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Development of Moxifloxacin Hydrochloride loaded *in-situ* gel for the treatment of periodontitis: *In-vitro* drug release study and antibacterial activity

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ABSTRACT

Periodontitis is one of the most widespread oral diseases. Medicated *in-situ* gels of Moxifloxacin Hydrochloride for extended period of retention in infected cavity were prepared for improved local action for the treatment of periodontitis. Medicated formulations were prepared using temperature sensitive (poloxamer 407), ion sensitive (gellan gum) and pH sensitive (carbopol 934P) polymers. 32 Full Factorial Design has been applied and prepared batches were characterized by FTIR, pH, syringeability, drug content, clarity, gelation temperature, gelling time, *in-vitro* gelling capacity, *in-vitro* diffusion study. Gelation temperature, (*in-vitro*) gelling time and the nature of gel formed in simulated saliva showed polymeric concentration dependency. Diffusion study of *in-situ* gel had been performed which showed augmented arrival of medication from 7-12 hours and the discharge was dependent on polymer utilized. The best fitted model was zero order kinetics which indicated that the formulation gave controlled delivery. All preparations were non-Newtonian and display pseudoplastic conduct. *Invitro* Antimicrobial study was carried out by utilizing *E. coli* and *S. aureus*. Optimized formulation containing 19.072 %w/v poloxamer 407 and 0.245 %w/v gellan gum exhibited desired characteristics for developing periodontal drug delivery systems.

Introduction

Periodontal diseases are one of the most prevalent oral diseases, where 80% of American adults and more than 50% of Indian centre of population suffers from this chronic inflammatory disease which demonstrates the harshness of the disease.[1]

Periodontal disease, is an infection of periodontal pocket which is caused by gram negative bacteria and is known by symptoms like sub gingival plaque, inflammation and degeneration of alveolar bones, teeth, dental cementum and periodontal ligaments. Gingivitis at an early stage is known by gingival swelling of gums, bleeding, bad breath and at severe stage it shows symptoms like degeneration and inflammation of gums, alveolar bone, and dental cementum which is normally identified as periodontitis. At the chronic phase of disease, degeneration of supporting collagen, periodontal ligament, resorption of alveolar bone and gingival epithelium occurs, that finally leads to the formation of periodontal pocket.

The cause of periodontal diseases are usually gram negative, facultative anaerobic bacteria species like B. intermedius and B. gingivalis; fusiform organisms: Actinobacillus, actinomycetemcomitans, Wolinella recta and Eikenella spp, various bacilli and cocci; spirochetes; amoebas and trichomonas. The situation gets worsened to a larger degree when the bacterial species releasetheir harmful by-products; mainly enzymes leukotoxins, collagenases, fibrinolysins, other proteases and chemicals that cause an immune reaction by activating the immune system into the periodontal pocket. All these actions occur simultaneously and ultimately lead to the loss of teeth.

Conventional therapies for the treatment of periodontit are used to reduce the bacterial flora, lessen inflammation and to discontinue bone desorption. Demerits of conventional therapies were insufficient drug concentration at target site, recurrent chances of bacterial resistance, incapability to provide sustained drug release effect and access deeper areas of periodontal pocket.[2] In recent times, most of the scientists, students, researchers were paying consideration on insertion an API or API loaded formulations at a specific site in the body by bio-adhesive water soluble polymers for controlled delivery of biologically active agents systemically or locally. Biodegradable excipients have been used in drug delivery system. Than the exploitation of bioabsorbable delivery approach represents a key pace ahead in the management of periodontal diseases. Later on, there was a call for the art of the delivery

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Table 1
-Selection of *in-situ* gelling polymer.

Ingredients	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17
Moxifloxacin Hydrochloride (% w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methyl cellulose (% w/v)	0.5	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Carbopol 934P (% w/v)	–	–	0.1	0.5	1.0	–	–	–	–	–	–	–	–	–	–	–	–
Poloxamer 407 (% w/v)	–	–	–	–	–	12	14	16	18	19	20	25	–	–	–	–	–
Gellan gum (% w/v)	–	–	–	–	–	–	–	–	–	–	–	–	0.05	0.1	0.2	0.3	0.4
Sodium citrate (% w/v)	0.1	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Disodium edetate (% w/v) <																	
Triethanolamine	–	–	To adjust pH			–	–	–	–	–	–	–	–	–	–	–	–
Deionized water up to (% ml)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
In all the formulations, 0.18 %w/v methyl paraben and 0.02 %v/v propyl paraben were added																	

device, which can be controlled more rapidly.[23] Therefore, controlled drug delivery approach of *in-situ* gel was used for the subgingival delivery of Moxifloxacin Hydrochloride. *In-situ* gel remains in sol form at non-physiological condition and forms gel at physiological condition under the control of stimuli such as temperature, pH and ions present at pocket site. *In-situ* gel provides the drug release at a controlled rate by way of directly accessing to the target site which reduces side effects, thus improving patient compliance.[3]

Moxifloxacin Hydrochloride is a broad-spectrum fluoroquinolone antibiotic. Moxifloxacin binds to in addition by inhibit the bacterial enzymes DNA gyrase (topoisomerase II) and topoisomerase IV, resulting in inhibition of DNA replication, repair and cell death in sensitive bacterial species.[4] Poloxamer 407 is a PEO-PPO-PEO triblock copolymer thermo-responsive polymer and it forms gel as soon as the temperature reaches to the body temperature.[5] Gellan gum is a natural polysaccharide which consists of double helical segments and these segments assemble and forms *in-situ* gel in presence of monovalent or divalent cations. Divalent cations are there in oral cavity which interacts to form gel.[6]

Materials and methods

Materials

Moxifloxacin Hydrochloride was obtained as a gift sample from Mankind Pharma. Ltd. Ahmedabad (India). Methyl cellulose, Carbopol 934P and triethanolamine were purchased from S.D Fine Chem Limited, Mumbai (India). Poloxamer 407 was purchased from Yarrow Chem Products, Mumbai (India). Gellan gum was purchased from Balaji Chemical Pvt. Ltd, Valsad (India). Sodium citrate was purchased from LobaChemiePvt. Ltd, Mumbai (India). Disodium edetate was purchased from Suvidhinath Laboratories, Vadodara (India). Methyl paraben and Propyl paraben were purchased from Research-Lab Fine Chem Industries, Mumbai, (India). Deionized water was purchased from Lemon water Co Pvt. Ltd, Surat (India).

Preparation Of In-Situ Gel[6]

Preparation of smart gel periodontal drug delivery system (SGPDDS) by hot process

Polymeric solution of gellan gum was prepared by dispersing the polymer in deionized water. Polymeric solution was heated between 60 and 90 °C with constant stirring using a magnetic stirrer with hot plate to assist the hydration of gelling agent. The necessary amounts of preservatives (methyl paraben, propyl paraben) and sodium citrate were added to it with continuous stirring. The solution was cooled below 40 °C. Finally, Moxifloxacin Hydrochloride was added into this polymeric solution.

Preparation of SGPDDS by cold process

Polymeric solution of poloxamer 407 was prepared by dispersing the polymer in deionized water at 4 °C by using magnetic stirrer. The partially dissolved polymer was stored in refrigerator for 24 h until the entire polymer was completely dissolved. The required amounts of preservatives (methylparaben, propyl paraben) were added with stirring. Finally, Moxifloxacin Hydrochloride was added to the polymeric solution.

Preparation of SGPDDS of poloxamer 407 and gellan gum using hot and cold process

Polymeric solution of altered concentrations of gellan gum were prepared by dispersing in required volume of deionized water with constant heating on hot plate and stirring by using magnetic stirrer. The required amounts of preservatives (methyl paraben, propyl paraben) and disodium edetate were added into it with constant stirring. The solution was cooled down to 4 °C. After cooling, the requisite quantity of poloxamer 407 was added into the previously prepared *in-situ* gelling solution maintained in cold condition. This poloxamer 407 solution was stored for 24 h at 4 °C in refrigerator to finally dissolve the polymer. Finally, the Moxifloxacin Hydrochloride was dissolved in this solution and further evaluation of various formulations were carried out.

