Research Article

Development and Evaluation of Novel Site Specific Periodontal Film of Minocycline Hydrochloride for Periodontal Diseases

Indira Raheja*[1], Sushma Drabu[1], Kanchan Kohli[2]
*[1]Maharaja Surajmal Institute of Pharmacy, Janakpuri, New Delhi, India.
[2]Department of Pharmaceutics, Jamia Hamdard, New Delhi, India.
*Corresponding author's E-mail: indu.raheja@gmail.com

ABSTRACT

Novel drug delivery system for the treatment of periodontitis was developed for site-specific delivery of Minocycline hydrochloride which has excellent activity against anaerobic microorganisms. Minocycline films were prepared by solvent casting technique using ethyl cellulose, HPMC K4M and EudragitRL100 as copolymer in chloroform: ethanol (1:1) solvent with di-butyl phthalate and PEG 400 as plasticizers. FT-IR and UV spectroscopic methods revealed no interaction between Minocycline hydrochloride and polymers. The films were evaluated for their thickness uniformity, folding endurance, weight uniformity content uniformity, surface pH, and *in vitro* antibacterial activity. *In vitro* release from periodontal films was fit to different equations and kinetic models like zero order, first-order equations and Hixson-Crowell, Higuchi models and korsmeyer peppas model to reveal drug release kinetics. The x-ray diffraction studies of the drug and the film showed that the classical peaks of the drug were depressed in physical mixture and more in the formulation. SEM studies indicated complete embedding of the drug in the film. The Formulation A6 released 91.37 % of drug at the end of seventh day and was considered as best formulation. The optimized formulation showed good antibacterial properties against *S.aureus* (MMTC3160) and *E.coli* (MMTC448). Ageing studies shows that the drug remained intact and stable in the periodontal films during storage. A short-term stability study shows that drug content decreased in various films and was ranging from 0.9% to 3.41%.

Keywords: *In vitro* release, Novel Drug Delivery system, Periodontitis, Periodontal film.

INTRODUCTION

Periodontal disease is a localised inflammatory response due to infection of a periodontal pocket arising from the accumulation of plaque. This produces an inflammatory response in adjacent tissues and these diseases may be broadly classified according to the extent of periodontal tissue involvement. The inflammatory response is confined to gingival in gingivitis but extends to deeper tissues in periodontitis. Progression of periodontitis results in loss of tooth support structure, increase pocket depth, clinical attachment loss, and destruction of alveolar bone.[1] The role of bacteria in the aetiology of these diseases has been well established. The bacteria accumulate in the space (or pocket) that develops between the roots of affected teeth and the soft tissues. Conventional methods for the removal of subgingival bacteria include periodic mechanical debridement of plaque from tooth surfaces and repeated topical or systemic administration of antibacterial agents.[2-6] The effectiveness of these conventional treatments is limited by the lack of accessibility to bacteria in the periodontal pocket. Systemic administration has been shown to achieve therapeutic concentrations at the site of infection.[7-13] These concentrations are, however, usually maintained for short periods of time after a single dose and the doses employed are capable of producing systemic side effects.[14] Generally, systemic administration is recommended for treatment of rapidly progressing or refractory periodontitis.[14-16] Pitcher et al.[17] showed that a plaque disclosing agent administered as a mouth rinse did not penetrate into periodontal pockets, indicating that this method of delivery is not suitable for the treatment of sub gingival infections. Another approach has been to administer antibacterial solutions directly into the periodontal pockets using specialised irrigating devices. This method of delivery has been reviewed by Greenstein[18] provides a natural reservoir bathed by gingival cervicular fluid which is easily accessible for the insertion of a delivery device. The gingival cervicular fluid provides a leaching medium for the release of a drug from the solid dosage form and for its distribution throughout the pocket. These features, together with the fact that the periodontal diseases are localized to the immediate environment of the pocket, make the periodontal pocket a natural site for treatment with local sustained-release drug delivery systems. The duration of action is generally short when antibacterial agents are administered in solution, and frequent application is required to maintain effective concentrations in the periodontal pocket. This makes patient compliance critical to ensure optimal clinical efficacy. Because of the shortcomings associated with the above methods of delivery, attention has focused on the development of prolonged release intra pocket delivery systems. As the average depth of a periodontal pocket is between 6 and 8 mm, the therapeutic drug delivery device therefore should be small and should not exposed beyond gingival margin when inserted in the periodontal pocket. Further, it is necessary that a small dosage of the active agent in the

