DESIGN AND DEVELOPMENT OF NOVEL MULTILAYER CHITOSAN INSERTS CONTAINING DOXYCYCLINE HYCLATE

A dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI- 600 032.

In partial fulfillment of the requirements for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted By

Reg No: 26118151

DEPARTMENT OF PHARMACEUTICS

EDAYATHANGUDY.G.S PILLAY COLLEGE OF PHARMACY

NAGAPATTINAM-611002

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Under the guidance of

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This is to certify that the dissertation entitled"Designand Development of Novel Multilayer ChitosanInsertsContaining Doxycycline Hyclate"submitted by **I.Ahila** (RegNo: **26118151**)in partial fulfillment for the award of degree of Master of Pharmacy to the Tamilnadu Dr. M.G.R Medical University, Chennai is an independent bonafide work of the candidate carried out under my guidance in the Department of Pharmaceutics,Edayathangudy.G.SPillay College of Pharmacy during the academic year 2012-2014.

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1.INTRODUCTION

Periodontal diseases are recognized as the major public health problem throughout the world. Daily oral hygiene plays a vital role in maintaining healthy teeth and gums. Periodontal disease can do occur in all age groups, ethnicities, races, genders and socioeconomic levels.

The term “Periodontal Disease” broadly defines several diseases associated with the periodontium. Changes in the microflora, histopathological variations, clinical symptoms, and the location of the inflammation help to further delineate periodontal disease. Gingivitis, the moderate stage of the disease, caused by an accumulation of supragingival plaque is characterized by swelling, light bleeding and redness of marginal gingiva. Gingivitis is associated with a change in microflora, shifting from a grampositive anaerobic flora to a more gram-negative flora. Periodontitis a more severe stage of periodontal disease, results in the resorption of the alveolar bone and detachment of the periodontal ligaments supporting the tooth.

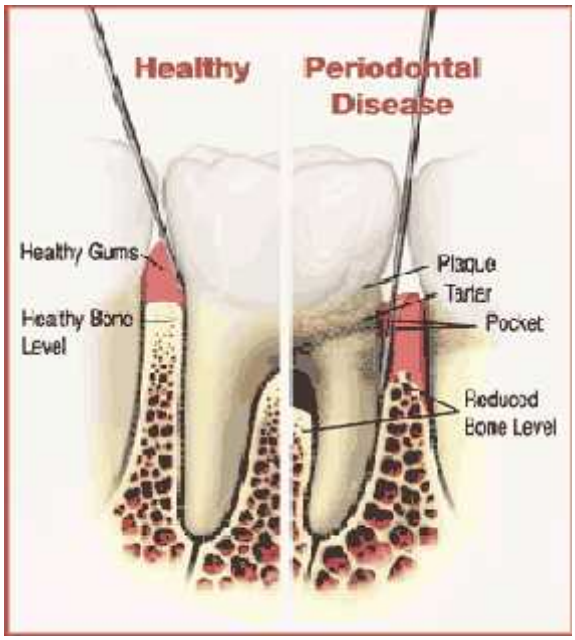


Figure No.1.1: Comparision of Healthy Periodontum/Gums and Periodontal Disease.

One of the clinical features of the periodontal disease is the formation of periodontal pocket, which is pathologically deepened sulcus. In normal sulcus, the gap between the gingiva and the tooth is normally between 1 and 3 mm deep. However, during periodontitis, the depth of pocket usually exceeds 5mm[1].

Causes of Periodontal Diseases:

The main cause of periodontal disease is bacteria plaque, a sticky, colorless film that constantly forms on teeth. However, factors like smoking/ tobacco use, genetics, pregnancy and puberty, stress, medication, clenching or grinding teeth, diabetes and poor nutrition also lead to periodontal diseases. Plaque (more plaque formation) Gingival inflammation Pocket formation Periodontal pathogens grow only where atmosphere and nutrient composition are strictly conductive to their requirements and the disease once established, causes major changes in the periodontal microenvironment. The gingival crevicular fluid (GCF) flow occurs at extremely low levels in healthy gingival sulci but increases enormously to 3.5ml/day or more. The most commonly grown anaerobic pathogenic bacteria are ***Actinobacillus actinomycetencomitans, Bacteroides gingivalis, Bacteroides melaninogenicus sub species intermedius, Porphyromonas gingivalis and Prevotella intermedia.*** Clinical signs such as bluish red thickened marginal gingiva, bluish red vertical zone from the gingival margin to the oral mucosa, gingival bleeding and localized pain are suggestive of the presence of periodontal pockets [2, 3].

Overview of the Pathogenesis of Periodontal Disease:

The normal healthy gingiva is characterized by its pink color, its firm consistency and free from histological evidence of inflammation, but this ideal condition is very rarely seen in microscopic tissue sections. This is because most human gingival tissues are, no matter how clinically healthy in appearance, slightly inflamed due to constant

presence of microbial plaque. Even in healthy state, the gingiva has a leukocyte infiltrate that is predominantly comprised of neutrophils or polymorphonuclear leukocytes. These leukocytes rephagocytose whose primary purpose is to kill bacteria in the gingival crevicular area or the gingival pocket. Neutrophils are recruited to the periodontal pocket or the gingival crevice because of attracting molecules, released by bacteria, called chemtactic peptides. Furthermore, as bacteria damage the epithelial cells, they cause epithelial cells to release molecules termed cytokines that further attract leukocytes to the crevice. The neutrophilscan phagocytose and digest bacteria within crevice and therefore remove these bacteria from the pocket. If the neutrophil becomes overloaded with bacteria, it degranulates or explodes. This causes tissue damage from toxic enzymes that are released from the neutrophils. Therefore, neutrophils can be viewed as being both helpful and potentially harmful. Depending on the individual, progression from gingivitis to periodontitis requires varying amounts of time. But in normal situation, more than 6 months may be needed for the lesion of gingivitis to change to periodontitis [4, 5, 6].

Microbiology of Periodontal Disease:

About 300 or 400 bacterial species are found in the human subgingival plaque samples. Of that number, possibly 10-20 species may play a role in the pathogenesis of destructive periodontal disease. These bacterial species must be able to colonize in order to survive and damage the periodontal tissues. To colonize subgingival sites, microbes must be able to,

1) Attach to periodontal tissues

2) Multiply

3) Compete with other microbes in their habitat and

4) Defend themselves from host defense mechanisms.

The gingival sulcus area is a lush place for microbial growth, but in order to colonize a subgingival site, a bacterial species must overcome a number of host-derived obstacles. If a species successfully enters underlying connective tissue, it faces a multitude of host immune cells including B and T lymphocytes, neutrophils, macrophages and lymphocytes. To be successful, bacteria must have the ability to evade all these host response mechanisms. The microbes involved in periodontal disease are largely gram-negative anaerobic bacilli with some anaerobic cocci and a large quantity of anaerobic spirochetes [4, 8, 9].

Histopathological Changes:

The major pathological features of periodontitis are the accumulation of an inflammatory infiltrate in the tissue adjacent to the periodontal pocket, breakdown of connective tissue fibers anchoring the root to gingival connective tissue and alveolar bone, apical migration of the epithelial attachment or junctional epithelium, and resorption of the marginal portion of the alveolar bone resulting in eventual tooth loss. Page & Schroeder developed a system to categorize the clinical and histopathological stages of periodontal disease and defined four histopathological stages of periodontal inflammatory changes.

- Health – Pristine Condition
- Initial lesion – Clinically healthy
- Early lesion – Early gingivitis
- Established lesion – Chronic gingivitis
- Advanced lesion – Chronic periodontitis

Following histopathological description by Kinane and Lindhe is based on model Proposed by Page & Schroeder is summarized in table below [4,7,10]

Table No.1.1: Histopathological Description

	Clinical condition	Histopathological condition
1.	Pristine gingiva	Histological perfection
2.	Normal healthy gingiva	Initial lesion
3.	Early gingivitis	Early lesion
4.	Established gingivitis	Established lesion with no bone loss nor apical epithelial migration (Plasma cell density between 10% and 30% of leukocyte infiltrate.
5.	Periodontitis	Established lesion with bone loss and apical epithelial migration from the cement enamel junction (Plasma cell density >50%).

The initial lesion appears within 4 days of plaque accumulation, characterized by an acute inflammatory response. The major features consist of an increased flow of crevicular fluid; inflammatory infiltrate occupies 5% to 10% of the gingival connective tissue below the epithelium and loss of collagen.

After approximately 7 days of plaque accumulation, an inflammatory infiltrate of mononuclear leukocytes develops at the site of initial lesion as it progresses to the early lesion. At this stage, the infiltrate occupies about 15% of the gingival connective tissue, with collagen destruction in the infiltrated area reaching 60-70%. Clinically the inflammatory changes are visible and present as edema and erythema.

After 2 to 3 weeks of plaque accumulation, the early lesion evolves into established

lesion, characterized by further increase in the size, predominance of plasma cells and lymphocytes at the periphery of lesion.

Features of advanced lesion include periodontal pocket formation, surface ulceration and suppuration, destruction of alveolar bone and periodontal ligament, tooth periodontitis is marked by the change in T-cell to B-cell predominance.

Mobility and drifting, and eventually tooth loss. The advanced lesion characterized by the same feature present in the established gingival lesion.

TREATMENT APPROACHES

Conventional Periodontal therapy:

The purpose of periodontal treatment is to cure the inflamed tissue, reduce the number of pathogenic bacteria and eliminate the depth of the diseased pockets and to stop bone resorption. The conventional methods of pocket elimination are more or less mechanical and are aimed at removal of supra and mechanical plaque and degenerated and necrotic tissue lining the gingival wall of periodontal pockets through scaling, root planning and curettage[11].

The mechanical debridement alone often leaves behind significant number of pathogens due to possible instrumentation or ability of microorganism to penetrate into deeper tissues. Inaccessibility and recolonization of pathogens can occur after scaling and root planning. With oral hygiene, a pathogenic subgingival microbial may reestablish within 42 - 60 days after a single periodontal debridement session[13]. Some deep periodontal pockets experience putative pathogen recolonization by 120 - 240 days despite multiple sessions of subgingival instrumentation and meticulous supra gingival plaque control[12].

Antibiotic Therapy:

The use of antibiotics in the treatment of periodontal diseases helps to reduce or eliminate bacteria that cannot be removed by scaling and root planning. Chemotherapeutic agents can be administered systemically or locally. Tetracycline's, imidazole derivatives, fluoroquinolones etc., are the most favored antibiotics.

Antimicrobials used to treat dental infections can be divided into two main categories, i.e., broad spectrum and narrow spectrum. Narrow-spectrum antimicrobials include penicillin, amoxicillin, cephalexin, macrolides and tetracyclines. These drugs are having a limited antimicrobial efficacy (Table1.2), as they are not effective against aerobic and anaerobic beta lactamase producers, as well as other specific organisms[14]

Systemic periodontal antimicrobial therapy is based on the premise that specific microorganism cause destructive periodontal disease and that the antimicrobial agent in the periodontal pocket can exceed the concentration necessary to kill the pathogens. Systemic antibiotics can reach microorganisms at the base of deep periodontal pockets and furcation areas via serum and may also affect organisms residing within gingival epithelial and connective tissue. With systemic antibiotic therapy there is a considerable variability in the therapeutic activity due to factors like poor absorption in the gastrointestinal tract, first pass metabolism, systemic distribution, bacterial sensitivity and resistance[15].

Some studies also report poor results due to the fact that the active product does not reach in adequate concentration at the site of action, as it is not retained locally for sufficient period of several thousand folds before it reaches the site of action

necessitating ingestion of large doses.

The increased toxic effects of these elevated dose levels make systemic administration unacceptable due to low benefit to risk ratio. Repeated long term use of systemic antibiotics is fraught with potential danger including resistant strains and super infections[17].

These draw backs can be markedly reduced if antimicrobial agent to be used locally. Because of the smaller dosage used and topical chemotherapy is much safer than systemic chemotherapy in avoiding the side effects of antibacterial agents[16]

Local Drug Delivery:

Local applications (as mouth rinse, gels, tooth paste etc,) control only supra gingival microbial plaque or periodontal disease involving pocket formation and also requires high initial concentrations and multiple applications in order to provide sustained effectiveness[18.]

Local application of antibiotics has been achieved either by subgingival irrigation or by incorporating the drug into different devices for insertion into periodontal pockets. Many drugs like chlorhexidine, tetracycline are tried as mouth rinses in the treatment of periodontal diseases. In-spite of its superior effects, chlorhexidine does not reach the periodontal pocket when administered as mouth rinse. Subgingival irrigation of antimicrobial involves local drug delivery but not controlled release.

Local drug delivery devices are of two types. In the first type, the drug delivery system is designed to deliver agent locally in the periodontal pocket but without any mechanism to retain therapeutic levels for a prolonged period of time. Such device generally exhibits exponential increase and decrease in drug concentration at the

site[19].

Second type is the controlled release local drug delivery devices which may secure antimicrobial effect for a prolonged period of time at the diseased site, than that can be achieved by systemic or local topical applications and also by passes the systemic complications[20].

