FORMULATION DEVELOPMENT AND EVALUATION OF DOXYCYCLINE INSITU-GEL FOR PERIODONTAL DRUG DELIVERY SYSTEMS

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ABSTRACT

By concentrating on the limitation of oral mucosal drug delivery system we have planned to overcome the problems and try to make the formulation more effective and efficient. Recently, in situ-gelling liquids have been investigated as a more convenient dosage form of topical applications.

Doxycycline is a semi-synthetic bacteriostatic tetracycline and a broad-spectrum antibiotic against Gram-negative and Gram-positive aerobic and anaerobic bacteria. Pharmacokinetics properties of doxycycline is superior than older tetracycline; in terms of higher lipid solubility, better tissue distribution, longer elimination half-life.

we have developed doxycycline with the optimum concentrations of xanthan/Pluronic solutions for use as an in-situ gelling vehicle for delivery of drug in the treatment of periodontitis which were 2.5% (w/w) and 15% (w/w). The procedure was adopted to prepare the solutions for characterization under physiological conditions (Phosphate buffer pH 6.6 and 37 °C). The absorbance of the solution measured at the wavelength of 274.5 nm.

Drug release profiles of doxycycline copolymer gels and Pluronic gels. All of which contained 4% (w/w) doxycycline. The Pluronic gel exhibited almost complete release of doxycycline within 6 hrs. Whereas the copolymer gels exhibited prolonged drug release up to 48 hrs. The release rate in 2.5 % xanthan/14% Pluronic solution was significantly lower. Only about 10% released after 1 hr., 41% after 6 hrs., 75% after 24 hrs., and about 98% after 48 hrs.

Analysis of the in vitro data reveals that delivery of the doxycycline in the form of a thermoreversible gel was clinically effective along with scaling and root planning.

Key words: Doxycycline, Poloxamer- 407, Xanthan gum, Pluronic gel, FTIR, DSC.

1) INTRODUCTION

The buccal portion of the mouth cavity is a desirable target for medication delivery. The administration of the desired medication via the buccal mucosal membrane lining of the mouth cavity is known as buccal delivery. The mucosal lining of buccal tissues offers a far more hospitable environment for drug absorption than oral drug delivery, which presents a hostile environment for medicines, notably proteins and polypeptides, due to acid hydrolysis and the hepatic "first-pass" effect. Other drug delivery methods, such as vaginal, nasal, ophthalmic, pulmonary, and rectal methods, have made it possible to distribute a wide range of substances.

Recently, in situ-gelling liquids have been investigated as a more convenient dosage form of topical applications. The liquids applied to the topical areas such as eyes can make transition to gels as a result of a chemical/physical change induced by the physiological environments.. There are several ways to sustain the release of a drug from gels in order to take full advantage of the contact time. The drug can be dispersed in the gel, giving a concentration that is higher than that corresponding to the solubility of the drug.

Doxycycline is a semi-synthetic bacteriostatic tetracycline and a broad-spectrum antibiotic against Gram-negative and Gram-positive aerobic and anaerobic bacteria, Rickettsiae, Chlamydiae, Mycoplasmas and some protozoa. Pharmacokinetics properties of doxycycline is superior than older tetracycline; in terms of higher lipid solubility, complete absorption, better tissue distribution, longer elimination half-life and lower affinity for calcium. The *In vitro* antimicrobial activity of doxycycline is more effective than other tetracycline for the treatment for respiratory, urinary and gastrointestinal tract diseases.

The present swot focuses on the development of insitu gel for periodontal drug delivery using temperature sensitive polymer and co-polymer to release the drug for a desired period of time.

2) MATERIALS AND METHODS

MATERIALS USED:

Doxycycline hyclate is the pure drug procured from Maan Pharmaceutical Ltd., Mehsana., India as gift sample. Poloxamer-407 procured from Signet chemicals, Mumbai, India. Xanthan Gum from Wellable pharmaceuticals. Pvt. Ltd., Mehsana, India. Cassia-tora gum powder is bought from Vinayaka Gum Industries, Ahmedabad, India.

