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Development and Evaluation of in Situ Gelling System for Treatment of Periodontitis

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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 gellan gum, poloxamer 407. 3^2full factorial design was applied for optimization. Selected dependent variables were concentration of gellan gum (X1) and poloxamer 407 (X2). Selected independent variables were viscosity (Y1) release at 1 hour (Y2), release at 8 hour (Y3). All the prepared formulations were evaluated for appearance, pH, viscosity, % drug content, syringeability, % drug release and effect of sterilization. By compatibility study drug was found to be compatible with formulation excipients. Gelation temperature and pH of all formulation found to be in the range of 29-40ºC and 5.34-6.83 respectively. Viscosity of all prepared formulations was found in the range of 310-692 centipoise. Both the independent variable had the significant effect on the entire three response variable ($P< 0.05$). All the formulations were developed using combination of gellan gum and poloxamer 407. The developed formulations showed satisfactory results for in-vitro gelling capacity, rheology and other physical properties. Based on maximum desirability and cost effectiveness formulation containing 0.5%w/v of gellan gum and 15 %w/v of poloxamer 407 was consider as an optimized batch.
Keywords*:* *In situ* gel; Periodontitis; Gellan gum; Poloxamer 407; Ion sensitive; Thermo-sensitive.

Introduction:

Periodontitis is an inflammatory response to the overgrowth of anaerobic organisms such as spirochetes and bactericides and in some cases, micro aerophillic organisms in the subgingival plaque. Periodontal disease, if unchecked result in the destruction of the bone and soft tissue supporting the tooth which causes tooth loss. The clinical sign of Periodontitis is changes in morphology of gingival tissues, gingival bleeding as well as periodontal pocket formation. This pocket provides an ideal environment for the growth and proliferation of anaerobic pathogenic bacteria[1-3].

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 cross linking 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 [4-7].

Figure 1 Healthy and Periodontal Disease

A polymer used to prepare *in situ* gels should have following characteristics:

- It should be biocompatible.
- It should be capable of adherence to mucus.
- It should have pseudo plastic behavior.
- It should have good tolerance and optical clarity.
- It should influence the tear behavior.

It should be capable of decreasing the viscosity with increasing shear rate there by offering lowered viscosity during blinking and stability of the tear film during fixation.

Tinidazole may be related to action of free nitro radical generated as a result of reduction by cell extracts. Tinidazole also causes DNA base changes in bacterial cells and DNA strand breakage in mammalian cells. Tinidazole is an antiprotozoal, antibacterial agent. The nitro- group of tinidazole is reduced by cell extracts of Trichomonas. The free nitro- radical generated as a result of this reduction may be responsible for the antiprotozoal activity. Chemically reduced tinidazole was shown to release nitrites and cause damage to purified bacterial DNA in vitro. Additionally, the drug caused DNA base changes in bacterial cells and DNA strand breakage in mammalian cells. The mechanism by which tinidazole exhibits activity against Giardia and Entamoeba species is not known[8].

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 2oomg. In situ gel formulations prepared by using carbopol 934, sodium citrate, methyl paraben and propyl paraben by cold process method. Thus the study aims to improve patient compliance, increase bioavilibility and sustained drug release[9].

Experimental

Materials

Tinidazole was obtained as a gift samples from cadila pharmaceutical Pvt. Ltd. Ahmedabad. Gellan gum and poloxamer 407 were 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

Preformulation study [10-12]

Preformulation testing is the first step in rational development of dosage forms of a drug substance. Preformulation study is the process of optimizing the delivery of drug through determination of physicochemical properties of the new compound that could affect drug performance and development of an efficacious, stable and safe dosage form. It gives the information needed to define the nature of the drug substance and provide a frame work for the drug combination with pharmaceutical excipients in the dosage form. Hence, Preformulation studies were performed for the obtained sample of drug for identification and compatibility studies.

1. Determination of melting point

Melting point of drug was determined by capillary method & compare with the reported value.