The controlled release delivery of antimicrobials directly into periodontal pocket has received greatest interest and appears to hold some promise in periodontal therapy. These delivery systems are produced by immobilizing antibiotic and antimicrobial agents with a carrier substance to provide controlled local release.

Local antimicrobial therapy in periodontitis involve direct placement of antimicrobial agents into subgingival sites minimizing the impact of the agents on non oral body sites. Local antimicrobial agents may be personally applied as a part of home care oral hygiene regimens and/or professionally applied as part of clinic based treatment procedures. Local antimicrobial therapy in periodontitis may be further classified as providing either non-sustained or sustained subgingival drug delivery. Non-sustained subgingival drug delivery provides high pocket concentrations of the antimicrobial agent over an extended time period within periodontal pockets. Controlled drug release can be provided with subgingival irrigation of an agent intrinsically substantive for both tooth surfaces or pocket placement of commercial antimicrobial fibers, gel or films. The potential application of these new concepts to periodontology and the treatment of periodontal infections was championed and developed into a viable concept primarily by Dr. J. Max Goodson[24].

Table 1.2:- Susceptibility of infective microorganisms to the major antimicrobials.

	Pen Ciln e	Oxacilln	Amoxicillin	Cef/1	Macolides	Clindamycin	Metronidazole	Tetracycline	Levofloxacin	Gatifloxacin
Aerobic bacteria										
Streptococc-us Group A	+	+	+	+	+	+	0	A±	+	+
Streptococc-us spp	+	+	+	+	+	+	0	+	+	+
Staphylococ-cus spp	0	+	+	+	+	+	0	A±	A±	+
Capnocytop-haa spp	+	+	+	A±	A±	+	0	+	+	+
Eikenella spp	+	0	+	0	A±	0	0	+	+	+
Anaerobic bacteria										
Peptostrepto-coccus spp	+	+	+	+	+	+	A±	A±	A±	+
Actinomyce-s spp	+	+	+	+	+	+	+	A±	+	+
Prevotella spp	+	A±	+	0	0	+	+	A±	A±	A±
Porphyromo-ns spp	A ±	A±	+	0	0	+	+	A±	0	A±
Fusobaceriu-m spp	A ±	A±	+	0	A±	+	+	A±	0	A±
Bacteoride	A ±	A±	+	0	0	+	+	A±	0	A±

A ±: Higher activity towards organisms

+: Moderate activity

0: No activity

Cef/1 – first generation cephalosporins

LOCAL ANTIMICROBIAL AGENT POCKET DELIVERY

Advantages of Local Controlled Release Devices:

- It is useful in controlling and monitoring the desired drug levels in the site.
- It allows local modification of tissue permeability inhibit protease activity or decrease immunogenic response.
- It is a useful means of delivery of drug to the oral cavity that is not absorbed into the gastro intestinal system (e.g. chlorhexidine).
- It bypasses hepatic first pass metabolism, therapy offering a greater bioavailability and reduction in dosage[21].
- The drug escapes the acidic environment of the stomach.
- Therapeutic serum concentrations of the drug can be achieved more rapidly
- Sustained release drugs offer a onetime application has an advantage over repeated application.

Disadvantages:

- There is difficulty in placing therapeutic concentrations of antimicrobial agent into deeper parts of periodontal pockets and frication lesions.
- Personal application of antimicrobial agent by patient's lack of adequate manual dexterity, limited understanding of periodontal anatomy and poor compliance and performance with recommended procedures.
- The task of professionally applying local antimicrobial agent in periodontitis patients with numerous advanced lesions distributed throughout their mouth is time consuming and labor intensive.

- Antimicrobial agents locally applied into periodontal pockets do not markedly affect periodontal pathogens residing within adjacent gingival connective tissues and on extra-pocket oral surfaces, which increases the risk of later reinfection and disease recurrence in treated areas.

Success of any drug system designed to target periodontal infection depends upon its ability to deliver the anti-microbial agent to the base of pocket at a bacteriostatic or bactericidal concentration. It must also facilitate retention of medicament long enough to ensure an efficacious result. Commonly used techniques[22] to administer antimicrobial with regard to attaining these criteria are compared in Table 1. 3.

TableNo.1.3: Comparison of Drug Delivery Systems for Management of Periodontitis.

	Mouth Rinse	Subgingival Irrigation	Systemic delivery	Controlled delivery
Reaches site of disease and activity	Poor	Good	Good	Good
Adequate drug concentration	Good	Good	Fair	Good
Adequate duration of therapy	Poor	Poor	Fair	Good

Local antimicrobial therapy in periodontitis involves direct placement of an antimicrobial agent(s) in to subgingival sites, minimizing the impact of the agent(s) on non oral body sites.

Types Of Local Antimicrobial Agent Therapy In Periodontal Diseases Include.

1. Personally applied (in-patient home self care)

- Non-sustained subgingival drug delivery (home oral irrigation)
- Sustained subgingival drug delivery (Not developed to date)

2. Professionally applied (in dental office)

- Non sustained subgingival drug delivery (Professional pocket irrigation)
- Sustained subgingival drug delivery (Controlled release devices)

Controlled drug delivery systems are designed to deliver the drug slowly for prolonged periods for sustaining drug action. These dosage forms are commonly referred as sustained-release, controlled-release, timed-release and slow release. These technologies assure therapeutic concentrations of the antimicrobial agents in the subgingival area, at least for 3 days following a single application. Most controlled release devices for periodontal application are polymer based, with diffusion of drugs across a rate controlling membrane.

CONTROLLED RELEASE LOCAL DELIVERY DEVICES

These devices employ the controlled release technologies to assure therapeutic concentrations of the antimicrobial in the sub gingival area for at least 3 days following a single application.

Different Types of Controlled Release Local Delivery Devices Fall Into Following Groups

Reservoir devices (membrane diffusion systems)

This includes dialysis tubing 3-5 mm long, 0.2 mm wide containing a core of drug

solution. This is left in the pocket for a week. Reservoir devices that lack rate control include hollow fibers, gels and dialysis tubing. These systems tend to release chemotherapeutic agents very quickly and only marginally qualify as sustained release devices. The problems associated with these devices are, irritation of the pocket, premature loss from the pocket and rapid drug release[23].

Monolithic devices

Bio absorbable, biodegradable materials can be left in situ. This eliminates the risk of disturbing a site after therapy. The controlled release local delivery devices (monolithic) are usually polymers (films/fibers) containing homogeneously dissolved or dispersed drug.

In these devices, the drug is dispersed in a solid polymer matrix, Examples include acrylic strips and ethylene vinyl acetate (EVA) fibers. Acrylic strips are typically 0-2 mm thick. Treatment is carried out over 2 - 4 weeks, with a replacement of new strips inserted each week. Drug release occurs over a period of 10 - 14 days. Strips tend to be lost from the pocket. This can be avoided by applying periodontal dressing. Other monolithic devices include strips made of ethyl cellulose (EC), poly ethylene glycol (PEG), Hydroxy propyl methyl cellulose (HPMC) and cross linked collagen films[23].

The two basic types are:

1. Fixed monolithic devices: Where the polymer maintains its integrity as the drug is lost.
2. Erodible monolithic devices: Which breaks up as the drug is released.

If the drug is present as dispersion, the release will be proportional to the square root of time and if the drug is present only dissolved in the constant release rates, then decay exponentially. It is possible to obtain more constant release rates by increasing

the concentration of drug towards the center of the monolithic core to produce a laminated device. The various polymers used for the monolithic device are ethylene vinyl acetate co-polymer, ethyl cellulose, methyl methacrylate, poly ethylene-2 hydroxy ethyl methylacrylate, poly (ortho esters), atellocollagen etc.

The device should be designed for rapid insertion and to minimize the pain and discomfort to the patient. The monolithic devices can be prepared conveniently by using simple polymer fabrication techniques.

Melt fabrication Technique

Films can be produced by extrusion in to thin film. Another process known as calendaring, where the polymer is squeezed between heated rollers to form film[23].

Solution casting

The polymer is dissolved in a suitable solvent to form a viscous solution, which is then spread on flat non-adhesive surface and the solvent is allowed to evaporate. The resultant film is peeled from the surface.

Polymerization In situ

A liquid polymer or pre polymerized inside a suitable mould. The release from monolithic devices depends on diffusion of drug through matrix. By manipulating the system, selecting the ideal polymer, adjusting the cross-linking, fillers, plasticizers and by using co-polymers, release of some low molecular drug can be achieved.For an antimicrobial agent to be successful the pathogen must be known, it must be susceptible to the drug. It should not readily develop resistance for an adequate period of time. Also the drug should have little or no side effects [24].

MECHANISMS OF DRUG RELEASE

The release of drug from a localized drug delivery system can be achieved by the following mechanisms.

1. Pure diffusion
2. Chemical reactions
3. Counter-current diffusion
4. Externally imposed controls

Controlled release polymeric systems can be classified on the basis of the mechanism controlling the release of the incorporated drug.

DIFFUSION CONTROLLED SYSTEMS

a) Reservoir systems

In these systems, core of the drug is surrounded by a swollen or non swollen polymer film and diffusion of the drug through the polymer is the rate limiting step. Reservoir devices are also of two types. Reservoir without a rate controlling system. Reservoirs that lack rate control include hollow fibers, gels and dialysis tubing. Reservoirs with rate controlling systems include erodible polymeric matrices, polymer membranes, monolithic matrices and coated particles[25].

b) Matrix systems

In these systems, the drug in uniformly distributed throughout a solid polymer. The drug diffusion through the polymer matrix is the rate limiting step.

CHEMICALLY CONTROLLED SYSTEMS

a) Bioerodable System:

In these systems, a drug is uniformly distributed throughout the polymer, and the drug is released by diffusion as the polymer phase decreases with time. As the polymer surrounding the drug resorbed, the drug escapes.

b) Pendant Chain System:

In these systems, the drug is chemically bound to a polymer back bone and drug release occurs via hydrolytic or enzymatic cleavage.

2.NEED FOR THE STUDY

In conventional mode of administration many drugs do not reach target areas in the body in sufficient concentration because of premature inactivation and excretion. This problem can be overcome by administering the drug directly to the intended site of action with lesser dose. In recent years, novel drug delivery systems are becoming popular as pharmaceutical technologists today are able to provide the drug delivery systems with very precise control over drug release for a prolonged period of time eliminating the need for frequent dosing and minimizing side effects, thereby increasing patient compliance and comfort.

The most common dental infections include dental carries, dental alveolar infections, gingivitis, periodontitis, deep facial face infections and osteomyelities. If untreated, dental infections spread and contribute to polymicrobial infections at other sites. Among the above mentioned dental infections periodontitis, a common cause of tooth lose is one of the chronic infections of oral cavity, primarily caused by gram positive, gram negative and anaerobic bacteria that reside in sublingual area.

The systemic administration of drugs for treating dental conditions can reach the systemic circulation via serum and hence the microorganisms at the depth of the periodontal sites and also the possible organisms residing within gingiva and capable of eliminating pathogens not only from periodontal lesion but from oral cavity as well.

The greatest disadvantage of systemic administration of antimicrobials for periodontitis is that, the drug is diluted several thousand folds before it reaches the site and exposes the rest of the body to potential side effects. In some patients, loss of

attachments develops very rapidly and continuously in spite of proper therapy.

A site specific system aims at delivering the therapeutic agent at sufficient levels inside the pocket and at the same time minimizing the side effects associated with systemic drug

3.AIMS&OBJECTIVES

The purpose of the present investigation is to develop the polymeric inserts (chitosan) containing different concentration of the anti-bacterial agent Doxycycline for local controlled drug delivery into periodontal pockets. Apart from this, an investigational study is planned to prolong the drug release for more number of days by formulating in to multilayer (polymer) inserts.

Specific objectives of the present investigation are as follows,

1. To develop a chitosan single layer with different concentration of doxycycline hyclate (10%, 20% and 30%).
2. To develop a chitosan multilayer inserts containing doxycycline hyclate.
3. Evaluation of prepared inserts for physical characteristics such as

- ✓ Thickness and Weight Variation
- ✓ Folding Endurance
- ✓ Percentage Moisture Loss
- ✓ Percentage Moisture Absorption
- ✓ Drug Content Uniformity
- ✓ Tensile Strength
- ✓ Differential Scanning Calorimetry
- ✓ Fourier Transfer – Infrared Spectroscopy(FT-IR)
- ✓ Water Uptake and Swelling Index

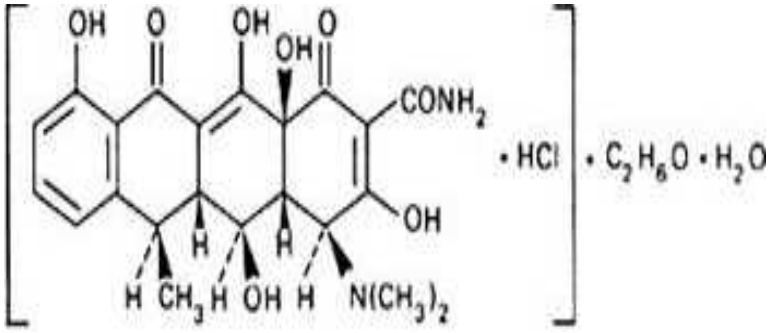
4. To study static dissolution pattern of the drug dosage forms.
5. *In-vitro* antibacterial activity.
6. Stability studies at different temperature as per ICH guidelines.

