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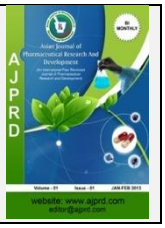

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Research Article

Formulation and Evaluation of In-Situ Gel for Periodontal Disease

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A B S T R A C T

Purpose: The main objective of present study is to formulate and evaluate Carbopol and Methyl cellulose based In-situ periodontal gel of Atorvastatin calcium. **Approach:** The In-situ gel was prepared by using different concentrations of Polymeric mixture of Carbopol and Methyl cellulose In-situ gel in solution state was evaluated for Gelling capacity, Gelling temperature, Gelling time, % Drug content and Syringeability and gel was evaluated for Physical appearance, pH, Viscosity and rheology, Spreadability, % Drug content, In vitro drug release and drug release kinetics. **Findings:** Compatibility study was performed using FTIR and DSC, so results showed there was no interaction between drug and other excipients. Gelation time and temperature was found in the range of 6-8 min and 34-37°C respectively.The Physical appearance of all formulations was cloudy except F3 and F3 was found to be clear. The pH of all formulations was found in the range of 5.6-5.8. All the formulation except formulation F6 showed satisfactory syringeability due to lower concentration of polymers.Viscosity of all formulations was found in the range of 1254.3 to 5680.8 centipoise at 37±1°C at 5 to 30RPM and all formulations exhibited pseudoplastic behaviour. Based on the results of release study, formulation F5 was found to be optimum formulation as it released 68.63% drug at the end of 18 hr. In vitro release study revealed that release rate of drug from the In-situ gel was concentration dependent; as concentration of Carbopol increased and Methyl cellulose decreased, the drug release rate was retarded upto 6 hr study. **Conclusion:** It can be concluded that formulation containing 2% w/v Gel base of 1.8g of Carbopol-940 and 0.2g of Methyl cellulose dissolved in 100ml of Distilled water gave optimized formulation.

Keywords: In situ gel, Atorvastatin, Gelation time, In vitro drug release, Pseudoplastic behaviour, Rheology, Syringeability, Spreadability.

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INTRODUCTION

Gel is the state which exists between solid and liquid phase. The solid component comprises a three-dimensional network of inter-linked molecules which immobilizes the liquid phase.[1] In situ gelation is a process of gel formation at the site of action after the formulation has been applied at the site. In situ gel phenomenon based upon liquid solution of drug formulation and converted into semi-solid mucoadhesive key depot.[2]

In situ gel forming formulations are currently a novel idea of delivering drugs to patients as a liquid dosage form, yet achieve sustained release of drug for the desired period. Different delivery systems based on polymers have been developed, which are able to increase the residence time of the formulation at absorption site of drugs.[3] Also its mucoadhesive property prevent it from getting washed by salivary fluid inside the buccal cavity. In recent years, there has been an increasing interest in water soluble polymers that are able to form gels after application to delivery site. These so called in situ gelling polymers are highly advantageous compared with other polymers because, in contrast to very strong gels, they can be easily applied in liquid form to the site of drug absorption. At the site of drug absorption, they swell to form a strong gel that is capable of prolonging the residence time of the active substance.

Hydrogels are the polymeric materials with three dimensional networks, which have gained much attention in biomedical fields as carriers for drugs, protein, cells, and others because of their good biocompatibility, solute permeability and tunable release characteristics.[4] The retaining ability of a large amount of water within their structures which results in high water content and soft-surface properties is the character that makes them compromised on the surrounding tissues and

leads to a good biocompatibility. Because of the present busy life system even in common man dental diseases are most widespread chronic disorders.[5-7] Periodontal disease refers to a group of problems that arise in the sulcus, the crevice between the gum and tooth the common causative organisms includes Actinobacillus, actinomycetemcomitans as a pathogen responsible for juvenile periodontitis, staphylococci subspecies epidermidis and aureus are responsible adult periodontal disease.[8] Site-specific therapy for periodontitis has three potential advantages, it decreased drug doses, increased drug concentration at the site of infection and reduced systemic side effects such as gastrointestinal distress.[9] Periodontal diseases treatment with a localized drug delivery system aims at delivering therapeutic agent at sufficient level inside the periodontal pocket and at the same time minimizing the side effects associated with systemic drug administration. Thus, it increases patient's compliance.[10]

MATERIALS AND METHODS

Materials:

Atorvastatin calcium trihydrate was purchased from Dhamtec Pharma and Consultants, Navi Mumbai. Sodium alginate was supplied by Research-Lab Fine Chem Industries, Mumbai. Triethanolamine and Paraffin liquid Light LR were supplied by SDFCL, Mumbai. Disodium hydrogen phosphate and Potassium dihydrogen phosphate were supplied by Pallav Chemicals and Solvents Pvt. Ltd, Boisar. Carbopol-940, Methyl cellulose, Methyl paraben, Propyl paraben and Methanol were supplied by Loba Chemie Pvt. Ltd, Mumbai.

