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FORMULATION AND EVALUATION OF *IN SITU* GEL CONTAINING ROSUVASTATIN IN THE TREATMENT OF PERIODONTAL DISEASES

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Objective: The main objective of present study was to formulate and evaluate methyl cellulose based *in situ* gel of rosuvastatin for the treatment of periodontal diseases.

Methods: The rosuvastatin *in situ* gel was prepared by using different concentration of methyl cellulose as gel base and gel was evaluated for pH, viscosity, rheology, drug content, syringeability, spreadability, drug release and drug release kinetics studies.

Results: Compatibility study was performed using FT-IR and results showed there was no interaction between drug and other excipients. Viscosity of all formulations was found in the range of 320-590 centipoise and all formulations exhibited shear thinning pseudoplastic behaviour. Gelation time and temperature was found in the range of 2-15 min and 26°C-39°C respectively. All the formulation except formulation F6, F7 and G8 passed syringeabilitytest, as these formulations easily gets expelled from the syringe. An *in vitro* release study was conducted using 1.2 pH buffer for 8 hours and results showed that formulation F5 containing 0.9% methyl cellulose was considered as optimum formulation as it released 54.33% drug at the end 8 hours. *In vitro* release study revealed that release rate of drug from the *in situ* gel was concentration dependent; as concentration of methyl cellulose increased the drug release rate was retarded. **Conclusion:**Thus, it can be concluded that formulation F5 containing 0.9%w/v of methyl cellulose as gel base was considered as an optimized formulation, as it release drug in sustain manner in the treatment of periodontal diseases.

Key words: *In situ gel, methyl cellulose, periodontal diseases, Rosuvastatin, syringeability.*

INTRODUCTION

It is estimated that approximately 10-30% of the total population suffers from periodontal disease[1]. Periodontal disease is inflammatory reaction caused by bacterial infection. It is characterized by inflammation and degeneration of gums, alveolar bone, and dental cementum[2]. Mainly there are two types of periodontal disease; namely gingivitis and periodontitis. Gingivitis is mildest form of periodontal disease which is relatively common and readily reversible by simple and effective oral hygiene. If gingivitis is not treated in time, it may proceed to chronic periodontitis, a continuous inflammatory process resulting in irreversible periodontal tissue destruction, occasional pain, discomfort, impaired mastication and leads to tooth loss[2,3]. Both of these diseases occur when bacteria from dental plaque invade surrounding tissues and generate destructive by-product and enzymes that break extracellular matrices as well as host cell membrane to produce nutrients suitable for bacterial growth. During that time they start damaging host medicated response directly or indirectly, which in turn induce an inflammatory response (self-injury)[2]. In early stage of periodontitis, scaling and root planning is effective in reducing bacterial count and probing depth. In case if probing depth increases the effectiveness of scaling and root planning is decreases significantly. Therefore, in recent years many drugs are used either topically or systemically in the treatment of periodontitis[1].

Local drug delivery is frequently utilized for the treatment of several localized disorders. The main advantages of this route of drug administration is that it can deliver the active agents directly to the site of action at bactericidal concentration and it can facilitate prolong drug delivery[1,2].

Therefore, some researchers had prepared and reported a newer drug formulation named as *in situ* gel, which is able to reside in oral cavity for a longer period of time and sustain drug release for desired period[4,5]. *In situ* gels are polymeric networks that absorb large quantities of water and remains insoluble in aqueous solutions due to the chemical or physical cross linking of individual polymer chains. This type of gel formulation is liquid at room temperature and gets converted to gel form after instillation into oral cavity due to phase transition trigger by temperature, pH change, ionic change and UV induced gelation[1,4,5]. In this way, the polymers which show sol-gel phase transition and thus trigger drug release in response to external stimuli are in first choice. The polymer used in preparation of *in situ* gel should be biocompatible, adhere properly to mucus, and have pseudo plastic behavior. Methyl cellulose is a cellulose

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derivative and available in wide range of molecular weights. Methyl cellulose is generally used in gel formulations due to its sustain release action, nontoxic, non-irritant, high mucoadhesive characteristics, easy incorporation with the drugs and stability at oral pH[6,7].

