_i - Y_{\min}}{Y_{\text{target}} - Y_{\min}} \text{ For } Y_i < Y_{\text{target}} \quad \dots(8)$$

$$d_2 = 1 \text{ for } Y_i > Y_{\text{target}}$$

Where d_2 = the individual desirability of Spreadability.

The CPR at 2 h value was minimized in the optimization procedure, as suitable *in situ* gel should have low CPR at 2 h. The desirability functions of this response were calculated using the following equation:

$$d_3 = \frac{Y_{\max} - Y_i}{Y_{\max} - Y_{\text{target}}} \text{ For } Y_i > Y_{\text{target}} \quad \dots(9)$$

$$d_3 = 1 \text{ for } Y_i < Y_{\text{target}}$$

d_3 = the individual desirability of % drug release at 2 h (CPR at 2 h).

The $t_{50\%}$ values were maximized in the optimization procedure, as suitable *in situ* gel should have high $t_{50\%}$. The desirability functions of these responses were calculated using the following equation:

$$d_4 = \frac{Y_i - Y_{\min}}{Y_{\text{target}} - Y_{\min}} \text{ For } Y_i < Y_{\text{target}} \quad \dots(10)$$

$$d_4 = 1 \text{ for } Y_i > Y_{\text{target}}$$

Where d_4 = the individual desirability of $t_{50\%}$.

The overall desirability values were calculated from the individual values by using the following equation: ^[39]

$$D = (d_1 d_2 d_3 d_4)^{1/4} \quad \dots(11)$$

Formulation of optimized formulation of *in situ* gel

From the desirability function was utilized to find out the best batch out of 13 batches. Batch F6 showed the highest overall desirability of 0.6282 [Table 8]. Therefore, this batch was considered to be the best batch, and the values of independent variables of this batch were considered to be optimum values for the preparation of *in situ* gel. The final optimized formulation was prepared using 19% w/v Poloxamer 407 and 0.2% w/v Carbopol 934P.

Comparison between observed and predicted results of checkpoint batches

In order to assess the reliability of the equations that describes the influence of the factors on the gelation temperature, spreadability, CPR at 2 h, and $t_{50\%}$ of *in situ* gel. The experimental values and predicted values of each response are shown in Table 9. The % relative error between predicted values and experimental values of each response was calculated using the following equation: ^[38]

$$\% \text{ Relative error} = \frac{|\text{Predicted value} - \text{Experimental value}|}{\text{Predicted value}} \times 100 \quad \dots(12)$$

The % relative error obtained from checkpoint batch was in the range of 1.9876-4.6353. It can be seen that in all cases, there was a reasonable agreement of predicted values and experimental values, since low values of the relative error were found. This confirmed the role of a derived reduced polynomial equation, proved the validity of the model, and ascertained the effects of Poloxamer 407 and Carbopol 934P on dependent variables.

Stability studies

In situ gel formulation optimized from statistical design application and was selected for the stability studies. The accelerated stability studies were carried out according to ICH guidelines by storing the samples at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH for 1 month using stability chamber (Remi, India). The optimized formulation were evaluated for clarity, pH measurement, drug content, gelation temperature, spreadability, CPR at 6 h, CPR at 2 h, and $t_{50\%}$. The results after the stability period are given in Table 10. Results of the stability study show no remarkable change in the release profile, assay, and other evaluation parameters of the periodontal *in situ* gel after exposing to accelerated stability conditions.

CONCLUSION

Chlorhexidine hydrochloride, a broad-spectrum anti-microbial agent used in the treatment of periodontal disease, was successfully formulated as temperature-sensitive *in situ* gel by

Table 8: Individual and overall desirabilities for *in situ* gel

Sr No.	d ₁	d ₂	d ₃	d ₄	D
F1	0	0.4486	0.2506	0.0236	0
F2	0	0.1553	0.4143	0.1702	0
F3	0.2225	0.0533	0.4133	0.2052	0.1781
F4	0.1108	0.6946	0.327	0.1879	0.2622
F5	0.25	0.1473	0.749	0.4946	0.3417
F6	0.5	0.6986	0.752	0.5931	0.6282
F7	0.5558	0.1486	0.7896	0.7213	0.4658
F8	0.9725	0.3506	0.6893	0.4072	0.5562
F9	0.3058	0.4133	0.451	0.2439	0.3434
F10	0.3608	0.4826	0.3966	0.1681	0.3282
F11	0.2775	0.4486	0.4113	0.195	0.3161
F12	0.3058	0.4146	0.4193	0.2051	0.3231
F13	0.2775	0.43	0.4456	0.2552	0.3413

Table 9: Comparison between observed and predicted results of checkpoint batches

Response parameters	Predicted value	Observed values	Relative error (%)
Gelation temperature	29.8203	30.5	2.1765
Spreadability	25.349	26.524	4.6353
CPR at 2 h	41.356	40.534	1.9876
$t_{50\%}$	215.424	208.52	3.2048

