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Biodegradable periodontal intrapocket device containing metronidazole and amoxycillin: formulation and characterisation

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The purpose of the study was to formulate biodegradable intrapocket dental films, which could be easily placed into the periodontal pocket, and be capable of delivering therapeutic concentrations of amoxycillin and metronidazole for prolonged period of time with a much lower dose, hence obviating untoward side effects. A biodegradable intrapocket device containing amoxycillin and metronidazole was prepared using 69.29 mg each of amoxycillin and metronidazole, 2.0% diethyl phthalate plasticizer and 750 mg poly (L-lactide-co-glycolide). The device was optimized on the basis of evaluation parameters such as weight variation, content uniformity, surface pH, and *in vitro* and *in vivo* release studies. The films showed sustained *in vitro* release for a period of 16 days. *In vivo* release studies showed that drug concentrations were maintained above the MIC value for the entire period of the release studies. The samples from this study were capable of inhibiting the growth of most test strains. The combination of amoxycillin and metronidazole in the carrier polymer poly (L-lactide-co-glycolide) not only showed an extended spectrum of antimicrobial activity but also showed a synergistic effect against *E. limosum*, which had been reported the resistant to metronidazole in earlier studies. Stability studies were conducted on the optimized formulation and the degradation rate constant was found to be 4.6×10^{-4}/day for metronidazole and 4.8×10^{-4}/day for amoxycillin. The drug was released locally and had a 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 present a better alternative.

1. 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 a periodontal pocket (lesions between teeth and junctional epithelium) (Heasman and Seymour 1994). The 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 an 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 a low benefit to high risk ratio (Gordon and Walker 1993; Heasman and Seymour 1994). 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 the periodontal pocket, release antimicrobial agents above the minimum inhibitory concentration for a sustained period of time. Thus intrapocket devices have a high benefit to low risk ratio (Pandit 1997). Combination therapy based on amoxycillin and metronidazole in conventional dosage forms has been widely investigated in clinical dental practice due to its activity against a wide range of anaerobes, facultative and aerobic bacteria. The combination of amoxycillin and metronidazole has synergistic action and covers a wide range of microflora, with metronidazole inhibiting the anaerobes and amoxycillin inhibiting the facultative aerobic bacteria. Both the drugs are bactericidal in nature and are administered systemically. This is important for complete elimination of subgingivally occurring periodontal pathogens (Schwach-Abdellaoni et al. 2000).

Poly (L-lactide-co-glycolide) is one of the most popular and well characterized biodegradable polymeric materials for use in controlled drug delivery. This polymer degrades slowly by hydrolysis to lactic acid and glycolic acid, which are body metabolites. It is biocompatible and produces little or no local and systemic toxicity on administration (Gordon and Walker 1993). Copolymers of lactide and glycolide have better acceptance as they may be tailored for any properties particularly bio-degradation kinetics.

In the present study our objective was to formulate biodegradable intrapocket dental films, which could be easily placed into the periodontal pocket, and be capable of deli-

Table 1: Formulae for drug loaded films

Formula code	Polymer I	Polymer II	Dichloromethane (ml)	Plasticizer (0.2 ml)	Mean surface pH
A-I	HPMC-E15 (1.3 g)	–	10	Diethyl phthalate	5.63 (± 0.023)
A-II	HPMC-E15 (0.8 g)	HPC-M (0.4 g)	10	Diethyl phthalate	6.08 (± 0.025)
A-III	HPC-L (0.8 g)	PLA (0.3 g)	10	Propylene glycol	5.53 (± 0.049)
A-IV	PLGA (0.75g)		10	Diethyl phthalate	6.04 (± 0.069)

vering therapeutic concentrations of amoxycillin and metronidazole for a prolonged period of time at a much lower dose, hence obviating untoward side effects. The antimicrobial activity of biodegradable dental films containing a combination of amoxycillin and metronidazole along with poly (L-lactide-co-glycolide) was also investigated against periodontal pathogens commonly found in periodontal infections. These include *Bacteroides melaninogenicus*, *Bacteroides oralis*, *Bacteroides fragilis, Peptostreptococcus assacharolyticus, Peptostreptococcus* species, *Eubacterium limosum, Propioniobacterium acnes*, *Staphylococcus aureus* and *Escherichia coli.*