Rosuvastatin used as a calcium salt is chemically bis[(E)-7[4(4-fluorophenyl)-6-isopropyl-2-[methyl (methyl-sulphonyl)amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt. Rosuvastatin is a synthetic drug that belongs to statin class of drug. It is a competitive inhibitor of the enzyme HMG-CoA reductase that is needed by the body to make cholesterol. So, is used for lowering serum cholesterol level and also used for treatment of atherosclerotic disease. Most recently multiple retrospective epidemiological studies have demonstrated that at higher dose statins reduces periodontal inflammation[8,9].

The objective of this study was to prepare a suitable mucoadhesive*in situ* gel formulation of rosuvastatin by using different concentration of methyl cellulose. As methyl cellulose possess appropriate mechanical and rheological properties it can adhere on oral mucosa for prolong period of time and sustain the drug release in the treatment of periodontal disease.

MATERIALS AND METHODS

Rosuvastatin was obtained as a gift samples from Yarrow Chem Products, Mumbai, India. Methyl cellulose and sodium citrate were procured from S.D. fine chemical, Mumbai. All other ingredients used were of analytical grade.

Pre-formulation studies:[3,10]

The pre-formulation studies like melting point determination and compatibility studies were done as per the procedure. Melting point of pure drug wasdetermined by capillary method and obtained data were compared with the reported value.Compatibility study by FT-IR was carried out to identify possible interaction between drug and polymer used as per the standard procedure.

Selection of Methyl cellulose concentration:[5]

Solution of different concentration ranging from 0.5-1.2 w/v % of methyl cellulose was prepared by cold process. Required amount of polymer was accurately weighed and dispersed in distilled water with continuous mild stirring for 5 m. The beaker containing partially dissolved methyl cellulose was sealed with aluminum foil and solution was kept aside till the entire polymer was completely dissolved (about 24 h.). The proper concentrations of methyl cellulose were selected on the basis of gelation temperature and gelation time.

Preparation of *in situ* gel:[3]

For the preparation of methyl cellulose containing *in situ* gel formulations, sodium citrate was first added to distilled water with continuous stirring until clear solution was obtained. Methyl cellulose was added to above solution with continuous stirring and allowed to hydrate overnight. Calculated amount of rosuvastatin (1.2% w/v) was dissolved in required quantity of methanol and 2-3 drops triethanolamine was added separately and then added to polymer solution under constant stirring. Finally, methylparaben and propylparaben were added to the above formulation mixture. The formulation design of rosuvastatin in situ gel was tabulated in Table 1. The optimization concentration of methyl cellulose was selected on the basis of gelation temperature and gelation time given in Table 2. Further, the prepared formulations were evaluated for various characterization studies.

Table 1: Composition design of various rosuvastatin in situ gel formulations

Batch Code	Rosuvastatin (%w/v)	Methyl cellulose (%w/v)	Sodium citrate (%w/v)	Methyl paraben (%w/v)	Propyl paraben (%w/v)	Tri ethanolamine	Distilled water
F1	1.2	0.5	0.1	0.15	0.02	Q.S	Q.S
F2	1.2	0.6	0.1	0.15	0.02	Q.S	Q.S
F3	1.2	0.7	0.1	0.15	0.02	Q.S	Q.S
F4	1.2	0.8	0.1	0.15	0.02	Q.S	Q.S
F5	1.2	0.9	0.1	0.15	0.02	Q.S	Q.S
F6	1.2	1.0	0.1	0.15	0.02	Q.S	Q.S
F7	1.2	1.1	0.1	0.15	0.02	Q.S	Q.S
F8	1.2	1.2	0.1	0.15	0.02	Q.S	Q.S

***Table 2:** Gelation temperature and time of various rosuvastatin in situ gel formulations*

Methyl cellulose Concentration (%)	Gelation temperature (°C)	Gelation time (m)
0.5	No gelation up to 45°C temperature	---
0.6	No gelation up to 45°C temperature	---
0.7	39	8
0.8	37	7
0.9	37	4
1.0	32	4
1.1	29	3
1.2	26	2

Characterization of *in situ* gel formulation:

Appearance:[3]

All prepared formulations were evaluated from the visual inspection.

