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PREPARATION AND EVALUATION OF IN-SITU GEL OF LEVOFLOXACIN HEMIHYDRATE FOR TREATMENT OF PERIODONTAL DISEASE

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Abstract

The aim of this study is to report the development, formulation and evaluation of thermo responsive in-situ gelling drug delivery system of Levofloxacin hemihydarte and its inference for the treatment of periodontal disease. A local controlled release system for direct placement of drug into the periodontal pocket without incision is developed using various concentrations of Polaxomer 407, which exhibits sol-to-gel phase transition converting to gel at body temperature (37^0 C) from liquid at room temperature (25^0 C). This type of drug delivery systems are considered as adjunctive to mechanical debridement viz. scaling and root planning benefiting more drugs at the target site. Each Formulation was evaluated for parameters like physicochemical properties, viscosity, gelation pH, gelation temperature, spreadability, *in-vitro* release, stability testing. Experimental part showed that viscosity of sols and gel strength was increased with increase in the concentration of polymers and the sustained release of drug was observed. The system thus developed was found to be clear and have good viscosity with prolonged release at 37°C, pH and drug content of all formulations was found to be satisfactory.

INTRODUCTION:

It is estimated that approximately 10-30% of the population suffers from periodontal diseases with pathological periodontal pockets [1]. Periodontitis is a chronic inflammatory disease of which the primary etiological factor is microbial dental plaque which causes an inflammatory response (Loe et al., 1965)[2]. Periodontitis destroys the periodontal tissue and eventually causes loss of teeth. Gingivitis, the mildest form of periodontal disease, is highly prevalent and readily reversible by simple, effective oral hygiene. Gingivitis affects 50–90% of adults worldwide. Inflammation that extends deep into the tissues and causes loss of supporting connective tissue and alveolar bone is known as Periodontitis. Periodontitis results in the formation of soft tissue pockets or deepened crevices between the gingiva and tooth root. Severe Periodontitis can result in loosening of teeth, occasional pain and discomfort, impaired mastication, and eventual tooth loss [3-5]. Disease occurs at individual periodontal sites and leaves an historical record of the damage to the periodontium in the form of periodontal attachment or bone loss [6]. It is initiated by bacteria that colonize the teeth and infect their surrounding soft tissues [7].

Periodontal disease, if not treated, results in the destruction of the bone and soft tissue supporting the tooth leading to tooth loss. In the early stage of Periodontitis, scaling and root planning is usually effective in removing calculus and plaque, thereby, reducing bacterial count and probing depth. As the probing depth increases, the effectiveness of scaling and root planning decreases. Therefore, in recent years, many antibiotics are either topically or systematically used in the treatment. Systemic antibiotic therapy has certain advantages. However, long-term use of systemic antibiotics is associated with several side effects such as development of resistance, hypersensitivity, and unwanted side effects [8].Controlled delivery of chemotherapeutic agents within periodontal pockets inhibit the pathogens and shows improvement in clinical signs of the disease. Local drug delivery systems provide several benefits such as the drug can be delivered to the target site at a bactericidal concentration and it can facilitate prolonged drug delivery[9].

In situ Gel systems are liquid at room temperature and gets converted to gel form when undergoes a phase transition triggered by temperature, pH change, ionic change & also UV induced gelation, Solvent exchange induced gelation and are capable of releasing drug molecule in a sustained manner with relatively constant plasma profiles.[10-13].Polaxomer is a triblock polymer consisting of polyoxyethylene–polyoxypropylene–polyoxyethylene units undergoes sol-gel transition mediated by temperature and forms micelles at low concentration and clear thermo reversible gel at a high concentration (14-30%)

Levofloxacin is a fluoroquinolone which is anti-infective and optically active L-isomer of ofloxacin with two fold more potency than ofloxacin and is reported to be more effective in the treatment of Periodontitis but in one of the survey need of the formulation was observed as it is currently not available in the market for local drug delivery system[14].

Hence, present work was taken up to develop and evaluate the in situ gel containing Levofloxacin hemihydrate as a local drug delivery within the periodontal pockets for the treatment of Periodontitis.