4. DRUG PROFILE

DOXYCYLINE HYCLATE:

Chemical Name:-(4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)1,4,4a,5,5a,6, 11,12aoctahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacene carboxamide monohydrochloride, compound with ethyl alcohol (2:1), mono-hydrate; [4*S*-(4α,4aα,5α,5aα,6α, 12aα)]-4-(dimethylamino)1,4,4a,5,5a,6,11,12a-octahydro-3,5,10, 12, 12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacene carboxamide monohydrochloride[26].

- **Structure:-**



- **Molecular formula :-** (C22H24N2O8 · HCl)2 · C2H6O ·H2O
- **Molecular weight :-** 444.435 g/mol
- **Melting point :-** 201-202 ^{0}C
- **Physical form :-** yellow crystalline powder
- **Solubility :-** Freely soluble in water acetic acid, 6.6 pH buffer and acetic acid
- **Pharmacology:**

Tetracyclines are readily absorbed and are bound to plasma proteins in varying degree. They are concentrated by the liver in the bile, and excreted in the urine and

feces at high concentrations and in a biologically active form. Doxycycline is virtually completely absorbed after oral administration.Following a 200 mg dose, normal adult volunteers averaged peak serum levels of 2.6mcg/ml of doxycycline at 2 hours decreasing to 1. 45 mcg/ml at 24 hours.

Excretion of doxycycline by the kidney is about 40%/72 hours in individuals with normal function (creatinine clearance about 75 ml/min). This percentage excretion may fall as low as 15%/72 hours in individuals with severe renal insufficiency (creatinine clearance below 10mL/min). Studies have shown no significant difference in serum half-life of Doxycycline (range 18-22 hours) in individuals with normal and severely impaired renal function.

Hemodialysis does not alter serum half-life. Results of animal studies indicate that tetracycline cross the placenta and are found in fetal tissues[27].

- **Mechanism of action:**

Doxycycline, like minocycline, is lipophilic and can pass through the lipid bilayer of bacteria. Doxycycline reversibly binds to the 30 S ribosomal subunits and possibly the 50S ribosomal subunit(s), blocking the binding of aminoacyl tRNA to the mRNA and inhibiting bacterial protein synthesis. Doxycycline prevents the normal function of the apicoplast of Plasmodium falciparum, a malaria causing organism[27].

- **Pharmacokinetics:**

1	Absorption	Complete, no interference by food
2	Protein binding	High
3	Bioavailability	90-95 %
4	Plasma half life	18-22 hrs
5	Metabolism	Hepatic
6	Excretion	Urine, feces

- **Elimination: Half-life :-** 18-22 hours

- **Drug interactions:**

If used in conjunction with the anesthetic methoxyflurane there can be severe or fatal kidney damage. May interact with anticoagulants and its effectiveness is lowered by over the counter antacids and bismuth subsalicylate, barbiturates, the anticonvulsants carbamazepine and phenytoin. Tetracycline blocks the action of bactericidal antibiotics such as penicillin and should not be given in combination with such antibiotics.

- **Contraindications:**

Doxycycline and other tetracyclines must not be given to women during the last half of pregnancy or to children under the age of eight. It can affect development of the skeleton in the fetus and can cause discoloration and malformation of the teeth. Any demonstrated sensitivity to tetracycline contraindicates use. Some people may show increased sensitivity to sunlight[28].

- **Side effects:**

Losses of appetite, nausea, vomiting and diarrhea have been observed. Hypersensitivity, anaphylaxis, and exacerbation of immune disorders have been seen. Hemolytic anemia and other disturbances of the white cell population of the blood are known[28].

5.POLYMER PROFILE

CHITOSAN

- **Synonyms :-** 2-Amino-2-deoxy-(1,4)-β-D-glucopyranan; deacetylated chitin; deacetylchitin; β-1,4-poly-Dglucosamine; poly-D-glucosamine; poly-(1,4-β-D-glucopyranosamine).
- **Chemical Name and CAS Registry Number :-** Poly-β-(1,4)-2-Amino-2-deoxy-D-glucose [9012-76-4]
- **Molecular weight :-** 10000-1000000
- **Origin of Chitosan**

One reason why chitosan has become of interest is undoubtedly because it can be obtained from natural sources that are abundant and renewable. Chitosan is prepared from chitin, the polymer second most abundant in nature after cellulose (Roberts, 1992). Chitin is the primary structural component of the outer skeletons of crustaceans, and of many other species such as molluscs, insects and fungi. The role played by chitin is similar to the roles played by cellulose in plants and collagen in higher animals. It is a reinforcing material, which occurs in three polymorphic forms, and chitin. Where hardness in needed chitin is found, where flexibility is required and chitin occurs. Chitin is inert in aqueous environment. This property limits the use of chitin as such. Chitosan is prepared from chitin to obtain a more reactive polymer[29].

Physical Properties

- Particle size < 30nm
- Density 1.35 – 1.49 g/cc
- Solubility: Insoluble in water but soluble in acids.

- **pH (1% w/v solution) :** 4.0–6.0

❖ **Solubility:**

Sparingly soluble in water; practically insoluble in ethanol (95%), other organic solvents, neutral or alkali solutions at pH above approximately 6.5.

❖ **Description:**

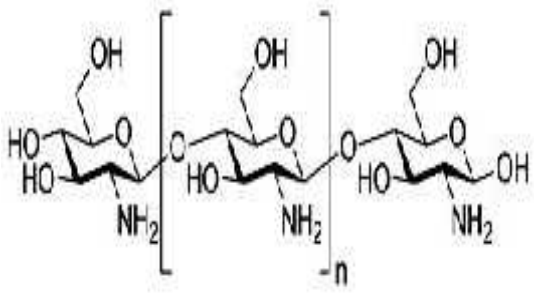
Chitosan occurs as odorless, white or creamy-white powder or flakes. Fiber formation is quite common during precipitation and the chitosan may look 'cottonlike'.

❖ **Functional Category:**

Coating agent, disintegrate, film-forming agent, mucoadhesive, tablet binder, viscosity increasing agent.

❖ **Structure:**

Chitosan is a linear polysaccharide consisting of ß (1-4)-linked 2-amino- 2-deoxy-D-glucose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (N-acetylglucosamine) units. The structure of chitosan is very similar to that of cellulose (made up of ß (1-4)-linked D-glucose units), in which there are hydroxyl groups at C2 positions of the glucose rings[30].



❖ **Mechanism of action:**

Chitosan formulations can easily be prepared using conventional tableting or granulating methods. The simplest way of achieving slow release of drugs by means of chitosan is to employ it as a gel-forming excipient in matrix-type formulations.

Retardant effects of chitosan on drug release were noticed as early as the 1980s. Chitosan was found to decrease rates of release of drugs from tablets during dissolution tests at acidic and slightly acidic pH levels (Kawashima et al., 1985; Acartürk, 1989). However, Akbua (1993a), who carried out studies at higher pH levels (pH 7.4), reported that chitosan exhibited no slow-release properties[29, 30].

❖ **Features:**

The Chitosan (CS) is known to be non-toxic and odorless. Chitosan has received considerable attention as a possible pharmaceutical excipient in recent decades Chitosan is an excellent polymer to be used for buccal delivery because it has muco/bioadhesive properties and can act as an absorption enhancer. Chitosan gives prolonged release of the drug in the buccal cavity improving the antimicrobial activity of the drug. Chitosan periodontal film with drug incorporated have antimicrobial activity due to the chitosan. The buccal bilayered devices (bilaminated films,) using a mixture of drugs and chitosan, with or without anionic crosslinking polymers has promising potential for use in controlled delivery in the oral cavity[30].

❖ **Properties of Chitosan:**

Cationic Polyamine

Chitosan has highly charged density at pH < 6.5 i.e. one charge per glycosamine unit. The ionic character along with reactive functional group in chitosan has made it a suitable polymer for utilization in controlled release technology. The molecular weight of chitosan varies from 50 KDa to 2000 KDa[29].

Amenable to Chemical Modifications

Chitosan is linear polyamine where amino groups are readily available for chemical reaction and salt formation with acids. The possibility of amino groups suggests that

chitosan could be used to formulate therapeutic system where the drug release kinetics depends on erosion pathway of polymeric network due to hydrolysis of cross-linking bonds.

Flocculation and Mucoadhesive Properties

Chitosan formulations exhibit ease of delivery [30, 31] a good retention at the application site, and a controlled release of the drug. Chitosan is shown to have an antimicrobial activity against *P. gingivalis* and higher activity with high molecular weight chitosan.The combination of chitosan with chlorhexidin showed a significant higher activity, when compared to that of chlorhexidine alone, which would provide chlorhexidine application at lower concentrations thus avoiding its unwanted side effects. Chitosan films and gels seem to be promising delivery systems for local therapy of periodontal diseases with its bioadhesive property and antimicrobial activity [32].

Biological Properties

Its biological properties include non toxicity, biodegradability and biocompatibility.

N-acyl chitosan showed the best blood biocompatibility due to increase of surface hydrophilicity and induction in hydrophilic and hydrophobic properties at the surface.

CLINICAL APPLICATION

Wound Healing

Chitin and chitosan are effective wound healing accelerators, in both animal and human tests, the surgical adjuncts of regenerated chitin are physiologically compatible, bioabsorbable and effective wound healing accelerators. N-Carboxybutyl

chitosan was studied in wound healing in rabbits; where complete re-epithelialization was observed with all the epithelial layers represented on 30th day of treatment, an interesting role in tissue reconstruction. Chitosan has also been used to prepare bandages for wound healing.

The physiological compatibility of chitin with living tissues, combined with its ability to form readily sulfate esters which are non thrombogenic appears to make chitin a most promising candidate for prosthetic structural devices of any desired shape or sizes[31, 33].

Haemostatic Agent

Chitosan solution formed a coagulum in contact with whole blood, where the possible mechanism appeared to be a reaction between red cell membrane and the chitosan solution leading to cross linkage or re-polymerisation. Chitosan is an effective and adequate homeostatic agent even under the most severe conditions of anticoagulation. Introduction of uronic acid carboxyl groups increases the anticoagulant activity of sulfacted chitosan.

Application in Dentistry

Many advantages are attainable by use of chitosan in periodontology such as decrease subjective symptomatology, good homeostatic action, and delayed release of antibiotics, wound healing acceleration and better condition for asepsis. Chitosan has been used in 24 patients all of which recovered completely. No allergic reactions or infections took place. Chitosan could be used as transparent membrane or preferably as a thin powder soaked in antibiotic solution, it accelerated wound healing, promoted regular fibrin formation and favored the epithelialization.

6. REVIEW OF LITERATURE

REVIEW OF PAST STUDY DONE ON DOXCYCLINE DRUG

- **Gaurav Tiwari1 et al.,** release studies of metronidazole and doxycycline from polycaprolactone films prepared by solvent evaporation. metronidazole and doxycycline with different antibacterial spectra are proposed to be formulated as combination therapy to have a broader antibacterial therapy, which is effective against both aerobic and anaerobic periodontal microflora. Simultaneous use of metronidazole and doxycycline is effective against wide range of periodontal pathogens. Metronidazole and doxycycline release from polycaprolactone films was zero-order after an initial period. Significant burst effects were observed with metronidazole and doxycycline (5, 10% w/w) [34].
- **Mohammed M Al-Abdaly, et al.,** studied the effects of topical application of Atridox (Doxycycline gel) in management of chronic periodontitis. This study was carried out on 15 patients (aged 25-55) with chronic periodontitis. They were received scaling and root planning (SRP) alone in one side and SRP plus Atridox (Doxycycline gel) in other side. Each individual was subjected to the following measurements. Evaluation of the clinical parameters pre and post treatment to detect the outcome of the treatment modality and Denial plaque samples initially and at 3, 6, 9 and 12 months were obtained for microbiological evaluation. Atridox (Doxycycline gel) delivered locally into periodontal disease sites reduced all subgingival bacteria. Both treatment and modality led to a highly statistically significant reduction in microbiological counts as well as clinical parameters applied. No clinical relevant side effects were observed[35].
- **Thawatchai Phaechamud et al.,** studied the chitosan sponges loaded with Doxycycline hyclate and their antibacterial activities. The pore density of

chitosan sponge prepared with freeze drying technique was increased as the higher concentration of chitosan solution was used. The sponge prepared from 10% w/w of the chitosan solution and crosslinking with glutaraldehyde solution was utilized for loading with doxycycline hyclate. The drug release and sustainable antibacterial activity of fabricated sponge were assessed using dissolution test and agar diffusion test, respectively. Drug release from non-crosslinked sponge into phosphate buffer pH7.4 was slower than that from crosslinked sponge since the former could absorb the medium and form gel to retard the initial drug diffusion. Sustainable antibacterial activity of developed sponge was evident against *S. aureus* and *E. coli*. Results indicated that the *in vitro* release profile and antibacterial efficiency of doxycycline hyclate could be sustained using chitosan sponge[36].