Gelling Capacity:[5]

All formulations were evaluated for gelling capacity in order to identify the compositions suitable for use as *in situ* gelling systems. The gelling capacity was determined by visual method in which coloured solution of prepared formulations were prepared. Gelling capacity was estimated by placing 2 ml 1.2 pH buffer in a 10 ml test tube and maintained at 37±1°C temperature. One millilitre of coloured formulation solution was added to the buffer solution. As the formulation comes into contact with 1.2 pH buffer it was immediately converted into a stiff gel-like structure. The gelling capacity of formulation was evaluated on the basis of stiffness of formed gel and time period for which formed gel remains as such. The *in vitro* gelling capacity was graded in three categories on the basis of gelation time and the time taken for the gel formed to dissolve.

pH measurement:[10]

pH is one of the most important parameter involved in the *in situ* gel formulation and it is measured directly with the help of digital pH meter.

Viscosity and rheological studies:[4,5]
Brookfield digital viscometer (Model LVDV–E, USA) was used for the determination of viscosity and rheological properties of rosuvastatin *in situ* gel using spindle no T-96.Gel weighing 50 g was taken in a beaker and the spindle was dipped in it. The viscosity of gel was measured at different angular velocities at a temperature of 25°C. A typical run comprised changing of the angular velocity from 10 to 50 rpm. The averages of two readings were used to calculate the viscosity.

Gelation temperature:[5]
A magnetic bead and 10 ml of the sample solution were put into a 30 ml transparent vial placed in a low temperature digital water bath. A thermometer was placed in the sample solution. The solution was heated at the rate of 1°C/m with the continuous stirring. The temperature at which the magnetic bead stopped moving due to gelation was considered as gelation temperature.

Gelation time:[5]
Gelation time of prepared *in situ* gel formulation was measured by placing 2 ml of the gel in 15 ml borosilicate glass test tube. This test tube was placed in water-bath (37±2°C) and gelation time was noted when there was no flow of the gel when test tube was inverted.

Drug content analysis:[8,11]
Accurately weighed amount of gel equivalent to 2 mg of drug was taken into a 100ml volumetric flask. They were lysed with 25 ml of medium (1.2 pH buffer) for 15 m. The clear solution was diluted to 100 ml of medium. Then 10 ml of this solution was diluted to 100 ml buffer. Aliquots were withdrawn and the absorbance was measured at 244 nm against 1.2 pH buffer by using UV-Visible Spectrophotometer-1800 (Shimadzu, Japan) and drug content was calculated from the calibration curve.

Syringeability:[3]
All prepared formulations were transferred into a 5 ml syringe placed with 20 gauge needle to a constant volume (2 ml). The solutions which were easily passed from syringe was termed as pass and difficult to pass were termed as fail.

Spreadability:[4]
For the determination of spreadability, excess of sample was applied between the two glass slides and was compressed to uniform thickness by placing 1000 gm weight for 5 m. Weight (50 g) was added to the pan. The time required separating the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of spreadability.Spreadability (g.cm/s) **(S)= M×L/T**

Where M = weight tied to upper slide, L = length moved on the glass slide, T= time taken.

***In vitro* drug release studies:**[4,11]
In vitro drug release study of Rosuvastatinfrom the *in situ* gel formulations was conducted for the period of 8 h using cellophane membrane. The diffusion medium was 1.2 pH buffer. Cellophane membrane, previously soaked overnight in the diffusion medium, was tied to one end of a glass cylinder. Then 1ml of the prepared formulation was placed in cellophane membrane tie in a glass cylinder and make the membrane just touched the receptor medium surface. The diffusion medium was stirred at required 50 rpm using magnetic stirrer. At pre-determined time interval one ml of the sample was taken and replaced by an equal volume of the receptor medium. The sample was analysed spectrophotometrically at 244 nm.

Drug release kinetics:[4,6]
To understand the drug release kinetics of the rosuvastatin *in situ* gel formulation, the drug release data were treated with zero order, first order kinetics and Higuchi equation. The release mechanism was understood by fitting the data to Korsmeyer-peppas equation $M_t / M_a = Kt^n$, where 'M_t / M_a'is fraction of drug released at time 't', 'K' is kinetic constant and 'n' is release exponent which characterized the drug release mechanism. If the value of 'n' is less than 0.45 then it is considered as Fickian release, values more than 0.45 and less than 0.89 is considered as anomalous (non-Fickian) transport and finally 'n' value greater than 0.89 follows super case-II release mechanism.