2. Investigation and results

The presented study was an attempt to develop a low dose biodegradable intrapocket device of amoxycillin and metronidazole for the treatment of periodontitis. The combination formulated was a biodegradable 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 a combination of drugs have been reported so far. This is the first time that an attempt was made to formulate a delivery system containing drugs effective against aerobes and anaerobes. Poly (L-lactide-co-glycolide) (PLGA, 85 : 15) was the carrier polymer in the intrapocket device. Films based on the biodegradable polymer poly (L-lactide-co-glycolide) (PLGA, 85 : 15) exhibited sustained release for a period of sixteen days. The optimized formulation contained 69.29 mg each of amoxycillin and metronidazole and films were 4.2 cm in diameter. From these films, slabs and cones of 1 cm side were punched out. Each carried 5 mg each of amoxycillin and metronidazole. 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 a decrease in the frequency of administration. The device being biodegradable released the drug in a sustained manner leaving no undisintegrated residual fragments. This was a significant achievement, as patients did not have to remove the device after predetermined time intervals. The films were white, smooth, non sticky, homogenous and flexible.

2.1. 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)	± S. D.	Range (mg)	Maximum Variation from average (%)
23.50	0.329	23.20–24.00	2.12

2.2. 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 a surface pH of 6.93.

2.3. In vitro release rate studies

Formulation A-I gave a release of 25.72% of metronidazole and 55.28% of amoxycillin in 8 days of study. Formulation A-II gave a release of 74.96% of metronidazole and 50.13% of amoxycillin in 8 days of study. Formulation A-III gave a release of 46.18% of metronidazole and 42.93% of amoxycillin in 8 days of study. The optimized formulation (A-IV) exhibited maximum drug release of 26.09% for metronidazole and 72.38% in case of amoxycillin in 16 days of study. Therapeutic drug concentrations were maintained for a period of 16 days (Fig. 1). Release of metronidazole 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. Similarly *in vitro* release of amoxycillin followed first order kinetics since the coefficient of variance for first order kinetics is less than that of zero order kinetics. A plot of percentage of drug released versus square root of time was linear for longer than seven days. The release profile of drugs dispersed from films can be treated using the Higuchi equation for diffusion-con-

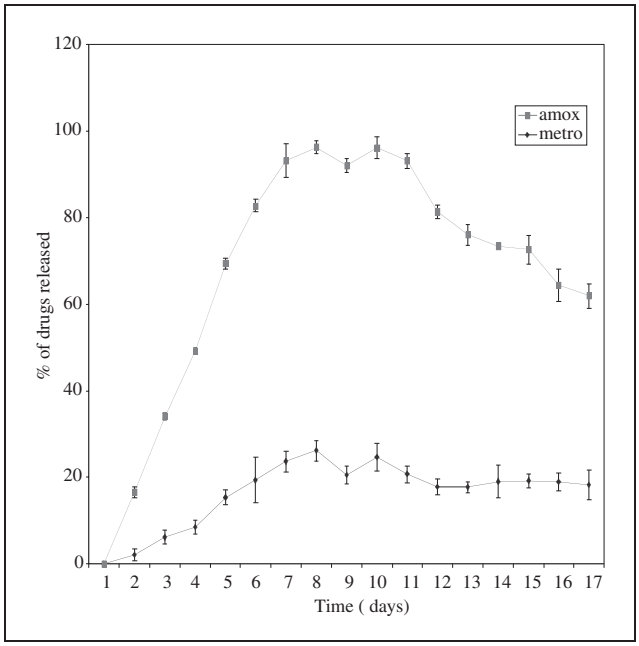

Fig. 1: *In vitro* release profile of optimized film

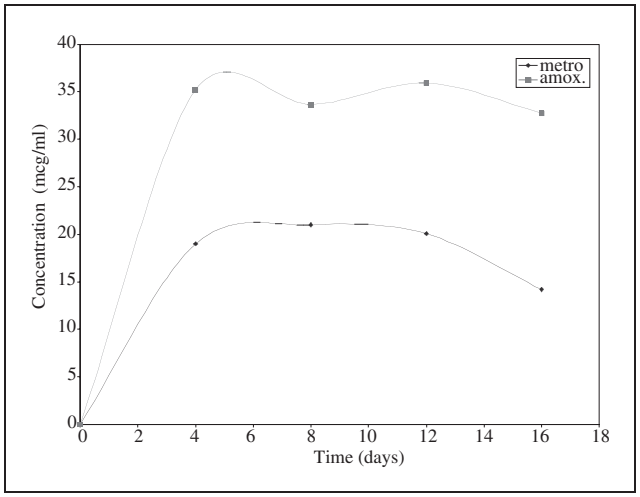

Fig. 2: *In situ* release profile of optimized film

trolled 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

$$Q = \frac{\varepsilon D(\tau A - \varepsilon Cs)\, Cst^{1/2}}{2\tau}$$

The correlation obtained between Q and $t^{1/2}$ indicated that the release was diffusion controlled for the initial 7 days and this was followed by a sustained release profile over the remaining duration.