2. Drug-interaction study

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) technique has been used to study the physical and chemical interaction between drug and excipients. Firstly DSC of a drug is taken and after a physical mixture of Tinidazole: Gellan gum: Poloxamer 407 was performed using a Shimadzu DSC-60. Samples were taken, sealed in aluminum pans, and analyzed in an atmosphere of air at flow rate of 25 mL/min. A temperature range of 50°C to 200°C is to be used, and the heating rate is 20°C/min.

3. Spectrophotometric Estimation of Tinidazole

Determination of λmax of Tinidazole

Tinidazole solution was prepared in phosphate buffer pH 6.8 and then dilute suitably. The UV spectrums of the solutions were taken on Shimanzu UV Spectrophotometer (Japan). The solutions exhibited UV maxima at 318.0nm in phosphate buffer pH 6.8.

Preparation of Standard curve of Tinidazole (UV)

Accurately weighed 100mg of tinidazole was transferred to a 100ml volumetric flask and dissolved in 100ml phosphate buffer pH 6.8 to prepared stock solution (1000µg/ml). Then 10ml of above solution was taken and diluted it with 100ml phosphate buffer pH 6.8 in 100ml volumetric flask to prepare the solution (100µg/ml). Then 10ml of above solution was taken and diluted it with 100ml phosphate buffer pH 6.8 in 100ml volumetric flask to prepare the solution (10µg/ml). Aliquots of working solution of tinidazole (4-24µg/ml) were transferred in to a series of 10 ml volumetric flask and volume was making up to the mark with phosphate buffer pH 6.8. Absorbance of the resulting solutions was measured at 318.0 nm against a reagent blank solution prepared similarly without drug using Shimanzu UV Spectrophotometer (Japan). Calibration curve was prepared by plotting concentration versus absorbance graph.

Preliminary study for selection of polymer

This study was done to get idea about the appearance, gelling capacity, pH, viscosity, gelation temperature, drug content uniformility, syringe ability and in vitro release study of the drug formulation. This study was aimed to select proper concentration of Gellan gum and poloxamer 407 were used as gelling agent.

Preparation of *In situ* gel formulations [13-15]

For the preparation of *in situ* gel formulations, Gellan gum was first added to distilled water with continuous stirring. Gellan gum was dissolved by warming the solution at 80 ºC for 15 min with continue stirring. poloxamer 407 was slowly added to cold water separately with continuous stirring and stirred until it completely mixed. The partially dissolved Poloxamer 407 solutions were stored in a refrigerator and stirred periodically until clear homogenous solutions were obtained (approximately 24 hrs).Then gellan gum formulations and poloxamer 407 formulations were mixed uniformly by stirring. Tinidazole was dissolved in required quantity of distilled water separately and then it was added to polymer solutions under constant stirring until a uniform solution was obtained. Finally Sodium citrate, Propyl paraben and Methyl 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 1).**

Table -1: Composition Of Drug And Excipients In Factorial Batches Of Periodontal *In Situ* Gel Of Tinidazole

Ingredients (%w/v)	Formulations								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Tinidazole	2	2	2	2	2	2	2	2	2
Gellan gum	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Poloxamer 407	10	10	10	15	15	15	20	20	20
Sodium Citrate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Methyl paraben	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Table - 2: Selection Of Levels For Independent Variables

Level	Variable	X_1 (Concentration of Gellan gum) %w/v	X_2 (Concentration of Poloxamer 407) %w/v
Low	-1	0.5	10
Medium	0	1.0	15
High	+1	1.5	20

Experimental design

A 3^2 randomized full factorial design was employed in the present study. In this design 2 factors were evaluated, each at 3 levels, and experimental trials were performed for all 9 possible combinations. The Concentration of Gellan gum (X_1) and concentration of Poloxamer 407 (X_2) were chosen as independent variables and viscosity, % cumulative drug release at 1 h. (Q_1) and % cumulative drug release at 8 h. (Q_8) were taken as dependent variables Shown in **(Table 2 and 3).**

Table - 3: Formulation Layout For 3^2 Factorial Batches

Batches	Coded value		Actual value	
	X_1	X_2	X_1 (Concentration of Gellan gum)	X_2 (Concentration of Poloxamer 407)
F1	-1	-1	0.5	10
F2	0	-1	1	10
F3	+1	-1	1.5	10
F4	-1	0	0.5	15
F5	0	0	1	15
F6	+1	0	1.5	15
F7	-1	+1	0.5	20
F8	0	+1	1	20
F9	+1	+1	0.5	20

Characterization of *in situ* gel [16,17]

1. Appearance

All developed formulations were evaluated from the visual inspection.