Stability study:[6]
Stability study of optimized formulation was carried out at 25± 2 ºC/ 60 ± 5% and 40 ± 2 ºC/75± 5% RH for a period of three months. During stability study *in situ* gel was analysed for pH, viscosity, drug content and *in vitro* drug release.

RESULTS

The melting point of rosuvastatin was found to be 156-158°C and compatibility studies from the FT-IR spectra of rosuvastatin pure drug and its physical mixture revealed that there was no significant change in peak of rosuvastatin in its physical mixture, indicating the compatibility of drug in formulation mixture.

***In vitro* gelling capacity:**
The main requirements for periodontal *in situ* gels were its viscosity and gelling capacity. Formulation F1 and F2 containing lower concentration of methyl cellulose showed weakest gelation after 12-15 m and dispersed rapidly on shaking. Formulation F3 and F4 showed (table 2) immediate gelation effect after 7-8 m but the formed gels are less stiff and does not remains for extended period of time. Formulation F5, F6, F7 and F8 showed immediate gelation after 2-4 m and formed gel was stiff and remained for extended period of time, this might be due to the presence of higher concentration of methyl cellulose.

Characterization of *in situ* gels:
The various characterization studies like appearance, clarity, gelling capacity, pH, drug content, viscosity, spreadability and syringeability was determined and the data is given in Table 3.

Appearance:
Formulations F1-F4 containing low concentration of methyl cellulose 0.5%-0.9% were clear, as the concentration of methylcellulose was increased from

Table 3: *Characterization of various in situ gel formulations*

Formulation Code	Clarity	Gelling capacity	pH	Viscosity (cps)	Drug content (%)	Syringeability	Spreadability g.cm/s
F1	Clear	+	5.9	320	98.78	Pass	28.11
F2	Clear	+	5.8	350	99.72	Pass	27.90
F3	Clear	++	6.0	380	98.53	Pass	27.55
F4	Clear	++	5.8	410	100.42	Pass	24.12
F5	Clear	+++	5.8	460	97.83	Pass	22.69
F6	Cloudy	+++	5.8	530	98.89	Fail	19.73
F7	Cloudy	+++	5.7	570	99.27	Fail	18.00
F8	Cloudy	+++	5.6	590	98.74	Fail	17.44

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

0.9% to 1%, 1.1% and 1.2% in formulation F5-F8 appearance to the gel became cloudy.

pH of prepared gel:
The pH of the prepared gel was found in the range of 5.6-6.0, which was required pH for periodontal gel preparation.

Drug content uniformity:
The data of drug content from all the prepared gel formulations was found in range of 97.83%- 100.42%.

Viscosity:
Viscosity of prepared *in situ* gel formulation was evaluated and values were found to be in the range of 320 to 590 cps.

Rheological studies:
The results of rheological studies indicated that prepared gel showed shear thinning pseudoplastic behaviour i.e. thin when exposed to higher shearing force and thick when shearing force was released (figure 1).

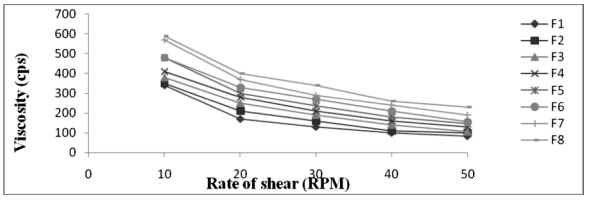

Fig. 1: *Rheological properties of in situ gel formulation batch F1-F8*

Syringeability of *in situ* gel:
Formulation F1-F5 pass syringeability test as the gel expelled quite easily from the syringe equipped with 20 gauge needle. Formulation F6, F7 and F8 fail the syringeability test, which may be presence of higher concentration of methyl cellulose.

Spreadability of *in situ* gel:
Spreading ability of prepared gel was determined by spreadability test. Spreadability test for all batches was performed and results were found in the range of 17.44-28.11 g.cm/s.

