

Research Article



A Novel Temperature Sensitive *In Situ* gel for Sustained Periodontal Drug Delivery of Tinidazole

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ABSTRACT

Tinidazole is well reported for the treatment of periodontal disease, they have better penetration into periodontal tissues, minimal bacterial resistance as compared to many of the other drugs used for the treatment. *In situ* gel-forming systems are viscous liquids that shift to a gel phase upon exposure to physiological conditions. Tinidazole periodontal gel was prepared by different concentrations of Poloxamer 407 (Temperature sensitive). All the prepared formulations were evaluated for appearance, pH, viscosity, Gelling capacity, Gelling Temperature, % drug content, syringeability, % drug release and effect sterility testing. By compatibility study drug was found to be compatible with formulation excipients. pH and Viscosity of all prepared formulations was found in the range of 5.6 – 6.2 and 378 - 834 centipoise respectively. The developed formulations showed satisfactory results for pH, viscosity, Drug content, in-vitro gelling capacity and other physical properties. Based on maximum desirability and cost effectiveness formulation containing 16% poloxamer 407 was considered as an optimized batch.

Keywords: *In situ* gel; Periodontitis; Poloxamer 407; Temperature sensitive.

INTRODUCTION

Periodontal disease is one of the most common microbial infections in adults. It is an inflammatory disease of bacterial origin that affects the tooth-supporting tissues. There are 2 major types of periodontal disease: gingivitis and periodontitis.

Gingivitis involves limited inflammation of the unattached gingiva and is a relatively common and reversible condition.

In contrast, periodontitis is characterized by a general inflammation of the periodontal tissues, which leads to apical migration of the junctional epithelium along the root surface and progressive destruction of the periodontal ligament and the alveolar bone.

Periodontitis progresses in cyclical phases of exacerbation, remission and latency, a phenomenon that is closely linked to the effectiveness of the host's immune response^{1,2}.

The classification of periodontal diseases has evolved a great deal over the years. In the most recent report of the American Academy of Periodontology, published in 1999, the various forms of periodontal disease were classified on the basis of cause, severity and site of disease.

Experts now distinguish among generalized and localized chronic periodontitis, generalized and localized aggressive periodontitis, periodontitis associated with systemic diseases, periodontitis associated with endodontic lesions and necrotizing ulcerative periodontitis. Of these, chronic periodontitis is the most frequently encountered in the adult population.

Epidemiologic studies have shown that 5% to 20% of the North American population suffers from a severe and generalized form of periodontitis³⁻⁷.

Distinguishing from preformed hydrogels, *in situ* forming gels are formulations, applied as a solution, which undergoes gelation after instillation due to physicochemical changes inherent to the biological fluids. In this way, the polymers which show sol-gel phase transition and thus trigger drug release in response to external stimuli are the most investigated. *In situ* hydrogels are providing such "sensor" properties and can undergo reversible sol-gel phase transitions upon changes in the environmental condition. These "intelligent" or "smart" polymers play important role in drug delivery since they may dictate not only where a drug is delivered, but also when and with which interval it is released. *In situ* gels are polymeric networks that absorb large quantities of water while remaining insoluble in aqueous solutions due to chemical or physical crosslinking of individual polymer chains.

They resemble natural living tissue more than any other class of synthetic biomaterials due to their high water content; furthermore, the high water content of the materials contributes to their biocompatibility. *In situ* gels show minimal tendency to adsorb proteins from body fluids because of their low interfacial tension. Further, the ability of molecules of different sizes to diffuse into (drug loading) and out of (drug release) *in situ* gels allow the possible use of dry or swollen polymeric networks as drug delivery systems for oral, nasal, buccal, rectal, vaginal, ocular and parenteral routes of administration. Preformed *in situ* gels can be defined as simple viscous



solutions which do not undergo any modifications after administration^{8,9}.

The main aim of research work is developed and evaluated of *in situ* gelling system for the treatment of Periodontitis for controlled drug delivery systems. The dose of tinidazole is 2%w/v. In *situ gel* formulations prepared by using poloxamer 407, sodium citrate, methyl paraben and propyl paraben by cold process method. Thus the study aims to improve patient compliance, increase bioavailability and sustained drug release¹⁰.