INSTRUMENTS USED:

UV/Vis Double Beam Spectrophotometer, Electronic Weighing Balance, Dissolution Test Apparatus, pH Meter, Differential Scanning Calorimeter (DSC), Fourier Transform Infrared Spectroscopy (FTIR), High Performance Liquid Chromatography (HPLC), Scanning Electron Microscope (SEM).

EXPERIMENTAL METHODS:

Infrared (IR) Spectroscopic Analysis

Fourier–transform infrared (FT–IR) spectra of moisture free powdered samples were obtained using a spectrophotometer (FTIR-8300, Shimadzu Co., Japan) by potassium bromide (KBr) pellet method (app. 5 mg sample in 200 mg KBr). The scanning range was 400–4000 cm^{-1} and the resolution was 1 cm^{-1}.

Differential Scanning Calorimetry (DSC) Analysis

DSC scans of the powdered samples were recorded using DSC- Shimadzu 60 with TDA trend line software. All samples were weighed (8-10 mg) and heated at a scanning rate of 10°C/min under dry nitrogen flow (100 ml/min) between 50 and 300° C. Aluminum pans and lids were used for all samples. Pure water and indium were used to calibrate the DSC temperature scale and enthalpic response.

Preparation of calibration curve

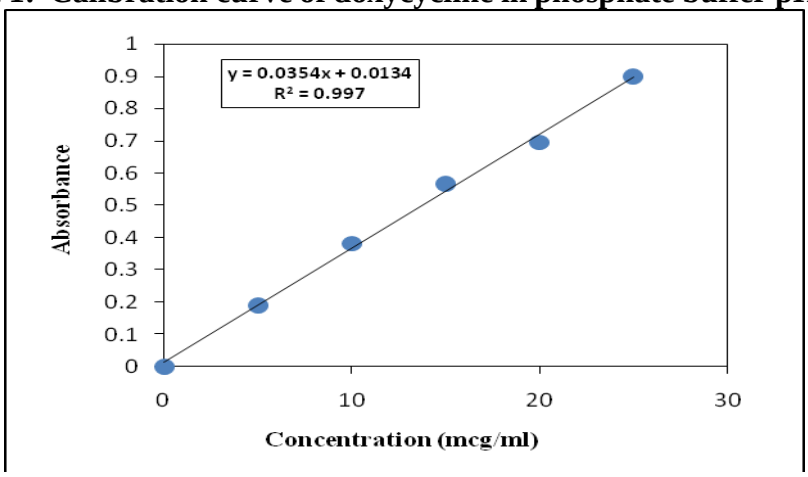
The calibration curve of doxycycline in Phosphate buffer pH 6.6 was prepared by measuring the absorbance of the solution in the rage of 5-25 g/ml. The absorbance of the solution measured at the wavelength of 274.5 nm.

Doxycycline (10mg) was dissolved in 10 ml of Phosphate buffer and volume was made up to 100 ml in volumetric flask. This stock solution (0.1 mg/ml) was further diluted with phosphate buffer pH 6.6 to obtained solution of 5-25 µg/ml. Absorbance of each solution was measured at 274.5 nm using Shimazdu-1700-UV/Vis spectrophotometer with phosphate buffer pH 6.6 as a reference standard. The standard curve was generated for entire range of 5-25 µg/ml. The experiment was performed in triplicate and based on average absorbance; the equation for the best line fit was generated.

Table 1: Calibration curve of doxycycline in phosphate buffer pH 6.6

Concentration(g/ml)	Absorbance
0	0.000
5	0.194 (0.002)
10	0.391 (0.005)
15	0.571 (0.006)
20	0.682 (0.004)
25	0.851 (0.008)
Correlation coefficient = 0.9953 Absorbance = 0.035 × concentration + 0.015	
Values in parenthesis indicates standard deviation (n = 3)	

Figure 1: Calibration curve of doxycycline in phosphate buffer pH 6.6



Preparation of insitu gel containing Poloxamer- 407 with natural polymers

The Poloxamer- 407 solutions were prepared by dispersing the required amount of Pluronic in the desired concentration of alginate solution; the resulting combination was mixed with a homogenizer at 12022 rpm for 3 min.Same processes were adopted for the xanthan gum and cassia tora gum containing formulation. The partially dissolved solutions were then refrigerated until thoroughly mixed (approximately 24 h). All the previous sample solutions were adjusted to pH 4.0 (0.1 by 0.5 M hydrochloric acid solution and then stored in the refrigerator before evaluation of their properties under non-physiological conditions (pH 4.0 and 25 °C).