***In vitro* drug release study:**
In vitro release profile of rosuvastatin from *in situ* gels containing different concentration of methyl cellulose is shown in figure 2. Formulation F1 to F4 containing lower concentration (0.5-0.8%)of methyl cellulose showed 96.32%, 94.11%, 89.88% and 87.33% respectively within 8 h. Formulations F5, F6, F7 and F8 containing 0.9%, 1%, 1.1% and 1.2% concentration of polymer showed 54.33%, 32.21, 28.85% and 23.73% drug release at the end of 8 h respectively. Among all formulations, formulation F5 showed 50% of drug released in sustained manner at the end of 8 hours, which is comparatively lower than F1, F2, F3 and F5 and higher than F6, F7 and F8; hence F5 was selected as optimized formulation for periodontal treatment.

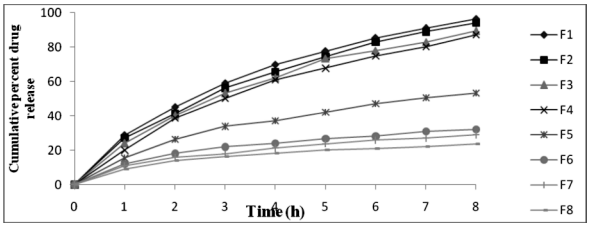

Fig. 2: *Comparative in vitro release profile of rosuvastatin in situ gel formulation F1-F8*

Drug release kinetics:
The result of *in vitro* release data was fitted to various kinetic models and results showed that drug release followed first order kinetics, as the values for first order (0.975-0.997) are higher in comparison to zero order (0.498-0.784) and Higuchi model (0.921-0.954). The release exponent value (n) for all formulation was found in the range of 0.335-0.442, which indicated that the drug release followed Fickian diffusion mechanism, the data is shown in Table 4.

Table 4: *Release kinetic values for different in situ gel formulations*

Formulation Code	Zero order	First order	Higuchi	Korsmeyer/peppas model	
	R^2	R^2	R^2	R^2	n
F1	0.672	0.992	0.921	0.981	0.411
F2	0.717	0.975	0.933	0.960	0.336
F3	0.562	0.989	0.945	0.958	0.349
F4	0.784	0.997	0.929	0.981	0.418
F5	0.498	0.988	0.954	0.966	0.335
F6	0.629	0.994	0.940	0.978	0.392
F7	0.597	0.986	0.924	0.972	0.442
F8	0.762	0.993	0.942	0.982	0.362

Stability study:
The optimized formulation F5 was selected for short term stability. During stability study formulation F5 was analysed for pH, viscosity, drug content and *in vitro* drug release and result showed no significant changes in any of these parameters. Thus, prepared formulation was stable throughout the study period; the data is shown in Table 5.

DISCUSSION
The melting point of rosuvastatin was similar with the values reported in official pharmacopoeia. Compatibility studies from the FT-IR spectra of pure drug and its physical mixture indicated that drug was compatible with other formulation mixture.

Table 5: *Results of short term stability studies of an optimized formulation F5*

Time Period	At 25±2 ºC/60±5%RH				At 40±2 ºC/75±5% RH			
	pH	Viscosity (cps)	Drug content (%)	% Drug release	pH	Viscosity (cps)	Drug content (%)	% Drug release
Initial	5.8	460	97.83	54.33	5.8	460	97.83	54.33
1 month	5.8	460	97.82	54.00	5.7	461	97.81	53.47
2 months	5.7	465	97.77	53.44	5.6	467	97.69	53.02
3 months	5.7	468	97.74	52.76	5.6	470	97.58	52.14

Methyl cellulose concentration:
Polymer plays an important role in release of drug from gel matrix. Concentration of polymer and type of polymer used in preparation of *in situ* gel affect the viscosity of gel and ultimately release of drug. For the selection of methyl cellulose concentration various solutions of methyl cellulose (0.5-1.2%) was prepared in distilled water and finalization of concentration was done on the basis of gelation temperature and gelation time. Gelation temperature of solution of 0.7-0.9% was observed in the range of desired gelation temperature (37-39°C). Among 0.7-0.9% solution, 0.9% solution showed shorter gelation time 4 m, so this concentration was selected for further study.

Characterization of *in situ* gels:
The prepared gel formulation was characterized for its appearance, clarity, gelling capacity, pH determination, drug content, viscosity, syringeability and spreadability.

Appearance:
Formulations with low concentration of methyl cellulose as gel base was clear, as the concentration of methyl cellulose was increased to more than 1% cloudy appearance was observed.

pH:
The pH of the formulations was found in the range of required pH suitable for periodontal treatment.

