Controlled Drug Delivery Using Bioerodible Polymeric Systems for the Treatment of Periodontitis

by

Kimberly Ann Gates

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Graduate Department of Pharmaceutical Sciences
University of Toronto

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Controlled Drug Delivery Using Bioerodible Polymeric Systems for the Treatment of
Periodontitis

Doctor of Philosophy (1999)

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ABSTRACT

Existing local drug delivery approaches for the treatment of periodontitis are; at times, unsatisfactory due to their rapid drug release and/or low biodegradability of the polymeric carrier. Bioerodible drug delivery systems may overcome these drawbacks and provide a more effective duration of treatment. In this study, bioerodible association polymer films were produced based on several blend ratios of cellulose acetate phthalate (CAP) and poly(oxyethylene-co-propylene oxide) (Pluronic) at different metronidazole concentrations. After a systematic study of polymer erosion of the sample blends, a 30/70 (w/w) ratio of the CAP/Pluronic L101 blend was selected as the base for the periodontal drug delivery device. These polymer films were evaluated for their thermal properties, association between CAP and L101, crystallinity, and stability at various humidity levels.

A single glass transition temperature was observed on the differential scanning calorimetry thermograms for CAP/L101 blends, typical of miscible polymers. In the Fourier transform infra-red studies, the wavenumber of the CAP carbonyl band increased with the Pluronic content, demonstrating an association between CAP and L101 via hydrogen bonding. Increased crystallinity was noted for the 30/70 blends from the x-ray diffraction studies. Stability
studies revealed that at 0 and 30% relative humidity, samples were stable for up to a month, while those stored at 100% relative humidity were not stable in terms of polymeric erosion and drug release rates.

The polymer films and drug release profiles were characterized in vitro (rotating disc apparatus), in vivo (dorsal rat model), and in pilot clinical trials. It was established that both the drug release and polymer erosion rates decreased with increasing hydrophobicity of the Pluronic component. Similar erosion and drug release profiles were observed in vitro, in vivo (animal model), and clinically, but varied in degree due to differences in their hydrodynamic conditions.

After the first pilot clinical trial, the inserts were reformulated to produce 30/70 CAP/L101 periodontal inserts containing 2% (w/w) metronidazole. Analysis of the metronidazole concentration in the gingival crevicular fluid during subsequent clinical trials indicated a reduction in the drug burst effect along with prolonged drug release, yielding therapeutic drug concentrations for up to 4 days. The inserts were readily accepted by patients with no apparent adverse effects. These inserts are suitable for the localized treatment of periodontal infections.
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1. INTRODUCTION

1.1 Statement of Problem

The traditional treatment of periodontal disease includes, in addition to physical methods, administration of systemic or topical antibiotics. Antimicrobial chemotherapy may be used either as monotherapy, or in conjunction with mechanical and/or surgical treatments.

Physical treatment methods include mechanical debridement of the tooth root surface, scaling and root planing (SRP), and it is done either as a separate procedure or during periodontal surgery. Periodontal diseases have fairly high rates of disease recurrence. The success or failure is greatly influenced by the patient’s oral hygiene habits, and compliance with treatment.

Mechanical methods of subgingival debridement accomplished by thorough scaling and root planing, and oral hygiene procedures, have served as the ‘gold’ standard of periodontal therapy for decades. Scaling and root planing procedures are technically demanding and very time consuming. Clinical trials have consistently demonstrated that scaling and root planing reduce gingival inflammation, reduce probing depth, and result in a gain of clinical attachment in most sites of patients with adult periodontitis. Probing depth and clinical attachment are well accepted treatment outcome measures. Further, available evidence supports that scaling and root planing result in a shift of the subgingival microbiota from one associated with disease towards one associated with health. Thus, mechanical therapy is, in most cases, the first mode of treatment recommended for most periodontal infections. Following an adequate post-scaling time for a healing response, the patient must be re-evaluated to determine if further mechanical, pharmacological and/or surgical treatment is required (Drisco, 1996).
There is evidence that mechanical non-surgical therapy may be effective in treating juvenile periodontitis (Wennstrom et al., 1986). However, even Actinobacillus actinomycetemcomitans (Aa), one of the putative pathogens of this disease, may not be eliminated by mechanical periodontal therapy alone (Cobb, 1996).

Supra-gingival irrigation, when used in conjunction with tooth brushing, helps reduce pathogenic gingivitis. Subgingival irrigation as a monotherapy, or as an adjunct to mechanical debridement, has been shown to reduce microbiota. However, there is no clear evidence that subgingival irrigation when used as a monotherapy or as an adjunct to scaling or root planing is of substantial long-term benefit in treating periodontitis (Cobb, 1996).

Tetracyclines (TCN) and metronidazole (MTZ) are the agents most frequently used in the management of periodontal disease, combined with mechanical treatments. Routine systemic use of these drugs in the management of chronic adult periodontitis is contraindicated, and is no substitute for root surface debridement and thorough supragingival plaque control. Both drugs can be given systemically or applied topically into the periodontal pocket. The latter route is preferred since the dose is reduced considerably, but the local tissue concentration is increased. The efficacy of local drug delivery is dependent upon the release kinetics of the drug from the delivery vehicle. Although local application can be time consuming, it reduces the risk of adverse reactions and drug interactions (Seymour and Heasman, 1995).

Existing systems for local drug delivery in the periodontal pocket are often unsatisfactory as they are either non-degradable, so the patient must return for removal of the spent device, or degrade too slowly compared to the rapid release of the drug. The presence of such non-degradable or slowly degradable systems, for prolonged periods of time, may cause irritation to the periodontal pocket.
Therefore, it is desirable to develop a novel bioerodible drug delivery system that would enhance patient compliance and maintain a localized, effective drug concentration at the site of infection for a predetermined duration. Such a system should simultaneously erode and release its drug so that the device disappears at the end of the releasing period, thereby eliminating the need for reapplication or device removal.

1.2 Review of Literature

1.2.1 Description of Periodontitis

A healthy periodontium is characterized by a gingival sulcus of approximately 1 to 3 mm in depth around the crown of the tooth. Healthy gingiva appears light pink in colour, with a firm stippled surface that does not bleed spontaneously or with mild instrumentation. Simple facultative bacterial populations such as Gram-positive, non-motile rods, cocci and filamentous forms of bacteria normally inhabit the gingival crevice or pocket. Pathogenic periodontal microflora, however, include a complex flora with increased numbers of spirochetes and Gram-negative motile rods. Undisturbed, these microbes tend to proliferate in the subgingival plaque, as evident by analysis of plaque collected from the diseased site, as shown in the Schematic Diagram of Plate 1-1 (Listgarten and Hellden, 1978). This leads to attachment loss, loss of alveolar bone, and potential need for periodontal surgery and/or tooth extraction. A clinical example of a patient with periodontal disease is included in Plate 1-2.

Microorganisms implicated in causing pathogenic periodontal conditions include: *Actinobacillus actinomycetemcomitans*, *Bacteroides* (Porphyromonas) *gingivalis*, *Bacteroides intermedius*, *B. melaninogenicus*, Capnocytophaga (spp.), *Eikenella corrodens*, *Fusobacterium*
nucleatum, Peptostreptococcus (spp.), Selenomonas sputigena and Wolinella recta. Researchers consider A. actinomycetemcomitans, and P. gingivalis to be the key pathogens found in the majority of diseased conditions (Slots and Rams, 1990).

In the current study, work was performed on the design and evaluation of devices used in the treatment of periodontitis.

The term periodontal disease refers to both diseases of the gingiva (gingivitis) and the tissues supporting the tooth (periodontitis). Gingivitis is a common disease of the periodontium associated with alterations in the gingiva, such as redness and swelling. The accumulation of microorganisms and ensuing inflammation makes the gum tissue painful to the touch and bleeding occurs during probing or tooth cleaning. Halitosis is also associated with this condition (Page, 1977). The transition from gingivitis to periodontitis is not well understood. When bacteria are allowed to accumulate at the gingival margin, a series of transformations takes place in the gingival tissues, leading from a healthy gingiva to the state of gingivitis. Gingivitis will eventually, but not necessarily, be followed by the loss of alveolar bone and collagen support for the affected teeth, leading to loosening of the teeth and ultimate loss of the affected teeth (Periodontitis) (Page and Schroeder, 1982).

As the disease progresses, there is an increase in pathogenic bacterial growth and gingival sulcus deepening (3-7 mm) to form a periodontal pocket. A pH shift in the periodontal microenvironment also occurs. The periodontal pocket becomes filled profusely with fluid due to an increased gingival crevicular fluid (GCF) flow. The actual volume of fluid from a given gingival sulcus is difficult to measure. The volume in an undisturbed sulcular location is generally less than 1.0 microlitre (Challacombe, 1980). In humans with a gingival index lower than 1, the mean fluid volume in approximal spaces ranged from 0.24 to 1.56 μL per tooth (0.24-
0.43 μL for anterior teeth and 0.43-1.56 μL for molars). In subjects with crowns, these values are higher. Challacombe (1980) also calculated that 0.5-2.5 mL of GCF is secreted into the oral cavity per day. The amount of fluid collected by means of a filter paper strip of 1.5 mm width, inserted 1.0 mm into a gingival sulcus of slightly inflamed gingiva, absorbs about 0.1 mg of fluid in 3 minutes (Cimasoni, 1983). Estimated GCF flow at individual sites can be measured using filter paper strips, as described above, or a Periotron. This estimation is influenced by the extent of the infection and other underlying dental diseases (Goodson, 1983).

It is unclear whether GCF results from physiological or pathogenic conditions since certain parameters (protein concentrations) resemble that of a physiological transudate, while others (Na⁺/K⁺ ratios) appear to be an inflammatory exudate (Alfano, 1974). Two mechanisms have been put forth to explain the origins of GCF: 1) the generation of a standing osmotic gradient and/or 2) the initiation of classical inflammation. These mechanisms are described below:

1. The osmotic gradient may arise from macromolecular bacterial by-products residing in the subgingival plaque. These macromolecules accumulate at the basement membrane resulting in a localized increased solute concentration and establishment of an osmotic gradient. Solvent molecules drawn across the tissue, raise the intercellular hydrostatic pressure and cause the exudation of GCF into the periodontal pocket (Alfano, 1974). The fluid produced by this mechanism may originate from gingival tissues which are histologically and clinically healthy.

2. If the bacteria plaque is not removed, its macromolecular byproducts will eventually penetrate the basement membrane. Depending on the enzymatic and toxic properties of these molecules, a classical inflammatory exudation may occur. Thus, gingival fluid may
progress, at different times, or in various areas of the dentition, from an initial osmotically
modulated exudate to a secondary inflammatory exudate, with consequent alterations in its
composition (Alfano, 1974).

The pH is suspected of playing a significant role in the structure and function of the
periodontal pocket as the microenvironment moves from a physiological to a pathogenic state.
Bickel and Cimasoni (1985) postulated that the formation of dental pellicles, subgingival plaque,
bacterial activity, lysosomal enzymes and their inhibitors may influence the pH of the GCF.

Although the relationships between subgingival plaque, pathogenic bacterial growth and
the development of periodontal disease have been well established, extensive investigation of
several forms of therapy has not lead to an optimal management of human periodontitis
(Soskolne et al., 1983). As mentioned previously, mechanical and surgical treatments were the
foundations of periodontal therapy until the 1970’s. These treatments include scaling and root
planing. However, pathogenic microbes quickly repopulate the periodontal pocket if good oral
hygiene is not maintained (Soskolne et al., 1983). For this reason, other treatments for better
results are to be explored.

1.2.2 Use of Antibiotics in the Treatment of Periodontitis

Strategies for choosing the type of drugs required for the treatment of periodontitis are
primarily aimed at suppression or elimination of specific periopathogens from the periodontal
pocket, thus resulting in a shift of bacterial strains towards those associated with normal
physiological flora. Since 1988, the drugs most frequently prescribed by periodontists include
tetracycline, doxycycline, metronidazole, penicillin, amoxicillin and chlorhexidine (CHX) (Slots
When providing pharmacological therapy to the periodontal pocket, the factors which must be considered are effective therapy, predictable clinical results, low incidence of drug side effects or interactions, decreased costs and patient acceptance of the drug. For a drug to be useful: 1) the periopathogens must be susceptible to the drug; 2) they must not develop resistance to the drug; and 3) they must be exposed to effective inhibitory concentrations of the drug for an adequate time period (Slots and Rams, 1990).

Systemic antibiotics have been shown to be useful in controlling subgingival plaque and related microbes (Listgarten and Hellden, 1978). A problematic group of periodontal patients are those with advance disease which do not respond to oral hygiene instructions. A weeks’ course of metronidazole resulted in significant improvements in pocket depth and attachment levels in these patients (Jenkins, 1989).

Elter et al. (1997) performed a meta-analysis of the effect of systemic metronidazole as an adjunct to scaling and root planing for adult periodontitis. The results suggested that systemic metronidazole combined with scaling and root planing may be a benefit over scaling and root planing alone, in the treatment of adult periodontitis patients with pockets of 4 mm or greater. Little benefit was evident if the initial probing depth was less than 4 mm, or follow-up was beyond 13 weeks.

In the treatment of refractory periodontitis associated with patients culture positive for *Bacteroides forsythus* and negative for *Actinobacillus actinomycetemcomitans*, systemic metronidazole (500 mg three times per day for 7 days) also produced a significant improvement in both the clinical and microbiological parameters when it is combined with supra and subgingival debridement (Winkel et al., 1997).
Bollen and Quirynen (1996) studied the microbial response to mechanical treatment (scaling and root planing) in combination with several adjunctive therapies (antibiotics and/or antiseptics) in the treatment of chronic adult periodontitis. They reviewed the 'additional' effect of a subgingival irrigation with chlorhexidine, or a local or systemic application of tetracycline or metronidazole, performed in combination with a single course of scaling and root planing. Generally, only the irrigation with chlorhexidine 2%, and the systemic use of metronidazole (in case of large proportions of spirochetes) or tetracycline (in case of large proportions of *A. actinomycetemcomitans*) seem to result in a supplementary effect when compared to scaling and root planing. Therefore, the routine use of systemic antibiotics in the treatment of chronic adult periodontitis should not be advocated, considering the increasing danger for the development of microbial resistance.

Early discontinuation of the systemic therapy may result in return of periopathogens, leading to reinfection. Risks associated with long term use of systemic antibiotics include the development of resistant bacterial strains, reduced bacterial sensitivity, superimposed infections and changes in gastrointestinal drug absorption (Goodson, 1984). The administration of systemic antibiotics may initially result in therapeutic drug levels at the gingival site, which decline to subtherapeutic levels over time. In order to maintain bactericidal drug concentrations in the periodontal pocket, higher systemic drug concentrations may be required. Due to the often poor biodistribution of some systemic antibiotics to the periodontal pocket, high serum drug concentrations are needed to produce effective therapy. This may lead to undesirable side effects. Metronidazole has been shown, in a limited number of cases, to produce side effects of diarrhea, dizziness, headaches, and nausea, as well as having drug interactions with alcohol...

Topical oral preparations such as mouthwashes and intra-pocket irrigants containing antimicrobial agents have been available to treat periodontitis for some time. These preparations, although associated with initial high drug concentrations, display low drug residence time at the site of the action. Other disadvantages include poor patient compliance, frequent dosing, disagreeable taste, and a tendency, in some cases, towards accumulation of a brown, black staining pigment on the teeth (Goodson, 1984).

Clinical evidence is available to support the effectiveness of sustained release antimicrobials locally delivered to sites that did not previously respond to treatment by scaling and root planing. Gains in clinical attachment levels and reduction in probing depth and bleeding on probing have been reported using a combination of scaling and root planing along with tetracycline fibers compared to root planing alone.

A study has demonstrated that metronidazole gels also produce beneficial adjunctive effective when compared to scaling and root planing (Pourtaghi et al., 1996 and Radvar et al., 1996). Noyan et al. (1997) evaluated the clinical and microbiological effects of systemic and local metronidazole delivery in adult patients with periodontitis; combined with scaling and root planing. Metronidazole was delivered locally using gel (Elyzol®) and systemically (Flagyl). All treatments resulted in clinical improvements (i.e., gingivitis, probing depth, attachment level). Scaling and root planing provided an initial clinical improvement with a selective reduction of periodontopathogens (92.6% obligate anaerobes, 42.9% capnophilic microorganisms), whereas the combination of local or systemic metronidazole with scaling and root planing were found superior in reducing capnophilic bacteria (93.7% and 93.4%, respectively). It is of critical
importance to have a treatment rationale since bacterial differences exist in the etiological subforms of periodontitis. Microbial testing should be performed before prescribing the adjunctive antibiotic and selecting the mode of delivery for the successful clinical management of periodontitis.

Awartani and Zulqarnain (1988) compared the clinical effects of subgingival application of a 25% metronidazole gel (Elyzol®) with scaling in the treatment of adult periodontitis. The treatments were effective in significantly reducing plaque index, gingival index and bleeding on probing over the 14 week observation period. No statistically significant differences were found between scaling alone and combined treatment. Scaling and combined treatment were better than metronidazole alone. Metronidazole produced transient effects, best noted during the first 4 weeks after treatment. No additional effect of metronidazole was noted in the combined treatment group. At week 14, only combined treatment sites and scaled sites showed statistically greater probing depth reduction than control sites. Thus, for the treatment of mild-to-moderate adult periodontitis, subgingival scaling alone was as effective as the combination of scaling and antibiotic therapy.

The effect of topical metronidazole, administered as a single treatment using a 95% collagen and 5% metronidazole device, combined with mechanical debridement on the subgingival flora in deep periodontal pockets was compared with mechanical debridement alone in the treatment of adult periodontitis (Hitzig et al. 1997). Plaque samples from test and control sites in cuspids and bicuspid were collected. After treatment, a significant difference in the test group in comparison with the control group was found for fusiforms, and a lower number of A. actinomycetemcomitans positive sites were also observed in the test group. These results show
that in this particular study a single application of topical metronidazole seems to be as effective as adjunctive antimicrobial treatment in adult periodontitis.

In this research, metronidazole was investigated for its use in a drug delivery system. One of the reasons for choosing metronidazole originates from a study by Addy et al. (1985), which demonstrated the effectiveness of acrylic strips delivering 50% (w/w) chlorhexidine, metronidazole and tetracycline. These systems produced high and sustained drug levels for seven days, leading to significant reductions of pathogenic bacteria for up to a three month period. Metronidazole was found to be the most effective in the treatment of chronic periodontal disease. Other reasons for selecting metronidazole include its low solubility (in the free base form) when dissolved in physiological fluids, and the ease of incorporation of this drug into the polymer delivery system.

Additional properties of metronidazole are included in Appendix A.

1.2.3 An ‘On Site’ Drug Delivery Approach

The anatomical structure of the periodontal pocket lends itself well to the insertion of a localized controlled drug delivery system. Such a device, in general, should have a reservoir of less than 1.0 milligrams (mg) of drug and a release rate of a few micrograms per hour. This rate should be sufficient to maintain the GCF drug concentration at therapeutic levels (Goodson, 1984).

Several drug delivery devices have been developed over the last ten to fifteen years to try to meet these goals. Existing major types of periodontal drug delivery systems are presented in Table 1-1, along with a description of the clinical study. These systems were chosen as they either employ the drug metronidazole or are biodegradable, in order to represent the state-of-the-
art in periodontal drug delivery systems. Device applications are detailed in Table 1-2. The behavior of each device is listed in Table 1-3.

The following criteria were selected for comparison of the different drug delivery systems: application technique, range of pocket size application, patient acceptance, drug delivery time, choice of therapeutic agent, composition and safety, and device degradation time. Each of these criteria are described below.

1.2.3.1 Application Technique

The drug delivery systems should be easy to place in the periodontal pocket. As substantial operator differences may be encountered, a learning curve is required as proper placement is important to the overall clinical success of the device (Kornman, 1993). The device should be easy to handle, and relatively quick and simple to apply, to ensure a high success of proper placement.

1.2.3.2 Range of Pocket Size Application

The delivery system should work equally well in all sites and all patients. The optimal use conditions have not yet been defined by investigators with experience in particular techniques, but have great variability from site to site.

1.2.3.3 Patient Acceptance

Survival times of drug bearing delivery devices in the periodontal pockets play a key role in determining the outcome of the treatment. In addition, such devices have to be aesthetically acceptable to the patient. In view of the vanity most people exhibit regarding their mouth, such devices should not extend above the gingival margin, must not be bulky or interfere with normal
daily oral hygiene, including tooth brushing and dental flossing, and should not require the patient to change their dietary patterns. Since the inflamed periodontium is very sensitive, the device should be amenable to rapid insertion into the pocket, and pain and discomfort to the patient during the treatment period should be minimized (Friedman and Steinberg, 1990).

