Research Article

Formulation, Evaluation and Clinical Assessment of Gemifloxacin *In Situ* Gel For The Treatment of Chronic Periodontitis

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ABSTRACT

A smart drug delivery system for localized controlled release of the broad spectrum antimicrobial agent, Gemifloxacin Mesylate (GM) following insertion into the periodontal pocket was developed using the thermosensetive polymer, poloxamer 407 and the ion activated polymer, gellan gum. Many drugs do not reach the site of action in the therapeutic concentrations intended. So, in the present study, works have been done for administering the drug directly to the target site so that the efficacy of treatment can be improved. This site specific delivery of drug can thus overcome the problems faced during systemic administration of antimicrobials for the treatment of chronic periodontitis, where the drug get diluted many times before it reaches the site of action. This also reduces frequency of administration and dose size, thereby, improves patient compliance and minimizes systemic side effects. GM *in situ* gels were prepared by different concentrations of polymers and evaluated for physical appearance, drug content uniformity, syringeability, rheological properties, pH, gelation time, gelation temperature, *in vitro* gelling capacity and *in vitro* drug release. Drug excipients compatibility study was done by FTIR. Results showed no evidence of interaction between the drug and excipients. The selected formulation was clinically tested and the results revealed that, GM *in situ* gel (Containing 18 % w/w poloxamer 407 and 0.8% w/w gellan gum) showed reasonable *in vitro* results and good clinical improvement.

Keywords: Periodontitis, Gemifloxacin Mesylate, poloxamer 407, gellan gum, *In situ*.

INTRODUCTION

Periodontal disease is a general term which includes several pathological conditions affecting the tooth supporting structures and mainly includes chronic periodontitis and aggressive periodontitis[1]. Periodontal disease is a result of local bacterial infection with apathogenic microflora within the periodontal pocket. The microflora found in periodontitis is complex and composed mainly of gram-negative anaerobic bacteria[2].

Scaling and root planning (SRP) remains the 'gold standard' as the non-surgical treatment of chronic periodontitis. SRP may, however, fail to reduce or eliminate the anaerobic infection at the base of the pocket, within the gingival tissue or in furcations which in turn may serve as reservoirs for periodontopathic bacteria from which re-colonization of treated root surfaces can occur. The bacterial reservoir which is inaccessible for by mechanical debridement alone can be further eliminated with the adjunctive use of chemotherapeutic agents[3].

Locally delivered antimicrobial therapy, in particular, has gained much interest because of the site-specific nature of periodontal infections, the higher concentration of anti-microbial agent delivered subgingivally and reduced side effects of systemic antibiotic use[4].

Two particular problems common to many periodontal drug delivery systems are short retention time and difficult as well as time consuming application[5-6].

Gemifloxacin Mesylate (GM) is a synthetic broad-spectrum antibacterial agent for oral administration related to the fourth generation of fluoroquinolone class of antibiotics that has a broad spectrum of activity against Gram-positive and Gram-negative periodontopathic bacteria[7-8]. Species variability was evident: Porphyromonas gingivalis and Prevotella spp. were susceptible to 0.5 mg/L of GM. These data suggest that GM may have a clinical role in the treatment of certain dental infections including chronic periodontitis[9]. Gemifloxacin is available as the Mesylate salt in the sesquihydrate form[10].

GM in the form of conventional dosage form such as tablets and capsules is available for the treatment of bacterial infection in a dose of 320 mg daily[11]. Limited studies have been carried out to examine the role of local GM formulated with rate controlling polymers in the management of chronic periodontitis although of its proved topical activity[12].

MATERIALS AND METHODS

Materials

Gemifloxacin Mesylate, Methyl and Propyl paraben were kindly supplied from (Hikma Pharmaceutical Co, Cairo, Egypt), Gellan gum and Poloxamer 407 was purchased from (Sigma Aldrich, USA), Propylene glycol, Sodium hydroxide and Dipotassium hydrogen orthophosphate were purchased from (El-Nasr Pharmaceutical Chemicals Co, Cairo, Egypt).