Preliminary screening

Selection of *in-situ* gelling polymer and its concentration were carried out on the basis of compatibility and novelty in polymer. The preliminary screening of polymer was performed to determine the effect of polymer concentration on formulation. As per Table 1.

Experimental design

A 3-level, 2-factor design was used statistically to optimize the formulation parameters and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on gelation temperature, gelling time, drug release at 1 h and time required to release 90% of drug. The computer generated quadratic model was given as general polynomial equation for full factorial design.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 + E \quad (1)$$

It was decided from the preliminary trials to use a 3^2 full factorial design in present study to evaluate the effect of independent variables and dependent variables. In this design, 2 factors were evaluated, each at 3 levels and experimental trials were performed for 9 possible combinations as shown in Table 2. The concentration of poloxamer 407 (X_1) and concentration of gellan gum (X_2) were chosen as independent variables in 3^2 full factorial design, while gelation temperature in °C (Y_1), gelling time in sec (Y_2), drug release at 60 min in % (Y_3), time required to release 90% of drug in hr (Y_4) were taken as dependent

Table 2
-3^2 full factorial design.

Run	Conc. of poloxamer 407 (%w/v) X_1	Conc. of gellan gum (%w/v) X_2	Gelation temperature (°C) Y_1	Gelling time (sec) Y_2	Drug release at 1 h (%) Y_3	Time required to release 90% of drug (hrs) Y_4
1	18	0.1	42 ± 0.5	176 ± 2.516	33.386 ± 0.319	5.8 ± 0.208
2	19	0.1	40 ± 0.5	168 ± 2.516	37.993 ± 0.204	6.4 ± 0.118
3	20	0.1	39 ± 0.5	151 ± 2.516	25.510 ± 0.315	6.7 ± 0.212
4	18	0.2	40 ± 0.5	137 ± 2.000	29.724 ± 0.192	7.7 ± 0.154
5	19	0.2	38 ± 0.5	124 ± 3.605	56.528 ± 0.214	8.3 ± 0.289
6	20	0.2	37 ± 0.5	104 ± 2.081	24.432 ± 0.316	8.7 ± 0.263
7	18	0.3	36 ± 0.5	88 ± 1.527	26.544 ± 0.326	9.1 ± 0.285
8	19	0.3	35 ± 0.5	71 ± 1.527	23.325 ± 0.177	10.2 ± 0.154
9	20	0.3	34 ± 0.5	51 ± 1.527	20.491 ± 0.315	11.2 ± .163

Each value represents mean ± SD, n = 3

variables.

Evaluation parameters

IR spectroscopy[7]

FT-IR spectrum of the drug was recorded (Bruker, alpha-E model) in the wave number ranging from 4000 to 400 cm^{-1}.The spectrum was scanned at a resolution of 0.15 cm^{-1} and scan speed was 20 scan/sec. Peaks of drug were identified, compared and interpreted from reference spectra.

Physical appearance[7]

The clarity of gelling solutions was determined visually against white and black background in light.

Determination of pH[7]

The pH of developed *in-situ* gel formulations were determined by using calibrated digital pH meter. The pH meter was calibrated before each use with standard pH 4, 7 and 9 buffer solutions. The temperature of formulations was maintained at 25 °C. pH was measured by placing electrode on surface of formulation.

Syringeability[6]

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

Measurement of viscosity[6]

The viscosity of the selected in situ gel formulation was determined using Brookfield Digital Viscometer LVDV-II + PRO Brookfield at 25 °C. 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. The viscosity measurements were made in triplicate.

Drug content[7]

The prepared *in-situ* gel formulations were analysed for drug content by transferring 1 ml of formulation in 100 ml of volumetric flask. Simulated salivary fluid of pH 6.8 was added followed by continuous shaking until the *in-situ* gel was totally disperse to give a clear solution. Final volume was adjusted to 100 ml with the help of simulated salivary fluid. Suitable dilutions were made, if necessary. Drug concentration of filtered solution was determined spectrophotometrically at λ_{max} of 288 nm using UV–Visible spectrophotometer (Shimadzu UV-1800 Double beam Spectrophotometer, Japan) for dissolved drug by regression equation of standard curve developed in same media in the linearity range of 0–12 µg/ml.

Gelation temperature[7]

Two ml of formulations were transferred to test tubes sealed with paraffin and immersed in a water bath at 4 °C. The temperature of the bath was augmented in increments of 1 °C. The samples were examined for gelation, which was deemed to have occurred when the meniscus would no longer move upon tilting at 90 °C. All measurements were performed in triplicate.

Gelling time[8]

Gelling time of prepared *in-situ* gel formulation was measured by test tube inversion method. In this method, 2 ml of the formulation was positioned in 10 ml glass test tube. This test tube was placed in water bath (37 ± 2 °C) and gelling time was noted.

In-vitro gelling capacity[6]

All formulations were evaluated for *in-vitro* gelling capacity to identify the compositions suitable to use as *in-situ* gelling systems. The *in-vitro* gelling capacity was determined visually, in which coloured solution of prepared formulations were prepared. Gelling capacity was estimated by placing two ml of 6.8 pH simulated salivary fluid in a 10 ml test tube and maintained at 37 ± 1 °C temperature. One millilitre of formulation solution was added to the simulated salivary fluid of pH 6.8. As the formulations have contact with phosphate buffer it immediately got converted into a stiff gel like structure. The *in-vitro* gelling capacity of formulation was evaluated on basis of stiffness of formed gel and time period for which formed gel remains as such.[21] The indications are represented in a way:

(+) Gels after few minutes, dispersed rapidly.
(++) Gelation immediate remains for few hours.
(+++) Gelation immediate remains for longer period.

In-vitro drug release studies[7]

The *in-vitro* drug release study of Moxifloxacin Hydrochloride from *in-situ* gel was determined by using a Franz-diffusion cell. The cellophane membrane was set on the receptor cell. The donor cell was packed with 1 g of *in-situ* gel formulation. The receptor compartment was filled with simulated salivary fluid pH 6.8 and constantly stirred using magnetic stirrer at a speed of 200–250 rpm throughout the experiments to prove homogeneity. The temperature was maintained at 37 ± 1 °C by circulating hot water through the jacket of Franz-

diffusion cell. 1 ml of sample was withdrawn at scheduled time intervals of 1hr and was replaced with same volume of pH 6.8 simulated salivary fluids to maintain the sink condition. Samples were analyzed at λ_{max} of 288 nm on UV–visible spectrophotometer (Shimadzu UV-1800 Double beam Spectrophotometer, Japan).

Release kinetics[9–11]

Different release kinetics was used to analyse the mechanism of drug release. Release rate data were fitted into different release kinetics mechanisms like zero order, first order, Higuchi model, Korsemeyer-Peppas. Based on R^2 value; the best fitted model was selected.

Evaluation parameters of the optimized batch

The optimized batch were evaluated for clarity, pH, syringeability, drug content, gelation temperature, gelling time, *in-vitro* gelling capacity, *in-vitro* drug release studies and release kinetics.

Texture profile analysis[5]

Mechanical properties like firmness, adhesiveness of formulated *in-situ* gels were evaluated with the help of Texture analyser Texture Pro CT V1.8 Build 31 (Brookfield Engineering Labs, USA). Temperature of formulation was maintained at room temperature before starting the experiment. The formulation was transferred into the beaker to a fixed height. The analytical probe of 10 mm diameter was inserted twice in each sample to a depth of 12 mm at a definite rate of 1.0 mm/s.

Rheological studies[6]

The rheological properties of the prepared formulations were measured using RST-CC Rheometer using CCT-14 cylinder as a measuring system. The viscosity of the sample solutions was measured at different RPM at a temperature of 37 ± 1 °C. Subsequently the viscosity was measured with respect to the change in RPM.