2.4. In situ release rate study

An *in situ* release study revealed that the concentration of amoxycillin and metronidazole released was maintained well above its minimum inhibitory concentration over a period of sixteen days (Fig. 2).

2.5. Surface characteristics

Surface Electron Microscopy revealed that the drugs appeared as white specks on the surface of the carrier matrix in the case of the optimized film (Fig. 3). The placebo film showed no such specks (Fig. 4). The surface characteristics of film from which drug had been released were also studied, showing pore formation in the polymer matrix indicating release of the drug (Fig. 5). SEM indicated that the films had a smooth surface prior to drug release while after release surface irregularities were evident.

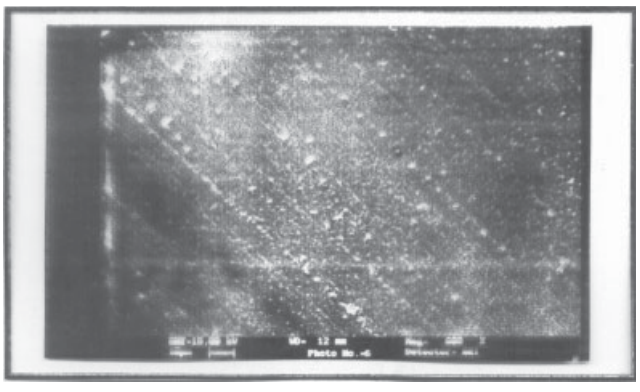

Fig. 3: Surface characteristics of drug loaded film

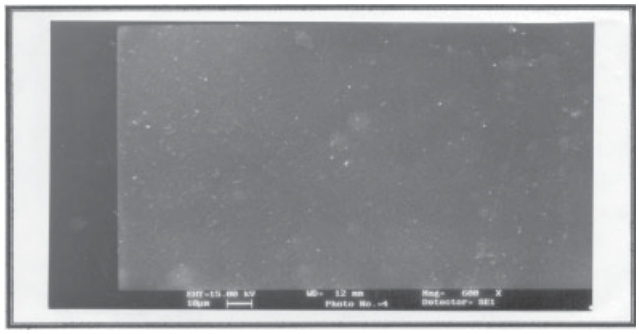

Fig. 4: Surface characteristics of placebo film

Some irregular pores were also seen. Formation of pores in the PLGA matrix indicated that the release of amoxycillin and metronidazole started with dissolution of the drugs and subsequently proceeded by diffusion through the pores.

2.6. Microbiological studies

The agar dilution technique revealed that the films were effective in inhibiting the growth of all the anaerobic strains after 72 h. However, poor inhibition was seen from the hydrophobic PLGA matrix. The *in vivo* release study samples were successful in inhibiting all the strains except *E. limosum*. Various dilutions of stock solutions were effective against all strains except *B. fragilis* and *E. limosum*. In the case of the broth dilution technique, the films were found to effectively inhibit all the test strains. However, in situ samples and stock solution dilutions were unable to inhibit the growth of *E. limosum. Eubacterium* and *B. fragilis* were found to be resistant even to the 5 μg metronidazole disk used in the AIIMS laboratory. However, the drug-loaded films were successful in inhibiting *Eubacterium limosum*, which has been reported to be resistant to metronidazole (Slots and Rams 1990). Thus, it can be concluded that an *in vivo* synergistic effect of amoxycillin and metronidazole was responsible for the activity against *Eubacterium limosum*. The cylinder plate method was used to test 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.

