

## Preparation and *in-vitro* Evaluation of Secnidazole as Periodontal In-situ Gel for Treatment of Periodontal Disease

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### Abstract

This study aims to develop a thermosensitive mucoadhesive periodontal in situ gel of secnidazole for local release of drug for treatment of periodontitis, in order to increase the drug residence time and to increase patient compliance while lowering the side effects of the drug.

Cold method was used to prepare 30 formulas of secnidazole periodontal in situ gel, using different concentrations of thermosensitive polymers (poloxamer407 alone or in combination with poloxamer 188) and methyl cellulose (MC ) or hydroxypropyl methylcellulose (HPMC K4M )in different concentrations used as mucoadhesive polymer and the resultant formulations were subjected to several tests such as gelation temperature GT, appearance and pH value. The formulas with the most appropriate GT were subjected to in-vitro drug release. Three formulas were chosen with appropriate release, F6 (15% P407, 1% MC), F29 (18%P407,3% P188, 0.8% HPMC) and F30 (18%P407,3% P188, 1% HPMC). These formulas were subjected to mucoadhesive force, viscosity, drug content, spreadability, gelation time and Fourier Transform Infrared (FTIR) compatibility studies.

The results indicates that formula F29 and F30 have best gelation temperatures (33°C, 32°C) gel strength (1.5h,2h) mucoadhesive force of (17.1, 23.4 dyne/cm<sup>2</sup>) and in-vitro drug release (98.2%, 100%) respectively during 3.5h and gelation, time about 10 seconds for both formulas and FTIR spectrum study show absence of important interaction between secnidazole and the polymers used.

**Keywords; in-situ gel, methylcellulose, HPMC, poloxamer, Secnidazole**

### التحضير والتقييم المختبري لـ سكيندازول هلام بالموقع لعلاج التهاب اللثة

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### الخلاصة

الهدف الرئيسي من هذه الدراسة هو تحضير هلام Secnidazole الذي يتصلب في الموقع وتحسينه لزيادة وقت تواجد الدواء وتسليم الدواء المحلي مما يزيد من امتثال المريض ويقلل من الآثار الجانبية للأدوية ويقدم معدل شفاء أسرع قد تم تحضير ٣٠ تركيبة للهلام (الذي يتصلب في الموقع) بالطريقة الباردة باستخدام بوليمرات متحسسة للحرارة (poloxamer 407 and poloxamer 188) مع بوليمرات تساعد على الالتصاق على الغشاء المخاطي (methylcellulose (MC) and hydroxypropyl methyl cellulose (HPMC)) واعتماد درجة الحرارة اللازمة للتحويل من السائل الى الهلام القريبة من درجة حرارة الجسم الطبيعية، كأساس في اختيار التركيبة المناسبة، تم اختيار التراكيب التي لها درجة حرارة تحول الى هلام قريبة من درجة حرارة الجسم. وقد خضعت هذه التراكيب الى تحرر الدواء خارج الجسم و كان احسن التراكيب التي أظهرت تحرر مناسب هي ثلاث تراكيب، التركيبة F6 التي تحوي (15% P407, 1% MC) و التركيبين ( F30 و F29 )الذين يحويان الاتي (F30 (18%P407,3% P188, 1% HPMC) F29 (18%P407,3% P188, 0.8% HPMC) وتم اختبارهم من حيث قوة الالتصاق للغشاء الهلامي واللزوجة والمحتوى الدوائي والانتشار ووقت التحول الى الهلام ودراسة امتصاص الطيف تحت الحمراء (FTIR) لبيان أي تعارض كيميائي بين المواد.

تتمتع الصيغتان F30 و F29 بأفضل درجات حرارة تحول الى الهلام (٣٣ و ٣٢ درجة مئوية) ، و قوة الهلام ( ١,٥ و ٢ ساعة ) ، قوة لاصق مخاطي ( ١٧,١ و ٢٣,٤ داين/سم<sup>٢</sup> ) تحرر العقار في المختبر (٩٨,٢% و ١٠٠%) على التوالي خلال ٣,٥ ساعة، ووقت تكون الهلام حوالي ١٠ ثوانٍ لكل منهما. دراسة طيف FTIR تشير إلى عدم وجود تفاعل كيميائي بين العقار سكيندازول والبوليمرات المستخدمة.  
الكلمات المفتاحية : هلام بالموقع، متحسس للحرارة، سكيندازول

### Introduction

Periodontitis is an inflammatory disease of supporting tissues of teeth caused by specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with periodontal pocket formation, gingival

recession or both <sup>(1)</sup>. Gingivitis, is the mildest form among periodontal disease<sup>(2)</sup>. Periodontitis affects nearly 60% of the world's elderly population and 50% of the adult population <sup>(2)</sup>. Male's susceptibility is more than females for chronic periodontitis<sup>(3)</sup>

