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FORMULATION AND EVALUATION OF LEVOFLOXACIN DENTAL FILMS FOR PERIODONTITIS

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ABSTRACT

A novel drug delivery system for the treatment of periodontitis was developed for site-specific delivery of Levofloxacin which has excellent activity against anaerobic microorganisms. The calibration curve for Levofloxacin was developed in pH 6.6 phosphate buffer at 287.6 nm in the range of 2 to 14 µg/ml. Levofloxacin films were prepared by solvent casting technique using ethyl cellulose and other co-polymers in chloroform: dichloromethane (1:1) solvent with dibutyl phthalate and PEG 400 as plasticizers. FT-IR and UV spectroscopic methods revealed no interaction between Levofloxacin and polymers. The films were evaluated for their thickness uniformity, folding endurance, weight uniformity, content uniformity, tensile strength, surface pH, and *in vitro* antibacterial activity. *In vitro* release from films was fit to different equations and kinetic models to reveal release kinetics. Kinetic models were studied for zero order, first-order equations, and Hixson-Crowell and Higuchi models. Formulation F_2 released 99.74% of drug at the end of tenth day and was considered as best formulation. A short-term stability study shows that drug content decreased in various films and was ranging from 0.9% to 3.41%.

Key words: Levofloxacin, Local delivery, I*n vitro* release, Periodontitis.

INTRODUCTION

Periodontal disease is a term that encompasses several pathological conditions affecting the tooth supporting structures. Periodontal disease includes conditions such as chronic periodontitis, aggressive periodontitis, systemic disease associated periodontitis, and necrotizing periodontitis[1]. These conditions are characterized by destruction of the periodontal ligament, resorption of the alveolar bone, and the migration of the junctional epithelium along with the tooth surface. The clinical signs of periodontitis are changes in the morphology of gingival tissues, bleeding upon probing as well as periodontal pocket formation. This pocket provides an ideal environment for the growth and proliferation of anaerobic pathogenic bacteria[2].

Conventional therapy, based on scaling, surgery and the use of antibiotics or antimicrobials has been proposed[3]. But due to bacterial resistance and toxic side effects of the administered antibiotics local delivery system are designed to maintain the antibiotic, in the gingival crevicular fluid at a concentration higher than that achieved by systemic administration[4].

Levofloxacin a fluoroquinolone anti-infective, optically active L-isomer of ofloxacin and two fold more potent than ofloxacin and reported to be more effective in the treatment of periodontitis was chosen for the present study.

Levofloxacin is available in the market as a conventional dosage forms such as tablets, capsules, and parenterals for the treatment of bacterial infections but not suitable means for the treatment of infection locally. Hence it was a challenge to develop periodontal films containing Levofloxacin with rate controlling polymers, which has a prolonged action and shows the antibacterial activity directly at the site

of infection without loss of dosage. Considering the above discussions it was decided to develop local controlled drug delivery system containing Levofloxacin.

MATERIALS AND METHODS

Materials

Levofloxacin was obtained as gift sample from Micro labs Pvt. Ltd., Bangalore., India. Ethylcellulose, Hydroxy Propyl Cellulose (HPC), and Hydroxy Propyl Methylcellulose (HPMC K_4M) were obtained from Loba Chemie Pvt. Ltd., Mumbai., India. Eudragit RL-100 and Eudragit E-100 were obtained from Micro labs Pvt. Ltd., Bangalore. Poly Vinyl Pyrrolidone (PVP K-30), from Ozone Internation, Mumbai. Other materials used in the study were of analytical grade.

Methods

Drug-polymer compatibility

Pure drug (Levofloxacin) and polymers were subjected to FT-I.R studies alone and in combinations. 3 mg of pure drug/ combination of drug-polymer were triturated with 97 mg of potassium bromide in a smooth mortar. The mixtures were placed in the sample holder and were analyzed by FT-IR to study the interference of polymers with the drug.