In-vitro antibacterial activity[12]

The *in-vitro* antibacterial study was carried out by using agar cup method. *S. aureus* and *E. coli* were inoculated in broth media and incubated for 3–4 days to gain its colony. Nutrient agar medium was ready 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 h at 37 °C. Finally, zone of inhibition was measured. Formulation with drug (F + D), Formulation without drug (F) and pure drug solution (P) were analysed for zone of inhibition.

Scanning Electron Microscopy[5]

Surface morphology of optimized formulation was done by SEM. Optimized formulation was freeze dried under vacuum for 48 h. The freeze-dried sample was coated with gold and evaluated under SEM.

Results and discussion

IR spectroscopy of drug[13]

All the prominent peaks of reference drug were present in the IR spectra of sample drug. Hence, the results of FTIR analysis confirms that Moxifloxacin Hydrochloride (sample) was pure and could be used for further study.

All the prominent peak of drug and excipients were present in the IR spectra of mixture indicating physical compatibility between drug and excipients. Four peaks at 3529 cm^{-1} (secondary N-H stretching), 1707.41 cm^{-1} (CO stretching of keto group), 1456.51 cm^{-1} (OH bending of COOH) and 1623.45 cm^{-1} (CO stretching) were found in FTIR spectra of Moxifloxacin Hydrochloride while 3524.01, 1706.57, 1445.17, 1601 cm^{-1} were observed in mixture. This indicates that the characteristic peaks of the drug were retained in the mixture. Hence, there was no interaction found between the drug and the excipients.

Preliminary screening

Batches P1 and P2 were formulated using methyl cellulose as a polymer, it was observed that there was no gelation beyond 65 °C. Therefore, this polymer was not used for further studies.[14]

Carbopol 934P is a well-known pH dependent anionic polymer and remains in solution form at acidic pH and forms gel at alkaline pH. For selection of carbopol 934P polymer concentration, various solutions of polymeric concentrations were prepared. All the batches of Carbopol 934P showed cloudy appearance. The pH of solution of F3-F5 batches showed in the range of 6.35–6.53. Batches P3 and P4 passes the syringeability criteria except P5 because the viscosity and concentration of carbopol 934P is relatively higher than in P3 and P4 batches which may be a disadvantage for formulation development of injectable periodontal gel. In the present study, for the prepared formulations, the gelling time was found to be 5 min in batch P3. Drug content was found to be between 97.106 and 98.119%. Formulations P3 and P4 slowly convert gel following contact with GCF fluid and dissolve rapidly because it contained the lower concentration of carbopol 934P. Formulation P5 converts into gel form instantly after contact with GCF fluid but does not remain for extended period.

Due to unacceptable results in different parameters, it was not possible to develop a Moxifloxacin Hydrochloride *in-situ* periodontal gel with combination of carbopol 934P polymer and it was omitted for further studies.

Poloxamer 407 was added to formulate thermo-reversible formulations. Gelation temperature is the temperature at which the liquid phase makes a transition to gel. The gelation temperature of SGPDDS would be higher than 25 °C. If the gelation temperature of the formulation is lower than 25 °C, then gelation occurs at storage temperature, leading to difficulty in manufacturing, handling and administering with the use of Poloxamer 407 as a gelling agent, the formation of gels occurs when the concentration of poloxamer is above critical micellar concentration. Poloxamer solution of 12–25% concentrations form clear liquid at cold temperature 4–5 °C and gel at room temperature. The gel can regain its consistency to liquid by cooling. Poloxamer formulations are prepared mainly using cold method. In the cold method, the poloxamer is added to cold water at 4–5 °C and stirred until homogenous solution is formed. When poloxamers was placed in cold water, hydration layer surrounds the poloxamer molecule. The hydrophobic portions of poloxamer are detached due to hydrogen bonding between water and hydrophilic chains. The hydrogen bonds fracture by increase in the temperature, resulting hydrophobic interactions among poly PO chains, thus forming gel. Poloxamer 407 is a thermo-responsive polymer and it was formulated in cold water because in hot water untimely hydrophobic interactions of poly PO blocks producing non-homogenous solution.[15]

Results of different concentration of poloxamer 407 were depicted in Table 1. All the batches were having clear appearance. pH of all the batches P6-P12 ranges from 7.00 to 7.02. Syringeability is unit number of force required to expel each formulation from the syringe equipped with a 20-gauge needle. All the batches from P6-P11 pass the syringeability criteria except P12. All the formulations showed concentration dependent increase in viscosity. Drug content was found to be in the range of 97.154–98.780%.

From the observations, Batch P6 does not form gel due to

insufficient concentration to form gel as it is temperature dependent polymer. When the concentration of polymer increases from P7-P12, gelation temperature decreases from 47 to 31 °C. Batches P7, P8 and P9 exhibits the gelation temperature were found to be higher than that of body temperature because poloxamer 407 is temperature dependent polymer. Results of the batch P12 revealed that the formulations have lower gelation temperature than body temperature. So, these formulations were not desirable to form *in-situ* gel at the site of periodontal pocket. Batches P10 and P11 of poloxamer 407 *in-situ* gels showed ability to form gel nearly at body temperature. So, it was selected for further studies.

During the formulation development, with rising temperature, the number of micelles formed increases as a result of negative coefficient of solubility of block copolymer micelles. Eventually, the micelles become so tightly packed that the solution become immobile and gel is produced. Conformational changes in the orientation of the methyl groups in the side chains of poly(oxypropylene) polymer chains, constitute the core of the micelle, with the expulsion of hydrating water from the micelles will contribute to gelation phenomenon.[6]

All the batches showed concentration dependent relative decreased in gelling time. From the batches P7-P12, gelling time decreases as the concentration of polymer was increased. Batches P7, P8 and P9 showed the weakest gelation and erodes rapidly, which could be due to the presence of low concentration of poloxamer 407. Batches P10, P11 and P12 showed instantaneous gelation but the formed gels did not remain for an extended period. This indicates that the formed gel might be less firm and erodes within few hours which might be due to relatively low concentration of poloxamer 407.

Potential drawbacks of poloxamer *in-situ* gels include their weak mechanical strength, rapid erosion and the non-biodegradability of PEO-PPO-PEO chains and thus it is necessary to formulate them with other gel forming polymers including gellan gum.

From Table 1, various concentrations of poloxamer 407 were prepared and finalization of concentration was done on the basis of gelation temperature. Therefore, batches P9, P10 and P11 were selected for further studies.

Influence of change in concentration of gellan gum was observed for its *in-vitro* gelling capacity and gelling time. The prepared gel formulations were characterized for its clarity, pH, syringeability, viscosity, drug content, gelling time and *in-vitro* gelling capacity.

All the formulations were having translucent appearance. All the batches from P13-P17, pH of the formulations ranges from 5.45 to 5.85. The results revealed that all the formulations were syringeable through the syringe equipped with 20-gauge needle except P17 as they have than relatively higher concentration in batches P13-P16. The viscosity data showed concentration dependent increase in viscosity. Drug content was found to be in the range of 97.521–100.015%.

The nature of the formed gel depends upon the polymer concentration. In batch P13, the concentration of gellan gum is less (0.05% w/v) which causes insufficient ionic interaction at the target site so it does not form the gel. All the batches, except P13, showed instantaneous gelation when contacted with the simulated saliva and remained for extended period of time. This indicates that the formed gel have sufficient stiffness and remained for extended period of time.

From Table 1, various concentrations of gellan gum were prepared and finalization of concentration was done on the basis of gelling capacity. Therefore, batches P14, P15 and P16 were selected for further studies.

From the individual polymer study, we select two polymers.