MATERIAL AND METHODS:

Materials:

Levofloxacin hemihydrate was kindly obtained as gift sample from J. Duncan Pvt. Ltd. PolaxomerL-407puriss was obtained as gift sample from Alpha Chemicals Laboratories, Germany. Benzalkonium chloride was procured from LobaChemie, Mumbai, India. All other materials used were of analytical grade. Dialysis Membrane MWCO 12000 Da .Purchased from Hi Media (Mumbai, India).

Methods:

Preparation of gel formulation:

Gel was prepared by two different methods, namely Cold method and hot method. Cold method is one of the preferred method as it provides clear solution for in situ gel as well as it does not form lumps of the polymer as it is reported and observed by hot process.

Cold process:

Cold method was performed as described by Schmolka et al[15] wherePolaxomer-

407was weighed accurately and was slowly added to cold water (5˚C) with constant stirring. Each dispersion was then refrigerated for five hours at 5˚C for complete polymer desolvation which result in to a clear solution. Levofloxacin hemihydrate was then added to the clear solution at 5˚C and mixed well to form a homogeneous mass. In the said experiment different concentration of polymers were used ranging from 14 to 22 %, Different formulations were prepared as shown in Table 1 where along with Drug and polymer, Benzalkonium chloride was added as preservative and triethanolamine was used to adjust pH of the formulation.

Table 1.

Composition of formulations

	Ingredients(%w/v)				
Formulation	Levofloxacin Hemihydrate	Polaxomer 407	Benzalkonium chloride	Triethanolamine	Water(ml)
F1	10	14	0.001	q.s.	100
F2	10	16	0.001	q.s.	100
F3	10	18	0.001	q.s.	100
F4	10	20	0.001	q.s.	100
F5	10	22	0.001	q.s.	100

EVALUATION:

Fourier transforms infrared spectrophotometry:

Drug, Polymer and different ratio of drug-polymer were subjected to FTIR analysis to study the purity and compatibility with respect to each other in formulation.

Clarity:

The clarity of the formulations after and before gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds.

Determination of drug content [16]:

The drug content of the formulations (F1 to F5) were analyzed by taking 1 mL of gel in 100 mL volumetric flask, dissolved and the volume was made upto 100 mL with 6.6 phosphate buffers. From the above solution 4 mL was pipetted out into a 10 mL volumetric flask and volume was adjusted with 6.6 phosphate buffer. Absorbance was measured at 288nm. Each formulation was subjected to pH measurement with previously calibrated pH meter using standard buffers of pH 4 and pH 7.

Determination of gelation temperature [17]:

The gelation temperatures of formulations (F1 to F5) were estimated by heating the solution (about 1-2 °C/min) in a test tube with gentle shaking until gel formed. The samples were examined for gelation when the meniscus would no longer move upon tiling through 90^0 and temperature was noted with the help of thermometer.

pH measurement:

The developed *in situ* gel formulations were evaluated for pH by using calibrated digital pH meter. The pH meter was calibrated before each use with standard.

Viscosity:

Viscosity of formulations was determined by using Brookfield's viscometer (model DV II, spindle no. 03, at 20 rpm) at Room Temperature.

***In-vitro* drug release [18, 19]:**

The dialysis technique using cellophane membrane was used to study *in-vitro* release. Prior to diffusion studies, the dialysis membrane was soaked overnight in pH 6.6 phosphate buffer solution. 1 mL of gel was placed in dialysis membrane (Mol. Wt. 12000 da), which was sealed on both

sides. The dialysis tube was placed in a glass beaker containing 18 mL of pH 6.6 phosphate buffer solution. The release studies were performed at 37±0.5°C for different time intervals. 1 mL of sample was pipetted out after every 15 min and was replaced with same volume of pH 6.6 phosphate buffer to maintain the sink condition. After suitable dilutions, samples were analyzed spectrophotometrically at 288 nm.

Spreadability[20]:

For the determination of spreadability (Harish et al., 2009), excess of sample was applied between the two glass slides and was compressed to uniform thickness by placing 100 g weight for 5 min. Weight (50 g) was added to the pan and the time required for separating the two slides, i.e. the time in which the upper glass slide moves over the lower plate was takenas measure of spreadability (S).