Table 10: Results of stability testing

Test parameters	Result*
Clarity	Clear
pH	6.95±0.020
Drug content (%)	98.82±0.158
Gelation temperature (°C)	31.33±1.154
Spreadability (gm-cm/sec)	25.62±0.315
CPR at 6 h	73.323±0.529
CPR at 2 h	39.23±0.350
$t_{50\%}$ (min)	209.89±0.860

cold method using Poloxamer 407 and Carbopol 934P as gelling agent. The clarity, pH, and drug content of all formulations were found to be satisfactory. All the formulations showed sustained drug release for a period of 6 h. The desirability function was utilized in order to find out the best batch out of all 13 batches of the central composite design. Formulation F6 showed the highest overall desirability of 0.6282. Therefore, this formulation was considered to be the best formulation, and the values of independent variables of this formulation were considered to be optimum values for the preparation of *in situ* gel. The results of the stability study show no remarkable change in the release profile, assay, and other evaluation parameters of the periodontal *in situ* gel after exposing to accelerated stability conditions.

REFERENCES

- Vyas SP, Sihorkar V, Mishra V. Controlled and targeted drug delivery strategies towards intraperiodontal pocket diseases. *J Clin Pharm Ther* 2000;25:21-42.
- Haffajee AD, Socransky SS. Attachment level changes in destructive periodontal diseases. *J Clin Periodontol* 1986;13:461-75.