Investigation of physicochemical compatibility of Gemifloxacin Mesylate and the polymers

The physicochemical compatibility between GM and polymers was studied using Fourier transform-infrared (Maltson, Genesis II FTIR, USA). The FTIR spectra were recorded in the wavelength region between 4000 and 400 cm^{-1}. The spectra of GM alone and physical mixtures (1:1 w/w) of GM with gellan gum and poloxamer 407 were compared with each other.

Preparation of Gemifloxacin *In situ* gel[13-14]

Dry gellan gum powder was dispersed in 25 ml of distilled water maintained at 95°C. The dispersion was stirred at 95°C for 2 minutes to facilitate the complete hydration of gellan gum. The specified amounts of preservatives (methyl paraben and propyl paraben) were added to gellan gum solution with continuous stirring. The solution was allowed to cool to room temperature. Then specified amount of poloxamer 407 was added with continuous stirring for 5 minutes. The formulation containing partially dissolved poloxamer 407 was stored in the refrigerator until entire polymer gets completely dissolved then volume was made to completion with distilled water and stored at refrigerator. The composition of different formulations was shown in table 1.

Physical Appearance

Formulations were subjected to visual inspection to determine the aspects of clarity, homogeneity and transparency.

Measurement of pH

An acidic or alkaline formulation is bound to cause irritation on mucosal membrane and hence this parameter assumes significance while developing a formulation[15]. An electronic pH meter was used for this purpose. It was calibrated using buffer solution having the pH of 4.0, 7.0 and 9.2. Each formulation was measured in triplicate.

Syringeability

All the prepared formulations were transferred into an identical 5 ml plastic syringe placed with 20 gauge needle to a constant volume (1 ml). The solutions which were easily passed from syringe were termed as pass and which fail to pass were termed as fail[16].

Drug content uniformity

About 1 gram of the formulation was transferred into 250 ml volumetric flask and 50 ml of simulated saliva, phosphate buffer (pH 6.8), was added. Vigorous shaking was done until gel was completely dispersed to give a clear solution. Final volume was adjusted to 100 ml with simulated saliva. Obtained solution was filtered through Whatman filter paper. First derivative measurements were carried out at wave length 258 nm, $\Delta\lambda$= 4 and scaling factor 10 using UV-Visible Spectrophotometer (Shimadzu, modal UV- 1601, Shimadzu, Japan)to determine drug concentration[17].

Rheological properties

The rheological properties of all prepared formulations were measured using a Brookfield viscometer DV-III Pro model viscometer using spindle no.40. The viscosity, shear rate and shear stress of each sample solution were measured at different speeds at a temperature of 25 ± 1°C. A typical run involved changing the speed from 1 to 100 rpm and then in a descending order.

Gelation time

After putting 2 ml of the formulation in 15 ml borosilicate glass test tube, the test tube was placed in a water-bath maintained at 37 ± 2°C. Gelation time was noted when there was no flow with test tube inversion.

Gelation temperature

A magnetic bead and 10 ml of the sample solutions were put into a 30 ml transparent vial that was placed in a low temperature digital water bath. A thermometer was placed in the sample solution. The solution was heated with continuous stirring at low rpm. The temperature was determined as gelation temperature, at which the magnetic bead stopped moving due to gelation[18].

***In vitro* gelling capacity**

To evaluate the formulation for its *in vitro* gelling capacity by visual method, colored solutions of different formulations were prepared. Two ml of simulated saliva was placed in a 15 ml borosilicate glass test tube and maintained at 37 ± 1°C temperature. One ml of colored formulation solution was added with the help of 1 ml pipette. The formulation was transferred in such a way that places the pipette at the surface of fluid in the test tube and formulation was slowly released from the pipette. As the formulation comes into contact with simulated saliva it was immediately converted into a stiff gel-like structure. The gelling capacity of formulation was evaluated on the basis of stiffness of the formed gel and the time period for which formed gel remained. Color was added to give a visual appearance to the formed gel. The *in vitro* gelling capacity was graded in three categories based on the durability of the formed gel. (+), gelation after few minutes, dispersed rapidly, (++), gelation immediate, remains for few hours and (+++), gelation immediate, remains for an extended period[19].