MATERIALS AND METHODS

Materials

Tinidazole was obtained as a gift samples from cadila pharmaceutical Pvt. Ltd. Ahmedabad. Poloxamer 407 was obtained as a gift sample from Corel pharmaceutical Pvt. Ltd. Ahmedabad. Methyl paraben, propyl paraben and sodium citrate were obtained from Seva fine chem. Ahmedabad. All other ingredients used were of analytical grade.

Methods

Preliminary Study¹¹⁻¹²

Compatibility Study

FT-IR study was carried out to identify the drug sample and to establish drug polymer compatibility.

Selection of Poloxamer 407 concentration

Solution of different concentration ranging from 8-20 % of poloxamer 407 was prepared by cold process. Required amount of polymer was accurately weighed and dispersed in distilled water with continuous mild stirring for 5 minutes. The beaker containing partially dissolved poloxamer 407 was sealed with aluminum foil and kept in refrigerator at 4°C until the entire polymer was completely dissolved (about 48 hrs.). For optimization of poloxamer 407 concentrations, different solutions of various concentrations were prepared and optimization was done on the basis of gelation temperature and gelation time.

Preparation of Temperature dependent gelling systems¹³

For the preparation of Poloxamer 407 containing *in situ* gel, it was first added to distilled water with continuous stirring and clear solution was obtained. Then Poloxamer 407 was sprinkled over this solution and allowed to hydrate overnight. Tinidazole was dissolved in required quantity of distilled water separately and then added to polymer solution under constant stirring until a uniform solution was obtained. Finally Sodium Citrate, Methyl paraben and Propyl paraben were added to the formulation under constant stirring until a uniform solution was obtained. Different concentrations of polymer were used to prepare *in situ* gel as per the composition shown in table. The prepared formulations were evaluated for mentioned parameters.

Table 1: Preliminary screening using Poloxamer 407 as a gelling polymer

Batch	Tinidazole (%w/v)	Poloxamer 407 (%w/v)	Sodium citrate (%w/v)	Methyl paraben (%w/v)	Propyl paraben (%w/v)	Distilled water
P1	1	8	0.1	0.15	0.02	Q.S
P2	1	10	0.1	0.15	0.02	Q.S
P3	1	12	0.1	0.15	0.02	Q.S
P4	1	14	0.1	0.15	0.02	Q.S
P5	1	16	0.1	0.15	0.02	Q.S
P6	1	18	0.1	0.15	0.02	Q.S
P7	1	20	0.1	0.15	0.02	Q.S

Formulation Optimization

To achieve the formulations with desired gelation temperature, viscosity, rheology, *in-vitro* drug release, and syringeability, the formulations prepared by using different concentrations of poloxamer 407 were evaluated.

In vitro 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 were determined by placing a drop of the system in a vial containing 2 ml of artificial GCF fluid freshly prepared and equilibrated at 37°C and visually assessing gel formation and noting the

time for gelation and the time taken for the gel formed to dissolve¹⁴.

pH

pH is one of the most important parameter involved in the periodontal formulation.

The two area of critical importance are the effect of pH on solubility and stability. The formulations were evaluated for pH by using digital pH meter.

Gelation Temperature

It was determined by using method described by Miller and Donovan technique.

A 2ml aliquot of gel was transferred to a test tube, immersed in a water bath.



The temperature of water bath was increased slowly and left to equilibrate for 5 min. at each new setting.

The sample was then examined for gelation.

Viscosity

The viscosity of all prepared formulations was measured using Digital Brookfield viscometer (DV-II+Pro, USA). The measurements were carried out using spindle no.62 at the speed of a 10 rpm in the sample.

Rheological Properties

The rheological properties of all prepared formulations were measured using a Brookfield viscometer DV-II+ Pro model viscometer using spindle no.62. The viscosity of the sample solutions was measured at different speeds at a temperature of $25 \pm 1^\circ\text{C}$. A typical run involved changing the speed from 10 to 100 rpm¹⁵.

