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Targeted retentive device for oro-dental infections: formulation and development

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Abstract

Fibers loaded with amoxycillin trihydrate were prepared for oro-dental infections using melt spinning technique. Ethylene vinyl acetate, a biocompatible polymer was used for providing controlled release effect over a period of several days. The fibers were evaluated for in vitro release in alkaline borate buffer pH 8.1 in a biological shaker which was rotated at 50 rpm at 37 °C. In situ studies were carried out in continuous flow through apparatus which simulated the conditions of periodontal pocket. Microbiological evaluation was carried out on strains commonly implicated in oro-dental infections namely *S. aureus*, *S. mutans*, and *Bacteroides cereus*. Results of in vitro release studies revealed that the effect was sustained over a period of 6 days and followed Fickian diffusion mechanism. In situ release study samples were well above the minimum inhibitory concentration of the drug. These samples were effective in inhibiting the growth of the above-mentioned strains. The optimized formulation was characterized for general appearance, content uniformity, and SEM. Stability studies carried out on the formulation showed the degradation rate constant value of 2.79×10^{-4} per day. Retentive fibers were found to be very effective in controlled delivery of amoxycillin, and hence can be feasible alternative to systemic administration.

Keywords: Retentive fiber; Periodontitis; Amoxycillin trihydrate; In vitro studies; In situ studies

1. Introduction

The oral cavity provides a diverse environment for colonization by a wide variety of microorganisms. Most localized or progressive oro-facial infections occur when pathogenic bacteria invade the surrounding tissues (William, 1990). Supragingival and subgingival plaque play an essential role in the causation of dental caries and periodontal disease, respectively (Ross, 1992). Control of bacterial plaque helps in slowing or arresting oro-dental infections. Conventional therapy has long sought the use of mechanical plaque control procedures, which are time consuming, require highly trained personnel to carry them out and result in varying amounts of discomfort to the patients (Heasman and Seymour, 1994). The bacterial composition of early plaque consists of mainly aerobic or facultatively anaerobic microorganisms, which later becomes colonized by strict anaerobes. Systemically applied antimicrobials have been advocated for the treatment of severe forms of oro-dental infections. However, systemic administration suffers

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from several disadvantages like hypersensitivity, gastrointestinal intolerance, and development of bacterial resistant strains. Moreover, adequate concentration at the site of action is not achieved as the drug gets diluted thousand fold times, thus leading to low benefit to high-risk ratio (Pandit, 1997). The use of amoxycillin trihydrate is most desirable since the drug is effective against facultative pathogens chiefly present at the sites where the infection initiates. At a later stage of infection the organisms are combination of anaerobic Gram-negative bacilli, facultative, and strict anaerobes chiefly *B. gingivalis*, *B. intermedius*, *P. gingivalis*, Fusobacterium, and Actinomyces species.

Several investigators have tried to treat oro-dental infections by local delivery of antimicrobials into the periodontal pocket. Each system tested had release half $t_{1/2}$ ranging from minutes to hours necessitating their replacement at regular intervals. In order to investigate the prolonged release effect a targeted sustained release device which could be inserted in the periodontal pocket and prolong the therapeutic levels at the site of action at a much lower dose is a need of today. The advantages offered by a targeted sustained release device include freedom from undesirable side effects to different parts of the body as the device is targeted to the periodontal pocket. Moreover, patient acceptability is high with such a system. The device can be placed within the periodontal pocket in a short time with minimal pain and discomfort and is therapeutically active at a much lower concentration (Goodson et al., 1985; Kornman, 1993; Schwach-abdellaoui et al., 2000).

The use of fibers was first introduced to deliver tetracycline (Goodson et al., 1979). He developed hollow cellulose acetate fibers of 0.25 mm diameter containing 300 μg tetracycline/cm. Rapid evacuation of drug took place within 24 h due to release from both the sides. Monolithic tetracycline fibers were developed for controlled delivery using melt extrusion technique (Goodson et al., 1983).

Main objective of the present study was to develop a low dose delivery system in the form of retentive fibers using ethylene vinyl acetate polymer for local delivery of amoxycillin trihydrate for periodontal infections and to maintain the concentration of the drug above its minimum inhibitory concentration value for a prolonged period of time at the site of infection, thus achieving high benefit to low risk ratio as compared to systemic delivery of amoxycillin trihydrate.