Preparation of cast film containing Levofloxacin

Periodontal films were prepared by solvent casting technique. Glass moulds were used for casting of the films. Formulations were designed as shown in the Table-1, in which Ethylcellulose was taken as the main non-biodegradable polymer in combination with different co-polymers for each cast films. Films were prepared by dissolving Ethylcellulose alone and with co-polymers (Eudragit RL-100, Eudragit E-100, PVP K-30, HPMC K4M, and HPC) in chloroform and dichloromethane (1:1) solution, using dibutyl phthalate and PEG-400 as plasticizers. Levofloxacin was added in to the polymeric solution and mixed homogenously using magnetic stirrer in a closed beaker. After complete mixing 10 ml of the solution was poured into the clean-leveled glass moulds of 15 sq. cm. The solvent was allowed to evaporate slowly by inverting a glass funnel with a cotton plug closed into the stem of the funnel at room temperature for 24 hours. After complete evaporation of solvent, cast films were obtained, which were then cut into pieces of 7×2 mm, wrapped in an aluminum foil and stored in a desiccator at room temperature in a dark place for further evaluation studies.

Evaluation of the films

Periodontal films were evaluated for physical characteristics as follows.

Thickness uniformity of the films: Thickness of the film was measured using digital screw gauge (Mitutoyo) at different areas of the film and the average was calculated.

Uniformity of weight of the films: Film (size of 7x2 mm^2) was taken from different areas of film. The weight variation of each film was calculated.

Surface pH: Periodontal films were left to swell for 1 hour on the surface of the agar plate, prepared by dissolving 2% (w/v) agar in warmed double distilled water with constant stirring and poured into the petridish to solidify at room temperature. The surface pH was measured by means of pH paper placed on the surface of the swollen film. The mean of three readings was recorded[5].

Viscosity: Aqueous solutions containing both polymers and plasticizers were prepared in the same concentration as

that of films. Viscosity was measured at 20 rpm at room temperature using Brookfield viscometer (LVDV-E model) attached to the helipath spindle number 18. The recorded values were mean of five determinations.

Folding endurance: As described by Khanna et al., the folding endurance of the films was determined by repeatedly folding the film at the same place up to 300 times till it broke or folded, which is considered satisfactory to reveal good film properties. This test was carried out on all the films[6].

Drug content uniformity of films: Film (size of 7x2 mm^2) was taken from different areas of the film and placed into a 10 ml volumetric flask, in to which 10 ml of ethyl alcohol was added and kept aside till the film is completely dissolved. Withdraw 1 ml of solution and diluted to 10 ml with pH 6.6 phosphate buffer. The absorbance of the solution was measured at 287.6 nm. The polymeric solution without drug served as blank. In case of HPMC & HPC films, combination of water and alcohol was used to dissolve the films.

Tensile strength of the films: Tensile strength of the films was determined by Universal strength testing machine. It consists of two load cell grips, the lower one is fixed and upper one is movable. The test film of specific size (4×1 cm^2) was fixed between these cell grips and force was gradually applied till the film breaks. The tensile strength of the film was taken directly from the dial reading in kilograms.

***In vitro* drug release:** Static dissolution method reported in the literature was adopted. Films of known weight and dimensions (size of 7×2 mm^2) were placed separately into small test tubes containing 1 ml of pH 6.6 phosphate buffer. The test tubes were sealed with aluminum foil and kept at 37°C for 24 hours. The buffer was drained off and replaced with fresh 1 ml of pH 6.6 phosphate buffer after 24 hours. The concentration of drug in the buffer was measured at 287.6 nm. The procedure was repeated for 10 days[7].

***In vitro* antibacterial activity:** The films (size of 2x2 mm^2) containing 26.6 µg of drug were taken for the study. Prepare and sterilize nutrient agar medium by autoclaving under aseptic condition and transfer the medium to sterile Petri plates. After solidification of nutrient agar medium, made a lawn with 0.1 ml microorganism i.e. S. aureus and E. coli in separate Petri plates, over that the films were placed and incubate for 48 hrs at 37^0 C. Measure the zone of inhibition using "Hi Antibiotic Zone Scale". Same procedure is followed by replacing the films over the next plates and measures the zone of inhibition[8].

Ageing: Ageing studies were performed on all periodontal films. Changes in the appearance and drug content of the stored films were evaluated at an interval of one week after storage. The data obtained were the mean of three determinations [9].