1. Gellan gum-Formulations with gellan gum exhibited translucent appearance and desired *in-vitro* gelling capacity and gelling time. It gel was formed immediately and remained for extended period.
2. Poloxamer 407-Poloxamer 407 gave desired gelation temperature. To get benefit of both, their combinations were tried which shows desired syringeability, *in-vitro* gelling capacity, drug content and remains for extended period. The formulation should also provide sustained drug release. Poloxamer 407 and gellan gum is temperature dependant and ion dependant polymers having different mechanism for gelation, it may be quite obvious to use their combination to achieve desired formulation.

Evaluation of 3^2 full factorial design batches

Preliminary investigations of the process parameters exposed that factors such as concentration of poloxamer 407 (X_1) and gellan gum (X_2) exhibited significant influence on gelation temperature, gelling time, drug release at 1hr and time required to release 90% of drug, as shown in Table 2. Experimental trials were performed for nine possible formulations suggested by 3^2 factorial design and results were evaluated for the selection of optimized batch.

Based on the experimental design generated, all the formulations were evaluated for clarity, pH, syringeability, drug content, gelation temperature, gelling time and *in-vitro* gelling capacity and the results were analysed. All the formulations were having clear appearance. The gel base was transparent at 4 °C whereas transparent semisolid gel was formed at body temperature. The normal physiological pH of GCF fluid ranges from 6.2 to 6.8. The pH of all formulations prepared by using poloxamer 407 and gellan gum was observed in the range of 6.26–6.74. Therefore, there was no need for pH adjustment by any external alkalinizing agent. All the batches were stable at this pH. These formulations forms gel at physiological pH due to the ions present in salivary fluid. All the formulations pass the syringeability test. This showed that the formulation can be easily administered in the solution form at the site of periodontal pocket. The drug content of all the batches from F1-F9 were found to be 97.154–100.084% which indicates that the drug was uniformly distributed throughout the formulation.

When poloxamer 407 and gellan gum were combined, the results of the batches F1-F9 showed significant decrease in gelation temperature owing to an increase in polymer concentration of poloxamer 407 and gellan gum. When the concentration of poloxamer 407 is increased, there is an increase in number and size of micelles at or above critical micellar concentration within the gel structure. The shorter intermicellar distance leads to a greater number of crosslinks between neighbouring micelles which allows the gel formation at lower temperature due to an increase in concentration of poloxamer 407. As the concentration of gellan gum was increased, it increases the formation of double-helical junctional zones followed by aggregation to form a 3D network by complexation with cations and hydrogen bonding with water which reduces the gelation temperature. Therefore, the combination of poloxamer 407 and gellan gum showed concentration dependent decrease in gelation temperature. Highest gelation temperature was observed in batch F1 which had least concentration of polymers and lowest gelation temperature was observed in batch F9 which had highest concentration of polymers. Batches F5, F6 and F7 showed the ability to form *in-situ* gel nearly at physiological temperature. So, these batches were most desirable for intrapocket-periodontal drug delivery.[6]

As the concentration of polymers was increased, there was an increased in number of cross-linking between the micelles within 3D structure of gel and thus reduced the gelling time. F1 batch showed highest gelling time whereas F9 batch showed least gelling time. F8 and F9 were most desirable for drug delivery at the pocket site.

The formulations of this study contained Na^+ ions within complexed form and the release of which in the slightly acidic conditions of the buccal cavity ensured reproducible gelation of the gellan gum. The quantities of the complexing agent disodium edetate should be such that there is no free sodium in free ionic form in the formulation to make sure that they are in fluid state prior to administration, but sufficient Na^+ ions must be released when the complex is broken down (due to dissociation) in presence of GCF fluid to cause gelation. Determination of the optimum amounts of complexing agent for gellan

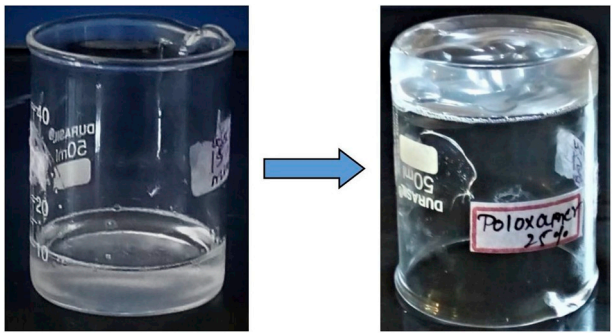

Fig. 1. Clarity of poloxamer 407 (25%w/v).

gum sols containing 0.1% disodium edetate was found to be acceptable to cause gelation. Low level of cations present in the solution was sufficient to embrace the molecular chains together and inhibit hydration. The use of poloxamer 407 for *in-situ* gel forming systems is substantiated by the property of its aqueous solutions form to stiff gels, when temperature is raised up to 37 °C.

Batches F1, F2 and F3 showed immediate gelation but the formed gels did not remain for an extensive period. This indicates that the formed gel might be not as much of stiff and erodes within few hours which could be due to comparatively lower concentration of gellan gum. Batches F4-F9 showed instantaneous gelation and the gel formed was stiff and remained for an extended period due to relatively higher concentration of gellan gum in these formulations (see Fig. 1).

The comparative diffusion study of all batches was showed in Fig. 2. From the results, it can be concluded that *in-vitro* drug release was clearly affected by concentration of gellan gum and poloxamer 407 (see Fig. 3).

The release of the drug decreased significantly as the concentration of polymers increased. The release from a variety of formulations can be ranked as follows at each time point: F1 > F2 > F3 > F4 > F5 > F6 > F7 > F8 and F9. This indicates that the structure of gel functioned as a resistant barrier to the rate of drug release as the concentration of polymers (poloxamer 407 and gellan gum) increased. The mechanism for barrier resistance with increase in poloxamer 407 concentration may be due to the fall in the number and dimension of water channels and to the increase in number and size of micelles within the gel structure, which resulted into cross-linked 3-D structure. This leads to lower rate of drug release.[6]

F1 batch contains lowest amount of gellan gum and poloxamer 407 therefore it showed drug release 92.809% in 7 h. F9 batch contains highest amount of gellan gum and poloxamer 407 and therefore it showed drug release up to 96.403% in 12 h. F1 batch showed 33.085% in 1 h and F9 batch shows 20.508%. The initial burst release of the drug from the prepared formulations could be explained the truth that these systems were formulated in an aqueous vehicle. The matrix formed on gelation has previously hydrated and water permeation could no longer liberate limit the drug release.[2] Therefore, from the results, it can also be concluded that the burst release effect decreases as the concentration of gellan gum increases. Formulations F1, F4 and F7 provides more drug release due to the least concentration of poloxamer 407(18% w/v) as compared to the other batches F2, F3, F5, F6, F8 and F9 that contains 20 %w/v poloxamer 407. Batches F2 & F3 does not show significant difference in drug release. Similarly, for batches F5 & F6 does not show any significant difference in drug release due to similar concentration of gellan gum in batches F2 & F3 (0.1% w/v) and between F5 and F6 (0.2% w/v). From the results, it can be clearly concluded that the drug release was affected by the concentration of gellan gum that was present in the formulation. This is due to their ability to alter or squeeze the extra micelle aqueous channels of poloxamer micelle through which the drug diffuses thereby, delaying the release process.

Prediction on release mechanism

The *in-vitro* drug release profile was fitted to various kinetics models to find out the release mechanism of drug release, as shown in Table 3. The regression coefficient (R^2) for all formulations except F5 was found higher for zero order. As, zero order is the best fit model for all the formulations (see Table 4).

Optimization of Moxifloxacin Hydrochloride *in-situ* gel by using 3^2 full factorial design

3^2 full factorial design was applied for optimization of Concentration of poloxamer 407 and gellan gum in the method development. Effects of selected independent variables on measured responses were analysed using multiple linear regression analysis with design expert version 11 (trial version).