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 studies by FTIR and DSC were carried out to identify possible interaction between drug and polymers used as per the standard procedure.[11]

Screening of polymeric mixture of Carbopol and Methyl cellulose:

Various concentrations of polymeric mixture of Carbopol and Methyl cellulose were chosen to conduct screening are mentioned in the Table No.01.

Table 1: Screening of polymeric mixture of Carbopol and Methyl cellulose

Sr. No.	(Carbopol + MC) 2% w/v
1	0.2g + 1.8g → 100 ml DW
2	0.4g + 1.6g → 100 ml DW
3	0.6g + 1.4g → 100 ml DW
4	0.8g + 1.2g → 100 ml DW
5	1.0g + 1.0g → 100 ml DW
6	1.2g + 0.8g → 100 ml DW
7	1.4g + 0.6g → 100 ml DW
8	1.6g + 0.4g → 100 ml DW
9	1.8g + 0.2g → 100 ml DW
10	2.0g + 0.0g → 100 ml DW

Method: Accurately weighed quantity of polymeric mixture of Carbopol and Methyl cellulose was taken in 100ml of beaker, then 100ml of distilled water added into it. This polymeric solution was stirred with mechanical stirrer until uniform solution was obtained. After this, sonicate this polymeric solution and kept aside till the entire polymers were completely dissolved (About 24hr). Finally, this solution evaluated for Gelling temperature and Gelling time.[12]After polymer screening, the batches of different polymeric mixture concentrations of Carbopol and Methyl cellulose were selected on the basis of Gelling temperature and Gelling Time.[12]

Method of preparation of Anti-inflammatory drug loaded In-Situ Gel:

For the preparation of Carbopol-940 and Methyl cellulose containing In-situ gel formulations, Carbopol-940 and Methyl cellulose was added to required quantity of distilled water with continuous stirring and allowed to hydrate overnight. Calculated amount of Atorvastatin calcium trihydrate (1.2% w/v) was dissolved in required quantity of methanol and then added to polymer solution under constant stirring and 2-3 drops of triethanolamine added separately. Finally, solution of Methyl paraben and Propyl paraben were added to the above formulation mixture under constant stirring until a uniform solution was obtained. The optimized concentration of Carbopol-940 and Methyl cellulose was selected on the basis of gelation temperature and gelation time. Further the prepared formulations were evaluated for various characterization studies.[11] Composition of formulations shown in Table No.02.

Table 02: Composition of In-situ gel formulations

Batch Code	Atorvastatin calcium trihydrate (%w/v)	Polymeric solution (2%w/v)	Methyl paraben (%w/v)	Propyl paraben (%w/v)	Triethanolamine	Distilled water
F1	1.2	Carbopol (1g) + MC (1g) in 100ml DW	0.15g	0.02g	2-3 drops	Q.S.
F2	1.2	Carbopol (1.2g) + MC (0.8g) in 100ml DW	0.15g	0.02g	2-3 drops	Q.S.
F3	1.2	Carbopol (1.4g) + MC (0.6g) in 100ml DW	0.15g	0.02g	2-3 drops	Q.S.
F4	1.2	Carbopol (1.6g) + MC (0.4g) in 100ml DW	0.15g	0.02g	2-3 drops	Q.S.
F5	**1.2**	**Carbopol (1.8g) + MC (0.2g) in 100ml DW**	**0.15g**	**0.02g**	**2-3 drops**	**Q.S.**
F6	1.2	Carbopol (2.0g) + MC (0.0g) in 100ml DW	0.15g	0.02g	2-3 drops	Q.S.