Table 2: Formulation of insitu gel containing Poloxamer-407 with naturalpolymers

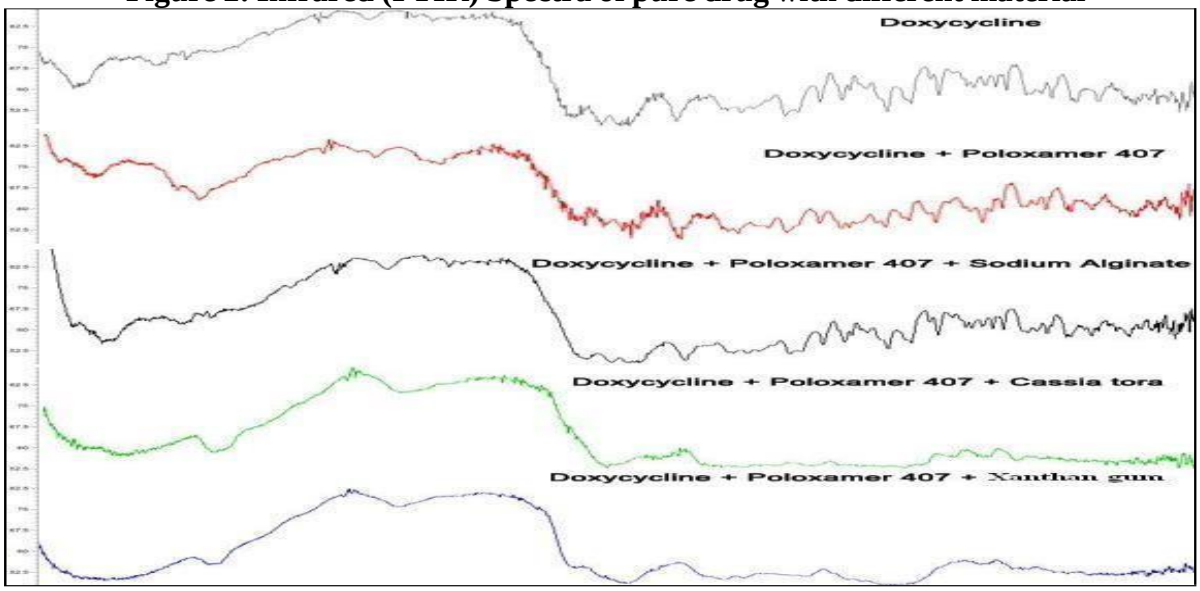
Ingredients(mg)	$F4_1$	$F4_2$	$F4_3$	$F4_4$	$F4_5$	$F4_6$	$F4_7$	$F4_8$	$F4_9$
Drug	20	20	20	20	20	20	20	20	20
Poloxamer-	75	75	75	75	75	75	75	75	75
Sod. alginate	0.5	1	2.5	-	-	-	-	-	-
Xanthan gum	-	-	-	0.5	1	2.5	-	-	-
Cassia-tora	-	-	-	-	-	-	0.5	1	2.5
Water q.s.	500	500	500	500	500	500	500	500	500

The same procedure was adopted to prepare the solutions for characterization under physiological conditions (Phosphate buffer pH 6.6 and 37 °C). To prepare the doxycycline-containing polymer solutions, the desired amounts of doxycycline were added to the alginate, xanthan and cassia-tora /Pluronic solutions with continuous stirring until thoroughly mixed.