Statistical analysis of gelation temperature

Regression equation was obtained from design expert version 11(trial version) and the polynomial equation was generated with it, which was given below:

$$Y_{1(\text{reduced equation})} = 37.89 - 2.67X_1 - 1.33X_2 \quad (2)$$

For gelation temperature, as seen from the equation and response surface plot revealed that a corresponding decrease in gelation temperature was observed with increase in concentration of poloxamer 407 and gellan gum. The coefficient value of X_1 is higher than X_2 which indicates poloxamer 407 has predominant effect on gelation temperature since its p value was < 0.05. Negative sign of coefficient X_1 and X_2 variable indicates that there is decrease in response Y_1 (i.e. gelation temperature) with the increase in concentration of poloxamer 407 and gellan gum. It was observed that X_1 and X_2 were significant model terms which affect the gelation temperature.

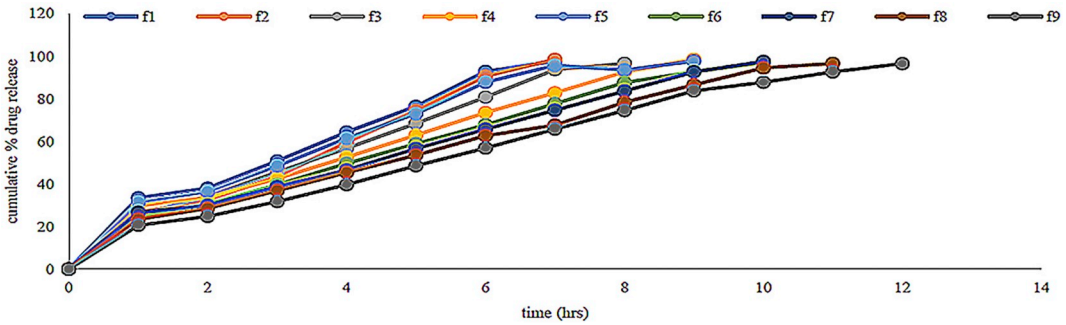

Fig. 2. Release profile from F1-F9 batches.

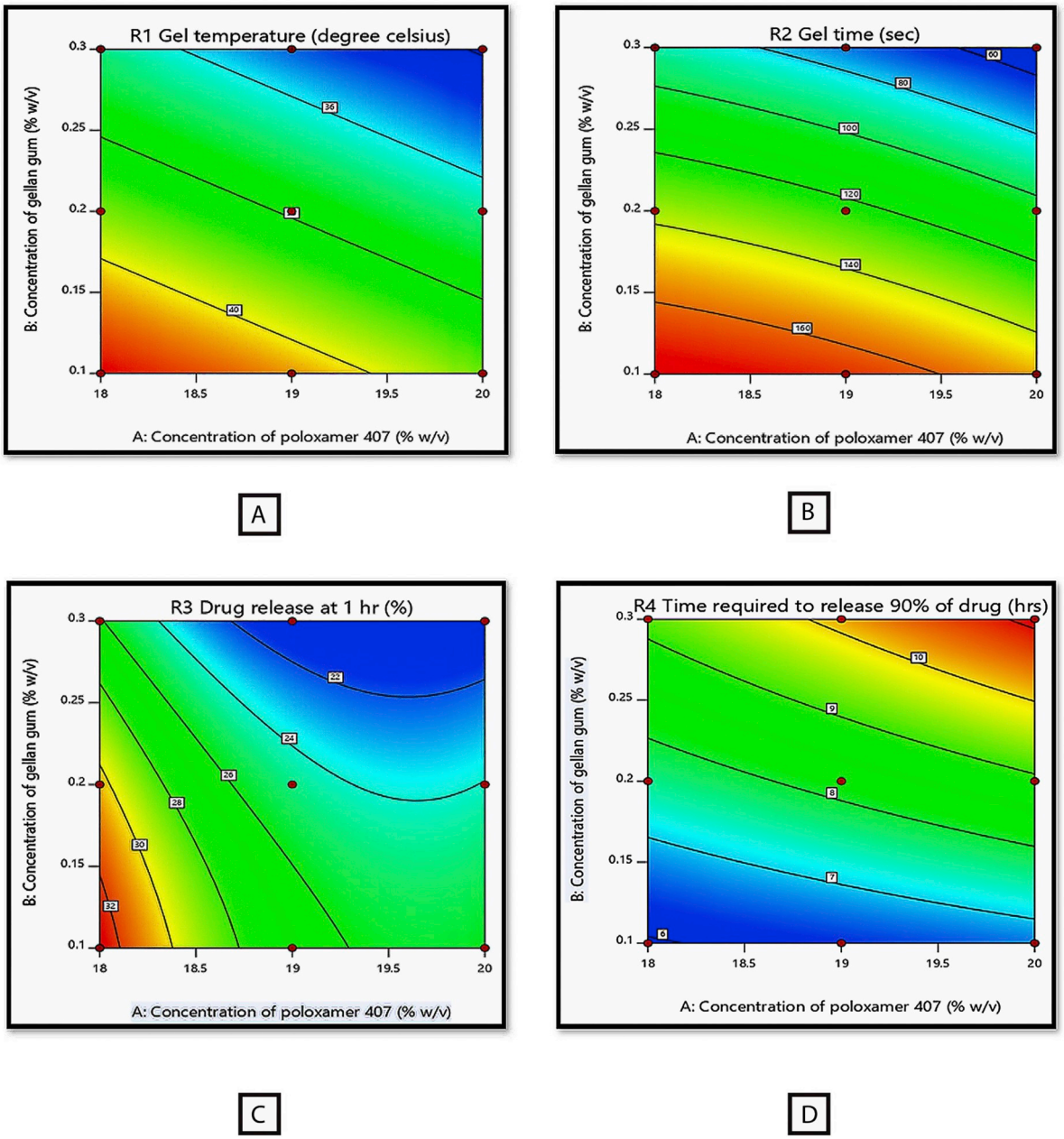

Fig. 3. Contour plots for gelation temperature (b) Contour plots for gelling time (c) Contour plots for drug release at 1hr (d) Contour plots for time required to release 90% of drug.

Table 3
-Drug release kinetics of various batches.

Batches	Zero order	First order	Hixon Crowell	KorsemeyerPeppas	Higuchi
	R^2	R^2	R^2	R^2	R^2
F1	0.9853	0.9776	0.9322	0.942	0.955
F2	0.9863	0.9808	0.9128	0.9488	0.9501
F3	0.9924	0.9619	0.9511	0.9818	0.9755
F4	0.9940	0.9775	0.9249	0.9492	0.9631
F5	0.9484	0.9143	0.9577	0.9592	0.9614
F6	0.9931	0.9534	0.9641	0.9773	0.9789
F7	0.9950	0.9738	0.9352	0.9525	0.9637
F8	0.9952	0.9584	0.9498	0.9743	0.9742
F9	0.9906	0.9465	0.9688	0.9701	0.9737

Table 4
-Formula for optimized batch from overlay plot.

Ingredients	Concentration
Moxifloxacin Hydrochloride	0.5 (%w/v)
Poloxamer 407	19.072 (%w/v)
Gellan gum	0.245 (%w/v)
Methyl paraben	0.18 (%w/v)
Propyl paraben	0.02 (%w/v)
Disodium edetate	0.1 (%v/v)
Deionized water	Up to 100 ml

Statistical analysis for gelling time

The gelling time was found to be in the range of 51–176 s. For gelling time, as seen from the equation and response surface plot revealed that a corresponding decrease in the gelling time was observed

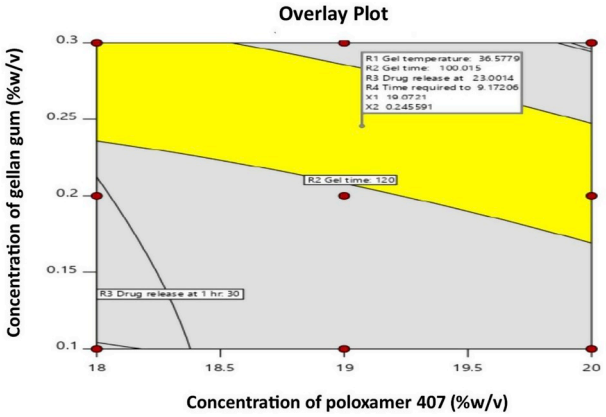

Fig. 4. Overlay plot for prediction of optimized batch.

with the increase in both the concentration of poloxamer 407 and gellan gum.

$$Y_{2(\text{full equation})} = 123.78–15.83\ X_1 – 47.50\ X_2 – 3.00X_1X_2 – 3.17\ X_1^2 – 4.17\ X_2^2 \quad (3)$$

The coefficient of X_2 was higher than X_1 which indicates that the concentration of gellan gum has predominant effect on gelling time as compared to poloxamer 407. Both the coefficient values show negative sign which indicates that there is negative effect in gelling time of increasing the concentration of both the variables. X_1 andX_2 were significant model terms which affect the gelling time since its p value $<$ 0.05. Interactive terms and quadratic terms were significant.