2. Materials and methods

2.1. Materials

Ethylene vinyl acetate was obtained from Sigma-Aldrich, USA. Amoxycillin trihydrate was obtained from Ranbaxy Laboratories Ltd., Gurgaon. All other reagents used were of analytical reagent grade.

A Shimadzu UV spectrophotometer UV 1601 (Japan) was used for spectrophotometric analysis. The HPLC system was of LC-10 Shimadzu model (Japan). Mechanical shaker was supplied by Veego (New Delhi, India). BOD incubators were also supplied by Veego.

2.2. Preparation of monolithic fibers

The polymer—amoxycillin mixture containing drug in concentration range of 0.5–3% was prepared and loaded into the plastometer (Fig. 1). Melting was carried out continuously in screw melters or extruders as these deliver a more homogenized and uniform melt. The melting temperature was maintained below 216–218 °C as above this temperature amoxycillin trihydrate tends to decompose. The melt was then transported under pressure to spinning blocks where an exact metering pump maintained an even issue of the melt. The melt was forced through capillaries in a plate, called the spinneret, and an endless, fine stream of fluid was formed (Fig. 2). The filaments were then quenched and solidified in the quench chamber and were drawn off from the bottom.

2.3. In vitro release of drug from fibers

Release of amoxycillin from the fibers was determined by immersing the sample in a container filled with 25 ml of alkaline borate buffer pH 8.1 containing 2.25% glycoproteins (Ali et al., 2002). The container was sealed and then incubated at 37 °C in a shaking water bath (Veego). Three-milliliter sample was withdrawn at suitable time intervals, filtered and analyzed for drug content at 226 nm on a Perkin-Elmer spectrophotometer. Same volume of fresh dissolution

