

DEVELOPMENT AND CHARACTERIZATION OF DENTAL FILMS CONTAINING TETRACYCLINE HCL FOR PERIODONTITIS

by

NIDHI GUPTA

(Under the Direction of J. C. Price)

ABSTRACT

Controlled release local drug delivery systems offer advantages compared to systemic dosage forms for periodontitis. The objective of this research was to design and evaluate sustained release dental films containing tetracycline HCL in a non-biodegradable carrier for targeted delivery of drug. Screening experiments revealed that the rate of drug release increased with an increase in the amount of drug loading in the films. Eight batches of dental films were prepared by varying three independent variables. In DSC studies, the glass transition temperature of the polymer decreased after its inclusion in the film. In vitro release studies showed that plasticizer had an effect on the rate of drug release. Tetracycline HCL films were successfully prepared using ethyl cellulose and these films were able to sustain the release of drug for one week.

INDEX WORDS: Periodontitis, Sustained release formulations, Dental films, Non-biodegradable film.

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DEDICATION

I dedicate this manuscript to the four people I most admire, my parents Dr. J. P Gupta and Pratima Gupta, and my brothers, Vikas and Vishal Gupta. Without them, I would not be the person that I am nor would I have been able to accomplish half of what I already have up to this point in my life.

“... The woods are lovely, dark and deep

But I have promises to keep

And miles to go before I sleep

And miles to go before I sleep”

- Robert Frost

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I would first and foremost like to thank my creator, God for His grace and presence. He helped me in the realization of my intellectual level and persistence in all my spiritual and academic endeavors. Without Him, there is no way I would have gotten through all the years of school.

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INTRODUCTION

The oral cavity provides a diverse environment for colonization of a wide range of microorganisms. Periodontal diseases are a general term encompassing several pathological conditions such as gingivitis and periodontitis. Periodontitis is a local infection with primary bacterial etiology in the gingival crevices, which affects the structural organs surrounding the teeth like periodontal ligament, connective tissue and bone. The warm and moist pocket environment fosters the growth of gram-negative, anaerobic bacteria that proliferate in the sub gingival space. Approximately 70 million American people suffer from adult periodontitis. The aim of dental healthcare is to control the population of microorganisms. Slowing or arresting of the oro-dental infections can be achieved by controlling bacterial plaque. The systemic administration of antibiotics is a useful method for controlling sub gingival flora but it has been found that discontinuation of systemic antibiotic therapy results in recolonization of pathogens. Therefore long term antimicrobial therapy is required for complete eradication of microorganisms. High oral doses are required to achieve effective concentrations in the pocket but high doses given for long periods of time eventually cause development of resistant strains of bacteria, super infection, gastrointestinal and central nervous system disturbances. To overcome the disadvantages of systemic chemotherapy with antibiotics, recent technical advancements have led to the development of new drug delivery systems that provide sustained therapeutic activity by targeting the delivery of a drug to a particular site. If a particular drug is targeted to a desired site, it minimizes the distribution of drug to other body organs. Controlled drug delivery

systems offer numerous advantages compared with conventional dosage forms, which include improved efficacy, reduced toxicity, improved patient compliance and convenience.

The primary objective of this research was to design and evaluate sustained release dental films containing tetracycline HCl for placement into the periodontal pockets of chronic periodontitis patients for targeted delivery of drug in order to reduce periodontal pathogens. The effects of various formulation variables on the drug release profiles from the films were studied to determine optimum formulations.

LITERATURE REVIEW

Dental diseases are considered as a major public health problem in the entire world (1). Most local oro-facial infections occur when pathogenic bacteria invade the surrounding tissues. These bacteria form biofilms called plaques on the tooth surfaces. This subgingival microbial ecosystem becomes complex with time and becomes inaccessible to oral hygiene by an individual. Over 400 species of bacteria have been isolated from plaques, but only a few species of bacteria release toxins and induce host inflammatory response resulting in the destruction of connective tissue and alveolar bone, which support the teeth resulting in a deepening of the sulcus, which is known as periodontal pocket formation (2). Approximately 70 million American people suffer from adult periodontitis, which is defined as periodontal pockets greater than 4mm (3, 4).

As shown in Figure 1, with the destruction of periodontium, patients often lose their teeth bringing about a decrease in dental function and esthetics (5, 6).



Figure 1. Genesis of Periodontitis. Reproduced with permission (6)

Bacterial invasion in the blood stream is not a predominant feature but may occur in certain situations. Bacterial endotoxins stimulate the release of pro-inflammatory cytokines, which can have systemic effects and may lead to low birth babies, and cardiovascular diseases such as atherosclerosis, myocardial infection and stroke. Smoking is a major risk factor for periodontitis and has a great impact on the progression of periodontal bone (Figure 2) and attachment loss (7). Patients should be encouraged to stop smoking to improve their periodontal health.

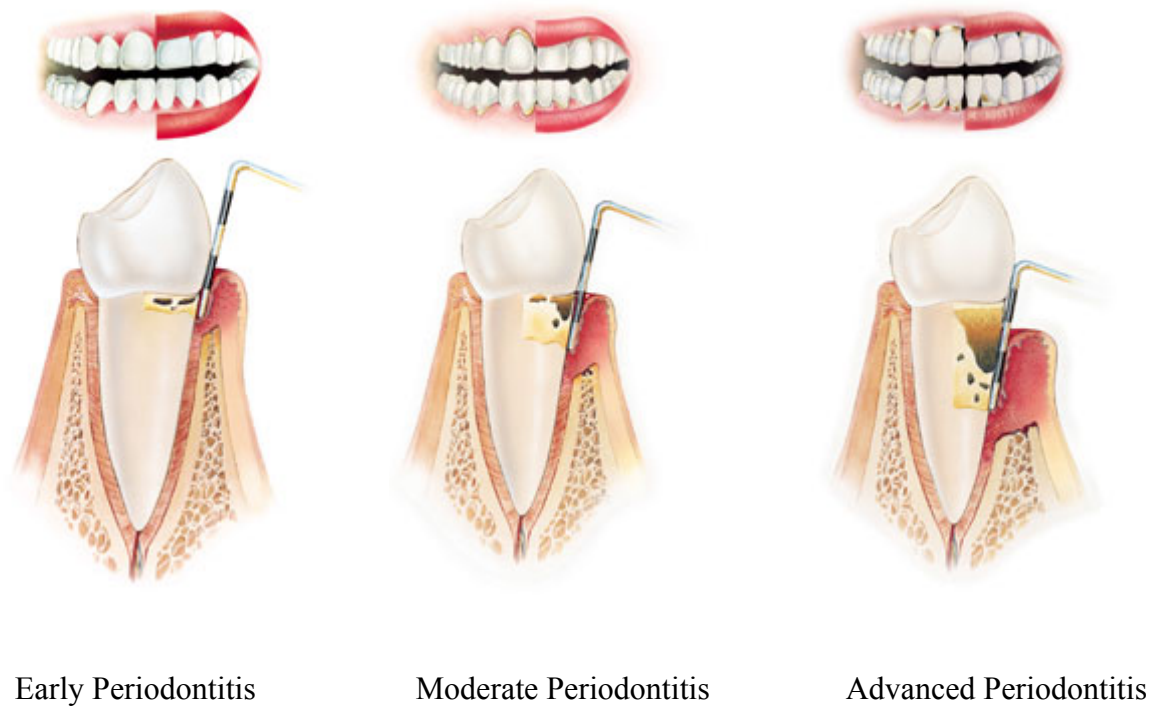


Figure 2. Various stages of Periodontitis. Reproduced with permission (7)

Supragingival and sub gingival plaque play a vital role in the causation of dental caries and periodontal infections respectively. In the diseased state, supporting collagen of the periodontium is destroyed and the alveolar bone begins to resorb. As shown in Figure 3, the epithelium of the gingiva begins to migrate along the tooth surface forming pockets, which provides an ideal environment for the growth and proliferation of microorganisms (8). The aim of dental healthcare is to control the population of microorganisms. Slowing or arresting of the oro-dental infections can be achieved by controlling bacterial plaque.

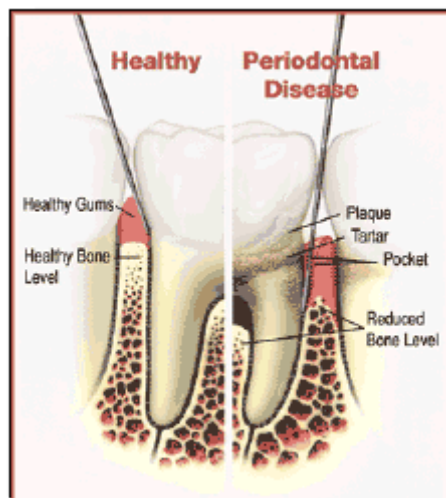
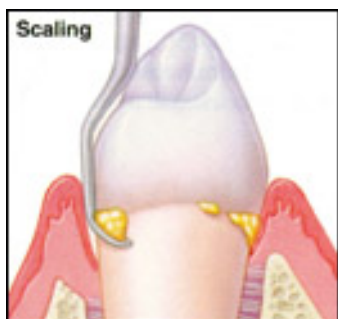


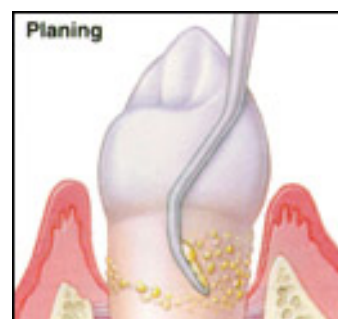
Figure 3. Comparison of a healthy tooth and periodontal diseased tooth. Reproduced with permission (8)

Mechanical plaque controlling procedures such as root planing and scaling (conventional therapy) which amount to scraping away the bacteria, calculus (calcified form of plaque), and biofilm are time consuming procedures and result in discomfort to the patient (9). There is high rate of recurrence of periodontitis, which further requires frequent visits to the periodontist. In deeper pockets, access and visibility are problematic and in these situations open scaling and root planing are more effective which involve surgical reflection (Figure 4) of the flap (10, 11).

Therefore, chemotherapeutic treatment of the infection is desirable.



Scaling



Root Planing

Figure 4. Conventional therapy in oral dental hygiene. Reproduced with permission (11)

Traditional dental delivery systems have not incorporated means of controlled release of drugs. As shown in Figure 5, the problem however is that with each dose of a non-controlled release drug, the concentration of drug available to the body immediately peaks and then declines rapidly (66). At times the drug concentration is very high, leading to adverse side effects, and at other times the drug concentration is too low to provide therapeutic benefit (12).

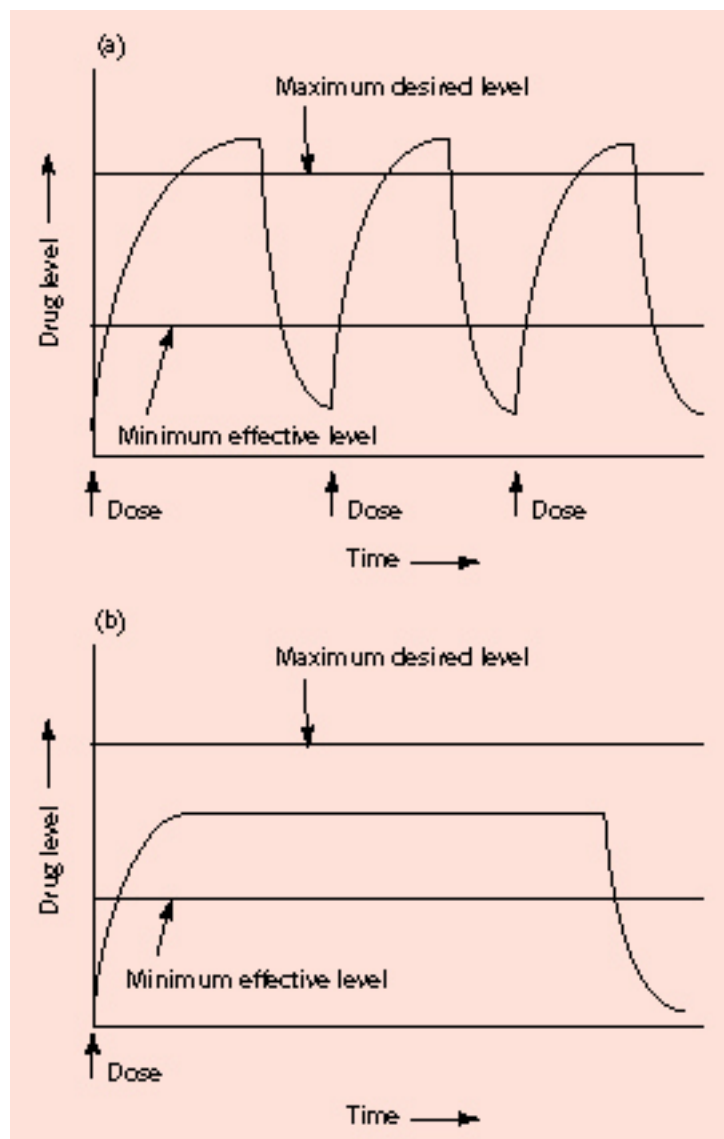


Figure 5. (a). Drug levels in case of traditional drug therapy; (b): Drug levels in case of controlled release drug delivery.

The following three mechanisms of controlled release drug delivery have been widely discussed in literature.

1. Diffusion controlled (membranes and matrices)
2. Chemically controlled (erosion and pendent chain)
3. Solvent activated (osmotic pressure and swelling)

The systemic administration of antibiotics is a useful method for controlling subgingival flora but it has been found that discontinuation of systemic antibiotic therapy results in recolonization of pathogens (13).

Unlike the majority of general infections, all the suspected pathogens for periodontitis are indigenous to the oral flora (14-16). This fact explains the chronic nature of these diseases and why they often require long treatment. In order for antibacterial agents to be effective for the periodontal diseases, an adequate concentration of the drug has to be present at the site of action and it has to be maintained at that level for a sufficient duration of time. The periodontal pocket is a significant site for this form of treatment since it is the source of continued local infection. Controlled drug delivery systems offer numerous advantages compared with conventional dosage forms, which include improved efficacy, reduced toxicity, improved patient compliance, and convenience. The aim of controlled release systems is to match the rate of drug release with the rate of drug elimination, and therefore maintain the drug concentration in within the therapeutic window for the vast majority of the dose interval. If a particular drug is targeted to a

desired site, it minimizes distribution of drug to other body organs. The means by which a drug is introduced into the body is almost as important as the drug itself.

Several antibiotics and antimicrobial drugs such as minocycline, spiramycin, metronidazole and chlorhexidine are known to be effective against periodontal flora (17). However, tetracycline HCl remains the drug of choice for treating periodontitis as it is a wide spectrum antibiotic, has low toxicity ($LD_{50} = 6443 \text{ mg/kg}$), and high efficacy against Gram-negative bacteria ($MIC_{90\%} = 8 \mu\text{gm/ml}$) (18-20). It also possesses tissue-regenerating effects including osteoblastic chemotactic effects. The concentrations of tetracycline in crevicular fluid achieved by controlled delivery are up to 100 times than those obtained from systemic dose ($1500 \mu\text{g ml}^{-1}$ vs. $15 \mu\text{g ml}^{-1}$). Tetracycline HCl inhibits matrix metalloproteinases and prevents the destruction of collagen in periodontal tissue (21). This inhibition of collagenase may be attributed to the drug's ability to bind with calcium and zinc ions (22-24). It may also be because of its ability to scavenge reactive oxygen radicals thus facilitating repair and new attachment formation. It inhibits bone resorption and it accumulates in the gingival fluid. Placing the film directly inside the pocket can prevent complications such as anorexia, nausea, vomiting, vulvo-vaginal candidiasis, diarrhea, glossitis, and dysphagia which are the side effects of systemic administration of tetracycline HCl.

The following factors are important in choosing a polymer for dental drug delivery from various pharmaceutical grade polymers available.

- 1.) Polymer should be stable during the time it takes for complete drug release.
- 2.) Polymer should be capable of forming self-supporting films when containing drug at high loading.

