Formulation and Evaluation of In situ Gel of Atorvastatin for the Treatment of Periodontitis

Mohammed Gulzar Ahmed*, Ravi Choudhari and Ankit Acharya
Department of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, B.G. Nagar, India.

ABSTRACT

Purpose: The main objective of present study is to formulate and evaluate methyl cellulose based in situ periodontal gel of Atorvastatin. Approach: The in situ gel was prepared by using different concentration of methyl cellulose and gel was evaluated for pH, viscosity and rheology, syringeability, drug content, in vitro drug release, and drug release kinetics. Findings: Compatibility study was performed using FTIR and results showed there was no interaction between drug and other excipients. Viscosity of all formulations was found in the range of 320-570 centipoise and all formulations exhibited pseudoplastic behaviour. Gelation time and temperature was found in the range of 6-17 min and 29°C-39°C respectively. All the formulation except formulation G6 and G7 showed satisfactory syringeability due to lower concentration of polymer. Based on the results of release study, formulation G5 was found to be optimum formulation as it released 96.87% drug at the end of 24 h. 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: It can be concluded that formulation containing 0.9%w/v of methyl cellulose gave optimized formulation.

Key words: Atorvastatin, Gelation time, In situ gel, In vitro drug release, Pseudoplastic behaviour, Syringeability.

INTRODUCTION

Periodontal disease is one of the most common microbial infections in man and affects the tooth-supporting structures, including the gingiva, the periodontal ligament, and alveolar bone in the adults.1 Mainly there are two types of periodontal disease; namely gingivitis and periodontitis. Gingivitis is relatively common and reversible condition where limited inflammation occurs in unattached gingiva. If gingivitis is not treated in time, it may proceed to chronic periodontitis, a continuous inflammatory process resulting in irreversible periodontal tissue destruction and leads to tooth loss, particularly in adults. Both of these diseases occur when bacteria from dental plaque invade surrounding tissues, which in turn induce an inflammatory response. This type of inflammatory events resulted in formation of periodontal pockets between gingiva and tooth and gingival bleeding. This pocket provides an ideal environment for the growth and proliferation of anaerobic bacteria responsible for the disease.2,3 When such anaerobic bacteria gain access to sterile body site, they can become opportunistic pathogens and cause serious and fatal infection. The main reason for incomplete eradication of these bacteria may be due to short residence time, time consuming application and degradation of an antibacterial agent in the oral cavity. Better stability and longer residence time will allow more of the therapeutic agent to penetrate through the oral mucosa to act for longer duration of time.

Therefore, some researchers had prepared and reported a newer drug formulation named as in situ gel, which is able to reside in an oral cavity for a longer period of time.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 formulations are applied as a solution, which undergoes gelation after instillation into oral cavity due to physicochemical changes inherent to the biological fluids. 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. Thus these “smart” polymers play an important role in drug delivery since they may dictate not only where a drug is delivered, but also when and with which interval drug is released. The polymer used in preparation of in situ gel should be biocompatible, adhere properly to mucus, and have pseudoplastic behaviour.

Atorvastatin is 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor and comes under statin class of drug. It 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 atorvastatin reduces periodontal inflammation.

In present study, an attempt was made to develop a sustained released in situ gel of atorvastatin to use in periodontal diseases by using different concentration of methyl cellulose. Thus the study aims to improve patient compliance, increase bioavailability and controlled the drug release by using biodegradable polymer network.

**Materials and Methods**

Atorvastatin was obtained as a gift samples from Microlabs, Bangalore. Methyl cellulose and sodium citrate were procured from S.D. fine chemical, Mumbai. All other ingredients used were of analytical grade.

**Preformulation studies**

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

**Selection of Methyl cellulose concentration**

Solution of different concentration ranging from 0.5-1.1w/w % 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 minutes. The beaker containing partially dissolved methyl cellulose was sealed with aluminium 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 \textit{In situ} gel

For the preparation of methyl cellulose containing \textit{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 Atorvastatin (1.2\% W/V) was dissolved in required quantity of methanol and 2-3 drops triethanolamine separately and then added to polymer solution under constant stirring. Finally methyl paraben and propyl paraben were added to the above formulation mixture under constant stirring until a uniform solution was obtained. The optimization concentration of methyl cellulose was selected on the basis of gelation temperature and gelation time given in Table 1. Further the prepared formulations were evaluated for various characterization studies.\textsuperscript{2}