***In vitro* drug release studies**

In vitro release studies were carried out using Franz diffusion cell using cellophane membrane soaked overnight in the receptor medium (simulated saliva, phosphate buffer pH 6.8). The diffusion medium was simulated saliva stirred at 50rpm at 37 °C ± 1°C. One end of the diffusion tube was covered by a cellophane membrane. The 1ml formulation was spread on the cellophane membrane and placed such that it just touches the diffusion medium present in the receptor

compartment. Aliquots of 1 ml were withdrawn periodically and each time equal volume was replaced with fresh phosphate buffer (pH 6.8) previously heated to 37 ± 1°C. The amount of drug release was estimated using UV spectrophotometer.

The experiment was performed in triplicate.

The cumulative percentage of drug released was plotted against time and release parameters were calculated to compare between investigated formulations.

Clinical study of selected Gemifloxacin Mesylate *in situ* gel

Subjects Selection

The current study included thirty patients with chronic periodontitis recruited from the outpatient clinic, Department of Oral Diagnosis, Oral Medicine and Periodontology, Faculty of Oral and Dental Medicine, Cairo University.

The clinical work in this study was approved by The Research Ethics Committee of Faculty of Oral and Dental Medicine, Cairo University.

Written informed consent was taken from each patient after explaining the procedure along with the risks and benefits.

All included patients were selected to be systemically healthy according to the modified Cornell Medical Index[20].

Chronic periodontitis patients (CP) were diagnosed on the foundation of the periodontal classification[21] of the American Academy of Periodontology (2000) with the following inclusion criteria: The patients had at least 20 natural teeth with a minimum of two sites of a probing depth (PD) ≥ 5 mm and clinical attachment level (CAL) ≥ 4 to 6 mm with radiographic evidence of bone loss ≥ 3 mm.

Exclusion criteria included: 1) patients with existing systemic disease that may influence the severity or progression of periodontitis, in particular Down syndrome, HIV infection, or diabetes mellitus type one or type two, 2) taking medications that may influence the periodontium (e.g., phenytoin, nifedipine, or non-steroidal anti-inflammatory drugs), 3) taking medication that may interact with GM (e.g., antiarrhythmics, coumarin derivates, tricyclic antidepressants, antimalarials, or antihistamines), 4) existing tendon diseases or damage as a result of previous quinolone therapy, 5) cardiac arrhythmia, 6) liver diseases, 7) antibiotic premedication required for dental interventions, 8) systemic administration or local application of antibiotics within the previous 6 months, 9) concurrent or planned extensive dental or orthodontic treatments, 10) pregnancy or lactation, 11) intraoral piercing or other intraoral body jewelry, 12) unable or not willing to comply with the study protocol, and 13) anticipated non-compliance with the examination and treatment appointments.

All patients were screened by comprehensive periodontal examination and full periodontal charts were obtained along with full mouth radiographic examination.

Chronic periodontitis patients (CP) were randomly allocated into one of two groups:

Group I: (n=15) received scaling and root planning followed by topical application of GM *in situ* gel and acted as test group.

Group II: (n=15) received scaling and root planning followed by topical application of plain *in situ* gel and acted as a control group.

Clinical Periodontal Assessment

The following clinical periodontal parameters were recorded at the most periodontally affected tooth at baseline and again 1 and 2 months after treatment for groups I and II: Plaque index (PI)[22], gingival index (GI)[23], pocket depth (PD) and clinical attachment level (CAL).

Gingival index and plaque index measurements were performed at 4 sites per tooth (buccal, mesial, distal, and lingual).

The scores from the four areas of the tooth were added and divided by four to give the GI and PI for the tooth.

PD was measured as the distance between the gingival margin and the apical end of the pocket utilizing the Michigan 0 with Williams' markings periodontal probe nearly in a line with the vertical axis of the tooth until the blunt end contacted the bottom of the pocket.

CAL was measured from the cemento-enamel junction (CEJ) till the apical end of the pocket utilizing the Michigan 0 with Williams' markings periodontal probe.

PD and CAL measurements were performed at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) all the measurements were approximated to the most elevated whole millimeters.

Statistical Analysis

Numerical results were presented as mean and standard deviation (SD) values. Data's normality was determined by utilizing Kolmogorov-Smirnov test of normality.