1.2.3.4 Drug Delivery Time

Delivery systems differ in several ways. It is uncertain at this time what other factors besides drug release kinetics, choice of drug and its carrier, and physical device placement and distention of the pocket are factors influencing clinical outcome and how long the device should remain in the pocket and release its drug. The singular aim of using antimicrobials as part of a treatment scheme is to achieve within the periodontal pocket a concentration of drug that is sufficient to kill (bactericidal) or arrest the growth (bacteriostatic) of pathogenic microorganisms over the required period of time. A poorly absorbed drug that has a low penetration through the mucosal tissues would enable the drug level to build up to a high concentration, and prolonged duration in the pocket. Ultimately, the concentration of the drug in the gingival crevicular fluid and the time that the drug concentration is maintained above the MIC in the pocket depends upon the drug's substantiveness and antibacterial potency. For instance, the placement of acrylic resin strips (Addy and Langeroudi, 1984) containing 40% (w/w) metronidazole into the periodontal pocket for two to three days reduced total microbial counts by more than 75% and achieved a shift in microbial patterns to a Gram-positive cocci dominance, which is generally considered associated with good health. It should also be noted that in most controlled release local delivery systems the level of antimicrobial release into the periodontal pocket far exceeds levels involved in normal antimicrobial mechanisms. It is very likely that these agents in such initial high
concentrations exert multiple effects on the local environment, only one of which may be antimicrobial in the traditional sense (Kornman, 1993).

1.2.3.5 Choice of Therapeutic Agent

Since the average depth of a periodontal pocket is between 6 and 8 mm, the therapeutic drug delivery device cannot be large. Thus, it is necessary that a small dosage of the active agent in the device should be highly effective as a therapeutic agent. Antibacterial drugs should be highly specific against the pathogenic bacteria in the pocket. The development of resistant strains of bacteria might occur due to the long duration of the antibacterial agent in the periodontal pocket. The drug choice should then be effective for a particular type of periodontal disease it is being used to treat, and should not lead to the development of resistance bacterial strains (Friedman and Steinberg, 1990). Using different drug delivery devices may be influenced by drug bioavailability, concentration, duration and spectrum of activity, resulting in differences in treatment results (Drisko, 1996).

1.2.3.6 Composition and Safety

A common approach to insuring the safety of a device is to establish its biocompatibility. Initially, when using biomaterials, the biocompatibility question was focused singularly on adverse effects, not on the performance of the device. Presently, the concerns involve both the aspects of performance and device adverse effects. The issue of biocompatibility is not whether there are adverse reactions associated with the biomaterial, but whether that material performs satisfactorily (i.e., in the intended fashion) in the application under consideration (Williams et al., 1981).
All implantable medical devices should meet two general criteria. They should be (1) biofunctional and (2) biocompatible during the period of implantation. If either criteria is not met, the device may present a threat to the patient and generally requires removal and replacement. Biofunctionability relates to the intended performance of the item, throughout the entire implantation period. Biocompatibility, as mentioned previously, refers to the absence of any adverse effects of the device during the implantation period, and is often associated with the broad toxicological aspects of the device or material (Williams et al., 1981).

1.2.3.7 Device Degradation Time

A degradable controlled release device would have several advantages over a nondegradable device. For example, elimination of a return visit to the periodontist to extract the device from the periodontal pocket would represent great time and cost savings. A degradable device should not be an obstacle during reattachment of the periodontal tissues to the tooth, thereby offering minimal interference in the reduction of pocket depth. A factor that needs to be taken into account when considering degradable devices is the problem of toxicity. The degradable components of the device have to be dissolved or absorbed from the site without causing any tissue irritation (Friedman and Steinberg, 1990). It is desirable to have the release system degrade during the time of drug release.

Soskolne et al. (1983) produced ethylcellulose strips containing chlorhexidine, which provided sustained drug release for up to seven days. Dunn et al. (1982) were able to show a persistent reduction of periopathogens for at least ten days using tetracycline loaded monolithic fibers composed of ethylene vinyl acetate co-polymer. Both of these devices and the fibers used by Goodson et al. (1991) are neither biodegradable nor bioerodible, and once depleted, the
patients are required to return to the practitioner for removal of the implants. Fiber devices of this type require elaborate manufacturing methods and take longer to apply than the strips.

Addy et al. (1988) demonstrated the effectiveness of acrylic strips which delivered chlorhexidine, metronidazole and tetracycline. These systems produced high and sustained drug levels for seven days leading to significant reductions in the count of pathogenic bacteria for up to three months. However, these strips did not degrade over time.

Friedman and Steinberg (1990) produced cross-linked gelatin delivery systems containing chlorhexidine. These were the first of the so called ‘biodegradable’ delivery systems, however, polymer degradation kinetics were very slow compared to the drug release.

Baker et al. (1988) produced a biodegradable microparticle (25-210 μm) system containing tetracycline. This system consists of poly(lactide-co-glycolide) (PLGA) microparticles in a thermoreversible gel base that is injected into the periodontal pocket in a liquid form which gels at body temperature. The release of drug from this system is quite rapid and the biodegradation of the poly(lactide-co-glycolide) microparticles occurs over a long period of up to several months.

The poly(lactide-co-glycolide) system has been used with other second generation tetracycline antibiotics. These include a commercial 10% (w/w) doxycycline hyclate biodegradable drug delivery system (Atridox™) (Polson et al., 1996), a 2% (w/w) minocycline•HCl powder encapsulated in poly(lactide-co-glycolide) (Dentomycin®), (Jones et al., 1994) and 25% (w/w) tetracycline in resorbable poly(lactide-co-glycolide) strips (Maze et al., 1995).

Noguchi and co-workers (1988) have produced hydroxypropyl cellulose strips containing chlorhexidine and tetracycline. These strips bioerode and release their drugs within twenty-four
hours. Although they are effective in reducing pathogenic bacteria when applied three times over seven days, but their drug delivery is not really prolonged.

Minabe et al. (1989) synthesized a tetracycline immobilized cross-linked collagen fiber. Similar to the cross-linked gelatin systems mentioned earlier, the cross-linking agents react to cause insolubility of the collagen and gelatin, thus making the collagen and/or gelatin very slow to erode. Surprisingly, however, the clinical ‘disappearance’ of the device was noted to occur as soon as one week.

The cross-linking agents (such as gluteraldehyde or formaldehyde) may also cause biocompatibility problems in the periodontal pocket. In addition, phagocytosis of non-biodegradable particles by macrophages induces release of lysosomal enzymes and other mediators of inflammation which results in tissue irritation (Williams et al., 1984).

Although the 25% (w/w) tetracycline poly(lactide-co-glycolide) system (Maze et al., 1995) seems to have a reasonably desirable drug release time of 10 days, the device degradation time is slow. There is concern over its detailed application technique, the loss of strips during treatment and the lack of reporting of the patient acceptance of the device. The growing resistance of some periopathogens to tetracycline (Goodson and Tanner, 1992) makes this drug questionable for use in a localized sustained release system.

The Elyzol® dental gel (25% (w/w) metronidazole benzoate in a mixture of mono- and triglycerides used in the studies of Ainamo et al., 1992, Stezel and Flores-de-Jacoby, 1996) initially looked like a desirable, exciting, new, sustained release periodontal drug delivery system. Unfortunately, it has several undesirable drawbacks. These include: a variable dose of drug in each periodontal pocket; bitter taste; gingival tenderness and pressure as the gel liquefies and then hardens (with some swelling) in the periodontal pocket over 20 minutes; a short drug
residence time (24 to 36 hours, Stoltze, 1995) in the pocket; polymer degradation time of 12 hours, and a large potential for drug loss both due to gel overflow and during crystal formation due to swallowing the gel. In fact, up to 70% of the dose is available to be swallowed. This may lead to a detectable systemic drug concentration, thus causing this 'localized' delivery system to now have some of the drawbacks associated with systemic metronidazole delivery.

1.2.4 Description of Bioerodible Polymers and Their Uses

Various polymer erosion mechanisms can be classified into three basic types. Type I erosion refers to water-soluble polymers insolubilized by covalent cross-links that solubilize as either the cross-links or the backbone undergo a hydrolytic cleavage. In type II erosion, polymers initially water insoluble are solubilized by hydrolysis, ionization or protonation of a pendant group. Type III erosion involves the conversion of water-insoluble polymers to small water-soluble molecules by backbone cleavage (Langer and Peppas, 1981). In this research, type II erosion is detailed in the following paragraphs.

Materials eroding by type II mechanisms have been mainly developed for enteric coatings. These coatings are designed to be insoluble in a low pH environment in the stomach, and to become soluble at an elevated pH in the intestine. Polymers eroding by type II mechanisms can be grouped into three general categories according to their dissolution mechanisms: (1) dissolution by hydrolysis of side groups; (2) dissolution by ionization of a pendant group (such as a carboxylic acid functional group); and (3) dissolution by protonation of an amine functional group (Heller, 1984).

The erodible polymer, cellulose acetate phthalate, CAP, (used in this research) dissolves by ionization of carboxylic acid functions and can generally be represented as a polyacid. This
polymer is insoluble in aqueous acidic media, but dissolves in aqueous bases upon ionization of
the free acid groups on the phthalates. Other examples of this type of erodible polymer are
systems such as esterified copolymers of methyl vinyl ether and maleic anhydride or partially
esterified copolymers of ethylene and maleic anhydride. These polymers have a narrow pH
threshold which changes linearly with the number of carbon atoms on the ester side group of the
copolymer (Heller et al., 1978). Additional properties of and CAP are included in Appendix A.

With relatively small ester groups, a low degree of ionization can solubilize the polymer,
therefore, the dissolution pH is low. As the size of the alkyl chain of the ester group increases so
does the hydrophobicity, and progressively higher degree of ionization is necessary to solubilize
the polymer, resulting in an increasingly high dissolution pH. This is also true for polymers
having the same number of ester groups but different degrees of esterification (Heller et al.,
1978).

Heller et al. (1978) showed that hydrocortisone release and polymer dissolution rate for
n-butyl half-ester polymer films containing dispersed hydrocortisone displayed excellent linearity
over the lifetime of the device. This provides strong evidence for supporting a surface-erosion
mechanism of drug release and negligible release due to diffusion. Because of the linear
correlation between polymer erosion and drug release, the distance eroded can be directly
correlated with the amount of drug released and has a significant dependence on the size of the
alkyl group. The erosion rate and drug release has also been shown to depend strongly on the
medium causing the erosion and a progressive decrease in erosion and drug release was observed
as the critical dissolution pH was approached (Heller et al., 1978).

The above experimental observations support an erosion model (Appendix C) which
views the polymer dissolution as a heterogeneous process, occurring only at the surface of the
device. The polymer is adjacent to a medium of high dielectric constant where the acid dissociation constant is normally measured in an aqueous medium. However, the interior of the polymer has a much lower dielectric constant, thus the acid strength is reduced considerably. Therefore, although water penetrates into the matrix, significant ionization can only occur at the 'outside' layer of the eroding device (Heller et al., 1978).

As ionization occurs, hydrogen ions accumulate at the polymer-water interface and the pH of the surface decreases until it reaches the polymer's dissolution pH ($H_D$), at which pH the polymer dissolution stops. Further polymer dissolution occurs only by a transfer of hydrogen ions into the bulk solution whose pH ($H_B$) is maintained at 7.4. Therefore, the rate at which the polymer dissolves is determined by the rate at which the hydrogen ions are transported away from the polymer into the bulk solution. Hydrogen ions are removed from the polymer surface by simple diffusion and by buffer assisted transport (Heller et al., 1978).

The buffer concentration used by Heller et al. was 0.1 M, making buffer-assisted transport the dominant process. At a constant buffer concentration, two factors influence the rate at which hydrogen ions are removed: (1) the pH gradient between the polymer surface ($H_D$) and the bulk solution ($H_B$); and (2) the thickness of the boundary layer ($l$). The thickness of the boundary layer increases as the stirring rate decreases, thus polymer erosion decreases as the stirring rate decreases.

Heller and Baker (1980) demonstrated the usefulness of the above system in vivo by placing hydrocortisone containing disc-shaped films in the lower fornical cul-de-sac of New Zealand rabbits. These devices were highly effective for zero-order drug delivery of hydrocortisone.
1.2.5 Release Profiles of Various Drug Delivery Systems

In some polymer drug delivery systems the polymer matrix can either erode or dissolve to release the entrapped drug into the surrounding environment. Erosion generally refers to polymers containing hydrolytically or enzymatically labile bonds which undergo hydrolysis or enzymatic cleavage in a given environment. Dissolution generally describes the result of physical disentanglement of polymer chains by a swelling solvent, with no chemical reaction taking place (Lee, 1991).

The development of controlled release bioerodible devices can have two fundamentally different approaches. One involves surrounding a drug core with a rate controlling polymer membrane and the other approach consists of dispersing the drug within a polymer to form a bioerodible monolithic device. In the latter case, if the drug is well immobilized in a solid matrix with minimal diffusional release and if the erosion is relatively fast, then drug release can be controlled by erosion (Heller, 1984).

In distinguishing between the two types of hydrolytic erosion of a solid hydrophobic polymer, bulk erosion refers to hydrolysis throughout the polymer matrix and surface erosion indicates erosion at the surface of the device. Surface erosion is more desirable as it leads to zero-order drug release, if the contribution of diffusion to drug release is minimal and overall surface area of the device is maintained constant (Heller, 1984) (Appendix C). The apparent zero-order release region has been attributed to the identical rate of both the diffusing and eroding fronts for an extended time period, in other words, to the synchronization of front velocities. This synchronization of front velocities is an important phenomenon common to all eroding drug delivery systems involving polymer surface erosion and dissolution. In general, the zero-order drug release from such systems will occur if $Ba/D$ (i.e., surface erosion rate constant,
B, multiplied by the half thickness of the slab, ‘a’, divided by the drug diffusion coefficient for the matrix, D) for the system is large enough for synchronization of front velocities to occur early in the release time. This can usually be achieved with a fast-eroding device (large B), a well packed polymer matrix (small D), or a sample with appropriate thickness (large a) (Lee, 1991, 1980).

The release of dissolved or dispersed drug from a polymer matrix into a constant, finite volume is an important consideration where a drug delivery system is placed in a body cavity containing a limited fluid volume (such as the periodontal cavity). In this situation, if the drug release rate from the device is in excess of the removal rate of the drug, the external drug concentration will be building up monotonically with time due to the condition of constant finite volume (Lee, 1991). Therefore, the assumption of perfect sink conditions is not realistic. This factor may be important to consider when looking at the possible design of a model to predict the rate of drug release from a drug delivery system placed in the periodontal cavity.

1.2.6 Selection of Bioerodible Polymer Systems

The pH sensitive bioerodible polymers suggested for use in this project are biocompatible, based on the association between a proton-donating polymeric carboxylic acid polymer and a proton-accepting ethoxylated non-ionic, surfactant polymer (Lee, 1988 and 1986). Such association polymers with intermolecular hydrogen bonding between ether oxygen and carboxylic acid groups can be totally insoluble at a low pH (where drug release will be totally diffusion controlled) and erodible at neutral pH (where drug release will be erosion controlled). The pH at onset of polymer erosion varies with the components of the association polymer. Cellulose acetate phthalate (CAP), a carboxylic acid containing polymer will be one of the
polymers of choice as it has been widely used in the pharmaceutical industry, as an enteric coating for tablets since the early 1940's, and is known for its oral safety. One of the family of Pluronics (a hydrophobic member in the family such as L101 or L121) form the second component of the association polymer system. Polyoxyethylene (hydrophilic domain) and polyoxypropylene (hydrophobic domain) block copolymer surfactants are known for their safety record as food additives and excipients in pharmaceutical tableting and injectable formulations. Additional properties of Pluronic L101 are included in Appendix A.

This information is useful as the pH of GCF varies from about 7 at healthy sites, to about 8 in the pathogenic inflamed states (Bickel et al., 1985). The combination of adjustable, pH-dependent erosion/diffusion and the capacity of this polymer system to slow down the drug release with the variation of the hydrophobic polymer component, makes this polymer system an ideal candidate for treatment of periodontal disease by localized, controlled drug delivery. An additional advantage is the thermoplastic nature of the polymer system which allows for simplification of the fabrication process.

1.3 Hypothesis

Clinically effective sustained release of antimicrobial agents into the periodontal pocket can be achieved over a predetermined time using a bioerodible polymer system in which the process of drug release occurs during the process of polymer erosion.
1.4 Objectives

Our objectives were to design and develop a novel bioerodible polymer system for the continuous delivery of antimicrobial agents into the periodontal pocket. The study had the following goals:

1. Characterization of a family of bioerodible association polymers based on compatible blends of cellulose acetate phthalate and hydrophilic block copolymers of polyoxyethylene and polyoxypropylene with respect to their physical and chemical properties.

2. Investigation of factors affecting the antimicrobial agent’s in vitro release profiles from various association polymer compositions.

3. Design of bioerodible devices (inserts) for the controlled delivery of antimicrobial agents in the periodontal pocket.

4. Determination of in vivo erosion characteristics, drug release and biocompatibility of the bioerodible devices by subcutaneous implantation using a rat model.

5. To detail the stability of 30/70 CAP/L101 films containing 10% (w/w) metronidazole, for storage at variable relative humidity conditions.


7. Reformulation and refinement of the insert design and drug loading concentration for optimization of the periodontal inserts.
<table>
<thead>
<tr>
<th>Method Name</th>
<th>Manufacturer</th>
<th>Description of Device</th>
<th>Reference and Description of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actisite® (25% (w/w) tetracycline•HCl)</td>
<td>ALZA Corp.</td>
<td>25% (w/w) tetracycline•HCl in a non-degradable ethylene vinyl acetate co-polymer (45% (w/w) vinyl acetate) periodontal monofilament fiber</td>
<td>Goodson et al. (1991). Randomized, single blind, 4 quadrant split mouth, placebo controlled, clinical trial comparing TCN fiber to placebo, no treatment, and SRP. Study conducted for 60 days.</td>
</tr>
<tr>
<td>25% (w/w) tetracycline PLGA strips</td>
<td>N/A</td>
<td>25% (w/w) tetracycline in a resorbable poly(D,L-lactide-co-glycolide) film strip</td>
<td>Maze et al. (1995). Randomized, 4 quadrant split mouth, 26 week trial.</td>
</tr>
<tr>
<td>Immobilized tetracycline containing cross-linked collagen film</td>
<td>N/A</td>
<td>Basic tetracycline powder contained in a resorbable cross-linked antelocollagen carrier film</td>
<td>Minabe et al. (1989). Randomized, placebo controlled, clinical trial comparing TCN film to placebo film (applied 4 times over 4 weeks). Study conducted for 7 weeks.</td>
</tr>
<tr>
<td>Atridox™ (10% (w/w) doxycycline hyclate)</td>
<td>Atix Laboratories Inc.</td>
<td>10% (w/w) doxycycline hyclate in a biodegradable drug delivery system (Atrigel®)</td>
<td>Polsen et al. (1996). Randomized, double blind, vehicle controlled and multi-centred trial over a 36 week period, one application every 12 weeks. 10% (w/w) doxycycline hyclate biodegradable system compared to 5% (w/w) sanguinarium chloride and vehicular control.</td>
</tr>
<tr>
<td>Dentomycin® (2% (w/w) minocycline)</td>
<td>Lederle</td>
<td>2% (w/w) minocycline•HCl powder, micro encapsulated in a biodegradable poly(DL-lactide-co-glycolide) polymer</td>
<td>Jones et al. (1994). Randomized, placebo controlled, single blind trial, where the drug encapsulated powder is evaluated alone or as an adjunct to SRP, in comparison to SRP or no treatment, over a 26 week period.</td>
</tr>
<tr>
<td>Acrylic strips containing 50% (w/w) MTZ, CHX and TCN</td>
<td>N/A</td>
<td>50% (w/w) MTZ, CHX and TCN in a non-resorbable acrylic resin vehicle</td>
<td>Addy et al. (1988). Randomized, controlled, single blind study with 50% (w/w) CHX, MTZ and TCN acrylic strips (applied twice within 2 weeks) compared with SRP over 12 weeks.</td>
</tr>
<tr>
<td>Elyzol® Dental Gel (25% (w/w) MTZ)</td>
<td>Dumex Limited</td>
<td>25% (w/w) metronidazole in a mixture of mono and triglycerides</td>
<td>Ainamo et al. (1992). Two applications of gel over two weeks. Comparison between gel and SRP. Randomized, 4 quadrant split mouth, multi-centred, 24 week trial. Stezel and Flores-de-Jacoby (1996). Comparison between gel and SRP. Randomized, split mouth, 24 week trial.</td>
</tr>
</tbody>
</table>
Table 1-2  Device Application.

