

Formulation and Characterization of Dental Film Containing Ofloxacin

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ABSTRACT

The aim of the study was to formulate intrapocket dental films, which could be easily placed into the periodontal pocket, and be capable of delivering therapeutic concentrations of ofloxacin for prolonged period of time at a much lower dose, hence obviating untoward side effects. Intrapocket device containing ofloxacin was prepared using 255.20 mg of ofloxacin, 2.0% plasticizer diethyl phthalate and 1.4g ethyl cellulose. The device was optimized on the basis of evaluation parameters such as weight variation, content uniformity, surface pH, *in vitro* and *in vivo* release studies. Films showed sustained *in vitro* release for a period of 11 days. *In vivo* release studies showed that drug concentrations were maintained above MIC value for entire period of release studies. The samples from this study were capable of inhibiting the growth of most test strains. Ofloxacin in carrier polymer ethyl cellulose showed extended spectrum of antimicrobial activity. Stability studies were conducted on optimized formulation and degradation rate constant was found to be 1.2056×10^{-4} /day. The drug released locally and had high benefit to low risk ratio as compared to systemic administration, which is unacceptable due to, low benefit to high-risk ratio. Hence low dose site-specific films is a better alternative.

INTRODUCTION

Periodontitis is an inflammatory response in which the structural support to the tooth is destroyed. The disease results in resorption of the alveolar bone, detachment of the periodontal ligament supporting the tooth and formation of periodontal pocket (lesions between teeth and junctional epithelium) (Heasman & Seymour, 1994; Sanz & van Winkelhoff, 2011). Pocket provides an ideal environment for the proliferation of a variety of pathogenic bacteria. Therapeutic approaches for the treatment of periodontitis include mechanical or surgical methods and administration of systemic antibiotics. However, for systemic administration the drugs must be given in high doses to maintain the effective concentration in gingival crevicular fluid (GCF).

High doses of antibiotics cause side effects such as gastrointestinal disorders, development of resistant bacteria and suprainfection. Systemic therapy has low benefit to high-risk ratio (Gordon & Walker, 1993). With advances in understanding of the

etiology and pathogenesis of periodontal disease, attention has been focused on local drug delivery systems. These include both sustained and controlled release polymeric systems which when inserted into periodontal pocket, release antimicrobial agents above minimum inhibitory concentration for a sustained period of time. Thus intra-pocket devices have high benefit to low risk ratio (Pandit, 2004). The drug is bactericidal in nature and is administered systemically. This is important for complete elimination of subgingivally occurring periodontal pathogens (Schwach-Abdellaoni *et al.*, 2000).

Ethyl cellulose is one of the most popular and well characterized polymeric materials for use in controlled drug delivery. It is biocompatible and produces little or no local and systemic toxicity on administration (Ahmed *et al.*, 2009).

In the present study our objective was to formulate intrapocket dental films, which could be easily placed into the periodontal pocket, and be capable of delivering therapeutic concentrations of ofloxacin for prolonged period of time at a much lower dose, hence obviating untoward side effects. Antimicrobial activity dental films containing ofloxacin with ethyl cellulose

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was also investigated against periodontal pathogens commonly found in periodontal infections. These include *Bacteroides melaninogenicus*, *Bacteroides oralis*, *Bacteroides fragilis*, *Peptostreptococcus assachrolyticus*, *Peptostreptococcus* species, *Eubacterium limosum*, *Propionibacterium acne*, *Staphylococcus aureus* and *Escherichia coli*.

MATERIALS & METHODS

Materials

Ofloxacin (lot #. M 10521601) was obtained as gift samples from Cadila Pharmaceuticals Ltd., Ahmadabad, India. Ethylcellulose was obtained from Ranbaxy Laboratories Ltd. Gurgaon. Boric acid, sodium hydroxide, dichloromethane, methanol were purchased from E.Merck (India) Ltd. (Mumbai, India). Potassium chloride, disodium hydroxide orthophosphate, acetone, and diethyl phthalate were obtained from CDH (P) Ltd. (Mumbai, India).

All other materials used were of analytical reagent grade. Shimadzu UV/Visible spectrophotometer, 1601 model with spectral bandwidth of 2nm and wavelength accuracy of 0.5 nm was used for spectrophotometric analysis. A 280-435 VP, PCV based scanning electron microscope was used to study the surface characteristics of the films. Biological shaker cum incubator and the peristaltic pump used in the *in vitro* and *in vivo* studies respectively were supplied by Metrex scientific instrument (P) Ltd. (New Delhi, India). The microbial strains used in the microbiological studies were obtained from Majeedia Hospital, New Delhi, India.