Spreadability (g.cm/s) (S)=M×L/T

Where M = weight tied to upper slide

L = length moved on the glass slide

T = time taken.

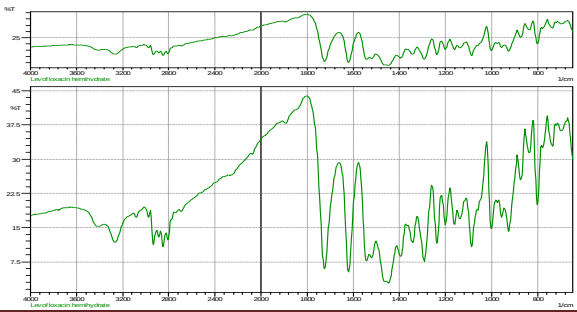
Measurement of gel strength:

Gel strength was measured by method reported by Choi et al. 50 g of gel was placed in a 100 ml graduated cylinder and gelled at 37°C using thermostat. A weight of 35g was placed onto the gelled solution and allowed to penetrate 5 cm in the gel. Time taken by weight to sink through the gel down by 5cm was measured.

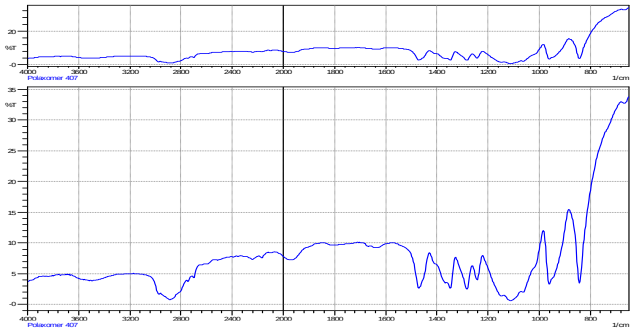
RESULTS:

FTIR spectroscopy:

Levofloxacin Hemihydrate



Polaxomer 407



Levofloxacin Hemihydrate and Poloxamer 407

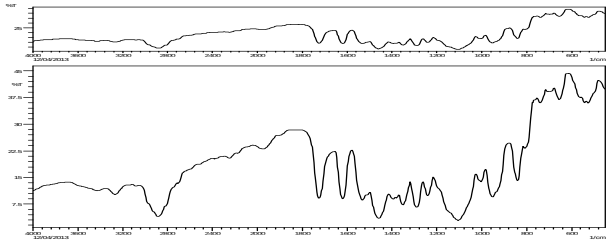


Figure 1: FTIR of levofloxacin, Poloxamer, and both levofloxacin plus Poloxamer.

The IR band of Levofloxacin shows the prominent peak at 3205 cm^{-1}(OH STRECHING), 1725 cm^{-1}

(C=O STRECHING). The bands of formulation of drug and the polymer shows the peaks at 3205 cm^{-1}(OH STRECHING), 1725 cm^{-1}**(** C=O STRECHING) shows no interaction between the drug and the poloxamer.

Clarity:

The formulations after and before gelling when observed under light alternatively against white and black backgrounds were found clear.

Drug content, pH, Gel strength and Gelation time:

Drug content of all formulation was found to be in range of 98-100%. The pH of all gel formulation was found to be in a range

of6.5-7.5 that is between physiological ranges of pH of mouth saliva. The gel strength of formulation at37^{0}C increased as the concentration of Polaxomer 407increased. Gelation temperature of formulation was in the range of 21 ± 0.3^{0}c to above 41±0.54^{0}c. Viscosity study of all formulation was in the range of 489±0.58to 9764±0.82.