Statistical analysis of drug release at 1 h

The % drug release at 1 h values for all batches were found to be in the range of 20–33%. This result clearly indicates that Y_3 is affected by the independent variables selected for the study. The response Y_3 obtained at various levels of two independent variables were subjected to multiple regression to give a quadratic polynomial equation. The full equation shown in equation 12 for drug release at 1hr is follow:

$$Y_{3(\text{full equation})} = 24.76–2.99\ X_1 – 3.18\ X_2 + 0.3715 \times 1 \times {}_2 + 2.49\ X_1^2 – 0.9135\ X_2^2 \quad (4)$$

For drug release at 1 h, as seen from the equation and response surface plot revealed that a corresponding decrease in the drug release at 1 h was observed with the increase in both the concentration of poloxamer 407 and gellan gum. The coefficient of X_2 was higher than X_1 which indicates that the concentration of gellan gum has

Table 5
-Evaluation of optimized batch.

Gel temperature	37 ± 1.0 °C
Gelling time	102 ± 1.529 s
pH	5.84 ± 0.015
Syringeability	Pass
Drug content	100.677 ± 0.010%
***In-vitro* gelling capacity**	+ + +
Drug release at 1hr	23.227 ± 0.180%
Time required to release 90% of drug	9.1 ± 0.213 h
Each value represents mean ± SD, n = 3	

(+) Gels after few minutes and dispersed rapidly.
(+ +) Gels immediate and remain for few hours.
(+ + +) Gels immediate and remains for extended period.

Table 6
-Release kinetics of optimized batch.

Optimized batch	Zero order	First order	Hixon Crowell	Higuchi	KorsemeyerPeppas
	R^2	R^2	R^2	R^2	R^2
	0.9967	0.9762	0.873	0.9617	0.9685

predominant effect on drug release at 1 h as compared to poloxamer 407. Both the coefficient values show negative sign which indicates that there is decrease in drug release at 1 h as the concentration of both the variables increases.

X_1 andX_2 were significant model terms which affect the drug release at 1hr since its p value $<$ 0.05. Quadraticand interactive terms were not significant. So, the full equation can be converted to reduced equation.

$$Y_{3(\text{reduced equation})} = 24.76–3.18\ X_1 – 2.99\ X_2 \quad (5)$$

Statistical analysis for time required to release 90% of drug

The time required to release 90% (t_{90}) of drug values for all batches were found to be in the range of 5.8–11.2 h. This result clearly indicates that Y_4 is affected by the independent variables selected for the study. The response Y_4 obtained at various levels of two independent variables were subjected to multiple regression to give a quadratic polynomial equation. The full equation for t_{90} was shown in equation 13 as follows:

$$Y_4 = 8.23 + 0.6667\ X_1 + 1.93\ X_2 + 0.3000X_1X_2 \quad (6)$$

For t_{90}, as seen from the equation and response surface plot

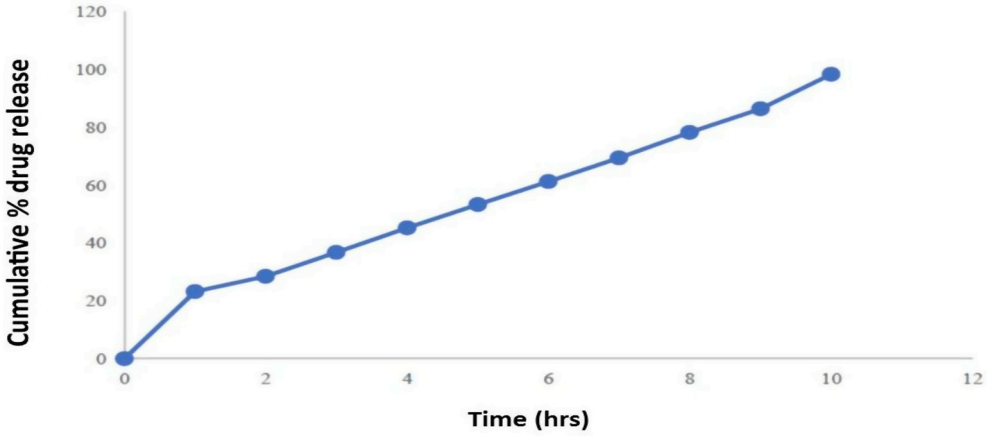

Fig. 5. In-vitro drug release profile of the optimized batch.

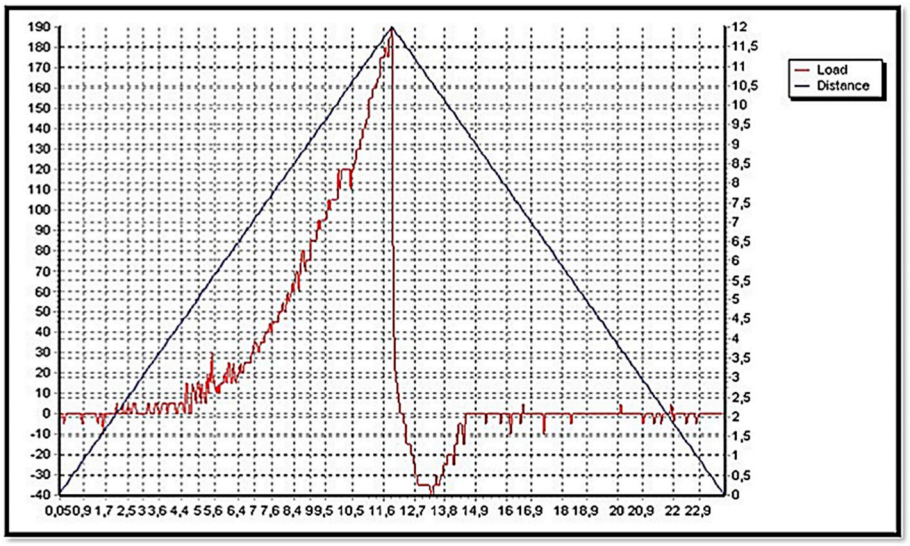

Fig. 6. Graph of load v/s time showing hardness and adhesiveness of optimized batch.