Fabrication of Dental Films by dispersion method

To determine the optimum combination of polymer, plasticizer and solvent placebo films were evaluated on the basis of homogeneity, flexibility, stickiness and smoothness. The films, which exhibited all the characteristics, were loaded with the drug and were taken up for further studies.

Table 1 gives the formulae for selected drug loaded films. The films were subjected to *in vitro* release studies. The formulation A-IV gave the best release both *in vitro* and *in situ*. The optimized formulation was prepared by using 1.4 g of ethyl cellulose in 10 ml of methanol containing 2.0% diethyl phthalate as plasticizer. 0.255 g, of ofloxacin were sieved through 80 to 120 mesh size and homogeneously dispersed in the polymer solution by vortexing for 5 to 10 minutes. Films were cast by pouring this dispersion into glass ring placed over aluminum foil. The films were initially dried at a temperature of -10 to -5°C for 8 to 10 hours and then at 25°C for further 20 to 24 hours. After drying, the films were cut into slabs of 10 mm size (Agarwal *et al.*, 1993; Kumar *et al.*, 2010).

20 films from each batch (carrying drug in different polymeric compositions) were weighed individually on a Sartorius electronic balance (AG 135 Mettler Toledo). Average weight range, standard deviation and maximum variation from the average were determined.

Content uniformity

The method involved analysis by UV spectrophotometer. Standard solutions of ofloxacin were prepared in alkaline borate buffer pH 8.1. From this standard solution, dilutions containing 10µg/ml of ofloxacin were prepared, scanned in the spectrum mode from 200-400 nm and the spectrum was recorded (Figure1). From the spectra it was observed that wavelengths that could be utilized for analysis of ofloxacin was 288 nm. Film samples consisting of ofloxacin placed in 10 ml methanol to dissolve the polymer ethyl cellulose. The volume was made up to 10 ml with alkaline borate buffer of pH 8.1. Aliquots of sample solution were diluted to get a final concentration of 10 µg/ml of drug. The resultant solutions were filtered diluted and was analyzed spectrophotometrically at λ_{max} of the drug (288nm). The concentrations of ofloxacin was determined by using spectral data of standards by the spectrophotometer.

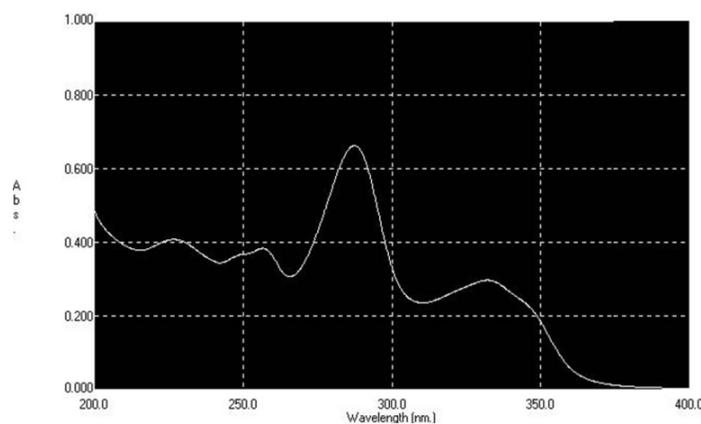


Fig. 1: UV scan of Ofloxacin in alkaline borate buffer pH 8.1.

Surface pH

The films were first allowed to swell in contact with 1 ml of distilled water (pH 6.5 ± 0.05) for 2 hours in specially fabricated glass tubes. The surface pH was noted by bringing a combined glass electrode near the surface of films and allowing it to equilibrate for one minute. The surface pH of the films was determined in order to investigate the possibility of any side effects, in the oral cavity. As acidic or alkaline pH is bound to cause irritation to the buccal mucosa, hence attempt was made to keep the surface pH close to the neutral pH (Bottenberg *et al.*, 1991).

Table. 1: Formulae for drug loaded films .

Formula Code	Polymer I	Polymer II	Methanol (ml)	Plasticizer (ml)	Mean Surface pH ±SD (n = 6)
A-I	HPMC-E15 (1.3 g)	--	10	Diethyl phthalate (0.2)	6.08 (±0.023)
A-II	HPMC-E15 (0.8 g)	HPC-M (0.4 g)	15	Propylene glycol (0.2)	5.63 (±0.026)
A-III	HPC-L (0.8 g)	EC (1.3 g)	15	Propylene glycol (0.4)	5.53 (±0.049)
A-IV	EC (1.4 g)	--	15	Diethyl phthalate (0.5)	6.04 (±0.069)

In vitro release rate study

The release of drugs from dental films was determined *in vitro* by placing the films in conical flasks with 50 ml of alkaline borate buffer of pH 8.1, which contained 2.25% glycoproteins (Ali *et al.*, 1998; Li, 1999; Ali *et al.*, 2002; Reddy *et al.*, 2011). Films were continuously shaken at 50 rpm using a biological shaker cum incubator (Metrex Scientific Instrument (P) Ltd., New Delhi, India) maintained at 37° C. 3ml samples were intermittently withdrawn and analyzed for ofloxacin using Shimadzu UV-1601 spectrophotometer. The volume of medium withdrawn was replaced by adding 3 ml buffer to the flask.