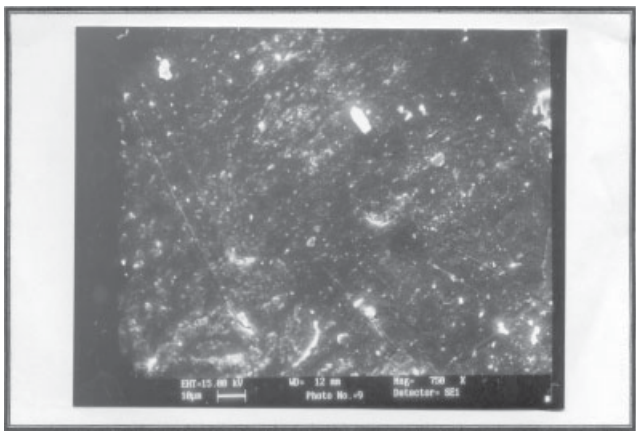

Fig. 5: Surface characteristics of film from which drug has been released

2.7. Stability studies

Stability studies on the films showed that no significant changes occurred in the ir physico-chemical properties at 40 ± 0.5 °C and $75 \pm 5.0\%$ R.H. The degradation rate constant was found to be 4.606×10^{-4}/day for metronidazole and 4.836×10^{-4}/day for amoxycillin. Since this value is very low, a tentative shelf life of 24 months was given to the formulation. No discernable change in physical appearance was seen in the samples. All films were white, smooth, non-sticky and flexible after the stability studies.

3. Discussion

In the present study an attempt was made to prepare a targeted retentive low-dose drug delivery device containing a combination of amoxycillin trihydrate and metronidazole, which could be applied directly to the periodontal pocket. Webber et al. 1997 prepared films containing tetracycline based on poly (L-lactide-co-glycolide). In that study the tetracycline drug loading was too low to reach the MIC but the drug was released from both sides within 24 h.

In the present study we attempted to load amoxycillin and metronidazole in the polymeric material for use in periodontal infections and characterize the films prepared. 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 16 days. Characterization of the films 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. The 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 its low benefit to high-risk ratio. Hence the low-dose site-specific films present a better alternative. The films had biodegradable characteristics thereby obviating the need to remove any undisintegrated device at the end of treatment. We also plan to study the effect of our device in patients after obtaining permission from the ethics committee.

4. Experimental

4.1. Materials

Amoxycillin and metronidazole (lot #.M 10521601) were donated by Lark Lab (India) Ltd., New Delhi, India. PLGA, 85 : 15 (Purasorb® lot# DL 672 FN) and PLA were obtained from PURAC Biochem BV. Boric acid, sodium hydroxide, dichloromethane, and 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.

A Shimadzu model 1601 UV/Visible spectrophotometer, with spectral bandwidth of 2 nm 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.

The 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 AIIMS and Majeedia Hospital, New Delhi, India.

4.2. Fabrication of dental films by dispersion method (Agarwal et al. 1993)

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 desired characteristics were loaded with the drug and were used in 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 0.75 g of poly (L-lactide-co-glycolide) in 10 ml of dichloromethane containing 2.0% diethyl phthalate as plasticizer. 146.18 mg each of amoxycillin and metronidazole were sieved through 80 to 120 mesh and homogenously dispersed in the polymer solution by vortexing for 5 to 10 min. Films were cast by pouring this dispersion into a glass ring placed over aluminum foil. The films were initially dried at a temperature of −10 to −5 °C for 8 to 10 h and then at 25 °C for a further 20 to 24 h. After drying, the films were cut into slabs of 10 mm size.

4.3. Weight variation

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

4.4. Content uniformity

A simple, accurate and reproducible method for the simultaneous estimation of amoxycillin and metronidazole in combined dosage forms was developed. The method involved analysis in multi-component mode by UV spectrophotometer (Rahman et al. 2004). Standard solutions of amoxycillin trihydrate (400 μg/ml) and metronidazole (100 μg/ml) were prepared in alkaline borate buffer pH 8.1. From this standard solution, dilutions containing 10 μg/ml of amoxycillin trihydrate and 10 μg/ml of metronidazole were prepared, scanned in the spectrum mode from 200–400 nm and the overlain spectra was recorded (Fig. 6). From the spectra it was observed that wavelengths that could be utilized for simultaneous analysis of amoxycillin and metronidazole, using the multi-component mode were 272 nm and 320 nm respectively. Film samples consisting of amoxycillin trihydrate and metronidazole were placed in 10 ml acetone to dissolve the polymer PLGA. The volume was made up to 100 ml with alkaline borate buffer of pH 8.1. Aliquots of sample solution were diluted to give a final concentration of 10 μg/ml of each drug. Four mixed standards of pure drug containing 40, 80, 120 and 160 μg/ml of amoxycillin and 4, 8, 16 and 20 μg/ml of metronidazole were prepared. These were scanned between 400 to 200 nm. The sampling wavelength (272 nm and 320 nm) and the concentration of