Paired t-test was used to compare between the two groups because this is a split mouth study. Paired t-test was used for comparisons regarding PD due to the normal (parametric) distribution of PD data.

CAL, GI and PI showed non-normal (non-parametric) distribution, so Wilcoxon-signed rank test was used for comparisons between the groups.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed with SPSS 16.0® (Statistical Package for Scientific Studies) for Windows.

RESULTS AND DISCUSSION

Physicochemical compatibility of Gemifloxacin Mesylate and polymers

FTIR spectrum of the intact drug shows a broad band at 3410 cm^{-1} indicating the stretching of O-H carboxylic group, it shows also C-H aliphatic group stretching at 2900 cm^{-1} and a strong band at 1670 cm^{-1} for C=O aryl ketone group. FTIR spectra of the physical mixtures between drug and each polymer (1:1 w/w) maintained all the bands of GM, which indicates that there was no change in the functional groups of GM in these mixtures. These results suggest that there is no interaction (incompatibility) between the drug and polymers used (data not shown).

Syringeability

The syringeability of each formulation is tested. As the concentration of gellan gum and poloxamer 407 increased, the viscosity of formulation was increased and increased force required to expel each formulation. It was revealed that all formulations were syringeable through the syringe equipped with 20 gauge needle except formulations FG3, FG6, FG8 and FG9 failed to pass the syringeability test because they contain higher concentrations of the polymeric material so they would be excluded for further investigations.

Physical appearance

All formulations were investigated for their physical appearance and revealed that, the prepared formulations have accepted appearance. They were homogenous, free from air bubbles, clear and transparent.

Measurement of pH

It was reported that the apparent viscosity of gellan gum solution can be markedly influenced by the pH[24]. Therefore, the pH of the formulation should be adjusted and maintained between (5 – 6) with the help of a non-ionic alkalinizing agent like Triethanolamine if necessary, but the pH of all prepared formulations was observed to be in the range of 5.85 ± 0.12 to 6 ± 0.4. Therefore, there was no need for pH adjustment by any external alkalinizing agent. The pH value of each formulation is presented in table 2. This pH range is suitable for periodontal pocket insertion with no irritation.

Drug content uniformity

Uniform distribution of active ingredient is important to achieve dose uniformity. Table 2 shows the result of percent drug content for the prepared formulations. The drug content was found to be in acceptable range for all formulations. Recovery was possible to the tune of 98.2 ± 0.022 to 104.1 ± 0.061. Result limits indicating that the drug was uniformly dispersed.

Rheological properties

Rheograms of the prepared formulations were plotted, Y axis was taken to represent the Shear Rate and X axis to represent the Shear Stress, as shown in Figure 1. The results revealed that GM *in situ* gels exhibited pseudoplastic flow. For selected *in situ* gels, 0.8% Gellan / 18% Poloxamer gel exhibited the highest viscosities at maximum and minimum rates of shear, the values were 43.2 cps and 1689.9 cps, respectively, compared to 6.8 cps and 252.6 cps, respectively, for the 0.6% Gellan / 16% Poloxamer *in situ* gel. Figure 2 shows the relation between the viscosity and shear rate of the formulated *in situ* gels. It could be noted that there is an inverse relationship between shear rate and viscosity confirming a typical pseudoplastic flow. As concentration was increased, the polysaccharides chains came closer and then their mutual entanglement occurred. Viscosity increases as the polymer concentration increases. All the formulations showed non-Newtonian flow and exhibited pseudoplastic property.