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Heavy smokers have a high risk of occurrence of chronic periodontitis<sup>(4)</sup>. In addition, it can be associated with other serious health conditions such as diabetes, cardiovascular disease and stroke<sup>(5)</sup>. Current concepts of the etiology of periodontitis implicate a bacterial infection as the primary cause of the disease<sup>(6)</sup>. The main cause of periodontitis is that bacterial flora gradually shifts to anaerobic status, in addition to periodontal pockets, which provide a favorable environment for the growth and proliferation of some anaerobic bacterial species<sup>(7)</sup>. *Porphyromonas gingivalis*, *Tannerella forsythia*, *Filifactor alocis*, (a gram-positive anaerobe)<sup>(8)</sup>, and *Treponema denticola* have shown to be the most common cause of periodontal disease<sup>(9,10)</sup>.

The non-surgical treatments are enough for patients with early or moderate disease which include scaling and root planning (SRP) (bacteria's mechanical removal). Supplemental use of local antibiotics, local antiseptic drugs, systemic antibiotics have been shown to provide some additional benefit compared with debridement alone<sup>(11)</sup>.

Nitroimidazoles (such as metronidazole) have excellent activity against anaerobic microorganisms due to their bactericidal activity, broad spectrum of activity and rapid onset of action<sup>(12)</sup>. While secnidazole, has a longer terminal elimination half-life than commonly used drugs in this class. Therefore, the treatment interval will be shorter and significantly more effective than the treatment using other imidazole drugs<sup>(13)</sup>.

Secnidazole is a second-generation of 5-nitroimidazole antimicrobials and has selective activity against many anaerobic Gram-positive and Gram-negative bacteria and protozoa<sup>(14)</sup>, it has molecular weight of 185.18g/mol, melting point is 76°C, a water solubility is nearly 34 mg/ml at 27°C, partition coefficient is 0.27 and half-life of 17 hours<sup>(15)</sup>, and is approved for dental infections<sup>(16)</sup>.

Local application into periodontal pocket could be very advantageous both in prolonged drug delivery, preventing systemic side effects<sup>(17)</sup>, ease of application; especially in situ gelling system, as they are applied as liquids that gel upon contact with the oral cavity, selectively targeting a limited number of diseased sites that were unresponsive to conventional therapy and possibly enhanced treatment results at due mucoadhesion and drug retention<sup>(18)</sup>.

Secnidazole vaginal in situ gel formulations were prepared by Karthick *et al.*, using 0.45% of Carbopol 940 with 0.35% HPMC K4M and the 0.35% of Carbopol 940 with 0.35% hydroxy propyl cellulose HPC these formulations also released less than 50% of drug in simulated vaginal fluid at the end of 8 hours<sup>(19)</sup>. While, Narayana *et al.*, formulated in situ vaginal gels of secnidazole, based on ion activated systems using gellan gum (0.1-0.75% w/v) and sodium carboxy methylcellulose to prolong the release of secnidazole (1% w/v)<sup>(20)</sup>.

For periodontal delivery, a previous study of Secnidazole and of Serratiopeptidase was performed by Priyanka *et al.* Using sodium alginate as an in-situ gel polymer (1%), and HPMC E50Lv (1-8%w/w) to modulate the gel strength and the bioadhesive force,<sup>(21)</sup> also, dento-oral gels of Secnidazole were prepared by Gad *et al.* by using 3% w/w MC and 5% w/w hydroxy ethyl cellulose HEC with HPMC or Carbopol 934 or Carbopol 971 (1 or 3% w/w)<sup>(22)</sup>. Also Secnidazole in situ implant to modify the release of secnidazole over 24hours using PLA(poly lactic acid) and PLGA(copoly lactic glycolic acid) polymers<sup>(23)</sup>.

Gelation temperature GT is the crucial criteria for the selection of the appropriate thermo-sensitive formula of poloxamers. Poloxamers are synthetic triblock copolymers of poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO), Poloxamer 407 (P407) has 70% PEO while poloxamer 188 (P188) has 30% PEO<sup>(24,25)</sup>. Poloxamer 407 (P407) as a thermosensitive polymer is mostly used in range 15-20 % (w/w) to achieve the desired sol-gel thermal transition near body temperatures (33-35°C)<sup>(26)</sup>. Due to their amphiphilic nature, at critical micelle temperature CMT, P407 molecules assemble to form spherical micelles at the critical micelle concentration CMC, with a dehydrated hydrophobic PPO core surrounded by hydrated swollen PEO chains, packing and entanglements of micelles will increase with temperature results in a 3D lattice structures<sup>(27,28)</sup>. Poloxamers form gel at the critical micelle concentration CMC and critical micelle temperature CMT, the higher the PEO % in the poloxamer, the higher is the CMC and CMT, thus P188 has a higher CMC and CMT than P407 and the crucial determining factor of GT is the type and concentration of the poloxamer<sup>(28)</sup>. Although the mucoadhesive polymer may alter the GT but this is secondary to the effect of poloxamer<sup>(29)</sup>.