3) RESULTS AND DISCUSSION

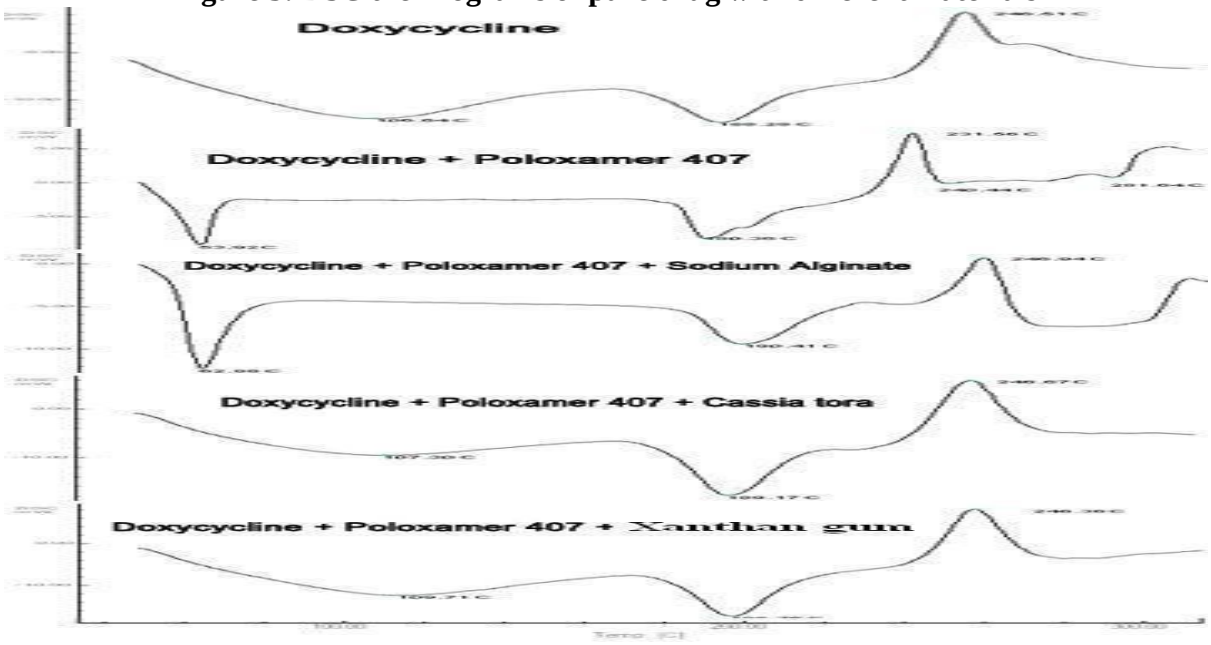
Infrared (IR) Spectroscopic Analysis

Figure 2: Infrared (FTIR) Spectra of pure drug with different material



Differential Scanning Calorimetry (DSC) Analysis

Figure 3: DSC thermograms of pure drug with different materials



Determination of Flow Behavior of Vehicles

Table 3: Flow Behavior of pluronic solution at different concentration

Pluronic concentration (%w/w)	20°C	37°C
10	+	+
11	+	+
12	+	+
13	+	+
14	+	+
15	+	++++
16	++	++++

+Liquid, very easy to flow; ++ liquid-gel like, flow less readily.
+++ gel, difficult to flow; ++++ strong gel, cannot flow.

Determination of Flow Behavior of Vehicles

Table 4: Flow Behaviors of 15% pluronic solution at different co-polymers concentration

Different combination (% w/w)		20 °C	37 °C
Sodium alginate + 15 % Pluronic Gel	0.1%	+	++++ +++
	0.2%	+	++++
	0.5%	++	++++
	0.8%	++	++++
	1.0%	+++	++++
Xanthan gum + 15% Pluronic Gel	0.1%	+	++++ +++
	0.2%	+	++++
	0.5%	++	++++
	0.8%	+++	++++
	1.0%	+++	++++
Cassia-tora gum + 15 % Pluronic Gel	0.1%	++	++++++ +
	0.2%	+++	++++
	0.5%	+++	++++
	0.8%	+++	++++
	1.0%	+++	++++

+ Liquid, very easy to flow ; ++ liquid-gel like, flow less readily; +++ gel, difficult to flow; ++++ strong gel, cannot flow

Invitro Release Studies

Table 5: Invitro drug release profile with different concentration of co- polymers