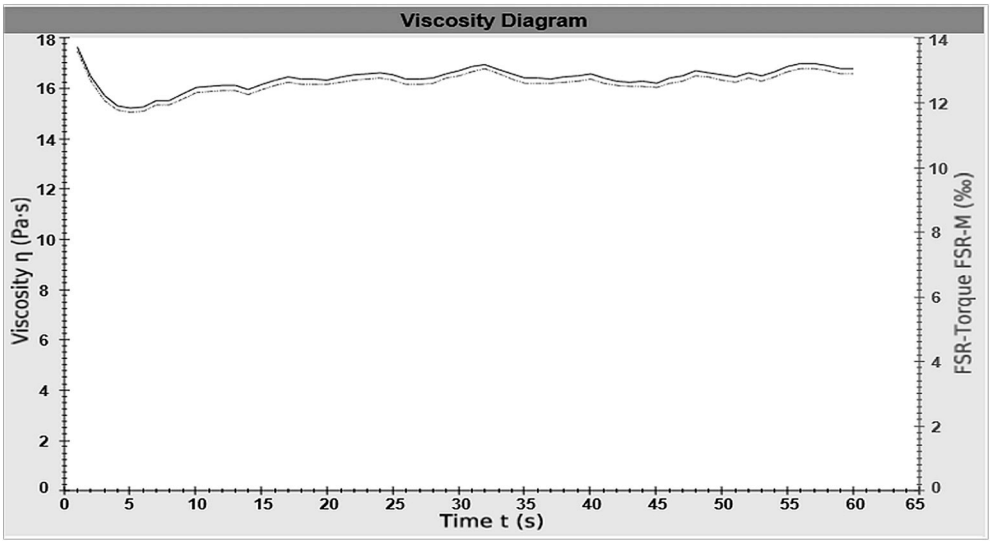

Fig. 7. Graph showing rheological behaviour of optimized batch.

Table 7
-Rheological profile of optimized batch.

Parameters	Minimum	Maximum	Average
Viscosity (cp)	15215.039	17630.175	16375.906
Time (s)	1.000	60.000	30.500
Temperature (°C)	36.940	36.990	36.973
Shear rate (1/s)	9.991	10.015	10.000

(Fig. 5.22) revealed that a corresponding increase in t_{90} was observed with the increase in both the concentration of poloxamer 407 and gellan gum. The coefficient of X_2 was higher than X_1 which indicates that the concentration of gellan gum has predominant effect on t_{90} as compared to poloxamer 407. Both the coefficient values show positive sign which indicates that there is increase in t_{90} as the concentration of both the variables increases. X_1 X_2 were significant model terms which affect the drug since its p value < 0.05. Interactive terms were significant.

Optimized batch from response optimizer by design expert 11 software

Optimized formulation was selected on the basis of following criteria: Gelation temperature, gelling time, drug release at 1hr and time required to release 90% of drug. Optimized batch was selected by using

Table 8
-*In-vitro* antibacterial activity of optimized batch.

Zone of inhibition(diameter)				
Type	Bacteria	Pure drug (P)	Without drug formulation (F)	With drug formulation (F + D)
Gram negative, aerobic	*E. coli*	21 mm	6 mm	20 mm
Gram positive anaerobic	*S. aureus*	20 mm	5 mm	20 mm

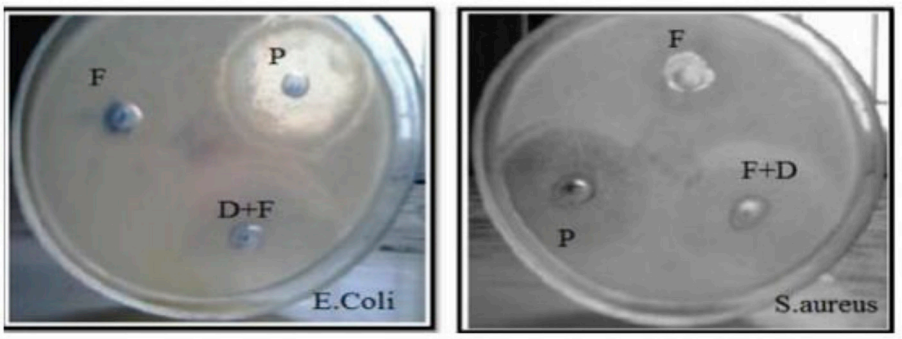

Fig. 8. Zone of inhibition of pure drug (P), with drug formulation (F + D) and without drug formulation (F) on different bacteria.

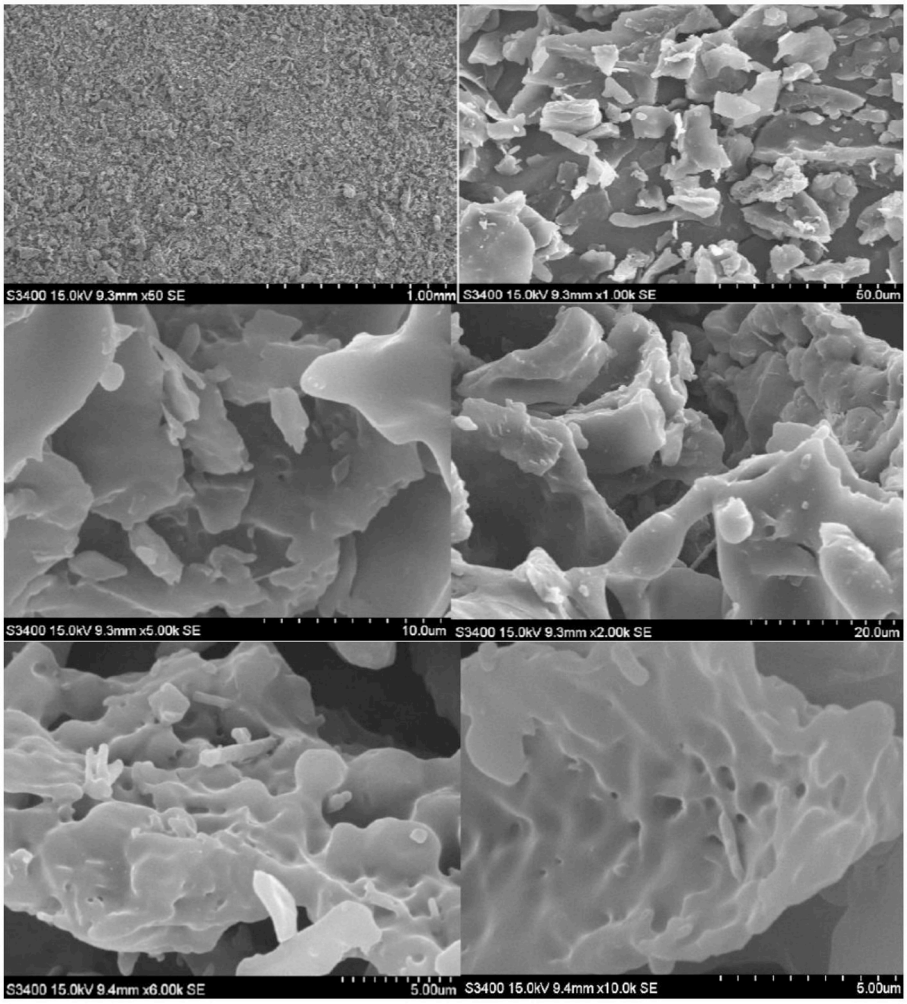

Fig. 9. SEM images of optimized formulation at different magnifications 1, 2, 5, 6 and 10 k.

design expert 11 and overlay plot was generated which was shown in Fig. 4. Based on the overlay plot, formulation containing 19.072% poloxamer 407 and 0.245% of gellan gum were used (see Table 5).

Evaluation of optimized batch

In-vitro drug release study

Release mechanism of optimized batch

The *in-vitro* release profile was fitted to various kinetics models in order to find out the release mechanism of drug release. The best fit model was evaluated using regression coefficient (R^2). The regression coefficient (R^2) of optimized formulation were found to be zero order, which depicts that the drug is independent of initial concentration of release reactants. So, the zero order is the best fit model for all the formulation as shown in Table 6.