Table 2

Gelation temperature, pH, Viscosity, %Drug Content, Gel Strengthand Gelation time of Formulation

Formulation	Gelation temperature(^{0}C)	pH	Viscosity in cps at 37^{0}C	Drug Content %	Gel Strength in Seconds	Gelation time (secs)
F1(14%)	41±0.54	6.9	489±0.58	98.76±0.22	41	240
F2(16%)	40±0.4	6.89	3156±0.6	99.13±0.46	68	155
F3(18%)	36±0.6	7.07	7448±0.25	99.98±0.86	92	62
F4(20%)	28±0.33	7	8056±0.7	100±0.43	130	43
F5(22%)	21±0.3	7.1	9764±0.82	98.56±0.45	147	34

***In vitro* release analysis:**

The prepared in situ gel implants are intended for placement in the periodontal pocket. Gingival crevicular fluid (GCF), inflammatory exudate flows continuously in the pocket. The pH of GCF 9 is 7.2 to 7.6 and a mouth saliva pH 6.4 to 7.4. Hence in the present study, phosphate buffer saline pH 7.4 was used for the in-vitro drug release studies of the gel formulations. The result of cumulative percent release is depicted in Figure 2.In-vitro drug release shows that F3 formulation released the drug completely within 2 hours. The results of in vitro release studies

showed that with increase in concentration of POLAXOMER 407, the rate of drug release decreased.

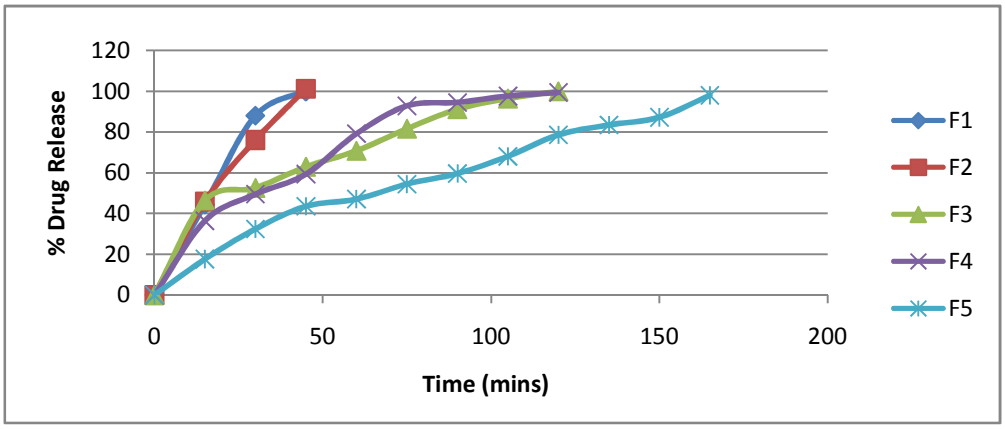


Figure 2: Drug release profile of formulations

Spreadability:

Table 3: Spreadability of formulations.

SR. No.	Formulation	Spreadability(gm.cm/sec)
1	F1	17.228
2	F2	12.635
3	F3	9.090
4	F4	5.526
5	F5	3.459

DISCUSSION:

IR spectra of drug and polymer show purity and compatibility of drug and polymer in formulation and also suggest no interaction between them. The clarity of all formulation shows solubility of drug in the solvent.The

mechanism of the increase gel strength might be related to hydrogen bonding between Polaxomer 407and Levofloxacin in the gel. It is observed that the thickening power of polaxomer in water increases as the hydrophobe molecule weight increases and as the ethylene oxide/propylene oxide ratio increases. Content uniformity studies showed that the drug was distributed uniformly in all formulations. Decrease in Gelation Temperature with increase in concentration of Polaxomer may be due to higher number of micelles formed at lower temperature. As concentration of Polaxomer increases, the structure becomes more closely packed and viscosity increases due to micellar entanglement which leads to gel formation. Polaxomer consist of micelles in aqueous phase which results in to slow *in vitro* release of the drug through in situ gel. Drug release from the gel follows korsemeyer- peppas model. Spreadability of gel decreases with increase in Polaxomer concentration. Formulations F4 and F5shows slow drug release which may be due to increase in viscosity were as formulations F1 and F2 shows faster drug release as the polymer concentration is less. F3 formulation is optimized as gelation temperature, release rate was sufficient for the intended purpose.

CONCLUSION:

The study shows that gelation temperature decreases with increase in polymer concentration. As the viscosity of the gel increases drug release decreases. The study also shows the mechanism of drug release through diffusion and it follows Korsmeyer-Peppas model. In present study single polymer was used and which gives satisfactory results but combination of polymers can be used in future studies for better in situ gel.

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