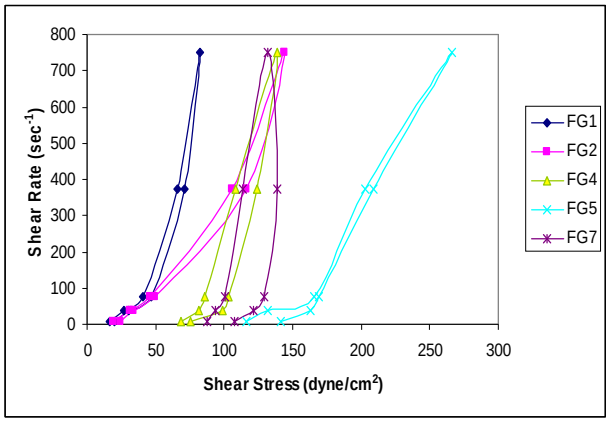

Figure 1: Rheogram of different formulated GM *in situ* gels

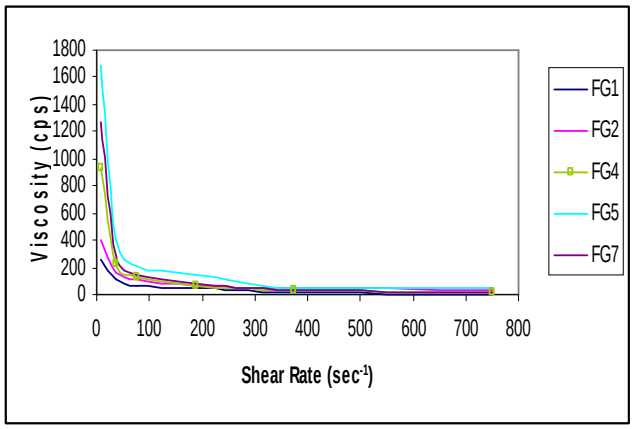

Figure 2: Viscosity value against shear rate of formulated GM *in situ* gels

Gelation Temperature

Gelation temperatures of different *in situ* gels are presented in Table 2. Gelation temperature ranged between (29 - 34) °C, so it is liquid that facilitates its syringeability during its application into the periodontal pocket while it will gel at the application site (physiological temperature, 37 °C) and thus controls its release.

Gelation time

The results of gelation time for each formulation were shown in Table 2. All formulations had gelation time as few minutes except formulation FG1 (containing 16% w/w poloxamer 407 and 0.6% w/w gellan gum) showed higher gelation time.

In vitro gelling capacity

The main pre-requisite for in situ periodontal gels were viscosity and gelling capacity. The formulation should undergo rapid sol to gel transition in simulated saliva due to ionic interaction. To facilitate sustaining the release of the drug to periodontal cavity, the formed gel should preserve its integrity without eroding or dissolving. Except formulation FG1 all the formulations showed instantaneous gelation when come in contact with simulated saliva maintained at 37 ± 1°C. However the nature of the gel formed depends upon the concentration of polymers.

Formulation FG1 showed weakest gelation and dispersed rapidly on moderate shaking, which may be due to presence of low concentration of gellan gum and poloxamer 407 while formulation FG5 which contain high concentration of poloxamer 407 and gellan gum showed immediate gelation and the formed gel was stiff and remained for extended period.

Table 1: Composition of GM *in situ* gel**.**

Components	Formula No.								
	FG1	FG2	FG3	FG4	FG5	FG6	FG7	FG8	FG9
Gemifloxacin Mesylate (mg)	400	400	400	400	400	400	400	400	400
Poloxamer 407(gm)	16	16	16	18	18	18	20	20	20
Gellan gum(gm)	0.6	0.8	1	0.6	0.8	1	0.6	0.8	1
Propylene glycol(ml)	2	2	2	2	2	2	2	2	2
Propyl paraben(mg)	20	20	20	20	20	20	20	20	20
Methyl paraben(mg)	180	180	180	180	180	180	180	180	180
Purified water to (gm)	100	100	100	100	100	100	100	100	100

Table2: pH, %Drug content, Gelation time, Gelation temperature and syringeability of formulated GM *in situ* gels.

Formulation code	pH Mean ±SD	%Drug content Mean ±SD	Gelation time (min)	Gelation temperature (°C)	Syringeability	*In vitro* gelling capacity
FG1	5.85 ± 0.12	104.1 ± 0.061	10	33-34	pass	+
FG2	6 ± 0.4	98.2 ± 0.022	9	31-32	pass	++
FG4	5.89 ± 0.07	99.1 ± 0.054	8	30-31	pass	++
FG5	6 ± 0.08	100.6 ± 0.004	7	30-31	pass	+++
FG7	6 ± 0.07	100.1 ± 0.008	8	29-30	pass	++

Table 3: Kinetic parameters of GM release data from different *in situ* gels according to Zero order, First order and Diffusion kinetics.