Characterizatio of In-Situ Gel (Solution state):

Gelling Capacity:

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 colored solution of prepared formulations were prepared. Gelling capacity was estimated by placing 2ml of 6.8 pH phosphate buffer in a 10ml test tube and maintained at 37±1° temperature. One milliliter of colored 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 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.[12]

Gelation temperature:

10ml of the sample solution and magnetic bead were put into a 30ml 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.[12]

Gelation time:

Gelation time of prepared in situ gel formulation was measured by placing 2ml of the gel in 15ml borosilicate glass test tube. This test tube was placed in water bath (37 ± 2^{O}) and gelation time was noted when there was no flow of the gel when test tube was inverted.[12]

Syringeability:

All prepared formulations were transferred into a 5ml syringe placed with 20 guage 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.[11]

Drug content analysis:

Accurately weighed amount of gel equivalent to 2mg of drug was taken in to a 10ml volumetric flask. They were lysed with 5 ml of medium (6.8 pH phosphate buffer) for 15 min. The clear solution was diluted to 10 ml of medium. Then filter this solution and filtrate was withdrawn in cuvette by pipette and the absorbance was measured at 246nm against phosphate buffer (pH6.8) by using UV-Visible Spectrophotometer-1800 (Shimadzu, Japan) and drug content was calculated from the calibration curve.[13]

Drug Content = (Practical value /Theoretical value) X 100

Characterization of In-Situ Gel (Gel state):

Appearance:

All prepared formulations were evaluated from the visual inspection.[11]

pH measurement:

pH is one of the most important parameters involved in the In-situ gel formulation and it is measured directly with the help of digital pH meter.[14]

Viscosity and rheological studies:

Brookfield digital viscometer (Model no. LMDV-200, LABMAA scientific instruments) was used for the determination of viscosity and rheological properties of atorvastatin in situ gel using spindle no. L3. After this 50g 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 37°C. A typical run comprised changing of the angular velocity from 5 to 30 rpm and similarly from 30 to 5 rpm for each batch of in situ gel formulations. [12,14]

Spreadability:

For the determination of Spreadability, excess of sample was applied between the two glass slides and was compressed to uniform thickness by placing 1000gm weight for 5min. Weight (50g) 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.[14]

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.

Drug content analysis:

Accurately weighed amount of gel equivalent to 2mg of drug was taken in to a 10ml volumetric flask. They were lysed with 5ml of medium (6.8 pH phosphate buffer) for 15min. The clear solution was diluted to 10 ml of medium. Then filter this

solution and filtrate was withdrawn in cuvette by pipette and the absorbance was measured at 246 nm against phosphate buffer (pH6.8) by using UV-Visible Spectrophotometer-1800(Shimadzu, Japan) and drug content was calculated from the calibration curve.[13]

Drug Content = (Practical value /Theoretical value) X 100

In vitro drug release studies:

In vitro release studies were performed using Franz diffusion cell. Dialysis membrane having pore size 2.4 nm, molecular weight cutoff between 12,000 –14,000, was used. Membrane was soaked in phosphate buffer (pH 6.8) for overnight before mounting in a Franz diffusion cell. Atorvastatin in-situ gel formulation equivalent to 10mg was placed in the donor compartment and the receptor compartment was filled with dialysis medium (phosphate buffer of pH 6.8). Required quantity (1 ml) of the medium was withdrawn at specific time periods (0, 2, 4, 6, 18 hr) and the same volume of dissolution medium was replaced in the flask to maintain a constant volume. The withdrawn samples were filtered and then 1 ml filtrate was made up to volume with 10 ml of phosphate buffer of pH 6.8. The samples were analysed for drug diffusion by measuring the absorbance at 246 nm using a UV/ visible spectrophotometer-1800 (Shimadzu Corporation, Japan). In-vitro drug release has been recognized as an important element in drug development. To analyse the mechanism for the release and release rate kinetics of the formulations, the data obtained from conducted studies was fitted into Zero order, First order, Higuchi matrix, Korsmeyer Peppas and Hixson Crowell model. In this way, by comparing the r-values obtained, the best-fit model was selected.[15]

Drug release kinetics

In vitro drug release has been recognized as an important element in drug development. To analysis the mechanism for the release and release rate kinetics of the formulated dosage form, the data obtained from conducted studies was fitted into Zero order, First order, Higuchi matrix, Korsmeyer-Peppas and Hixson Crowell model. In this by comparing the r- values obtained, the best-fit model was selected.

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 $M_t / M_a = Kt^n$, 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. [14,16]

Stability study:

Stability study of optimized formulation F5 was carried out at 25^{o}/60% and 40º/75% RH for a period of three weeks (21 days). During stability study in situ gel was analysed for pH, viscosity, drug content and in vitro drug release.[16] It was observed that optimum formulation F5 was stable throughout the study, since no significant changes showed in any parameters.

Table 3: Composition of F5 batch

Batch Code	Atorvastatin calcium trihydrate (%w/v)	Polymeric solution (2%w/v)	Methyl paraben (%w/v)	Propyl paraben (%w/v)	Triethanolamine	Distilled water
F5	1.2	Carbopol (1.8g) + MC (0.2g) in 100ml DW	0.15g	0.02g	2-3 drops	Q.S.