RESULTS AND DISCUSSION

FT-IR spectrum of Levofloxacin alone and in combination with polymers are were studied. FT-IR spectrum of the Levofloxacin and the drug-polymer mixture have characteristic bands at 1723 cm^{-1} (carbonyl group), 1884 cm^{-1} (carbonyl group of quinolone moiety), 2935 cm^{-1} (aromatic C-H stretching), and 3275.5 cm^{-1}(O-H group of carboxyl moiety) indicating that Levofloxacin is not involved in any chemical reactions with the polymers used. Further, the interference was also verified using UV-spectrophotometric method.

In the present study, periodontal films of levofloxacin were formulated using the polymer matrix of Ethyl cellulose

and the effect of Eudragit RL100, Eudragit E100, PVP-K30, HPMC K4M, and HPC as rate-controlling polymers. The prepared films were translucent and smooth surfaced with good tensile strength. The procedure developed to prepare the films was reproducible.

Table 1: Composition of different formulations containing Levofloxacin

Ingredients	F1	F2	F3	F4	F5	F6
Ethylcellulose (mg)	590	500	500	500	500	500
Eudragit RL-100 (mg)	*	90	*	*	*	*
Eudragit E-100 (mg)	*	*	90	*	*	*
PVP K-30 (mg)	*	*	*	90	*	*
HPMC K4M (mg)	*	*	*	*	90	*
HPC (mg)	*	*	*	*	*	90
Dibutyl phthalate (ml)	0.15	0.15	0.15	0.15	0.15	0.15
PEG 400 (ml)	0.05	0.05	0.05	0.05	0.05	0.05
Levofloxacin (mg)	10	10	10	10	10	10

* No ingredients added; PVP = Poly vinyl pyrolidone; HPMC = Hydroxypropyl methylcellulose; HPC = Hydroxypropyl cellulose; PEG = Polyethylene glycol; F_1 to F_6 = Films codes

All the films have uniform thickness throughout with the standard deviation of ± 0.00339 mm (n = 6).

The films of all the batches were found to be of uniform weight, ranging from 4.5752 ± 0.00164 mg to 4.8336 ± 0.00152 mg. (n = 5).

The surface pH of all the films was found to be neutral and hence no periodontal pocket irritation is expected.

The viscosities of the solutions were ranging from 13.66 to 44.26 cps for films F_1 to F_6. Viscosity of the film F_2 solution was more when compared to other films, could be due to complete solubility of polymers in Chloroform and Dichloromethane (1:1) mixture.

Folding endurance of the films was > 250 times indicate that the formulations have good film properties.

Content uniformity studies of the films shows that the drug was uniformly dispersed and recovery was possible to the tune of 93.01 to 99.09 % for formulations F_1 to F_6 (Table 2).

The tensile strength of all drug-loaded films was studied (Table 2). The effective cross linking was observed on addition of Eudragit RL 100 as a co-polymer, which also shows higher tensile strength when compared to all other formulations. The tensile strengths of films were in the order of $F2 > F_3 > F_1 > F_4 > F_6 > F_5$.

Table 2: Physicochemical characteristics of periodontal films containing Levofloxacin

Films	Thickness uniformity (mm)	Weight uniformity (mg)	Tensile strength (kg)	Content uniformity (%)
F_1	0.3880 ± 0.00089	4.6264 ± 0.00134	1.3494 ± 0.0320	95.7481 ± 1.2716
F_2	0.4547 ± 0.00339	4.8336 ± 0.00152	1.9410 ± 0.0512	99.0917 ± 1.7329
F_3	0.4357 ± 0.00273	4.7862 ± 0.00164	1.1598 ± 0.0580	98.1798 ± 1.2716
F_4	0.3913 ± 0.00361	4.6506 ± 0.00152	1.2727 ± 0.0313	93.0124 ± 1.3594
F_5	0.4213 ± 0.00186	4.5752 ± 0.00164	1.1861 ± 0.0242	93.9243 ± 1.8614
F_6	0.4253 ± 0.00314	4.5970 ±0.00122	1.1886 ± 0.0243	95.4441 ± 1.0747