In situ release study

In situ release study was performed using a flow through apparatus, which simulated the *in vivo* conditions. It consisted of a cavity 1.2 cm in length and 1 cm in depth for placement of bovine buccal mucosa and the film. Alkaline borate buffer of pH 8.1 simulating the pH of GCF was continuously pumped at a flow rate of about 15ml/day, using a peristaltic pump. Since the GCF is an inflammatory exudate, its flow rates increases to 3.5 ml/day or more (Ahuja *et al.*, 2003a) Buccal Mucosa was cut into strips (1.5 cm long and 1.5 cm wide) and was placed in the central cavity of the cell and was stabilized with the buffer in order to remove soluble components.

After stabilization, films were stuck onto the mucosal membrane using 25 µl of alkaline borate buffer and a weight of 10g for 30 sec. (Ahuja *et al.*, 1998). Samples were withdrawn from the central cavity using a micropipette at regular intervals. The volume of sample was made up to 3 ml using alkaline borate buffer, filtered through a Whatman filter paper no.42 and analyzed by UV-1601 spectrophotometer.

Surface characterization of films by SEM

Surface characteristics of drug loaded films, placebo films and films from which drug had been released, were compared using SEM. Films were sputter coated with gold (30 nm thick) under vacuum and microscopy was done using a LEO 435 VP, PC based digital scanning electron microscope.

Microbiological evaluation of drug loaded dental films

Dental films containing ofloxacin were tested on aerobic strains commonly found in periodontitis. These included aerobic organisms *Staphylococcus aureus* and *Escherichia coli* (Li, 1999; Ali *et al.*, 2002). For this purpose Agar Dilution Method was used. Mueller Hinton agar was used for aerobes Microbiological response of stock solution, *in situ* release samples and drug loaded films were tested. All microbiological studies were performed in an aseptic area in a laminar flow hood.

Stability studies

Stability studies were carried out to determine the effect of temperature and humidity on the content of the drug and also to determine the stability of the formulation under accelerated storage

conditions of temperature and humidity. Stability studies were carried out according to ICH guidelines. Dental films were kept in sealed petridishes lined internally with aluminium foil. These petridishes were then placed in desiccators containing saturated solution of sodium chloride in order to maintain relative humidity condition of 75% ±5.0%.

The whole assembly was kept inside a hot air oven at a temperature of 40° ±0.5° C. Samples were withdrawn at 0, 15, 30, 60 and 90 days. The films were triturated with methanol in a glass pestle and mortar. The resultant solution was filtered and diluted. The samples were analyzed for their drug content by HPTLC analysis using the standard curve ($y = 26.684x$). Concentration of the drug was calculated from the calibration curve of the pure drug (Abounassif *et al.*, 1991).

RESULTS AND DISCUSSION

The present study was an attempt to develop a low dose intrapocket device of ofloxacin for the treatment of periodontitis. The ofloxacin formulated was a matrix, which had a better release profile and patient compliance compared to earlier formulations containing other polymer combinations and could easily be placed in the periodontal pocket. No such formulation containing of drugs is reported so far. This is the first time that attempt was made to formulate a delivery system containing drugs effective against aerobes and anaerobes.

Ethyl cellulose was the carrier polymer in the intrapocket device. Films based on polymer ethyl cellulose exhibited sustained release for a period of eleven days. The optimized formulation contained 255.20 mg ofloxacin and films were 5.7 cm in diameter. From these films, slabs and cones of 1 cm side were punched out. Each carried 10 mg each of ofloxacin. The dental films had a significant advantage in terms of dose reduction and better therapeutic efficacy because of their high benefit to low risk ratio compared to conventional treatment. Our new matrix system had better patient compliance because of decrease in the frequency of administration. The films were white, smooth, non sticky, homogenous and flexible.

Weight Variation

Table 2 gives the average weight, range and maximum variation from the average weight of the films.

Table 2: Weight variation of intrapocket films.