RESULTS AND DISCUSSION

Preformulation studies:

The melting point of Atorvastatin calcium was found to be 156-160ºC and compatibility studies from the FT-IR spectra of Atorvastatin calcium pure and its physical mixture with polymers revealed that there was no significant change in peak of Atorvastatin calcium in mixture. From the DSC study, it was observed that there was no significant change in melting point of drug and polymers.

Selection of polymeric mixture of Carbopol and Methyl cellulose:

So, by using polymer screening method, the batches of polymeric mixture of Carbopol and methyl cellulose were selected for the preparation of In-situ gel formulation. The batches mentioned in Table No.04 were selected to prepare final In-situ gel formulation. The reason behind using this combination of Carbopol and methyl cellulose polymer was lesser gelling time (8, 8, 7, 7, 6-7 and 6min for 2% w/v mixture of Carbopol and MC for different batches mentioned in Table No.04) which was essential to convert gelling solution into stiff gel matrix for sustained release at 34-37ºC. Selected batches of polymeric combinations are given in Table No.04.

Table 4: Gelling temperature and time of selected batches of polymeric mixture of Carbopol and Methyl Cellulose

Sr. No.	(Carbopol + MC) 2% w/v	Gelling Temp. (ºC)	Gelling time (min)
1	1.0g + 1.0g → 100 ml DW	35-37	8
2	1.2g + 0.8g → 100 ml DW	34-37	8
3	1.4g + 0.6g → 100 ml DW	35-37	7
4	1.6g + 0.4g → 100 ml DW	34-37	7
5	1.8g + 0.2g → 100 ml DW	34-37	6-7
6	2.0g + 0.0g → 100 ml DW	35-37	6

Among all the selected combination polymeric concentrations, Carbopol 1.8g and methyl cellulose 0.2g dissolved in 100ml distilled water i.e. 2%w/v polymeric mixture showed shorter gelation time (6-7 min), So this concentration was selected for further study.

Characterization of In-SituGel (Solution state):

The various characterization studies such as Gelling capacity, Gelling temperature, Gelling time, Syringeability and drug content analysis were performed.

Gelling capacity:

In vitro capacity studied and it was found that F1 batch required highest gelling time (8min), The F6 batch showed lowest gelling time (6min). The batches F2 and F3 required 7min. Finally, batches F4 and F5 required moderate time 6-7 min for gelation. The obtained data shown in Table No.05. The optimum batch F5 was selected for further studies.

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 F1, all remaining formulations showed instantaneous gelation when coming in contact with phosphate buffer (pH) maintained at 34-37ºC. However, the nature of the gel formed was dependent on the concentration of polymers used. Formulation F1 containing lower concentration of polymer showed weakest gelation and dispersed rapidly on shaking. Formulation F2 and F3 showed immediate gelation effect but the formed gels were less stiff and did not remain for extended period of time. Formulation F4, F5 and F6 showed immediate gelation, formed stiff gel and remained for extended period of time. This was due to the presence of higher concentration of Carbopol.

Gelling temperature and Gelling time:

F1 formulation showed highest gelling time (8 min) as it consists lowest Carbopol and highest MC. Formulations F2 and F3 showed moderate gelling time (7min) as these consist moderate Carbopol and MC. Formulations F4, F5 and F6 showed lowest gelling time (6-7 min) as these formulations contain highest concentration of Carbopol and lowest MC.The Gelling temperature for all batches was found to be 34-37ºC which is essential for gelation. The optimum batch F5 was selected for further studies. The optimum batch selected on the basis of Gelling temperature and time. The obtained data showed in the following Table No.05

Syringeability:

Formulations F1 to F5 expelled quite easily from syringe equipped with 20gauge needle and passes syringeability test. Formulation F6 failed in syringeability test may be because it contains higher concentration of Carbopol. The obtained data showed in Table No.05

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.

Drug content analysis:

The data of drug content from all the prepared formulations showed that the values range between 95.75% and 100.2%. The obtained data showed in Table No.05.