In vitro release studies of Levofloxacin was carried out in pH 6.6 phosphate buffer for 10 days which shows that there was an abrupt release observed in first three days, and there after the release of drug was found to be controlled. Average amount of drug release per day after fourth day is found to be above the minimum inhibitory concentration of levofloxacin (MIC ≤ 2 µg/ml). *In vitro* release studies shows that the drug release was more sustained in case of film F_2 followed by $F_3 > F_5 > F_1 > F_4 > F_6$. The regression values of films F_1 to F_6 are higher with first order and therefore the release kinetics followed first order from all the films. The release data of levofloxacin films (F_1 to F_6) were given in Table 3 and fig 1 & 2.

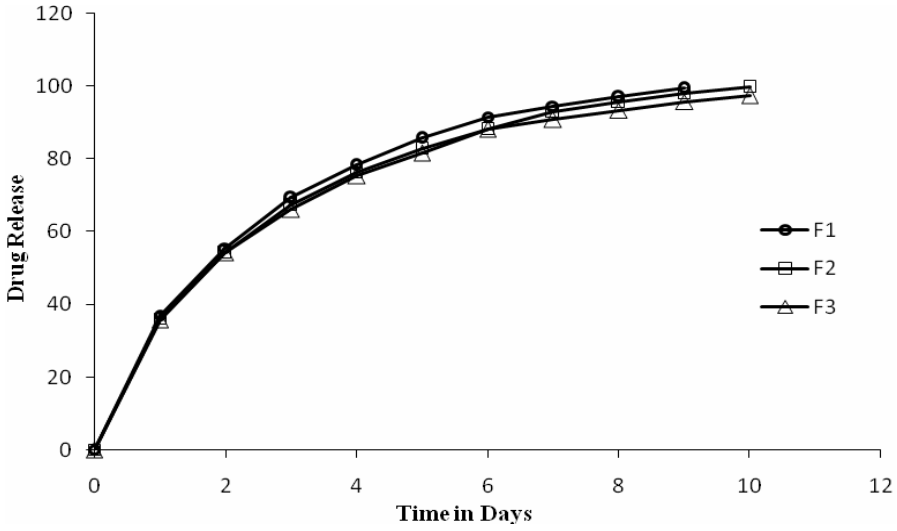

Fig. 1: *In vitro* release profile of Levofloxacin from film F_1 to F_3

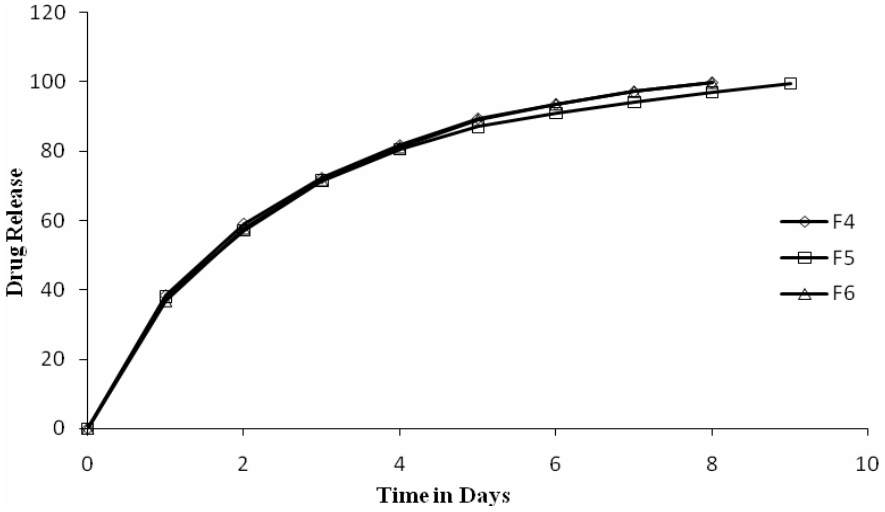

Fig. 2: *In vitro* release profile of Levofloxacin from film F_4 to F_6

Table 3: *In vitro* release profile of Levofloxacin films from F_1 to F_6

Time (Days)	% of drug release					
	F_1	F_2	F_3	F_4	F_5	F_6
0	0.00	0.00	0.00	0.00	0.00	0.00
1	36.90	36.11	35.76	38.48	38.10	36.80
2	55.49	54.08	54.13	59.14	57.16	57.74
3	69.32	67.19	66.16	72.34	71.53	71.61
4	78.29	76.25	75.33	81.79	80.62	81.08
5	85.71	82.77	81.59	89.49	87.16	89.01
6	91.56	88.05	88.05	93.64	91.14	93.51
7	94.40	92.98	90.72	97.24	94.17	97.16
8	97.00	95.65	93.39	99.87	96.99	99.74
9	99.39	98.06	95.70	-	99.53	-
10	-	99.74	97.32	-	-	-

Table 4: Comparison of release mechanism based on regression coefficient (R^2) values

Film code	Regression coefficient (R^2) values			
	Zero order	First order	Hixson-Crowell model	Higuchi's model
F_1	0.8292	0.9419	0.9781	0.9909
F_2	0.8223	0.9033	0.9757	0.9906
F_3	0.8106	0.9967	0.9708	0.9817
F_4	0.8366	0.8430	0.9814	0.9876
F_5	0.8090	0.9240	0.9705	0.9845
F_6	0.8463	0.8852	0.9833	0.9919

Hixson Crowell cube root law and Higuchi's model were applied to test the release mechanism. The R^2 values are higher for Higuchi's model compared to Hixson Crowell cube root law for all the films. Hence Levofloxacin release from all the films followed diffusion rate controlled mechanism. Data are as shown in Table 4.

In vitro antibacterial activity was performed as mentioned in the methodology on *S.aureus* and *E.coli* organisms. The zone of inhibition of the prepared formulations was found to be effectively higher in 48 hrs and then declined at 96 hrs. The study indicates that the formulated polymeric films containing Levofloxacin retained their antibacterial activity.