- 3.) There should be non-appreciable swelling of the polymer in water and it should have a softening point above 37°C.
- 4.) There should be ease of fabrication.
- 5.) Low cost.

Ethyl cellulose was chosen based on these requirements. Ethyl cellulose is an inert, hydrophobic polymer and is essentially tasteless, odorless, colorless and non caloric. It has been extensively used as a pharmaceutical vehicle in a number of dosage forms. It has been widely used for its application as a matrix forming material in films.

Dibutyl sebacate (water insoluble plasticizer) was chosen to assure approximately constant drug diffusivity from the polymer. On the contrast, water-soluble plasticizers leach from the delivery system, resulting in decreasing plasticizer contents and thus changing diffusivities.

Generally, with increasing plasticizer level the diffusivity of the drug increases due to the higher mobility of the polymer chains. The plasticizing effect of a substance is based on the reduction of attractive forces between the polymer chains. Decreasing attractive forces leads to increased mobility of the macromolecules.

Periodontal local delivery device

A wide variety of specialized local delivery systems (i.e. intrapocket devices) have been designed to maintain the drug in the gingival crevicular fluid (GCF) at a concentration higher than the MIC. Fibers, films, strips and micro particles made up of biodegradable or

nonbiodegradable polymers have been reported as effective methods to administer antibacterial agents for periodontal therapy.

Fibers

Fibers are thread like devices used for sustained release of drug into the periodontal pocket. They can either be hollow or matrix delivery devices.

Hollow fiber

The open spaces within the fibers are filled with a therapeutic agent and the agent is released simply by diffusion through the reservoir wall (25). There is no rate control in these types of fibers.

First delivery devices by Goodson were composed of cellulose acetate filled with tetracycline (26). These fibers released 95% of the drug in the first 2 hrs and the release followed first order kinetics. Although GCF levels of tetracycline remained within the therapeutic range for 24 hrs, the study was viewed mainly as an evaluation of drug delivery.

As shown by Goodson, hollow fiber systems used in periodontal pockets release drug quite rapidly. They can be qualified only marginally as sustained release devices. Studies have been conducted and have demonstrated the clinical efficacy of these fibers. However actual value in patient therapy is somewhat difficult to interpret as clinicians have found the fiber placement technique challenging. A considerable number of fibers become dislodged from the periodontal pocket during the course of 10-day treatment period (27). The fiber is tied around the tooth below the gingival margin so that the periodontal pocket is packed with the drug releasing

material. The intricacies of winding the fiber into place, keeping it in the pocket, and then removing it after 7-10 days may limit wide acceptance by periodontists and their patients.

Strips and compacts

A controlled release strip coded PT-01 made up of polymethacrylic acid and hydroxypropyl-cellulose containing 10% ofloxacin has been reported in studies by Kimura et al. (28). Data showed that ofloxacin could be found in higher concentrations than the MIC of most periodontal bacteria in GCF over 7 days by a single application of PT-01 in the human periodontal pocket. Although the weekly application of PT-01 on days 0-35 showed some further shift in the proportion and reduction in subgingival microbial flora, no significant differences in the microbiological results between the strip group and the control groups were noticed. Consequently the authors suggested that the application of PT-01 might have a beneficial effect as an adjunct to conventional therapy. The controlled release strips that gave long-term improvement were chlorhexidine strips (29). Controlled trials involving chlorhexidine in ethyl cellulose strips used every 3 months in place of routine supportive periodontal therapy have shown significant clinical benefits lasting up to 2 years.

Because of the non-biodegradable nature of polymeric carriers and the only temporary clinical improvements after treatment completion, no product has been marketed yet.

Injectable systems

The application of an injectable system is easy and does not take long, is without pain and is carried out with the help of a syringe. Moreover, an injectable delivery system can fill the pocket, thus reaching a large proportion of pathogens. Two types of injectable delivery systems

have been assessed in the treatment of periodontal diseases, biodegradable micro particles and gels (56).

Microparticles

Microparticles based on poly (α -hydroxyacids) such as polylactide or poly (lactide-co-glycolide) containing tetracycline have been designed for periodontal disease therapy (67). The *in vitro* release rate is influenced by the polymer choice and by the pH of the medium; the release rate is increased as the pH increases. PLGA microspheres have been suggested for the delivery of histatines (68). The stability of peptide release has been maintained at 100% through out release by the addition of non-ionic surfactant. *In vitro* release of total peptide lasts for one month. Some questions related to the retention of such formulations in the periodontal pocket need clarification.

Gels

Mucoadhesive, metronidazole-containing gel systems designed for periodontal treatment based on hydroxyethylcellulose, Carbopol 974P and Polycarbophil have been described (69). *In vitro* drug release was significantly decreased with the increasing concentration of the polymer, due to both the concomitant increased viscosity of the formulation and additionally, the swelling kinetics of polycarbophil following contact with dissolution fluid. The optimal choice of bioadhesive formulation for use in periodontal disease involves a compromise between achieving the necessary release rate of the drug and the mechanical characteristics of the formulation.

Microcapsules

They are being used for the delivery of encapsulated antibacterial agents in treating periodontal disease. These systems release the drug over a prolonged period of time in the salivary or crevicular fluid (57).

Films

They are matrix delivery systems in which the drug is distributed through out the polymer and drug release occurs by diffusion and/or matrix dissolution or erosion. The dimensions and shape of the film can be controlled to correspond to the dimensions of the pocket where the film is to be inserted. It can be rapidly inserted into the pocket with minimal pain to the patient. It can be totally submerged in the pocket and can be inserted to the base of the pocket. If the thickness of the film is reduced to less than 400 μ m, it carries sufficient adhesiveness, thus not interfering with the oral hygiene of the patient. Both degradable and non-biodegradable films have been developed. The films that release drug by diffusion alone are prepared using water insoluble or non-degradable polymers (30, 31), whereas those that release drug by diffusion and matrix erosion use soluble (32, 33) or biodegradable polymers in the matrix (34, 35).

Non-degradable films

The first descriptions of an intra pocket, non-biodegradable matrix delivery device was made by Addy and coworkers (36) in 1982. They described the use of matrix films of polymethacrylates for the intrapocket delivery of tetracycline, metronidazole and chlorhexidine. Suitable sized films were prepared. *In vitro* release profile and duration of drug release was studied and was shown to be dependent on the drug loading in the delivery systems. It also depended on the nature of drug incorporated. Films containing 30% w/w chlorhexidine, tetracycline and metronidazole released

57.0, 40.0 and 96% of the drug load. They further described formulations delivering *in vitro* therapeutic levels of all three drugs over a 14-day trial period. Clinical assessment of films containing 30% w/w drug have shown metronidazole containing strips to be more effective but no evaluation of *in vivo* drug release rates was done (37,38). In later studies these systems were found to be associated with slower rate of relapse of clinical parameters and have not yet been developed commercially (39).

Ethyl cellulose matrix films for intrapocket drug delivery have been described (58). These films were made by casting ethanol or chloroform solutions of the polymer into molds and allowing the solvent to evaporate. The appropriate drug and plasticizing agent were incorporated into the solution prior to casting. The dried films were then cut into desired shapes. Films containing chlorhexidine (59), metronidazole (60) and minocycline (61) have been developed and tested to various degrees. The release of the therapeutic agent from these films is dependent on the solvent used, the presence of the plasticizer, the nature and concentration of the drug in the film. Films cast from ethanol solutions containing 5% w/w chlorhexidine released 95% of the drug over a period of 10 days, whereas chloroform-cast films released 20% of drug load over a 205-day period (62). This could be due to the differential solubility of the drug in the casting solvents. Drug release from chloroform-cast films was modified by the addition of PEG to the formulation. Golomb et al. (63) described metronidazole-bearing films cast with PEG 3000 and concluded that the amount of crystalline water bound to the surface of the films increased with the inclusion of PEG. It was further suggested that enhanced release of drug was due to improved water binding to the surface of the matrix films containing PEG. Stabholz et al. (64) assessed the efficacy of periodic treatment with chlorhexidine-containing films in a 2-year study of maintenance of periodontal pocket. Treatment showed significantly lower incidence of

bleeding on probing, pocket depths and attachment levels when compared to the conventional maintenance treatment (65).

The limitations of such delivery devices include the removal and replacement as they do not degrade. On the other hand, less expertise is required than for scaling and plaque removal (40).

Degradable/Soluble films

These systems dissolve or erode in the gingival crevice so that removal after treatment is not required. Drug release occurs by erosion or dissolution and drug diffusion through the matrix. Sustained release profile can be obtained by appropriate manipulation of one or more release mechanisms.

A number of biodegradable polymers are used for the delivery of antimicrobial agents in the treatment of periodontal diseases. Some of these polymers are hydroxypropylcellulose (41), polyesters (42, 43), cross-linked collagens and protein films (44). Device erosion probably accounts for the more gradual release seen from the device from 2 to 24 h.

OBJECTIVES

The objectives of this research were as follows –

1. Design and evaluate sustained release dental films containing tetracycline HCl.
2. Simultaneously study the effect of various formulation variables on the drug release profile from films using design of experiments.
3. Study potential physical interactions between the drug, polymer, and plasticizer.

MATERIALS AND METHODS

Materials**Table 1.** Materials

Material	Lot Number	Manufacturer
Tetracycline HCl	SL0041	Spectrum Chemicals Gardena, CA
Ethyl cellulose (46%ethoxycontent)	-	Scientific Polymer Products, Inc ONTARIO, New York
Dibutyl sebacate	42F-0188	Sigma Chemical Company ST. Louis, MO
Methanol	031334	Fisher Chemicals Fair Lawn, New JERSEY

Methods

Choice of Substrate for film casting

Any material chosen as a substrate for film casting should be non-reactive and preferably non-toxic. It should be easy to handle and should yield uniformly thick films. It should have smooth surface to permit easy film removal. The most commonly employed substrates for film casting include mercury and glass. Teflon has some advantages over both mercury and glass.

Mercury is toxic and difficult to handle. Residual contamination of film with mercury may occur. Teflon on the other hand is non-toxic, inert and easy to handle. With the use of glass, adhesion of the film to substrate may occur which may present a problem. When glass is used, long periods of drying are employed. In preliminary studies, smooth Teflon surface permitted quick and easy film removal and a leveled surface of Teflon gave uniformly thick films. In view of these advantages, Teflon was chosen as a substrate for film casting. Film casting apparatus can be seen in Figure 6.

Preparation of films

An accurately weighed amount of ethyl cellulose was slowly added to methanol and dissolved under constant stirring using a magnetic stirrer. Mixing was continued until a clear solution of polymer in solvent was obtained. After the complete dissolution of the polymer, plasticizer was added to the polymer solution. An accurately weighed amount of tetracycline hydrochloride was homogenously dispersed in the above polymer solution under stirring. This dispersion was then poured into a Teflon mold. The mould was placed on a level plate to ensure uniform thickness of the film. The solvent was allowed to slowly evaporate by covering the mold with a glass beaker, and set aside for 12 hours in a dark place. The initial slow drying was necessary to prevent

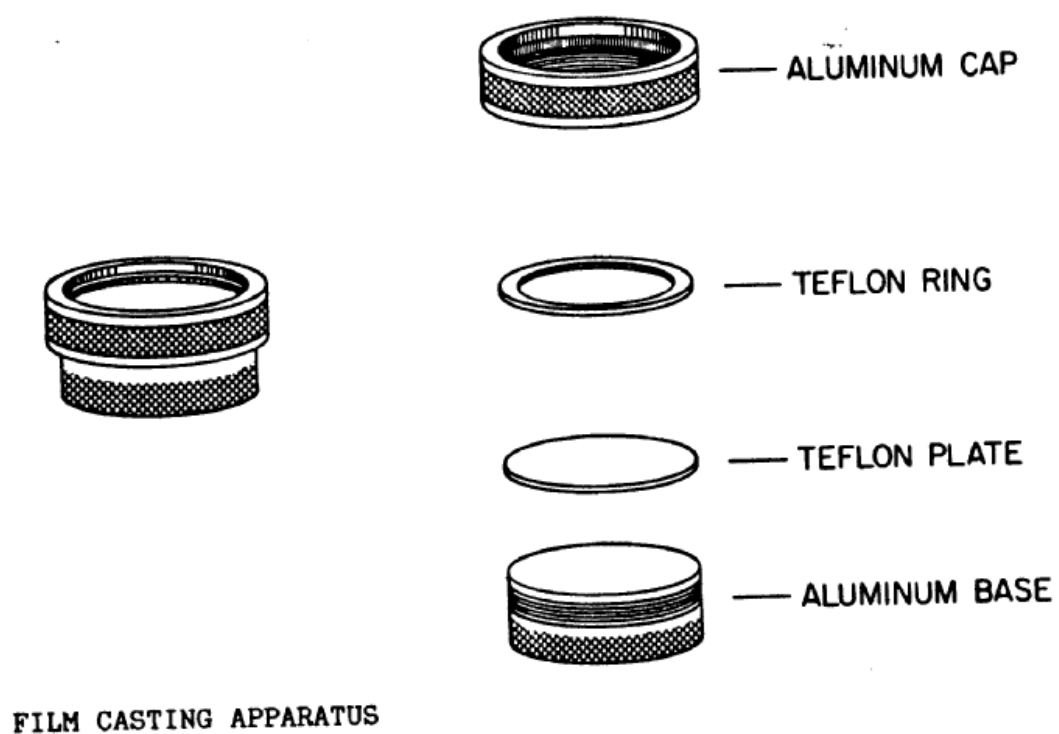


Figure 6. Film Casting Apparatus.

formation of gas bubbles in the film, which may be caused by initial rapid evaporation of methanol. After drying the film overnight, the films were carefully removed from the Teflon mold and stored in a light-resistant container because the drug is light sensitive.

Size of tetracycline HCl films

In chronic periodontitis patient, the pocket depth of inflamed gingiva ranges from 5 to 8 mm and is around 2 mm in thickness (45). In this study, uniform cast films 0.5mm thick were cut into squares of 5mm length and 5mm width, so that they can be easily introduced into the periodontal pocket. Film thickness was measured at 10 different places with a digital caliper. The thickness of the film did not vary by more than 5% over the film surface.

Film weight and thickness variation

Five samples from each batch of films were weighed and the mean weights calculated. Film thickness of each sample was measured using a digital caliper. Variation in the size and weight of individual films was determined.

Dosage amount calculation

The minimum inhibitory concentration (MIC) of tetracycline HCl to the pathogens which cause periodontitis, such as bacteroides, *P. melaninogenicus*, *B. fragilis*, *F. necrophorum*, *A. actinomycetem comitans* and spirochetes, *P. aeruginosa*, *E. coli*, *Entetobacter*, *S. sanguis*, *S. salivarius*, etc., is 8 µg/ml (25). This factor was considered in deciding the size of films. The films of 5 mm × 5 mm × 0.5mm are expected to release the drug well above the minimum inhibitory concentration required for oral pathogens.

Calibration curve of tetracycline HCl in methanol

A stock solution of 1mg/ml was prepared by dissolving one gm of tetracycline HCl into a 1000 ml volumetric flask. About 50ml of methanol was added to the volumetric flask and tetracycline HCl was dissolved in it. The volume was made up to 1000ml with methanol in the volumetric flask. From this solution, successive dilutions of 1 μ g/ml, 2 μ g/ml, and 5 μ g/ml and so on were prepared. The absorbances of these solutions were recorded and a standard curve was prepared (Figure 7).