device should be highly effective as a therapeutic agent. Ideally, these systems should deliver the antibacterial agent for prolonged periods to the affected pocket(s) at levels in excess of the minimum inhibitory concentration for the causative organisms Minocycline is a semi synthetic derivative of tetracycline. It has broader spectrum of activity than other members of this group. Minocycline is used primarily to treat acne and other similar skin diseases, but as it also has the broader spectrum therefore it also acts against periodontal pathogens. Minocycline-loaded microcapsules have been studied in periodontal treatments.[19-21] It has been effectively used for treatment of periodontitis and related infections in periodontal diseases.[22-23] The major advantage of Minocycline is its anticollagenase properties and ability to reduce soft tissue destruction and bone resorption which is very important in the treatment of periodontal disease. In addition, Minocycline is a good candidate for local antibiotic delivery.[24-25] Minocycline is available in the market as a conventional dosage forms such as tablets and capsules for the treatment of bacterial infections and in form of microsphere for the local antibacterial treatments. So the objective was to develop periodontal films containing Minocycline with rate controlling polymers, which has a prolonged action and shows the antibacterial activity directly at the site of infection without loss of dosage.

MATERIALS AND METHODS

Materials

Minocycline HCL was obtained as gift sample from Ranbaxy laboratories Limited, Gurgaon, India. Ethyl cellulose was obtained from Loba Chemie Pvt Ltd, Mumbai., India. Hydroxy Propyl Methylcellulose (HPMCK4M) and Eudragit RL-100 were obtained as gift sample from Ranbaxy Research lab Gurgaon, India. Other materials used in the study were of analytical grade.

Methods

Drug- Polymer compatibility studies

Pure drug (Minocycline hydrochloride) and polymers were subjected to FT-I.R studies alone and in combinations. For the study 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 analysed by FT-IR (Shimadzu co., Japan) to study the interference of polymers with the drug.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) analysis was performed for pure drug, ethyl cellulose (EC) alone and with HPMCK4M and eudragitRL-100 as copolymer in a physical mixture using a DSC, Shimadzu instrument. Physical mixture (1:1) of drug and excipients were mixed thoroughly for five minutes in mortar. Each sample was accurately weighed (~1-3 mg) in an aluminium pan which was crimped and hermetically sealed, similarly an empty pan of the same type was used as a reference. The samples were scanned at the heating rate of 200ºC/min over a temperature range of 100 to 300ºC under the nitrogen atmosphere. The DSC graph was obtained.

Preparation of cast film

Periodontal films were prepared by solvent casting technique. Films were prepared by dissolving ethyl cellulose (EC) and hydroxy propyl methyl cellulose (HPMCK4M) alone and with copolymers eudragit RL-100 (EU), in chloroform and ethanol (1:1) solution, using di butyl phthalate or polyethylene glycol (PEG400) as plasticizer (Table 1). Minocycline was added in to the polymeric solution and mixed homogenously using magnetic stirrer in a closed beaker. After complete mixing the solution was poured into the clean glass moulds. 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. Two batches A and B were prepared using 15% and 20%w/w of Minocycline HCL (MH) with that of dry weight of polymer (Table 1). After complete evaporation of solvent, cast films were obtained, which were then cut into pieces of 5×5mm wrapped in an aluminium 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[26]

Thickness of the film was measured using screw gauge at different areas of the film and the average thickness was calculated.

Uniformity of weight of the films[27]

Film (size of 5×5 mm^2) was taken from different areas of film. The weight variation of each film was calculated.

Surface pH[26]

Periodontal films were left to swell for 1 hour on the surface of the agar plate, prepared by dissolving 2% (w/v) agar in warm double distilled water with constant stirring and then poured into the Petridis 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.

Viscosity

Polymeric 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 number18. The recorded values were mean of three determinations.

Folding endurance[28]

The folding endurance of the films was determined by repeatedly folding the film at the same place up to 200 times till it broke or folded, which is considered satisfactory to reveal good film properties. This test was carried out on all the films.