Characterization of \textit{In situ} gel formulation

\textbf{Appearance}

All prepared formulations were evaluated from the visual inspection.\textsuperscript{2}

\textbf{Gelling Capacity}

All formulations were evaluated for gelling capacity in order to identify the compositions suitable for use as \textit{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 of 6.8 pH phosphate buffer in a 10 ml test tube and maintained at 37 ± 1° temperature. One millilitre of coloured formulation solution was added to the phosphate buffer. As the formulation comes into contact with phosphate 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 \textit{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.\textsuperscript{3}

\textbf{pH measurement}

pH is one of the most important parameter involved in the \textit{in situ} gel formulation and it is measured directly with the help of digital pH meter.\textsuperscript{4}

\textbf{Viscosity and rheological studies}

Brookfield digital viscometer (Model LVDV–E, USA) was used for the determination of viscosity and rheological properties of atorvastatin \textit{in situ} gel using spindle no T-96.50 g of the gel 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°. A typical run comprised changing of the angular velocity from 5 to 25 rpm. The averages of two readings were used to calculate the viscosity.\textsuperscript{4,5}

\textbf{Gelation temperature}

10 ml of the sample solution and a magnetic bead 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°/min with the continuous stirring. The temperature at which the magnetic bead stopped moving due to gelation was considered as gelation temperature.\textsuperscript{5}

\textbf{Gelation time}

Gelation time of prepared \textit{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°) and gelation time was noted when there was no flow of the gel when test tube was inverted.\textsuperscript{3}

\textbf{Drug content analysis}

Accurately weighed amount of gel equivalent to 2 mg of drug was taken into a 100 ml volumetric flask. They were lysed with 25 ml of medium (6.8 pH phosphate buffer) for 15 min. The clear solution was diluted to 100 ml of medium. Then 10 ml of this solution was diluted

\begin{table}
\centering
\caption{Composition design of various Atorvastatin \textit{in situ} gel formulations}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Batch code & Atorvastatin (%w/v) & Methyl cellulose (%w/v) & Sodium citrate (%w/v) & Methyl paraben (%w/v) & Propyl paraben (%w/v) & Deionized water \\
\hline
G1 & 1.2 & 0.5 & 0.1 & 0.15 & 0.02 & Q.S \\
G2 & 1.2 & 0.6 & 0.1 & 0.15 & 0.02 & Q.S \\
G3 & 1.2 & 0.7 & 0.1 & 0.15 & 0.02 & Q.S \\
G4 & 1.2 & 0.8 & 0.1 & 0.15 & 0.02 & Q.S \\
G5 & 1.2 & 0.9 & 0.1 & 0.15 & 0.02 & Q.S \\
G6 & 1.2 & 1.0 & 0.1 & 0.15 & 0.02 & Q.S \\
G7 & 1.2 & 1.1 & 0.1 & 0.15 & 0.02 & Q.S \\
\hline
\end{tabular}
\end{table}
to 100 ml of phosphate buffer (pH 6.8). Aliquots were withdrawn and the absorbance was measured at 246 nm against phosphate buffer (pH 6.8) by using UV-Visible Spectrophotometer-1800 (Shimadzu, Japan) and drug content was calculated from the calibration curve.¹

**Syringeability**

All prepared formulations were transferred into a 5 ml syringe placed with 20 gauge needle to a constant volume (2 ml). The solutions, which easily passed from syringe were termed as pass and difficult to passed were termed as failed.²

**In vitro drug release studies**

In vitro drug release study of Atorvastatin from the in situ gel formulations was conducted for a period of 24 h using cellophane membrane. The dissolution medium was phosphate buffer (pH 6.8). Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a glass cylinder. Then 1 ml of the prepared formulation was placed in cellophane membrane tied to the glass cylinder and made the membrane just touch the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Sample was withdrawn at regular interval and replaced by an equal volume of the receptor medium. At predetermined time interval one ml of the sample was taken and analysed spectrophotometrically at 246 nm.³,⁴

**Drug release kinetics**

To understand the drug release kinetics of atorvastatin 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 \[ M_t/M_a = K t^n \], 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.⁴,⁸

**Stability study**

Stability study of optimized formulation was carried out at 25º/60% and 40º/75% 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.⁸

**RESULTS**

The melting point of atorvastatin was found to be 158-160º and compatibility studies from the FT-IR spectra of atorvastatin pure and its physical mixture revealed that there was no change in significant peak of atorvastatin in mixture.