Slope	46.97
Intercept	0.0588
Correlation coefficient	0.9986

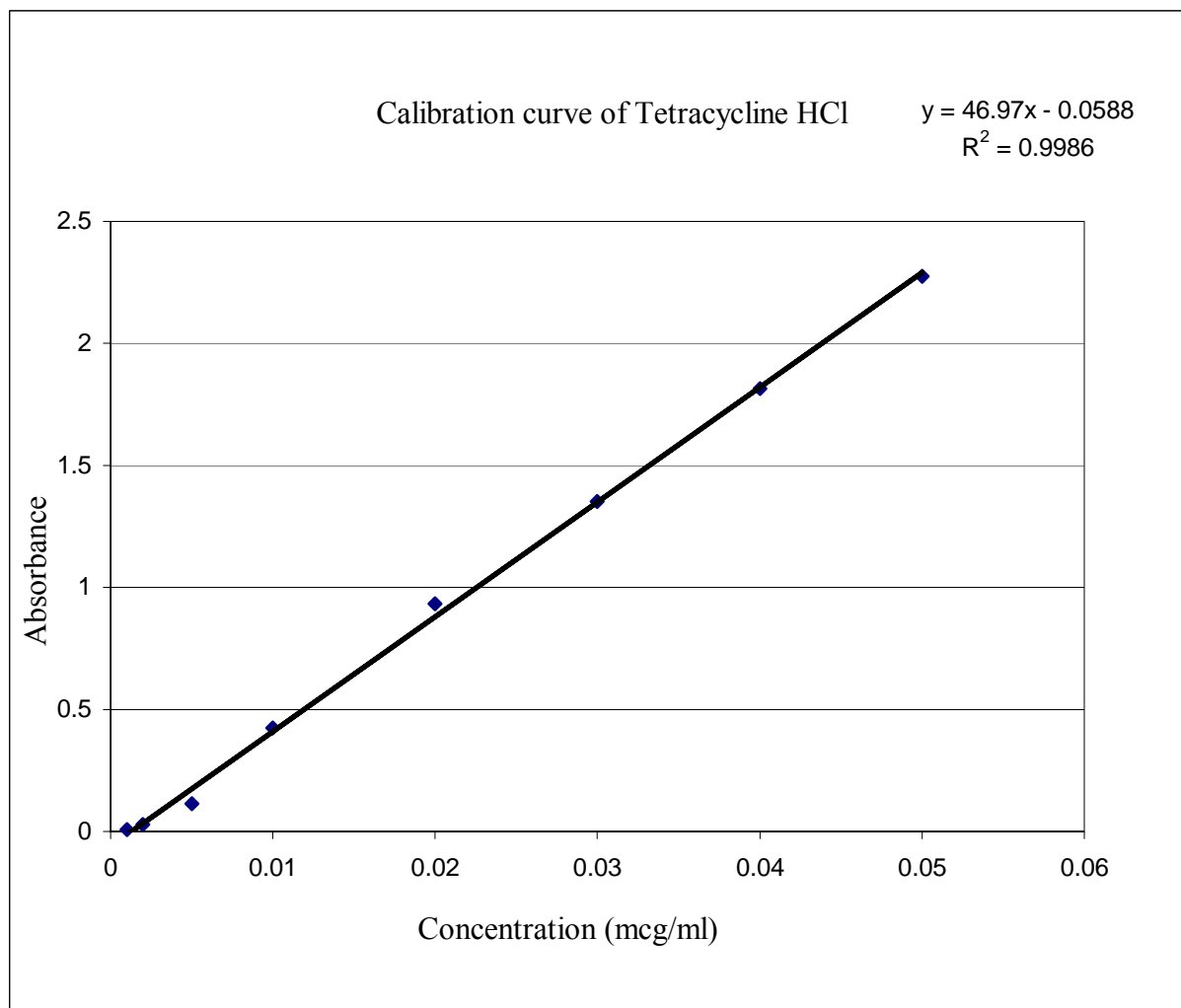


Figure 7. Calibration curve of Tetracycline HCl.

Scanning electron microscopy (SEM)

A Scanning electron microscope (LEO 982) was used to study the surface characteristics of the films before and after dissolution. Samples were attached to aluminum stubs with 12mm diameter carbon adhesive tabs (Electron Microscopy Sciences). Each membrane piece was also attached to the adhesive tabs by placing conductive carbon cement (Electron Microscopy Sciences) on two opposite sides. Samples were coated for 90 sec with gold palladium in a sputter

coater (Structure Probe Inc) for a coating thickness of approximately 23.0 nm. Samples were stored in a dessicator until imaged on a Leo 982 field emission scanning electron microscope at 5.0 kV with a working distance of 8 to 10 mm. SEMs were taken at 5000X magnification.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is a technique used for measuring the energy that is necessary to establish nearly zero temperature difference between a sample and an inert substance, as both of these are subjected to similar temperature conditions in an environment heated or cooled at a controlled rate. Two commonly used types of DSC systems are:

1. Power compensation DSC
2. Heat flux DSC

In power compensation DSC, the temperature of the sample and the reference are controlled independently using identical, similar furnaces. The temperatures of the sample and reference are made identical by varying the power input to the two furnaces; the energy required to do this is a measure of the enthalpy or heat capacity changes in the sample relative to the reference.

In heat flux DSC, a low resistance heat flow path connects the sample and reference. The whole assembly is enclosed in a single furnace. The heat capacity changes in the sample cause a difference in its temperature relative to the reference. The resultant heat flow is small because the sample and the reference are in good contact. The temperature difference is recorded and related to enthalpy change using calibration experiments. The difference in energy required to maintain them at a nearly identical temperature is provided by the heat changes in the sample. Any excess energy is conducted between the sample and the reference through the connecting metal disc.

The experiments can be conducted either isothermally or with the temperature changing at a constant rate. In the former case, the ordinate value would be plotted against time or isothermal temperature. In the second case, it could be plotted against time or temperature. The height of the curve at any given time is a measure of the difference in temperature between sample and the reference.

Thermal analysis techniques have the advantage that only small amount of material is necessary. This ensures uniform temperature distribution and high resolution. The sample can be encapsulated in an inert atmosphere to prevent oxidation, and low heating rates lead to higher accuracies. Heating and cooling through critical temperature range can check the reproducibility of the transition temperature. During a first order transformation, a latent heat is evolved, whereas second order transitions do not accompany latent heat.

In this study, DSC was employed to study any potential change in tetracycline HCl that the drug may have experienced during its processing into periodontal films. DSC thermograms were obtained using TA instruments and the model was 2920 differential scanning calorimeter fitted to a 2100 thermal analyst. Prior to analysis, the instrument was calibrated using an indium standard. Samples were crumpled in aluminium cells and were heated at a rate of 5°C in an atmosphere of nitrogen.

Determination of drug content

Weighed samples of films from each batch were placed in 50 ml of methanol to dissolve ethyl cellulose and drug. After the complete dissolution of the polymer and the drug in the solvent, aliquots were removed for the quantitation of tetracycline HCl by UV spectrophotometric analysis at 276nm (Bausch and Lomb Spectronic 2000). The drug content in the films was

expressed as percent w/w of tetracycline HCl in the film calculated from triplicate samples. Quantification of ethylcellulose was also done using UV spectrophotometric analysis at 276nm. It showed minimal absorbance, and therefore it was not taken into account in calculations.

Preparation of buffer solutions pH 7.4 and pH 6.8

Phosphate buffer solutions of pH 7.4 and 6.8 were made according to USP-19 (55) using monobasic potassium phosphate, sodium hydroxide, and water.

Dissolution studies

The films (1 ± 0.2 mg) were placed in glass vials containing 20 mL of dissolution medium at 37°C (de-ionized water for screening studies; pH 6.8 and 7.4 phosphate buffer thereafter). The glass vials were mounted on a bath shaker and shaken at 75 oscillations per minute. Sink conditions were ensured by taking the solubility of drug and amount of release medium into account. Samples (4 mL) were withdrawn at regular time intervals, filtered and analyzed for drug content at 276 nm using a spectrophotometer. Release studies were carried out for 7-days. The percent cumulative release of tetracycline HCl into the release medium was determined from the replicates of five films for a period of seven days.

Although a serum rich medium would mimic the gingival crevicular fluid of the periodontal pocket with respect to its protein composition, phosphate buffer of pH 6.8 and 7.4 was chosen as the release medium as its pH is similar to that found in the periodontal pocket in the initial stages of the periodontal disease. Watanabe et al. have shown that patients with gingivitis have a mean pH ranging from 6-7 (46). Moreover the presence of proteins in the release medium would make it difficult to establish the baseline release characteristics of the delivery systems.

SCREENING EXPERIMENTS

Screening experiments were carried out by varying drug to polymer ratio in order to get the limits of independent variables for the dissolution runs. Three formulations were studied for screening studies as follows:

1. Low drug

Amount of drug	5% of polymer
Amount of polymer	20% of solvent
Amount of plasticizer	10% of polymer

2. Medium drug

Amount of drug	10% of polymer
Amount of polymer	20% of solvent
Amount of plasticizer	15% of polymer

3. High drug

Amount of drug	15% of polymer
Amount of polymer	20% of solvent
Amount of plasticizer	15% of polymer

STATISTICAL DESIGN OF EXPERIMENT (DOE)

Preliminary screening experiments were done to determine the experimental region where the complete release of tetracycline HCl would occur for a period of 3-7 days. In these experiments, the amount of drug was varied between 5-15 % and the amount of polymer and plasticizer was kept constant. After the screening studies, the simultaneous effect of three formulation factors was studied using a mixture experiment design. Mixture design was selected for this study because of its appropriateness for the objectives of this study.

The properties of a mixture are almost always a function of the relative proportions of the ingredients rather than their absolute amounts. In experiments with mixtures, a factor's value is its proportion in the mixture, which falls between 0 and 1. The sum of the proportions in any mixture recipe is 1 (100%). Designs for mixture experiments are fundamentally different from those for screening. Screening experiments are orthogonal. That is, over the course of an experiment, the setting of one factor varies independently of any other factor. The interpretation of screening experiments is simple, because the effects of the factors on the response are separable.

With mixtures it is impossible to vary one factor independently of all the others. When the proportion of one ingredient is changed, the proportion of one or more other ingredients must also change to compensate. This simple fact has a profound effect on every aspect of experimentation with mixtures: the factor space, the design properties, and the interpretation of the results.

Because the proportions sum to one, mixture designs have an interesting geometry. The feasible region for a mixture takes the form of a simplex. The plane where the sum of the three factors

equals to one is a triangle-shaped slice. This plane can be rotated to see the triangle faces and the points in the form of a ternary plot.

Three main types of mixtures designs are:

1. Simplex centroid
2. Simplex lattice
3. Extreme vertices

The extreme vertices design was chosen for this study because it is the most flexible of all mixture designs.

The three independent factors studied were: amount of drug, amount of polymer and amount of plasticizer. These factors were considered important for drug release. These factors and their range are shown in Table 2. The following two response variables were studied.

Response 1: Time for 50% drug release (t_{50})

Response 2: Amount of drug released in 7-days

These two responses were chosen for this study to identify formulations that provide nearly zero-order drug release and complete release of drug in 7-days.

Based on these factors and their range, a DOE table was created that consisted of eight experimental runs (Table 3). A ternary diagram of this mixture design is shown in Figure 8.

Table 2. Three independent factors and their low and high values used for DOE.

Factor	Low Value	High Value
	% (fraction)	% (fraction)
Amount of drug	5 (0.05)	30 (0.3)
Amount of polymer	45 (0.45)	90 (0.9)
Amount of plasticizer	5 (0.05)	25 (0.25)

Drug = Tetracycline HCl; Polymer = Ethyl Cellulose; Plasticizer = Dibutyl Sebacate

Table 3. Various DOE runs based on Extreme Vertices Mixture Design.

Run #	Drug (% in film)	Polymer (% in film)	Plasticizer (% in film)
1	30	65	5
2	5	70	25
3	5	90	5
4	5	80	15
5	17.5	77.5	5
6	30	55	15
7	17.5	57.5	25
8	30	45	25

RESULTS AND DISCUSSION

Screening experiments

Screening experiments were carried out in order to determine the range of the independent variables to be chosen for the DOE experiments. Three different batches of films were prepared using different drug to polymer ratio (D/P) in order to study the effect of D/P ratio on the rate of drug release from the films. The D/P ratios chosen for the three experiments were .05 (low drug), .1 (medium drug) and .15 (high drug). As shown in Figure 9, for films that had D/P ratio of 0.05, 90% of the drug was released in 10 days and the release was almost linear with time, which indicates that it follows zero order release pattern. For films with D/P ratio as 0.1 and 0.15, there appear to be two phases of drug release -- an initial rapid release for 48 hrs followed by slow drug release (Figure 10,11).

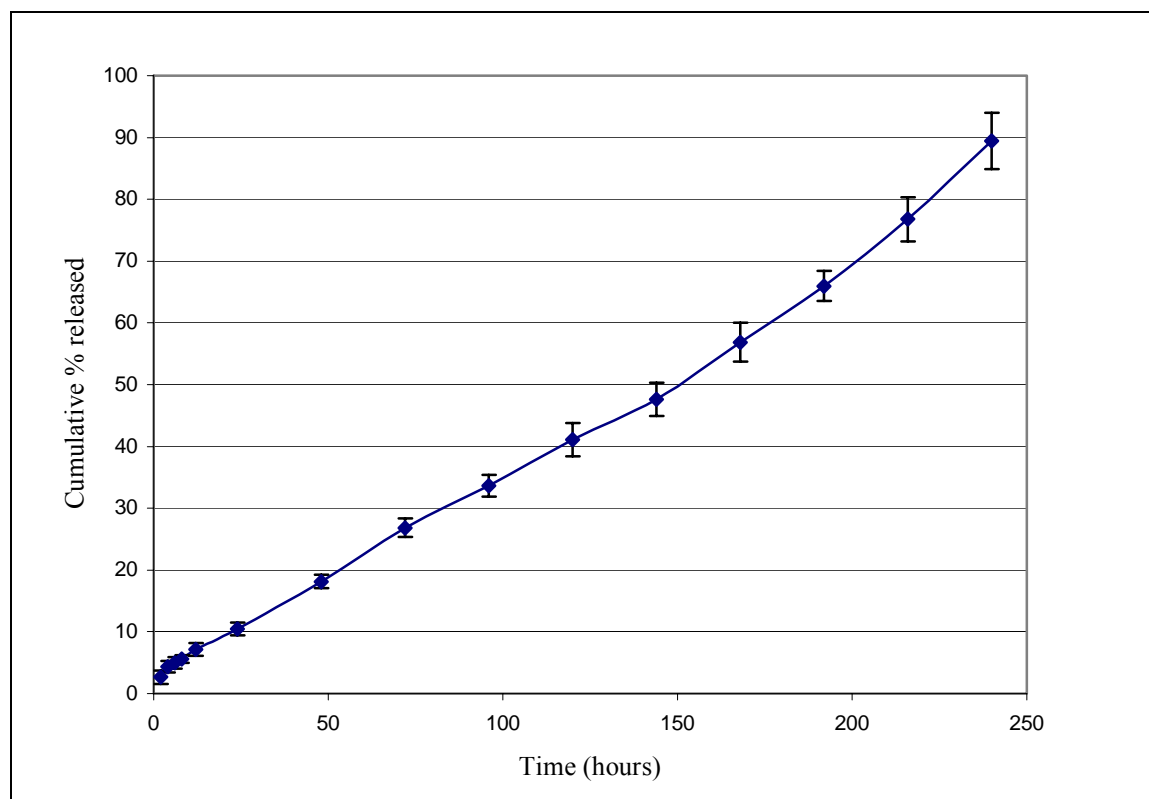


Figure 9. Dissolution profile of films containing low amount (5%) w/w Tetracycline of polymer and 10% plasticizer