<table>
<thead>
<tr>
<th>Method Name</th>
<th>Application Technique</th>
<th>Range of Pocket Size Application</th>
<th>Patient Acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actisite®</td>
<td>Following SRP, a blunt instrument is used to place the fibers in the pocket, and a thin layer of cyanoacrylate adhesive is placed circumferentially to keep the fibers in place.</td>
<td>The amount of TCN fiber used depends on the depth of each pocket, and the number of teeth used in the study.</td>
<td>Discomfort on fiber placement 10% (over packing the pocket causes pressure and discomfort), and local erythema following fiber removal 11%. Fiber worked out prior to planned removal in 29% of cases. No way to determine if any fiber is missing prior to, or during removal of the fiber. Treatment site does not have an improved appearance upon fiber removal. Initially, the tissue appears inflamed and distended, causing patient disappointment.</td>
</tr>
<tr>
<td>25% (w/w) tetracycline PLGA strips</td>
<td>Strips (0.5 x 2 x 10 mm) inserted to base of pocket, and cut at 2 to 3 mm above gingival margin. Coronal portion of strip tied to tooth crown with 4.0 silk suture. Cyanoacrylate adhesive applied to suture and coronal aspect of strip.</td>
<td>Strip placed at base of pocket, and cut to fit pocket.</td>
<td>Loss of some strips. No oral or systemic side effects reported.</td>
</tr>
<tr>
<td>Immobilized tetracycline containing cross-linked collagen film</td>
<td>Films were inserted into the periodontal pocket.</td>
<td>There is not enough information given about the film to judge.</td>
<td>There is no information reported on this subject.</td>
</tr>
<tr>
<td>Atridox™</td>
<td>Applied as a fluid and molds to the pocket shape, then solidifies. Two applications required, one every 4 months.</td>
<td>Amount of fluid adjusted to suit pocket size.</td>
<td>Patients commented that it was less painful and required less treatment time than SRP.</td>
</tr>
<tr>
<td>Dantomycin®</td>
<td>Administered with a disposable plastic syringe into the pocket at a depth to accommodate all 4 mg of powder (1 mg of minocycline base). The volume is tapped into the pocket with the tip of a syringe.</td>
<td>All 4 mg injected into pocket, but not to the base of the pocket. Small pockets may be over-filled, large pockets under-filled.</td>
<td>Local adverse effects, such as mild pain, were no more common in the test group than with the placebo gel. Drug levels suggest that systemic absorption was minimal.</td>
</tr>
<tr>
<td>Method Name</td>
<td>Application Technique</td>
<td>Range of Pocket Size Application</td>
<td>Patient Acceptance</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Acrylic strips containing 50% (w/w) MTZ, CHX and TCN</td>
<td>Strips were placed in the pocket using college tweezers. Strips were prepared to be 1 mm longer than the respective pocket and maintained <em>in situ</em> with a small amount of periodontal dressing. After 1 week, the strips were removed and replaced with another set for an additional week.</td>
<td>Depending on the width of the pocket, a minimum of 2 and a maximum of 3 strips were placed at each site.</td>
<td>Not reported.</td>
</tr>
<tr>
<td>Elyzol® Dental Gel</td>
<td>Viscous gel is applied into the pocket using a Parojet® syringe with a blunt cannula. The gel is liquified by body heat and hardens to form crystals in contact with water. Removal of overflow gel is required.</td>
<td>The gel can more or less be applied to fill the pocket to the gingival margin. Adjustable for variable pocket sizes.</td>
<td>Bitterness reported by 66%, and 30% reported gingival tenderness. Short drug residence time in the periodontal pocket, and large potential for drug loss from gel overflow and during crystal formation due to swallowing the gel.</td>
</tr>
</tbody>
</table>
Table 1-3 Device Behavior.

<table>
<thead>
<tr>
<th>Method Name</th>
<th>Drug Delivery Time</th>
<th>Choice of Therapeutic Agent</th>
<th>Composition and Safety</th>
<th>Device Degradation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actisite®</td>
<td>Tetracycline conc. of 4 to 8 µg/mL is inhibitory to most periodontopathic organisms. The mean GCF tetracycline conc. over a 10 day period was 1590 µg/mL, which declines exponentially when removed. There is a near zero order drug release profile.</td>
<td>25% (w/w) tetracycline•HCl</td>
<td>Ethyl vinyl acetate is a biocompatible non-irritating co-polymer which is elastic enough to mold to the pocket. Actisite fibers must be removed after 10 days. Fibers should not be tightly packed into a draining acutely abscessed periodontal pocket. When the entire 9 inch fiber is not used, remaining material must discarded. The cyanoacrylate sealant is difficult to remove. Systemic drug conc. similar to that of a 250 mg TCN capsule were noted.</td>
<td>The fibers do not degrade, but must be removed after 10 days.</td>
</tr>
<tr>
<td>25% (w/w) tetracycline PLGA strips</td>
<td>Released therapeutic drug conc. for 10 days. 145 µg/mL mean drug conc. during first 2 weeks of trial. No drug detected after 1 month.</td>
<td>25% (w/w) tetracycline</td>
<td>Loss of strips through swallowing during the first 2 weeks.</td>
<td>Strips intact after two weeks, but degraded to particles which dissolved prior to 1 month.</td>
</tr>
<tr>
<td>Immobilized tetracycline containing cross-linked collagen film</td>
<td>An effective MIC dose of drug was reported in the GCF for up to 10 days. The <em>in vivo</em> slow release study reports 17µg/mL of TCN on day 10. Goodson <em>et al.</em> (1985) reported that a TCN GCF conc. of 4µg/mL is needed for up to 48 hours to suppress periopathogen growth.</td>
<td>Basic tetracycline powder</td>
<td>No patient side effects were reported in this study. Antelocollagen (purified from bovine dermis, solubilized with pepsin) is reported to have excellent features as a carrier for immobilized active substances, drugs and enzymes.</td>
<td>Clinical observation revealed that the film, once inserted into the pockets, dissolves in about a week.</td>
</tr>
<tr>
<td>Atridox™</td>
<td>The drug is released over 7 days. No periodontal pocket drug concentrations are given.</td>
<td>10% (w/w) doxycycline hyclate</td>
<td>No information reported.</td>
<td>No information reported.</td>
</tr>
<tr>
<td>Method Name</td>
<td>Drug Delivery Time</td>
<td>Choice of Therapeutic Agent</td>
<td>Composition and Safety</td>
<td>Device Degradation Time</td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>-----------------------------</td>
<td>------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Dentomycin</td>
<td>Drug conc. in the periodontal pocket above MIC₉₀ for 3 days.</td>
<td>2% (w/w) minocycline•HCl</td>
<td>No information reported.</td>
<td>Poly(DL-lactide-co-glycolide) is a biodegradable polymer. No information regarding degradation given in study.</td>
</tr>
<tr>
<td>Acrylic strips</td>
<td>The strips were left in place for a total of 2 weeks but no GCF drug concentrations were reported.</td>
<td>50% (w/w) metronidazole, chlorhexidine acetate and tetracycline•HCl incorporated into the powder phase of the polyethyl methacrylate</td>
<td>The control in this study was no treatment. No drug-free strips were used since there is evidence that they may aggravate the existing condition. After 2 week application of the TCN system, some tetracycline resistant organisms were noted.</td>
<td>The device does not degrade.</td>
</tr>
<tr>
<td>Elyzol® Dental Gel</td>
<td>128 µg/mL metronidazole in pocket during first 8 hours. 90% of patients had conc. above MIC₉₀ up to 12 hours, but only 50% of the patients had a conc. above MIC₉₀ after 24 hours</td>
<td>40% (w/w) metronidazole benzoate (pro-drug) leading to 25% (w/w) metronidazole in gel mixture</td>
<td>70% of dose available to be swallowed, which may lead to detectable drug conc. systemically. Gel conversion to glycerol and oleic acid distends periodontal pocket, causing pressure.</td>
<td>No glyceryl mono-oleate (vehicle) noted after 12 hours.</td>
</tr>
</tbody>
</table>
Plate 1-1 Schematic Diagram of Periodontal Pocket.
Plate 1-2 Clinical Example of Patient with Periodontal Disease as Illustrated Through Probing of the Periodontal Pocket.
2. EXPERIMENTAL

2.1 Delivery Systems

2.1.1 Materials and Equipment

The materials and equipment listed in this section were used in this study.

2.1.1.1 Materials

A) Polymers

1. Pluronic L64 (average molecular weight 2000, melting point 16°C), lot number WPDJ-506D, was donated by BASF, Parsippany, NJ.

2. Pluronic F127 (average molecular weight 12600, melting point 56°C), lot number WPYL-591B, was donated by BASF, Parsippany, NJ.

3. Pluronic L101 (average molecular weight 3800, melting point -23°C), lot number WPNJ-516C, was donated by BASF, Parsippany, NJ.

4. Pluronic L121 (average molecular weight 4400), lot number WPNJ-516C, was donated by BASF, Parsippany, NJ.

5. Cellulose Acetate Phthalate (CAP) (molecular weight 2000-8000), lot number 90504, was donated by Eastman Chemicals, Kingsport, TN.

6. Cellulose Acetate Trimellitate (CAT), lot number T002-1088, was donated by Eastman Chemicals, Kingsport, TN.

7. Poly(Acry1ic Acid) (PAA) (average molecular weight 250000, CH₂CH[CO₂H]ₙ), catalogue number 18,128-5, lot number 03422JV, Sigma-Aldrich Corp., St. Louis, MO.
8. The drug containing inserts (mean dry weight = 7.6 mg), 30/70 CAP/L101, inserts containing 2, 5 and 10% (w/w) metronidazole, prepared as part of this study at the Faculty of Dentistry, University of Toronto.


B) Drugs

1. Metronidazole (99% free base), lot number PX00618DX, from Sigma-Aldrich Corp., St. Louis, MO.

2. Sodium pentobarbital (Somnotol), Department of Comparative Anatomy, University of Toronto.

C) Chemicals

The following buffer salts were used to prepare Sørenson isotonic sodium phosphate buffer (Gennaro, 1980).

1. Monobasic sodium phosphate (NaH₂PO₄•H₂O from Sigma-Aldrich Corp., St. Louis, MO.

2. Dibasic sodium phosphate (Na₂HPO₄), from Sigma-Aldrich Corp., St. Louis, MO.

3. Sodium chloride (NaCl), from Sigma-Aldrich Corp., St. Louis, MO.

D) Solvents

1. Methanol (methyl alcohol) (CH₃OH), reagent grade, from BDH Chemicals, Toronto, Ontario.

2. Ethanol (ethyl alcohol) (C₂H₅OH), reagent grade, from BDH Chemicals, Toronto, Ontario.

3. Acetone (CH₃COCH₃), reagent grade, from BDH Chemicals, Toronto, Ontario.

4. The mobile phase used for HPLC determination of metronidazole content in gingival crevicular fluid samples consisted of 93% (v/v) 0.0144 M triethylamine adjusted to pH 2.5 with 14.7 M phosphoric acid, 4% (v/v) methanol and 3% (v/v) acetonitrile. These
materials were obtained by the Control Unit of the Sunnybrook Health Science Centre, Toronto, Ontario.

E) Animals

1. Male Sprague Dawley rats, received live from Charles River Canada, St. Constant, Quebec, each 250 to 350 g, and housed at the Faculty of Pharmacy, Animal Care Room, University of Toronto.

2.1.1.2 Equipment

1. The rotating-disc apparatus consisted of a modified Hannson, Northridge, CA, dissolution apparatus, with the paddles replaced by rotating spindle sample holders.


3. The UV absorbance of CAP and metronidazole were determined at wavelengths of 240 and 320 nm, respectively, using the Quantitation II software package with a Hewlett-Packard, Fort Collins, CO, 8452 Diode Array Spectrophotometer, with a multicell-transport system for flow-through UV cuvettes.

4. Perkin-Elmer, Norwalk, CT, model DSC-2 differential scanning calorimeter, equipped with a dual-stage cooler (Intercooler II) and a thermal analysis data station (TADS).

5. Siemens D5000 multi-purpose, fully automated diffraction system was used for data collection of Cu Kα radiation (L = 1.5406 Å), monochromatized by a Solid State Kevex 4608 detector (EDS, L = 8.04 KeV, width = 420 eV). Data were processed with Diffrac-AT V3.0 software on a 486 PC work station. Polymer films and gels were submitted, for analysis to the X-ray Diffraction Service, at the Department of Chemistry, University of Toronto.

7. 1 Lambda micropipette, by Drummond Microcaps, Broomall, PA.

8. High pressure liquid chromatograph, (HPLC) at the Quality Control Unit of the Sunnybrook Health Science Centre, Toronto, Ontario. The chromatographic system consisted of an isocratic pump (Waters, model 510) which pumped the mobile phase through a C18 reverse phase column (Beckman Ultrasphere: 4.2 mm x 250 mm) at 1.0 mL/minute. Each sample or standard was injected into the system using an automatic sample injector (Waters, WISP 712). The column effluent was monitored with a variable wavelength UV detector (ABI, 759A absorbance detector) set at 220 nm. A chromatographic integrator recorded and integrated each chromatogram, which were subsequently archived on computer disk for permanent storage using Chromstation/2 software, by Spectra Physics, San Jose, CA.

9. Stainless steel (Unified Numbering System 30400) ‘dumbbell’ shaped punches, fabricated by Mr. Andras Nagy, Faculty of Pharmacy, University of Toronto.


11. Fourier transform infra-red spectrophotometer, model IFS85, from Bruker, Billerica, MA. This machine resides at the Centre of Biomaterials, University of Toronto. The following settings were used: collective sensitivity = 1.0, resolution = 4, scan number = 100, and spectral range = 650 - 4000 nm.
2.1.2 Preparation and Characterization of Cellulose Acetate Phthalate (CAP)

Prior to preparation of the association polymers, cellulose acetate phthalate (CAP), was precipitated in water from an acetone solution to remove extractable impurities. After filtering and washing thoroughly with distilled water, the CAP was air dried for 12 hours, then dried under vacuum at room temperature for 72 hours.

In order to shorten the preparation time, a polymer extraction process was also adopted. In a separatory funnel, the CAP was extracted using a methanol:water 1:2 mixture. This procedure was repeated twice, each time using fresh distilled water and a fresh methanol:water 1:2 mixture. After the final extraction, the CAP was washed thoroughly with distilled water and dried for 48 hours under vacuum at room temperature.

The resulting CAP was characterized for its free acid, phthalyl, and acetyl content in triplicate (3) samples, using USP assay procedures (Eastman, 1980a). The results are given in Section 3.1.1.

2.1.3 Association Polymer Film Preparation

Purified CAP and a Pluronic non-ionic surfactant were dissolved in acetone (reagent grade, containing a trace amount of distilled deionized water) to form a 10% (w/v) solution (i.e., 10% of total solid). All other reagents were analytical grade. To produce drug loaded films, an appropriate amount of metronidazole (free base) was dissolved in the casting solution to give samples with 2, 5, 10 or 20% (w/w) of drug loading on a dry basis.
The polymer films were initially cast in aluminum dishes, but to avoid any possible reactions between the aluminum and components (McEvoy, 1991) in the drug loaded polymer films, previously cleaned and sterilized glass casting dishes were later employed.

Glass rings 1.0 cm thick and 5.0 cm in diameter were placed on glass plates to form casting dishes. 10 mL of the polymer solution were deposited gradually (layer by layer) in the dish within a six hour time period. The acetone was allowed to evaporate at room temperature for 12 hours after which the films were dried under vacuum for 24 hours, again at room temperature. The glass casting dishes were then placed in a fume hood and loosely covered with paper towel to avoid exposure to varying air currents and to slow down the drying process. Afterwards, film samples were carefully separated from the glass and stored individually, at room temperature, in a light protected desiccator prior to use.

In order to determine the most suitable Pluronic compound for producing an association polymer with a 5 to 7 day erosion time, CAP/Pluronic films were prepared with several Pluronic non-ionic surfactants (Pluronic F127, L64, L101 and L121). The Pluronic family members mentioned above are listed in decreasing order of their hydrophilic/hydrophobic balance (HLB) values which corresponds to an increase in their hydrophobicity (Appendix B).

A series of polymer blends based on different polycarboxylic acid contents were examined to fully explore the potential of the present bioerodible association polymer system for other possible applications. These polymer blends include cellulose acetate phthalate (CAP), CAP/L101 (90/10, 70/30, 50/50, 40/60 and 30/70 (w/w) blends), CAP/L121 (90/10, 70/30, 50/50, 40/60 and 30/70), cellulose acetate trimellitate (CAT), CAT/L101 (100% CAT, 90/10, 80/20, 60/40, 50/50, 40/60, 30/70, 20/80 and 10/90 (w/w) blends), and poly(acrylic acid) (PAA), PAA/L101 (100%, 90/10, 80/20, 70/30, 60/40, 40/60, and 30/70 (w/w) blends).
2.1.4 *Polymer Film Dissolution and Drug Release*

2.1.4.1 *In Vitro* Experiments

The polymer erosion and drug release rates were determined using a rotating-disc apparatus as depicted in Figure 2-1. A single spindle rotating disc apparatus was initially setup with a temperature controlled dissolution bath system. In order to increase the efficiency of data collection, the polymer dissolution tests were performed in a 6-vessel temperature controlled bath, based on a modified Hannson dissolution apparatus.

The UV absorbency of CAP and metronidazole were monitored continuously using a flow rate controlled system and a multicell-transport system for flow-through UV cuvettes. The UV absorbance of CAP and metronidazole were determined at wavelengths of 240 and 320 nm, respectively, using the Quantitation II software package on a diode array spectrophotometer.

Drug loaded polymer discs 1.74 cm in diameter and approximately 0.3 mm thick (mean dry weight = 77.9 mg) were die cut from the polymer films and mounted in the sample chamber. To aid retrieval and gravimetric determination at the end of the dissolution, a mylar backing disc approximately 3.0 mm wider in diameter than the polymer film, was placed between the polymer and the stainless steel ring in the sample chamber.

During a typical experiment, the disc was rotated at 60 rpm in a glass vessel containing 400 mL of pH 7.0 Sørenson isotonic sodium phosphate buffer (buffer salts). The vessel was maintained at 37°C using an external circulating water bath. Triplicate runs were carried out for each sample.
2.1.4.2 In Vivo Experiments

While the in vitro studies were performed using four different series of association polymer films with great variation of both composition and the extent of loaded drug, in the in vivo experiments the initial measurements were reduced to one type of polymer film (i.e., blends of CAP and Pluronic L101).

The in vivo polymer erosion and drug release studies were conducted in a rat dorsal implant model. Polymer implants with 10% (w/w) metronidazole were produced from CAP/L101 films with the following blend ratios: 50/50, 40/60 and 30/70. Implants with dimensions of 1 mm wide, 10 mm long and 0.3 mm thick, with a mean dry weight of 3.46 mg, were utilized in all implant studies.

Prior to the implant surgery, male Sprague Dawley rats, with an average weight of 350 g, were anesthetized with a 2.86 mg/100 g intraperitoneal injection of sodium pentobarbital. The rat’s dorsal torso was shaved and the dermis disinfected with a 70% (v/v) aqueous ethanol solution (Plate 2-1). The implants were injected subcutaneously into the back of 8 anesthetized rats using a 14 gauge cannula disinfected with a 70% (v/v) aqueous ethanol solution. A total of 6 implants were placed in each rat (Plate 2-2). There were 5 sample times for each of the 3 polymer blends.

At predetermined times, the rats were sacrificed with sodium pentobarbital. The dorsal skin layers were cut away to expose the polymer implants (Plate 2-3 and Plate 2-4). The implants were then carefully removed and blotted free of any blood and/or tissue. The cleaned implants were dried at room temperature for 24 hours followed by drying in a vacuum oven for an additional 24 hours. The weight of each retrieved implant was then determined gravimetrically.
To determine the percentage of metronidazole remaining in the implants, the dried samples were extracted with 3 mL of a methanol:water 1:1 solution for 24 hours using a hematology mixer. The metronidazole concentration in the extracting solvent was then determined at a wavelength of 320 nm using the UV-visible spectrophotometer.

2.1.5 Reformulation of the Inserts

During the preliminary pilot clinical study, it was found that the 1.0 g periodontal insert prototype (30/70 CAP/L101 with 10% (w/w) metronidazole - 0.3 mm thick) released 80% of its drug within 3 hours. This drug release profile was considered too short for treatment of periodontitis. Thus, the insert was reformulated.

Various methods were employed to reduce the rate of release of metronidazole. This was achieved by two methods, namely surface extraction and dip-coating, to provide a drug free surface layer.