Drug content uniformity of films

Triplicate sample from film from each batch (size of 5×5 mm^2) was taken from different areas of the film and placed into a 10 ml volumetric flask, in to which 10 ml of methanol was added and flask was vigorously shaken till the film is completely dissolved in order to extract the drug. Then 1 ml resulting solution was diluted to 100 ml with phosphate buffer saline. The absorbance of the solution was measured using spectrophotometer at 248.5nm. The polymeric solution without drug served as blank. In case of HPMC films combination of water and methanol was used to dissolve the films and rest procedure was same. The drug content was studied in triplicate and mean was reported.

Table 1: Composition of periodontal films

Film codes	Drug (mg)	Ethyl cellulose (mg)	HPMCK4M (mg)	EudragitRL100 (mg)	PEG 400 (ml)	Dibutylphthalate (ml)
A1	225	---	1500	---	1.0	---
A2	225	---	1350	150	1.0	---
A3	225	---	1200	300	1.0	---
A4	225	1200	300	---	---	0.75
A5	225	1200	---	300	---	0.75
A6	225	1350	150	---	---	0.75
A7	225	1350	---	150	---	0.75
A8	225	1500	---	---	---	0.75
B1	300	---	1500	---	1.0	---
B2	300	---	1350	150	1.0	---
B3	300	---	1200	300	1.0	----
B4	300	1200	300	---	---	0.75
B5	300	1200	---	300	---	0.75
B6	300	1350	150	---	---	0.75
B7	300	1350	---	150	---	0.75
B8	300	1500	---	---	---	0.75

Table 2: Data of thickness, weight variation, surface pH, % moisture loss of the formulations A1-A8 and B1-B8

Periodontal Film code (PF)	Thickness (mm) Mean ± S.D (n=3)	Weight uniformity (mg) Mean± S.D (n=3)	Surface pH	% Moisture loss Mean ± S.D* (n=3)
A1	0.29 ± 0.04	10.32± 0.15	6-7	11.03 ± 0.22
A2	0.33 ± 0.05	10.35±0.12	6-7	10.94 ± 0.54
A3	0.34 ± 0.02	10.39±0.05	6-7	10.15 ± 0.33
A4	0.39 ± 0.01	10.48±0.04	6-7	09.45 ± 0.21
A5	0.38 ± 0.05	10.43±0.05	6-7	09.25 ± 0.43
A6	0.35 ± 0.04	10.45±0.02	6-7	07.86 ± 0.37
A7	0.37 ± 0.06	10.44±0.03	6-7	09.55± 0.27
A8	0.41 ± 0.02	10.51±0.03	6-7	08.84 ± 0.35
B1	0.29± 0.07	10.45+0.12	6-7	13.67± 0.56
B2	0.35± 0.01	10.48 ±0.05	6-7	11.43±0.24
B3	0.36 ± 0.03	10.53±0.03	6-7	10.79 ± 0.21
B4	0.37 ± 0.02	10.58±0.03	6-7	10.45 ± 0.42
B5	0.41 ± 0.05	10.61±0.04	6-7	08.24 ± 0.32
B6	0.38 ± 0.03	10.60±0.02	6-7	08.89 ± 0.13
B7	0.39 ± 0.05	10.55±0.06	6-7	09.72 ± 0.28
B8	0.42 ± 0.02	10.62±0.04	6-7	07.64 ± 0.33

In vitro drug release[29]

Static dissolution method reported in the literature was adopted. Films of known weight and dimensions (size of 5×5 mm^2) were placed separately into small test tubes with closure containing 1 ml isotonic phosphate buffer saline (pH 7.4).The test tubes were sealed with aluminium foil and kept at 37°C for 24 hours. One ml of the isotonic phosphate buffer saline was withdrawn from 1st to 7th day and immediately replaced with 1 ml fresh IPBS . The drug content was estimated by measuring the absorbance using UV spectrophotometer (Shimadzu-UV1700, Japan).

In vitro antibacterial activity[30-31]

The films (size of 5×5 mm^2) containing of drug were taken for the antibacterial studies Nutrient agar medium was prepared and sterilized by autoclaving under aseptic condition and the medium was transferred to sterile Petri plates. After solidification of the nutrient agar medium, a lawn culture was prepared with 0.1 of bacterial stain i.e. *S. aureus* (MMTC 3160) and *E. coli* (MMTC448) in separate Petri plates. The films (0.5cm^2) were placed over the medium and plates were incubated for 48 hours at 37°C. Zone of inhibition was measured after incubation of the films. Then the films were transferred onto freshly seeded agar plates for an additional 48 h for incubation. The zone of inhibition area on the agar plate was measured and the procedure was repeated after 96hrs and 144hrs.