- **N. Damodharan et al.,** developed delayed release doxycycline tablets for targeting to small intestine tablets were prepared by wet granulation method and enteric coating of tablets (conventional standard coating technique). Using pH depended polymers like Eudragit and HPMC Phthalate. Preformulation studies like angle of repose, bulk density, tapped density, porosity, Carr's index, Hausner's ratio were performed. Six batches (F1 to F6) were formulated and evaluated for hardness, friability, weight variation, drug content, disintegration and *in-vitro* dissolution. Among the six batches, batch F4 was showed 94% drug release and was considered as best formulation[37].
- **S.Shanmuganathan N et al.,** prepared Doxycycline loaded

chitosan microspheres were developed using a novel water-in-oil emulsion technique, involving oil phase ionic gelation. Microspheres were prepared by using 6% v/v of chitosan (3% w/v in acetic acid), soya oil–n-octanol oil mixture (1:2 v/v) as continuous phase and 5% span 80 as emulsifier. Doxycycline was entrapped by equilibrium swelling method with 8.4% total entrapment. The drug-loaded spheres were spherical with smooth surface morphology. The MTT assay showed that doxycycline-loaded microspheres were able to improve the percentage cell viability in comparison to the pure drug. In vitro release studies showed that a burst release of 42% in 6 h was achieved and maintained an equilibrium concentration of 72% in 24 h. Assessment of antibacterial activity showed that doxycycline was able to exhibit a minimum microbicidal concentration (MIC) of 16.5, 17.4, 11.2 and 98.3µg against ***Klebsiella pneumoniae*** **(ATCC15380),** ***Escherichiacoli*** **(ATCC25922),** ***Staphylo coccusaureus*** **(ATCC9144) and** ***Pseudomonas aeruginosa*** **(ATCC 25619),** respectively. Gelatin zymography studies revealed that it could inhibit MMP 2 and MMP9 at sub-antimicrobial concentration. The present investigation provides scope for using doxycycline-loaded chitosan microspheres for healing infected wounds[39].

- **MarcoAntonio Botelho etal.,** aim of this study was to test the efficacy of a locally applied 8.5% nanostructure doxycycline (DOX) gel in preventing alveolar bone loss in experimental periodontal disease (EPD) in rats by using the tapping mode atomic force microscopy (AFM). Material and method EPD was induced in 24 Wister rats. Animals were treated with the doxycycline gel topically, immediately after EPD induction, and 3 times a day during 11 days. Four groups

(n=6) were formed as follows: each group (animals not subjected to EPD nor treated); non-treated (NT) group (animals subjected to EPD, but not treated); vehicle gel (VG) group (animals subjected to EPD and treated with topical gel vehicle); and DOX group (test group) animals subjected to EPD and treated with the 8.5% DOX gel. In order to investigate topographical changes in histological sections, a novel simple method was used for sample preparation, by etching sections from paraffin-embedded specimens with xylol. Results: comparing the AFM images, several grooves were observed on the surface of the alveolar bone and other periodontal structures in the NT and VG groups, with significantly greater depths when compared to the DOX group[40].

- **Narayana Charyulu et al.,** developed strips of chitosan polymer 2% containing ciprofloxacin Hcl, norfloxacin and doxycycline by solution casting method using 1% acetic acid as casting solvent. Macroscopical features revealed that drugs were dissolved in the polymer. The DSC and UV scan studies confirmed the absence of any chemical interaction between the drugs and polymer. Short term stability studies were carried out at different temperature. The static dissolution showed a burst release effect initially followed by a progressive fall in the release. Mass balance studies did not deviate by more than 3%. It was observed from the above studies that the chitosan strips have an ability to sustain the release of drugs and more useful as slow release device for periodontal disease[41.]
- **Heba A. Gad et al.,** formulated *in situ* implants containing doxycycline hydrochloride and/or secnidazole that could be used in the treatment of periodontitis by direct periodontal intrapocket administration. Biodegradable polymers [poly (lactide) (PLA) and poly (lactide-co-glycolide) (PLGA)], each polymer in two concentrations 25%w/w, 35%w/w were used to formulate the

insitu implants. The rheological behavior, *in vitro* drug release and the antimicrobial activity of the prepared implants were evaluated. Increasing the concentration of each polymer increases the viscosity and decreases the percent of the drugs released after 24 h. PLA implants showed a slower drugs release rate an PLGA implants in which the implants composed of 25% PLGA showed the fastest drugs release. The in vitro drug release and antimicrobial activity results were compared with results of Atridox®. Results revealed that the pharmaceutical formulation based on 25% PLGA containing secnidazole and doxycycline hydrochloride has promising activity in treating periodontitis in comparison with Atridox[42].

PAST WORK DONE ON POLYMER (CHITOSAN)

- **Shankraiah et al.** were prepared Flexible films of sparfloxacin for periodontitis treatment for easy insertion into periodontal pockets. The optimum concentration of chitosan used for the preparation of strips was found to be 2% w/v, because at this concentration the strips were flexible and easily removable from the die. An optimum concentration of drug to be loaded was found to be 30% w/v to the polymer or less than that. For the present investigation, chitosan strips containing sparfloxacin with three different concentrations, i.e.10, 20, and 30% to the weight of the polymer, were prepared using the solvent casting method. All the formulations were found to contain almost uniform quantity of drug as per content uniformity studies, indicating reproducibility of the technique[43].
- **Dan Mei et al.,** The objective of this work was to assess and compare the absorption promoting effect of different molecular-weight chitosans, trimethyl chitosans and thiolated chitosans for intranasal absorption of 2,3,5,6-tetramethylpyrazine phosphate (TMPP). An in situ nasal perfusion technique in rats was utilized to test the rate and extent of TMPP absorption in situ. *In vivo* studies were carried out in rats and the pharmacokinetic parameters were calculated and compared with that of intravenous injection. All the chitosan derivatives investigated could enhance the intranasal absorption of TMPP significantly. However, thiolation could not improve the absorption-enhancing capacity of chitosan remarkably even when the thiolation ratio was as high as 152 lmol/g. In contrast, trimethylated chitosan exhibited stronger absorption-enhancing ability than the homopolymer chitosan. The permeation enhancing effect of chitosan increased with increasing molecular weight up to Mw 100 kDa. In vivo studies indicated that chitosan 100 kDa and TMC 50 kDa had comparable

absorption-enhancing effect but chitosan 100 kDa functioned for more than 120 min versus 90 min for TMC. A good correlation was found between the in situ absorption data and plasma concentration in vivo for the polymers investigated. This study demonstrated that both chitosan structural features and chitosan molecular weight play a key role on promoting the intranasal absorption of TMPP. Taking safety reason into account, chitosan 100 kDa is the most promising as an intranasal absorption enhancer.[44].

- **Barat R et al.,** chitosan based metronidazole inserts were fabricated by the casting method and characterized with respect to mass and thickness uniformity, metronidazole loading and *in vitro* metronidazole release kinetics. The fabricated inserts exhibited satisfactory physical characteristics. The mass of inserts was in the range of 5.63 ± 0.42 to 6.04±0.89 mg. The thickness ranged from 0.46 ±0.06 to 0.49 ±0.08 mm. Metronidazole loading was in the range of 0.98 ±0.09 to 1.07±0.07 mg except for batch CM3 with MZ loading of 2.01V± 0.08. The inserts exhibited an initial burst release at the end of 24 hr, irrespective of drug to polymer ratio plasticizer content or cross linked. However further drug release was sustained over the next 6 days, cross linking to 10% (m/m) of glutaraldehyde inhibited the burst release by~30 % and increased the mean dissolution time (MDT) from 0.67 to 8.59 days. The decrease in drug release was a result of reduced permeability of chitosan due to cross linking[45].
- **Senel S, IkicI G.et.al.,** developed the formulation containing chitosan for local delivery of chlorhexidine gluconate (chx) to the oral cavity. Gels at (1 or 2% concentration) or film forms of chitosan were prepared containing 0.1-0.2% chx (chlorhexidine gluconate) and there *in vitro* release properties were studied. The

antifungal activity of chitosan itself as well as their various formulations containing chlorhexidine gluconate (chx) was also examined. Release of chlorhexidine gluconate (chx) from gels was maintained for 3hr. A prolong release was observed with film formulation. No lag time was observed in release of chx from either gels or films. The highest antifungal activity was obtained with 2% chitosan gel containing 0.1% chlorhexidine gluconate (chx.)[46].

- **Amal Hassan El-Kamel et al**., developed a local, oral mucoadhesive metronidazole benzoate (MET) delivery system that can be applied and removed by the patient for the treatment of periodontal diseases. Mucoadhesive micromatricial chitosan poly(ε-caprolactone) (CH/PCL) films and chitosan films were prepared. Thermal behavior, morphology, and particle size measurements were used to evaluate the prepared films. The effect of different molar masses of CH and different ratios of medium M.wt.molar mass chitosan (MCH): PCL on water absorption, *in vitro* bioadhesion, mechanical properties, and *in vitro* drug release was examined[47].
- **Ritu B. Dixit et al.,** developed cefadroxil drug loaded biopolymeric films of chitosan-furfural schiff base were prepared by reacting chitosan with furfural in presence of acetic acid and perchloric acid respectively for the external use. Prepared films were evaluated for their strength, swelling index, thickness, drug content, uniformity, tensile strength, percent elongation, FTIR spectral analysis and SEM. The results of *in vitro* diffusion studies revealed that the films exhibited enhanced drug diffusion as compared to the films prepared using untreated chitosan. The films also demonstrated well to moderate antibacterial activities against selective gram positive and gram negative bacteria[48].

- **Mohammed Gulzar Ahmed et al,** developed chitosan strips containing Gatifloxacin (10%, 20% and 30% to the weight of polymer) were prepared by solution casting method using 1% v/v acetic acid in water. Further strips containing 30% gatifloxacin were cross-linked by exposing to the vapours of 2% v/v glutaraldehyde in water intended to extend the release. The prepared films were evaluated for their thickness, content uniformity, weight variation, tensile strength, hardness and *in-vitro* dissolution. The average weight and thickness of both the cross linked and uncross-linked strips were uniform. There was a reduction in the tensile strength and increase in hardness when the films were cross-linked. Static dissolution studies showed a burst release initially followed by a progressive fall in the release of the drug and extended up to 19 days once the strips were cross-linked. Release kinetics of gatifloxacin from chitosan strips followed the higuchi's diffusional model and also showed zero order release profile[49].

- **S.Sangeetha et al.,** Nanoparticle made up of biodegradable carrier such as chitosan have advantage of providing stearic interference in the systemic circulation. Cytarabine nanospheres were prepared by ionic gelation method with an objective of improving its intracellular targeting and thereby targeting the cancer cells. The average particle size determined through SEM was found to be 466.45 ± 5.32 nm. The average drug loading was found to be 62% for the batch loaded with 1.50 mg/ml of drug when compared to other batches. The cumulative percentage of drug release from all the drug loaded batches at 18 hours was found to be in the range of 86.73 % to 93.50%.Among the four batches of nanoparticles formulated the batch containing 1.50mg of drug/ml of polymer showed the highest of about 93.50% of release. Release of drug from the matrix was by non-

Fickian analomous diffusion mechanism. *Invivo* bio distribution studies showed that the Cytarabine in the form of nanoparticles was having a greater bio distribution when compared to free drug in different organs like liver, spleen, lungs and kidney[50].

- **K. Bansal et al.,** develop satranidazole-containing mucoadhesive gel for the treatment of periodontitis. Different mucoadhesive gels were prepared, using various gelling agents like sodium carboxymethylcellulose (SCMC), poloxamer 407, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl methyl cellulose, and the mucoadhesive polymer carbopol 934P. The selected formulations were studied for different mechanical properties, such as mucoadhesive strength, hardness, compressibility, adhesiveness, and cohesiveness through texture profile analyzer. *In vitro* satranidazole release from the prepared formulations was also determined and compared with marketed preparation of metronidazole. The formulation SC30 (Containing SCMC 3% w/v) showed maximum mucoadhesive strength (167.72 ± 3.76 g) and adhesiveness (−46.23 ± 0.34 Nmm), with low hardness (9.81 ± 0.04 N) and compressibility (40.05 ± 0.48 Nmm) and moderate cohesiveness (0.87 ± 0.01). SC30 formulation exhibited long-term release. Thus, SC30 gel was evaluated for its clinical effectiveness along with marketed metronidazole gel. At the end of the study (42 days of clinical studies), both formulations were found to significantly reduce the probing depth, plaque index, gingival index, calculus criteria, and bleeding index. However, the SC30 gel was more effective in reducing the above parameters than marketed metronidazole gel. This study confirmed the acceptability and effectiveness of satranidazole gel for treatment of

periodontitis.[51].