The aim of this study is the preparation of a thermo-sensitive in-situ periodontal gel for the local intra-pocket administration of secnidazole, that will gel near physiological temperature, using the thermo-responsive polymers (Poloxamer 407 and Poloxamer 188) with mucoadhesive polymers (Methyl cellulose or Hydroxypropyl methylcellulose).

## Materials and Method

Secnidazole, poloxamer 407 P407, poloxamer 188 P188 and methyl cellulose MC (63000 Da) were purchased from Baoji Guo Kang Bio-technology Co., Ltd. China. Hydroxypropyl methyl cellulose HPMC (K4M) from Hangzhou Hyper chemicals limited, Zhejiang, China. All other chemicals and solvent were of analytical reagent grade.

### Method of in situ gel preparation

*In situ gel* of Secnidazole SC was prepared by cold method<sup>(30)</sup>. A predetermined amount of poloxamer P407 (15-18% w/v) alone or in combination with P188 (2-4% w/v) was gradually added to cold water at 4 °C in a beaker with continuous agitation at a speed of 500 rpm for 2 hours using a magnetic stirrer, then the poloxamer solution was left overnight for complete hydration (at 4 °C) to ensure the formation of a clear and

viscous solution of poloxamer. Secnidazole at 1% w/v<sup>(16,20)</sup> was then added to poloxamer solution in the next day, using magnetic stirrer.

The viscosity enhancement polymer dispersions were prepared beforehand, HPMC was added with continuous mixing to hot water (70°C), while MC was added slowly and gradually to water at room temperature with continuous mixing and their dispersion was left overnight for complete hydration.

Preservative (methylparaben MP and glycerin GL) and the viscosity enhancement polymers HPMC and MC were added to the poloxamer- drug mixture with continuous stirring. The final dispersion was kept in refrigerator for another night at 4°C. Finally, the volume was completed with purified water<sup>(31)</sup>. Secnidazole periodontal in-situ gel components are shown in Table 1

**Table 1. Composition of SC Periodontal in-situ gel**

Formula No.	P 407 (g)	P188 (g)	MC. (g)	HPMC (g)	MP (g)	GL (mL)	SC g	H <sub>2</sub> O To (mL)
1.	15			0.2	0.01	2	1	100
2.	15			0.4	0.01	2	1	100
3.	15			0.6	0.01	2	1	100
4.	15		0.25		0.01	2	1	100
5.	15		0.5		0.01	2	1	100
6.	15		1.0		0.01	2	1	100
7.	15		1.5		0.01	2	1	100
8.	15	4	0.25		0.01	2	1	100
9.	14	4	0.5		0.01	2	1	100
10.	17			0.2	0.01	2	1	100
11.	17			0.4	0.01	2	1	100
12.	17			0.6	0.01	2	1	100
13.	17			0.8		2	1	100
14.	17		0.25		0.01	2	1	100
15.	17		0.5		0.01	2	1	100
16.	17	4	0.25		0.01	2	1	100
17.	17	4	0.5		0.01	2	1	100
18.	17	4		0.2	0.01	2	1	100
19.	17	4		0.4	0.01	2	1	100
20.	17	3		0.2	0.01	2	1	100
21.	17	3		0.4	0.01	2	1	100
22.	17	2	0.1		0.01	2	1	100
23.	17	2	0.2		0.01	2	1	100
24.	18		1		0.01	2	1	100
25.	18	2	0.1		0.01	2	1	100
26.	18	2	0.2		0.01	2	1	100
27.	18	3		0.2	0.01	2	1	100
28.	18	3		0.4	0.01	2	1	100
29.	18	3		0.8	0.01	2	1	100
30.	18	3		1	0.01	2	1	100

### Characterization of prepared SC periodontal in-situ gel

#### Determination of Sol -Gel transition temperature

To determine the gelation temperature test tube tilting method was employed, two mL of the formula was placed in a test tube with 1 cm diameter and sealed with parafilm, to be immersed in cold water (at 4 °C) in a water bath. (Medical Sources Co., Ltd., China), the temperature was increased 3°C at the beginning, then when the temperature approached the desired gelation temperature (around 30°C) it was increased by 1°C and kept constant in each temperature degree for ten minutes. When the test tube was tilted 90° and there is no flow of the formula, this was recorded as the gelation temperature<sup>(32)</sup>.

#### Appearance and pH determination

All formulations were checked visually for their clarity, color and if there are any suspended particles and this is done against black and white background<sup>(33)</sup>. In addition, the pH of all the formulas was measured using a digital pH meter (Hanna Instruments). The pH meter probe was immersed in each formula and this is achieved in triplicate and takes the average as the pH of in-situ gel formula<sup>(34)</sup>.