Surface characterization of films by SEM

A Scanning electron microscope (Zeiss model EVO 40) was used to study the surface characteristics of the films before and after dissolution. Films were sputter coated with gold (30 nm thick) under vacuum and sample were stored in desiccator until studied on a field emission scanning electron microscope at 5.0kv with working distance of 8 to 10 mm. Scans was taken at 500X magnification and shown in figure 5.

Release kinetics studies

In order to study the mechanism and kinetics of drug release, the data of *In vitro* drug release studies were fitted in various release kinetic equations[13] such as zero order, first order, Hixon Crowell model, Higuchi matrix model and Peppas-Korsmeyer equation and the best fit model was determined..

### X-ray Diffraction analysis

For the x-ray diffraction studies of drug and formulation were submitted for analysis to the X-ray Diffraction Studies center, at the Advanced Instrumentation Research Facility (AIRF) Jawaharlal Nehru University, New Delhi. With the diffraction system (X Pert Pro model, P analytical) a slow step scan mode (0.02 degree per 5 second) was used to include the most informative range of the diffraction pattern for drug, drug and polymers and the film.

Ageing

Optimized medicated films were subjected to stability testing. The stability of the film was studied at 40°C± 5°C with RH 75%± 5%. The periodontal film of size (5×5) were weighed and wrapped in aluminium foil and placed in petri plates. These plates were stored for a period of three months. All the films were observed for any physical changes such as colour, appearance, flexibility, or texture. The drug content and *In vitro* drug release was estimated at an interval of each month. The data presented were the mean of three determinations.

RESULTS AND DISCUSSION

Drug polymer interaction plays a vital role with respect to release of the drug from the formulation. The drug–polymer interactions was ruled out as there were no major shifts in the absorption bands (peaks) of Minocycline HCL (MH)with polymer combinations with HPMCK4M and ethyl cellulose. Differential Scanning Calorimetry has been employed to access any possible interaction between drug and polymer. The DSC thermogram for pure drug, drug + ethyl cellulose + HPMC was determined and shown in figures 1. The DSC thermogram of Minocycline with polymer exhibited an endothermic peak at 202.715º. A slight shift in Minocycline peak in the thermograph of drug film could be due to the presence of moisture in the film. Considering the compatibility of Minocycline with polymer it was used for further preparation of various periodontal films.
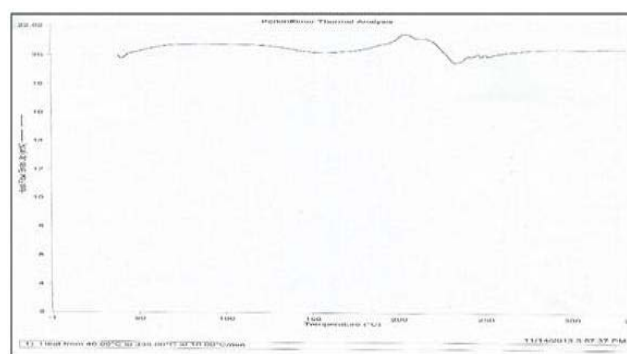

(A)
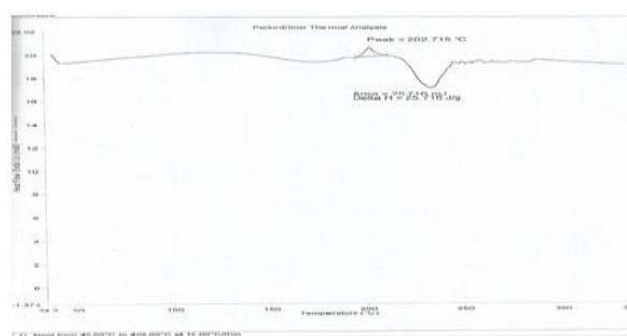
(B)

Figure 1: (A) DSC thermogram of Minocycline HCL (MH) (B) DSC thermogram of MH+EC+HPMCK4M