- **Mohammed Gulzar Ahmed et al.,** prepared Chitosan films containing tetracycline in three different concentrations were prepared by the solution casting method, using 1% v/v acetic acid solution for therapy of periodontal disease. The prepared films were evaluated for various properties. The stability studies did not show any significant changes. Static dissolution studies showed a burst release initially followed by a progressive fall in the release of the drug, and also showed extended release when cross-linking was attempted. The *in-vitro* release kinetics of tetracycline followed the zero order patterns[52].

REVIEW STUDY DONE ON LOCAL DRUG DELIVERY SYSTEM

- **Shankraiah et al.,** developed a sustained release device containing ornidazole for insertion within periodontal pockets. Cast films of ethyl cellulose with dibutyl phthalate as plasticizer, containing ornidazole were prepared. The films were evaluated for thickness, folding endurance, weight variation, content uniformity, tensile strength, *in vitro* antibacterial activity and *in vitro* release study. The release data obtained were subjected for release kinetics study. The study revealed that drug release was found to be diffusion controlled with sustained release of ornidazole over a period of nine days within the periodontal pocket[53].

- **G.L.Prabhushankar et al.,** developed a levofloxacin dental films for periodontitis between levofloxacin and polymers.The films were evaluated for their thickness uniformity, folding endurance, weight uniformity, content uniformity, tensile strength, surface pH and *in vitro* antibacterial activity[54].

- **Mastiholimath V.S et al.,** site specific one time delivery of ornidazole an antimicrobial compound with excellent activity against anaerobic micro-organism in the treatement of periodontal disease was prepared by solvent casting technique using ethyl cellulose, hydroxyl propyl cellulose, hydroxypropylmethyl cellulose K4M and eudragit RL-100 With dibutyl phthalate is plasticizer.The physiochemical parameters like thickness, weight variation, content uniformity and release characteristics were evaluated. The drug release was initially high on day one to achieve immediate therapeutic level of drug in pocket, followed by marked fall in release by day two and progressive moderate release profile to maintain therapeutic level following anomalous transport release profile mechanism. Formulation V6 released 97.07 % of drug at the end of 120 hour and was considered as best formulation. *In vitro* antibacterial activity was carried

out on *Streptococcus Mutans*[55].

- **Hong Hua., Chi ping.et al.,** prepared multilayer membranes which were loaded with drug for guided tissue regeneration were prepared using an immersed precipitation phase inversion technique, single layer, bilyer and trilayer mechanism were fabricated with chitosan used as carrier and tinidazole as medicine model which was loaded on the membrane. The influence of layer on structure and properties of membrane were studied by SEM, UV spectrometer and mechanical test. Drug release properties of three types of layer membrane also investigated. The result showed that release rate could be shown in both bilayer and trilayer membrane (11 days and 14 days respectively) and trilayer membrane lastest the longest. After a process of rapid release, the concentration of tinidazole which was released by the membrane was maintained at an efficient dosage level compared with single layer and bilayer membranes, be found trilayer membrane could play a role in controlling low rate drug release especially at the early stage of release, and keep an efficient dosage at affected part for a long period of time. The loss of drug which loaded on membrane decreased from 84.6% for Single layer to 13.04% for trilayer the mechanical strength of three types of membrane were detected an showed that it could meet the requirements of clinical practice the membranes specially with trilayer could be more valuable in application[56].

- **Sujatha Muchalambet al.,** Specific one time continuous delivery of sparfloxacin an antimicrobial compound with excellent activity against anaerobic micro-organisms in the treatment of periodontal disease was prepared by using solvent casting technique using hydroxy propyl cellulose, hydroxy methyl cellulose,

eudragit RL-100 and ethyl cellulose with dibutyl phthalate as plasticizer. The physicochemical parameters like thickness, weight variation, content uniformity and release characteristics were evaluated. The drug release was initially high on day one to achieve immediate therapeutic level of drug in periodontal pocket followed by marked fall in release by day two with progressive moderate release profile to maintain therapeutic level following anamolous transport mechanism. Formulation F4 released 90.24% of drug at the end of 120 hr and was considered as best formulation. *In vitro* antibacterial activity was carried out on *Streptococcus mutans*[57].

- **K. Schwach-Abdellaoui et al.,** Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by groups of specific microorganisms. Aggressive forms of periodontitis can be localized or generalized. The concept that localized problem sites may be treated by local drug delivery appears attractive as the antimicrobial agent is delivered within periodontal pockets and the therapy is targeted on specific pathogenic microorganisms. Local delivery of antimicrobial agents using controlled release systems should be considered as adjunctive to mechanical debridement for the treatment of localized forms of periodontal destruction. This article reviews various types of delivery systems evaluated in practical periodontal therapy. Despite the large number of studies showing an enhanced effectiveness of local antibiotherapy, there are insufficient Comparative data to support any of the local delivery system[58].

- **Vineet Bhardwaj et al.,** Chitosan films of different concentrations containing ofloxacin were prepared by solvent casting method. Some of the drug-loaded films were crosslinked with 2% gluteraldehyde for 1,2 and3 hrs, respectively. The films were then evaluated for their physicochemical properties including

weight variation, thickness, moisture loss, tensile strength, elongation, *in vitro* anti bacterial activity and *in vitro* release. I*n vitro* drug release data indicate that the films showed an initial burst release followed by sustained release of the drug. The drug-loaded films that were not crosslinked had effect on for up to 9 days and the films which are crosslinked for different duration shows release depended on concentration of chitosan in different films (1%, 2%, 3%)[59].

- **Steinberg et al.,** developed a degradable sustained release device composed of a cross linked protein containing chlorhexidine. The in-vitro release profile of chlorhexidine (from the degradable films) was altered by the amount of chlorhexidine loaded, cross- linking density of the polymer and type of chlorhexidine salt. This work demonstrated the release of chlorhexidine and showed that the degradation of the matrix can be controlled by factors in the formulation[60].

- **Hitzig et al.,** observed 28 patients suffering from adult periodontitis for a span of 3 months. They treated the patients with 5% metronidazole in collagen device in periodontal pocket more than 5 mm, keeping mechanical root debridement as control. Analysis of data from 28 patients indicated that both debridement and metronidazole therapy decrease pocket depth, bleeding on probing and gingival index, but results were significantly better with metronidazole[61].

- **Kurien et al.,** evaluated the efficacy of ciprofloxacin and norfloxacin when used in a sustained release system in periodontal pockets. Sustained release inserts were prepared using ethyl cellulose (EC) and hydroxypropyl methyl cellulose as (HPMC) co-polymers along with polyvinyl pyrrolidine (PVP), drug and ßeta cyclodextrin complex. The rate of release of the drug was controlled and

prolonged for 14 days period. It was found that both norfloxacin and ciprofloxacin appeared to be equally effective in reducing periodontal pathogens and in controlling the inflammation compared with the placebo treated control sites[62].

7. MATERIAL AND METHODS

MATERIALS

Drug, Polymer and Chemicals:

S.No	Name of the Chemicals	Supplier Name
1.	Doxycycline hyclate	Micro labs, Banglore.
2.	Chitosan	Central Institute of Fisheries Technology , Metysapuri,Cochin.
3.	Acetic acid	S-difine Chemicals., Mumbai
4.	Potassium hydrogen ortho Phosphate	S-difine Chemicals., Mumbai
5.	Sodium hydroxide	S-difine Chemicals., Mumbai
6.	Glutaraldehyde	S-difine Chemicals., Mumbai
7.	Calcium chloride	S-difine Chemicals., Mumbai
8.	Anhydrous aluminum chloride	S-difine Chemicals., Mumbai
9.	Acetone	S-difine Chemicals., Mumbai

All the other chemicals used in this study are of analytical reagent grade (A.R. grade).

List of Instruments Used For Formulation and Evaluation

S.No	Instruments	Supplier
1.	UV/Visible Spectrophotometer 1700	Shimadzu
2.	Vortex mixer VM 301	Remi., Mumbai
3.	Digital pH meter	HANNA Instruments, Italy
4.	Electronic weighing Balance	Essaie-Teraoka Ltd., USA
5.	Tensile strength tester	Digital testing apparatus
6.	Digital screw gauge	Mitutoyo., Japan
7.	FT-IR	Shimadzu
8.	Levelled glass moulds	Designed in our laboratory

4. EXPERIMENTAL METHODS:

1. PREFORMULATION STUDIES

Preformulation testing is the first step in the rational development of dosage form of the drug. It can be defined as an investigation of physical and chemical properties of drug substances, alone and when combined with excipents. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be mass produced.

A thorough understanding of physicochemical properties may ultimately provide a rational for formulation design or support the need for molecular modification or nearly conform that there are more significant barriers to the compounds development. The goals of the program therefore are to establishment the necessary physicochemical characteristics of new drug substances

- Determine its kinetic release rate profile.
- To establish its compatibility with different excipents.

Hence, preformulation studies on obtained drug sample include physical tests and compatibility studies.

- **Melting Point determination:**

Melting point was determined by taking small amount of drug in a capillary tube whose one end was sealed by melting. The capillary tube was placed in the Digital melting point apparatus. The temperature was slowly increased with simultaneous observation of the sample. The temperature at which the drug starts melting was recorded as melting point. This process was performed three times for Doxycycline. The mean of three readings was recorded.

2. COMPATIBILITY STUDIES

Drug- Excipient interaction studies:

The successful formulation of a stable and effective solid dosage form depends on the careful selection of the excipients that are added to facilitate administration, promote the consistent release and bioavailability of the drug.

FTIR and DSC studies can be used to investigate and predict any physicochemical interactions between components in a formulation and can therefore be applied to the selection of suitable chemically compatible excipients.

a. Fourier transforms infrared (FTIR) Spectral studies:

FTIR spectra were taken on a Shimadzu instrument to investigate the possible chemical interactions between the drug and the blend matrix. FTIR spectra of plain doxycycline and physical mixture were scanned in the range between 4000 and 500 cm^{-1}. The spectra's are compared with the peak intensities found in a standard reference.

Sample preparation

Samples are taken in the ratio of 1:100, where 1 is the amount of sample taken and 100 was the amount of potassium bromide taken and triturated with due precaution not to contact with the moisture, which may interfere with the test. Samples were crushed with KBr to get the pellets by applying a pressure of 300 kg/cm^2. The sample along with blank (KBr), reference standard drug are placed in order on the disk provided. Peaks obtained in FTIR are compared to that of standard peaks for any significant change[64].

b. Differential scanning calorimetry (DSC):

The DSC measurements were performing on a DSC – 61000 (Seiko Instruments, Japan) differential scanning colorimeter with thermal analyzer. All accurately weighed samples (about 5 mg of doxycycline and chitosan) were placed in a sealed aluminum pans, before heating under nitrogen flow (20 ml/min) at a scanning rate of 20^0C per min from 100 to 300^0C. An empty aluminum pan was used as reference[65].

3. ANALYTICAL METHODS

a. PREPARATION OF REAGENTS

Preparation of 1% v/v acetic acid:

Accurately measured 1 ml of concentrated acetic acid was dissolved in 100 ml of distilled water.

Potassium dihydrogen orthophosphate 0.2M:

Dissolved 27.218 grams of potassium dihydrogen orthophosphate in distilled water and volume adjusted to produce 1000 ml.

Sodium hydroxide 0.2 M:

8.0 grams of NaOH was dissolved in distilled water and volume adjusted to produce 1000 ml[63].

Table no.4.1: Preparation of 6.6 pH phosphate buffer solution

pH	Potassium hydrogen phosphate solution 0.2M(ml)	NaOH 0.2M Solution (ml)	Adjusted (ml) to
6.6	50	16.4	200

b. Preparation of Standard Stock Solution:

Standard stock solution of doxycycline hyclate was prepared by dissolving accurately weighed 100mg of doxycycline hyclate in small quantity of 6.6 pH phosphate buffer in 100ml volumemetric flask. The volume was made up to 100ml by using 6.6 pH buffer to obtaine the solution of 1000µg/ml.

c. Determination of Analytical Wavelength:

Scanning of Doxycycline Hyclate by UV Spectrophotometer:

From the standard stock solution 4ml was pipetted out into 100ml of volumemetric flask. The volume was then made up to 100ml with 6.6 pH phosphate buffer. The resulting solution containing 40µg/ml was scanned between 200-400nm and scan is shown in Figure no.6.1.

Calibration Curve of Doxycycline Hyclate in 6.6 pH Phosphate Buffer:

From the standard stock solution of doxycycline hyclate (40µg/ml), Appropriate liquates were taken in to different volumetric flask and volume made up to 10ml with 6.6 pH phosphate buffer, so as to get solution with drug concentration of 4 to 24 µg/ml. The absorbencies of the drug solution were measured at 274nm.These procedure was performed six times to validate the calibration curve. The data are given in Table no.6.1. The calibration curve constructed is showed in Figure no.6.2.