Average Weight (mg) (n = 3)	± SD	Range (mg)	Maximum Variation from average (%)
23.50	0.329	23.20-24.00	2.12

Surface pH

Surface pH of films was taken into consideration so that the films do not cause irritation to the buccal mucosa. Earlier workers have not considered this concept and no mention of such work has been made anywhere in the literature. The optimized formulation exhibited surface pH of 6.04.

In vitro release rate studies

Formulation A-I gave a release of 42.11% of ofloxacin in 5 days of study. Formulation A-II gave a release of 55.90 % of ofloxacin in 6 days of study. Formulation A-III gave a release of 44.77 % of ofloxacin in 7 days of study. The optimized formulation (A-IV) exhibited maximum drug release of 36.48% of ofloxacin in 11 days of study. Therapeutic drug concentrations were maintained for a period of 11 days (Figure.2). Release of drug from the optimized formulation followed zero order kinetics since the coefficient of variance for zero order release is less than the coefficient of variance for the first order release. Plot of percentage drug released versus square root of time was linear for over four days. The release profile of drugs dispersed in films can be treated using the Higuchi equation for diffusion –controlled transport in a polymer matrix. For a granular matrix the amount of drug Q, liberated per unit surface area of matrix into the external medium in time t is given by

$$Q = \frac{[\varepsilon D (\tau A - \varepsilon C_s) C_{ST}]^{1/2}}{\tau}$$

Where C_s is the solubility

D is the diffusion coefficient of drug in the polymer

ε is the porosity

τ is the tortuosity of the matrix respectively

The linear correlation obtained between Q and $t^{1/2}$ indicated that the release was diffusion controlled for initial 4 days and this was followed by a sustained release profile over the remaining duration.

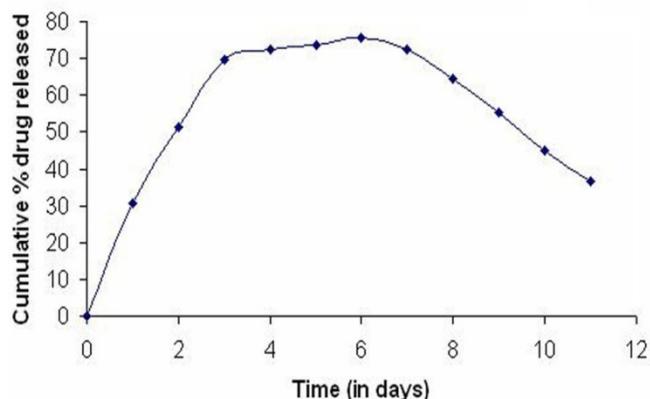


Fig. 2: *In vitro* release profile of optimized film.

In situ release rate study

In situ release study revealed that the concentration of ofloxacin released was maintained well above its minimum inhibitory concentration over a period of eleven days (Figure 3).

Surface characteristics

Surface Electron Microscopy revealed that the drugs appeared as white specks on the surface of the carrier matrix in case of optimized film (Figure 4). The placebo film showed no such specks (Figure 5). Surface characteristics of film from which

drug had been released was also studied and it showed pore formation in the polymer matrix indicating release of the drug (Figure 6). SEM indicated that the films had a smooth surface prior to drug release while after release surface irregularities were evident. Some irregular pores were also seen. Formation of pores in the ethyl cellulose matrix indicated that the release of ofloxacin started with dissolution of the drugs and subsequently followed diffusion through the pores.

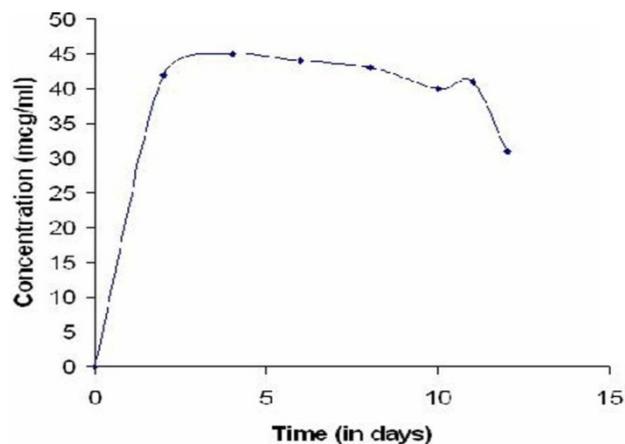


Fig. 3: *In situ* release of ofloxacin from optimized film.

