

Thermoreversible *in situ* gel for subgingival delivery of simvastatin for treatment of periodontal disease

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Abstract

Objective: The aim of this *in vitro* study was to formulate an *in situ* thermoreversible injectable gel with poloxamer (PM) and methylcellulose (MC) to deliver simvastatin (SMV) in a controlled manner.

Subjects and Methods: Preformulation studies (Fourier transform infrared and differential scanning calorimetry) to assess the interaction between SMV and MC and PM were performed before gel formulation. Keeping the concentration of SMV at 2.2%, the concentration of PM and MC was altered to formulate *in situ* thermosensitive gel at 37°C. Rheological studies were carried to analyze the physical property of the various formulations. Drug release profile and stability studies were done for the selected formulation. The *in vitro* drug release profile was carried out for using open end tube method and ultraviolet spectroscopy.

Results: The preformulation studies showed that there is no interaction between the polymer and drug based on the rheological studies of different formulation, the formulation. F8 gels at 37°C and attains a viscosity of 4150 cps.

Conclusions: PM 25% and MC 5% formed an ideal thermosensitive injectable gel at 37°C for subgingival delivery of SMV and also show controlled drug release for the period of 10 days *in vitro*.

Keywords: *In situ* gel, periodontitis, simvastatin

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INTRODUCTION

Periodontal diseases are a group of inflammatory conditions affecting the supporting structures of the teeth characterized by destruction of periodontal hard and soft tissues resulting in pocket formation, mobility, and in turn leading to tooth loss.^[1] The deepened gingival sulcus serves as an ideal environment for the growth and proliferation of periodontopathic bacteria leading to periodontitis.^[2] The host immune and inflammatory response is activated by the components of host immune

system in a sequential manner. The immune response aims to protect the host tissue from bacterial aggression, but it also acts as a mediator of the periodontal destruction.^[3] Apart from bacterial degradation of host tissues directly, Birkedal-Hansen suggest that the host plays a major role in the degradation of host connective tissue.^[4] Current periodontal treatment strategies target the bacterial deposits on the tooth surface by mechanical debridement which aids in shifting the pathogenic microflora to a healthier environment.^[5] To maintain the balance between tissue destruction and inhibition, treatment strategy was

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focused on the modulation of host. Simvastatin (SMV), a potent prodrug of hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, belongs to the statin family that blocks conversion of HMG-CoA to mevalonic acid, which is needed for cholesterol biosynthesis, used in the treatment of hyperlipidemia.^[6] It had been proved that SMV has additional properties such as anti-inflammatory,^[7] bone regenerative,^[8] and promotion of new blood vessels.^[9] Hence, SMV could be used in the treatment of periodontitis by modulating the host response and control inflammation, thus maintaining homeostasis. The local drug delivery systems use biodegradable polymers that can deliver and achieve a sustained release of the drug over days to combat the periodontal bacteria in the pocket.^[10] The advantage of using such a system is the self-elimination of carrier medium. The periodontal pocket which is the target site for local drug delivery is a complex region where there is constant the flow of crevicular fluid. An injectable *in situ* sustained release gel would be an ideal option for the drug to reach the complex environment and deliver its desired effects. Hydroxypropyl methylcellulose (HPMC) and methylcellulose (MC) are nonionic cellulose ethers used in the topical application of oral sustained release drug delivery system.^[11,12] Poloxamers (PMs) are nonionic, polyoxyethylene-polyoxypropylene-polyoxyethylene (PEO-PPO-PEO) triblock copolymers. Their amphiphilic nature depends on the concentration and temperature.^[13-15] The ability of these hydrogels to carry a significant amount of a drug, and biodegradable, nontoxic, and stable characteristics made them suitable to use as controlled release agents.^[16] Lower concentrations of PMs with polymers such as collagen, MC, and HPMC have been used in the formulation of thermosensitive ocular gels.^[17] At a lower concentration between 20% and 30% and lower temperatures (4°C–5°C), PM 407 (P407) or Pluronic F 127 remains liquid and turns into gel at particular temperature, this gel can be reversed to liquid when the temperature is lowered and again to gel at room temperature.^[18] This thermoreversible gelation property of P407 (18%–35%) has been used as drug delivery system for nasal application.^[19-21] Hence, MC and PMs can be combined to achieve a thermosensitive *in situ* gel for controlled drug release. The aim of this *in vitro* study was to formulate an *in situ* thermoreversible injectable gel with PM and MC to deliver SMV in a controlled manner.