Drug Content Uniformity

The container containing formulations were properly shaken for 2–3 min. One milliliter of the formulation was transferred into a 50 ml volumetric flask. Twenty five milliliters of GCF fluid was added. The formed gel was completely crushed with the help of a glass rod followed by vigorous shaking until the formed gel was completely dispersed to give a clear solution. Final volume was adjusted to 50 ml with GCF fluid. Obtained solution was filtered through Whatman filter paper. One milliliters of this solution was transferred to a 10 ml volumetric flask and volume was adjusted with GCF fluid and the drug concentration was determined at 316.5 nm by using UV-Visible Spectrophotometer-1800 (Shimadzu, Japan).

Syringeability

All 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 was termed as pass and difficult to pass were termed as fail.

In vitro Drug Release Studies^{16,17}

The *in vitro* release of Tinidazole from the formulations was studied through cellophane membrane using a modified USP II dissolution testing apparatus.

The dissolution medium was artificial GCF fluid (pH 6.8). Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 5 cm diameter).

A selected volume of the formulation was accurately pipette into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium maintained at $37 \pm 0.5^\circ\text{C}$ so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at required 50 rpm using magnetic stirrer. Aliquots, a sample was withdrawn at regular intervals and replaced by an equal volume of

the receptor medium. The aliquots were diluted with the receptor medium and were analyzed by UV-VIS spectrophotometer at 316.5 nm.

Sterility Testing¹⁸

The sterility test was performed according to Indian Pharmacopoeia. Direct inoculation method was preferred to use. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically transferred to fluid thioglycolate medium (20 ml) and soyabean casein digest medium (20 ml) separately. The liquid was mixed with the media. The inoculated media was incubated for not less than 14 days at 30°C to 35°C in the case of fluid thioglycolate medium and 20°C to 25°C in the case of soyabean-casein digest medium.

RESULTS AND DISCUSSION

Compatibility study

From the FT-IR spectra of tinidazole and mixture it, can be seen that there no change in significant peak of tinidazole in mixture indicating stability of drug in formulation mixture.

Results of selection of Poloxamer 407 concentration

Table 2: Gelation temperature of poloxamer 407 solutions

Poloxamer 407 concentration (%)	Gelation Temperature ($^\circ\text{C}$)
8	No gelation up to
10	40
12	38
14	38
16	37
18	34
20	28

For the selection of poloxamer 407 concentration various solutions of different concentration ranging from 8-20% was prepared and finalization of concentration was done on the basis of gelation temperature. Gelation temperature of solution of 10 to 18% was observed in the range of desired gelation temperature ($35\text{--}40^\circ\text{C}$) so this range was selected for further study.

pH

It was reported that the apparent viscosity of poloxamer 407 solutions can be markedly influence by the pH (13). Therefore, the pH of the formulation was adjusted and maintained between 5–6 with the help of a non-ionic alkalizing agent like triethanolamine if necessary. The pH of all prepared formulations was observed in the range of 5.6–6.2. Therefore, there was no need for pH adjustment by any external alkalizing agent.

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 GCF fluid due to temperature interaction shown in Table 3. To facilitate the sustained release of the drug to periodontal cavity, the formed gel should preserve its integrity without eroding or dissolving. Except the formulation P1 and P2 all the batches shows instantaneous gelation when come in contact with GCF fluid maintained at $37 \pm 1^\circ\text{C}$. However the nature of the gel formed depends upon the concentration of polymers. Formulation P1 and P2 shows weakest gelation and dispersed rapidly on moderate shaking, which may be due to presence of very low concentration of poloxamer 407. Formulation P3 and P4 shows immediate gelation but the formed gels are less stiff and does not remains for extended period of time. Formulation P5, P6 and P7 shows immediate gelation and formed gel was stiff and remained for extended period of time, this is due to the presence of higher concentration of poloxamer 407.

(+), gels after few minutes, dispersed rapidly;

(++), gelation immediate, remains for few hours; and

(+++), gelation immediate, remains for an extended period

Syringeability

The syringeability of each formulation is represented in Table 3. As the concentration of poloxamer 407 increases, the viscosity of formulations were increased and increase the force required to expel each formulation from the syringe equipped with 20 gauge needle. Formulation P6 and P7 fail the syringeability test because they contain higher amount of polymer concentration.

Drug Content

Drug content of all batches is shown in Table 3. This indicates the uniformity of drug content.