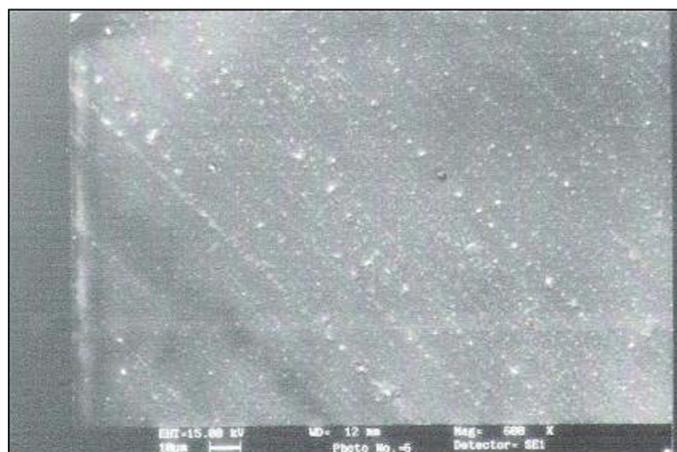


Fig. 4: Surface characteristics of optimized film (600X).



Fig. 5: Surface characteristics of placebo film (600X).

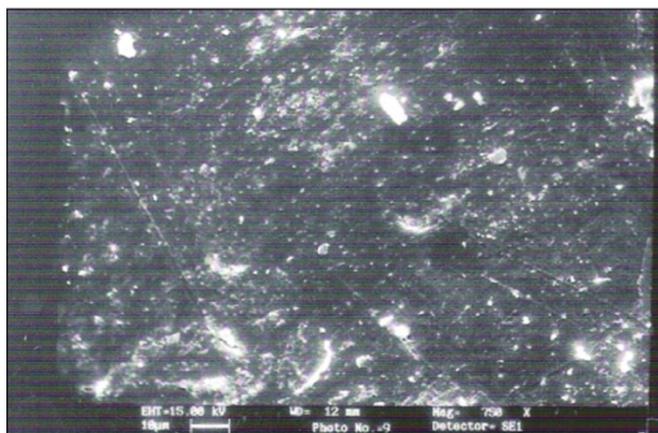


Fig. 6: Surface characteristics of film from which drug has been released (600X).

Microbiological studies

Agar dilution technique revealed that the films were found to be effective in inhibiting the growth of all the aerobic strains after 72 hours. However, poor inhibition was seen from the ethylcellulose matrix. Cylinder plate method was used for testing the microbiological responses of stock solutions, *in vivo* release study samples and drug loaded films against the aerobes: *S. aureus* and *E. coli*. Inhibition of growth was seen in all the samples tested (Figure 7).

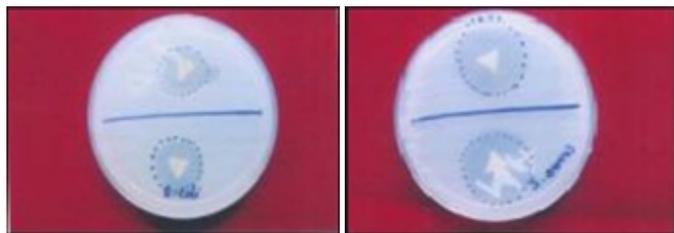


Fig. 7: Anti-microbial activity of drug-loaded film against *E.coli* & *S.aureus*.

Stability Studies

The stability studies of the films showed that no significant changes occurred in the physico-chemical properties at $40^{\circ} \pm 0.5^{\circ}\text{C}$ and $75\% \pm 5.0\%$ R.H. The degradation rate constant was found to be $1.2056 \times 10^{-4}/\text{day}$. Since this value is very low, a tentative shelf life of 2.31 years was given to the formulation. No discernable change in the physical appearance was seen in the samples. All films were white smooth, non-sticky and flexible after the stability studies.

CONCLUSION

In the present study we attempted to load ofloxacin in the polymeric material for use in periodontal infections and characterize the prepared films. After evaluating these for various parameters we concluded that it is an excellent system for drug delivery. The films were smooth, homogenous, non-sticky and flexible. The films were capable of maintaining therapeutic concentrations (above the MIC for causative organisms) for a period of 11 days. These films when characterized by SEM clearly

showed that the drug was present on the surface of the films and embedding had taken place completely. The films were capable of inhibiting the growth of aerobic and anaerobic strains commonly found in periodontal disease. Stability studies showed that our formulation was very stable after loading the drug and could be stored without degradation for more than two years. Films were developed to a satisfactory level in terms of drug content, drug release, mechanical properties, *in vitro* release, *in situ* release and microbiological evaluation. Since the drug release occurred locally, it had high benefit to low risk ratio as compared to systemic administration, which is unacceptable due to, low benefit to high-risk ratio. Hence low dose site-specific films are a better alternative. We also plan to study the effect of our device in patients after obtaining permission from ethical board.

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