4. PREPARATION OF INSERTS

Inserts were prepared by using antimicrobial agent Doxycycline hyclate and polymer chitosan by solvent casting method.

a. Preparation of drug loaded chitosan inserts:

The general flow diagram for the preparation of chitosan inserts was given in figure 4.1. Chitosan (2% w/v) was soaked in acetic acid (1% v/v in water) for 24 hours to get a clear solution. This dispersion was filtered through a muslin cloth to remove undissolved portion of the polymer (chitin). Required amount of the drug was added and vortexed for 1-2 hrs to dissolve the drug in chitosan solution. The viscous dispersion was kept aside for complete expulsion of air bubbles. The films were casted by pouring the drug-polymer dispersion into the center of leveled glass moulds and allowed to dry at room temperature (30°C) for 24 hours. After drying, films were cut into inserts of required size (7 × 2 mm). Inserts containing zero percent (placebo), 10%, 20% and 30% w/w of the drugs to the weight of polymer were prepared These were wrapped in aluminum foil and stored in desiccators until further use [56].

b. Preparation of bi-layer inserts:

15 ml 2% chitosan cast solution without drug was poured into mould and dry at room temperature to form the first layer of bi-layer membrane. 20 ml chitosan cast solution with 30% drug was cast on the first layer and it was dried to room temperature. Then the dried inserts were kept in desiccators for further study[56].

c. Preparation of tri-layer membrane:

15 ml of 2% chitosan cast solution without drug was cast into mould and then dried to room temperature to form the down layer of the tri-layer. 20 ml chitosan cast solution with 30% drug was cast on the down layer after it was dry to form the middle layer of the tri-layer. And then 15 ml of 2% cast solution without drug was cast onto the

previous membrane also after it had been dry again to form the upper layer of the tri-layer. Then dried inserts were kept in desiccators for further use.

Table No. 4.2: Composition of different periodontal inserts

Inserts	**Inserts code**	**% of drug loaded**
Plain Inert	CP	0
Single layer	DSL-10	10
Single layer	DSL-20	20
Single layer	DSL-30	30
Bilayer insert	DBL-30	30
Trilayer insert	DTL-30	30

CP = Chitosan polymer, DSL-10, 20, 30= Doxycycline single layer with 10%, 20% and 30%, DBL-30 =Doxycycline bilayer with 30% and DTL-30= Doxycycline trilayer with 30%.

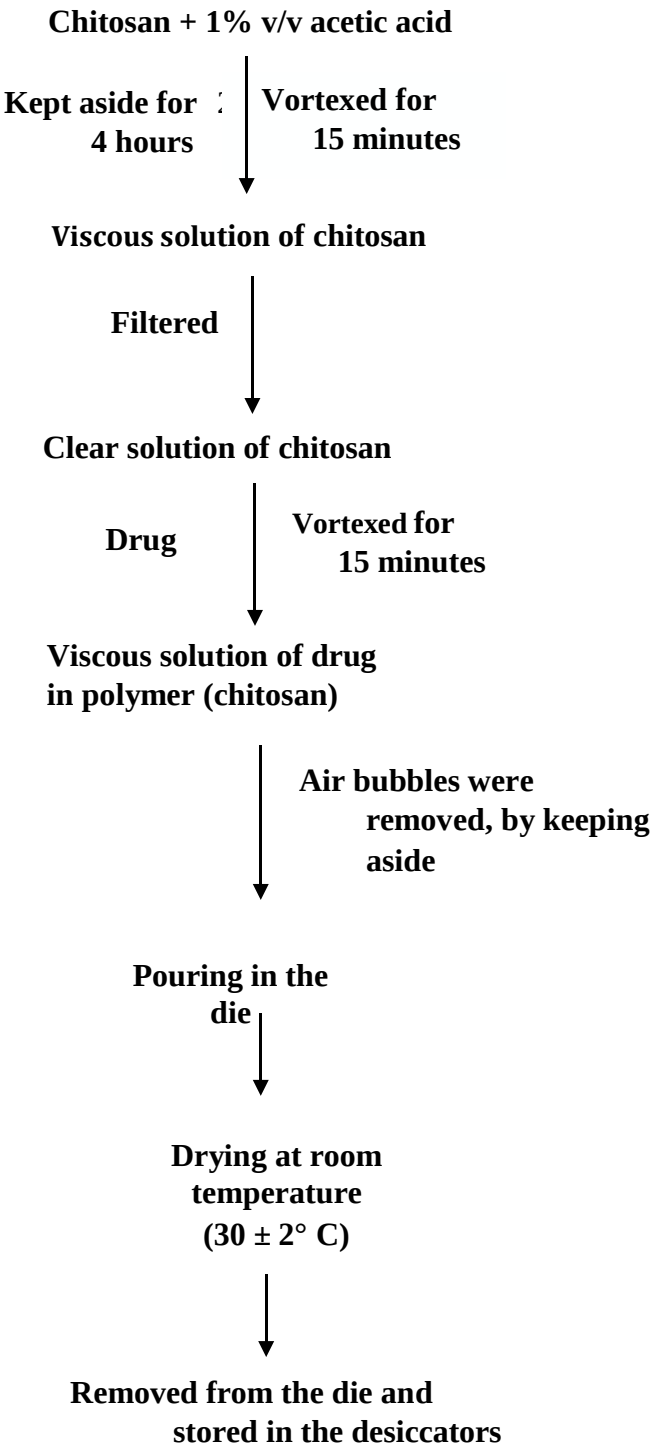


Figure No. 4.1: Protocol for the Preparation of Drug Loaded Chitosan Inserts

5. CHARACTERIZATION OF THE POLYMERIC INSERTS

All the prepared periodontal inserts were evaluated for various parameters like thickness, weight variation, folding endurance, tensile strength, drug content, *in vitro* release, swelling index, *in-vitro* anti microbial activity and stability studies.

a) Thickness Measurement:

The thickness of the polymer inserts (2x7 cm) was measured by using a digital screw gauge (Mitutoyo) at different areas of the inserts and the average was calculated[53].

b) Weight Variation:

The weight variation test was carried out by weighing 6 inserts cut from different places of same formulation and their individual weights were determined by using the digital balance. The mean valve was calculated. The standard deviations of weight variation were computed from the mean value[53].

c) Folding Endurance Studies:

Folding endurance of the inserts was determined by repeatedly folding the insert at the same place till it broke or folded. The number of times, the insert could be folded at the same place, without breaking, gave the value of folding endurance[53].

d) Tensile Strength Measurement:

Tensile strength of the inserts was determined by Universal strength testing machine. It consists of two load cell grips, the lower one is fixed and upper one is movable. The test inserts of specific size (2 × 2 cm) was fixed between these cell grips and force was gradually applied till the film breaks. The tensile strength of the inserts was taken

directly from the dial reading in kilograms Measurements were run in triplicate for each film[53].

Figure No. 4.2: Digital Tensile Strength Measurement Instrument.

e) Estimation of Drug Content:

The drug loaded chitosan insert of known weight (7 × 2 mm) were dissolved in small volume of 1% (v/v) acetic acid and drug solution was suitably diluted with 6.6 pH phosphate buffer and the absorbance was measured at 274nm[53].

f) Percentage Moisture Absorption:

The percentage moisture absorption test was carried out to check physical stability or integrity of the inserts. Periodontal inserts of known size were weighed and placed in a dissector containing 100ml of saturated solution of aluminum chloride and 79.5% humidity was maintained. After three days the inserts were taken out and reweighed. The percentage moisture absorption was calculated using the formula.

$$\% \text{ Moisture absorbance} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

g) Percentage Moisture Loss:

The percentage moisture loss test was carried out to check physical stability or integrity of the inserts. Periodontal inserts of known size were weighed and placed in a dissector containing 100ml of saturated Calcium chloride and 79.5% humidity was maintained. After three days the inserts were taken out and reweighed. The percentage moisture loss was calculated using the formula.

$$\% \text{ Moisture Loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

h) Determination of Water Uptake and Swelling Behavior:

Water uptake was determined gravimetrically. Drug loaded inserts were placed on a filter paper. The lower side of filter paper was immersed in a beaker containing 6.6 pH phosphate buffer, and incubated at 30 ^{0}C. Weight of each inserts was determined with digital weight balance at predetermined time points. The size changes of the inserts due to swelling investigated macroscopically.

i) *In-Vitro* Antibacterial activity:

In-vitro antibacterial activity was performed on all formulation by placing the insert (2x7) on agar plates seeded with oral bacteria *steptococuss mutans*. After 48 hrs of incubation at 37 ^{0}C, the inserts were transformed to freshly seeded agar plates and incubated for additional 48 hrs. This procedure was repeated until no inhibition of bacterial growth was detected on agar plate. The growth inhibition zone on the agar plate was measured.

j) *In-vitro* Release Studies:

Since the pH of the gingival fluid lies between 6.5 – 6.8, phosphate buffer pH 6.6 was used as simulated gingival fluid for the dissolution studies and the inserts remains immobile in the periodontal pocket, a static dissolution model was adopted.

A static dissolution method reported in the literature was adopted in this thesis. Sets of 3 inserts of known weight and dimension (7 × 2 mm) were placed separately into small test tubes containing 1.0 ml phosphate buffer, pH 6.6. The tubes were sealed and kept at 37°C ±1 for 24 hours. The buffer was then drained off and replaced with a fresh 1.0 ml phosphate buffer pH 6.6. The concentration of drug in the buffer was measured at 274nm .The procedure was continued for consecutive days[53].

6. DEPENDENT-MODEL METHOD (KINETIC MODELING):

The results obtaining *in vitro* release studies were plotted in different models of data treatment as follows:

ZERO ORDER KINETIC

It describes the system in which the drug release rate is **independent** of its concentration.

$$Qt = Qo + Ko\ t$$

Where,

Qt= Amount of drug dissolved in time t

Qo = Initial amount of drug in the solution, which is often zero and

Ko = zero order release constant.

If the zero order drug release kinetic is obeyed, then a plot of **Qt versus t** will give a straight line with a **slope of Ko** and an **intercept at zero**.

FIRST ORDER KINETIC:

It describes the drug release from the systems in which the release rate is concentration dependent.

$$\mathbf{Mt / M\infty = ktn}$$
$$\mathbf{Log\ [Mt / M\infty] = \ log\ k + n}$$

Where,

Qt = amount of drug released in time t.

Qo = initial amount of drug in the solution

k = first order release constant

If the first order drug release kinetic is obeyed, then a plot of **log (Qo- Qt) versus t** will be straight line with a **slope of kt / 2.303** and an **intercept at t=0 of log Qo.**

HIGUCHI MODEL:

It describes the fraction of drug release from a matrix is proportional to square root of time.

$$\mathbf{Log\ Qt = log\ Qo + kt / 2.303}$$

Where,

Mt and M∞ are cumulative amounts of drug release at time t and infinite time, and **kH** = Higuchi dissolution constant reflection formulation characteristics.

If the Higuchi model of drug release (i.e. Fickian diffusion) is obeyed, then a plot of Mt / M∞ versus $\mathbf{t^{1/2}}$ will be straight line with slope of kH.

KORSMEYER-PEPPAS MODEL (POWER LAW):

The power law describes the drug release from the polymeric system in which release deviates from Fickian diffusion, as expressed in following equation.

$$\mathbf{Mt / M\infty = kHt^{1/2}}$$

Where,

Mt and M∞ are cumulative amounts of drug release at time t and infinite time

(i.e. fraction of drug release at time t),

k = constant incorporating structural and geometrical characteristics of CR d

device,

n = diffusional release exponent indicative of the mechanism of drug release

for drug dissolution.

- To characterize the release mechanism, the dissolution data {Mt / M∞ <0.6} are evaluated.

- A plot of **log {Mt / M∞} versus log t** will be linear with **slope of n and intercept gives the value of log k**.
- Antilog of log k gives the value of k.
- Peepas used the **n value** in order to characterize different release mechanisms as shown in the table below,

Table No.4.3: Release Mechanism of Peppas

'n'	**MECHANISM**
0.5	Fickian diffusion
0.5 < n <1	Non- fickian diffusion
1	Class II transport

Stability Studies:

The stability of the drug loaded polymer inserts were studied at different temperatures using the reported procedure. The 3 inserts of size (7 × 2 mm) were weighed. The strips were wrapped in aluminum foil and placed in petridishes. These container was stored at ambient humid conditions, at room temperature (27 ± 2°C), oven temperature (40 ± 2°C**)** and in refrigerator (5 - 8°C) for a period of 3 months. The samples were analyzed for physical changes such as color and texture.

The drug content was estimated at an interval of 1 month using the procedures reported earlier in this thesis[53].

The results obtained from these methods and the discussion of the obtained results is given in the following chapter "Results and Discussion".

8.RESULT AND DISCUSSION

Periodontal inserts were prepared by solvent casting method and inserts were characterized for various parameters like thickness, weight variation, swelling index, moisture absorption, folding endurance, tensile strength, percentage moisture loss**,** drug content, stability study and *in-vitro* release studies. Table no.4.2 shows composition of various formulations of periodontal inserts with aimed at providing local long term release of Doxycycline (DCL).