The first method was carried out by extracting previously prepared film samples containing 10% (w/w) metronidazole with distilled deionized water. Film samples were extracted for up to one minute at either 7°C or 27°C (identified as 'cold' or 'warm' extraction, respectively) to determine the effect of the temperature of extraction medium on the drug release profiles (Lee, 1984).

The second method consists of dip-coating the film samples in an acetone solution of 30/70 CAP/L101, followed by quick drying of the solvent. Two different dipping solutions were prepared in acetone, consisting of either 5 or 20% (w/v) polymer, and are identified as low viscosity and high viscosity solutions, respectively. In both cases the extracted and dipped
polymers were dried in a vacuum oven for 24 hours before in vitro drug release characterization (Lee, 1984).

Another approach applied to modify the release rate of metronidazole consisted of decreasing the drug loading in the polymer. Films of 30/70 CAP/L101 with 2, 5 and 10% (w/w) metronidazole were produced and their in vitro drug release profiles determined using the previously described rotating disc apparatus (Figure 2-1).

2.1.6 Differential Scanning Calorimetry (DSC) Analysis of Polymer Films

By using the DSC measurements, one can obtain thermal properties which can be used to infer polymer structural morphology (Mathot, 1994). The DSC analysis was used in this study to check for compatibility between the two selected polymer components, CAP and Pluronic L101 blends.

Polymer film samples were analyzed for their glass transition temperatures (Tg) and other thermal transitions using a differential scanning calorimeter, equipped with a thermal analysis data station (TADS).

DSC thermograms were obtained for a range of CAP/Pluronic L101 blends (70/30, 40/60, 50/50, 30/70), as well as for pure CAP, Pluronic L101 and metronidazole. Typical samples of approximately 15 mg were placed in small stainless steel pans, sealed, and dried in a desiccator for 2 days. These samples were then placed in the DSC sample holder for analysis, at a scanning rate of 10 K/minute over a temperature range of 210-500 K (as required). Analysis of the resulting thermograms was carried out on a thermal analysis data station. Plots of the sample
heating thermograms were obtained and examined to determine the thermal transitions and T_g's of the drug, individual polymers and the various CAP/L101 blends.

### 2.1.7 Fourier Transform Infra-Red (FTIR) Spectroscopy for Characterization of Polymers and Polymer Blends

The infrared spectrum is a unique molecular 'fingerprint' for a compound, which can be distinguished from other compounds. The spectrum is an arrangement of electromagnetic radiation ordered according to wavelength. FTIR provides a more definitive test for detection of function groups than does any other chemical analysis (Smith, 1979).

As a preliminary check to determine the evaporation time for the acetone solvent, a KBr disc was scanned to obtain a background spectrum and then 50 μL of acetone was applied onto the disc. The disc was scanned immediately, then once every 5 minutes for 20 minutes, and the scanning was repeated at 6, 12, 24, and 48 hours. The disc was then stored in a desiccator to prevent any water absorption. After a 10 minute evaporation period, no difference was observed between the background spectrum for the twenty potassium bromide (KBr) disc and the spectrum for the disc containing acetone.

For the FTIR studies, KBr discs were produced using a Carver Press, then dried in a desiccator for 3 days. A background spectrum was scanned using FTIR and stored on a computer disk for each KBr disc. Two percent (2%) (w/w) solutions of the following polymers and/or polymer blends in acetone were prepared for this study: CAP/L101 (30/70, 50/50, and 80/20), L101 100%, CAP 100% and CAP/L101 30/70 (w/w) blends with 2% (w/w) metronidazole.

Fifty μL of the test solution was applied to a KBr disc, then dried in a desiccator for 3 days to rule out any absorption contribution from the acetone solvent or water. The polymer
coated KBr discs were scanned on a Fourier transform infra-red spectrophotometer. This machine resides at the Centre of Biomaterials, University of Toronto, and is operated under the supervision of Mr. S. Lagousky. Shifts in the carbonyl stretching band were tracked as a function of blend composition. Representative spectra were obtained for each of the samples applied to the KBr discs. These spectra were examined for both the characteristic functional groups and evidence of association between the CAP and Pluronic L101 polymers.

2.1.8 X-ray Diffraction for Characterization of Polymers and Polymer Blends

X-ray diffraction method is a widely used analytical approach to distinguish ordered from disordered states of solid and semi-solid materials (Alexander, 1969). A small amount of powdered sample (or polymer films and gels in the case of this study) is exposed to a high density x-ray beam. The reflected spectrum is then collected by an appropriate detector. The yield diffraction pattern can be used to determine the following information about the sample: its crystallinity, phase composition (both qualitative and quantitative), macro and micro-structural features, and crystal chemical peculiarities.

For a crystal, x-rays which penetrate beneath the surface are scattered by the parallel layers of atoms. Each of these layers acts as a separate weak x-ray source. According to Bragg’s law, constructive interference of the x-rays occurs if the spacing of the layers is related to the wavelength of the radiation as follows (Considine, 1976):

\[ n\lambda = 2d \sin \theta \]

where:

- \( n \) = is an integer
- \( \lambda \) = wavelength of x-ray (metre)
\[ d = \text{distance between layers (i.e., spacing between crystal planes);} \]
\[ \text{this is also reported as d-spaces (metre)} \]
\[ \theta = \text{angle measure relative to crystal face, as opposed to the} \]
\[ \text{perpendicular (radians)} \]

In addition, x-ray energy will also be diffracted at an angle equal to the incident angle. The angle 2 theta (2\(\theta\)) reported on the diffractogram is equal to twice the incident angle of the x-rays, as measured from the crystal surface.

Diffraction patterns (diffractogram) for crystals consist of sharp peaks at several d-spacings. Amorphous solids, on the other hand, exhibit one or more broad peaks of relatively low intensity, without the appearance of any sharp peaks. Polymers have diffraction patterns varying between those for crystals and amorphous solids.

A uniform spacing of polymer chains is analogous to a uniform spacing of atoms within a crystal. For this reason, it is stated that the 'crystallinity' of a polymer increases as the intensity of a peak on a diffractogram increases, and the width of the peak decreases. Two peaks is an indication of uniform spacing in two directions.

Polymer films and gels were submitted, for analysis to the X-ray Diffraction Service, at the Department of Chemistry, University of Toronto, managed by Dr. S. Petrov. With the diffraction system, a slow step scan mode (0.02 degree/1.5 second) was used to include the most informative range of the diffraction pattern for polymer materials, namely: 3-40 degree two-theta (running time of 0.8 hour per sample).

For example, the d-distance at \(2\theta\) of 20 degrees is calculated as follows:

\[ d = \frac{n\lambda}{2 \sin (\theta / 2)}, \text{ for example} \]

\[ d = \frac{1 \ (1.5405 \ \text{Å})}{2 \sin (20 / 2)} = 4.436 \ \text{Å} \]
The L101 polymer gels were originally placed in a standard sample holder. The association polymer films, and later the glass filters containing L101 gels, were mounted on a special low background quartz plate.

The following series of CAP/L101 polymer blends were analyzed at room temperature: 30/70, 40/60, 50/50, 60/40, 70/30, 80/20 and 90/10, using pure CAP films as a control.

As a means to study the association of Pluronic L101 with water (i.e., to determine if L101 combined with water forms a liquid crystalline structure), 1.0 g of L101 was examined without water and then with the addition of 2, 10 or 50 µL water stirred into the gel. When small amounts of water were added to the L101 liquid (10 µL of water per 1 g of sample), the viscosity increased significantly and a gel was formed. When larger amounts of water were added, viscosity of the L101 decreased, making it difficult to maintain a constant sample thickness on the mounting plate. A low viscosity fluid sample flows across the mounting plate as it is rotated during testing.

For additional liquid samples, x-ray diffraction spectra were obtained using glass fiber filters as a support in order to minimize flow related effects previously experienced with L101 gels on a flat sample holder. A glass fiber filter was chosen as it does not interfere with the diffraction spectrum of the polymer, as can be seen in Figure 2-2. Since the sample (in the sample holder) is rotated around a fixed x-ray source, a non-participating support media, specifically the glass fiber filter, is needed to prevent the flow of samples with varying viscosity. The samples were layered onto the glass fiber filter and continuously weighed to ensure that a constant amount of polymer was placed on each filter (i.e., to achieve a constant thickness across the sample). Three aliquots of the sample gel were scanned, and their spectra compared for reproducibility.
To study the effects of water on 30/70 CAP/L101 polymer films, samples were compared both before and after immersion in distilled, deionized water. The films were immersed for one hour in distilled water to ensure good wetting.

Pure metronidazole base powder and 30/70 CAP/L101 films containing 0, 2, 5, and 10% (w/w) metronidazole were also examined to study the x-ray diffraction characteristics of drug loaded polymer films and the pure drug.

2.1.9 Humidity and Stability Studies of Association Polymer Blends

In this study, polymer phase separation, drug crystallization, and liquid beading on sample surface were experienced as a result of prolonged sample exposure to high relative humidity. The stability of the 30/70 CAP/L101 films containing 10% (w/w) metronidazole was assessed after 1, 3, 6 and 12 months. Film samples (7 for each different humidity condition) were protected from light and stored undisturbed at different relative humidity levels in desiccators containing either silica gel (close to 0% relative humidity, the control samples), a saturated solution (approximately 50% w/w) of calcium carbonate•H₂O (30% relative humidity) or water saturated air (approximately 100% relative humidity). In addition to physical observations, resulting samples were evaluated with respect to in vitro drug release, polymer erosion profiles, and thermal transition profiles using DSC. Film samples were not returned to the desiccator after evaluation.
2.2 Pilot Clinical Studies

2.2.1 Initial Clinical Study

The pilot clinical trials were conducted at the Graduate Clinic of the Faculty of Dentistry, University of Toronto, based on a protocol approved by the University’s Human Subjects Review Committee. The trials were carried out under the supervision of periodontist, Dr. Peter Birek.

In order to be included in the study, patients in good health had at least 6 periodontal pockets with a probing depth of at least 6 mm. Participants were excluded from the study if they were pregnant, lactating, diabetic, currently taking metronidazole, had a history of an allergy to metronidazole, possessed an infectious disease, or had a condition(s) requiring the administration of prophylactic antibiotics in conjunction with dental procedures.

For the determination of the important material parameters relevant to the clinical performance of the present polymer system, prototype periodontal inserts were produced from 30/70 CAP/Pluronic L101 polymer films containing 10% (w/w) metronidazole. These film samples were prepared in a disinfected laminar flow hood, then were allowed to air dry for 24 hours before being dried for an additional 6 hours in a slab gel dryer. Refer to Appendix D for examples of the insert manufacturing information and insert placement data sheet.

The shelf-life of the inserts was determined. Before an insert was placed in a patient, a UV analysis (using the previously mentioned in vitro association polymer sample dissolution technique, Section 2.1.4) was performed to compare the stability of drug release from freshly prepared inserts with those manufactured previously. Films from which the inserts were produced were cut in half. One half of the film was used to prepare the inserts and the other half for in vitro drug and polymer dissolution studies. Periodontal inserts (9 mm x 3 mm x 0.3 mm)
were die cut from the films using a disinfected, stainless steel ‘dumbbell’ shaped punch (Plate 2-6), then stored in a vacuum desiccator.

The drug containing inserts (mean dry weight = 7.6 mg) were placed (Plate 2-5) in at least 6 sites of 7 volunteer patients (5 females and 2 males), exhibiting periodontal pocket depths of at least 6 mm. The patients ranged from 34 to 52 years of age, with an average of 46. Two inserts were retrieved from each patient after 60, 120, and 180 minutes, during which time the patients abstained from eating and drinking (Birek et al., 1994). Gingival crevicular fluid (GCF) samples were obtained for the above mentioned time periods using a 1 Lambda (1 μL) micropipette.

The microcapillary tubes containing the crevicular fluid were weighed then expelled into 0.5 mL of sterile, distilled water. The samples were stored at -80°C up to 2 weeks to await further analysis. The metronidazole concentration was determined using an HPLC assay at the Quality Control Unit of the Sunnybrook Health Science Centre, managed by Mr. S. Walker.

The retrieved inserts were dried and their erosion rates determined gravimetrically. To determine the percentage of metronidazole remaining in the retrieved inserts, the dried samples were extracted with 3 mL of a methanol:water 1:1 solution for 24 hours using a hematology mixer. The metronidazole concentration in the extracting solvent was subsequently determined at a wavelength of 320 nm using the UV-visible spectrophotometer, and the amount of drug remaining was calculated.

The metronidazole concentration in GCF samples was determined by means of a modified HPLC assay (Kaye et al., 1980). A 200 μL aliquot of the diluted GCF sample was centrifuged for 10 minutes before being injected onto the HPLC column. The chromatographic system consisted of an isocratic pump which pumped the mobile phase through a C18 reverse phase column at 1.0 mL/minute. The mobile phase consisted of 93% (v/v) 0.0144
M triethylamine adjusted to pH 2.5 with 14.7 M phosphoric acid, 4% (v/v) methanol and 3% (v/v) acetonitrile. Two hundred microlitres of each sample or standard were injected into the system using an automatic sample injector. The column effluent was monitored with a variable wavelength UV detector set at 220 nm. A chromatographic integrator recorded and integrated each chromatogram, which were subsequently archived on computer disk. The concentration of metronidazole was determined by interpolation from a standard curve. Standard curves were linear in the range from 69 ng/mL to 51 mg/mL. The detection limit of this assay was a concentration of 200 ng/mL of metronidazole in the GCF.

2.2.2 Clinical Studies with Reformulated Inserts (Part 1 and Part 2)

In the two subsequent studies (clinical trial Part 1 and Part 2) of the prototype periodontal inserts, the drug loading was decreased from 10 to 5% (w/w) (Part 1) and then to 2% (Part 2) (w/w) metronidazole. A new punch (Plate 2-6) was obtained to die cut smaller inserts (7 mm x 2 mm) (Plate 2-7). Inserts were stored in a vacuum desiccator until clinical insertion. The thickness of the inserts increased from 0.3 mm for samples containing 10% (w/w) metronidazole, to 0.6 mm for inserts loaded with 5 and 2% (w/w) metronidazole. The drug containing inserts (mean dry weight = 1.03 mg) were placed in at least 6 sites of 12 volunteer patients (6 females and 6 males), exhibiting periodontal pocket depths of at least 6 mm. The patients ranged from 33 to 53 years of age, with an average of 43.

Using a 1 Lambda micropipette, GCF samples were obtained from the periodontal pocket before placement of the insert. Samples of GCF were also obtained at 1 and 4 hours after insert placement, then daily until the end of the study at 4 days. The metronidazole concentration in the GCF samples was determined using the HPLC assay mentioned previously (Section 2.2.1).
the end of the pilot clinical studies, patients were given a questionnaire (Appendix D) to determine their response to the inserts in terms of taste and comfort. Any additional complaints were recorded. The patients were also asked if they would undergo repeated therapy with the drug containing polymer inserts.
Plate 2-1 Anesthetized Male Sprague Dawley Rat Prepared for Implantation of the Bioerodible Drug Delivery Device. The dorsal tissue was shaved and the dermis disinfected with a 70% (v/v) ethanol solution.

Plate 2-2 Implants Injected Subcutaneously into the Back of the Anesthetized Rat (Dorsal Rat Back Model).
Plate 2-3 Dorsal View of Sacrificed Rat. After 4 days of implantation of bioerodible association polymers, no inflammation or infection was noted and implant sites were healing well. No adverse tissue reactions were noted.

Plate 2-4 *In Vivo* Polymer Implant. The dorsal skin layers were cut away from the rat to expose the subcutaneously visible, partly eroded polymer implants (cream colour masses).
Plate 2-5  The Placement of Polymer Insert into Periodontal Pocket. The insert is visible as it still must be placed at the bottom of the periodontal pocket.
Plate 2-6 Specially Designed Stainless Steel Punches Used to Prepare Inserts for Pilot Clinical Studies. Punches ‘A’ and ‘B’ are for the original and reformulated inserts, respectively.

Plate 2-7 Prototype Bioerodible Periodontal Inserts for Pilot Clinical Studies. ‘A’ and ‘B’ are the original and reformulated inserts, respectively.
Figure 2-1 Schematic Representation of a Rotating Disc Apparatus Used for Studying In Vitro Polymer Erosion and Drug Release. Samples rotated at 60 rpm in an isotonic phosphate buffer solution and maintained at 37°C by a jacketed water bath.
Figure 2-2  X-ray Diffraction Spectra of Various Fiber (paper or glass) Filters. A glass fiber filter (spectrum 4) was chosen as it has little absorption in the range of the L101 gels (spectra 1, 2, 3).
3. RESULTS

3.1 Delivery Systems

3.1.1 Preparation and Characterization of CAP

Prior to the formation of the association polymers, cellulose acetate phthalate (CAP) was prepared by either extraction or precipitation (Section 2.1.2). Characteristics of the CAP samples prepared by both precipitation and extraction techniques are presented in Table 3-1. All of these values conform to USP standards. The extracted CAP had a lower moisture and free acid content, but higher phthalyl and acetyl content compared with the precipitated CAP.

3.1.2 Characterization of Association Polymer Films

The association polymer films prepared were transparent, without any phase separation or Pluronic seepage onto the surface of the polymer (except in polymer blends containing more than 70% Pluronic (L101 or L121) or during times of storage at high ambient humidity). The most frequently used 30/70 CAP/Pluronic L101 blends were well plasticized and flexible.

Freshly prepared CAT/L101 films initially appeared to be compatible at a concentration of CAT as low as 20% (possibly due to the extra carboxylic acid available for the formation of potential additional association bonds to the CAT). However, upon storage, seepage of L101 on the surface of 20/80 CAT/L101 film began to appear, accompanied by apparent phase separation. Films prepared with poly(acrylic acid), especially those with a high L101 content, were very sticky and had a poor handling capability when samples were prepared for in vitro dissolution.
Association polymers prepared in this study were transparent with a drug loading up to 2% (w/w). However, films loaded with 5 and 10% (w/w) metronidazole were opaque due to the presence of dispersed drug in the polymer matrix. Samples above 10% (w/w) drug loading appeared to be opaque and had visible crystals and granularity on the surface of the film. These films were more brittle than polymer films containing 10% (w/w) or less metronidazole.

3.1.3 Polymer Film Dissolution and Drug Release

Highly hydrophilic members of the Pluronic series (e.g., F127) were initially investigated for their polymer erosion profiles. These substances, when combined with CAP and metronidazole, erode and release their drug over 5 to 7 days. Typical erosion in vitro profiles for two association polymers, the very hydrophilic CAP/F127 and the less hydrophilic CAP/L101, at the 50/50 blend ratio are plotted in Figure 3-1. From this graph it can be noted that CAP/F127 has a much faster erosion rate, reaching 100% at 60 minutes compared to the CAP/L101 sample which eroded completely after only 90 minutes.

The erosion of association polymers as a function of the hydrophobicity of the Pluronic component was also investigated. The erosion rates of Pluronic members F127, L64 and L101 at a 50/50 CAP/Pluronic blend ratio are shown in Figure 3-2, from the most to the least hydrophilic substances, respectively. It is evident that the association polymer erosion rate decreases with increasing hydrophobicity of Pluronics.

In order to determine the range of applicability, several association polymer blend systems based on polycarboxylic acids other than CAP were investigated. The individual series of polymer blends (including CAP, Figure 3-1 to Figure 3-4, CAT, Figure 3-5, and PAA, Figure 3-6) typically exhibit decreased erosion rates with decreasing CAP content for the more
hydrophobic L101 (Figure 3-3). The same is true for PAA/L101 blends. However, the erosion rates of the CAP/L121 blends increased from 90% to 70% CAP. As the level of CAT decreased in the blends, the erosion rate decreased, with a sharp drop at a CAT content of 50%. As a general trend, erosion rates of all of the polymer blends decrease in the following order: CAP/L121 (Figure 3-4) ≥ CAP/L101 (Figure 3-3) ≥ CAT/L101 (Figure 3-5).

Blends of poly(acrylic acid) with Pluronic L101 were also tested for erosion rate (Figure 3-6). It was noted that poly(acrylic acid) containing polymer blends swell to approximately 20 times their original thickness (especially those with high amounts of L101).

*In vitro* and *in vivo* studies were conducted to characterize the drug release and polymer erosion kinetics of the association polymer blends. Figure 3-7 and Figure 3-8 represent typical profiles for *in vitro* results of both polymer (CAP) erosion (%) and metronidazole release (%) from 50/50 and 30/70 CAP/Pluronic (L101) film samples, respectively, loaded with 10% (w/w) metronidazole. By comparing the time scales minutes in Figure 3-7 and Figure 3-8, it is evident that it takes twice as long for the metronidazole to be released when the CAP/L101 blend ratio changes from 50/50 to 30/70 (i.e., 100% metronidazole released after 60 minutes for the 50/50 blend, 47% metronidazole released after 1200 minutes for the 30/70 blend). The corresponding calculated metronidazole release rate decreased from 1.758 to 0.19 %/minute initially, then 0.0087 %/minute after 95 minutes. In addition, calculated CAP erosion rate decreases from 1.737 to 0.003%/minute. Consequently, the projected duration of metronidazole release is prolonged from 1 hour (60 minutes) to well over 20 hours (600 minutes), and that of polymer erosion from 1 hour to approximately 10 days.