#### In vitro release studies

Secnidazole release from the selected SC periodontal *in situ gel* formulations were done by placing 5mL of the prepared formulation (F6, F7-F10 F11, F29, F30) into the dissolution jar filled with 500 ml simulated saliva fluid SSF pH 6.8<sup>(35)</sup> using USP dissolution apparatus type II (paddle type) for a period of 3.5 h with rotation rate 50 rpm at 37°C. Volume of (5mL) were taken at different time intervals and checked spectrophotometrically at 320 nm<sup>(22,23)</sup>. The formulas that showed acceptable release were chosen for further evaluation.

#### Kinetic modeling of Secnidazole release

The mechanism of release of secnidazole from the selected formulas that showed acceptable release were analyzed by fitting the release data into Zero, First, Higuchi, and Korsmeyer Peppas equations. Using a DDSolver Excel Microsoft Add-in program and k and R<sup>2</sup>, were obtained for each equation, and n value for Korsmeyer Peppas equation at 60% of release<sup>(36)</sup>.

#### Drug content

Accurately, 1 ml of the formulation (equivalent to 10mg/mL SC) from the selected SC periodontal in situ gel formulas were diluted to 10 ml with SSF<sup>(37)</sup>, and 1 ml of this solution was diluted again to 10 ml with SSF. Finally, the absorbance was measured at the maximum absorption wavelength using UV Spectrophotometer.

#### Determination of gelling capacity

The gelling capacity depend on the formulation properties like gelling time and time required for the formed gel to dissolve in a specific

environment. Gelling capacity was measured by placing a drop of the formula in a vial containing two mL of freshly prepared simulated saliva fluid equilibrated at 37 °C and assessing visually the gel formation and record the time for gel to be formed and the time required for the formed gel to dissolve. Gelation time was classified in three groups depending on the gel stiffness, gelation time and duration<sup>(38)</sup>.

#### Viscosity determination

The selected formulas were maintained at physiological temperature (at 34-37 °C) with the aid of the water bath (Medical Sources Co., Ltd., China) to form a gel.<sup>(20,34)</sup> The viscosity was measured using Fungilab Smart Viscometer fitted with spindle R2, the spindle was rotated from 6 to 100 rpm, and the rotation speed was increased gradually and allowed to rotate for two minutes before viscosity measurement were recorded in Pascal per second (Pa. s<sup>-1</sup>).

#### Determination of mucoadhesive force

Mucoadhesive force is the adherence power of the formulations to the epithelial mucosa. The modified balance method was used with a plastic beaker at one facet and on the other facet of the balance, then a vial was geared up with the oral mucosa of the sheep (with a thickness of 0.6 mm obtained from slaughtered sheep) taken immediately after sacrifice. The oral mucosa was outfitted on the backside of the vial. The vials were maintained at temperature (32–34 °C) for 10 minutes, then 1 mL of the SC periodontal in-situ formula was placed in a watch glass beneath the vial fitted with the oral tissue, then the vial was pressed down onto the gel for one minute as preliminary contact time. Water was added to the beaker on the other side of the balance gradually until complete separation of the mucosa from the formula as shown in Figure (1). The mucoadhesive force was measured by the weight of water needed to separate the dental in-situ formula from the oral mucosa by Equation (1):

$$\text{Detachment force} \left( \frac{\text{dyne}}{\text{cm}^2} \right) = \frac{m \times g}{A} \dots\dots\dots \text{Eq (1)}$$

Where, m is the required weight (in g), g is the acceleration (980 cm/s<sup>2</sup>) due to the gravity, A is the exposed oral tissue area which is 3.14 cm<sup>2</sup> in all preparations<sup>(39)</sup>.



**Figure 1. Mucoadhesive force modified balance method.**

#### **Determination of gel strength**

A sample of 3 g of each selected SC periodontal in-situ gel formula was placed in a 5 ml cylinder, after complete sol to gel transition and kept in water bath at (33°C). A mass of 5g was placed on the upper surface of the gel. The gel strength was recorded as the time required by the mass to move down 0.5 cm into the formed gel<sup>(33)</sup>.

#### **Spreadability test**

The spreadability was checked for the selected formulas after gelation was completed, by dropping 0.1 g of gel on the center of glass slide (20 x20 cm in size), and another glass slide with the same dimensions, covered the initial slide. The initial gel diameter was measured (in cm.) and after placing a 100 g weight on upper slide glass for 5 minutes, The final diameter of spread gel after

weight was removed, the difference between the two diameters is the measure of spreadability<sup>(40)</sup>.

#### **Statistical analysis**

The results of the statistical analysis showed that the value of F calculated for the tests conducted is smaller than the level of significance ( $p < 0.05$ ), which leads to the rejection of the null hypothesis and thus there are significant or substantial differences between the mean of the treatments.