2. 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 phosphate buffer pH 6.8 freshly prepared and equilibrated at 37^{o}C and visually assessing gel formation and noting the time for gelation and the time taken for the gel formed to dissolve.

3. 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.

4. 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.02 at the speed of a 10 rpm in the sample.

5. Gelation temperature

Ten milliliters of the sample solution and a magnetic bead 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 at the rate of 1°C/min with the continuous stirring at lower rpm. The temperature was determined as gelation temperature.

6. Drug content uniformity

Accurately weighed amount gel equivalent to 2mg of drug was taken in a 100ml volumetric flask. Phosphate buffer (pH 6.8) was added to it and kept on magnetic stirrer to dissolve the drug. The volume was made to 100ml with Phosphate buffer (pH 6.8) and filtered using 0.45µm filter paper. 10ml aliquot of the above solution will be taken and diluted to 100ml with Phosphate buffer (pH 6.8). The absorbance of sample solution was determined at 318 nm against Phosphate buffer (pH 6.8) by using UV-Visible Spectrophotometer-1800 (Shimadzu, Japan).

7. Syringeability

All prepared formulations were transferred into an identical 5 ml 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.

8. In vitro Drug Release Studies

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 phosphate buffer (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± 0.5°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.

9. Sterility Testing

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 soyabeancasein 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.

Result and Discussion:

Preformulation Study

1. Determination of melting point

The melting point of Tinidazole was found to be 124-128 ºC which was similar with the reported value Shown in **(Table 4).**

Table - 4: Melting Point Of Tinidazole

Drug	Practically obtained	Theoretical range
Tinidazole	124-128 ºC	124-130ºC

2. Drug- interaction study

Differential Scanning Calorimetry (DSC)

The DSC results provided both qualitative and quantitative information about the physicochemical state of the drug present in formulation. The thermograph of pure Tinidazole showed a melting endothermic peak at 128^0C while in the thermograph of mixture peaks it was observed at 128^0C. The DSC thermo grams of the mixture showed distinct endothermic peaks for Tinidazole and the polymer. This corresponds to the peaks of individual drug and polymer without exhibiting any modification which indicates that the drug did not interact with excipients used in the tablets. This confirmed that the presence of other excipients did not affect the drug stability.