Figure 3-9 and Figure 3-10 are graphical summaries of the *in vivo* results of polymer erosion and metronidazole release from 50/50 and 30/70 CAP/Pluronic (L101) blends after
implantation in a dorsal rat back model. A significant reduction in both the calculated rates of polymer erosion and metronidazole release occurs as the blend ratio is changed from 50/50 to 30/70 (changing from 1.51 to 0.0134%/minute and 1.55 to 0.067%/minute, respectively); this trend is similar to the in vitro results of Figure 3-7 and Figure 3-8. The resulting in vivo duration of metronidazole release increased from about 1 hour (60 minutes) to over 60 hours (3600 minutes), while polymer erosion was extended from about 1 hour to over 100 hours (6000 minutes).

3.1.4 Reformulation of the Inserts

To slow down the drug release, the drug carrier films had to be reformulated. The overall metronidazole release rate profiles of the extracted and dip-coated samples did not show significant differences from that of the 30/70 CAP/L101 control containing 10% (w/w) metronidazole (Figure 3-11). The dip-coated samples (referred to on the graph as the low viscosity and high viscosity solutions) and extracted samples (referred to on the graph as ‘cold’ and ‘warm’ water extractions) had a slower rate of release of metronidazole relative to the control sample (at 170 minutes). Only the cold and warm water extracted films showed any differences in their metronidazole rate at the 600 minute experimental time period. The dip-coated samples were too thick for insertion into the periodontal pocket, whereas the extracted films had a very uneven surface compared to the control samples. Therefore, samples produced by either extraction or dip-coating did not result in a formulation suitable for periodontal applications.

To decrease the metronidazole release rate (%/minute) and prolong the drug release duration, the approach of decreasing the percentage of drug loading in the polymer films was
examined. The release characteristics of films of 30/70 CAP/L101 with 2, 5 and 10% (w/w) metronidazole are summarized in Figure 3-12. Although the metronidazole released (%/minute) increased as the drug loading level decreased, the actual amount released (mg/minute) was less as the drug loading level decreased.

Based on these results, inserts containing 5 and 2% (w/w) metronidazole were produced. The sample thickness for the reformulated inserts was increased from 0.3 mm to 0.6 mm. The inserts with both the 2 and 5% (w/w) drug loading were reduced in size compared to the original inserts by using a smaller, redesigned, stainless steel punch.

3.1.5 DSC Analysis of Polymer Films

Figure 3-13 shows typical DSC thermograms for (a) pure CAP, (b) Pluronic L101, (c) metronidazole (base), and (d) a 30/70 CAP/L101 blend. Values of $T_g$ were calculated in accordance with the convention described in Appendix E (i.e., half way up the slope of the glass transition) and are listed in Table 3-2.

The thermograms display the following: (a) the $T_g$ of CAP at 340 K; (b) an exothermic (crystallization) peak of Pluronic L101 at 234 K; and (c) an endothermic (melting/fusion) peak of metronidazole at 279 K, followed by decomposition after 376 K; and (d) the $T_g$ of the 30/70 CAP/L101 blend is 255 K, with a broad exothermic peak at 390 K.

The thermal transitions of various CAP/L101 polymer blends are summarized in Table 3-2. The $T_g$ of the 50/50 CAP/L101 blend (271 K) appeared to be reduced from that of pure CAP (340 K) by approximately 69 K. However, at a low CAP content (30%), the $T_g$ is reduced from that of pure CAP by 85 K. The largest single reduction of $T_g$ was 60 K, which occurred between pure CAP and the 70/30 CAP/L101 blend.
3.1.6 Use of FTIR to Study Association Between CAP and Pluronic Polymer Blends

Free and hydrogen bonded functional groups can be readily identified based on their corresponding IR stretching vibrational bands (i.e., stretch vibrational mode of molecular bond). Figure 3-14 shows typical IR spectra of (a) pure CAP, (b) Pluronic L101, (c) metronidazole (base), and (d) a 30/70 CAP/L101 blend. The FTIR spectrum for CAP is characterized by a strong carbonyl (C=O) stretching band (Meites, 1963, Brown, 1975) at 1728 cm⁻¹, whereas that of Pluronic L101 showed a distinctive stretching bands at 1111 cm⁻¹ attributed to ether oxygen groups (-O-). These groups appear in the 30/70 CAP/L101 blend IR spectrum, but the peak at 1728 cm⁻¹ has shifted to 1744 cm⁻¹.

The peak at 2870 cm⁻¹ is due to the CH₂ stretching band on the hydrophobic poly(propylene oxide) (PO) unit of L101 (Park et al., 1992). Additional peaks for the L101 are detected at 2970 and 2930 cm⁻¹, due to CH antisymmetric stretching and CH₃ stretching bands, respectively. In this study, each of these bands is visible for all polymer blends containing L101.

To assess the effects of hydrogen bonding between the carbonyl and ether oxygen groups, representative spectra in the carbonyl stretching region (1630-1850 cm⁻¹) of pure CAP, various CAP/L101 blends (80/20, 50/50, 30/70), and a 30/70 CAP/L101 blend with 2% (w/w) metronidazole are shown in Figure 3-15. The pure CAP is characterized by a major carbonyl band at 1728 cm⁻¹, which develops a slight shoulder and shifts from 1728 to 1742 cm⁻¹ as the CAP percentage decreases from 100 to 30% (Xu and Lee, 1993). An additional shift of the carbonyl band towards a higher wavenumber is noted as 2% (w/w) metronidazole is added to the 30/70 CAP/L101 polymer blend. The frequency (and wavenumber) decrease as the number of
the electron receptor groups increases (i.e., as the double bond character increases) (Brown, 1975).

3.1.7 X-ray Diffraction Studies on Polymer Films and Pluronic L101 Gels

The x-ray diffraction patterns for association polymer blends CAP/L101 70/30 and 30/70 are plotted in Figure 3-16. The single peak for the 30/70 blend is indicative of a uniform spacing in one direction only and is suggested as an indicator of crystallinity (Petrov, 1993). The 70/30 blend has two peaks, which shows a tendency towards uniform spacing in two directions, not just one. This corresponds to a greater level of order compared to 30/70. For this reason, the 70/30 blend is classified as more highly ordered than the 30/70 blend, but the 30/70 blend has a higher degree of crystallinity.

X-ray diffraction patterns for CAP/L101 blends 100/0 (i.e., pure CAP), 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70 and 25/75 are plotted in 3 and 2 dimensions in Figure 3-17 and Figure 3-18, respectively. In both figures, one can see the transition from pure CAP to 25/75 blend as the two peaks are gradually replaced by one peak. There is not a sharp transition, the change in order gradually decreases as the amount of L101 increases.

To determine the effect of water on the 30/70 CAP/L101 films, the sample of association polymer blend CAP/L101 30/70 was tested before and after immersing the sample in deionized water for 1 hour. The diffractograms for this sample with and without immersion in water are plotted in Figure 3-19. Differences between these two diffractograms are slight, which may be an indication of either surface effects or it may be within the range of preparation error for the equipment.
Samples of L101 with increasing amount of added water displayed an increase in intensity of the diffraction spectrum. It was hypothesized that this effect could be due to the different flow behaviors of the gel across the sample plate as the sample holder is rotated. The viscosity of the gel decreases with the amount of water added. To test this hypothesis, a non-participating support medium was sought. A silica glass fiber filter was selected (Section 2.2), as its peak of 26.5 is not in the range of the peaks for the association polymers of 8 to 21 2 theta (29). Tests conducted on L101 with varying amounts of water, but mounted on glass silica fiber filter, displayed little difference in intensity between the spectra (Figure 3-20; note that the spectra have been offset vertically for clarity).

Figure 3-21 is an x-ray diffractogram for metronidazole (base). The intense, distinct peaks are typical for crystals, compared to the less intense, broad peaks for the amorphous polymers. Figure 3-22, Figure 3-23 and Figure 3-24 are diffractograms of 30/70 CAP/L101 films with 10, 5 and 2% (w/w) metronidazole, respectively. Pure 30/70 CAP/L101, without any metronidazole, is also plotted in Figure 3-24. As the amount of metronidazole was reduced, the intensity of the many sharp peaks decreased. For the cases of 10 and 5% (w/w) metronidazole, the magnitude of the peaks was less than for pure metronidazole, but the 2θ (d-spaces) did not change. At 2% (w/w) metronidazole, the characteristic peaks for metronidazole disappeared.

3.1.8 Humidity and Stability Studies of Association Polymer Blends

The stability of the metronidazole loaded polymer films was characterized under normal storage conditions (i.e., in a desiccator at near to zero relative humidity) and at different levels of humidity, over a twelve month period.
Polymer erosion and drug release rates for CAP/L101 blends with 10% (w/w) metronidazole are plotted in Figure 3-25 and Figure 3-26, for samples stored for 1 and 3 months, respectively. These figures include results for samples stored at 0, 30 and 100% relative humidity.

Results after one month show that for 0 and 30% relative humidity, samples were stable in their release profiles (Figure 3-25). However, increased polymer erosion and drug release rates were noted in samples stored at high relative humidity. On the surface of these samples, a large number of drug crystals were also noted. These crystals were assumed to be metronidazole. The surface of these samples were covered with beads of an oily liquid, likely to be L101. The samples were sticky and also extremely difficult to prepare for in vitro film dissolution testing.

After one month, the samples stored at 100% relative humidity did not present the same characteristic DSC spectrum (Figure 3-27) as samples stored at 0 and 30% relative humidity. This DSC spectrum, altered by humidity, lacked the characteristic T_g at 255K and the exothermic thermal transition at 390K, associated with the control 30/70 CAP/L101 polymer film (Figure 3-13 (d)).

After three months, polymer films stored at 100% relative humidity had completely fragmented and were unsuitable for sample testing. Samples stored at 30% relative humidity for three months were very similar to those stored at 100% relative humidity for 1 month, with respect to the DSC thermograms (Figure 3-27), polymer erosion, sustained (long term) drug release profiles (Figure 3-26), and visible polymer integrity. After six months, films stored at 30% relative humidity could not be sampled due to their deteriorated state. Exposure to moisture had a significant effect on the association polymer films. Polymer erosion and drug release increased with the degree and duration of exposure to humidity.
At all sampling times, (1, 3, 6 and 12 months) the 30/70 CAP/L101 films loaded with 10% (w/w) metronidazole and stored at close to zero percent relative humidity, were found to be the most stable samples. They retained their appearance of a thin, flexible, opaque polymer film. These films experienced no phase separation and/or L101 beading on the surface of the polymer, and all possessed thermograms similar to Figure 3-13 (d).

3.2 Pilot Clinical Studies

3.2.1 Initial Pilot Clinical Study

Before the newly prepared inserts were utilized in patients, an *in vitro* UV analysis of the 30/70 CAP/L101 association polymer films (from which the inserts were prepared) was performed to compare the stability and drug release of freshly prepared inserts (sample #31) with those manufactured 3 months earlier (sample #7). Drug release and polymer erosion profiles and rates can be found in Figure 3-28. No significant difference was observed in either the erosion or drug release characteristics of the two lots.

Preliminary results from the initial pilot clinical trials (shown in Figure 3-29) indicate that metronidazole was present in the crevicular fluid during the three sampling periods (60, 120 and 180 minutes). Levels of metronidazole remained well above the 90% minimum inhibitory concentrations (MIC$_{90}$) for most periodontal pathogens (47.45, 25.10 and 21.20 mg/mL, for the three sampling periods, respectively). The dry weight of the retrieved inserts (Figure 3-30) over the three sampling times was 6.56, 4.54 and 5.31 mg, respectively. The removal of inserts containing metronidazole in the initial study was, at times, complicated by the fact that upon hydration, the polymers became mildly adhesive. The reported dry weight of the retrieved inserts
are underestimated, in some cases, due to incomplete retrieval of the hydrated inserts from the inflamed periodontal pockets.

After a significant release (burst) of metronidazole during the first hour after their placement, the inserts continued to release the drug. Although the erosion of the periodontal inserts showed an average of 30% lost during the sampling period, the corresponding metronidazole release reached about 80%, over the entire sampling time (up to 3 hours).

3.2.2 Pilot Clinical Studies with Reformulated Inserts (Parts 1 and 2)

Information gained from the two subsequent clinical studies using reformulated inserts (to extend the time of drug release) loaded with 5 and 2% (w/w) metronidazole (Part 1 and Part 2, respectively) are shown in Figure 3-31 and Figure 3-32, respectively. In comparing the two graphs, a large difference is noted between the drug release concentrations on the y-axis for the two inserts of different drug content. For example, at the sampling time of 2 hours, metronidazole concentrations of 432 and 134 µg/mL are noted for inserts containing 5 and 2% (w/w) drug loading, respectively.

Median results from the questionnaire regarding patients perception of the periodontal insert’s taste and comfort are shown in Figure 3-33 and Figure 3-34, respectively. Six patients detected a slight bitterness during the first 45 minutes after the insert placement, but this decreased after a couple of hours. Slight to moderate discomfort (depending on the individual patients’ level of pain perception) was reported by 4 individuals after insert placement. This discomfort either decreased or disappeared completely by the second patient recall. No major irritation was reported by the test subjects. Overall, patients found the insert highly acceptable and all agreed to additional future treatment using the periodontal pocket inserts.
Table 3-1 Characterization of Precipitated and Extracted CAP.

<table>
<thead>
<tr>
<th>Sample or Standard</th>
<th>Free Acid Content (% w/w)</th>
<th>Phthalyl Content (% w/w)</th>
<th>Acetyl Content (% w/w)</th>
<th>Loss on Drying (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitated CAP</td>
<td>0.65</td>
<td>30.03</td>
<td>23.15</td>
<td>0.45</td>
</tr>
<tr>
<td>(Precipitated CAP</td>
<td>(0.71)</td>
<td>(35.54)</td>
<td>(20.09)</td>
<td>(0.56)</td>
</tr>
<tr>
<td>after 3 months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extracted CAP</td>
<td>0.31</td>
<td>34.40</td>
<td>21.97</td>
<td>2.40</td>
</tr>
<tr>
<td>(Extracted CAP</td>
<td>(0.41)</td>
<td>(33.50)</td>
<td>(19.44)</td>
<td>(1.33)</td>
</tr>
<tr>
<td>after 3 months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USP Standard</td>
<td>≤ 3.0</td>
<td>30.0-36.0</td>
<td>19.0 - 23.5</td>
<td>≤ 5.0</td>
</tr>
</tbody>
</table>
Table 3-2 Thermal Properties of CAP/L101 Polymer Blends.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_g$ (K)</th>
<th>Thermal Transition (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure L101</td>
<td>-</td>
<td>234 (exothermic)</td>
</tr>
<tr>
<td>30/70 CAP/L101</td>
<td>255</td>
<td>390 (exothermic)</td>
</tr>
<tr>
<td>40/60 CAP/L101</td>
<td>260</td>
<td>-</td>
</tr>
<tr>
<td>50/50 CAP/L101</td>
<td>271</td>
<td>-</td>
</tr>
<tr>
<td>70/30 CAP/L101</td>
<td>280</td>
<td>-</td>
</tr>
<tr>
<td>Pure CAP</td>
<td>340</td>
<td>-</td>
</tr>
<tr>
<td>Metronidazole Powder</td>
<td>-</td>
<td>279 (endothermic)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>376 (endothermic)</td>
</tr>
</tbody>
</table>

The transition temperatures were determined from DSC thermograms, using a Perkin-Elmer DSC-2 differential scanning calorimeter.
Figure 3-1  *In Vitro* Dissolution Profiles of Association Polymers CAP/F127 and CAP/L101 at a 50/50 CAP/Pluronic Blend Ratio. Dissolutions were performed using a rotating disc apparatus in pH 7.0 isotonic phosphate buffer. The polymer erosion time was faster for CAP/F127 films than those composed of CAP/L101.
Figure 3-2 Erosion Rates of Association Polymers CAP/F127, CAP/L64 and CAP/L101 at a 50/50 CAP/Pluronic Blend Ratio. Dissolutions were performed using a rotating disc apparatus in pH 7.0 isotonic phosphate buffer. CAP/Pluronic films composed of hydrophobic Pluronics (such as L101) had a much slower rate of polymer erosion than those containing moderately hydrophobic Pluronics (L64) or very hydrophilic Pluronics (such as F127).
Dissolutions were performed using a rotating disc apparatus in pH 7.0 isotonic phosphate buffer. The erosion rate for CAP/L101 blends decreased very rapidly in samples containing less than 50% CAP.
Figure 3-4 Erosion Rates of CAP/L121 Association Polymers at Various Blend Ratios.

Dissolutions were performed using a rotating disc apparatus in pH 7.0 isotonic phosphate buffer.

The erosion rate for CAP/L121 blends decreased very rapidly in samples containing less than 70% CAP.
Figure 3-5 Erosion Rates of CAT/L101 Association Polymers at Various Blend Ratios.

Dissolutions were performed using a rotating disc apparatus in pH 7.0 isotonic phosphate buffer.

The erosion rate for CAT/L101 blends decreased very rapidly in samples containing less than 60% CAT.
Figure 3-6 Erosion rates of Poly(acrylic acid)/L101 Association Polymers at Various Blend Ratios. Dissolutions were performed gravimetrically using a rotating bottle apparatus in pH 7.0 isotonic phosphate buffer. The erosion rate for poly(acrylic acid)/L101 blends decreased rapidly in samples containing less than 80% poly(acrylic acid). However, the poly(acrylic acid)/L101 blends of association polymers had the fastest erosion rates of the association polymer blends investigated.
Figure 3-7 Characteristics of *In Vitro* CAP Erosion and Metronidazole (MTZ) Release from 50/50 CAP/Pluronic L101 Films with 10% (w/w) Metronidazole Loading. Dissolutions were performed using a rotating disc apparatus in pH 7.0 isotonic phosphate buffer. *In vitro* drug release occurred synchronously with polymer erosion over a 60 minute time period.
Figure 3-8 Characteristics of *In Vitro* CAP Erosion and Metronidazole (MTZ) Release from 30/70 CAP/Pluronic L101 Films with 10% (w/w) Metronidazole Loading. Dissolutions were performed using a rotating disc apparatus in pH 7.0 isotonic phosphate buffer. Drug release occurred over 12 hours as the polymer slowly eroded.
Figure 3-9 Characteristics of *In Vivo* Polymer Erosion and Metronidazole (MTZ) Release from 50/50 CAP/Pluronic L101 Films (with 10% (w/w) Metronidazole Loading) in a Dorsal Rat Model. *In vivo* drug release occurred synchronously with polymer erosion over a 60 minute time period.
Figure 3-10 Characteristics of *In Vivo* Polymer Erosion and Metronidazole (MTZ) Release from 30/70 CAP/Pluronic L101 Films (with 10% (w/w) Metronidazole Loading) in a Dorsal Rat Model. *In vivo* drug release occurred over three days as the polymer slowly eroded over a 4 to 5 day period.
Figure 3-11 *In Vitro* Rate of Metronidazole Release from Treated Polymer Films Prepared with Low Viscosity Versus Higher Viscosity Coating Solutions and Warm Versus Cold Water Extraction Techniques Are Compared with a Control 30/70 CAP/L101 Film Containing 10% (w/w) Metronidazole. Although some difference in the rate of drug release was observed between the treated and control samples at 170 and/or 600 minutes, the overall drug release rate of the treated samples was not improved enough over the control for these samples to be suitable for clinical applications.
Figure 3-12 *In Vitro* Metronidazole Release Rates Profiles From 30/70 CAP/L101 Polymer Blends with 10%, 5% and 2% (w/w) Metronidazole Loading. Dissolutions were performed using a rotating disc apparatus in pH 7.0 isotonic phosphate buffer.
(b) Pluronic L101

Mcal/second Endothermic $\rightarrow$

(a) CAP

Mcal/second Endothermic $\rightarrow$

Temperature (K)

Temperature (K)
Figure 3-13 Representative DSC Thermograms. These thermograms were obtained using a Perkin-Elmer Model DSC-2 differential scanning calorimeter equipped with a dual stage cooler.
Figure 3-14 Representative FTIR Absorbance Spectra. The spectra were obtained from a polymer coated KBr discs using a Bruker Fourier transform infra-red spectrophotometer.