Formula number	R^2			Release order	K mg $min^{-1/2}$	$T_{50\%}$ (min)
	Zero	First	Diffusion			
FG1	0.989625	0.93865	0.993813	Diffusion	3.248508	236.9039
FG2	0.989038	0.9471	0.993413	Diffusion	3.243689	237.6082
FG4	0.989208	0.95043	0.9934	Diffusion	3.241621	237.9115
FG5	0.986097	0.96258	0.991163	Diffusion	3.212056	242.3113
FG7	0.991535	0.9794	0.994224	Diffusion	3.216426	241.6534

Table 4: The means, standard deviation (SD) values and results of paired t-test for the comparison between PD in the two groups and results of Wilcoxon signed-rank test for the comparison between CAL, GI and PI in the two groups.

Clinical Parameters	Time Period	Group I (Test) (mean ± SD)	Group II (Control) (mean ± SD)	*p*-value
PI	Baseline	1.4± 0.5	1.3± 0.5	**0.414**
	1 month	0.5 ± 0.5	1.1 ± 0.3	**0.020***
	2 month	0.3± 0.5	0.8 ± 0.5	**0.014***
GI	Baseline	1.5± 0.5	1.4 ± 0.5	**0.705**
	1 month	0.4 ± 0.5	1 ± 0	**0.008***
	2 month	0.3 ± 0.5	1 ± 0.5	**0.0079**
PD (mm)	Baseline	6.8 ± 1.5	7.7 ± 1.4	**0.128**
	1 month	3.9 ± 0.8	5 ± 1.1	**0.002***
	2 month	2.6 ± 0.6	3.9 ± 1.3	**0.004***
CAL (mm)	Baseline	6.5 ± 2	7.2 ± 0.82	**0.410**
	1 month	3.7 ± 1.4	4.8 ± 0.63	**0.099**
	2 month	2.4 ± 0.67	3.8 ± 0.85	**0.044***

*Statistically significant different, *p*-value ≤ 0.05.

In vitro drug release studies

The cumulative amount of GM released vs. time for the selected formulations is shown in Figure 3. One hour time for first sampling was selected in order to evaluate the effect of increasing polymer concentration on the cumulative amount of drug released. The results showed that the amount of drug released in the first hour decreased with increasing polymer concentration, and the trend continued for the entire duration of the study. The initial burst release of the drug from the prepared formulations could be explained by the fact that these systems were formulated in an aqueous vehicle. The matrix formed on gelation was already hydrated and hence hydration and water permeation could no longer limit the drug release. The release of drug decreased markedly as the concentration of polymer increased. The release from various formulations can be ranked as follows at each time point: FG1 > FG2 > FG4 > FG5 > FG7. This indicates that the structure of gel becomes more closely packed and functioned as an increasing resistant barrier to drug release as the concentration of polymer increased. In general there was a reduction in drug release as the concentration of polymers increases. The Slowest drug release was observed from formulation containing 0.8% (w/w) of gellan gum and 18% (w/w) of poloxamer 407, and relatively faster drug release was observed from formulation containing a different concentration of gellan gum 0.6% (w/w) and 16% (w/w) poloxamer 407.

In order to determine the release model which describes the pattern of drug release, the *in vitro* release data were analyzed according to zero-order, first-order and diffusion controlled mechanism according to the simplified Higuchi model. The prevalence of a certain mechanism was based on the determination coefficient (R^2) for the parameters studied, where the mechanism of release that possessed the highest coefficient is referred to the order of release from such formula. Complete drug release was attained after 720 minutes. The drug release was best fitted to diffusion technique and can be arranged in a descending order according to their K as FG1 > FG2 > FG4 > FG7 > FG5 (3.248508, 3.243689, 3.241621, 3.216426 and 3.212056% mg $min^{-1/2}$, respectively) as shown in Table 3.