—◆— 5% drug

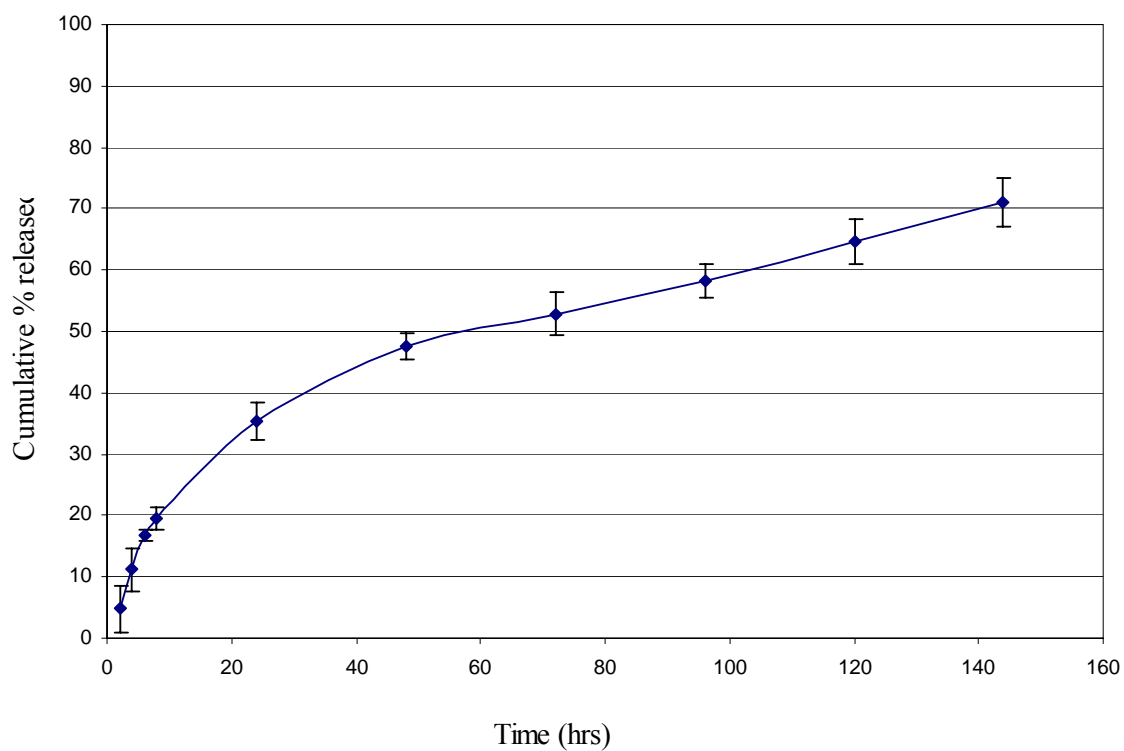


Figure 10. Dissolution profile of films containing medium amount (10%) of drug and 15% plasticizer

—◆— 10% drug

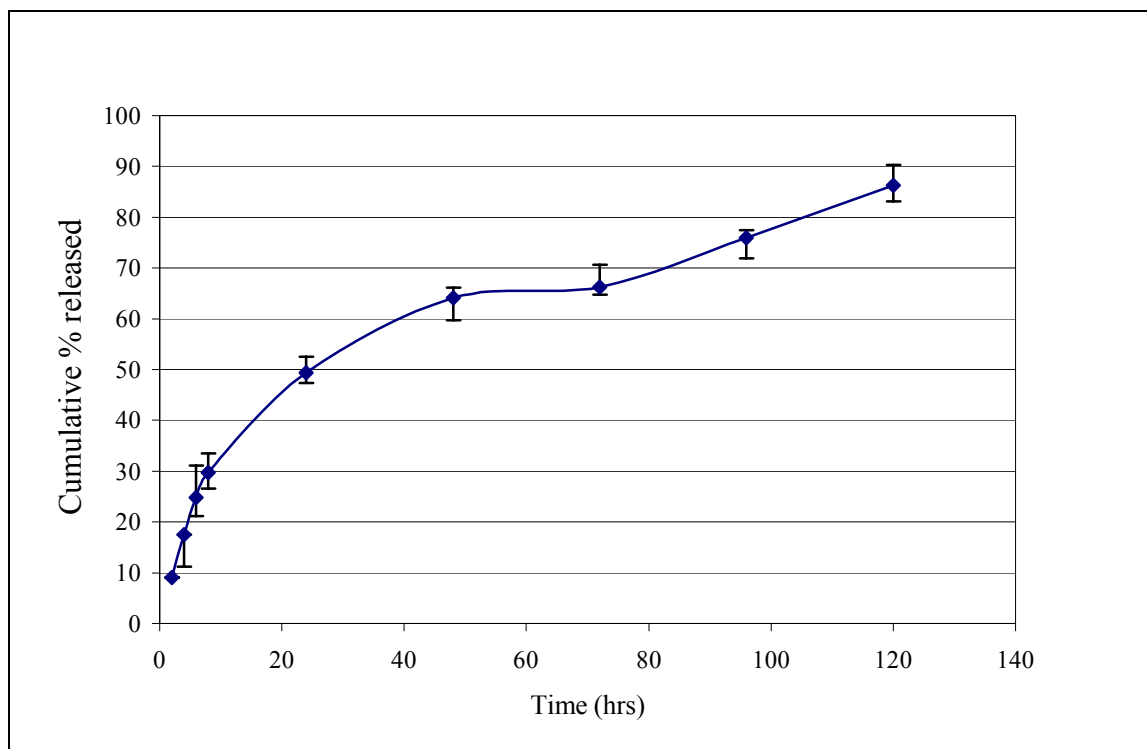


Figure 11. Dissolution profile of films containing high amount (15%) of drug and 15% plasticizer

—◆— 15% drug

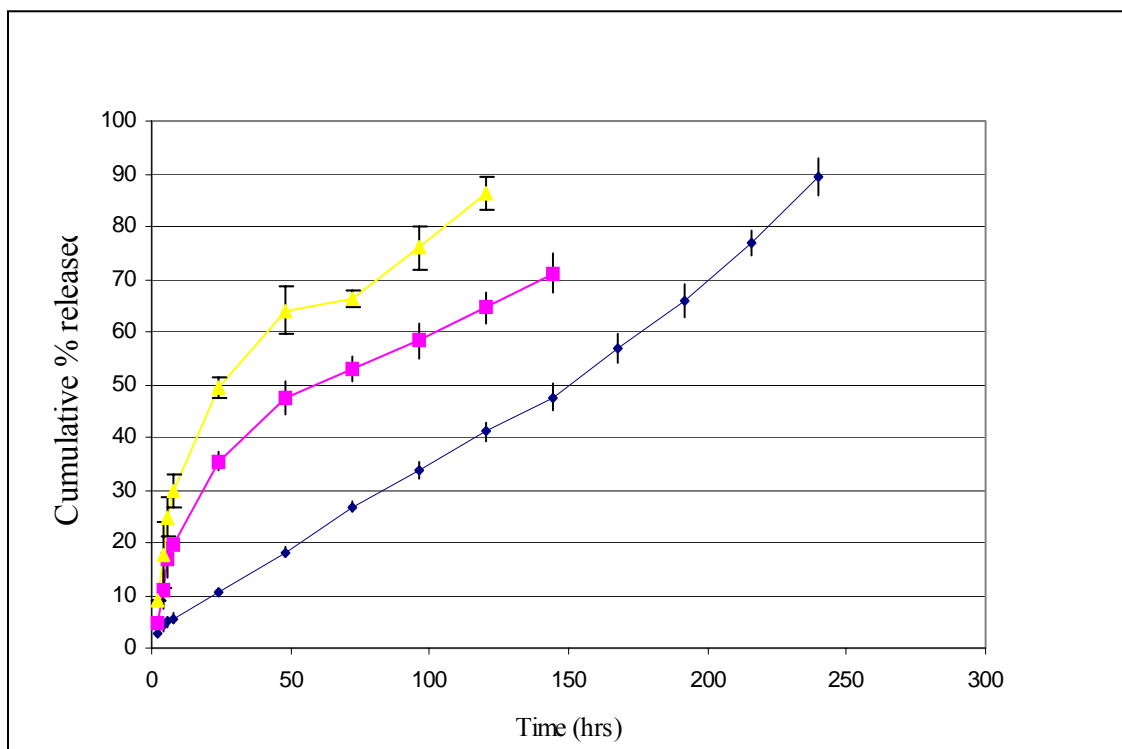


Figure 12. Effect of drug loading on dissolution from films containing tetracycline HCl

—◆— 5% drug —■— 10% drug —▲— 15% drug

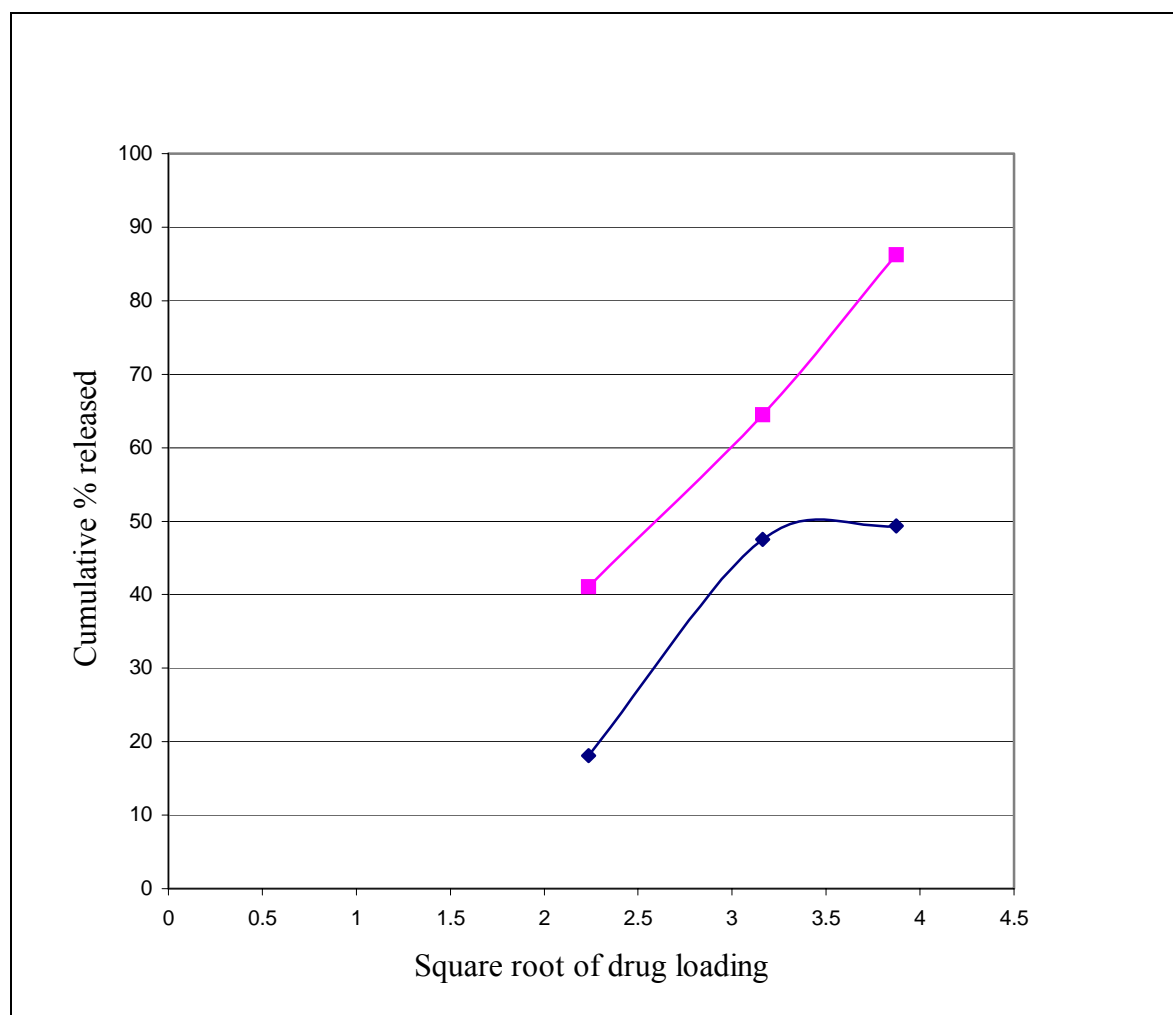


Figure 13. Effect of square root of drug loading from tetracycline HCl as seen after 2-days and 5-days

—◆— Average% (2 days) —■— Average% (5 days)

The initial rapid release is due to drug that is more concentrated near or on the surface of the film. Once the surface drug dissolves, rate of release slows down because of the increase in diffusion path length. The dissolution medium has to travel longer through the matrix to reach the drug present deep inside the matrix. Also during the evaporation of the solvent, film dries and the solvent carries drug molecules to the surface of the film. This causes the top of the film to have a higher concentration of drug molecules than the center or the bottom of the film. Therefore dissolution rate is higher initially. As shown in Figure 12, the rate of release increases with an increase in D/P ratio. Some amount of drug was retained in the films at the termination of the dissolution studies. This unavailable drug was considered to be entrapped within the hydrophobic, polymer matrix. At higher drug loadings the chances of the drug particles in contact with each other are high. This results in the formation of particular aggregates, which extends into the interior of the polymer matrix. Following dissolution of these drug aggregates, the polymer matrix porosity increases and therefore, more drug could be dissolved. However, at low drug loading, some of the drug particles could be completely entrapped and only those present at the surface could be released (47). Also, as the drug loading increases, dissolution media gets exposed to more drug per unit area of the film, resulting in an increase in the porosity of the polymer matrix due to the dissolution of the water-soluble drug.

For a homogeneous matrix, the amount of drug released (Q) at any time t is linearly dependent upon the square root of the initial amount of drug present in the matrix (A), as described by Equation 1 (49)

$$Q = [D \epsilon / \tau (2A - \epsilon C_s) C_s t]^{1/2} \quad (1)$$

Where D is the diffusion coefficient of the drug in the leaching medium and C_s is the solubility of the drug in the dissolution medium, ϵ is the porosity and τ is the tortuosity of the matrix.

Conversely, for a granular, porous system, the term A/ρ is present as can be seen in Equation 2, where ρ is the drug density.

$$Q = A [D/\rho\tau (2 - C_{s/\rho}) C_{st}]^{1/2} \quad (2)$$

Equation 2 illustrates that the amount of drug released at a time (Q) is linearly dependent upon the initial amount of drug present in the matrix (A). Since the percent of tetracycline HCl released by the 5th day was linearly related to the square root of initial drug loading (figure 13), the ethyl cellulose matrix in this study can be considered to follow homogenous diffusion matrix model in the later stages of dissolution. This plot is also useful to determine the drug to polymer ratio required to prepare films for desired extent of release within specific time duration.

Based on the results of these screening studies, the limits for the independent variables to be used in the experimental design were chosen.

DOE experiments

In vitro characterization of films

Tables 4 and 5 summarize the results of the film weight, thickness and assay of drug content. The mean weight of the films used for dissolution studies at pH 6.8 and 7.5 were 15.127 ± 2.85 mg 15.866 ± 2.85 mg respectively. Both sets of films had standard deviations less than 5.0% for all batches. The mean thickness of the films used for dissolution studies was 0.6 ± 0.07 mm. The experimentally determined drug content of the films was within 73.8-91% of the theoretical drug content of all the eight DOE runs. The reason for decreased drug content was that the drug had the tendency to move towards the periphery of the film in Teflon mould. The films used for experimental work were taken from the center of the mould.

Table 4. Physical characteristics of films prepared for DOE experimental runs at pH 6.8.

Expt. No.	Theoretical drug loading (% w/w)	Experimental^a drug loading (% w/w)	Percent theoretical drug content (%)	Weight^b (mg)	Thickness^b (mm)
1	30	22.14±0.04	73.8	12.58±. 237	0.432±.021
2	5	4.55±0.017	91	15.34±. 53	0.61±.0291
3	5	4.45±0.0016	89	16.64±. 65	.64±.035
4	5	3.69±0.002	73.8	15.84±. 722	0.572±.043
5	17	14.09±0.172	82.8	17.11±. 423	.654±.02
6	30	22.9±0.144	76.3	16.9±.804	.678±.072
7	17	14.91±0.211	87.7	16.968±.531	.634±.013
8	30	22.63±0.124	75.4	16.61±.718	.63±.025

^aEach value is the mean±SD of three determinations

^bEach value is the mean±SD of five determinations

Table 5. Physical characteristics of films prepared for DOE experimental runs at pH 7.5.