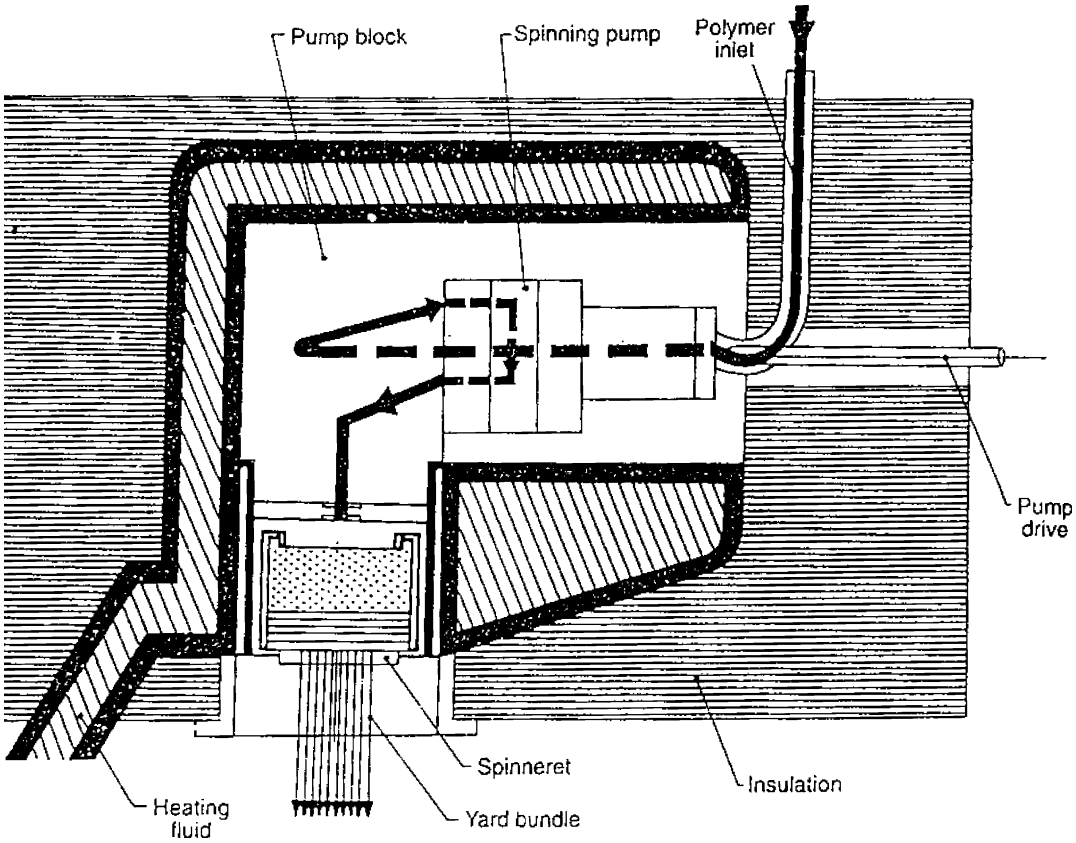

Fig. 1. Extruder of lab spinning line.

media was replaced. The amount of drug released was determined at different time intervals. The tests were performed in triplicate.

2.4. Analysis of dissolution data

Dissolution data was analyzed using the equation proposed by Ritger and Peppas to describe the relative availability of the drug from the matrix (Ritger and Peppas, 1987).

$$\frac{M_t}{M_\infty} = Kt^n \quad (1)$$

where M_t corresponds to the amount of drug released in time t, and M_∞ is the total amount of drug released after infinite time, K denotes a constant, and n is the release exponent indicating the type of drug release mechanism. Different kinetic equations (zero order, first order and Higuchi's equation) were applied to interpret the release rate of drug from the fiber at pH 8.1.

2.5. Characterization of fibers

Scanning electron microscopic analysis was carried out with LEO S-440 PC based Digital SEM, on placebo as well as on drug loaded fibers.

2.6. Optimization and evaluation of the optimized fibers

Drug loaded fibers were optimized on the basis of in vitro drug release studies and their physical characteristics. The optimized fibers of amoxycillin trihydrate were subjected to various tests including, their general appearance, dimensional analysis, physical characteristics, SEM analysis, etc.

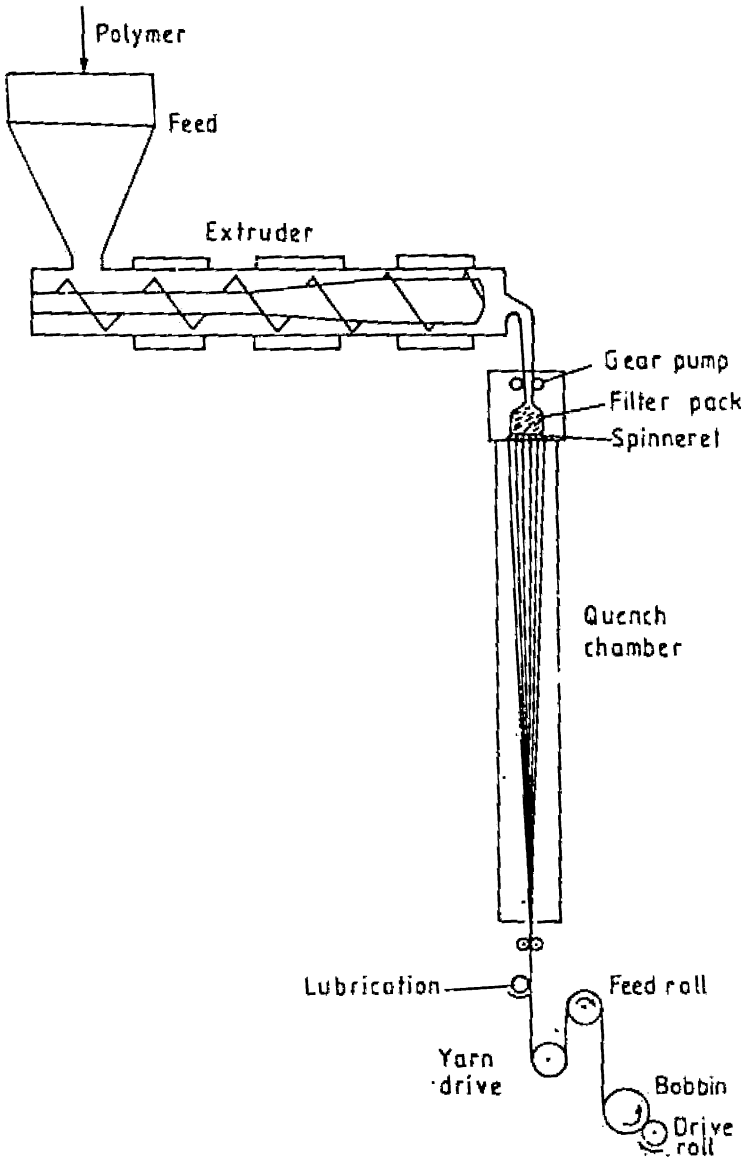

Fig. 2. Laboratory scale melt spinneret.