SUBJECTS AND METHODS

This *in vitro* study was conducted in Department of Pharmaceuticals, Faculty of Pharmacy, Sri Ramachandra University. MC 4000 cps was purchased from Central Drug House, New Delhi. SMV was purchased from Microlabs,

Mumbai. PM was purchased from Sigma Aldrich, Mumbai. All the chemicals used were analytical grade. Preformulation studies (Fourier transform infrared [FT-IR] and differential scanning calorimetry [DSC]) to assess the interaction between SMV and MC and PM were performed before gel formulation.

Differential scanning calorimetry

Thermal characterization of pure drug and physical mixture was performed with DSC. Samples were weighed (2.00 ± 0.5 mg) and placed in sealed aluminum pans. The samples were scanned at 20°C/min from 25°C to 300°C.

Fourier transform infrared

The interaction between drug and the polymer was assessed using FT-IR (Shimadzu FT-IR 8400S, Japan). A physical mixture (5 g) of each polymer and drug was taken in a ratio of 1:1 and mixed properly with 100 mg of KBr. Pellets were made from the mixture by taking 50 g of the mixture and compressing using a hydraulic press at 15 tonnes pressure. The prepared pellets were scanned from 4000 to 400 cm^{-1} using FT-IR spectrophotometer.

Hydrolysis of simvastatin

SMV powder was converted to the active SMV hydroxyl acid by dissolving weighed amount of SMV powder in 95% ethanol and 0.1M NaOH and heated at 50°C for 2 h and then the pH was adjusted to 7.4 by adding 0.1M HCl and stored at -20°C .^[22,23]

Formulation of simvastatin gel

A total of 13 different formulations were developed with varying concentration of MC and PM keeping the SMV as constant (2.2%). In brief, the desired concentration of PM was dissolved in 10 ml of cold water by continuous stirring until the polymer is completely dissolved and stored at 4°C. MC and SMV were prepared in the same manner. From this, different concentrations of formulations were prepared [Table 1]. The polymers were stirred continuously with a mechanical stirrer until a homogeneous mixture was achieved. All the formulations were stored in 4°C overnight.

Rheology

The rheological analysis was performed using a stress- and strain-controlled rheometer model (RHEOPLUS MCR301). Flow measurements were performed with single gap cylinder CC27 geometry, the diameter of rheometer plate and interplate was 20.05 and 0.052 mm, respectively, whereas the cone angle was 1°, max shear rate was 10,000 1/s, and max shear stress was 22,260 Pa. To evaluate the exact gelling temperature,

change in the loss modulus and storage modulus was observed in response to gradual change in temperature from 4°C to 40°C.

Viscosity

The complex viscosity and the viscosity in relation to shear rate were also observed from the rheological studies.

Stability studies

Stability testing for 3 months as per the International Conference on Harmonisation norms at a temperature of 40°C ± 2°C was performed. At various time intervals, drug content and pH were analyzed.^[24]

In vitro drug release studies

Drug release for selected formulation alone was done using an open end tube method as described in our previous study.^[24] Briefly measured quantity of a gel is placed in the open end tube, using phosphate buffered saline of pH 7.4 as the medium at temperature 37°C. Samples were withdrawn at frequent intervals and assessed in ultraviolet spectrometer.

RESULTS

Fourier transform infrared spectroscopy

FTIR analysis revealed that the functional groups of the MC, PM, and SMV remained unaltered when SMV and MC and PM were mixed and assessed [Figure 1].

Differential scanning calorimetry

In thermal characteristic study, it was observed that the melting point of PM started at 46.2°C and ends at 62.7°C with the peak at 56.9°C. When the physical mixture of PM, MC, and SMV exposed to highest temperature (200°C), the individual meeting point of each component remained unchanged [Figure 2].