Time	Invitro drug release profile of prepared gel formulation									
	F4	$F4_1$	$F4_2$	$F4_3$	$F4_4$	$F4_5$	$F4_6$	$F4_7$	$F4_8$	$F4_9$
0	0	0	0	0	0	0	0	0	0	0
1	24.23	17.65	14.25	14.01	13.71	12.02	10.34	15.11	12.79	11.9
2	38.03	26.34	22.09	19.98	22.63	20.13	18.23	23.87	20.74	18.76
3	52.17	41.21	36.12	32.15	30.04	28.24	24.1	36.98	31.24	27.91
4	68.36	53.89	46.74	41.1	34.20	33.82	30.28	42.35	39.04	36.16
5	84.27	67.19	58.12	52.36	40.16	38.48	33.61	51.67	46.12	41.13
6	98.47	81.18	72.81	59.87	46.24	44.62	40.61	69.36	54.08	52.76
12	98.21	96.13	88.98	71.41	54.74	52.17	46.11	84.76	73.2	58.11
18		98.12	96.12	84.29	71.32	68.92	62.23	91.27	79.35	69.99
24		98.15	96.40	91.72	84.81	81.15	75.29	98.27	88.13	80.12
30				98.13	99.67	94.43	83.21	98.22	98.24	88.91
36				98.10	99.50	99.12	89.07		98.14	97.69
48						99.02	98.61			97.45

Effect of different concentrations of doxycycline

Table 6: Invitro drug release profile with different concentration of drug

Time (Hrs)	Different concentration of doxycycline		
	4%	6%	8%
0	0	0	0
2	10.34	12.9	14.01
4	18.23	19.76	21.98
6	24.1	29.91	36.15
8	30.28	38.16	46.1
10	33.61	43.13	57.36
12	40.61	55.76	64.87
18	46.11	63.11	76.41
24	62.23	76.99	89.29
36	75.29	85.12	94.72
48	82.21	91.01	99.13

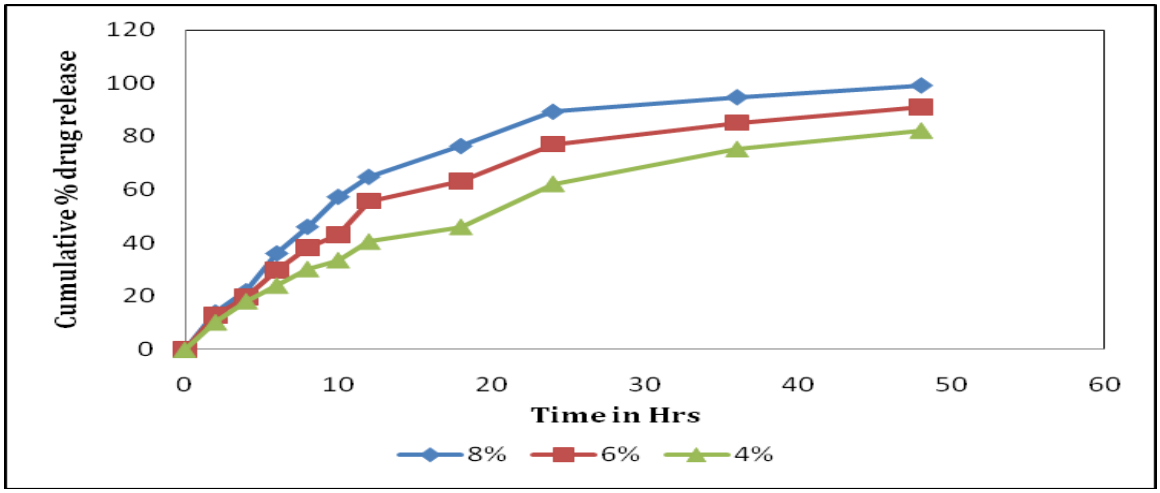
Viscosity Measurements

Table 7: Viscosity as a function of temperature with different concentration of copolymers

Formulation code	Different temperature interval							
	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	37 °C
F4	0.148	0.155	0.155	0.495	0.597	0.777	0.935	1.16
$F4_1$	0.155	0.16	0.163	0.514	1.028	1.837	2.916	2.987
$F4_2$	0.18	0.187	0.188	0.593	1.174	2.046	3.173	3.389
$F4_3$	0.19	0.195	0.197	0.965	1.568	2.434	3.134	3.18
$F4_4$	0.184	0.189	0.189	0.555	1.314	2.126	3.157	3.235
$F4_5$	0.202	0.206	0.207	0.682	1.235	2.383	3.262	3.364
$F4_6$	0.211	0.214	0.214	1.347	2.057	2.764	3.335	3.458
$F4_7$	0.212	0.217	0.217	0.592	1.076	1.757	3.248	3.382
$F4_8$	0.23	0.235	0.235	0.938	1.257	2.321	3.462	3.539
$F4_9$	0.261	0.25	0.267	1.546	2.283	2.997	3.629	3.692