**Methyl cellulose concentration**

For the selection of methyl cellulose concentration various solutions of methyl cellulose (0.5-1.1%) were prepared and gelation temperature of solution of 0.7-0.9% was observed. The 0.9% solution showed shorter gelation time 6 min, the data is given in Table 2.

**Characterization of in situ gels**

The various characterization studies like appearance, clarity, gelling capacity, pH determination, drug content and viscosity was determined and the data given in Table 3.

---

### Table 2: Gelation temperature and time of Atorvastatin in situ gel

<table>
<thead>
<tr>
<th>Methyl cellulose Concentration (%)</th>
<th>Gelation temperature (°)</th>
<th>Gelation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>No gelation up to 50° temperature</td>
<td>--</td>
</tr>
<tr>
<td>0.6</td>
<td>No gelation up to 50° temperature</td>
<td>--</td>
</tr>
<tr>
<td>0.7</td>
<td>39</td>
<td>9</td>
</tr>
<tr>
<td>0.8</td>
<td>37</td>
<td>8</td>
</tr>
<tr>
<td>0.9</td>
<td>36</td>
<td>6</td>
</tr>
<tr>
<td>1.0</td>
<td>32</td>
<td>14</td>
</tr>
<tr>
<td>1.1</td>
<td>29</td>
<td>17</td>
</tr>
</tbody>
</table>

### Table 3: Characterization of in situ gel

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Clarity</th>
<th>Gelling capacity</th>
<th>pH</th>
<th>Viscosity (Cps)</th>
<th>Drug content (%)</th>
<th>Syringeability</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Clear</td>
<td>+</td>
<td>5.8</td>
<td>320</td>
<td>98.78</td>
<td>Pass</td>
</tr>
<tr>
<td>G2</td>
<td>Clear</td>
<td>+</td>
<td>5.6</td>
<td>350</td>
<td>99.72</td>
<td>Pass</td>
</tr>
<tr>
<td>G3</td>
<td>Clear</td>
<td>++</td>
<td>5.7</td>
<td>380</td>
<td>98.53</td>
<td>Pass</td>
</tr>
<tr>
<td>G4</td>
<td>Clear</td>
<td>++</td>
<td>5.6</td>
<td>410</td>
<td>100.42</td>
<td>Pass</td>
</tr>
<tr>
<td>G5</td>
<td>Clear</td>
<td>+++</td>
<td>5.8</td>
<td>460</td>
<td>97.63</td>
<td>Pass</td>
</tr>
<tr>
<td>G6</td>
<td>Cloudy</td>
<td>+++</td>
<td>5.9</td>
<td>530</td>
<td>98.89</td>
<td>Fail</td>
</tr>
<tr>
<td>G7</td>
<td>Cloudy</td>
<td>+++</td>
<td>5.5</td>
<td>570</td>
<td>99.27</td>
<td>Fail</td>
</tr>
</tbody>
</table>

(+) gels after few minutes, dispersed rapidly; (++) gelation immediate, remains for few hs; and (+++) gelation immediate, remains for an extended period.
Appearance
The low concentration of methyl cellulose 0.5–0.9% in formulations G1-G5 were clear, as the concentration of polymer was increased from more than 1% (G6 and G7) results with cloudy appearance.

pH of prepared gel
The pH of the formulations was found in the range of 5.5-5.9, which was required pH for periodontal treatment.

Drug content uniformity
The data of drug content from all the prepared formulations show that the values range between 97.63% and 100.42%.

In vitro gelling capacity
Formulation G1 and G2 containing lower concentration of polymer showed weakest gelation after 10-12 min and dispersed rapidly on shaking. Formulation G3 and G4 showed immediate gelation effect after 6-8 min but the formed gels are less stiff and does not remains for extended period of time. Formulation G5, G6 and F7 showed immediate gelation after 2-4 min and formed gel was stiff and remained for extended period of time, this is due to the presence of higher concentration of methyl cellulose.

Rheological studies
The results indicated that prepared gel showed (Figure 1) pseudoplastic behaviour i.e., thin when exposed to shearing stress and thick when shearing stress is released.

Syringeability of in situ gel
Formulations G1 to G5 expelled quite easily from the syringe equipped with 20 gauge needle and passes the syringeability test. Formulation G6 and G7 fail the syringeability test may be because they contain higher concentration of methyl cellulose.