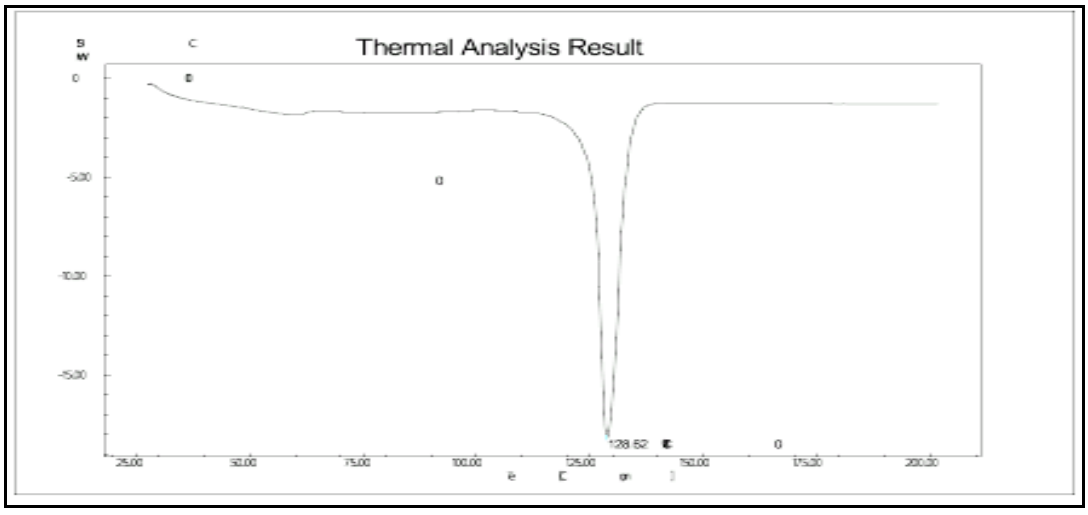

Figure 2 Dsc Of Tinidazole

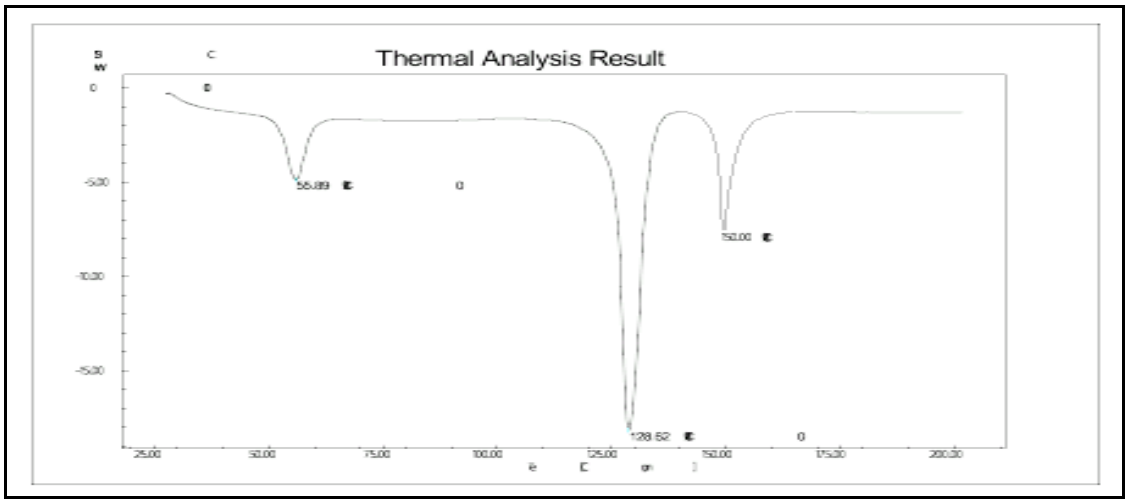

Figure 3 Dsc Of Tinidazole With Polymer Mixture

3. Spectrophotometric Estimation of Tinidazole

Determination of λmax of Tinidazole

The wavelength maximum for Tinidazole was found to be 318.0 nm (shown in figure: 4).

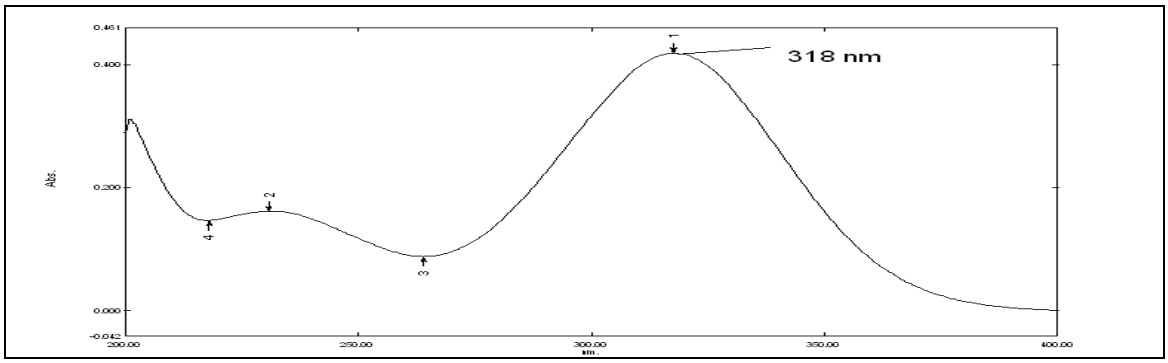

Figure 4 λmax OF TINIDAZOLE

Preparation of Standard curve of Tinidazole (UV)