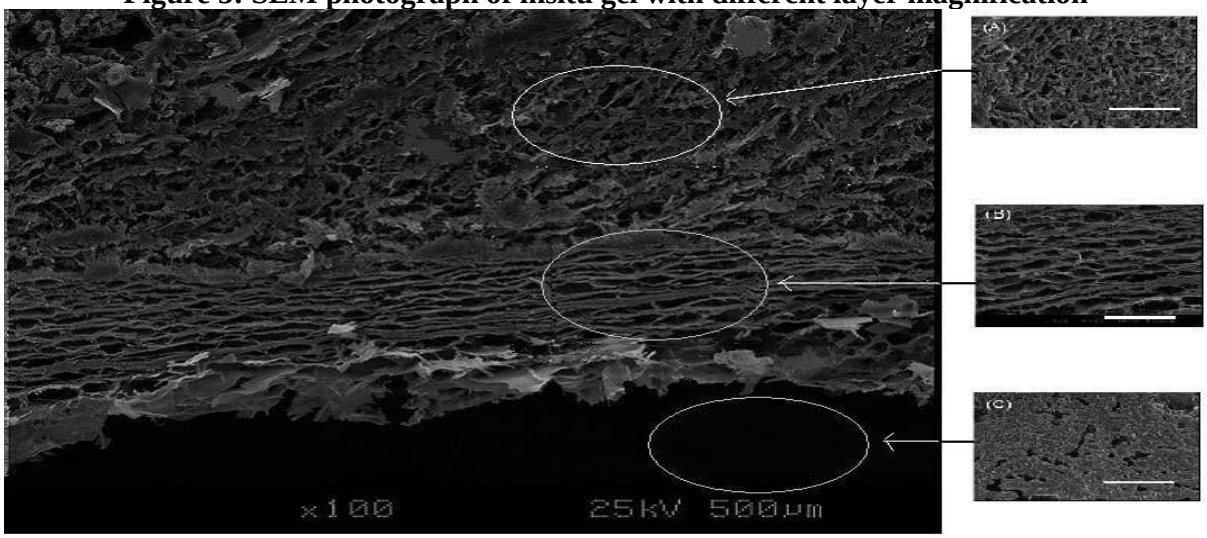
Viscosity was measured in $\eta = 10_3$ cps

Figure 4: Amount of doxycycline released from gel containing different concentrations of doxycycline



SEM Measurements of in Vivo Gel State

Figure 5: SEM photograph of insitu gel with different layer magnification



In vitro antimicrobial efficacy

Table 8: Zone of inhibition produced by the optimized formulation F4

Micro organism	Area of zone of inhibition (mm^2) after 48 hrs. of incubation		
	Formulation $F4_6$	Marketed gel (Atridox^R)	Control
S. Aureus	595±2.0	590±2.0	585±3.0
Porphyromonas gingivalis	669±1.5	665±1.2	660±1.8

Number of observation n=3

4) ACKNOWLEDGEMENT

We are special thanks to JNTUA-OTPRI Anantapuram for doing the project work.

5) CONCLUSION

In this work, we have developed the optimum concentrations of xanthan gum / Pluronic solutions for use as an in-situ gelling vehicle for delivery of drug in the treatment of periodontitis which were 2.5% (w/w) and 15% (w/w), respectively. It has released the drug for a 75% after 24 hrs., and about 98% after 48 hrs. Which make suitability for the treatment of periodontitis in terms of convenience to patient and doctor. The results indicated combined polymeric systems performed better release than individual. The system may be used a better alternative to conventional solution and rinses and also reduces the frequency of visit for the patient. Local delivery of the doxycycline in the form of a thermoreversible gel was clinically effective along with scaling and root planning. The cost involved in the manufacturing of insitu gel can also be minimized by altering the content without changing the efficacy.

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