Rheology

All the formulations were semisolid to liquid in consistency, at a minimum temperature of 4°C. Whereas upon

warming the formulation turned to liquid and to gel, few formulations attained gelation from their semisolid state. All the different formulation attained different gelation temperature. The storage modulus G [Figure 3a], “the loss modulus G,” and the complex viscosity (H×) are shown in Figure 4a and b. The temperature at which g0 suddenly varied corresponded to the temperature of the sol-gel transition of F8 [Figure 3b] was observed. The viscosity decreased as the shear rate increased [Figure 4c].

Drug release study

The drug release profile was controlled release of formulation F8 and was in a controlled manner for a period of 10 days. The release pattern is shown in Figure 4d.

Stability study

The stability of the *in situ* gel for the formulation F8 was assessed at 0, 1, 3, and 6 months' postformulation. The drug content was 99.2% ± 0.06% at baseline, 99.1% ± 0.15% at 1 month, 98.5 ± 0.17 at 3 months, and 98.1 ± 0.17 at 6 months. No change was observed in the pH due to storage; however, change in the drug content was elicited as storage time increased. The appearance of the gel remained clear throughout the observation period [Table 2].

DISCUSSION

SMV-based gel has been formulated using MC in different concentrations and its efficacy has been widely studied *in vivo*.^[24,25] Different concentrations of SMV, namely, 1.2%, 1.7%, and 2.2% have been used to formulate injectable gel using 4% MC.^[25,26] In our previous study, we evaluated 4%, 5%, and 6% MC and 1.2%, 1.7%, and 2.2% SMV gel *in vitro* and found that 6% MC and 2.2% SMV had a controlled release for a longer period compared to 4% and 5%.^[27] Hence, for this study, 2.2% SMV was chosen. Furthermore, the results indicated that the release of the drug in a controlled manner is directly proportional to the

Table 1: Formulation Details

Name	Methyl cellulose (%)	Poloxamer (%)
F1	4	30
F2	4	35
F3	4	40
F4	5	30
F5	5	35
F6	5	40
F7	5	20
F8	5	25
F9	5	10
F10	6	10
F11	4	-
F12	5	-
F13	6	-