Expt. No.	Theoretical drug loading (%w/w)	Experimental drug loading (%w/w)	Percent theoretical drug content (%)	Weight (mg)	Thickness (mm)
1	30	22±0.04	73.3	12.492±. 6	0.432±.021
2	5	4.55±0.016	91	15.938±.53	0.61±.0291
3	5	4.34±0.004	86.8	17.23±.36	0.62±.021
4	5	3.7±0.002	74	14.246±.644	0.572±.043
5	17	14.09±0.1766	82.8	17.582±.691	0.654±.02
6	30	23±0.156	76.6	18.65±.557	0.678±.072
7	17	14.9±0.228	87.6	18.27±.324	0.634±.013
8	30	22.61±0.137	75.3	18.394±.772	0.63±.025

^aEach value is the mean±SD of three determinations

^bEach value is the mean±SD of five determinations

Scanning electron microscopy (SEM)

Scanning electron micrographs indicate that prior to drug release, the top surface of the films have a smooth surface where as bottom surface exhibits pores as can be seen in Figure 14, 16. This might be due to the reason that solvent when evaporates, takes some amount of drug along with it to towards the top surface of the film. More over these pores are more evident in films that have higher drug loading as can be seen in Figure which can be seen by comparing figure 14 and 16. Irregular pores are evident after the release of the drug as can be seen in Figure 15, 17. Bottom surface exhibits larger pores as compared to top surface may be because of the coalescence with the previous pores which can be seen in Figure 15 (b) and 17 (b). The formation of pores in the ethyl cellulose matrix supports the premise that the release of tetracycline HCl from these ethyl cellulose films was by dissolution of the drug followed by diffusion from the pores.

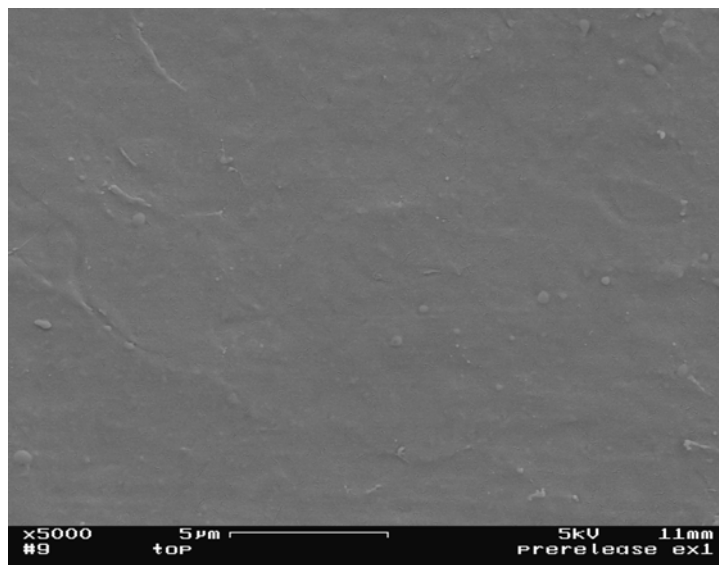


Figure 14. (a) SEM of top surface of film with 30% drug loading before dissolution.

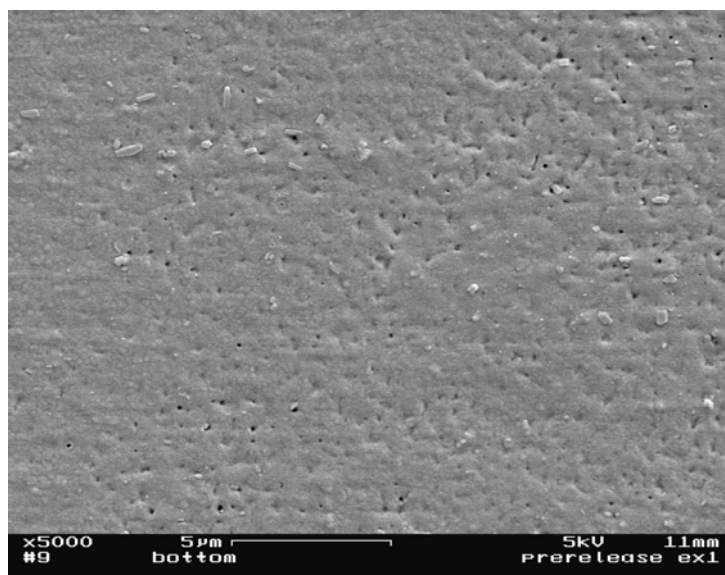


Figure 14. (b) SEM of bottom surface of film with 30% drug loading before dissolution.

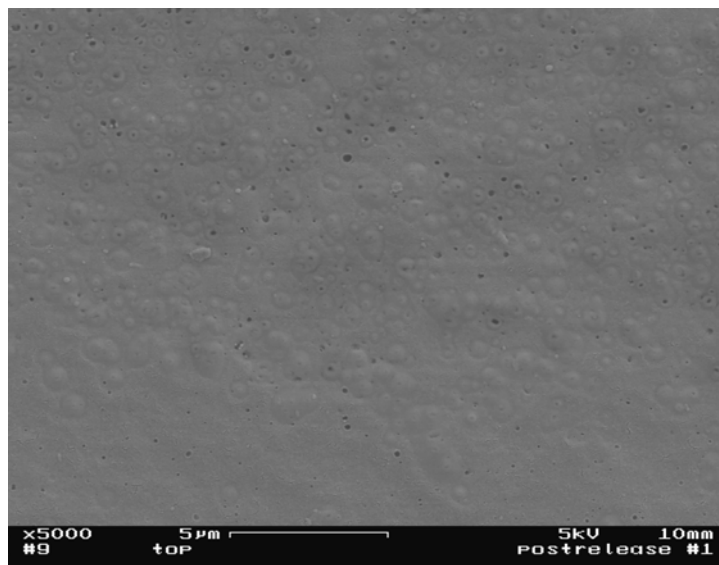


Figure 15. (a) SEM of top surface of film with 30% drug loading after dissolution.

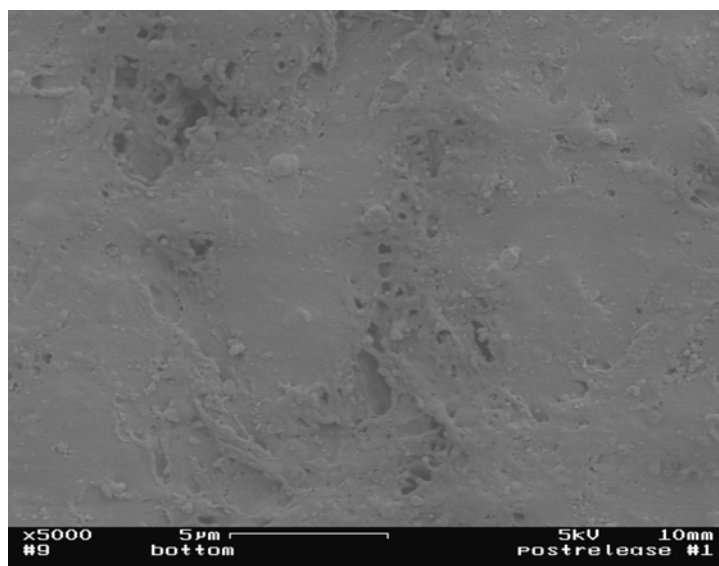


Figure 15. (b) SEM of bottom surface of film with 30% drug loading after dissolution.

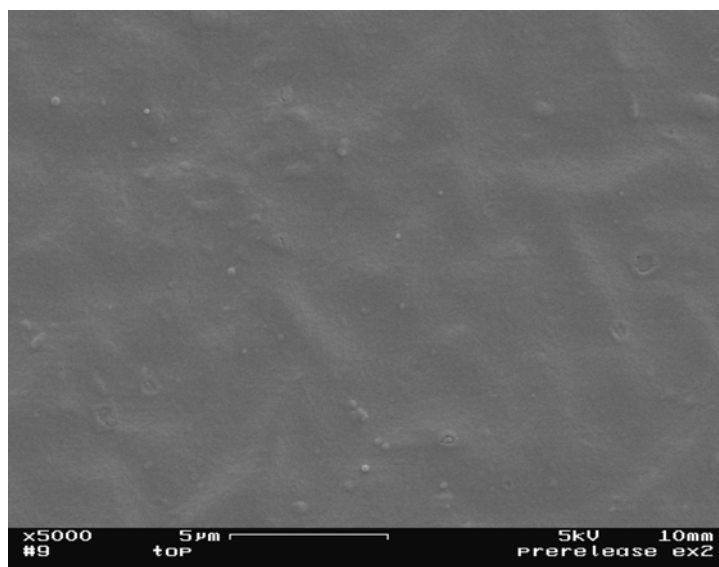


Figure 16. (a) SEM of top surface of film with 5% drug loading before dissolution.

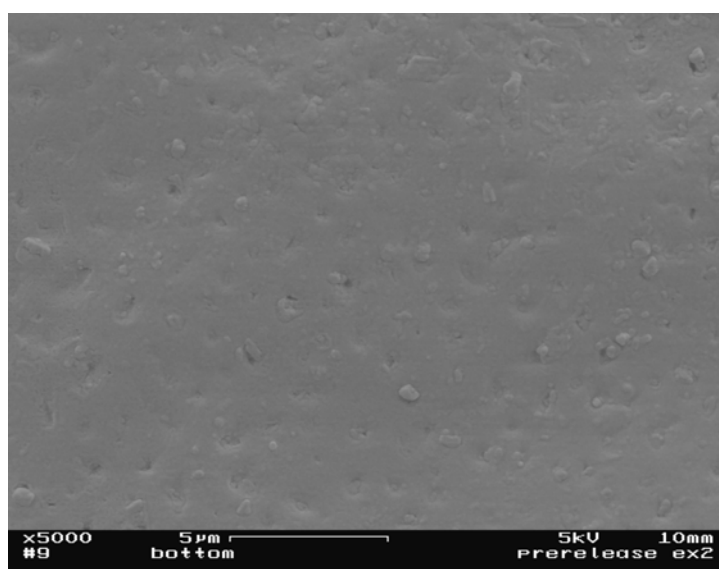


Figure 16. (b) SEM of bottom surface of film with 5% drug loading after dissolution.

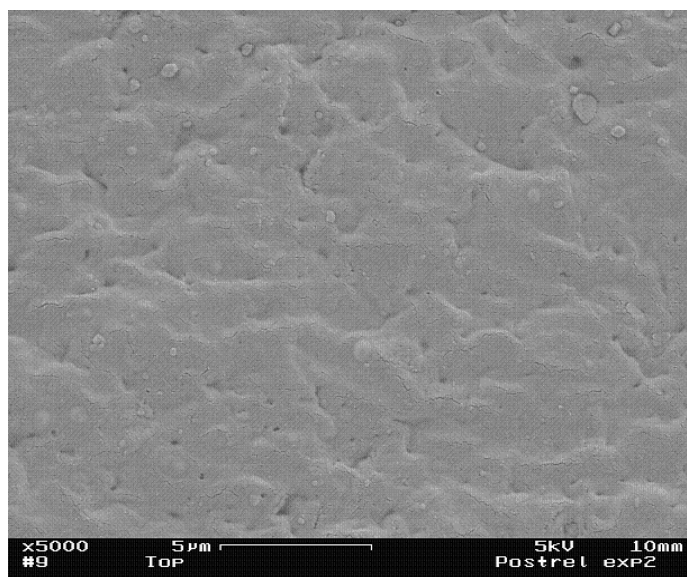


Figure17. (a) SEM of top surface of film with 5% drug loading after dissolution.

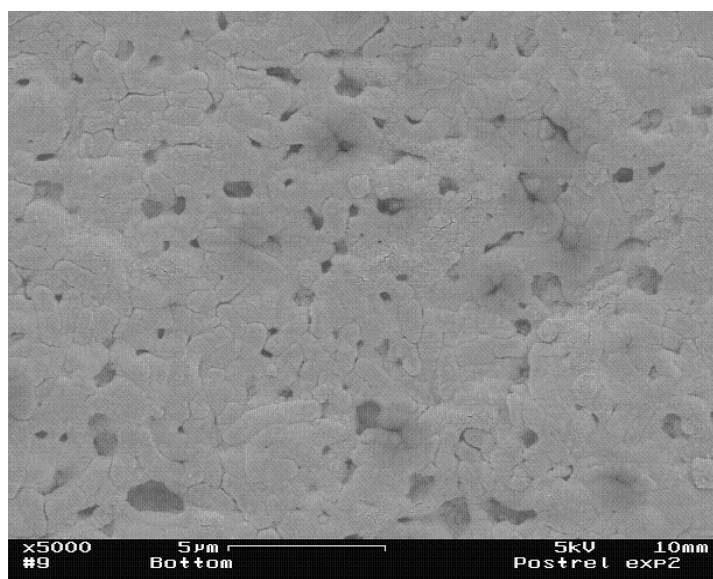


Figure 17. (b) SEM of bottom surface of film with 5% drug loading after dissolution.

Differential Scanning calorimetry

Thermal analysis of the drug and the polymer was done before and after their incorporation into the film. The glass transition temperature of the polymer decreased from 125.98°C to 50.79°C after incorporation in the film containing 25% plasticizer and can be seen if we compare figures 18 and 19. This is because plasticizer inside the film increases the mobility of the polymer chains and aids in lowering the glass transition temperature. Thermal analysis of the pure drug was done to see the melting point of drug. It 234.38°C as can be seen in Figure 20. If we see Figure 21, the endothermic peak is at 200°C. It might be the decomposition point of the polymer or it can be the melting point of the drug. Since this film had far more polymer loading (70%) as compared to that of drug loading (5%), we would consider it to be the decomposition point of the polymer. Thermal analysis of the pure polymer was done to confirm its decomposition point. Its decomposition point is 204°C as can be seen in Figure 22.

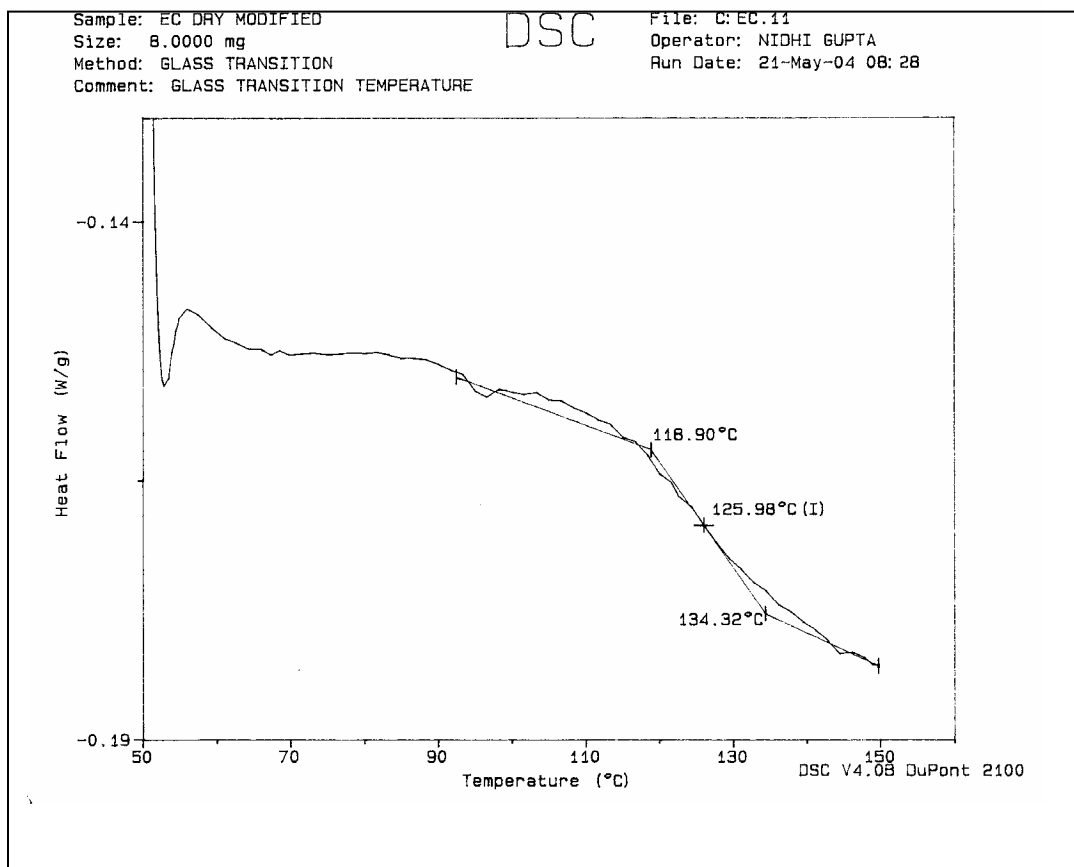


Figure18. DSC curve showing glass transition temperature of pure ethyl cellulose.