The linear regression data obtained for the calibration curve showed a good linear relationship over the concentration range 4-24 µg/ml with respect to Absorbance shown in **(Table 5).**

Table - 5: Standard Plot Of Tinidazole In Phosphate Buffer (Ph 6.8) At λmax 318nm

Concentration(µg/ml)	Absorbance
0	0.000
4	0.121
8	0.248
12	0.362
16	0.485
20	0.589
24	0.743

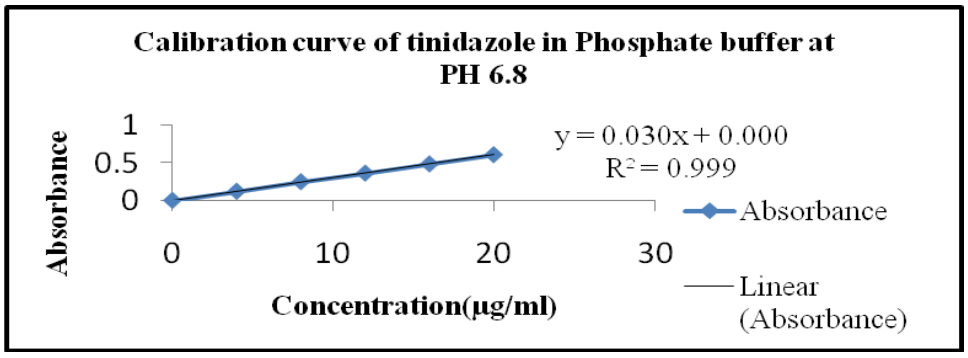

Figure 5 Regressed Calibration Curve For Tinidazole, Ph 6.8

Characterization of 3^2 full factorial batches

In the present study, 3^2 factorial design was applied in which Gellan gum and Poloxamer 407 polymer in combination were fixed from the preliminary trials. In this design two factors were evaluated, each at three levels, and experimental trials were carried out at all nine possible combinations. The factors were selected based on preliminary study. The concentration of Gellan gum (X_1) and concentration of Poloxamer 407 (X_2) were selected as independent variables. Viscosity (cps), Drug release at 1 h. (Q_1) (%) and Drug release at 8 h. (Q_8) (%) were selected as dependent variables. The evaluation of factorial batches (F1 to F9) has been shown in **(Table 6).**

Table - 6: Evaluation Of Factorial Batches F1-F9

Batch	Clarity	Gelling capacity	pH	Gelation temperature (^{o}c)	Viscosity (in cps)	Drug Content (%)	Syringeability
F1	Clear	+	6.37	37	503.4 ± 4.3	97.25 ±0.12	Pass
F2	Clear	++	6.21	32	710.2 ± 3.6	94.83± 0.23	Pass
F3	Clear	++	6.08	31	1045.9 ±5.3	96.68± 0.15	Fail
F4	Clear	+++	6.28	33	607.3 ±6.6	95.82±0.26	Pass
F5	Clear	+++	6.12	29	753.0 ±5.4	97.08±0.26	Pass
F6	Clear	+++	6.02	31	1093.2± 3.8	97.30± 0.31	Fail
F7	Clear	++	5.92	36	670 ± 2.9	97.93± 0.31	Pass
F8	Clear	+++	5.72	33	845 ± 3.8	95.73± 0.14	Pass
F9	Clear	+++	5.61	28	1070± 4.6	96.24± 0.24	Fail

1. Appearance

All the formulations were checked for the appearance. From batch F1 to F9, all other batches were found to be clear

2. Gelling Capacity

The gelling capacity o all the formulations were shown in **(Table 6)**. The gelling capacity of all formulation was found to be satisfactory.

3. pH

The formulations were evaluated for pH as per described in material and methods. pH of all the formulations was shown in **(Table 6)**. The pH of the formulations was in the range of 5.34 - 6.83 which was satisfactory.

4. Gelation temperature

It was determined as described in material and methods. Gelation temperature of all the formulations was shown in **(Table 6)**. The satisfactory results were found which is quite beneficial for gelation.