2.7. In vivo release studies on the optimized fiber (Mumtaz and Ch'ng, 1995; Ali et al., 1998)

Continuous flow through apparatus was designed for release studies in vivo. It simulated the in vivo conditions of periodontal pocket. The cell was made of glass and had a length of 10.5 cm and diameter of 2.1 cm. It was closed at one end but was open from the other end. A small inlet tube of 0.5 cm diameter was attached to one end of the cell. Another outlet tube of the same diameter was attached to the opposite end. In the center of the lower chamber, there was a cavity of 8 mm length and 3 mm width for placement of fiber. Bovine cheek pouch was used as the mucosal membrane. The animal was sacrificed in the slaughterhouse and the cheek pouch was excised. It was washed thoroughly with distilled water and was dipped in ammonia solution (0.2 M). This treatment

lead to the separation of buccal mucosa from underlying tissues. The mucosal membrane so separated was cut into strips of 3 mm × 3 mm (3 mm length and 3 mm width). A strip of mucosal membrane was washed with isotonic phosphate buffer of pH 8.1 and was kept in the central cavity. It was stabilized with alkaline borate buffer pH 8.1 in order to remove soluble components. After stabilization, the fiber was placed on the mucosal membrane. One end of the flow through cell was attached to the reservoir containing alkaline borate buffer of pH 8.1 simulating the GCF pH. It was continuously pumped at a flow rate of 0.65 ml/min using flow regulators (Schneyer and Levin, 1965). The flow rate chosen corresponded with the mean GCF flow rate. The whole assembly was maintained at 37 °C. The samples were collected from the cavity at different time intervals and analyzed spectrophotometrically at 226 nm for drug content.

2.8. Microbiological evaluation of in situ release samples

Sterilized petri dishes were taken and 25 ml of media was poured into petri dishes aseptically and were allowed to solidify. Before solidification it was inoculated with about 0.2 ml culture of the microbes which are commonly implicated in such infections like *E. coli* and *S. aureus*. Blood agar media was inoculated with *S. mutans* and *B. cereus*.

The samples obtained from in situ release studies were filtered through sterilized Millipore membrane filters (0.2 μm) and added in cups bored in inoculated solidified media. These were incubated at 37 ± 0.5 °C for a period of 48 h in an incubator. Same procedure was adopted for placebo formulations.

2.9. Permeation studies across animal membrane (Gummer et al., 1994)

A modified version of Franz diffusion cell was used to study the permeation of drug across bovine mucosal membrane into systemic circulation. The assembly consisted of two chambers. The upper cylindrical chamber was open from above and harbored the bovine buccal mucosa at its base. The fiber was placed on top of it. The lower chamber was in the form of a closed cylinder and contained a sampling port and a Teflon coated magnetic needle at its base. The junction between the two chambers was tightly secured by placing buccal mucosa in between the two chambers. Fifteen milliliters of isotonic phosphate buffer of pH 7.4 was added to the lower chamber containing Teflon coated needle. Upper chamber contained 10 ml of alkaline borate buffer of pH 8.1. The cell was placed on a magnetic stirrer. The whole set up was kept at 37 ± 0.5 °C in an oven. Three milliliters of sample was withdrawn at different time intervals over a period of 12 days from the lower chamber. The samples were filtered and diluted suitably. The samples were analyzed spectrophotometrically at 226 nm for the amount of drug permeated.