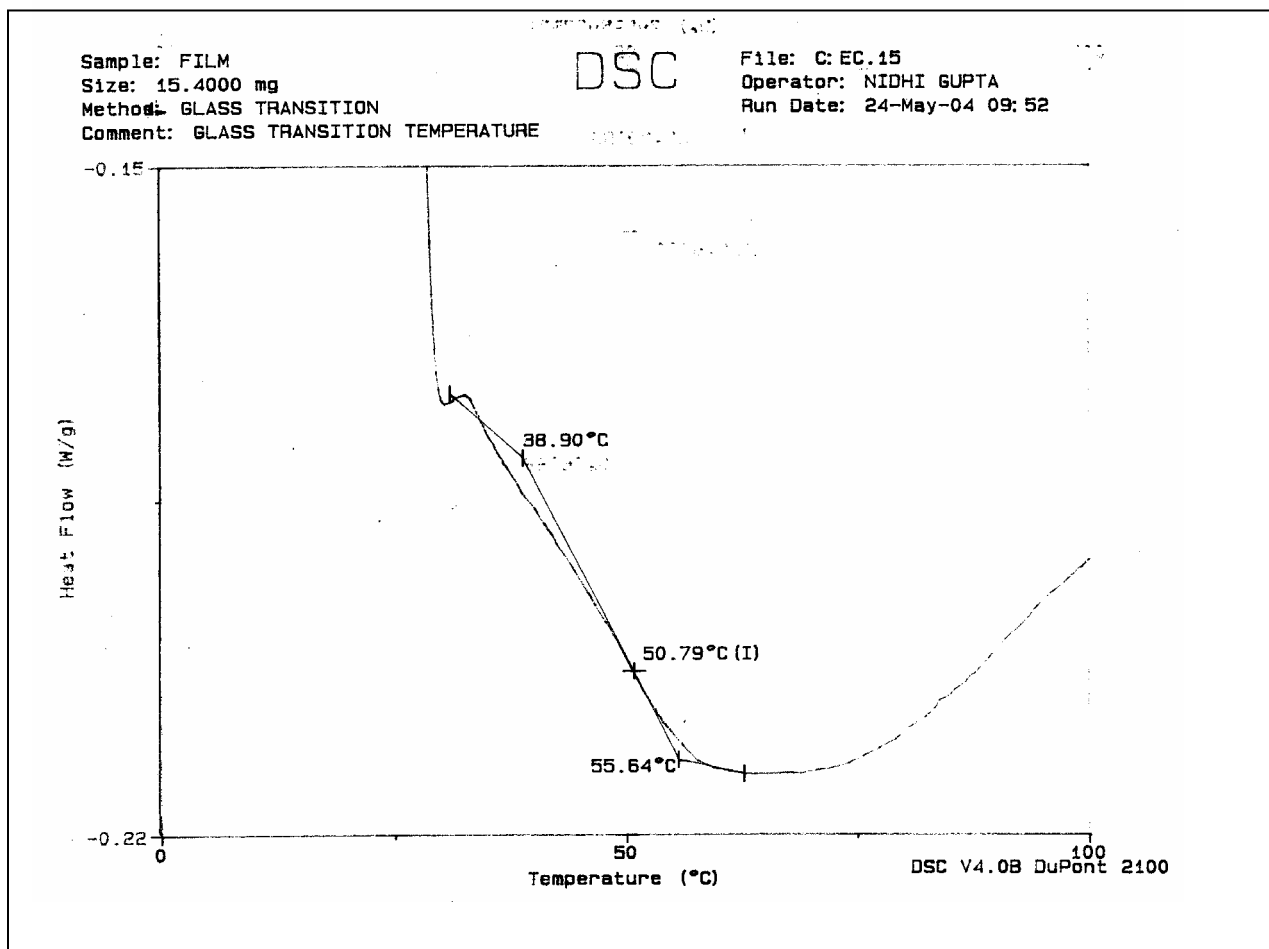


Figure19. DSC curve showing glass transition temperature of ethyl cellulose in a film containing 25% plasticizer.

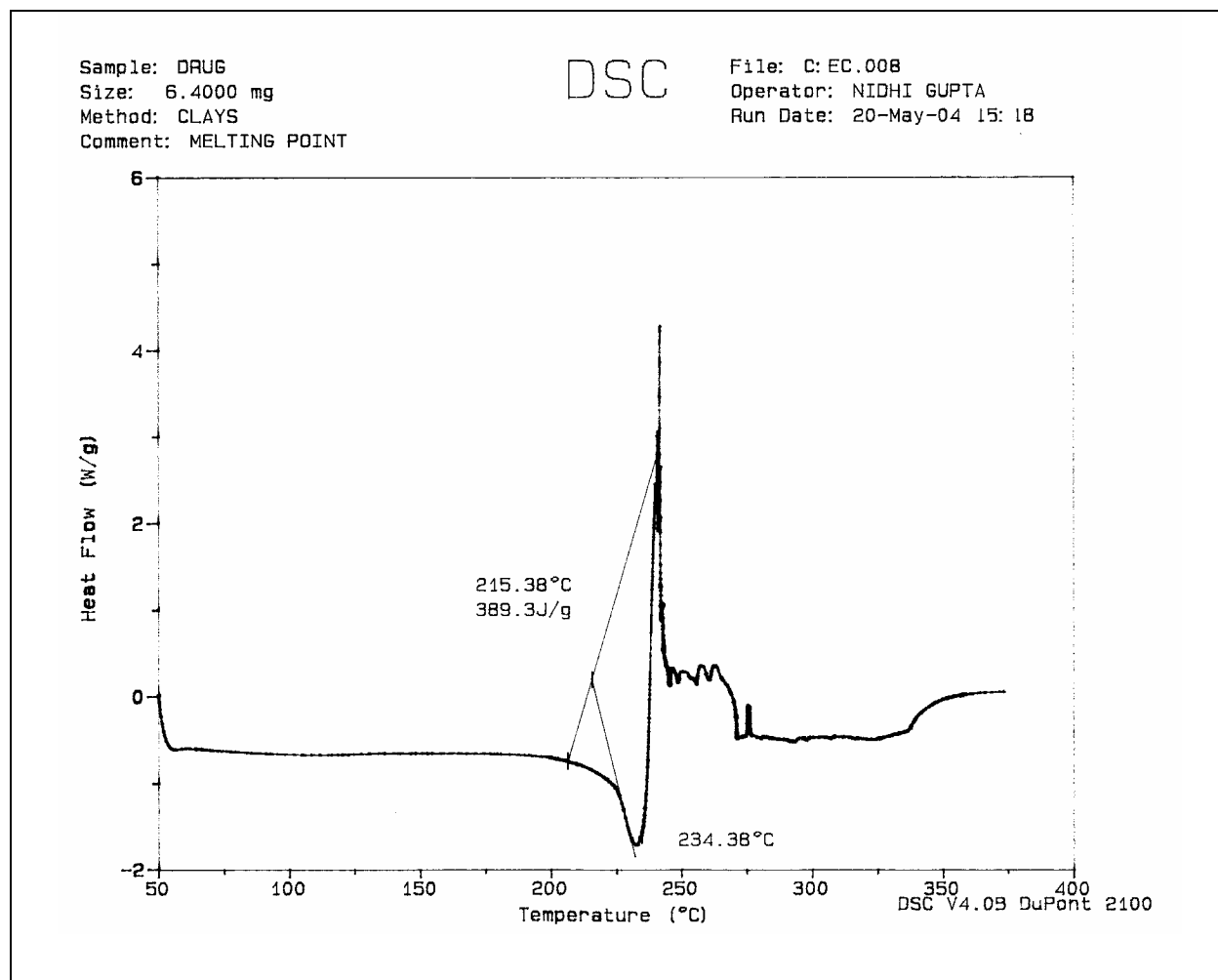


Figure20. DSC curve showing melting point of pure tetracycline HCl.

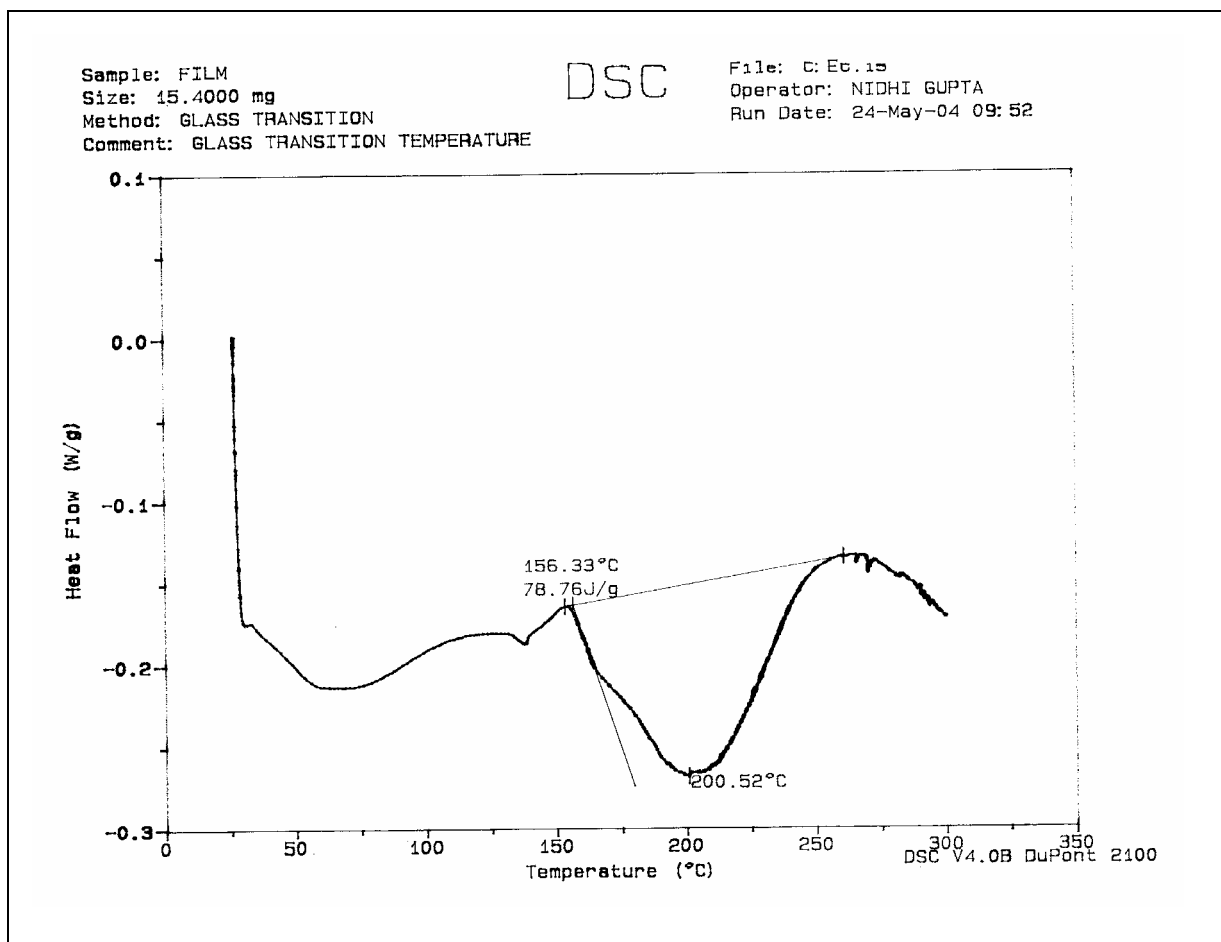


Figure21. DSC curve showing decomposition temperature of ethyl cellulose containing 5% drug, 70% polymer and 25% plasticizer.

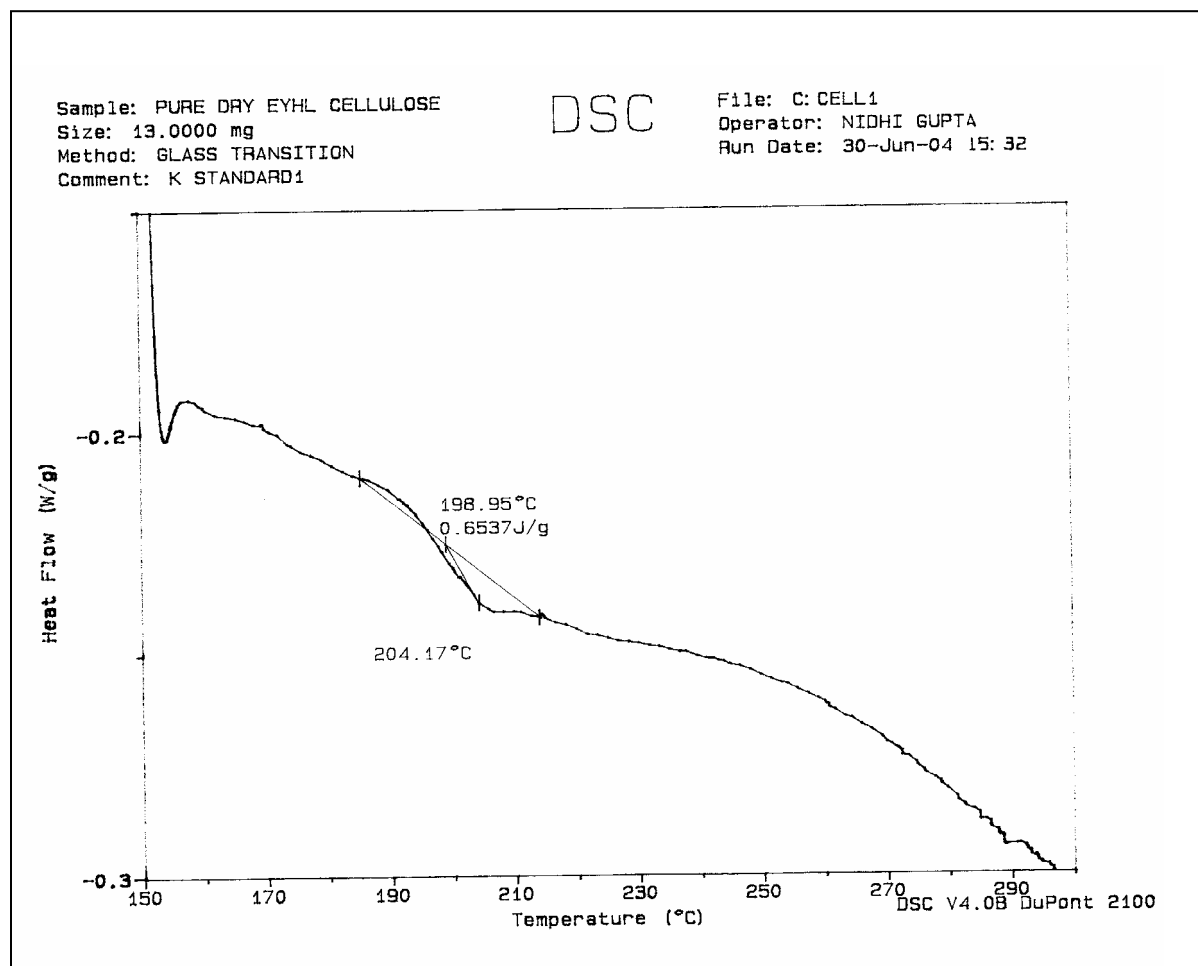


Fig 22. DCS curve showing decomposition temperature of pure ethyl cellulose.

In vitro release of tetracycline HCl

The cumulative percent drug released from the films of DOE runs at pH 6.8 and 7.5 is shown in Figure 23 and 24. The rate of drug release from the ethyl cellulose films increased with an increase in plasticizer amount but drug loading and polymer amount had minimal effect on the rate of drug release (Figure 25-28). Also the pH of the dissolution media had minimal effect on the rate and extent of drug release as can be seen in Figure 23 and 24.