5. Viscosity

Viscosity of all formulations was measured using Brookfield digital Viscometer DV-II+ Pro viscometer. The gel under study was placed in the spindle S61 at 50 RPM for liquid formulations and gels. Viscosity of batch E1 to E9 was 503.4 ± 4.3, 710.2 ± 3.6, 1045.9 ± 5.3, 607.3 ± 6.6, 753.0 ± 5.4, 1093.2 ± 3.8, 659.2 ± 2.9, 875 ± 3.8, 1065.2 ± 4.6 respectively. Highest viscosity was found in batch F9. Figure 6 shows that increase in concentration of polymer causes increase in viscosity of the formulation.

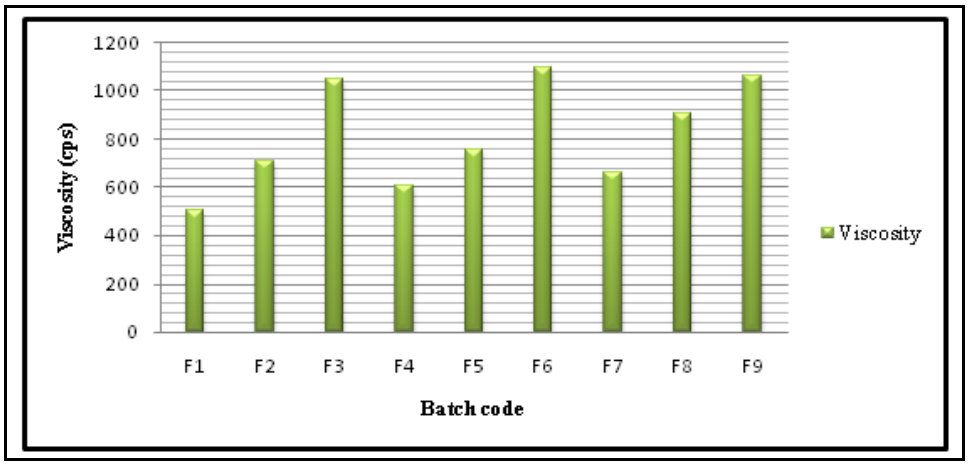

Figure 6 Viscosity Of All Formulations

6. Drug content

Drug content of all factorial batches were found to be 97.25 ±0.12, 94.83 ± 0.23, 96.68 ± 0.15, 95.82± 0.26, 97.08± 0.26, 97.30 ± 0.31, 97.93 ± 0.31, 95.73 ± 0.14 and 96.24 ± 0.24 for batch F1 to F9 respectively. This indicates the uniformity of drug content.

7. Syringeability

Syringe ability of the formulations was determined as per material and methods. Syringe ability of all the formulations was shown in **(Table 6)**. In that F3, F6 and F9 batches are fail and all other batches were passed the syringe ability.

8. *In vitro* drug release studies

The in vitro release of Tinidazole from the formulations was studied and evaluated. Shown in **(Table 7).**