2.10. Stability studies on the optimized fiber

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 WHO guidelines. Fibers were kept in sealed petri dishes lined internally with aluminium foil. These petri dishes were then placed in a desiccators containing saturated solution of NaCl 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 fibers were triturated with the mobile phase in a glass pestle and mortar. The solution was then filtered through Millipore membrane filter (0.45 μm). Mobile phase used was a mixture of methanol, phosphate buffer 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 done at 235 nm. Twenty microliters of filtered samples were injected into the column and mobile phase was run for 10 min. Concentration of the drug was calculated from the calibration curve of the pure drug (Abounassif et al., 1991).

3. Results

3.1. Mechanical properties of fibers

Physical evaluation of the fibers showed that 3% drug loaded fibers were deep yellowish in color and

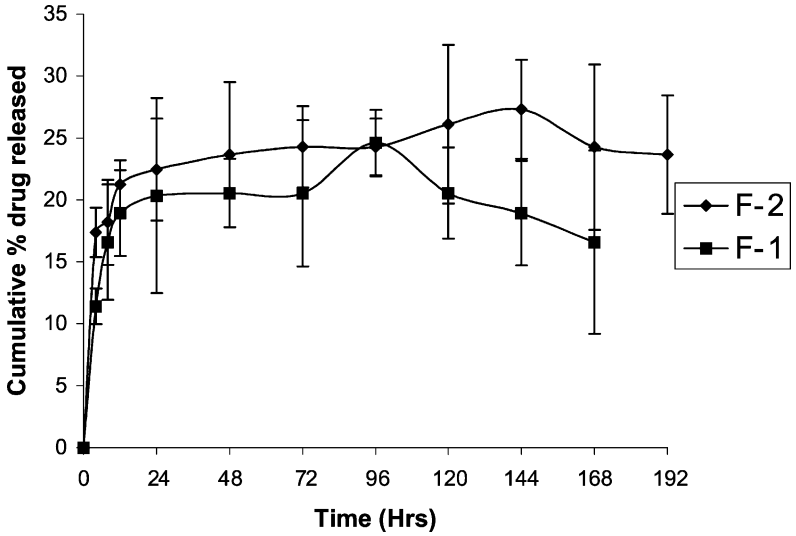

Fig. 3. In vitro release from F-1 and F-2.

were very thick (1 mm). Five percent loaded fibers broke into pieces while they were drawn out. Fibers containing 0.5% drug could not be properly evaluated. Only 1 and 2% drug loaded fibers were subjected to evaluation and they were flexible and elastic. These fibers were yellowish in color and were very slightly bitter. Diameter was found to be 0.56 mm and length was 10.0 cm. Folding endurance was <50.

3.2. In vitro release rate studies

One percent drug loaded fiber (F-1) showed release up to a period of 168 h whereas 2% drug loaded fiber (F-2) showed sustained release up to a period of 192 h. Both the formulations released the drug by diffusion mechanism. Best correlation was observed with the formulation F-2. So the formulation selected for the further study was F-2 (Fig. 3).

3.3. Characterization of fibers

Drug-embedding characteristics of drug loaded fibers were compared with that of placebo fiber with the help of SEM. The optimized fibers prepared from a mixture of EVA and amoxycillin showed drug embedding. The drug appeared as white specks on the surface of the optimized fiber. These specks were not present in the placebo fiber (Figs. 4 and 5).

3.4. Analysis of release rate data

Different kinetic equations were applied to interpret the release rate from the optimized fiber at pH 8.1. The best fit with the highest correlation was achieved with the Higuchi's equation. In Eq. (1) when n approximates 0.5, a Fickian diffusion-controlled release is implied, whereas for $0.5 < n < 1.0$ non-Fickian transport is implied. When value of n approaches.1.0, one can conclude that release is approaching zero order. From Eq. (1) value of n is calculated as 0.266 for optimized formulation. On this basis, we can conclude that release of drug from the optimized fiber followed Fickian diffusion.

3.5. In vivo release studies on the optimized fiber

From the release rate data obtained using flow through apparatus it was found that concentration of drug released remained above its MIC value over a period of 12 days as shown in Fig. 6.