## **Results and Discussion**

#### **Determination of Sol-Gel transition temperature**

As seen in Table 2A, increasing the concentration of HPMC caused a reduction in GT when comparing formulas containing P407 15% F1, F2 and F3 (HPMC 0.2, 0.4, and 0.6%), with formulas containing P407 17% F10, F11, F12, and F13, (HPMC 0.2,0.4, 0.6 and 0.8%), formulas containing P407 17%, P188.3% , F18, F19 (HPMC 0.2,0.4%), formulas containing P407 17%, P188.4% F20, F21 (HPMC 0.2,0.4%), formulas containing P407 18%, P188.3% F27, F28, F29 and F30 (HPMC 0.2, 0.4, 0.6, 0.8 and 1%). The gelation temperature lowering effect due to HPMC K4M could be attributed to the capability of HPMC to join to the chain of poly-oxyethylene in the poloxamer moieties<sup>(41)</sup>. That will lead to increase the dehydration of poloxamer, resulting in an increase in the complexity of neighboring molecules as well as intermolecular hydrogen bonding drastically increasing which produce gelation at much lower degree of temperature<sup>(28,37,41)</sup>.

**Table 2. A gelation temperature GT, appearance, pH of the formulated SC periodontal in-situ gel with HPMC as mucoadhesive polymer**

No.	P407	P188	HPMC	GT	pH	Appearance at Refrigerator Temperature (4°C)
F1	15		0.2	38±0.70	6.42±0.15	Clear liquid
F2	15		0.4	36±1.00	6.62±0.10	Clear liquid
F3	15		0.6	26±0.97	6.68±0.10	Clear liquid
F10	17		0.2	34±0.50	6.52±0.12	Clear liquid
F11	17		0.4	32±0.59	6.65±0.05	Clear liquid
F12	17		0.6	24±0.90	6.81±0.05	Clear liquid
F13	17		0.8	21±0.86	7.07±0.12	Clear liquid
F18	17	4	0.2	41± 1.25	6.60±0.08	Clear liquid
F19	17	4	0.4	39± 1.00	6.78±0.08	Clear liquid
F20	17	3	0.2	35± 1.00	6.03±0.15	Clear liquid
F21	17	3	0.4	33± 1.32	7.02±0.15	Clear liquid
27	18	3	0.2	37± 1.3	6.43±0.10	Clear liquid
28	18	3	0.4	35± 1.15	6.76±0.15	Clear liquid
29	18	3	0.8	33± 1.00	6.94 ±0.05	Clear liquid
30	18	3	1	32± 1.04	7.13 ±0.10	Clear liquid

While the effect of MC on GT is concentration and molecular weight dependent, as seen in Table 2-B. This may be due to MC hydrophobic interaction with poloxamer molecules will enhance CMC formation and decrease GT and enhance gel formation<sup>(42)</sup>. Opposite to expectation, increasing MC concentration increased the GT of the formulation,<sup>(42)</sup> due to the high molecular weight of MC (63000 Da) as seen in Table 2B, according to these results different polymers used alter GT significantly, since the gelation of methylcellulose is an entropy-driven process<sup>(43)</sup>. At first while increasing the concentration of MC it will undergo intermolecular hydrogen bonding through its unmodified hydroxyl groups with hydrophilic part

of poloxamer 188 and water molecules forming a network of macromolecular structure that interfere with CMC formation and increasing GT which is responsible for the formation of a stiff gel and cause drastic increment in gelation temperatures in formulation containing poloxamer 188 and MC combinations. MC being a thermosensitive polymer with a GT between 50-70°C (depending on the molecular weight of MC). It is impossible to have sol.-gel transition at temperatures lower than 51 °C because the hydrophobic association between the methyl groups of MC are not formed at this temperature range, this is not obvious at lower concentrations of MC<sup>(43)</sup>.

**Table 2 .B Gelation temperature GT, appearance, pH of the formulated SC periodontal in-situ gel with MC as mucoadhesive polymer**

No.	P407	P188	MC.	GT	pH	Appearance at 4°C	Appearance at high temperature (> 50°C)
F4	15		0.25	26±0.76	7.10±0.05	Clear liquid	
F5	15		0.5	28±1.10	7.00±0.15	Clear liquid	
F6	15		1.0	32±1.00	6.93 ±0.15	Clear liquid	thick (2 layers)
F7	15		1.5	Above 60	5.92±0.06	Clear liquid	thick (2 layers)
F8	15	4	0.25	Above 50	6.43±0.065	Clear liquid	thick (2 layers)
F9	5	4	5	Above 50	6.12±0.05	Clear liquid	thick (2 layers)
F14	17		0.25	20±1.00	6.48±0.12	Clear liquid	
F15	17		0.5	22±0.76	6.44±0.02	Clear liquid	
F16	17	4	0.25	41±0.76	6.33±0.10	Clear liquid	
F17	17	4	0.5	51± 1.25	6.11±0.06	Clear liquid	
F22	17	2	0.1	32± 1.65	6.92±0.05	Clear liquid	
F23	17	2	0.2	45± 1.24	6.64±0.05	Clear liquid	
F24	18		1	25± 1.08	6.33±0.10	Clear liquid	
F25	18	2	0.1	32± 1.08	7.07±0.05	Clear liquid	
F26	18	2	0.2	33± 1.3	6.71±0.05	Clear liquid	