(e) Metronidazole

(d) 30/70 CAP/L101 Blend
Figure 3-15  Representative FTIR Spectrum of Samples in the Carbonyl Stretching Region of Pure CAP (CAP 100), CAP/L101 Polymer Blends (CAP 80/20, CAP 50/50 and CAP 30/70) and a CAP/L101 Blend Containing 2% (w/w) Metronidazole. By observing the peak shoulder wavenumber (located in brackets) a leftward shift in the carbonyl stretching area is noted.
Figure 3-16  X-ray Diffraction Spectra of Association CAP/L101 Polymer Blends 70/30 and 30/70. These spectra were obtained using a Siemens D5000 automated diffraction system.
Figure 3-17 A 3-Dimensional X-ray Diffraction Spectra from Various CAP/L101 Blends and Pure CAP Films. These spectra were obtained using a Siemens D5000 automated diffraction system.
Figure 3-18 X-ray Diffraction Spectra of Pure CAP and CAP/L101 Blends 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70, 25/75. These spectra were obtained using a Siemens D5000 automated diffraction system.
Figure 3-19 X-ray Diffractograms of 30/70 CAP/L101 Before and After Immersion in Deionized Water for 1 Hour. The top curve is after immersion in water, and the bottom curve is before immersion in water.
Figure 3-20  X-ray Diffraction Spectra of L101 Gels Containing Water. Plots (top, middle, bottom) are for 2, 10 and 50 μL of water, respectively, for each 1.0 g of L101. These plots were offset for clarity. The gel samples were applied to glass fiber filters for support while they were being scanned. Little difference between the spectra was observed.
Figure 3-21  X-ray Diffraction Spectrum for Metronidazole (Base) Powder. The peaks are marked with appropriate Miller indices.
Figure 3-22 X-ray Diffraction Pattern of 30/70 CAP/L101 Polymer Film Containing 10% (w/w) Metronidazole. The peaks that belong to metronidazole are marked with appropriate Miller indices.
Figure 3-23  X-ray Diffraction Pattern of 30/70 CAP/L101 Polymer Film Containing 5% (w/w) Metronidazole. The peaks that belong to metronidazole are marked with appropriate Miller indices.
Figure 3-24 X-ray Diffraction Patterns of 30/70 CAP/L101 Polymer Blend Film with 2% (w/w) Metronidazole (top) and Pure Film Without Metronidazole (bottom).
Figure 3-25 Characteristics of *In Vitro* CAP Erosion and Drug Release from 30/70 CAP/Pluronic L101 Films with 10% (w/w) Metronidazole Loading Stored at 0, 30 and 100% Relative Humidity for One Month. Dissolutions were performed using a rotating disc apparatus in pH 7.0 isotonic phosphate buffer. Samples stored at control (0%) and 30% relative humidity remain stable during storage for one month. However, the samples stored at 100% relative humidity exhibited increased polymer erosion and an altered drug release profile.
Figure 3-26 Characteristics of *In Vitro* CAP Erosion and Drug Release from 30/70 CAP/Pluronic L101 Films with 10% (w/w) Metronidazole Loading Stored at 0 and 30% Relative Humidity for Three Months. Dissolutions were performed using a rotating disc apparatus in pH 7.0 isotonic phosphate buffer. Samples stored at control humidity (0%) remained stable during storage for twelve months. However, the samples stored at 30% relative humidity exhibited increased polymer erosion and altered drug release profiles after 3 months of storage.
Figure 3-27 Thermogram of a 30/70 CAP/L101 DSC Tracing from Samples Stored at 100% Relative Humidity After 1 Month. This diagram lacks a characteristic exothermic peak associated with the 30/70 CAP/L101 control (0% relative humidity) film shown in Figure 3-13 (d). This thermogram was obtained using a Perkin-Elmer Model DSC-2 differential scanning calorimeter equipped with a dual stage cooler.
Figure 3-28 *In Vitro* Stability Study Showing Drug Release and Polymer Erosion Profiles for Freshly Prepared Inserts (Sample #31) and Samples Aged for 3 Months (Sample #7). No significant change was observed in the drug release or polymer erosion profiles of the two lots.
Figure 3-29  Metronidazole Concentration in the Gingival Crevicular Fluid (GCF) During Drug Release from the Original Periodontal Insert Containing 10% (w/w) Drug Loading.

After an initial burst in drug release, no significant change in drug concentration was noted during the next 2 hours of the study.
Figure 3-30  Dry Weights of Retrieved Inserts.  Approximately 30% of the polymer erosion occurred throughout the sampling period.
Figure 3-31 Metronidazole Concentration in the Gingival Crevicular Fluid During Drug Release from the Periodontal Insert Containing 5% (w/w) Drug Loading. Results from pilot clinical study with reformulated inserts (Part 1). A decreased initial drug release was noted compared to the original inserts and drug release was extended to over three days. At all times the drug concentration remained above that which is inhibitory for most periodontal pathogens.
Figure 3-32 Metronidazole Concentration in the Gingival Crevicular Fluid During Drug Release from the Periodontal Insert Containing 2% (w/w) Drug Loading. Results from pilot clinical study with reformulated inserts (Part 2). A decreased initial drug release was noted compared to the inserts with 5% (w/w) metronidazole. The concentration in the GCF remains steady after 24 hours. At all times the drug concentration remained above that which is inhibitory for most periodontal pathogens.
Bitter Taste

Figure 3-33 Results from the Questionnaire on How Patients Perceived the Insert Tasted

During the Pilot Study. Some patients noticed a bitter taste immediately after insert placement, however this taste disappeared after a few hours.
Figure 3-34 Results from the Questionnaire on How Patients Perceived the Insert Felt During the Pilot Study. Some patients felt a slight discomfort immediately after insert placement, however this sensation disappeared before the second patient recall.
4. DISCUSSION

4.1 Delivery Systems

4.1.1 Preparation and Characterization

In keeping with the goal of creating a clinically effective bioerodible insert for localized metronidazole delivery to the periodontal pocket, a novel, drug loaded polymeric association system was prepared and examined.

The localized release device was examined as a function of its two polymer components and their formulation, physical and chemical properties. The in vitro and in vivo characterization has led to the progressive insert design to meet the clinical requirements of the drug delivery system.

The association polymer blends (i.e., a physical and/or chemical association of the two polymers CAP and Pluronic L101) exhibit advantageous physical and mechanical properties that each individual polymer lacks.

4.1.2 In Vitro Polymer Erosion and Drug Release

The influences of Pluronic’s hydrophobicity and concentration on polymer erosion and drug release are presented in Figure 3-1 to Figure 3-3 for L101, L64 and F127. The most hydrophobic Pluronic L101 was chosen for this study as it provides a slower erosion time, and hence a slower drug release, compared to the most hydrophilic F127 (Figure 3-1). It was also noted that non-drug loaded polymer blends eroded more slowly than the 50/50 CAP/L101 blend containing 10% metronidazole (Figure 3-7). When comparing 10% (w/w) metronidazole loaded
CAP/Pluronic L101 polymer blends, with varying blend ratios, the in vitro results for the 50/50 CAP/L101 blend ratio demonstrate that metronidazole release and polymer erosion curves were nearly linear and superimposable, supporting the model of a primary surface erosion controlled release mechanism (Figure 3-7) (Gates et al., 1994). On the other hand, the corresponding in vitro results for the 10% (w/w) metronidazole loaded 30/70 CAP/L101 blend ratio show an initial metronidazole release faster than polymer erosion, suggesting a diffusion controlled release process with limited modulation from polymer erosion (Figure 3-8) (Gates et al., 1994).

4.1.3 In Vivo Drug Release and Polymer Erosion

The use of an animal model (rat dorsal implant model) was explored to determine polymer erosion and drug release characteristics in an in vivo setting. Also, the rat dorsal implant model was used to determine biocompatibility and seek out any adverse tissue reaction that may have been associated with the implantation of the polymer inserts.

The in vivo results of polymer erosion and metronidazole release from 50/50 and 30/70 CAP/L101 blends after implantation in a dorsal rat back model are summarized in Figure 3-9 and Figure 3-10. Similar to the in vitro results shown in Figure 3-7 and Figure 3-8, a significant reduction in both the rates of polymer erosion (approximately 100 times) and metronidazole release (approximately 100 times) occurred when the blend was changed from 50/50 to 30/70 CAP/L101. There was a reduction in in vitro polymer erosion and drug release rates, as a result of increasing Pluronic L101 content in the polymer blend (from Figure 3-7 to Figure 3-8). This reduction was reflected in the in vivo dorsal rat model, but to varying degrees (Figure 3-10).

Due to polymer hydration while in the rat tissue, the implants became somewhat adhesive and eventually turned to a liquid-gel like state before eroding completely. The fragmentation
which occurred on contact with the polymer implants led to incomplete retrieval of implants during their removal and may be responsible for some overestimation of the metronidazole release and polymer erosion in the \textit{in vivo} results. In such cases, the effective rate for the \textit{in vivo} drug release and polymer erosion may be smaller and the effective duration may be longer than those presented in Figure 3-9 and Figure 3-10. In the \textit{in vivo} setting, the placement of implants subcutaneously in a rat model was chosen because it permitted implants to be exposed to an environment supplied with a limited amount of interstitial fluid, similar to that found in the diseased periodontal pocket. Gingival crevicular fluid is released in response to inflammation in the periodontal pocket (both GCF and interstitial fluid resemble a physiological plasma exudate). It appears that certain areas of the rats dorsal subcutaneous tissue (those in close proximity to a blood supply) may be bathed in more interstitial fluid that others. This insert placement effect may also account for some of the variability in the \textit{in vivo} results as well as differences in erosion and drug release when comparing \textit{in vivo} and clinical trial results.

During the \textit{in vivo} rat experiment, no visible signs of adverse tissue reactions were observed. Previously in our lab (Xu \textit{et al.}, 1991) CAP/Pluronic F127 blends were implanted subcutaneously in the dorsal rat model. In addition to gross morphological examinations, tissue samples surrounding the implants were sized and fixed in a 10\% (w/v) sodium phosphate buffered formalin solution. After embedding in paraffin and microtome sectioning, the tissue samples were stained for histological examination. Preliminary histological examination of the tissue sections from the implant sites showed no visible tissue reaction or macrophage accumulation around the implant during the entire period of implantation.

The \textit{in vivo} metronidazole release rate appeared to be slower than that for \textit{in vitro} release in a primarily diffusion controlled system such as the 30/70 blend. This could be due to
differences in their respective local hydrodynamic conditions which directly affected the rate of mass transfer. *In vivo* polymer erosion was observed to be faster than that of the *in vitro* erosion in both blend compositions, possibly due to differences in local pH and ionic compositions (Gates *et al.*, 1994).

In summarizing the *in vivo* results, it was found that the duration of metronidazole release and polymer erosion in both the *in vitro* and *in vivo* experiments favours the use of the 30/70 polymer blend composition containing 10% (w/w) metronidazole as a bioerodible insert for the delivery of metronidazole to human periodontal patients.

4.1.4 DSC, FTIR and X-ray Diffraction Characterization of Polymer Films and Their Components

4.1.4.1 DSC

"The DSC behavior of individual random copolymers in the glass transition region is like that for homopolymers. The transition to block copolymers presents the potential for two $T_g$'s (amorphous blocks), which corresponds to two individual components" (Mathot, 1994). For blends, the existence of one or more regions (for amorphous components) implies incompatibility (Mathot, 1994).

The $T_g$ of the CAP/L101 polymer blend decreases nearly linearly from 280 K to 255 K (Table 3-2) as the Pluronic L101 content increases from 30 to 70 percent. The change in value (60 K) of $T_g$ is more significant from pure CAP to the 70/30 CAP/L101 blend. The presence of only a single $T_g$ (Table 3-2) in all CAP/L101 blends corresponds to the existence of a single
miscible phase (a compatible blend) (Xu and Lee, 1993, Park et al., 1992). The presence of two $T_g$'s would suggest a two-phase heterogeneous system.

Pluronic L101 has a well defined exothermic thermal transition (crystallization peak) at 234 K, whereas CAP does not. Metronidazole (base) is the only material which displayed a noticeable fusion/melting peak (endothermic peak).

It has been suggested that a more ordered liquid crystalline phase develops as the L101 content increases (Park et al., 1992). The term 'liquid crystalline' has been defined by others as "any self assembled and ordered molecular structure which include the 'gel' formation, often found in hydrophilic Pluronic surfactant/water systems above a certain temperature range" (Park et al., 1992). PEO/PPO/PEO triblock copolymers exhibit a complicated self-aggregation behavior in aqueous solutions due to micelle formation (Park et al., 1992). Self-aggregation or micelle formation is a necessary environment for development of liquid crystals. There may be insufficient aqueous content in the polymer blend films during manufacture for the formation of liquid crystalline phases. After exposure to gingival crevicular fluid in the periodontal pocket, however, the potential for formation of liquid crystalline phases increases.

The $T_g$ of the CAP/L101 polymer blends decrease as the amount of CAP decreases. None of the polymer blends in Table 3-2 containing CAP displayed a defined crystallization peak as for Pluronic L101 was found (Figure 3-13). The 'crystallization peak' for the 30/70 blend is possibly the result of an increased level of intermolecular hydrogen bonding between the CAP carbonyl and the Pluronic ether oxygen groups, leading to a more ordered structure, or it may be due to decomposition of the CAP.
4.1.4.2 FTIR

Quantitative analysis using FTIR is rarely performed on cast polymer films as they are not highly reproducible (Smith, 1996); for this reason, the IR spectra prepared from our film samples were evaluated qualitatively.

Xu and Lee (1993) detected a carbonyl (C=O) stretching band for CAP at 1735 cm\(^{-1}\). The position of the stretching band is also influenced by other functional groups (Brown, 1975). The range for the carbonyl stretching band might vary from 1430 to 1950 cm\(^{-1}\) (Smith, 1979). In the current study, a carbonyl stretching band was detected with a value of 1728 cm\(^{-1}\). This band was observed for all CAP/L101 polymer blends combinations containing CAP.

Metronidazole does not display any of the same stretching bands as either CAP or L101. Hence, its presence does not interfere with identification of the functional groups for either CAP or L101.

There is a shift in the carbonyl stretching band with increasing Pluronic L101 content. As polycarboxylic acids are strongly self-associating in the solid state through intermolecular carboxylic dimers (Harthcock, 1989), it is probable that the primary intermolecular interaction in pure CAP involves the hydrogen bonding between the carbonyl group of the carboxycylic acid moiety with the acid (O-H) group of another molecule (Xu and Lee, 1993). On the other hand, polyethoxylated polymers are inherently weakly self-associating. They do, however, associate more strongly with polycarboxylic acids. Therefore, as the percentage of Pluronic increases in the blend, additional sites capable of hydrogen bonding are provided by the ether oxygen group. Formation of each hydrogen bond between an acid and an ether oxygen group will liberate a 'free' carbonyl group, thereby resulting in the observed shift in the characteristic stretching frequency (Xu and Lee, 1993). Similar shifts in carbonyl stretching bands as a result of
intermolecular hydrogen have been reported in other miscible polymer blends (Moskala et al., 1984, Lee et al., 1988). It is believed that such intermolecular hydrogen bonding also plays a key role enhancing the compatibility of the present polymer blends.

4.1.4.3 X-ray Diffraction

The single peak on the x-ray diffractogram for the 30/70 polymer blend (Figure 3-16) is indicative of a uniform spacing in one direction only. The intensity of the peak is taken as a measure of crystallinity (Petrov, 1993). Hence, the single peak refers to a larger degree of crystallinity when compared to the two less intense peaks of 70/30 blends which have a greater level of order. The transition from pure CAP to 25/75 is not sharp, as the two peaks are gradually replaced by the single peak (Figure 3-17 and Figure 3-18). In other words, the change in order gradually decreases as the amount of L101 increases.

Silica glass fiber filter proved to be an effective mounting media, as it did not interfere with IR peaks for the association polymers. When tests were conducted on L101 with varying amounts of water, but mounted on a glass silica fiber filter, the small difference in intensity between the spectra was within the error of variability for sample preparation (Figure 3-20). The slight variations noted for films dipped in water were not suspected to be related to changes in overall polymer crystallinity.

The intense, distinct peaks on the x-ray diffractogram for metronidazole (base) were typical for crystals (Figure 3-21), compared to the less intense, broad peaks typical for amorphous solids and semi crystalline polymers (Alexander, 1969, Petrov, 1993).

With 10% (w/w) metronidazole added to a 30/70 CAP/L101 Pluronic blend, there was a strong peak related to metronidazole at 12 degrees (2θ). However, when 5% (w/w)
metronidazole was added to the polymer, the magnitude of the peak at 12 degrees decreased, and there was a large peak observed at 25 degrees. This could be due to very strong additional preferred orientations of metronidazole crystallites along the \{011\} and \{103\} faces (Petrov, 1993).

At 2% (w/w) drug concentration, the characteristic peaks for metronidazole were no longer visible, which indicated that crystals of metronidazole were no longer heterogeneously mixed (suspended) in the polymer blend, but were dissolved within the polymer matrix. It is also possible that the sample with a 2% (w/w) metronidazole loading level contains a drug concentration which is less than the detection limit of this x-ray diffractometer.

4.1.5 Stability of CAP/L101 Polymer Blends

Exposure to humid conditions has a deleterious effect on the association polymer films, as noted in the drug release and polymer erosion profiles of samples stored or produced in humid conditions (Figure 3-25 and Figure 3-26). The stability of the metronidazole loaded polymer films was characterized under normal storage conditions (nearly 0% relative humidity) and at different levels of relative humidity, over a twelve month period. Samples stored for twelve months at 0% relative humidity were stable. Results after one month show that samples stored at 0 and 30% relative humidity had stable release profiles (Figure 3-25), and had DSC profiles associated with freshly prepared 30/70 CAP/L101 polymer films (Figure 3-13). However, increased polymer erosion and long term drug release rates were observed for samples stored at high relative humidity (100%) after one month, or after three months of prolonged exposure at 30% relative humidity.
4.2 Pilot Clinical Studies

4.2.1 Evaluation of Study Inserts

The efficacy and design of periodontal pocket inserts was studied via a series of pilot clinical trials. The periodontal insert was redesigned, after a short clinical study of polymer erosion and metronidazole release rates, to improve the ease of application and retention of inserts into the periodontal pocket. The redesigned insert was then tested for efficacy and drug release in additional exploratory trials.

The 30/70 CAP/L101 blend containing 10% (w/w) metronidazole was chosen for use in the pilot clinical studies for the following reasons: (a) its in vivo rates of drug release and polymer erosion, and biocompatibility characteristics, as previously investigated in a rat model (Gates et al., 1994); (b) the pharmacological safety of its components; (c) and the ease of handling of the polymer film product.

Data from the initial pilot clinical study were consistent with the in vitro results in which the polymer erosion lagged behind the metronidazole release (Figure 3-29 and Figure 3-30). However, the extent of insert erosion (30% in 3 hours) and the corresponding metronidazole release (80% in 3 hours) were more rapid than those observed in vitro (Figure 3-8). Variations between results from the clinical study and the in vitro rates of erosion and drug release may be due to the differences in the local bioactive enzyme environment and ionic compositions or their respective local hydrodynamic conditions. In addition, the higher pH of human gingival crevicular fluid at the inflamed site (pH = 8.0) (Bickel et al., 1985) can result in a higher erosion rate of the association polymer than that carried out at physiological pH of 7.4.
The removal of inserts containing metronidazole in the initial study was, at times, complicated by the fact that upon hydration, the polymers became mildly adhesive, and eventually turn into a liquid-gel like state before completely eroding. Therefore, incomplete retrieval of the hydrated inserts from the inflamed periodontal pockets (similar to the retrieval problems encountered in removing subdermal implants from the rat model) may, in part, account for an overestimation of the rate of drug release and polymer erosion. The inserts were also often fragmented and dispersed during removal. For this reason, the effective rate of clinical drug release may be smaller, and the effective duration may be longer than those presented in Figure 3-29 and Figure 3-30, due to this phenomenon. If it is indeed true, the effectiveness of this periodontal delivery system may actually increase.

In the clinical study, the inserts containing 10% (w/w) metronidazole were readily accepted by patients with no apparent adverse effects. However, the drug release profiles were not favorable (i.e., there was an initial burst of rapid release followed by a period of slow drug release (for 90 minutes) that fell short of a sustained release profile). Based on the data acquired during this pilot clinical study, further investigations were carried out to refine the insert design and to reduce the rates of drug release and polymer erosion. The changes were expected to increase the overall period of sustained drug release as well.

During the redesign process, the original 30/70 CAP/L101 blend ratio was maintained for the matrix of the device since it possessed a desirable rate of polymer erosion, good handling properties, and preliminary patient acceptance. Several attempts were made to modify the device as illustrated in Figure 3-11 and Figure 3-12.