3.6. Microbiological evaluation

In situ release study samples when tested against *S. aureus*, *S. mutans*, *B. cereus*, and *E. coli* showed that samples inhibited the growth of all above-mentioned

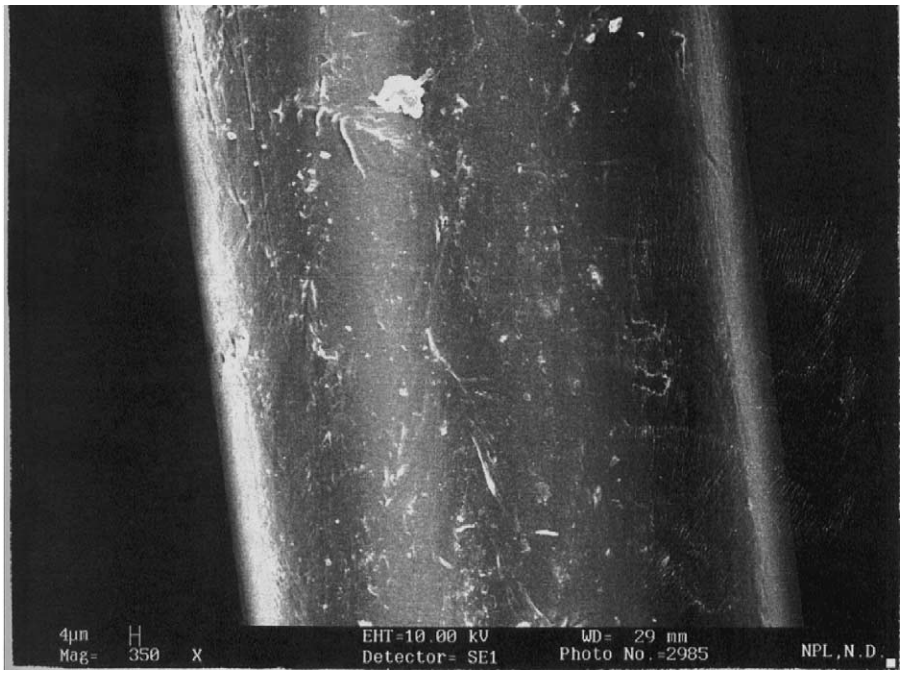

Fig. 4. Surface characteristics of placebo fiber (350×).

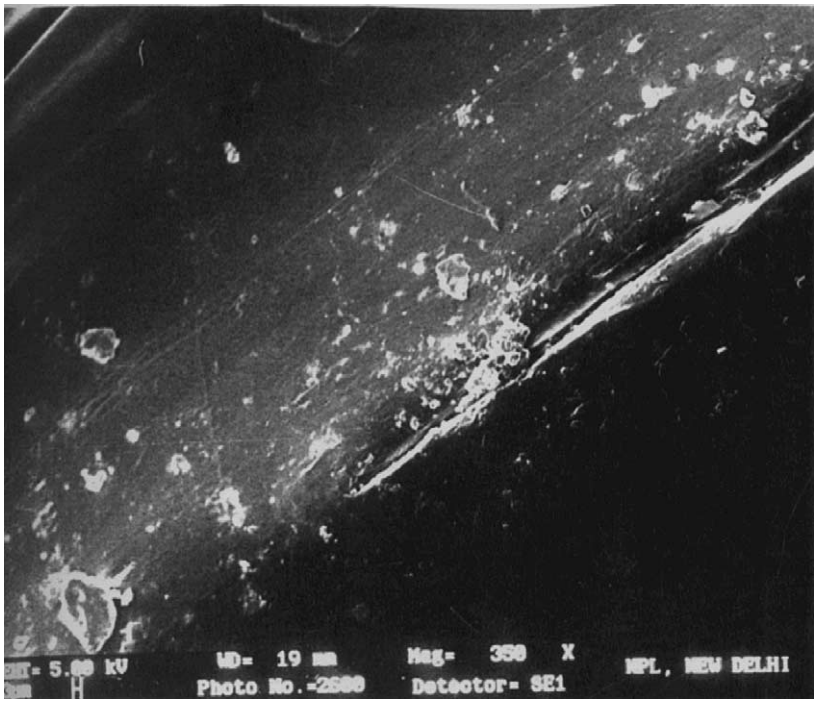

Fig. 5. Surface characteristics of optimized fiber (350×).