1. PREFORMULATION STUDIES

The following preformulation studies were performed for drugs and polymer.

Determination of Melting Point:

Melting point was determined and it was found to be **202^0- 203^0C** $\pm 2^0$C (N =3). This value is very close to that of literature citation. Thus, indicating purity of the drug.

2. EXPERIMENTAL METHODS

a) Analytical Methods

Determination of λ max:

Absorption spectrum of doxycycline hyclate was obtained using phosphate buffer pH 6.6 by scanning the sample in UV spectrophotometer in the range of 200-400 nm. The absorption maxima were found to be at 274 nm and the spectra is shown in the figure no.6.1.

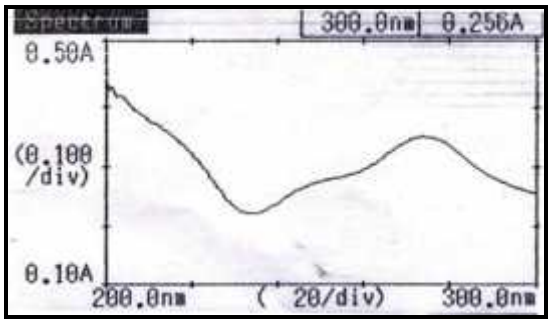


Figure No.6.1: Analytical Wavelength of Doxycycline Hyclate.

Calibration Curve of Doxycycline:

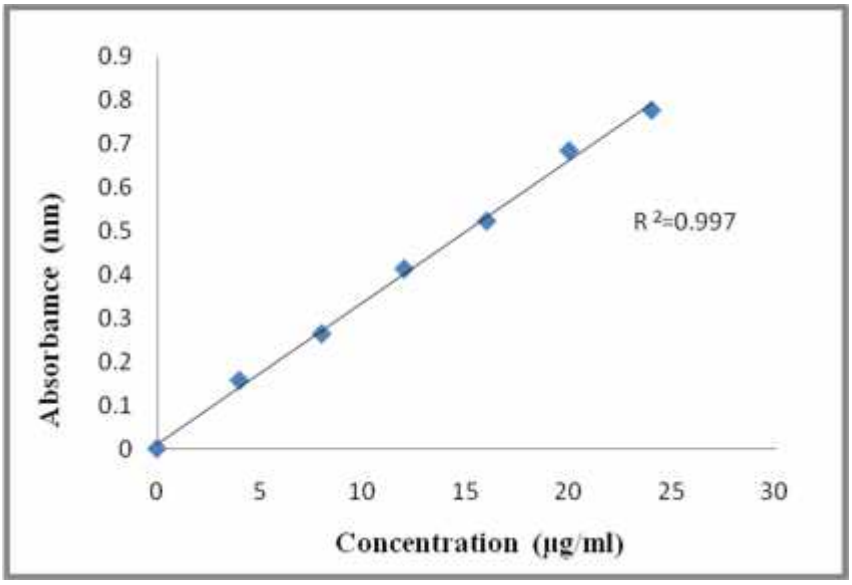
Table no.6.1 shows the absorbance reading of DCL. Standard solutions containing 4- 24 µg/ml in phosphate buffer pH 6.6. Figure no. 6.2 shows standard calibration curve for DCL with the correlation coefficient of 0.997. The drug content uniformity and *in vitro* drug release study are based on this calibration curve. The absorbance was measured at λ max of 274 nm.

Table No. 6.1.: Calibration Data of Doxycycline hyclate in 6.6 pH Phosphate Buffer.

SL.NO.	CONCENTRATION (µg/ml)	ABSORBANCE* AM ±SD
1	4	0.157 ± 0.002
2	8	0.264 ± 0.005
3	12	0.413 ± 0.006
4	16	0.523 ± 0.001
5	20	0.684± 0.028
6	24	0.778 ± 0.007

Each value is an average of six replications* R^2=0.997

Figure No.6.2: Calibration Curve of Doxycycline in pH 6.6 Phosphate Buffer.



3. DRUG EXCIPIENT COMPATIBILITY STUDIES

- **Fourier Transform Infrared Spectroscopy (FT-IR) studies:**

The preformulation studies between the drug and formulation component under the experimental conditions were done by using IR spectrum and was recorded on shimadzu FT-IR by preparing KBr disc. The entire characteristics peak obtained in the spectra's of the formulation correlate with peaks of drug spectrum. This indicates that the drug is compatible with all the formulation components. The spectra of all formulations were shown Figure no. from 6.3-6.5.

The results of IR spectral analysis showed the following major peaks for DCL, chitosan and their formulation components are tabulated in the table no.6.2.

Figure No.6.3: Fourier Transform Infrared Spectroscopy (FT-IR) of Doxycycline.

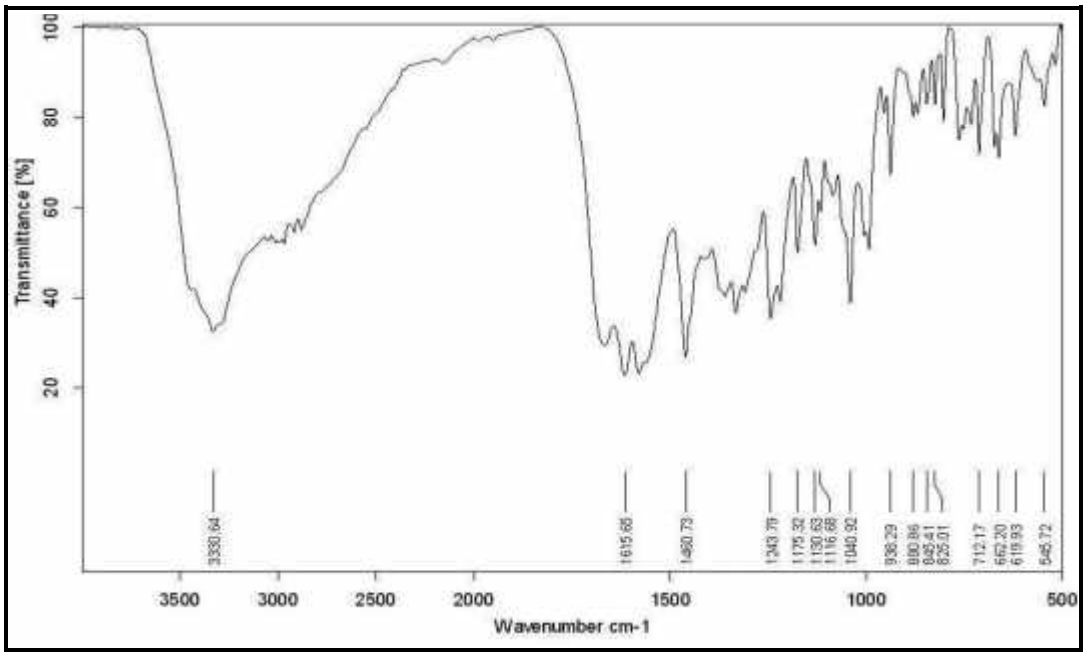


Figure No. 6.4: Fourier Transform Infrared Spectroscopy (FT-IR) Of Chitosan.

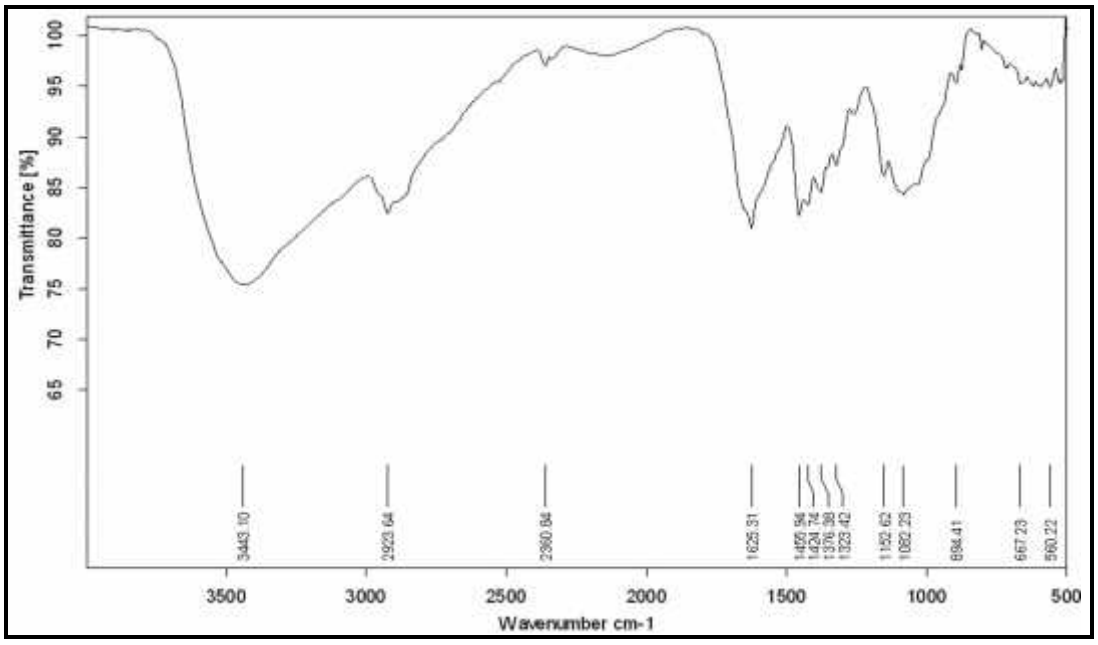
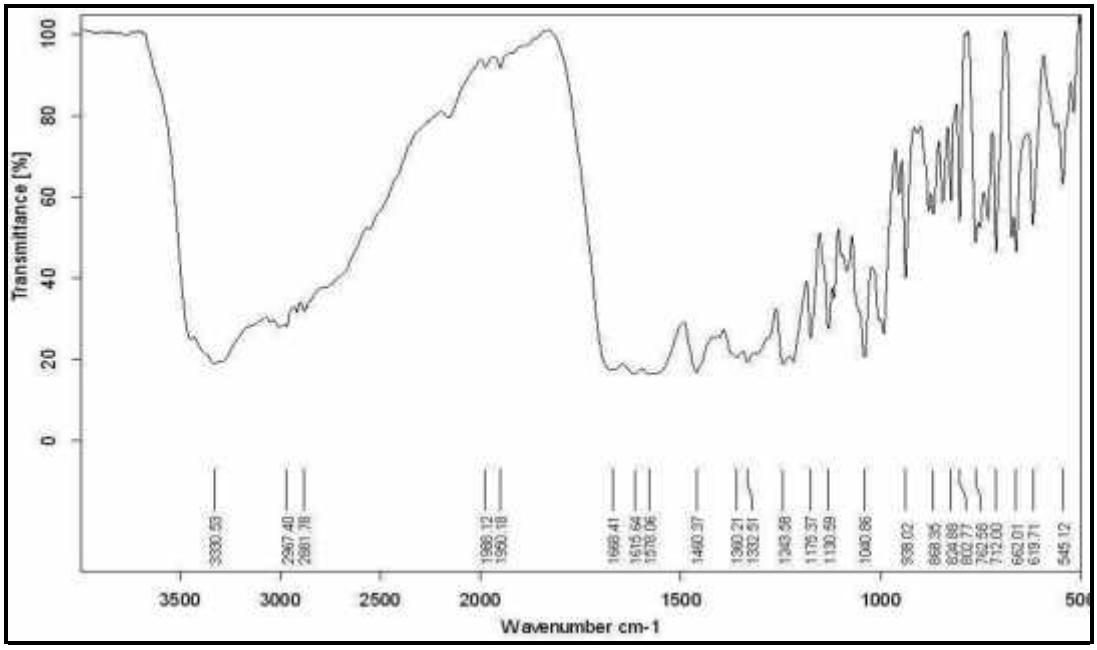


Figure No.6.5: Fourier Transform Infrared Spectroscopy (FT-IR) of formulation.



Fourier Transform Infrared Spectroscopy:-

The peaks of formulation were compared with the peaks of the drug and we found the maximum similarities. Finally we conclude that the drug retains its originality even after formulation, so the drug may not get disturbed in the formulation.

Table No.6.2: Data of the FT-IR Spectra of Pure Doxycycline(DCL) and Polymer Chitosan Peaks of Functional Group (Cm^{-1}).

FORMULATION	C=O Stretching cm^{-1}	Ar-CH Stretching cm^{-1}	-OH stretching cm^{-1}	N-H bending cm^{-1}	-OH bending cm^{-1}	C-N Stretching cm^{-1}	C-C-C O Stretching cm^{-1}
DCL	1615.65	2900	3330	1570	1460.73	1243.79	1175.32
DCL+ Chitosan	1615.64	2967	3330.33	1578.06	1460.37	1243.58	1175.37

- **Differential Scanning Calorimetry:**

DSC is useful in the investigation of solid state interactions. Thermograms are generated for pure drug and physical mixtures of drug along with other excipients. In the absence of any interaction, the thermogram of mixtures show endothermic peak corresponding to that of pure drug. In the event that interaction occurs, this is indicated in the thermogram of a mixture by the shift in the value of melting endotherm of the pure drug.