Unfortunately, all of these attempts were unsuccessful as the resulting devices had similar drug release rates compared to the original (10% metronidazole) drug loaded inserts. In some
cases, the inserts were clinically unsuitable due to their increased thickness, making them impractical and perhaps too unpleasant to the patient when placed in the periodontal pocket. These devices could be suitable for other drug delivery situations where the thickness of the device is not limited by the anatomical constraints of the periodontal pocket.

In the original clinical investigation, drug levels measured in the gingival crevicular fluid (GCF) were several magnitudes greater than the minimum inhibitory concentration (MIC) needed to eradicate susceptible periodontal pathogens. In order to minimize the burst effect, the drug loading level (i.e., drug/polymer ratio) needed to be decreased. This technique has been reported in literature on drug loaded hydrogels (Lee, 1984). A reduction in drug content of the polymer blend decreases the formation of microscopic pores in the film which occur when the drug diffuses out of the polymer matrix, hence polymer erosion decreases with reduction in drug content.

In Figure 3-12, the *in vitro* drug release rates (%/minute) at 170 and 600 minutes for 5 and 2% (w/w) drug loaded films, were higher compared to films containing 10% (w/w) metronidazole. Because the amount of drug in each polymer sample differs, the actual amount released (mg/minute) was less as drug loading level deceased.

The encouraging results of *in vitro* testing of the polymer films with reduced metronidazole loading (Figure 3-12), needed to be verified to ensure that the same trend was present clinically. The initial burst effect of the metronidazole concentration was reduced, so the decision was made to test this approach in a small clinical study. Along with the reduction of drug loading from 10 to 5% (w/w) metronidazole, the device's physical design parameters were also changed in order to create smaller inserts which could be used in periodontal pockets with shallower probing depths. The thickness of the device (Plate 2-7) was increased from 0.3 to 0.6
mm and the contacting surface area was decreased in an effort to prolong the drug release time of the insert. Care was taken when changing the insert thickness in order to produce a product which could be comfortably placed in the periodontal pocket. The reduced size of the insert was accomplished by designing a new stainless steel punch (Plate 2-6), which was used to produce the inserts (Plate 2-7) from the polymer film.

For the first pilot clinical study, using the reformulated inserts (Part 1), the drug loading level used was 5% (w/w). Favorable results were obtained, including a lower initial burst of metronidazole, and an extended release time up to 4 days. However, the initial burst release still exceeded metronidazole concentrations of 600 µg/mL which is far above the MIC₉₀ for most periodontal pathogens (Figure 3-31).

A second pilot study (Part 2) was undertaken using inserts with the same size, thickness, and polymer blend composition as used in the first clinical trial (Part 1), except inserts were prepared with 2% (w/w) metronidazole to further reduce the initial drug burst. Information gained from the clinical trial using inserts containing 2% (w/w) metronidazole was very favorable. The initial burst of drug release was reduced to approximately 250 µg/mL, and the drug concentration plateaus were less than 20 µg/mL after 24 hours, where the concentrations remained above the MIC₉₀ for an additional 2 days (Figure 3-32).

It is worth noting that prior to placement, inserts containing 2% (w/w) metronidazole were nearly transparent, compared to the opaque appearance of the inserts containing 5 and 10% (w/w) metronidazole. The shift from transparent to opaque may be due to the solubility limit of metronidazole in the matrix, which was consistent with patients reporting an absence of bitter taste using the 2% drug containing samples, even during the first hour after insert placement,
The periodontal inserts were well tolerated by the patients, as indicated in their responses to the questionnaires (Figure 3-33 and Figure 3-34). No major adverse effects were reported.

Drug concentration in the GCF may have been underestimated due to the difficulty in obtaining GCF samples from the periodontal pocket near the end of the study. The reason for this is that the inflammation in the periodontal pocket is significantly decreased, which reduces the flow of GCF. At times, less than 1 µL of GCF could be obtained from the pocket, which made the analysis of drug concentration difficult and perhaps inaccurate.

Any remains of the insert in the periodontal pocket were rarely observed after two days into the study. However, the drug concentrations in the GCF remained above MIC90 at the end of the trial (Figure 3-32). This may be due to one of the following reasons: The insert became a clear, colourless gelatinous mass on hydration, thus blending in, and becoming indistinguishable from the gingival tissue. It is also considered that the drug may adhere to the enamel of the tooth root or to the gingival crevicular tissues, or be concentrated in the GCF (Giedrys-Leeper et al., 1985) after it has been released from the insert. This was observed (Baker et al., 1985) with the antibiotic tetracycline, which adhered to the tooth root surface. The gingival tissues, or enamel of the tooth root, may then act as their own sustained metronidazole release system. Sink conditions (in regards to the amount of GCF in the periodontal pocket and the flow of GCF out of the pocket) were not maintained in the periodontal pocket microenvironment. Therefore, the drug release from the insert might not be as constant or as fast as model systems which contain an excess of fluid (Lee, 1991).
4.2.2 Comparison of Inserts with Other Periodontal Drug Delivery Devices

In the Introduction, several types of periodontal drug delivery devices were presented from the current literature and their performances were given in detail (Table 1-1, Table 1-2 and Table 1-3). In Table 4-1, Table 4-2 and Table 4-3, the same parameters for comparison were used regarding the present study on bioerodible association polymer inserts, containing 2% (w/w) metronidazole.

The bioerodible association polymer inserts can be easily applied. They are placed to the base of the periodontal pocket with tweezers. In the clinical studies with the reformulated inserts (Part 2), a small amount of cyanoacrylate adhesive is applied to the edges of the periodontal pocket to maintain the already somewhat bioadhesive insert in place. This technique is fast and easy, and does not need a significant learning curve for optimum placement such as for the periodontal delivery systems Actisite® or 25% (w/w) tetracycline PLGA strips.

The choice of metronidazole as the therapeutic agent takes advantage of the excellent antibacterial activity of the drug against anaerobic cocci and Gram negative bacilli. Metronidazole has been shown to be concentrated in the GCF (Giedrys-Leeper et al., 1985). This drug also has a very low risk for the development of resistant bacteria, in comparison to tetracycline and some of its derivatives (Giedrys-Leeper et al., 1985). It was found that systemic metronidazole appears to be most useful as an adjunct to non-surgical management in cases of advanced or refractory periodontitis and advanced adult periodontitis (Loesche et al., 1991 and 1987).
Depending on the size and shape of the periodontal pocket, the bioerodible association polymer periodontal insert's unique design and flexibility allowed the insert to be used as supplied or trimmed immediately before placement into the periodontal pocket. This option allowed the inserts to be tailored for periodontal pockets of variable depth.

Although slight bitterness and gingival tenderness on insertion of the device was reported, these symptoms disappeared one half hour after the device was applied. In a questionnaire given to the patients at the end of the range finding trials, all patients stated that they would agree to application of additional inserts in the future.

Initial metronidazole burst concentrations in the GCF were approximately 250 µg/mL. The drug concentration reached a plateau at 20 µg/mL after 24 hours, and was above the MIC_{90} for most periodontal pathogens at the end of the 4 day study. This system allows for a longer drug residence time in the periodontal pocket compared to the Elyzol® system, where only 50 percent of the patients maintained an MIC_{50} after 24 hours. The optimal time for metronidazole delivery to the periodontal pocket is not known. In the UK, severe periodontal infections are treated by a systemic dose of metronidazole of 400 mg twice per day (BID) for 3 to 5 days. This systemic drug level equates to crevicular fluid concentrations of 3 to 4 µg/mL (Seymour and Heasman, 1995).

The association polymer components CAP and Pluronic L101 have been widely used in the pharmaceutical industry for the last 15-20 years, with no adverse side effects reported. This is in contrast to cross linked collagen films containing immobilized tetracycline, in which the cross linking agents gluteraldehyde and formaldehyde may cause biocompatibility problems (Williams et al., 1984). Acrylic strips may also adversely affect and aggravate an existing
periodontal condition. In addition, these strips may cause difficulties leading to periodontal abscesses when packed too tightly into the periodontal pocket.

The bioerodible association polymer periodontal inserts have been shown to clinically 'erode completely' after placement for 2 days in the periodontal pocket. However, this same device was visible for 5 days in the *in vivo* rat model. As the inserts become a clear, colourless gelatinous mass on hydration, they may have blended and become indistinguishable from the gingival tissue. This would account for the discrepancies in polymer erosion between the *in vivo* and the clinical environments.

This bioerodible association polymer periodontal insert system appears to achieve the goal of concurrent drug release and polymer erosion.

### Table 4-1 Study Drug Delivery System.

<table>
<thead>
<tr>
<th>Method Name</th>
<th>Description of Device</th>
<th>Reference and Description of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioerodible association polymer periodontal inserts</td>
<td>2% (w/w) metronidazole base in a biocompatible bioerodible association polymer system (30% (w/w) CAP and 70% (w/w) Pluronic L101)</td>
<td>A range finding study to determine GCF metronidazole levels and polymer erosion.</td>
</tr>
</tbody>
</table>
Table 4-2 Study Device Application.

<table>
<thead>
<tr>
<th>Method Name</th>
<th>Application Technique</th>
<th>Range of Pocket Size Application</th>
<th>Patient Acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioerodible association polymer periodontal inserts</td>
<td>The insert was placed at the base of the periodontal pocket.</td>
<td>Depending on the size and shape of the pocket, the unique insert design and flexibility permitted the insert to be used as supplied or trimmed immediately before placement.</td>
<td>Some patients reported a slight bitterness and gingival tenderness during the first half hour after insert placement, when using inserts with 5% drug loading, but not with 2% drug loading. All patients agreed they would have additional inserts applied in the future.</td>
</tr>
</tbody>
</table>

Table 4-3 Study Device Behavior.

<table>
<thead>
<tr>
<th>Method Name</th>
<th>Drug Delivery Time</th>
<th>Choice of Therapeutic Agent</th>
<th>Composition and Safety</th>
<th>Device Degradation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioerodible association polymer periodontal inserts</td>
<td>Metronidazole concentrations in the GCF were above MIC₉₀ up to the end of the 4 day study.</td>
<td>2% (w/w) metronidazole base in a bioerodible association polymer system</td>
<td>The association polymer components CAP and Pluronic L101, have been widely used in the pharmaceutical, cosmetic and food industries.</td>
<td>The device was visible for 5 days in the in vivo dorsal rat model. In the clinical setting, the insert was not observed 2 days after placement.</td>
</tr>
</tbody>
</table>
5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 Drug Release and Polymer Erosion

Among the various bioerodible association polymers studied, the CAP/L101 polymer blends were found to be the most suitable for the present purpose of producing a periodontal insert. Below a blend ratio of 40/60 CAP/L101, there is a significant decrease in polymer erosion and drug release, possibly due to the existence of increased crystallinity in the polymer or the high hydrophobic content of (70%) Pluronic L101. The optimal in vitro polymer erosion and drug release was obtained with the model polymer blend of 30/70 CAP/L101, containing 10% (w/w) metronidazole loading.

In vitro and in vivo drug release from 50/50 CAP/L101 appear to be regulated by a surface erosion-controlled release process. However, in vitro and in vivo results for the 30/70 CAP/L101 blend ratio containing 10% (w/w) metronidazole had a rate of drug release greater than that for polymer erosion, suggesting a diffusion-controlled release process with modulation from polymer erosion. Although the in vivo and clinical polymer erosion and drug release profiles reflected those obtained in vitro, these trends are not directly comparable due to differences in their hydrodynamic conditions.

5.1.2 Physical Characterization and Polymer Stability

In the FTIR study, a shift of the carbonyl stretching band (in CAP polymer blend) was observed with increasing Pluronic L101 content, which is likely due to intermolecular hydrogen
bonding. This finding provides confirmation that the CAP/L101 blends form an association polymer.

From the DSC results, only one $T_g$ was detected for each CAP/L101 blend. This indicates the presence of a single miscible phase, and that the CAP/L101 blend is a compatible co-polymer system.

From the x-ray diffractograms, it was noted that the degree of crystallinity increased as the percentage of L101 increased in the CAP/L101 polymer blend. The characteristic x-ray diffraction pattern for metronidazole was visible in the 30/70 polymer blends loaded with 10% (w/w) metronidazole, which indicated that the drug crystals were heterogeneously distributed within the polymer matrix. The opacity of polymer blends loaded with 10% (w/w) drug is likely due to the presence of distinct crystals of metronidazole. At 2% (w/w) drug loading, the films became transparent and the characteristic x-ray diffraction pattern for metronidazole was no longer evident. The disappearance of the x-ray diffraction pattern suggests that the metronidazole no longer existed as distinct crystals within the association polymer phase, but are solubilized in the association polymer matrix.

The stability of the 30/70 CAP/L101 polymer blend loaded with 10% (w/w) metronidazole was adversely affected by humid storage conditions. With increasing relative humidity and duration of exposure, the polymer erosion and drug release rates of the polymer blends increased and these changes were reflected by the DSC thermograms.

5.1.3 Clinical Achievements

A new sustained release delivery device for metronidazole has been developed and characterized. It exhibits properties suitable for the localized treatment of periodontal infections.
The main advantages of the newly developed inserts are (a) studies demonstrated that the reformulated inserts containing 2% (w/w) metronidazole provided drug concentrations in the GCF above MIC\textsubscript{90} for up to 4 days, and (b) the reformulated inserts had a decreased ‘burst effect’ and a prolonged drug release compared to those results obtained in the initial study.

Patient acceptance of the periodontal insert was very high and no adverse effects were reported during the study. The present bioerodible drug delivery system is adequate for the treatment of periodontal infection, as it achieves the goal of device erosion concurrent with drug release. However, some aspects of the device design and the clinical trial testing of the insert still need to be improved.

5.2 Recommendations

Although the composition and drug release characteristics of the inserts containing 2% metronidazole are favorable, additional drug delivery device design modifications, characterization and clinical trials need to be considered.

1. Additional future modifications to the insert design should include the following:

a) The use of the pro-drug metronidazole benzoate instead of metronidazole (free base) is highly recommended, as it has a much lower aqueous solubility of 1:7000, compared to metronidazole which has an aqueous solubility of 1:100 (Norling et al., 1992). This may lead to a further decrease in the drug burst effect, and prolong the drug release time.

b) New ways of securing the insert in the periodontal pocket should be explored in order to ensure that the insert does not leave the pocket before releasing its drug
and eroding completely. Several possibilities of maintaining insert placement include:

i) Enhancing the present bioadhesive properties of the insert by applying a thin coat of biodegradable poly(acrylic) acid or other suitable substance to the insert before placement in the periodontal pocket.

ii) A thin layer of cyanoacrylate adhesive could be applied circumferentially to the edges of the periodontal pocket to keep the insert in place. This technique is used in Actisite® fiber placement (Goodson et al., 1991).

iii) The insert could be tied to the tooth crown (similar to the procedure used by Maze et al., 1995) with biodegradable sutures.

iv) The use of a small amount of periodontal dressing is another alternative to keep the insert secured (Addy et al., 1985).

2. To further the understanding of the film's surface and bulk characteristics and metronidazole distribution throughout the film, electron microscopy and scanning electron microscopy techniques should be employed to study the polymer film. The 30/70 CAP/L101 blend should be examined to determine if these bioerodible films possess liquid crystalline properties. This should be accomplished by applying low angle x-ray diffraction procedures and examining the appearance of the polymer film in polarized light (Norling et al., 1992). Stability studies should be carried out on the final optimized 30/70 CAP/L101 insert loaded with 2% (w/w) metronidazole. Drug release and polymer erosion studies on this optimized drug loaded polymer blend should be carried out to simulate non-sink conditions, in order to more thoroughly compare the in vitro and clinical profiles. The 30/70 CAP/L101 blends containing 2, 5 and 10%
metronidazole should be characterized (using DSC, FTIR, and x-ray diffraction) to determine if the metronidazole affects the physical properties of the polymer blends.

3. Larger scale, clinical trials using the optimized periodontal insert need to be performed to establish the drug delivery device's efficacy and clinical performance. These clinical trials should include the following:
   a) a large number (e.g., 100) of patients (with at least 6 sites per patient qualifying for insert placement) to increase the statistical power of the study;
   b) the use of a control such as the 30/70 CAP/L101 inserts with no drug; and
   c) comparison of clinical parameters between use of the inserts and the control, both with and without scaling and root planing.

   The measurement of clinical indices during the study should include measurement of bleeding on probing (BOP), crevicular fluid flow (CFF), changes in probing depth, and shifts in the periodontal microflora from those associated with periodontal infections to those of a healthy periodontium. These clinical parameters should be tested at baseline and weekly for six weeks, on a monthly basis for the next six months, then four times annually. The total study length should be two years, similar to the studies performed by Pavicic et al. (1994) and Papli and Lewis (1989). GCF sampling for metronidazole content should be frequent to fully detail drug release from the periodontal inserts. Sampling should occur at 0.5, 1.0, 2.0, 4.0, 12, 24, 48, 72, 96, 120, 168 and 216 hours after insert placement to ensure the sustained release of metronidazole over one week. Both the drug release and polymer erosion kinetics should be followed.
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7. APPENDICES

7.1 Appendix A - Physical Properties of Metronidazole, CAP and L101

7.1.1 Metronidazole

7.1.1.1 Physical Properties

Metronidazole is 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole. Its chemical structure and physical characteristics are given in Appendix A. It has a creamy coloured crystalline appearance and lacks a discernible odour. Metronidazole solubilities in various solvents at 25°C are as follows: water (10.5 mg/mL), ethanol (15.4 mg/mL), methanol (32.5 mg/mL), and chloroform (3.8 mg/mL). The melting point ranges from 158 to 160°C. The molecular weight of metronidazole base is 171.16. Metronidazole exhibits an infrared absorption maxima around 274 nm, when using a 0.1 molar sulfuric acid in methanol as a solvent. Additional physical properties for metronidazole (e.g., infrared spectrum absorption bands and molecular formula) are included in Appendix A (Florey, 1976, Budavari, 1989).

Metronidazole is a low molecular weight uncharged molecule, which is relatively inactive. The drug is not ionized at physiologic pH. Its bactericidal effect is not altered by pH changes within the range of 5.5 to 8.0, this makes the drug ideal for incorporation into pH sensitive bioerodible association polymers.
7.1.1.2 Mode of Action

Metronidazole was selected as the antimicrobial agent for this study because it has been shown to be highly effective against anaerobes which are the prime periodontal pathogens in adult periodontal disease (Slots and Rams, 1990).

Metronidazole is bactericidal, amebicidal and trichomonacidal in action. Its antimicrobial effects depend upon its selective reactivity in the unstable and reduced form. After drug ingestion, metronidazole readily permeates mammalian cells as well as anaerobic and aerobic bacteria cell membranes by diffusion, to achieve a steady state intracellular concentration. When the nitro group of the compound is reduced by nitroreductase (ferrodoxin-like electron transport protein), a concentration gradient is created and more drug enters the cells. Reduction of metronidazole leads to the release of decomposition products with toxic properties (nitro, nitroso, nitroso-free radicals and hydroxylamine derivatives) (Lockberry et al., 1984). It is believed that these intermediates interfere with DNA synthesis.

Once in the cell, metronidazole binds to the DNA strands and disrupts the helical structure of the molecule. DNA strand breakage and inhibition of nucleic acid synthesis occurs, which ultimately leads to cell death. This process results in rapid killing of anaerobic microorganisms.

The hydroxymetabolite of metronidazole also exhibits antimicrobial activity. This suggests that there may be synergism between the parent drug and its metabolite, which may account for the greater than expected clinical efficacy of the drug in treating A. actinomycetemcomitans (Aa) infection (Jousimies-Somer et al., 1988).

The drug is primarily metabolized in the liver and its metabolites are excreted in the feces (Plaissance et al., 1988). Prior to drug degradation, metronidazole enters tissues, cerebrospinal
fluid, saliva, and can be detected in the GCF. The concentration of metronidazole in the GCF varies depending on the assessment technique, drug loading dose and the number of ingested doses. Metronidazole's bactericidal action has also been shown to be effective in controlled drug delivery devices for treatment of periodontitis (Addy et al., 1988).

7.1.1.3 Spectrum of Activity

Metronidazole was developed in France during the 1950's to treat protozoan infections (Scully, 1988). Shinn (1962) observed that when this drug was administered to treat Trichomonas vaginalis infection, ulcerative gingivitis was also resolved.

It is equally effective in dividing and non-dividing cells (McEvoy, 1991). Metronidazole is a nitroimidazole compound with broad spectrum activity against protozoa and anaerobic bacteria. It is used in the treatment of trichomonal genital infections, as a prophylactic agent before abdominal surgery and in the management of severe anaerobic infections.