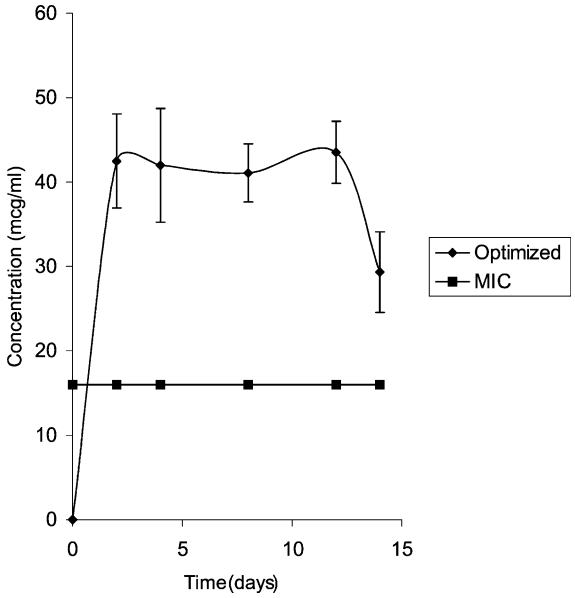

Fig. 6. In situ release of amoxycillin trihydrate from optimized fiber.

microorganisms except *E. coli*. Amoxycillin acts by inhibiting the cell wall synthesis of bacteria by inhibiting the enzyme transpeptidase and carboxypeptidase. Cell wall of Gram-positive bacteria is essentially made up of peptidoglycan whereas in case of Gram-negative bacteria it is alternative layer of lipoproteins and mucopeptides. This is possibly the cause of negligible activity of amoxycillin towards *E. coli*. Placebo fibers were also tested against the above-mentioned microorganisms and it was found that these were not effective against the same.

3.7. Permeation studies

Permeation studies across bovine cheek pouch membrane using modified Franz diffusion cell showed that only 1.49% drug permeated per centimeter square in 6 days, indicating that low amount of drug is expected to go into systemic circulation.

3.8. Stability studies

Stability studies revealed that no significant changes occurred in the physico-chemical properties of the optimized fiber at $40 \pm 0.5\,^{\circ}C$ and $75 \pm 5.0\%$ R.H. No significant change occurred in the drug content of the formulation at the storage temperature of $40 \pm 0.5\,^{\circ}C$ and $75 \pm 5.0\%$ R.H. Hence, storage temperature not exceeding $45\,^{\circ}C$ and moisture proof packing are necessary to ensure stability of the batch. The degradation rate constant was found to be 2.79×10^{-4} per day. Since this value is very low, a tentative shelf life of 24 months was given to the formulation.

4. Discussion

In the present study, an attempt was made to prepare drug loaded fibers containing amoxycillin trihydrate. Scientists attempted in late 1970s to prepare such fibers. In 1979, Goodson et al. (1979) prepared hollow fibers containing tetracycline, but the drug released from both the sides within 24 h. In 1983, when Goodson prepared loaded monolithic fibers of tetracycline, the effect was retained for a longer period of time (Goodson et al., 1983). There is no scientific evidence of any work after Goodson. We attempted to load amoxycillin in the polymeric material for use in periodontal infections and characterize the prepared fibers. After evaluating them for various parameters we concluded that it is an excellent system for drug delivery. From the mechanical properties of the fibers prepared by us, we can say that as drug concentration increased, the fibers changed their colour. Only at 2% loading, the fibers retained their elasticity and flexibility. With high loading of the drug, the elasticity was lost. In vitro release studies showed that 2% loading helped in sustaining the drug release up to a period of hours and the release followed Fickian diffusion mechanism. These fibers when characterized by SEM clearly showed that the drug was present on the surface of the fibers and embedding had taken place completely. In vivo studies proved that the release was sustained for more than 12 days, as desired. All samples inhibited the growth of microbes tested in the present study. From the permeation studies, we can infer that very less drug has gone into the circulation thus showing that drug is available in therapeutic concentrations in the cavity. Stability studies showed that our formulation was very stable after loading the drug and could be stored without degradation for more than 2 years.

The present study was an attempt to develop local targeted drug delivery device in the form of a fiber. It

was developed to a satisfactory level in terms of drug content, drug release, mechanical properties, in vitro release, in vivo release, and microbiological evaluation. Since the drug release occurs locally, it has 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 fiber is 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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