Table 5: Characterization of In-situ gel (Solution state)

Batch Code	Gelling capacity	Gelling Temp. (ºC)	Gelling time (min)	Syringeability	% Drug content
F1	+	34-37	8	Pass	98 %
F2	+ +	34-37	7	Pass	95.75 %
F3	+ +	34-37	7	Pass	100.2 %
F4	+ + +	34-37	6-7	Pass	96.85 %
F5	**+ + +**	**34-37**	**6-7**	**Pass**	**99.1 %**
F6	+ + +	34-37	6	Fail	100.2 %

Gelling capacity codes and their meaning: + (gels after few min, dispersed rapidly); + + (gelation immediate, remains for few hours); + + + (gelation immediate, remains for extended period)

Characterization of In-Situ Gel (Gel state):

The various characterization studies like Physical appearance, pH, viscosity, spreadability, drug content and In vitro drug release studies were done.

Physical Appearance:

Physical appearance of each batch was checked by visual inspection.

Table 6: Physical appearance of In-situ gel

Sr. No.	Batch Code	Physical appearance
1	F1	Cloudy
2	F2	Cloudy
3	F3	Clear
4	F4	Cloudy
5	**F5**	**Cloudy**
6	F6	Cloudy

All the formulations of in situ gels were cloudy in appearance except for F3 batch and Most of the batches was cloudy as

shown in Table No.06 may be due the presence of Carbopol polymer.

pH of prepared gel:

The pH of all formulation batches was found in the range of 5.6 to 5.8 which is required. The obtained data shown in Table No.07.

Table 7: pH of In-situ gel

Sr. No.	Batch code	pH
1	F1	5.7
2	F2	5.8
3	F3	5.6
4	F4	5.8
5	**F5**	**5.8**
6	F6	5.8

The pH of the all the formulations were found in the range of required pH suitable for periodontal disease treatment shown in Table No.07.

Viscosity and rheological studies:

Viscosity of all prepared batches (Table No.08) were evaluated by using Brookfield digital viscometer and the values ranges from 1254.3 to 5680.8 cps at 37 ± 1ºC at 5 to 30 RPM.

Table 8: Viscosity values of In-situ gel for ascending order of RPM

Sr. No.	RPM (Ascending order)	Viscosity (mPa.s or cps)					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
1	5	2848.4	3156.8	3658.7	4545.6	**4965.8**	5680.8
2	10	2086.6	2575.2	3168.1	3796.9	**4422.1**	4886.2
3	15	1755.2	2323.5	2805.8	3299.4	**3899.2**	4339.2
4	20	1584.9	2096.2	2556.4	3058.9	**3512.9**	4001.6
5	25	1365.7	1801.4	2196.5	2666.1	**3096.2**	3808.9
6	30	1254.3	1742.8	2096.3	2589.4	**2899.1**	3711.5

Table 9: Viscosity values of In-situ gel for descending order of RPM

Sr. No.	RPM (Descending order)	Viscosity (mPa.s or cps)					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
1	30	1252.4	1735.5	2089.2	2553.5	**2896.4**	3720.1
2	25	1356.4	1851.8	2194.6	2655.8	**3092.4**	3815.4
3	20	1583.5	2085.4	2563.4	3065.4	**3528.6**	4015.6
4	15	1756.1	2322.2	2812.9	3289.5	**3890.5**	4332.8
5	10	2058.6	2578.6	3162.5	3792.4	**4425.9**	4889.4
6	5	2846.9	3158.1	3655.8	4553.8	**4952.4**	5688.9

Table 10: Shear stress and shear strain data of optimized batch F5

Sr. No.	RPM	Viscosity (Pa. s)	Shear strain (1/s)	Shear stress (Pa or N/m^2)
1	0	0	0	0
2	5	4.9658	17.15	96.08823
3	10	4.4221	39.05	174.938276
4	15	3.8992	59.85	227.089408
5	20	3.5129	75.36	250.294125
6	25	3.0962	89.98	267.263984
7	30	2.8991	97.59	269.181435