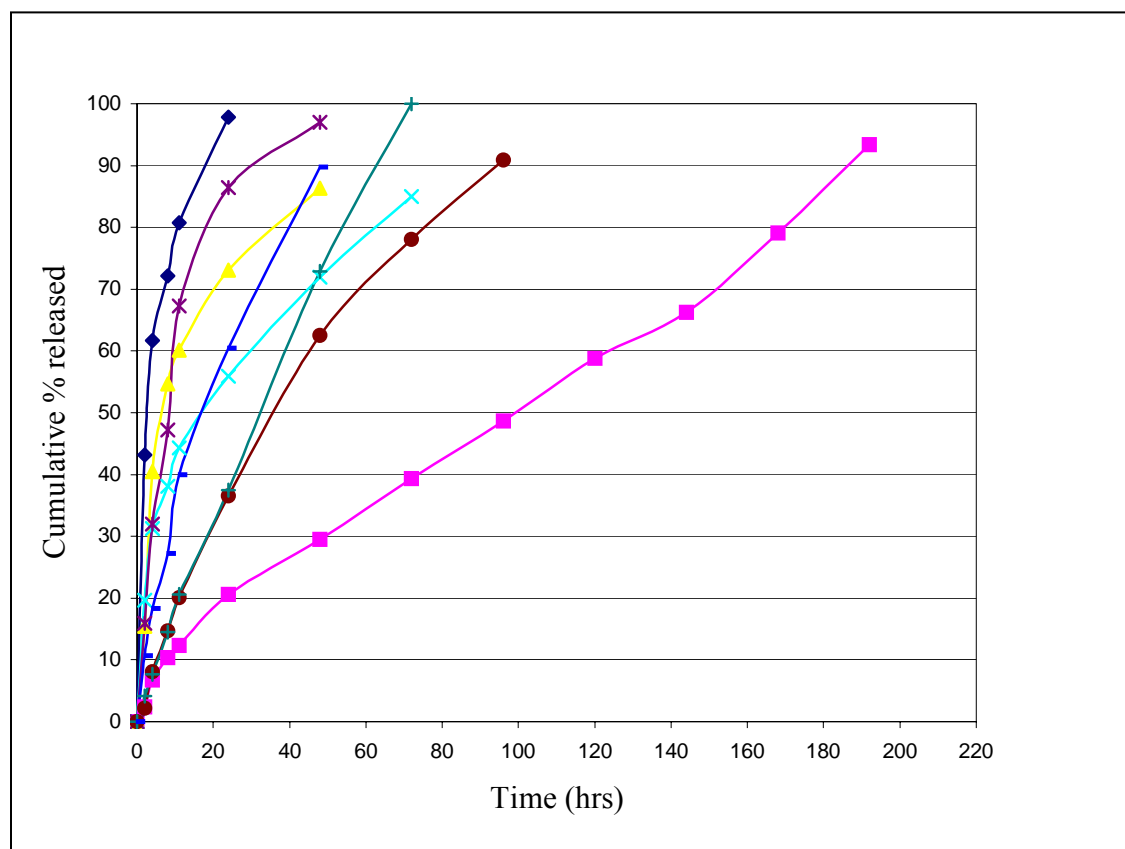
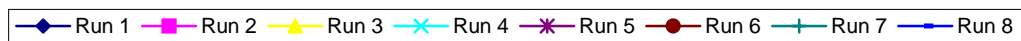


Figure 23. Dissolution profile of film from 8 dissolution runs at pH 6.8



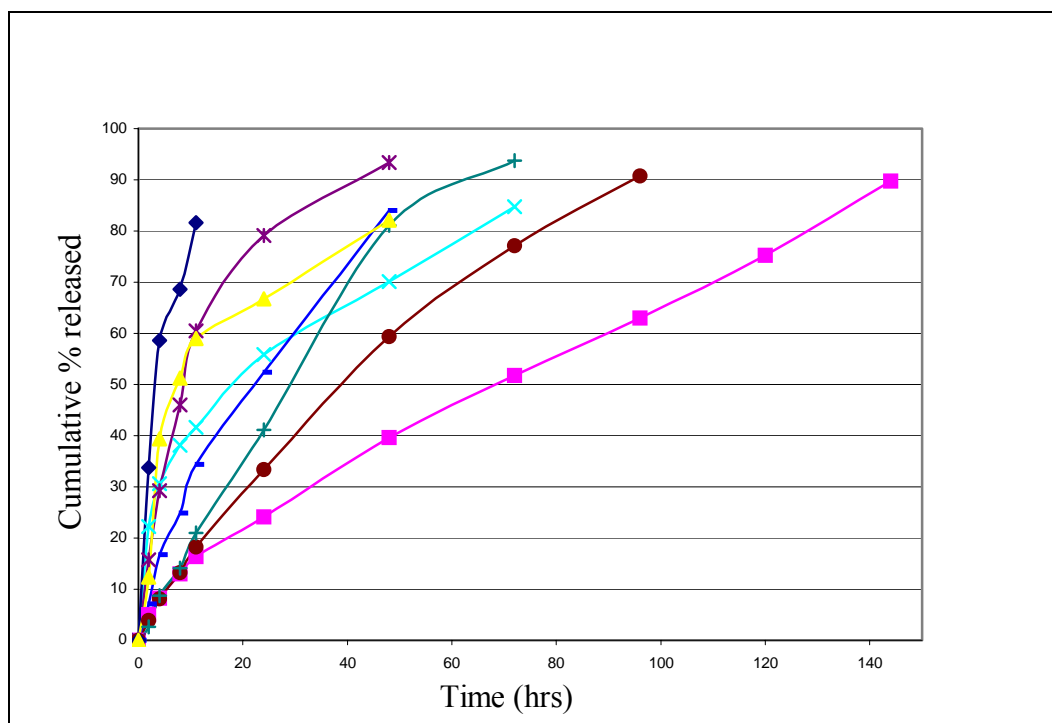
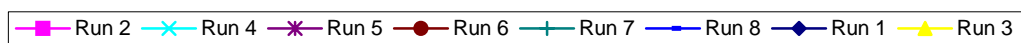


Figure 24. Dissolution profile of films from 8 dissolution runs at pH 7.5



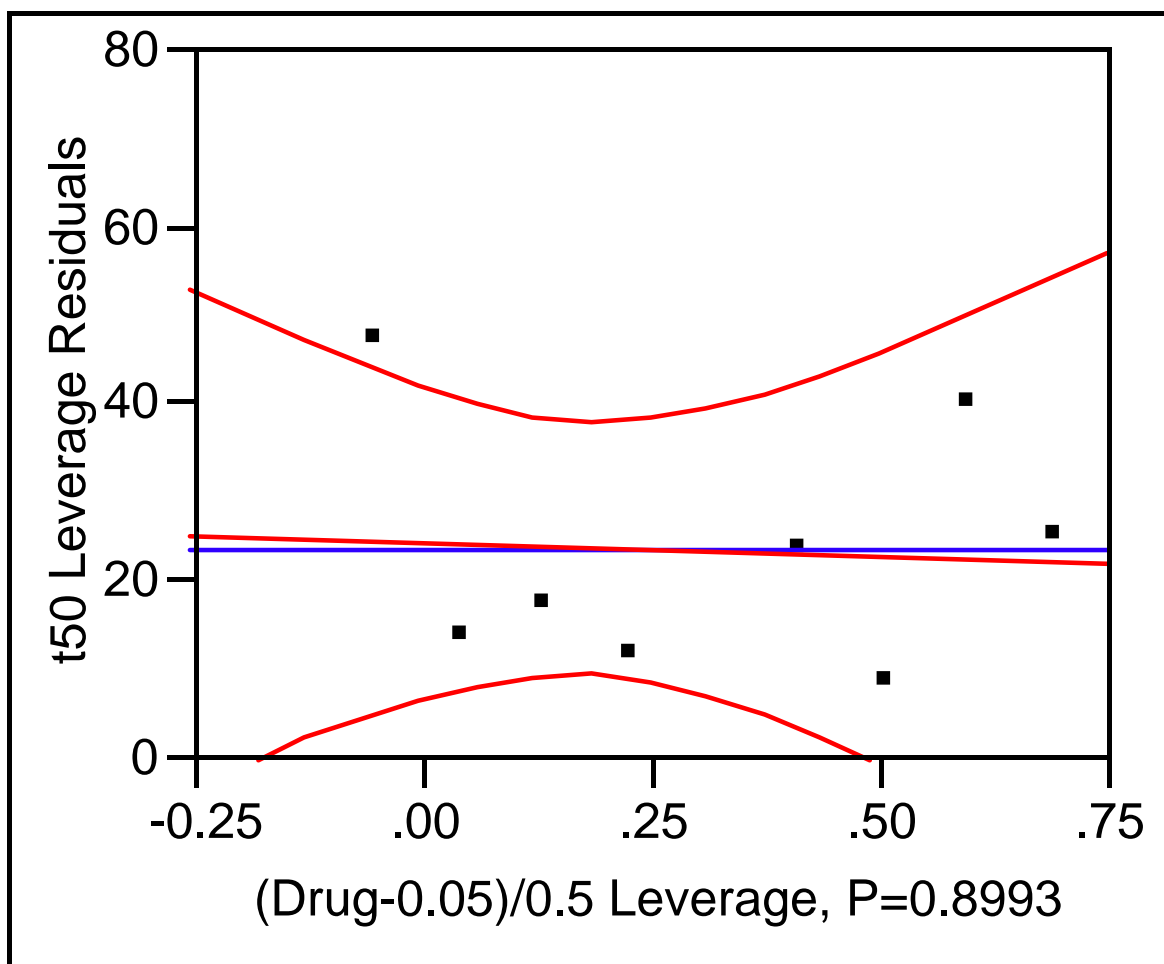


Figure 25. Effect of amount of drug in films on t50 in the DOE study.

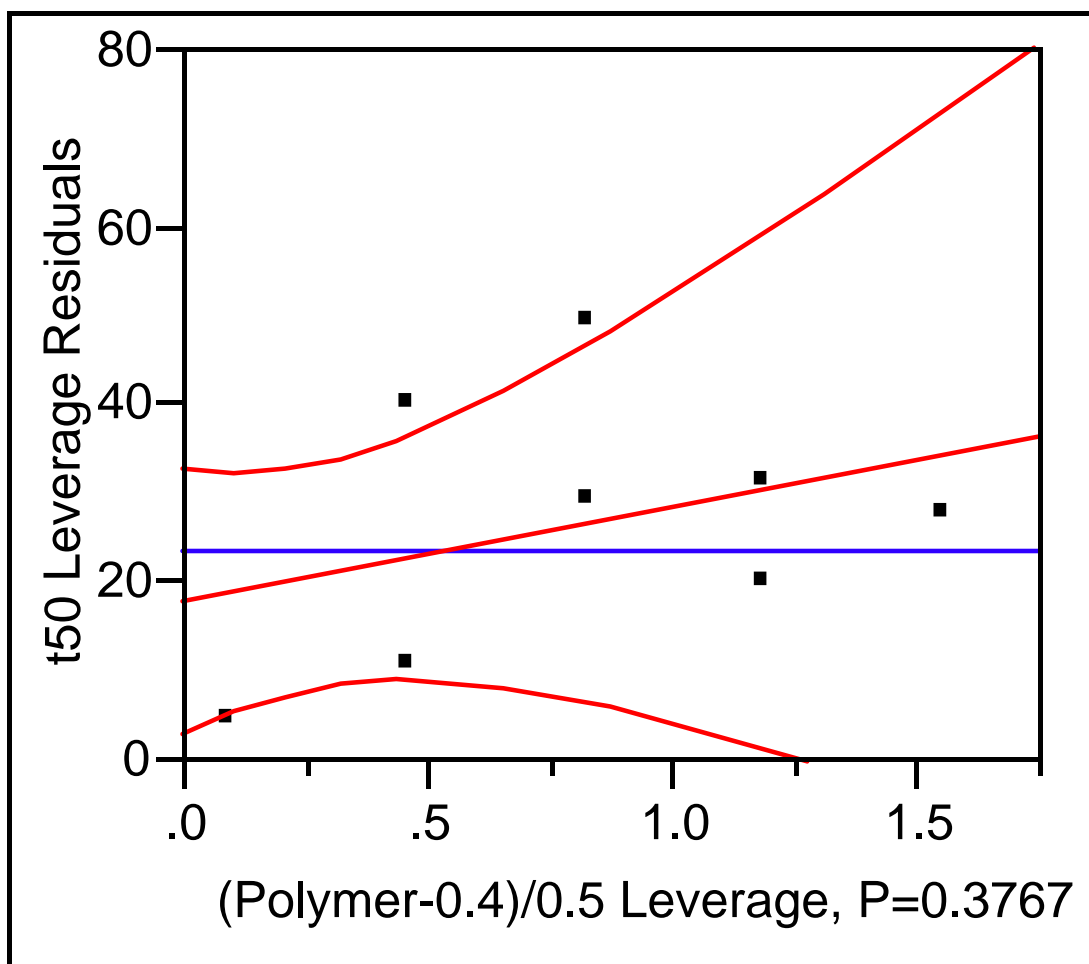


Figure 26. Effect of amount of polymer in films on t50 in the DOE study.

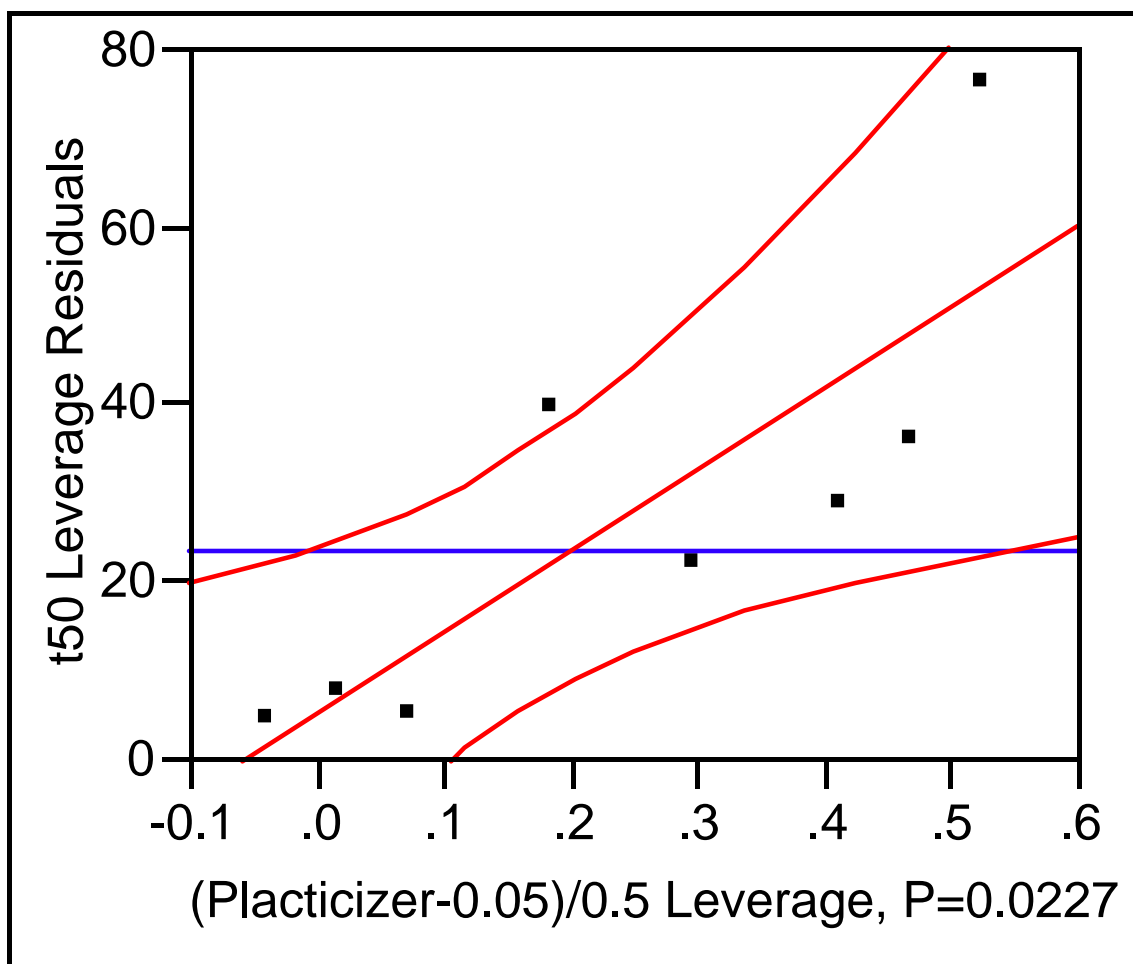


Figure 27. Effect of amount of plasticizer in films on t50 in the DOE study.