The percentage drug content in various formulations ranged from 91.56-98.30%. The viscosities of the solutions were ranging from 16.54-28.34 cps for films in the two batches A and B. Viscosity of the polymeric solution A8 containing ethyl cellulose was more when compared to other films which could be due to complete solubility of polymers in chloroform and ethanol (1:1) mixture. It was observed from the drug content data that there was no significant difference in the uniformity of the drug content. However, when compared with the theoretical drug content the estimated drug content was slightly less; it is the indication of drug loss during fabrication of the films. The films had uniform thickness throughout with low value of standard deviation given in the table 2. The ranges of thickness of the various films were found to be 0.29±0.04-0.42±0.02. The order of thickness of the film codes was A1<A2<A3<A6<A7<A5<A4<A8 and in the batch II the order was B1<B2<B3<B4<B6<B7<B5<B8. Moisture loss studies were conducted on all the formulations and reported in table 2 and table 3. Moisture loss was found to be in range of 7.64 ± 0.33 and13.67 ± 0.56 and it was observed that formulation B1 and A1 showed maximum amount of moisture loss because of more concentration of hydroxyl propyl methyl cellulose K4M undergoing moisture loss in dry condition. Formulation A8 and B8 showed minimum percentage moisture loss in their respective set because of hydrophobic ethyl cellulose. The formulations were found to be of uniform weight, ranging from 10.45 ± 0.12mg to 10.67± 0.07 mg. The surface pH of all the films was found to be in between 6-7 and hence no periodontal pocket irritation is expected. Folding endurance of the most of the films was > 150 times indicate that the formulations have good film properties.. The tensile strengths of drug loaded film were found to be higher than plain (dummy) films. This is because of dissolved drug strengthened the bonding of polymer chains. The results are also in agreement with the viscosity determinations. The cross linking was observed on addition of eudragit RL 100 as a copolymer, which also shows higher tensile strength when compared to all other formulations.

In vitro release studies of Minocycline was carried out in isotonic phosphate buffer saline for seven days which showed that there was an abrupt release observed on first day, and there after the release of drug was found to be controlled. Overall release of drug from the polymeric films was in range of 76.45%-92.33% (Figure 3 and 4). Complete release of MH was not obtained as drug particles were entrapped within the hydrophobic ethyl cellulose matrix. Average amount of drug release per day was found to be above the minimum inhibitory concentration of Minocycline (MIC ≤ 8µg/ml). Film code A1 and B1 (HPMC films) released the drug for three days and film code A2, B2 for four days and A3, B3 for five days which may be due increase in the concentration of co polymer eudragit in these films. As ethyl cellulose is more hydrophobic zit releases the drug more slowly therefore

ethyl cellulose films A4-A8 and B4 B8 released the drug for seven days. Similar release profile was observed for B set of formulation as shown in graph. Further it was also observed that on increasing the concentration of Minocycline in the formulations, there was slight increase in drug release. The film was studied for drug release order and for drug release kinetics studies. The release kinetics indicated zero order release from most of the films. Hixson-Crowell cube root law and Higuchi's model were applied to study the release mechanism. R^2 values were found to be higher for Higuchi's model as compared to Hixson–Crowell for all the films. Hence Minocycline release from all the films followed diffusion rate controlled mechanism. Study of Korsmeyer-Peppas model showed that the film codes A2-A8 and B2 - B8 indicated non fickian transport and the mechanism of transport was anomalous transport and Film code A1 andB1 showed n values more than one which indicated case II transport mechanism which may be due to a higher initial burst release from these formulations.
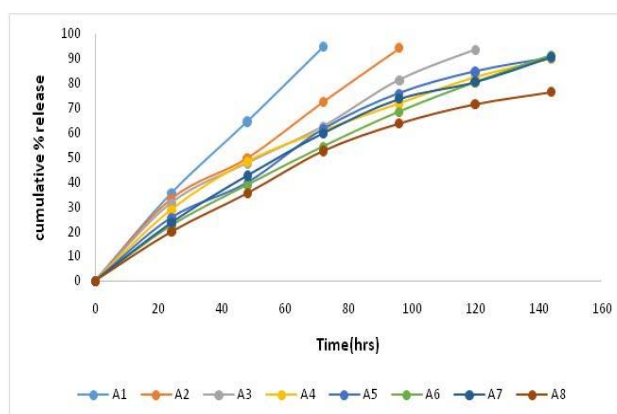