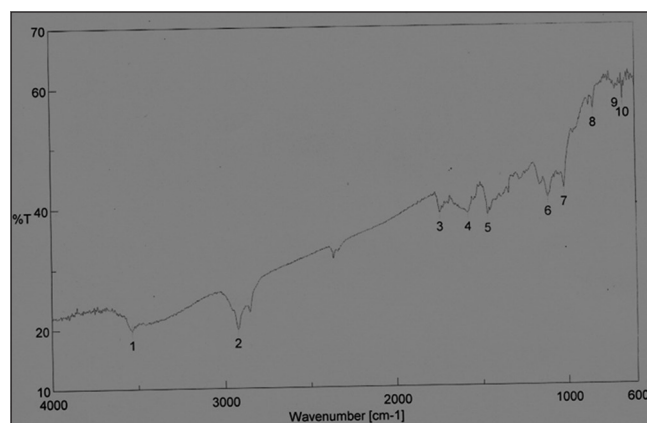


Figure 1: Fourier transform infrared spectroscopy analysis

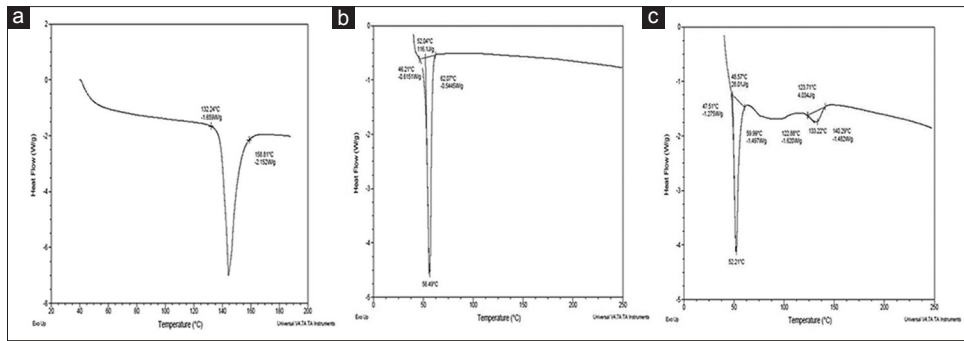


Figure 2: Differential scanning calorimetry

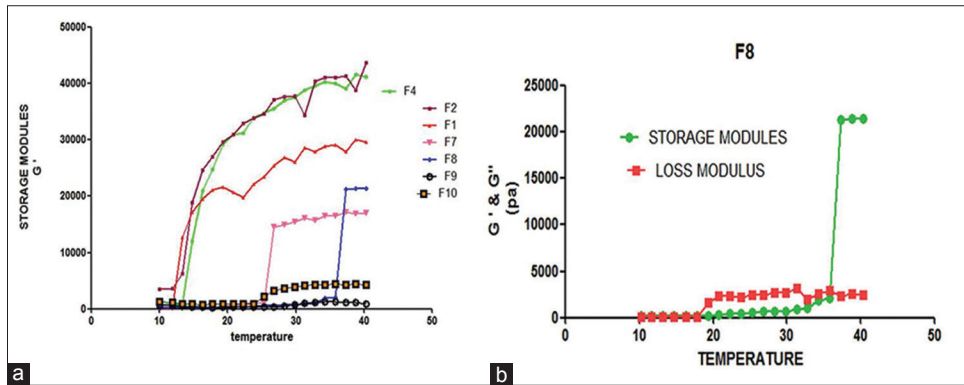


Figure 3: Rheology study

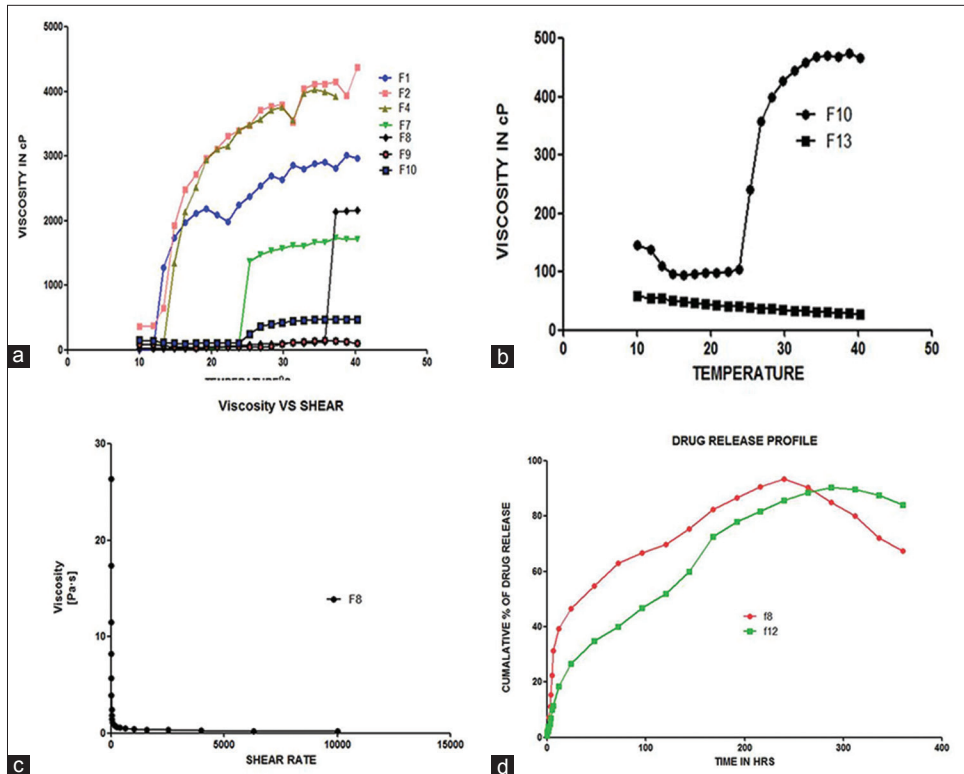


Figure 4: Viscosity and drug release profile

concentration of the MC. Although 6% MC gave higher rate of controlled release, its effect on the thermoreversible

gelation property of PM has to be evaluated. Hence, to achieve optimum gelling property close to body