In vitro drug release study
Formulations G1-G4 containing lower concentration of polymer (0.5%-0.8%) showed (Figure 2) that the whole amount of drug was released within 12-18 h. Formulations G5-G7 containing higher concentration of polymer (0.9%-1.1%) showed release of drug up to 24 h. Among all the formulations, it was observed that 96.87% of drug released in sustained manner from G5 at the end 24 h.

Drug release kinetics
The results show (Table 4) that the drug release followed first order kinetics, as the values for first order (0.986-0.995) are higher in comparison to zero order (0.498-0.776) and Higuchi’ model (0.21-0.954). The release exponent value (n) for all formulations was in the range of 0.337-0.448.


During stability study formulation G5 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; the data is shown in Table 5.

**DISCUSSION**

The melting point of atorvastatin was similar with the reported value. Compatibility studies from the FT-IR spectra of atorvastatin pure and its physical mixture indicating the compatibility of drug in formulation mixture.
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 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 (36-39°). Among 0.7-0.9% solution, 0.9% solution showed shorter gelation time 6 min, so this concentration was selected for further study.

Characterization of in situ gels
The various characterization studies like appearance, clarity, gelling capacity, pH, drug content and viscosity were done.

Appearance
The low concentration of methyl cellulose formulations were clear, as the concentration of polymer was increased to more than 1% cloudy appearance was observed.

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

Drug content uniformity
The data of drug content from all the prepared formulations are acceptable and indicate uniform drug content in all the formulations.

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 formulation G1 and G2 all remaining formulations showed instantaneous gelation when coming in contact with phosphate buffer (pH) maintained at 37 ± 1°. However the nature of the gel formed was dependent on the concentration of polymer used. Formulation G1 and G2 containing lower concentration of polymer showed weakest gelation and dispersed rapidly on shaking. Formulation G3 and G4 showed immediate gelation effect but the formed gels were less stiff and did not remain for extended period of time. Formulation G5, G6 and F7 showed immediate gelation, formed stiff gel and remained for extended period of time. This was due to the presence of higher concentration of methyl cellulose.

Viscosity
One of the important requirements for a periodontal gel was 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 higher viscosity in order to stay at the site of application for longer time.

Rheological studies
The rheological characteristic of all prepared gel formulations were understood by plotting viscosity of gel vs speed of rotation and results indicated that viscosity of the prepared gel decreased with increase in shearing force.

Syringeability of in situ gel
Syringeability of any gel formulation depends on the concentration of polymer. As the concentration of polymer increased the viscosity of formulation also increased and greater force was required to expel gel from the syringe.

In vitro drug release study
In vitro release profile of atorvastatin from in situ gels containing different concentration of methyl cellulose showed that release of the drug from these formulations was concentration dependent, as the concentration of methyl cellulose increases release rate of drug was retarded. Initially release of drug from these formulations was higher due to the bursting effect and as the time period increases gelation effect was seen and finally release rate was retarded. Formulations containing lower concentration of polymers release the drug quite faster when compared to higher concentrations.

Drug release kinetics
The results of in vitro release data was fitted to various kinetic models in order to know the drug release mechanism. The data were processed for regression analysis using MS Excel-10 statistical function, which 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, 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 G5 was selected for short term stability study (3 months) at 25°/60% and 40°/75%
RH. The prepared formulation was stable throughout the study.

**CONCLUSION**

In the present study *in situ* gel of atorvastatin for the clinical treatment of periodontal diseases was successfully formulated using methyl cellulose as gel base. This *in situ* gel formulation possesses mucoadhesive properties, which prolongs residence time of the drug at the site of application, and in turn better therapeutic effect. 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 is an optimized formulation and provided sustained drug release over an extended period of time i.e. more than 90% of drug released in 18 h. This may lead to better patient compliance. Further clinical trials are on to study the effect of these *in situ* gels on patients when administered locally for better treatment with respect to periodontal diseases.

**ACKNOWLEDGEMENTS**

The authors sincerely thank the Principal Sri Adichunchanagiri College of Pharmacy, B.G. Nagara for providing infrastructure facilities and moral support to carry out this research work. The authors also express their sincere thanks to Microlabs, Bangalore for providing atorvastatin drug as gift sample.

**REFERENCES**