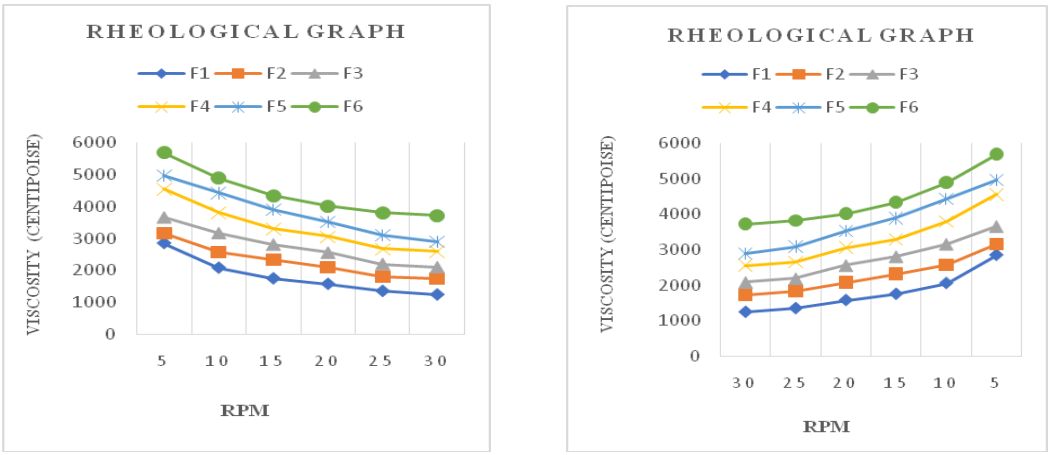

Figure 1: Rheological properties of prepared batches of Atorvastatin calcium In-Situ gel

From theFig No.01, it is observed that viscosity decreases with increase in angular velocity (RPM) as well as viscosity increases with decrease in angular velocity (RPM).The rheological characteristics 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. From the Table No.10, it is observed that as the shear strain increases, shear stress increases. The plot of shear stress verses shear strain in depicted in Fig No.02 and obtained data of shear stress and shear strain shown in Table No.10.

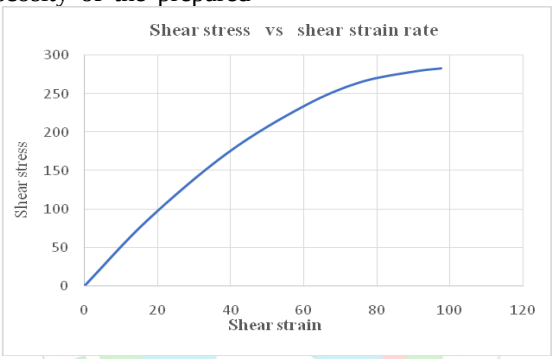

Figure 2: Plot of shear stress vs shear strain of optimized batch F5

The results indicated that prepared gel showed (Fig No.02) pseudoplastic behaviour i.e. thin when exposed to shear stress and thick when shear stress is released.

Table 11: Rheological profile of In-situ gel at 37º C

1	**Viscosity**	High at 37º C, due to gelation
2	**Shear behaviour**	Pseudoplastic/ Shear-thinning
3	**Flow behaviour**	Non-Newtonian

One of the important requirements for a periodontal gel was viscosity of the formulation. The prepared gel should be such that it should have 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. Rheology profile is shown in Table No.11.

Spreadability:

Spreadability was measured by Glass slide method, the obtained data shown in the following Table No.12.

Table 12: Spreadability test of In-situ gel

Batch code	M (g)	L (cm)	T (s)	S (g.cm/s)
F1	1000	4.5	85	52.94
F2	1000	4.2	84	50
F3	1000	3.5	85	41.17
F4	1000	3.2	87	36.78
F5	**1000**	**3**	**84**	**35.71**
F6	1000	2.8	82	34.14

The Spreadability of gel formulation was found in the range of 34.14 to 52.94 g.cm/s. The Spreadability of optimum batch F5 was found 35.71 g.cm/s and it is satisfactory to achieve desired treatment of periodontal disease.

Drug content analysis:

The data of drug content from all the prepared formulations showed that the values range between 95.75% and 100.2%. The obtained data showed in Table No.13.

Table 13: Drug content analysis of In-situ gel

Sr. No.	Batch code	% Drug content
1	F1	98 %
2	F2	95.75 %
3	F3	100.2 %
4	F4	96.85 %
5	**F5**	**99.1 %**
6	F6	100.2 %

The optimum formulation F5 showed 99.1% of drug content and it is satisfactory to achieve treatment of Periodontal disease. Drug content for all batches is depicted in Fig No.03

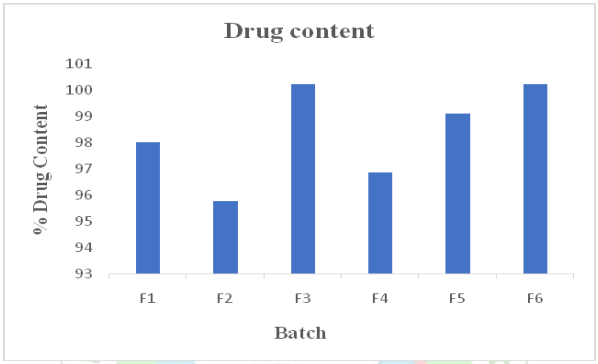

Figure 3: Comparison of Drug content of Atorvastatin calcium In-situ gel

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

In vitro Drug Release studies:

From the data (Table No.14) obtained from In vitro drug release study by using Franz diffusion cell, it was observed that, from batches F1 to F5 drug release was in increasing order for 18 hr, this is due to increase in Carbopol concentration (1, 1.2, 1.4, 1.6 and 1.8g) and decrease in MC concentration (1, 0.8, 0.6, 0.4 and 0.2g). For batch F6, it was observed that it showed lowest drug release (46.12% CDR) at 18 hr as compared to remaining batches, since it contains only Carbopol even if its concentration is maximum (2g or 2% w/v) than others. So, the batches F1 to F5 consist combination polymer showing better result as compared to F6 batch. So optimum batch can be selected through F1 to F5.