3. Iqbal Z, Jain N, Jain GK, Talegaonkar S, Ahuja A, Khar RK, et al. Dental therapeutic systems. *Recent Pat Drug Deliv Formul* 2008;2:58-67.
4. Nagarwal RC, Srinatha A, Pandit JK. *In situ* forming formulation: Development, evaluation and optimization using 3³ factorial design. *AAPS Pharm Sci Tech* 2009;10:977-84.
5. Mahajan H, Shaikh H, Gattani S, Nerkar P. *In situ* gelling system based on thiolated gellan gum as new carrier for nasal administration of dimenhydrinate. *Int J Pharm Sci Nanotech* 2009;2:544-50.
6. Krishna MK, Ravindran SK, Vivekanandan G, Navasivayam A, Thiagarajan R, Mohan R. Effect of a single episode of subgingival irrigation with tetracycline HCl or chlorhexidine: A clinical and microbiological study. *J Indian Soc Periodontol* 2011;15:245-9.
7. Wannachaiyasit S, Phaechamud T. Development of chlorhexidine thermosensitive gels as a mouth antiseptic. *J Miner Met Mater Soc* 2010;20:165-8.
8. Schwach-Abdellaouia K, Vivien-Castionib N, Gurny R. Local delivery of antimicrobial agents for the treatment of periodontal diseases. *Eur J Pharm Biopharm* 2000;50:83-99.
9. Ruel-Gariépy E, Leroux JC. *In situ*-forming hydrogels: Review of temperature-sensitive systems. *Eur J Pharm Biopharm* 2004;58:409-26.
10. Klouda L, Mikos AG. Thermoresponsive hydrogels in biomedical applications. *Eur J Pharm Biopharm* 2008;68:34-45.
11. Nirmal HB, Bakliwal SR, Pawar SP. *In situ* gel: New trends in controlled and sustained drug delivery system. *Int J Pharm Tech Res* 2010;2:1398-408.
12. Jelvehgaria M, RashidbMR, Samadia H. Mucoadhesive and drug release properties of benzocaine gel. *Iranian J Pharm Sci Autumn* 2006;2:185-94.
13. Ji QX, Zhao QS, Deng J. A novel injectable chlorhexidine thermosensitive hydrogel for periodontal application: Preparation, antibacterial activity and toxicity evaluation. *J Mater Sci Mater Med* 2010;21:2435-42.
14. Jayaraj Kumar K, Jayachandran E, Srinivas GM, Giridhar B, Nair R, Jayakandan M. Formulation of thermoresponsive and buccal adhesive *in situ* gel for treatment of oral thrush containing itraconazole. *J Pharm Sci Res* 2010;2:116-22.
15. Singh G, Pai RS, Devi VK. Response surface methodology and process optimization of sustained release pellets using taguchi orthogonal array design and central composite design. *J Adv Pharm Technol Res* 2012;3:30-40.
16. Garala K, Patel J, Patel A, Dharamsi A. Enhanced encapsulation of metoprolol tartrate with carbon nanotubes as adsorbent. *Appl Nanosci* 2011;1:219-230.
17. Harrington J. The desirability function. *Ind Qual Control* 1965;21:494-8.
18. Derringer R, Suich R. Simultaneous optimization of several response variables. *J Qual Technol* 1980;12:214-9.
19. Gupta H, Sharma A, Shrivastava B. Pluronic and chitosan based *in situ* gel system for periodontal application. *Asian J Pharm* 2009;94-6.
20. Dabhi MR, Nagori SA, Gohel MC, Parikh RK, Sheth NR. Formulation development of smart gel periodontal drug delivery system for local delivery of chemotherapeutic agents with application of experimental design. *Drug Deliv* 2010;17:520-31.
21. Nanjawade BK, Manvi FV, Manjappa AS. *In situ* forming hydrogels for sustained ophthalmic drug delivery. *J Control Release* 2007;122:199-34.
22. Miller SC, Donovan MD. Effect of poloxamer 407 gel on the miotic activity of pilocarpine nitrate in rabbits. *Int J Pharm* 1982;12:147-52.
23. Badgujar SD, Sontakke MA, Narute DR, Karmarkar RR, Tupkar SV, Barhate SD. Formulation and evaluation of sumatriptan succinate nasal *in situ* gel using fulvic acid as novel permeation enhancer. *Int J Pharm Res Dev* 2010;2:1-8.
24. Kumar P, Awasthi R, Kumar PR, Kumar M, Kumar MP. Mucoadhesive *in situ* gels of local anaesthetic for periodontia. *Der Pharm Lettre* 2010;2:28-39.
25. Patel RP, Baria AH, Pandya NB, Tank HM. Formulation evaluation and optimization of stomach specific *in situ* gel of ranitidine hydrochloride. *Int J Pharm Sci Nanotech* 2010;3:834-43.
26. Wamorkar V, Varma MM, Manjunath SY. Formulation and evaluation of stomach specific *in situ* gel of metoclopramide using natural, bio-degradable Polymers. *Int J Res Pharm Biomed Sci* 2011;2:193-201.
27. Harish NM, Prabhu P, Charyulu RN, Gulzar MA, Subrahmanyam EV. Formulation and evaluation of *in situ* gel containing clotrimazole for oral candidiasis. *Indian J Pharm Sci* 2009;71:421-7.
28. Kumar L, Verma R. *In vitro* evaluation of topical gel prepared using natural polymer. *Int J Drug Del* 2010;2:58-63.
29. Scherlund M, Welin-Berger K, Brodin A, Malmsten M. Local anaesthetic block copolymer system undergoing phase transition on dilution with water. *Eur J Pharm Sci* 2001;14:53-61.
30. Shams MS, Alam MI, Ali A, Sultana Y, Aqil M. Pharmacodynamics of a losartan transdermal system for the treatment of hypertension. *Drug Dev Ind Pharm* 2010;36:385-92.
31. Gui-Ling L, Mei L. Preparation and evaluation of ophthalmic thermosensitive *in situ* gels of penciclovir. *J Chinese Pharm Sci* 2007;16:90-5.
32. Majithiya RJ, Ghosh PK, Umrethia ML, Murthy SR. Thermoreversible-mucoadhesive gel for nasal delivery of Sumatriptan. *AAPS Pharm Sci Tech* 2006;7:67.
33. Gonjari ID, Hosmani AH, Karmarkar AB, Godage AS, Kadam SB, Dhale PN. Formulation and evaluation of *in situ* gelling thermoreversible mucoadhesive gel of fluconazole. *Drug Discov Ther* 2009;3:6-9.
34. Abdel-Mottaleb MM, Mortada ND, Elshamy AA, Awad GA. Preparation and evaluation of fluconazole gels. *Egypt J Biomed Sci* 2007;23:266-86.
35. Vijayalakshmi A, Tripura A, Ravichandiran V. Development and evaluation of anti-acne products from terminalia arjuna bark. *Int J Chem Tech Res* 2011;3:320-7.
36. Patel RP, Dadhani B, Ladani R, Baria AH, Patel J. Formulation, evaluation and optimization of stomach specific *in situ* gel of clarithromycin and metronidazole benzoate. *Int J Drug Del* 2010;2:141-53.
37. Bhardwaj R, Blanchard J. Controlled release delivery system for the α -MSH analog melanotan-1 using poloxamer 407. *J Pharm Sci* 1996;85:915-9.
38. Paterakis PG, Korakianiti ES, Dallas PP, Rekkas DM. Evaluation and simultaneous optimization of some pellets characteristics using 3 (3) factorial design and the desirability function. *Int J Pharm* 2002;248:51-60.
39. Mashru RC, Sutariya VB, Sankalia MG, Parikh PP. Development and evaluation of fast-dissolving film of salbutamol sulphate. *Drug Dev Ind Pharm* 2005;31:25-34.

How to cite this article: Garala K, Joshi P, Shah M, Ramkishan A, Patel J. Formulation and evaluation of periodontal *in situ* gel. *Int J Pharma Investig* 2013;3:29-41.

Source of Support: Nil. **Conflict of Interest:** None declared.