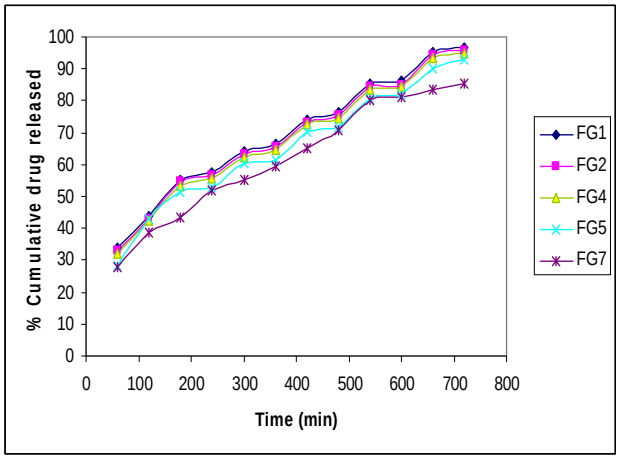

Figure 3: Release profile for GM from formulated in situ gels.

Clinical study of selected Gemifloxacin Mesylate *in situ* gel

This study included a total of thirty patients, the mean and SD values for the age in the test group and control group were 37.92 ± 9.39 and 40.82 ± 8.01 respectively. There was an insignificant difference in mean age values between the two groups (p=0.436).

The test group had included 15 patients (7 males and 8 females) and the control group had included 15 patients (5 males and 10 females). There was no statistically significant difference in gender distributions among the two groups.

There were no reported adverse effects (complaints, signs of allergy, inflammation, irritation and pus formation) or any other complications were reported by patients included in the study suggesting that the formulation was well tolerated.

Clinical parameters

1-Probing depth (PD)

The mean and standard deviation values of PD in test group were 6.8 ± 1.5 mm at baseline, 3.9 ± 0.8 mm after 1 month and 2.6 ± 0.6 mm after 2 months.

The mean and standard deviation values of PD in control group were 7.7 ± 1.4 mm at baseline, 5.0 ± 1.1 mm after 1 month and 3.9 ± 1.3 mm after 2 months.

At baseline, there was no statistically significant difference between the two groups. After 1 and 2 months, Test group showed statistically significantly lower mean PD than Control group.

2-Clinical attachment level (CAL)

The mean and standard deviation values of CAL in test group were 6.5 ± 2 mm at baseline, 3.7 ± 1.4 mm after 1 month and 2.4 ± 1.3 mm after 2 months.

The mean and standard deviation values of CAL in control group were 7.2 ± 2.1 mm at baseline, 4.8 ± 1.7 mm after 1 month and 3.8 ± 1.8 mm after 2 months.

At baseline and after 1 month there was no statistically significant difference between the two groups. After 2 months, Test group showed statistically significantly lower mean CAL than Control group.

3-Gingival Index (GI)

The mean and standard deviation values of GI in test group were 1.5 ± 0.5 at base line, 0.4 ± 0.5 after 1 month and 0.3 ± 0.5 after 2 months.

The mean and standard deviation values of GI in control group were 1.4 ± 0.5 at baseline, 1 ± 0 after 1 month and 0.3 ± 0.5 after 2 months.

At baseline, there was no statistically significant difference between the two groups. After 1 month, Test group showed statistically significantly lower mean GI than Control group. After 2 months, there was no statistically significant difference between the two groups.

4-Plaque Index (PI)

The mean and standard deviation values of PI in test group were 1.4 ± 0.5 at baseline, 0.5 ± 0.5 after 1 month and 0.3 ± 0.5 after 2 months.

The mean and standard deviation values of PI in control group were 1.3 ± 0.5 at baseline, 1.1 ± 0.3 after 1 month and 0.8 ± 0.5 after 2 months.

At baseline, there was no statistically significant difference between the two groups. After 1 and 2 months, Test group showed statistically significantly lower mean PI than Control group.

The previous results of clinical parameters are listed in Table 4.

CONCLUSION

In the present research GM *in situ* gel was developed with combination of gellan gum and poloxamer 407. By doing compatibility study, drug was found to be compatible with formulation excipients, it is concluded that the selected polymers are likely to be suitable for the preparation of GM *in situ* gel. The developed formulations showed satisfactory results for physical appearance, % drug content, gelation time, gelation temperature, syringeability, pH, *in vitro* gelling capacity and *in vitro drug release*. Formulation containing 0.8% w/w gellan gum and 18% w/w of poloxamer 407 was considered the best formulation as it gives satisfactory *in vitro* and clinical results.

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