Figure 2: *In-vitro* drug release of periodontal film codes A1-A8
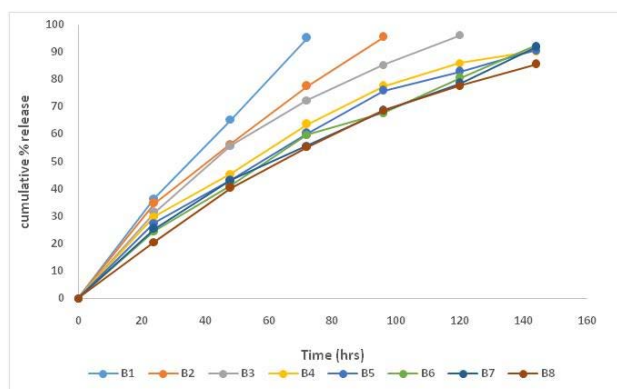

Figure 3: *In-vitro* drug release of periodontal film codes B1-B8

The x-ray diffraction profile of drug showed the classical peaks indicating the crystalline nature of drug and polymer mixture with drug indicated more amorphous nature of the drug. The classical peaks of the drug were depressed in physical mixture and more in formulation. Further no major difference in the diffractogram of physical mixture and the drug was observed as shown in Figure 4. Scanning electron micrographs indicated that

the drug was completely embedded in the polymer matrix prior to drug release (Figure 5). SEM study also indicated that the top surface of the films have a smooth surface whereas bottom surface exhibits pores. This might be due to the reason that solvent when evaporates, takes some amount of drug along with it to towards the top surface of the film. More over these pores are more evident in films that have higher drug loading.

(A)　　　　　　　　　(B)
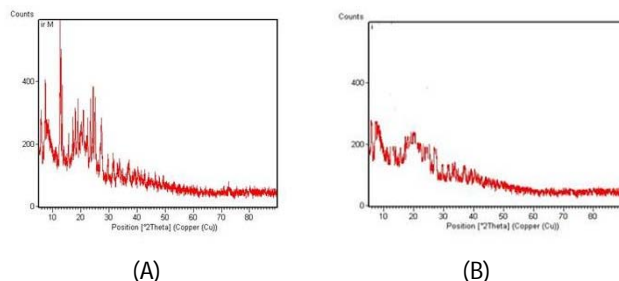
Figure 4: (A) X-ray diffraction study of Minocycline hydrochloride. (B) X-ray diffraction study of periodontal film.
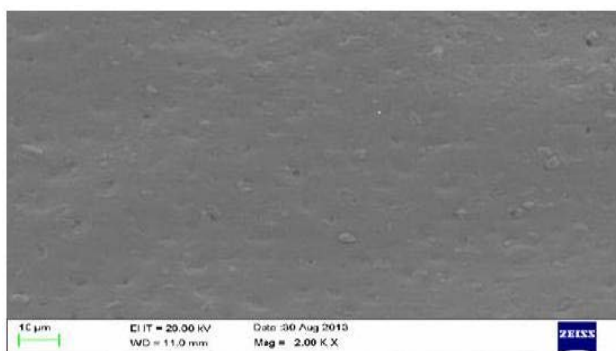
Figure 5: SEM of Minocycline film (A6)

In vitro antibacterial activity was performed as mentioned in the methodology using *S.aureus* (MMTC3160*)* and *E.coli* (MMTC448) microorganisms. The zone of inhibition of the prepared formulations was found to be effectively higher in 48 hours and then declined at 144 hrs. The study indicated that the formulated polymeric films containing Minocycline retained their antibacterial activity for six day above MIC. Periodontal film (A6) was found to be best formulation and look promising for the treatment of periodontitis. Ageing studies performed on all prepared periodontal films indicate that decrease in the drug content from the films ranged from 0.90% to 3.41%. It was found that the drug loss is less during the storage for three months. The films were also observed for their physical appearance and texture. It was noted that these properties did not change in films during the entire period of study.

CONCLUSION

The local delivery of antimicrobials in form of site specific periodontal films has opened up a new arena for the management of periodontal diseases. Efficacy of such delivery systems is dependent upon the sustain release of the drug from the system and its penetration into the

base of the periodontal pocket and adjacent connective tissue. In the study, Minocycline HCl films were successfully prepared using polymer ethyl cellulose and hydroxy propyl methyl cellulose. The films were smooth, homogenous, non-sticky and flexible. These films were able to sustain the release of drug for about one-week and showed good antimicrobial activity against test microorganism studied. Ageing studies shows that the drug remained intact and stable in the periodontal films during storage. Overall studies indicated that site-specific periodontal films having low dose of antibacterial drug and sustained effects are a better alternative to systemic therapy in treatment of periodontal diseases.

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