### Appearance

All prepared formulas were translucent, and clear. The turbidity observed during preparation was found to disappear and regain clarity after overnight standing at refrigerator<sup>(44)</sup>. All the HPMC containing formulas were clear at low temperature and formed a translucent gel at higher temperature. While some of the MC containing formulas (F6, F7, F8 and F9) separated into two phases when the temperature was raised.

In formula F8 (15 % P407 4% P188 0.25% MC) and F9 (15 % P407 4% P188 0.5% MC) poloxamer 407 concentrations was (15%) which is too low for appropriate gelation<sup>(45)</sup>, poloxamer 188(4%) further delay gelation temperature of this mixture<sup>(45)</sup>, for this reason high temperature is required for poloxamer solution to reach gelation temperature and this high temperature concomitantly with low concentration of MC used

(0.25-0.5) the hydrogen bonding between MC and water will be weaker, leads to decrease water retention ability of MC and instead MC chain to chain aggregate through its hydrophobic parts, simultaneously stronger network of hydrogen bonds between poloxamer and water is formed, diluting the poloxamer, and poloxamer fails to reach CMC<sup>(43)</sup> so when the water returns to the poloxamer solution and at this stage we can see two separated layers one with clear appearance (phase separation), which represent the poloxamer solution and more thick and turbid layer represent the MC semi gel solution as seen in Table (2). Figure 2 shows the phase separation of poloxamer solution with MC, the same concept applies for F6 formula which almost converted to gel at 32°C but when the temperature is increased to more than 65°C the gel collapse and phase separation occur.



**Figure 2.**Phase separation of F6 when the temperature was raised to 65° C

#### ***pH Measurement***

The pH value is important in oral in-situ gel formulations, for good patient acceptance and compliance<sup>(46)</sup>. The pH of selected oral in-situ gel formulas (F6, F29 and F30) ranged between (6.93) and (7.13) as shown in Table (2). The pH range is acceptable with oral pH.

The pH of all formulations was within the range that would not cause any irritation upon administration; and close to oral pH<sup>(47)</sup>.

#### ***Selection of the appropriate formula***

According to the results of the sol-gel transition temperature 7 formulas were chosen for further evaluation with the appropriate sol-gel transition temperature near body temperature with MC (F6, F26) and with HPMC (F10, F20, F21, F29 and F30)

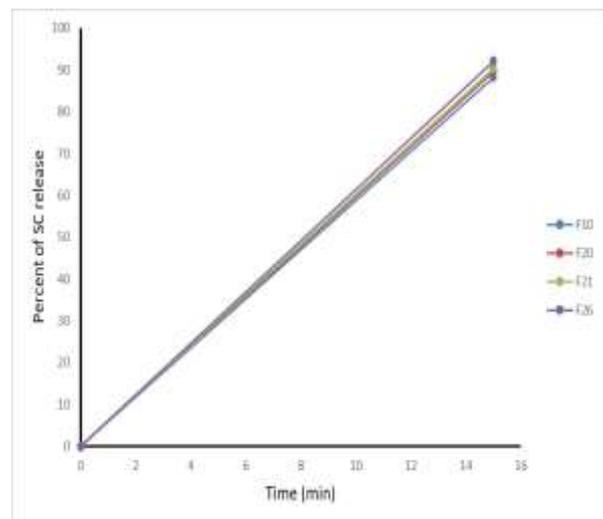
#### ***In vitro release studies***

The release of SC from the selected formulas in SSF is seen in Figure 3. Mucoadhesive polymer used at low concentration F10, F20 (0.2% HPMC), F21 (0.4% HPMC) and F26 (0.2% MC) showed rapid drug release time.

Increasing the concentration of the mucoadhesive polymer, as in formulas F6 (1%MC), F29 (0.8% HPMC) and F30 (1% HPMC) figure (4), the release of SC was slower due to the higher concentration of the cellulosic mucoadhesive polymer in these formulations which may be explained to their ability to increase the formulations viscosity as well as their ability to distort or squeeze the extra-micellar aqueous channels of poloxamer micelles through which the drug diffuses thereby delaying the release process as seen in previous studies<sup>(48)</sup>. For this reason, at low HPMC or MC concentration the gel layer surrounding poloxamer micelles are thinner and easily penetrated by the dissolution medium, while at high concentration this

gel layer is more condensed and needs time for erosion and dissolution, that's to say, polymer concentration has a significant effect on secnidazole release<sup>(49)</sup>.