Texture analysis

Texture profile analysis (TPA) permits to evaluate the mechanical properties of *in-situ* gel to gather information about the physical structure of *in-situ* gel. TPA is important to predict a sample's behaviour under different environmental and physiological conditions. The mechanical parameters that can be evaluated through TPA are hardness,

compressibility, adhesiveness and cohesiveness and elasticity.[16]

Hardness or firmness is the force required to attain a given deformation and the altitude of first peak gives the hardness value. It is shown as positive peak in the graph of load-time curve. This parameter expresses the applicability of gel to the desired site. Higher the hardness value, the thicker and higher the consistency of the sample. The hardness of the formulation increases as the concentration of polymer increases. Hardness value of the optimized formulation was found to be 190 g.[17–19]

Adhesiveness or stickiness is the work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe. It is shown as negative peak in the graph of load-time curve. It determines the proper gel contact and retention at mucosal surface thereby leading to enhanced bioavailability of drug. Adhesiveness value of the optimized formulation was found to be 0.60 mJ.[20]

Rheological profile

Rheological profile of optimized batch of *in-situ* gel was shown in Fig. 7 and in Table 7(see Fig. 6. All the gel formulation demonstrated pseudoplastic flow with thixotropy. Shear thinning phenomenon, an advantageous property of periodontal gel, was observed for all the gel tested. In this flow the molecule at rest entangled with the association of the immobilized solvent. Under the influence of shear, the molecule tends to become disentangled and align themselves in the direction of flow. The molecules thus offer less resistance to flow and this together with the release of entrapped water account for the lower viscosity.Thedatashowed increase in viscosity with increment of shear rate and temperature which depicts the Non-Newtonian pseudoplastic behaviour of formulation.

In-vitro antibacterial activity

Antimicrobial studies were carried out to assess the performance of MX loaded *in-situ* gel. The results of antimicrobial activity were shown in Table 8 and Fig. 8. The study indicated that MX retained its antimicrobial activity when formulated as gel forming system for periodontal system against selected *S. aureus* and *E.coli*. Difference in the zone of inhibition in vehicle and medicated *in-situ* gels obtained from the *in-vitro* antimicrobial study indicated that the formulation contains active drug moiety (see Fig. 9).

Scanning electron microscopy

SEM images of medicated in-situ gel of optimized batch at different magnifications 1,2,5,6 and 10k showed that the morphology medicated *in-situ* gels appeared similar indicating homogeneous mixing of polymers with drugs as no drug particles were observed on the surface of sample gels. Further, the rough appearance of liquid gels could be attributed to Pl407 as it is present in large amount showed the Scanning electon microscopy images of freeze dried batch at different magnifications 2, 5 and 10 KX which exhibited cracks, pits, lumps formed due to loss of water during drying process concluding towards large water content of gel and supporting their hydrogel nature and it also showed porosity.

Conclusion

Moxifloxacin Hydrochloride loaded thermosensitive smart gel was successfully formulated by combination of poloxamer 407 and gellan gum. FTIR study indicates no sign of incompatibility between drug and excipient. So the selected polymers were likely to be proper for the preparation of periodontal smart gel. The formulations remain in liquid at non-physiologic conditions (10–25 °C) and forms gel at physiologic condition (37 °C). The developed formulations showed acceptable results for gelation temperature, gelling time, syringeability and drug release which were dependent on concentrations of poloxamer 407 and gellan gum. Amongst the various formulations (F1-F9) assorted, optimized batch contains 19.072 %w/v poloxamer 407 and 0.245%w/v gellan gum, which have desired gel temperature 36 °C, gelling time 102 s, 98% of drug release at 9 h and 90% of drug release takes 9.1 h. Texture analysis indicated that hardness of the formulation increases as the concentration of polymer increases. Hardness value of the optimized formulation was found to be 190 g. Adhesiveness value of the optimized formulation was found to be 0.60 mJ. Antimicrobial studies indicates that MX retained its antimicrobial activity when formulated as in situ gel delivery for periodontal system.

Declaration of conflict of interest

There is no conflict of interest.

References

1. Borderwala K, Patel S, Gandhi J, Naik H, Pillai L, Shah P. Advanced local drug delivery approaches for periodontitis: a strategic intervention. *Res Rev J Pharmaceut Sci.* 2008;9:4–11.
2. Jain N, Jain GK, Javed S, Iqbal Z, Talegaonkar S, Ahmad FJ. Recent approaches for the treatment of periodontitis. *Drug Discov Today.* 2008;13:932–943.
3. Joshi D, Garg T, Goyal AK, Rath G. Advanced drug delivery approaches against periodontitis. *Drug Deliv.* 2016;23:363–377.
4. https://www.drugbank.ca/drugs/DB00218.
5. Bansal M, Mittal N, Yadav SK, Khan G, Gupta P, Mishra B. Periodontal thermoresponsive, mucoadhesive dual antimicrobial loaded in-situ gel for the treatment of periodontal disease: preparation, in-vitro characterization and antimicrobial study. *J Oral Biol Craniofac Res.* 2017;5:2–12 2017.
6. 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;17:520–531.
7. Garala K, Joshi P, Shah M, Ramkishan A, Patel J. Formulation and evaluation of periodontal in situ gel. International Journal of Pharmaceutical Investigation 1013; 3:29-31.
8. Rajendran S, Kumar KS, Ramesh S, Rao SR. Thermoreversible in situ gel for subgingival delivery of simvastatin for treatment of periodontal disease. *Int J Pharmaceut Investig.* 2017;7:101–105.
9. Shaikh HK, Kshirsagar RV, Patil SG. Mathematical models for drug release characterization: a review. *World J Phamraceutical Sci.* 2015;4:324–338.
10. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modelling on drug release from controlled drug delivery system. *Acta Pol Pharm.* 2010;67:217–223.
11. Singhvi G, Singh M. Review: in-vitro drug release characterization models. *Int J Pharm Sci Res.* 2011;2:77–84.
12. Parvathy S, Unnikrishnan A, George P, Nair C. Thermoreversible-pH sensitive cephalexin insitu gel for treating periodontal disease. *J Chem Pharm Res.* 2015;7:555–567.
13. Sabitha K, Sajeeth C, Santhi K. Chitosan nanoparticles: a novel vehicle for the enhanced ocular delivery of moxifloxacinHCl. *Res J Pharmaceut Biol Chem Sci.* 2012;3:534–538.
14. Ahmed M, Acharya A, Chaudhari R, Panicker K, Reddy R. Formulation and evaluation of in situ gel containing rosuvastatin in the treatment of periodontal diseases. *J Pharm Res.* 2015;14:45–50.
15. Bachhav H, Savkare A, Karmarkar R, Derle D. Development of poloxamer based in situ gel ocular gel of betaxolol hydrochloride. *Int J Pharm Pharm Sci.* 2015;7(6):287–291.
16. Baloglu E, Karavana S, Senyigit Z, Guneri T. Rheological and mechanical properties of poloxamer mixtures as a mucoadhesive gel base. *Pharmaceut Dev Technol.* 2011;16:627–636.
17. Enyoyt Z, Karavana SY, Gursel Y. Evaluation of chitosan based vaginal bioadhesive gel formulations for antifungal drugs. *Acta Pharm.* 2014;64:139–156.
18. Ranch K, Patel H, Chavda L, Koli A, Maulvi F, Parikh RK. Development of in situ ophthalmic gel of dexamethasone sodium phosphate and chloramphenicol: a viable alternative to conventional eye drops. *J Appl Pharm Sci.* 2017;7:101–108.
19. CevherE, Taha MA, Orlu M, Araman A. Evaluation of mechanical and mucoadhesive properties of clomiphene citrate gel formulations containing carbomers and their thiolated derivatives. *Drug Deliv.* 2008;67:15–57.
20. Basu S, Kumar A. Development and characterization of mucoadhesive in situ nasal gel of midazolam prepared with ficuscarica mucilage. *AAPS PharmSciTech.* 2010;11:1223–1231.
21. Nasra MMA, Khiri HM, Hazzah HA, Abdallah OY. Formulation, in-vitro characterization and clinical evaluation ofCurcumin in-situ gel for treatment of periodontitist. *Drug Deliv.* 2017;24(1):133–142.
23. Gorle A, Yadav R, Rathod M, Mali P. Formulation and evaluation of in-situ gel containing ciprofloxacin hydrochloride in the treatment of periodontitist. *Asian J Pharmaceut Clin Res.* 2017;10(6):154–159.