Table 3: Gelling capacity, drug content and syringeability of prepared formulations

Batch Code	Clarity	Gelling Capacity	Drug Content	Syringeability
P1	Clear	+	99.12	Pass
P2	Clear	+	100.20	Pass
P3	Clear	++	99.68	Pass
P4	Clear	++	101.12	Pass
P5	Clear	+++	99.45	Pass
P6	Clear	+++	101.65	Fail
P7	Clear	+++	98.78	Fail

Viscosity of all Prepared formulations

One important pre-requisite for a periodontal gel was viscosity of the formulation.

As indicated, a formulation suitable for application to the periodontal pocket should ideally have a low viscosity when applied, and after administration should have a high viscosity in order to stay at the application site.

All the formulations of batches P1–P7 showed a polymer concentration-dependent rise in viscosity shown in Table 4. **Rheological properties of all prepared formulations**

The flow curve (viscosity against speed) of all prepared formulations indicates that, at the examined polymer concentration pseudoplastic systems were obtained. The prepared formulations tend to thin when being exposed to shearing force. Viscosity of formulations increases with increased concentration of polymer.

Table 4: Rheological properties of prepared formulations

RPM	P1	P2	P3	P4	P5	P6	P7
5	378	467	523	598	653	793	834
10	323	434	490	498	585	677	756
20	267	367	412	409	502	592	670
50	212	288	345	356	464	512	557
100	190	221	290	290	396	466	445

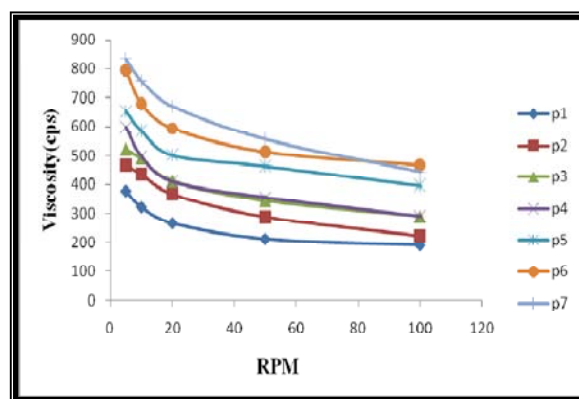


Figure 1: Rheological properties of prepared formulations

In vitro drug Release study

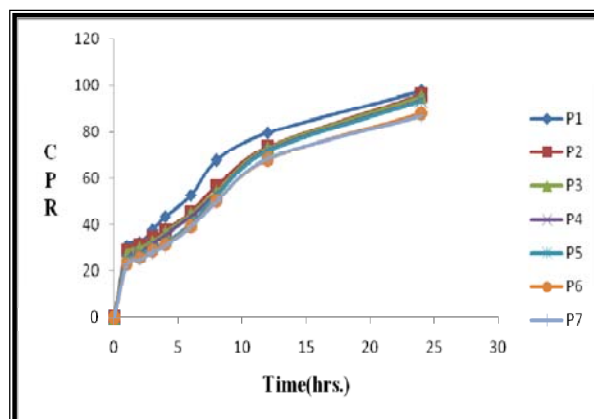


Figure 2: In vitro drug release profile of in situ gel formulation

The cumulative amount of tinidazole released vs. time profile for the selected formulations. First sampling was done 1 h after the formulation placed in test tube. 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 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 significantly as the concentration of polymer increased. In general there was a reduction in drug release as the concentration of polymers increases.

Sterility Testing



Figure 3: Soyabean casein medium



Figure 4: Fluid thioglycolate medium

Formulation P5 passed the sterility test as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for a period of not less than 7 days at 30-35°C in case of fluid thioglycolate medium and at 20-25°C in the case of soya bean casein digest medium.

CONCLUSION

In this study *in situ* periodontal gel of Tinidazole was successfully formulated using Poloxamer 407 (Temperature sensitive) approaches.

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 preparation of *in situ* periodontal gel formulation.

The developed formulations show satisfactory results for gelation temperature, pH, Viscosity, *in vitro* gelling capacity, Syringeability and other physical properties.

Based on maximum desirability the formulation containing 16% Poloxamer 407 was considered as an optimized formulation.

The developed formulation was therapeutically efficacious, stable and provided sustained release of the drug over extended period of time.

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