Another factor is HPMC is more hydrophilic, than MC resulting in a faster rate of polymer swelling and hydration and a large increase in drug release<sup>(49)</sup>.



**Figure 3.** The percent release of SC with time in SSF (pH 6.8) at 37°C.

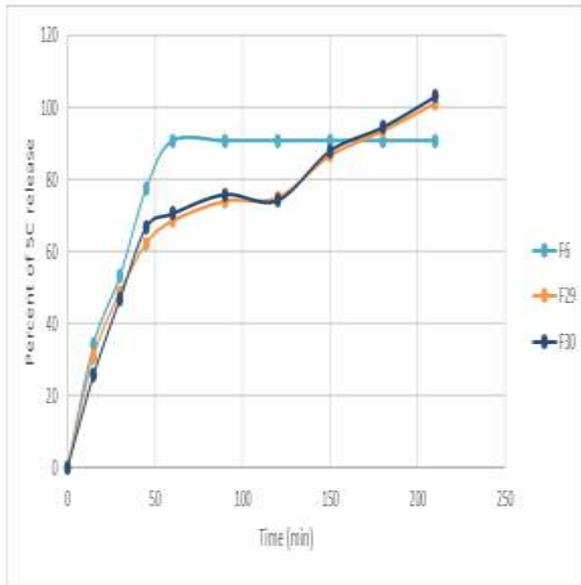


Figure 4. The percent release of SC with time in SFF (pH 6.8) at 37°C

Several studies in which mucoadhesive polymers such as MC or HPMC were used and increasing the concentration of these polymers leads to extension of time of drug release and enhances gel strength and each of these characterizations differ widely in each study depending on concentration and type of polymer responsible of gelation process used beside the effect of mucoadhesive polymer (30,48,50,51).

**Kinetic modeling of Secnidazole release**

According to the results obtained from the release data seen in Figure 3, the kinetic data modeling was performed for F6, F29 and F30, is shown in Table (3). The release of Secnidazole obeys Korsmeyer Peppas non Fickian diffusion according to the n value. Drug release from hydrophilic polymers like (MC and HPMC) will be based upon all factors that influence the swelling and erosion of the gel such as the composition of the formulation, the characteristics of the drug itself, as well as the properties of the polymers in the product (52). The progressive swelling of polymers leads to changes the porosity of the polymers that affect the diffusional release of a drug (53).

Table 3. Mathematical release kinetics of secnidazole from oral in-situ gel .

		F6	F29	F30
<b>First order</b>	<b>K1</b>	0.029	0.018	0.019
	<b>R<sup>2</sup></b>	0.9594	0.9480	0.9509
<b>Zero order</b>	<b>K0</b>	0.618	0.589	0.597
	<b>R<sup>2</sup></b>	-0.1712	0.4110	0.4447
<b>Higuchi</b>	<b>KH</b>	7.952	7.377	7.471
	<b>R<sup>2</sup></b>	0.7046	0.9412	0.9303
<b>Korsmeyer- Peppas to 60% release</b>	<b>K KP</b>	4.083	5.643	2.482
	<b>n</b>	0.769	0.632	0.865
	<b>R<sup>2</sup></b>	0.9967	0.9999	1.0000

**Drug content**

The drug content of the selected SC oral in-situ gel formulas (F6, F29 and F30) was in the range of (88.2%–101%) which is acceptable according to the USP (54), indicating high content, uniformity of the in-situ gel formulas and suitability of the preparation method. The results are presented in Table (4).

Table 4. The drug content in the selected sc periodontal in situ gel

Formulas	Drug Content
F6	88.2%
F29	98.2%
F30	100%

**Gelation time and gel capacity**

Gelling time was measured to test the time the formula will remain in gel form before been dissipated by the SSF, and the gelation time was inspected by visual examination. The grading and gelling capacity are shown in Table 5. All the formulas showed good gelation time and capacity due to the higher concentration of both the poloxamer and the HPMC. The selected SC oral in situ formula F29 and F30 containing HPMC (K4M) (from 0.8 to 1%) showed excellent gelation time and gel capacity, due to the high concentration of poloxamer 407 (18%) and high concentration of HPMC which possess high molecular weight. In addition, F6 has good gelation capacity this is, due to high concentration of MC (1%) used which enhance viscosity and improve gelation properties but take more time to form in-situ gel this may be due to the lower poloxamer 407 concentrations, 15% used in this formula, so both gelation time and

gelation temperature are affected significantly by types and concentration of polymer used.

**Table 5. The gelation time and gelling capacity of sc periodontal in situ formulas**

Formula Code	Gelation time (sec.)	Gelling Capacity**
F6	10	++
F29	10	+++
F30	10	+++

(+)gelation occurs after few minutes, remains for few minutes, and dispersed rapidly.

(++)gelation occurs at once and remains for 8 hours

(+++)  
gelation occurs at once and remains for more than 8 hours.