Drug content uniformity:
The results of drug content from all the prepared formulations are acceptable and indicated uniform drug content.

***In vitro* gelling capacity:**
The main requirements for *in situ* periodontal gels were viscosity and gelling capacity. The *in situ* gel formulation should undergo rapid sol to gel transition in phosphate buffer due to ionic interaction. To facilitate the sustained release of the drug to periodontal cavity, the formed gel should preserve its integrity without eroding or dissolving in periodontal cavity. Except the F1 and F2 remaining all formulations were showed instantaneous gelation when come in contact with buffer (pH 1.2) maintained at 37 ± 1°C. However, the nature of the gel formed depends upon the concentration of polymer used. Formulation F1 and F2 containing lower concentration of polymer showed weakest gelation after 10-12 m and dispersed rapidly on shaking. Formulation F3 and F4 showed immediate gelation effect but the formed gels are less stiff and does not remains for extended period of time. Formulation F5, F6, F7 and F8 showed immediate gelation and formed gel was stiff and remained for extended period of time, this might be due to the presence of higher concentration of methyl cellulose.

Viscosity:
One of the important requirements for a periodontal gel is viscosity of the formulation. The prepared gel should be such that it should have a low viscosity when applied to the periodontal pocket, and after administration it should have a high viscosity in order to stay at the site of application.

Rheological studies:
The rheological characteristic of all prepared gel formulation was understood by plotting viscosity of gel vs speed of rotation and results indicated that prepared gel decreased viscosity with increase in shearing stress.

Syringeability:
Syringeability of gel formulation depends upon the concentration of polymer used, as the concentration of methyl cellulose increased the viscosity of formulations was also increased, which in turn required greater force to expel gel from the syringe.

Spreadability:
Spreadability of gel formulation is also concentration dependent i.e. spreadability increases with decrease in the concentrations of polymer and vice versa. Formulation containing lower concentration of methyl cellulose was more spreadable compared to formulation containing higher concentration of methyl cellulose.

***In vitro* drug release study:**
In vitro release profile of methyl cellulose based rosuvastatin*in situ* gels formulation showed that release of the rosuvastatin from these formulations was concentration dependent, as the concentration of methyl cellulose increases release rate of drug was retarded significantly. Initially, release of drug from these formulations was higher due to the brusting effect and as the time period increases gelation effect was seen and finally release rate was retarded. Formulations containing lower concentration of methyl cellulose release the drug quite faster when compare to formulations containing higher concentration methyl cellulose. Formulations containing methyl cellulose beyond certain level retarded the drug release in such a way that expected amount of drug did not release at predetermined period of time.

Drug release kinetics:
The results of *in vitro* release data was fitted to various kinetic models in order to know the drug release mechanisms. The results drug release kinetics showed that the drug release followed first order kinetics. The release exponent value (n) for all formulation used to characterize the drug release mechanism and the obtained 'n' values for all formulations was less than 0.45. The values of 'n' were found in the range of 0.337-0.448, which indicated that the drug release followed Fickian diffusion mechanism. This might be due to swelling property of the methyl cellulose used in gel.

Stability study:
The optimized formulation F5 was selected for short term stability study for the period of 3 months at 25± 2 ºC/ 60 ± 5% and 40 ± 2 ºC/75± 5% RH . The results

showed that prepared gel formulation was physiochemically stable throughout the study period.

CONCLUSIONS

In this present study *in situ* gel of rosuvastatin for the clinical treatment of periodontal diseases was successfully formulated using methyl cellulose as gel base. This *in situ* gel formulation possesses muco-adhesive properties, results of which prolong residence time at the site of application, which in turn exhibited better therapeutic effects. In addition, *in situ* gel provides intimate contact between the drug and the absorbing tissue which may result in high drug concentration in local area. Thus, based upon obtained results it can be concluded that the formulation containing 0.9% methyl cellulose was considered as an optimized formulation and provided sustained drug release over an extended period of time i.e. more than 50% drug was released in 8 h this may leads to better patient compliance. Further, clinical trials have to be conducted to study the effect of these *in situ* gels on patients when administered locally for the better treatment with respect to periodontal diseases.

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