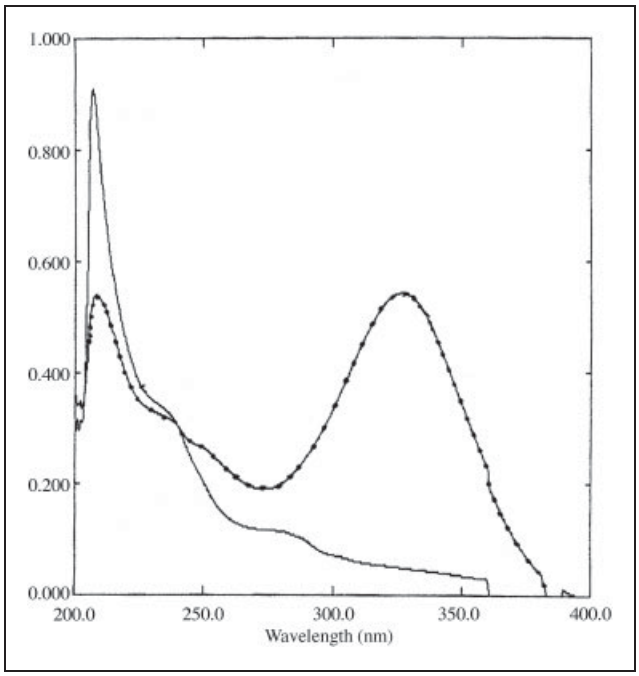

Fig. 6: Overlain spectra of amoxycillin and metronidazole

the two components in each of the mixed standards were fed into the multi-component mode of the instrument. The concentrations of amoxycillin trihydrate and metronidazole in the sample solution were determined by using spectral data of mixed standards from the spectrophotometer.

4.5. Surface pH

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

4.6. In vitro release rate study

The release of drugs from the 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). 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. Samples 3 ml were intermittently withdrawn and analyzed for amoxycillin trihydrate and metronidazole using the mutli-component mode of the Shimadzu UV-1601 spectrophotometer. The volume of medium withdrawn was replaced by adding 3 ml buffer to the flask.

4.7. In situ release study

An *in situ* release study was performed using a flow-through apparatus, which simulated *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 15 ml/day, using a peristaltic pump. Since GCF is an inflammatory exudate, its flow rate increases to 3.5 ml/day or more (Ahuja et al. 2003). Buccal mucosa was cut into strips (1.5 cm long and 1.5 cm wide), placed in the central cavity of the cell and 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 10 g for 30 s. (Ali et al. 2002). 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 no. 42 filter paper and analyzed using the multi-component mode of the UV-1601 spectrophotometer.

4.8. 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 µm thick) under vacuum and microscopy was done using a LEO 435 VP, PC based digital scanning electron microscope.

4.9. Microbiological evaluation of drug loaded dental films

Biodegradable dental films containing amoxycillin and metronidazole were tested on aerobic and anaerobic strains commonly found in periodontitis. These included the obligate anaerobes *Bacteroides melaninogenicus*, *Bacteroides oralis*, *Bacteroides fragilis, Peptostreptococcus assacharolyticus, Peptostreptococcus* species, *Eubacterium limosum* and *Propioniobacterium acnes*. Aerobic organisms tested were *Staphylococcus aureus* and *Escherichia coli* (Ahuja et al. 1998; Ahuja et al. 2003a, 2003b).

For this purpose methods were developed based on modifications of the following standard techniques: agar dilution method, broth dilution method, cylinder plate method. Agar dilution and broth dilution methods were used for anaerobic organisms and cylinder plate method was used for aerobic organisms.

Mueller Hinton agar was used for aerobes and Brain Heart Infusion agar and broth were used for culturing the anaerobes. 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.

4.10. 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 petri dishes lined internally with aluminium foil. These petri dishes were then placed in desiccators containing a saturated solution of sodium chloride in order to maintain a relative humidity 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 the mobile phase in a glass pestle and mortar. The solution was than filtered through a millipore membrane filter (0.45 µm). For analysis of metronidazole, methanol, acetonitrile and potassium dihydrogen phosphate (0.005 M) in the ratio of 4 : 3 : 93 was used as the mobile phase. Flow rate was adjusted to 2.0 ml/min. For analysis of amoxycillin, the mobile phase used was methanol, phosphate buffer of pH 6 and water (HPLC) mixed in the ratio of 15 : 1 : 84. Flow rate was adjusted to 1 ml/min. The column used was µ-Bondapack C18, 10 µm (30 cm × 4 mm). Detection was performed at 320 nm for metronidazole and 272 nm for amoxycillin. Concentration of the drug was calculated from the calibration curve of the pure drug (Abounassif et al. 1991).

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