**Viscosity determination**

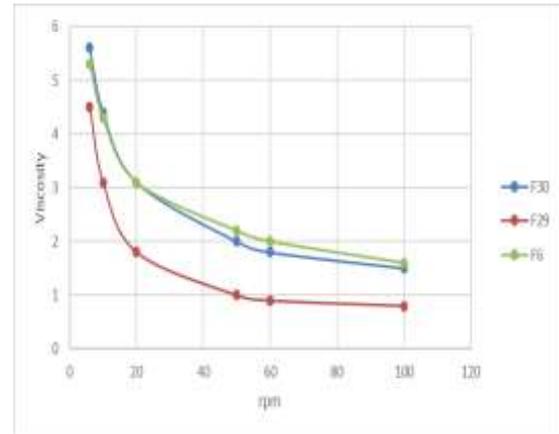
The viscosity is important since lower viscosity formulas will drain from the oral cavity. The viscosity measurements for selected formulations F29, F30, and F6 at different rpm are shown in Figure 5. The formulations showed pseudoplastic rheology, shear thinning and decrease in viscosity with increase velocity, the viscosity was significantly dependent on the polymeric content and effected by their concentration, 0.8 % HPMC (F29), 1% HPMC (F30) or 1% MC (F6) with relatively high concentration of poloxamer 407, which assist formulation to be fluids before and during administration at room temperature, they can be easily injected by means of a periodontal syringe, allowing the formulation to get access to the entire pocket<sup>(28)</sup>.

**Table 6. Gel Strength, mucoadhesive force and spreadability of SC oral in-situ gel selected formulas (mean ±SD) (n=3)**

Formula code	Gel strength (h)	Mucoadhesive force (dyne/ cm <sup>2</sup> )	Spreadability (cm)
F6	24±0.03	8991.04 ± 862.54	3.7 ±0.3
F29	1.5±0.06	5338.34± 359.54	3.3 ±0.3
F30	2±0.06	7305.22± 511.54	2.8 ±0.2

**Determination of mucoadhesive force**

Table 6 shows the mucoadhesive force of the selected formulas. Formula F30 has a much higher mucoadhesive force than F29 due to higher concentration of HPMC in F30. The mucoadhesive force is the result of hydrogen bonding between the polymers and oligosaccharide chain of the mucus, lining the mucus membrane. The mucoadhesive force increases significantly due to an increase in the number of penetrating hydrophilic chain to mucus glycoprotein as the concentrations of polymer increase. Mucoadhesive forces depends on the nature and the concentration of viscosity enhancement polymers, as the polymer concentration increases<sup>(57-59)</sup>.



**Figure 5. Viscosity (Pa/s) of F6, F29 and F30 at different rpm at 37°C**

**Determination of gel strength**

Formulations with higher concentration HPMC (F30) relative to poloxamer shows higher gel strength than those with lower concentration of HPMC (F29) relative to poloxamer polymers<sup>(55,56)</sup>, while, MC (F6) showed more gel strength in comparison with HPMC K4M polymer (F29, F30), this might be related to the formation of more interlocking forces (hydrogen bond, Van der Waals) between the poloxamer and MC polymer more than with poloxamer and HPMC<sup>(56)</sup>, which means the polymer type and concentration have significant effects on gel strength.

The high molecular weight of MC and the high concentration of MC in F6 may allow extensive adhesion to the oral mucosa, since high concentration of MC enhances the rheological synergy between MC and mucin<sup>(60)</sup>. When the temperature increases, over and around 30 °C, non-Newtonian behavior occurs corresponding to chain-chain interaction due to the hydrophobic character of methylcellulose. This behavior is in good agreement with the hypothesis of Kobayashi *et al* (61). A high molecular weight of MC have the more effect on muco-adhesion<sup>(62)</sup>. The mucoadhesive force of the oral in-situ gel formulas should be enough to resist saliva fluid flow in oral cavity to maintain good contact with oral mucosal

membranes, especially when placed in the dental pocket and is useful for treatment of periodontitis.

#### Spreadability test

From the results shown in Table 6, all the selected formulas have spreadability from 2.3 to 3.7 cm, in F29 and F30 at high concentration of the poloxamer and viscosity enhancement polymer HPMC, the viscosity of the SC periodontal in-situ formula increase and the spreadability decreased significantly (63), while in F6 showed higher spreadability, which may be due to low poloxamer 407 concentration 15% .

#### Conclusion

Intra pocket drug delivery system is an attractive and promising way to deliver antimicrobial agent into periodontal pocket to achieve local enhanced gingival crevicular drug concentration combined by lower systemic side effect.

While in situ gelling is favored as the formula can be applied in a syringable form, and gel at the site. Poloxamers P407 P188 were selected as thermosensitive in situ gelling polymers for mucosal drug delivery, in combination with mucoadhesive polymers (MC or HPMC).

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#### Conflict of interest

There is no conflict of interest.

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