Table 2: Stability Study

Formulation Code	Duration (Months)	pH	Drug Content (%)	Appearance
F8	0	7.19±0.03	99.2±0.06	Clear
	1	7.16±0.02	99.1±0.15	Clear
	3	7.14±0.01	98.5±0.17	Clear
	6	7.11±0.01	98.1±0.17	Clear

temperature, three concentrations of MC (4%, 5%, and 6%) and five concentrations of PM (10%, 20%, 25%, 35%, and 30%) were chosen. The physical mixture of MC, PM, and SMV was assessed using DSC and IR, and the results revealed that there was no drug and polymer interaction. The rheology study of formulation F1 and F2 showed that the concentration of PM decreases from 35% to 30% and the gelation temperature increases (11.8°C-F2–14.8°C-F1). F4 attained gelation at 13.3°C, which was less than F1. This could be attributed to the higher viscosity in F4 (5% MC) which had decreased the gelation temperature. Further, the gelation of F7 was at 25.3°C and F8 at 35.8°C, which is closer to the body temperature. Although F9 starts gelation 26.6°C and completes gelation 35.8°C beyond this temperature, it loses its storage modulus. Further to increase the gel strength F9, F10 was formulated with 6% MC. This resulted in higher storage modulus at 10°C and it reduced as the temperature increased close to 25°C. Although F10 exhibited high gel strength, it failed to form *in situ* gel at body temperature. At lower temperature, water serves as good solvent for PEO and PPO units of P407. The hydrophobic portion of PM is kept separated by the hydrogen bonding between water and PPO chain at lower temperature. When the temperature is high, these hydrogen bonds are disrupted lead to hydrophobic interaction and gel formation. Thus, gelling properties of PM are dependent on proportion of hydrophobic portion.^[21] This could be the one of the reasons for gel formation at lower temperature in F1, F2, and F3 where the availability of hydrogen bonds was more as the concentration of MC was only 4% compared to F8. Comparing F10 with F13, we found that in the absence of PM, the MC gel decreased its viscosity as the temperature increases. Both the storage and loss modulus are higher in the presence of PM. The storage modulus being higher than loss modulus. The complex viscosity of all the formulations increased as the temperature increases and it reached the peak along with the storage modulus. In F7, F8, F9, and F10 formulation, the complex viscosity remained stable after gelation till the temperature reached 40°C. Among these four formulations, only F8 had the highest viscosity of 2140 cps whereas F1, F2, and F4 showed higher viscosity than F8 and F1 being the highest of 4150 cps at 37°C. This could possibly be due to overall higher polymer percentage. All the formulations

demonstrated shear thinning as the shear rate increased. The high viscosities of the gel at low shear rates would aid in maintaining a good contact between the tooth surface and gingiva, and this will leads to uniform distribution of the gel into the pocket. This property is similar to the shear rate and viscosity of *in situ* ocular gel.^[28]

The storage modulus and loss modulus of F8 formulation till 17.8°C were almost at the same level. From 19.3°C to 31.3°C, the loss modulus was higher which makes the gel less viscous and increases the flowability as well as helps in better handling at room temperature [Figure 3b].

In vitro drug release of F8 and F12 formulation showed a control drug release. Compared to F12, the rate of drug release was faster in the F8 and after 240 h the percentage of drug release reduced whereas F12 started to reduce only after 288 h. This shows the presence of P407 which increases the percentage of drug release from the gel and MC which can promote sustained release. The results of our study are in accordance with study performed by Desai and Blanchard^[29] where the authors showed addition of polyethylene glycol (PEG) or polyvinylpyrrolidone. PEG/poly(lactic-co-glycolic acid) block copolymers accelerated pilocarpine release while the addition of MC slowed the release rate. This indicates that PM alone cannot be used for sustained release system of any drug. Hence, combining both these polymers would lead to sustained release in an *in situ* gel formulation.

Based on the gel strength, the gelling temperature and release profile of F8 formulation formed the ideal gel for thermosensitive controlled release gel. Further, the gel strength could be enhanced by addition of high viscosity polymers and additives. Although injectable SMV has been used in pilot study in the management of periodontal defect and alveolar ridges,^[30] an *in situ* gel system for delivery of SMV into periodontal pockets could prove to be clinically effective.

CONCLUSIONS

PM 25% and MC 5% formed an ideal thermosensitive injectable gel for subgingival delivery of SMV. However, studies have to be carried out to assess whether addition of another polymer like Carbopol can increase the gel strength after gelation which could help the gel to reach the subgingival region for the longest period.

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Conflicts of interest

There are no conflicts of interest.

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