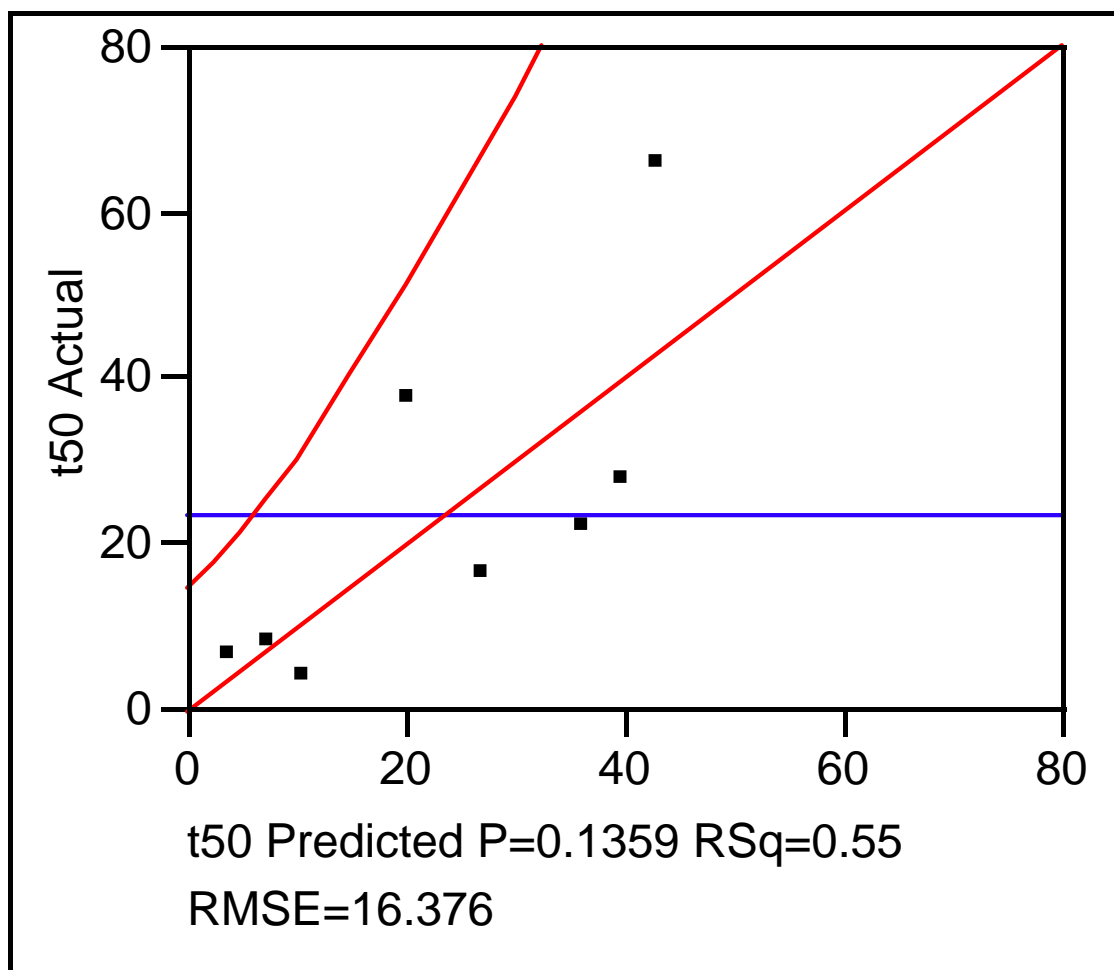


Figure 28. The actual and predicted values of t50 generated by DOE.

An initial rapid release was observed for most runs. This burst effect can be attributed to rapid dissolution of the water soluble drug from the surface of the films.

The percent tetracycline HCl released from the films containing varying amounts of drug, polymer and plasticizer is summarized in Table 4, 5. The entire available drug was not released. This unavailable drug was considered to be entrapped within the hydrophobic polymer matrix.

For all drug loadings, the plots of the percent TH released versus square root of time were linear ($r^2 \geq 0.95$) except for Run 3 at pH 7.5 as can be seen in Figure 24. At pH 6.8, the values of r^2 were ≥ 0.93 for all the experiments as can be seen in Figure 23. Values of r^2 can be seen in Tables 6, 7. The plots were linear for more than initial 60% of the drug released. The linear relationship between the percent tetracycline HCl released and the square root of time from the films with the same surface area indicates a matrix-controlled release (48).

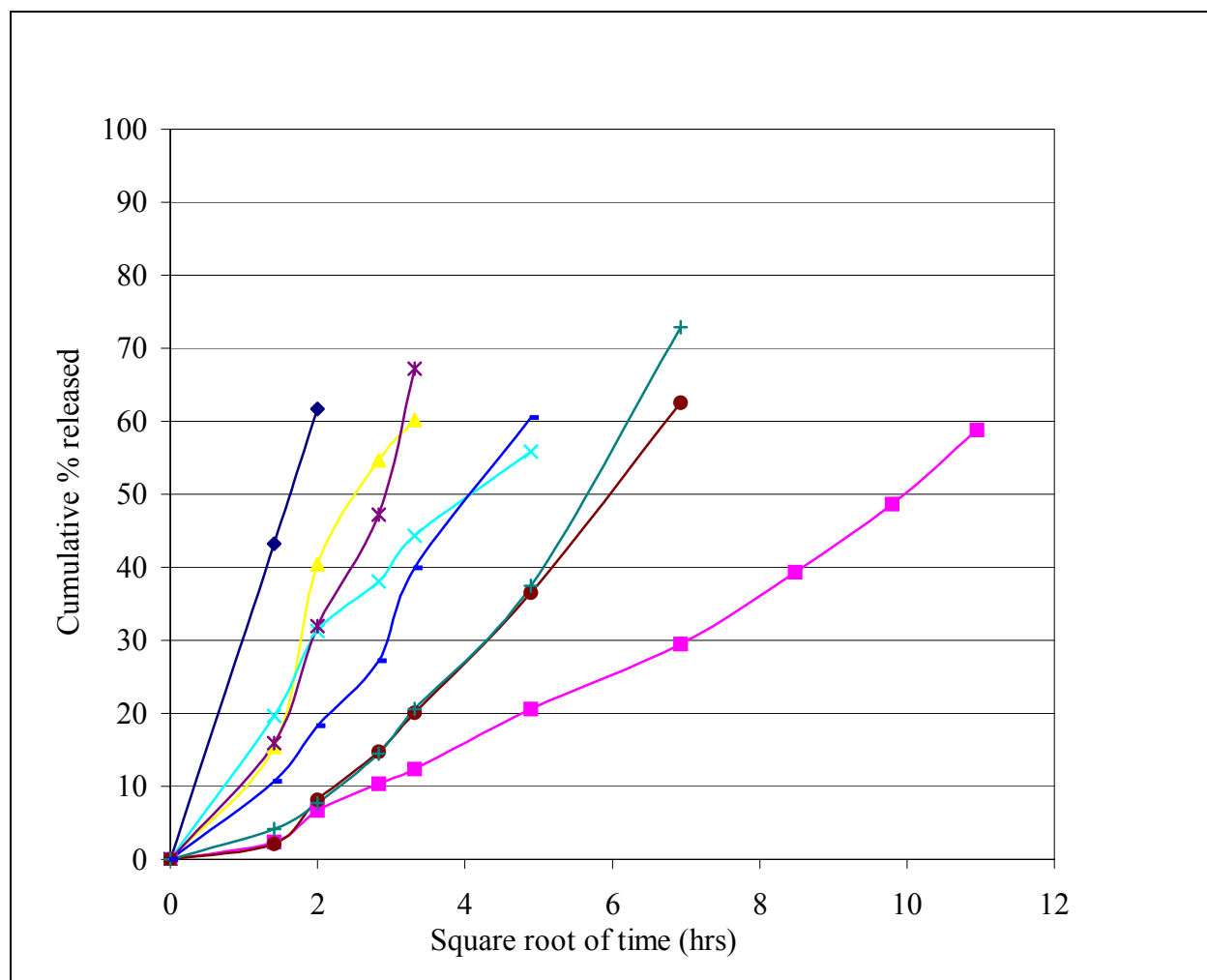
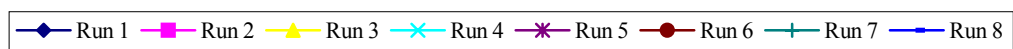


Figure 29. Higuchi equation fit for runs at pH 6.8



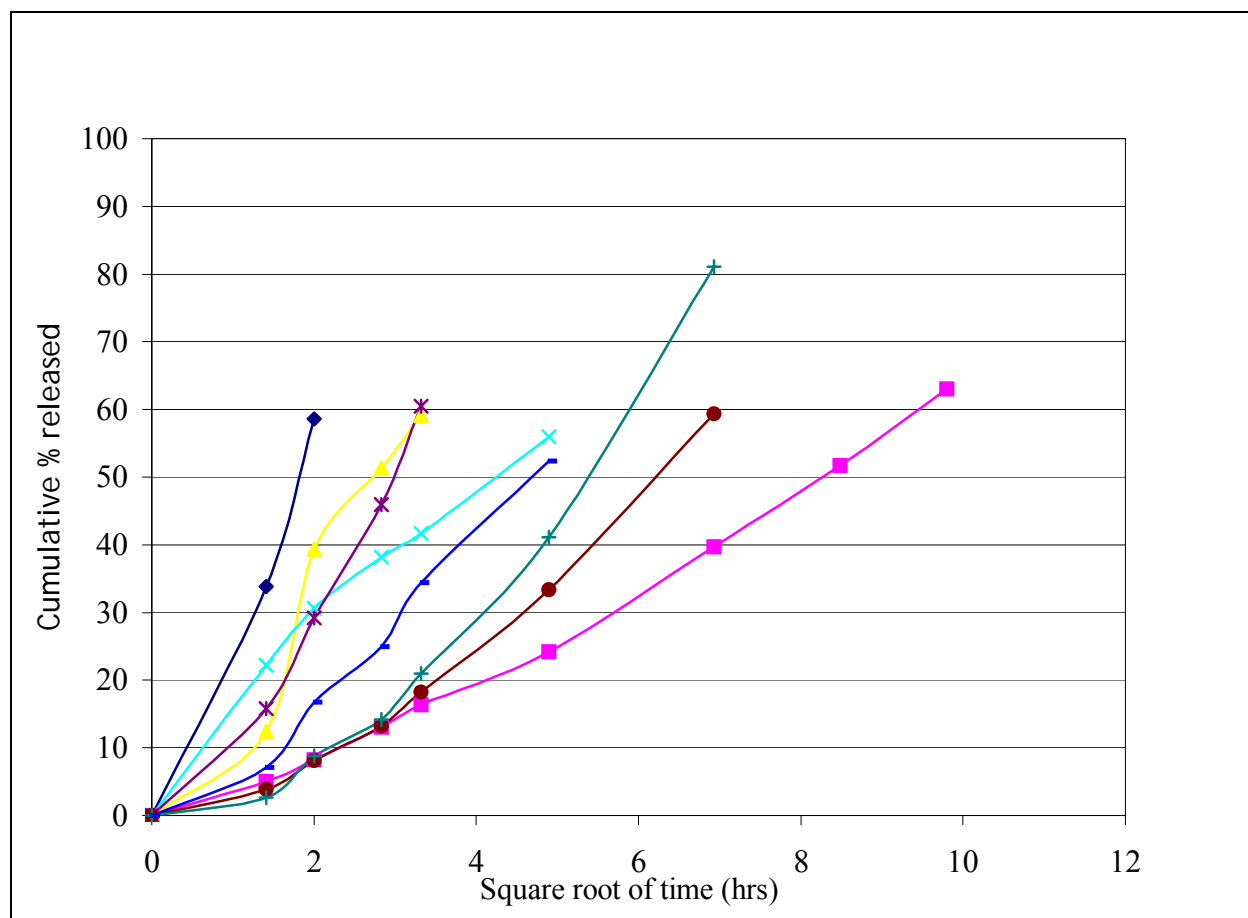


Figure 30. Higuchi equation fit for runs at pH 7.5



Table 6. Values of r square for DOE runs at pH 7.5 after fitting with Higuchi square root kinetics and zero order equation.

Expt no	R ² at pH 7.5 (Higuchi Equation)	R ² at pH 7.5 (Zero order fit)
1	0.9809	0.859
2	0.9801	0.988
3	0.8901	0.6767
4	0.9766	0.8384
5	0.9578	0.7905
6	0.9785	0.9868
7	0.9557	0.9713
8	0.9806	0.9607

Table 7. Values of r square for DOE runs at pH 6.8 after fitting with Higuchi square root kinetics and zero order equation.

Expt no	R ² at pH 6.8 (Higuchi Equation)	R ² at pH 6.8 (Zero order fit)
1	0.992	0.8594
2	0.9647	0.9914
3	0.9309	0.6875
4	0.9743	0.8305
5	0.938	0.7593
6	0.9831	0.9669
7	0.9503	0.9958
8	0.9862	0.9456

Table 8. Values of n and k at pH 6.8.

Expt no	n	k
1	0.5739	6.33
2	0.7057	48.91
3	0.51	30.24
4	0.4091	6.215
5	0.807	10.52
6	1.0055	64.81
7	0.8989	44.60
8	0.7043	14.92

Table 9. Values of n and k at pH 7.5.

Expt no	n	k
1	0.7947	5.13
2	0.6406	29.88
3	0.8794	12.13
4	0.3623	5.62
5	0.7732	10.56
6	0.8445	43.21
7	1.0698	65.343
8	0.7895	21.018

Analysis of dissolution data

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

$$M_t/M_\infty = Kt^n$$

Where M_t corresponds to the amount of drug released in time t , M_∞ is the total amount of drug released after infinite time, K denotes a kinetic constant, and n is the release exponent indicating the type of drug release mechanism. In the equation when n approaches 0.5, a Fickian diffusion controlled release is implied, whereas for values $0.5 < n < 1.0$, non-Fickian transport is implied. When value of n approaches 1.0, one can conclude that release is approaching zero order. From the above equation, values of n were calculated for all the formulations of the DOE. At pH 6.8, runs one, three and four showed release pattern according to Fickian diffusion, runs two, five, seven and eight showed non-fickian diffusion, and run six showed zero order release as can be seen in Table 8. At pH 7.5, run four showed fickian diffusion, runs one, two, three, five, six, and eight showed non-fickian diffusion, and run seven showed zero order release as can be seen in Table 9. Various other kinetic equations like zero order, first order, and Higuchi's square root were applied to interpret the release of drug from the films at both pH 6.8 and 7.5.

In the matrix diffusion system of this study, the drug is dispersed within a polymer matrix. Typically the course of events is as follows: water diffuses into the matrix, the drug dissolves, and finally the dissolved drug diffuses out of the polymer. The rate of drug release depends upon the amount of drug present at a particular time, thus the rate of release is time dependent (49).

The ethyl cellulose film was capable of delivering therapeutic concentrations of tetracycline HCl as desired for duration of one week into the intra-crevicular fluid within the inflamed pocket. It is assumed that the matrix was in contact with a well-mixed dissolution medium, the volume of which is large enough to ensure that the drug concentration is virtually zero at all times (sink condition).

DISCUSSION

The local delivery of antimicrobials into the periodontal pocket has opened up a new arena for the management of periodontal diseases. Efficacy of such delivery systems is dependent upon the slow release of the drug from the system and its penetration into the base of the pocket and adjacent connective tissue. Application of antimicrobials into the local delivery system has been considered an important adjunct to conventional periodontal treatment although it may be time consuming. In the study, tetracycline HCl films were successfully prepared using ethyl cellulose as the rate controlling polymer. These films were able to sustain the release of drug for about one-week.

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