Ageing studies performed on all prepared periodontal films. Decrease in the drug content from the films ranged from 0.90 to 3.41%. It was found that the drug loss is less, though the films were stored for one month. The films were also observed for their appearance and texture. These properties did not change in films during the period of study.

CONCLUSION

Periodontal films containing Levofloxacin were prepared. *In vitro* characterization studies revealed that Levofloxacin can be incorporated in a slow release device for the treatment of periodontitis. Ageing studies shows that the drug remained intact and stable in the periodontal films during storage. Spectroscopic data shows there is no significant chemical interaction between the drug and polymers. Further, detailed investigation is required to establish *in vivo* efficiency of these films.

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REFERENCES

1. Colin B.W, Edward E.P. The Periodontal Disease Classification System of the American Academy of Periodontology. J Can Dent Assoc 2000; 66:594-7.
2. Marish PD. Host defenses and microbial homeostasis: role of microbial infections. J Dent Res 1989; 68:1567-75.
3. Van DOFJ. Anti-plaque agents. Rationale and prospectus for prevention of gingivitis and periodontal disease. J Clin Periodontal 1991;18:447-54.
4. Kornman K. Controlled-release local delivery antimicrobials in periodontics prospects for the future. J periodontal 1993;64:782-91.
5. Noha AN, Nabila AB, Fatima A, Ismail, Lobna MM. Design and characterization of mucoadhesive buccal patches containing cetylpyridinium chloride. Acta Pharm 2003; 53:199-212.
6. Khanna R, Agrawal SP, Ahuja A. Preparation and evaluation of buccal films of clotrimazole for oral Candida infections. Indian J Pharm Sci 1997; 59:299-305.
7. Mastiholimath VS, Dandagi PM, Gadad AP, Patil MB, Manvi FV and Chandur VK. Formulation and evaluation of ornidazole dental implants for periodontitis. Indian J Pharm sci 2006; 68(1): 68-71.
8. Chapter in a book: Friedman M and Steinberg D. Sustained release drug delivery devices for local treatment of dental diseases. In: Type P Drug delivery devices, fundamentals and applications. vol 32.New York: Marcel Dekker Inc.; 1988:491-515.
9. Patrick J.C. International Stability Testing. Pharmaceutical Science & Technology 1999; 227-8.