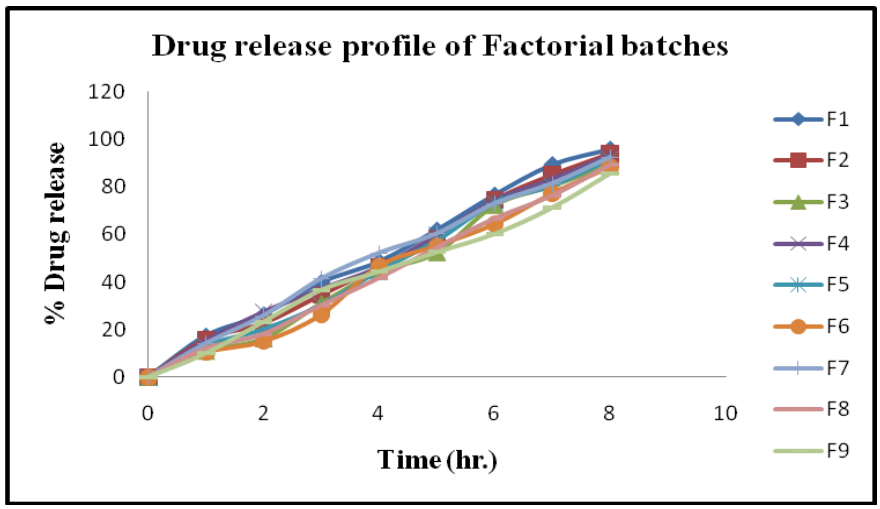

Figure 7 % Drug Release Profile Of Factorial Batches Of Periodontal In-Situ Gel

The release profile of a drug predicts how a delivery system might function and gives valuable insight into its in vivo behavior. The in vitro release of Tinidazole from the formulations was studied as described in the material and methods. In vitro release profile of the selected formulations was shown in **(Table 7)**. The in vitro release profile represented that the *in situ* gelling formulation could provide sustained and controlled release of drug. The comparison of release profile of factorial batches shows there were significant effect of both the factors concentration of Gellan gum and Poloxamer 407. The release pattern also showed that the concentration of Gellan gum is more significant than the concentration of Poloxamer 407 on the variable % drug release. So the controlled release of Tinidazole is more significant on the concentration of Gellan gum.

Table - 7: % Drug Release Of Factorial Batches Of Periodontal In-Situ Gel

Batch code --> / Time (hour)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	17.37	15.84	11.02	15.31	13.21	10.41	14.22	12.07	10.02
2	26.31	22.69	16.22	27.44	20.14	15.07	25.88	18.22	23.21
3	39.86	34.52	31.04	37.17	30.24	26.31	41.35	30.2	36.57
4	48.52	46.36	44.34	46.21	44.61	46.69	52.14	41.64	44.2
5	61.78	57.77	52.35	59.67	57.34	55.28	60.27	54.44	52.39
6	76.47	74.32	72.21	74.37	73.22	64.31	72.89	66.54	60.04
7	89.17	84.96	80.39	83.28	80.42	77.21	81.29	76.25	71.02
8	95.64	93.83	89.31	93.48	91.47	88.35	92.27	89.07	85.31

9. Sterility testing

As Per the material and methods sterility testing was determined. Formulation F4 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.

Kinetic Modeling of Dissolution Data

Based on the r^2-value, the best-fit model was selected. The n value was 0.5-1 so the drug transport mechanism was non Fickian diffusion. The highest R^2 value was obtained in the Higuchi model so the release kinetics followed by higuchi model as best fit model shown in **(Table 8).**

Table - 8: Kinetic Modeling Of Drug Release Data

Batch no.	Higuchi	Zero order	First order	K-Peppas
	r^2	r^2	r^2	r^2
F4	0.994	0.963	0.9504	0.9818

Discussion:

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. The excipients used in this present work are Gellan gum and Poloxamer 407 as gelling agent which form immediate gel. The Methyl paraben and Propyl paraben is used as preservatives. Preformulation studies were carried out in order to establish the compatibility between the drug and polymers by DSC. The studies revealed that drug and polymers were compatible. From the results, two factors were selected i.e. concentration of Gellan gum (X1) and concentration of Poloxamer 407 (X2) as independent variables for 3^2 full factorial design. Dependent variables selected were Viscosity (CPS), Drug release after 1hr (%) and Drug release after 8hrs (%). The prepared formulations were evaluated for different parameters like pH, appearance, drug content, *in vitro* gelation study, viscosity, *in vitro* release study, and sterility testing. From the *in vitro* study, it was found that the developed formulation was provided sustained release of the drug over 8 hrs. On the bases of Desirability

approach, formulation containing Gellan gum and Poloxamer 407 in concentration of 0.5 and 15% respectively was selected as optimized batch. The release profile of the formulation follows Higuchi order model (r^2=0.994) and release mechanism was non-Fickian diffusion. 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 shows satisfactory results for gelation time, gelation temperature, Viscosity, Syringeability and other physical properties. Based on maximum desirability the formulation containing 0.5%w/v of Gellan gum and 15%w/v of Poloxamer 407 was consider 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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