Metronidazole has been used extensively in clinical practice for years, and its uses have expanded. The drug has excellent bioavailability and good penetration in most tissues (including penetration into the gingival tissue), cerebrospinal fluid and brain abscess contents. It is usually well tolerated with few side effects. With a few exceptions, the percentage of anaerobes, including B. fragilis group, that are resistant to metronidazole remains low (Falagas, 1995).

In a study to evaluate the use of metronidazole along with two other antibiotics for the treatment of Helicobacter pylori infections, 29% of the patients had cultures with a resistance to metronidazole (Lim et al., 1997). However, in developing countries H. pylori resistance can occur in up to 95% of those treated (Goodwin, 1997). “Inappropriate use of antibiotics
needlessly increases drug expenditures, enhances the emergence of antimicrobial resistance in hospitals and heightens the risk of toxicity, especially in elderly patients” (Lutters et al., 1998).

Metronidazole is bactericidal at low concentrations for most anaerobes, such as bacteroides, fusobacteria, treponemes and certain parasites. It is most active against Gram-negative anaerobic bacilli. Affective organisms are usually killed at concentrations of 0.25 to 4.0 μg/mL. However, Walker (1985) noted that this concentration varied, 16 μg/mL was needed to eliminate black-pigmented bacteroides in vitro, while others found that less than 1.0 μg/mL was sufficient to eliminate 90% of these microbes. Bacteria often unaffected by metronidazole included aerobic facultative and microaerophilic microorganisms (Walker, 1985).

Its antibacterial activity against anaerobic cocci, Gram-negative and Gram-positive bacilli has led to its use in the treatment of periodontal disease (Seymour and Heasman, 1995). In periodontal treatment, metronidazole is given in various systemic doses. In the UK, the common dosage is 200 mg, 3 times per day (TID) for 3 to 5 days. More severe infections may need an increased dosage of 400 mg, 2 times a day (BID) for 3 to 5 days. Metronidazole is absorbed from the gastrointestinal tract and is widely distributed throughout the body. Levels in the crevicular fluid peak at between 3 and 4 μg/mL approximately 4 hours after dosing (Britt and Phlod, 1986). After 5 days of oral dosing with 250 TID, the level of metronidazole in the GCF shows a much greater range and can be nearly 50% higher than the serum concentration. This presumably is due to the accumulation of drug in the GCF (Giedrys-Leeper et al., 1985).

7.1.1.4 Adverse Effects

Metronidazole was studied extensively for 20 years and has proven to be safe in humans (Mahmood and Dolby, 1987, Roe, 1982). Negative aspects of drug administration that need to
be addressed are physical effects, drug interactions and teratogenicity. Administration of metronidazole can, on occasion, result in side effects which include gastrointestinal discomfort, diarrhea, nausea, loss of appetite, metallic taste, urticaria and discoloured urine (McEvoy, 1991). Gastrointestinal distress can be minimized if the medication is taken with meals. Concurrent consumption of metronidazole with food does not inhibit its bioavailability, however, absorption is slowed (McEvoy, 1991). In general, short term utilization of metronidazole is well tolerated, and results in few side effects which resolve upon cessation of drug intake.

Metronidazole may cause a disulfiram (antabuse) like effect when alcohol is ingested. The response is proportional to the amount of alcohol imbibed, and can result in severe cramps, nausea and vomiting. These side effects can be avoided if the patient refrains from using alcohol products during therapy, and for at least 1 day after. Individuals undergoing anticoagulant therapy should also not be prescribed metronidazole, because it inhibits warfarin metabolism, prolonging prothrombin time (McEvoy, 1991).

Studies involving pregnant women, who received metronidazole during pregnancy, have not reported an increased incidence of teratogenicity (Chaco et al., 1987). Burtin et al. (1995) performed a meta-analysis to determine the safety of metronidazole in pregnancy. They concluded that metronidazole does not appear to be associated with an increase teratogenic risk. In a later meta-analysis (Caro-Paton et al., 1997), no relationship between metronidazole exposure during the first trimester of pregnancy and birth defects was found. In fact, metronidazole is currently being recommended to treat bacterial vaginosis during pregnancy. Thirty to 50% of women experiencing bacterial vaginosis during pregnancy have a recognized risk of pre-mature delivery. However, treatment of bacterial vaginosis with oral metronidazole
has been shown to return the vaginal flora to normal helping to prevent increased incidences of pre-mature delivery (Lanouette, 1997).

7.1.1.5 Infrared Spectrum Absorption Bands

Table 7-1 Infrared Spectrum (Adapted from Florey, 1976).

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<td>OH stretch</td>
</tr>
<tr>
<td>3105</td>
<td>C=CH; C-H stretch</td>
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<tr>
<td>1538 and 1375</td>
<td>NO₂; N-O stretch</td>
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<td>1078</td>
<td>C-OH; C-O stretch</td>
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<tr>
<td>830</td>
<td>C- NO₂; C-N stretch</td>
</tr>
</tbody>
</table>

7.1.1.6 Molecular Formula

C₆H₉N₃O₃

Figure 7-1 Molecular Formula of Metronidazole (Florey, 1976).
7.1.2 Cellulose Acetate Phthalate (CAP)

7.1.2.1 Physical Properties

Cellulose is the most plentiful organic product of photosynthetic cells and it is widely distributed in the plant kingdom where its crystallinity influences its structural functions (Dyess and Emert, 1976).

Cellulose is a polymer of glucose, with each glucose unit containing three hydroxyl groups. The glucose units of cellulose have a β-1,4 linkage (Dyess and Emert, 1976). Approximately one half of these groups are acetylated, and about one fourth are esterified, with one of the two acid groups being phthalic acid. The other acid group is free (APA, 1986). Cellulose acetate phthalate (CAP) is a white, free flowing, hygroscopic, tasteless powder prepared by reacting a partial acetate ester of cellulose with phthalic anhydride (APA, 1986, Chambliss, 1983, Reynolds, 1982). It contains between 30 to 36% combined phthalyl and 19 to 23.5% combined acetyl components (Chambliss, 1983). The cellulose polymer used for production of CAP has a molecular weight ranging from 2000 to 8000.

CAP is a pH sensitive long chain polymer with ionizable carboxyl groups. CAP has been used extensively as an enteric coating for tablets, to resist decomposition of tablets by gastric fluid, enabling them to reach the intestinal track. As the pH shifts to 6 and above, the percentage of ionized acid groups on the polymer increases, which causes a charge repulsion and stretching of the polymer chain. This results in increased water solubility. The pH of the GCF varies between 7 and 8, which is very similar to the pH of the intestinal tract (e.g., 6.5-7.6 in duodenum, 7.9-8.0 in colon) (Chambliss, 1983).
Long term toxicity studies of CAP in rats and dogs demonstrated that CAP was very safe regarding its toxicity (Chambliss, 1983, Hodge, 1944). Workmen continuously exposed to the dust of CAP for many years, where skin contact was unavoidable, showed no adverse airway or dermal effects from such exposure (Eastman Chemical, 1980a). The contents of free acid, phthalyl, and acetyl for CAP are listed in Table 3.1.

The structure and geometric shape of the polymer molecules affect the strength of CAP films. Branched macromolecules with regular unhindered shapes form the most cohesive films. CAP produces a strong cohesive film, due to its ring structure and the large number of functional groups which enable the formation of hydrogen bonds and lead to a plasticizing effect (Chambliss, 1983).

CAP has been widely used in the pharmaceutical, cosmetic and food industries, and as an enteric coating material (Luce, 1977). Eastman Chemical (1987, 1980b) also markets cellulose acetate trimellitate (CAT) as an enteric coating material. CAT contains 29% trimellityl, 22% acetyl acid, and 0.5% free trimellitylic acid. CAT is compatible, and can be blended with CAP, for customized delivery systems. The CAT enteric coating material erodes at a pH of approximately 5.2 (Kumar and Banker, 1987).

Plasticizers may be added to a polymeric film to reduce its brittleness, increase the flexibility, and improve flowability. Plasticizers are generally low-volatile liquids that change the physical and mechanical properties of the polymer (Ellis et al., 1976). The plasticizer must be miscible with the polymer (Chambliss, 1983). It is assumed that plasticizers influence the polymer-polymer attachments, thus reducing molecular rigidity. The film’s cohesiveness is reduced with a resulting increase in the flexibility and toughness.
The addition of plasticizers to CAP improves the water resistance of the material. CAP was found to be compatible with the following plasticizers: dimethyl phthalate, diethyl phthalate, ethyl phthalylethyl glycolate, butyl phthalyl-butyl glycolate, tripropionin, dibutyl tartrate, glycerin, propylene glycol, polyethylene glycol, and triacetin (APA, 1986).

7.1.2.2 Molecular Formula

\[
R = \text{CH}_3 - \text{C} \quad R^1 = \text{phenyl}
\]

Figure 7-2 Molecular Structure of CAP (APA, 1986).

7.1.3 Pluronic L101

7.1.3.1 Physical Properties

Block copolymers of ethylene oxide (polyoxyethylene) and propylene oxide (polyoxypropylene) were introduced commercially in the early 1950's. Pluronic nonionic surfactants are 100% active and relatively nontoxic and non-irritating. The ability to selectively vary the length of both the hydrophobic (polyoxypropylene) and hydrophilic (polyoxyethylene) parts of the molecule results in the Pluronic series with their broad range of properties. Members
of this series are used as emulsifiers, detergents, dispersants, binders, stabilizers, gelling agents, rinse aids, wetting agents, and chemical intermediates in such industries as cosmetics, drug, textile, detergent, paper, and metal cleaning (BASF, 1987).

Several physical forms are represented within the Pluronic grade structure, ranging from mobile liquids of varying viscosities, through pastes, prills, and cast solids. The Pluronic series includes a molecular weight range of 1100 to 14000 (BASF, 1979). The selected surfactants, Pluronic L101, is a viscous translucent liquid, with a slight 'pearl' like reflection. It has an average molecular weight of 3800, with 10% repeating ethylene oxide units.

In synthesizing Pluronic surfactants, the first step is the creation of a hydrophobic component of desired molecular weight by the controlled addition of propylene oxide to the two hydroxyl groups of propylene glycol. Ethylene oxide is then added to sandwich this hydrophobic component between hydrophilic groups, controlled by length to constitute 10 to 80% (w/w) of the polymer molecule (BASF, 1979).

The Pluronic grid (Appendix B) was developed to provide graphic representation of the relationship between copolymer structure, physical form and surfactant characteristics. The basic grid is formed by plotting the molecular weight ranges of the hydrophobic against the percentage of hydrophilic components of the polymer molecule (BASF, 1987).

The toxicology properties of BASF block copolymers have been investigated since 1952. These products have no acute oral toxicity and low potential for causing irritation or sensitization. Pluronic block copolymers have been analyzed in a variety of subchronic and chronic studies. In general, the studies show that the toxicity of these blocks copolymers is very low and decreases as the molecular weight and ethylene oxide content increase. The oral LD$_{50}$ for the Pluronic family of copolymers is 2 to $\geq 15$ g/kg. The dermal LD$_{50}$ is $> 5$ g/kg, and the eye
and skin irritation levels are non to slightly irritating (BASF, 1987). Rats fed 3 or 5% Pluronics in their diet, for 2 years, did not develop significant symptoms of toxicity.

Topical irritation is caused by many factors and many mechanisms. Manufacturers have taken careful steps to eliminate irritation in the Pluronic family and to ensure the ‘mildness’ of the Pluronic products. Ethylene oxide compounds have been shown to be mild. Studies indicate that propylene oxide produces an even milder compound (BASF, 1987).

A Draize Eye Score value of 0 is classified as non-irritating, while a score of 15 is classified as severely irritating. An aqueous solution of 12% sodium lauryl ether sulfate has a Draize Eye Score 14.2. When 3% Pluronic F127 is added to this aqueous solution, the Draize Eye Score is reduce to 1.3 (i.e., practically non-irritating). The entire series of Pluronics have a Draize Eye Score value of 0.0 to 0.5, which classifies them as non-irritating substances (BASF, 1987).

Members of the Pluronic series, including L101, have been used safely in many clinical applications. Four members of this series (P85, F64, L68 and L101) have been used in drug delivery systems based on their self-assembled, supra-macromolecule forming structures. ‘Micro-containers’ for drug targeting to the brain were prepared using polymeric surfactants (i.e., poly(oxyethylene)-poly(oxypropylene) copolymers). Drug molecules were solubilized in Pluronic micelles, and were incorporated into their inner hydrophobic core formed by poly(oxypropylene) chain blocks (Kabanov et al., 1992). Specific targeting to the brain was observed when antibodies to the antigen of brain, glial cells (α2-glycoprotein) were incorporated into Pluronic micelles.

The hydrophobic surface-active agent Pluronic L101 was shown to be a potent in vitro inhibitor of human pancreatic lipase when administered as a 1 or 3% dietary admix to meal-fed
rats. It is also known that Pluronic L101 produced a significant and dose-dependent decrease in body weight gain while not affecting food consumption. Excretion of dietary fat in the feces was enhanced significantly in a dose-dependent manner during Pluronic L101 treatment, while the serum levels of cholesterol, triglyceride and glucose remained unchanged. During Pluronic L101 treatment, no overt signs of toxicity were observed (Comai and Sullivan, 1980).

In medical applications, Pluronic L101 has been used as a wetting agent, a drug carrier for targeting blood in the brain, and an immunological adjuvant (Schmolka, 1977, Kabanov et al., 1992, Hunter and Bennett, 1984).

7.1.3.2 Molecular Formula

Chemical Structure (BASF, 1987):

\[
\text{EO} \quad \text{PO} \quad \text{EO} \\
\text{HO-} (\text{CH}_2\text{CH}_2\text{O})_a (\text{CH}_2\text{CHO})_b (\text{CH}_2\text{CH}_2\text{O})_a - \text{H} \\
\quad \text{CH}_3
\]

where, EO = ethylene oxide, molecular weight = 44.05
PO = propylene oxide, molecular weight = 58.08
\(a = 7\)
\(b = 54\)

For ‘a’ of 7 and ‘b’ of 54, the molecular weight of L101 can be approximated as follows:

\[
\text{MW} = 2(7)(44.05) + 54(58.08) + 18.02 \\
= 3771
\]

For L101, propylene oxide constitutes approximately 80% of the total molecule weight. The average molecular weight of L101 is reported as 3800 (BASF, 1987).
7.1.4 CAP/Pluronic Association Polymer Blend

Pluronic: poly (ethylene oxide co-propylene oxide) block co-polymer
(EO)\textsubscript{a} - (PO)\textsubscript{b} - (EO)\textsubscript{a}

Figure 7-3 Molecular Structure of CAP/Pluronic Association Polymer Blend (BASF, 1987).
Typical Molecular Weight of Poly(oxypropylene) hydrophobe

Figure 7.4: The Pluronic Grid. A graphical representation of the relationship between poly(oxyethylene) hydrophilic unit in local molecule (%) and typical molecular weight of poly(oxypropylene) hydrophobe.
7.3 Appendix C - Heller Polymer Erosion Model

Figure 7-5 Model for Erosion Mechanism (adapted from Critical Reviews in Therapeutic Drug Carrier Systems, Heller, 1984).
7.4 Appendix D - Data Forms

7.4.1 Periodontal Insert Data Form

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Average weight of capillary tubes for trial = _________________ g
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Each Eppendorf & insert will be labeled before weighing with a lot number as follows:

- Insert number 01 from film number 011093A will be assigned insert lot # of 011093A01.
### Film Manufacturing Record

<table>
<thead>
<tr>
<th>Date</th>
<th>wt. L101 (g)</th>
<th>wt. CAP (g)</th>
<th>wt. metronidazole (g)</th>
<th>% metronidazole (w/w)</th>
<th>Lot Number</th>
</tr>
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<tbody>
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### Insert Manufacturing Record

<table>
<thead>
<tr>
<th>Date</th>
<th>Film Lot Number</th>
<th>Insert Number</th>
<th>wt of Eppendorf &amp; insert (g)</th>
</tr>
</thead>
<tbody>
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7.4.2 Letter of Intent

Clinical Trials
Department of Periodontics
Faculty of Dentistry
University of Toronto

Research Team: Dr. Peter Birek, Dr. Ping Lee
              Helen Grad, Kimberly Gates, Lucie Nadeau

December 6, 1994

Patient Name
Patient Address
Dec. 13, 1994, 1:30 pm
Dec. 15, 1994, 2:00 pm
Dec. 16, 1994, 9:30 am

Dear Patient:

Thank you for your interest in our pilot study. Our overall objective is to develop a better method of delivering antibiotics to the gum pockets in our effort to treat gum infections. This method may prove to be more effective than administering the medication in form of tablets. So far, the plastic inserts were developed under the guidance of Professor Ping Lee from the Faculty of Pharmacy, University of Toronto. Although they are rather small in size, the inserts contain enough antibiotic to affect the gum infection.

The study you will be involved in will assess whether the plastic inserts containing an amount of antibiotic would release the drug in the pockets at a desired rate. The plastic in which the antibiotic is incorporated is one that is commonly used in coating tablets that thousands of people take every day.
The antibiotic used is a very common one. Unless you have had a reaction to metronidazole (Flagyl), your chances of having a reaction during our study is remote. Please look at the days and times set aside for your participation. During the first day you will have the inserts placed into your gum pockets. You will have to return in 1 hour for taking of a gum-fluid sample. You will then return in two days for a 15-20 minute appointment for the same. On Friday, you will have to return again for a short appointment. In addition to the taking of a gum-fluid sample, we'll ask you to complete a short questionnaire.

During the study, in addition to placement of the plastic inserts around six of your teeth, we'll make measurement of your saliva gum-fluid. Neither the tests nor the insertion of the plastic material will be likely to cause pain or discomfort. This study has been approved by the Human Experimentation Committee of the University of Toronto as one that is likely to lead to important results. At the completion of your participation you will then receive $60.00 for your efforts along with our sincere thanks for participating in our study.

Should you have any questions regarding the study please do not hesitate to call me (979-4900, extension 4406). You can access this extension by punching in “1” on your touch tone dial after your call of 979-4900 was answered by an answering machine). You can then leave a message on my voice mail after you dialed my extension of 4406.

Again, I would like to thank you for your interest and co-operation.

Sincerely,


Associate Professor, Department of Periodontics

University of Toronto.

P.S. Prior to your participation in this study you will be required to sign a Consent Form. For your information I have included it with this note.
7.4.3 Consent Form

I, ........................................... Hereby agree to participate in the study of the effects of a slow release antibiotic (metronidazole) on by gums.

I understand that I will have small discs of an experimental material made of biomedical plastic containing small amounts of metronidazole inserted in several pockets around my teeth at the Faculty of Dentistry. I will then return in about one hour thereafter for an assessment of my gums, lasting about ten minutes. I will then return in two days for another assessment of my gums, lasting about twenty minutes and then again for a final time the following day for another assessment of my gums. I am likely to experience a bitter taste due to the metronidazole and may have minor gum irritation after insertion or retrieval of the inserts.

Any information about me learned during the study will be confidential and neither my name or other identifying information will be made available to anyone other than the investigators nor appear in any publication without my prior approval. I understand that if I do not choose to participate in this project, or withdraw from it, my treatment or position as a patient at the Faculty of Dentistry will not be affected.

Signed: ...........................................

Witness: ...........................................

7.4.4 Patient Questionnaire

Patient # ______

For the following question place a slash like this (|) on the line to indicate how you feel about the insert.

1. How would you best describe the taste of the insert?
   (A) During the first hour:
      no taste   bitter
      1 _________________________________ 10
   (B) During the first day:
      no taste   bitter
      1 _________________________________ 10
   (C) Until the end of the study:
      no taste   bitter
      1 _________________________________ 10

2. Was there any discomfort experienced when the insert was in place?
   (A) During the first hour:
      no discomfort   very uncomfortable
      1 _________________________________ 10
   (B) During the first day:
      no discomfort   very uncomfortable
      1 _________________________________ 10
(C) Until the end of the study:

no discomfort   very uncomfortable

1 _______________________________ 10

3. Do you have any complaints about the insert?

YES _____ no _____

If you answered YES please describe the complaint(s).

__________________________________________

__________________________________________

4. Would you have another insert placed at the site if it was needed?

YES _____ no _____

If you answered NO please state your reason(s).

__________________________________________

__________________________________________

5. Any additional questions or comments?

__________________________________________

__________________________________________

__________________________________________

Questionnaire administered to patients at the end of the study to determine their perceptions in regards to the metronidazole containing periodontal insert.
The convention used for this study is for endothermic and exothermic heat flow rates at the top and bottom of the figure, respectively. For polymers, it is customary that the glass transition temperature \( T_g \) be taken at the point one half way up the slope of the glass transition (i.e., at the point labeled glass transition) (Mathot, 1994).
7.6 Appendix F - List of Author’s Presentations and Publications

7.6.1 Presentations


### 7.6.2 Publications