Table 14: In vitro drug release study of In-situ gel

Time (hr)	% Cumulative Drug Release					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
0	0	0	0	0	0	0
2	26.31	24.97	24.08	22.31	20.97	17.86
4	32.51	30.67	28.84	26.53	25.58	24.09
6	36.74	34.80	33.34	31.36	29.92	28.36
18	49.99	55.96	59.76	62.59	68.63	46.12

The formulation F5 showed highest drug release i.e. 68.63% as compared to F1, F2, F3 and F4 for 18 hr. Finally, it was observed that, among all the formulations 68.63 % of drug released in sustained manner from F5 at the end of 18 hr.

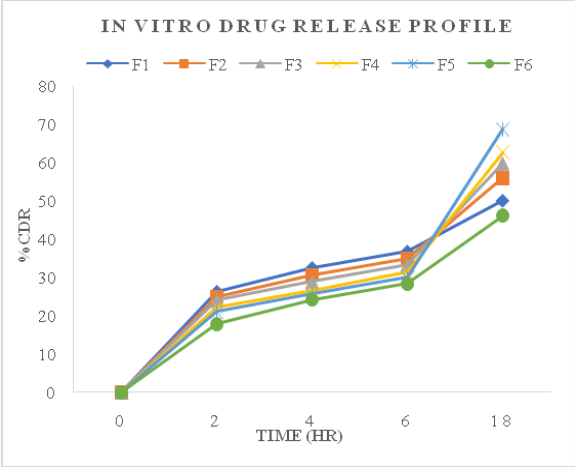

Figure 4: In vitro drug release profile of Atorvastatin calcium In-situ gel

In vitro release profile (Fig No.04) of Atorvastatin calcium from in situ gels containing different concentration of combined polymeric mixture of Carbopol and methyl cellulose showed that release of the drug from these formulations was concentration dependent, as the concentration of Carbopol increases and MC decreases, the release rate of drug was retarded upto 6hr study. 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 upto 6 hr study.

Drug Release kinetics:

The results showed (Table No.15) that the drug release following Higuchi model, as the values of $R^2$for Higuchi model (0.9307-0.9946) are higher in comparison to Zero order kinetics (0.6781-0.9525) and first order kinetics (0.7843-0.9872). The coefficient of determination i.e. R^2 for Korsmeyer/Peppas model and Hixson Crowell model are in the range of 0.6174- 0.7357 and 0.7496- 0.9825 respectively.

Table 15: Release kinetic values of In-situ gel

Batch code	Zero order	First order	Higuchi	Korsmeyer/Peppas		Hixson Crowell
	R^2	R^2	R^2	R^2	n	R^2
F1	0.6781	0.7843	0.9307	0.6174	1.1534	0.7496
F2	0.8037	0.9092	0.9819	0.6546	1.1912	0.8765
F3	0.8664	0.9526	0.9916	0.6781	1.2128	0.9295
F4	0.9152	0.9769	0.9877	0.7061	1.2318	0.9626
F5	**0.9525**	**0.9872**	**0.9698**	**0.7357**	**1.2651**	**0.9825**
F6	0.8406	0.913	0.9946	0.6965	1.155	0.8911

The results of in vitro release data were fitted to various kinetic models in order to know the drug release mechanism. The data were processed for regression analysis using Microsoft® Excel® 2021 MSO statistical function, which showed that the drug release followed Higuchi model kinetics. In the Korsmeyer/Peppas model, the release exponent value 'n' is greater than 1,it indicates super case II transport, which is characterized by the release rate being controlled by both diffusion and relaxation of the polymer chains. This means that the release is not only dependent on diffusion but also on the swelling and deformation of the polymer matrix, this might be due to swelling and deformation of polymer matrix of Carbopol and methyl cellulose.

Table 16: Drug release kinetic values of batch F5

Sr. No.	Time (T) (hr)	Sq. rt. of T	Log T	% Cum. Drug Released	% Cum. Drug Retained	Log % Cum. Drug Released	Log % Cum. Drug Retained	Cube rt. (% Cum. Drug Retained)
1	0	0	0	0	100	0	2	4.641588
2	2	1.414213	0.301029	20.97	79.03	1.321598	1.897791	4.291383
3	4	2	0.602059	25.58	74.42	1.407900	1.871689	4.206264
4	6	2.449489	0.778151	29.92	70.08	1.475961	1.845594	4.122854
5	18	4.242640	1.255272	68.63	31.37	1.836513	1.496514	3.153829

Table 17: Regression Coefficient of F5 batch

Batch code	Regression coefficient (R^2)				
	Zero order	**First order**	**Higuchi**	**Peppas**	**Hixson Crowell**
F5	**0.9525**	**0.9872**	**0.9698**	**0.7357**	**0.9825**

For F5 formulation (optimum batch), drug release study showed that the release of drug from In-situ gel by First order kinetics followed by Hixson Crowell and Higuchi model.

Stability study:

Stability study for optimized formulation was performed to check various parameters shown in the Table No.18.

Table 18: Stability studies of F5 batch

Time Period	At 25º/60% RH			At 40º/75% RH				
	Ph	**Viscosity (cps)**	**Drug content (%)**	**% Drug release**	**pH**	**Viscosity (cps)**	**Drug content (%)**	**% Drug release**
0 week	5.8	4239.5	98.20	68.62	5.8	4241.9	98.15	68.61
1 week	5.8	4241.4	98.12	65.86	5.7	4289.5	98.05	65.85
2 weeks	5.7	4281.2	98.05	57.29	5.7	4312.5	98.03	57.24
3 weeks	5.6	4295.8	97.64	56.28	5.6	4384.9	97.55	56.10

The optimized formulation F5 was selected for short term stability study (3 weeks) at 25º/60% and 40º/75% RH. Formulation F5 was analyzed 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 No.18.

CONCLUSION

Atorvastatin calcium-loaded In-situ Gel represent a promising drug delivery system for atorvastatin, a widely used antihyperlipidemic, anti-inflammatory and bone-regenerative medication. This formulation of In-situ gel is composed of polymeric solution that are liquid at room temperature and can convert into Gel at body temperature (36ºC). In-situ Gel offer several advantages for drug delivery, including enhanced drug stability, sustained release kinetics, and improved bioavailability. The gel matrix of In-situ gel protects the drug from degradation and allows for sustained release over an extended period, leading to improved therapeutic efficacy and reduced side effects. Formulation of Atorvastatin calcium-loaded In-situ gel has demonstrated their potential for various applications, including the treatment of inflammation at tooth gums and regeneration of tooth loss by periodontal disease. This formulation has shown that In-situ gel can improve the pharmacokinetic profile of Atorvastatin calcium, prolonging its circulation time and enhancing its accumulation at the target site. Different type of polymers was used for the formulation of In-situ gel, these polymers were screened for Gelling temperature and Gelling time. Combination of Carbopol and Methyl cellulose showed better result so used for preparing desired atorvastatin loaded In-situ gel for periodontal disease. Prepared drug loaded In-situ gel were evaluated for pH, gelling temperature, gelling time, rheological properties, % Drug content, syringeability and In vitro drug release. Results revealed that there were no possible interactions in their IR spectra. And stability studied was carried out in different temperature for 3 weeks (21 days) and physical appearance was determined. It was found that In-situ gel was in solution form at room temperature (27ºC) and in get state at 34-37ºC.

FUTURE ASPECT

The Atorvastatin also has antimicrobial properties; in future it may also be studied for antimicrobial study to reduce the chronic periodontal infection due to periodontopathic bacteria. The statins also possess a remarkable beneficial effect on chronic periodontitis, alveolar bone loss, osseointegration of implants, dental pulp cells, orthodontic tooth movement and subsequent relapse, tissue healing (wound/bone healing), and salivary gland function, as well as exhibiting anti-cancer properties in the oral cavity. In the future, it is strongly suggested that larger clinical trials be conducted to assess the pleiotropic effects of statins on dental and oral health, focusing on determination of the ideal duration, dose and specific statin (atorvastatin, fluvastatin, lovastatin, rosuvastatin or simvastatin) for the treatment of each particular dental or/and oral condition. However, in the meantime, and based on the findings contained in the present research work, it is probably safe to suggest that local use of atorvastatin should be considered as a novel, safe, inexpensive and very accessible therapeutic agent with which to improve various aspects of overall dental and oral health.

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