The DSC thermogram of pure DCL and its mixture with different excipients are shown in figure. Pure DCL showed a sharp endotherm at 219.78C corresponding to its melting point/transition temperature. There was no appreciable change in endotherm peaks of formulation compared to pure drug. This observation further supports the IR spectroscopy results, which indicated the absence of any interaction between drug and additives used in the preparation.

Figure No.6.6: Differential Scanning Calorimetric (DSC) Thermogram of Doxycycline Hyclate Drug.

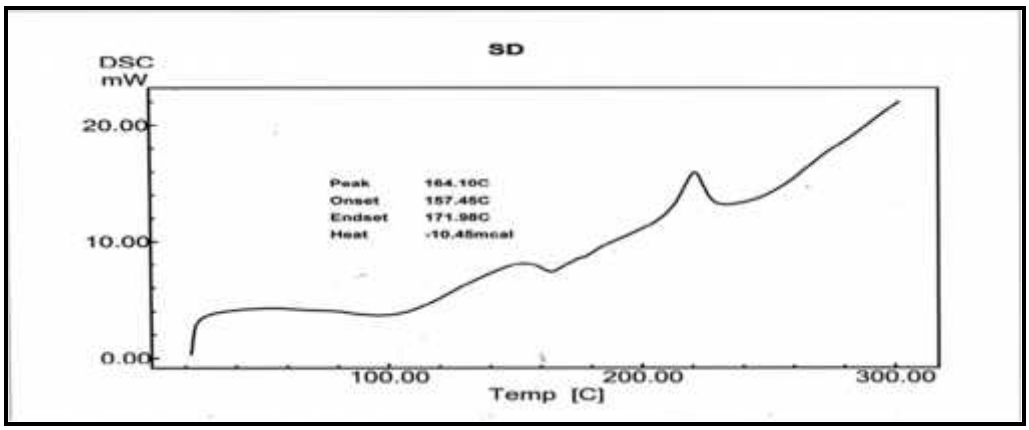


Figure No.6.7: Differential Scanning Calorimetric (DSC) Thermogram of Chitosan Polymer.

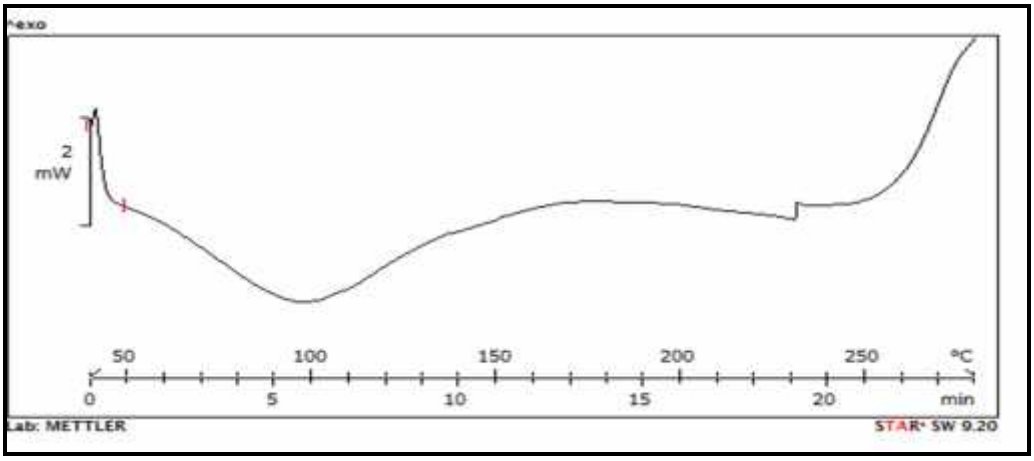
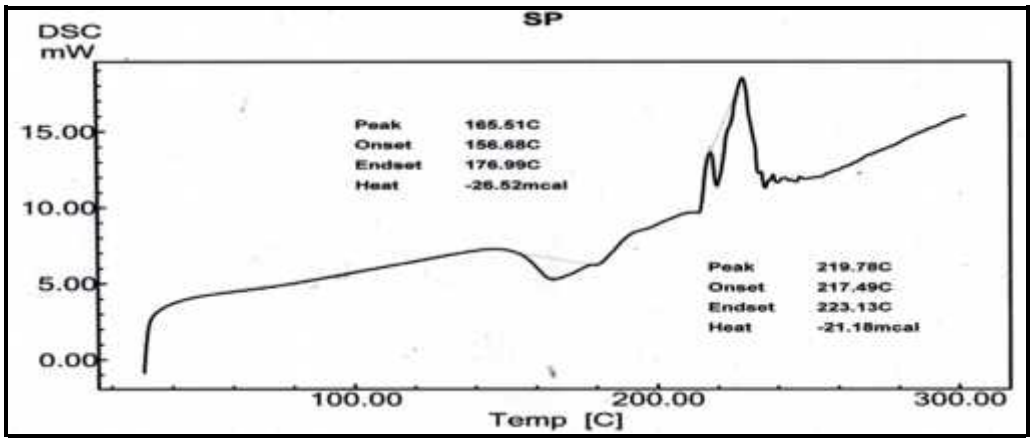


Figure No.6.8: Differential Scanning Calorimetric (DSC) Thermogram of Formulation.



4. Preparation of Drug Loaded Chitosan Inserts:

The optimum concentration of chitosan used for the preparation of inserts was found to be 2% w/w, because at this concentration the inserts were flexible and easily removable from die, at higher conc. of polymer, the polymer dispersion was highly viscous and difficult to filter using muslin cloth, inserts were brittle and difficult to remove from die.

The amount of drug added to polymer solution changes the inserts characteristics an optimum conc. of drug to be loaded was found to be less than 30% w/w to polymer, at higher drug conc. (i.e. >30%) the inserts were stiff and brittle. Plasticizer was not used because inserts obtained were flexible with plain chitosan. There was no visible sign of crystallization.

5. Evaluation of Physical Parameters of DCL Loaded Chitosan Inserts:

a) Physical appearance:

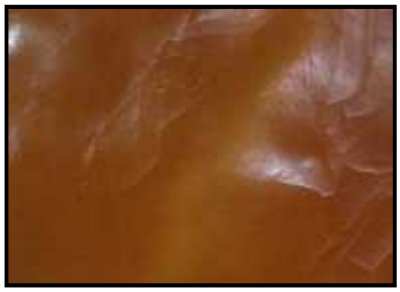
Both drug and polymer combination used for formulation of periodontal inserts showed good film properties and reproducibility. The fabricated inserts were thin, flexible, elastic, smooth and non transparent.

Photography of doxycycline hyclate was shown in the figure no.6.6.

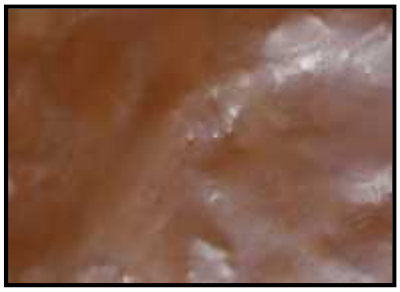
Figure No.6.8: Photograph Showing Doxycycline Hyclate Periodontal Inserts.

1) Plain Chitosan Insert

2) Single Layer With 10% of DCL

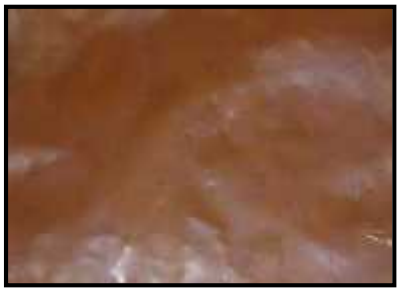


3) Single Layer With 20% of DCL

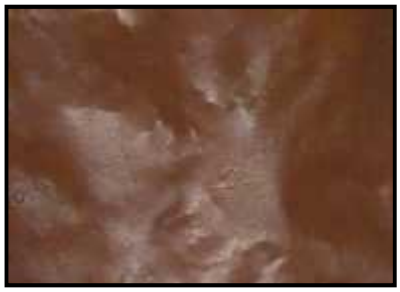


4) Single Layer With 30% of DCL

5) Bilayer Layer With 30% of DCL



6) Trilayer Layer With 30% of DCL



b) Thickness Measurement:

Thickness of each insert was measured at six different points and average thickness with standard deviation was calculated. The thickness of the various inserts as given in the table no.6.3. The data of inserts thickness indicates that there was no much difference in the thickness within the formulation. The thickness of the inserts was increased as increased in the drug concentration and more thickness was obtained in bilayer and trilayer respectively.

c) Weight variation test:

Drug loaded inserts (2X7) were test for uniformity of weight, the results of weight uniformity are given in the table no.6.3 with less standard deviation values which indicates that the inserts were uniform in weight. The data of weight uniformity indicates that as the concentration of the drug increases there is an increase in the weight of the insert. This is an agreement with uniformity of thickness of the film as shown in the table no.6.3.

d) Folding endurance:

Table no.6.3. Shows the value of folding endurance. There replication of each test was carried out. It was found that folding endurance of the inserts was decreased by increase in the drug concentration. Bilayer and trilayer inserts exhibits minimum folding endurance as compared to the other inserts. It is evident from the literature that the films having folding endurance having more than 100-150 is considered as optimum. In the present study the folding endurance was reduced in all inserts upon the increase in concentration of drug and also formulating into bilayer and trilayer inserts. However, the folding endurance of the bilayer and trilayer inserts containing 30% drug was also more than 150. Therefore the inserts of the study were having very good folding endurance and can be considered as optimum.

Table No. 6.3: Data of Physical Measurement values of Doxycycline Inserts.

Insert Code	Thickness (mm)AM ± SD	Folding Endurance	Weight Uniformity (mg)
CP	0.103±0.0157	340±2.581989	0.8±0.208167
DSL-10%	0.124 ±0.049	280±14.719	1.0± 0.208
DSL-20%	0.171 ±0.278	260.75±13.50	1.5 ±0.346
DSL-30%	0.197 ±0.024	201.75±19.431	2.2 ±0.057
DBL-30%	0.360 ±0.010	187±9.128	2.9 ±0.30
DTL-30%	0.456±0.0030	155.25±9.844	3.9 ±0.888

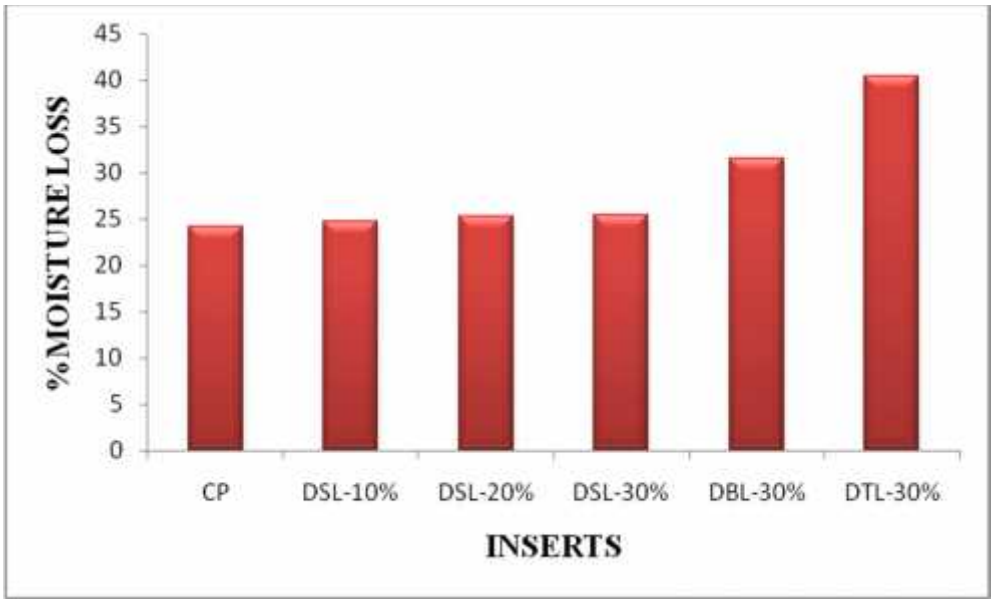
e) Percentage Moisture Loss:

This test is of great significance as variation in moisture content causes a significant variation in mechanical properties of the inserts especially when inserts comprises of hygroscopic components. Moisture loss studies were conducted on various formulations and reported in the Table no.6.3. It was observed that formulations containing maximum amount of drug showed maximum amount of moisture loss because of more concentration of drug which undergoing moisture loss in dry condition.

Table No.6.4: Data for Percentage Moisture Loss of Doxycycline Inserts.

INSERT CODE	MOISTURE LOSS (%) AM ± SD
CP	24.155±7.35
DSL-10%	24.77 ±12.365
DSL-20%	25.318±12.744
DSL-30%	25.37± 6.4593
DBL-30%	31.498±12.879
DTL-30%	40.43±11.737

Figure No.6.9: Graph for Percentage Moisture Loss of Doxycycline Inserts.



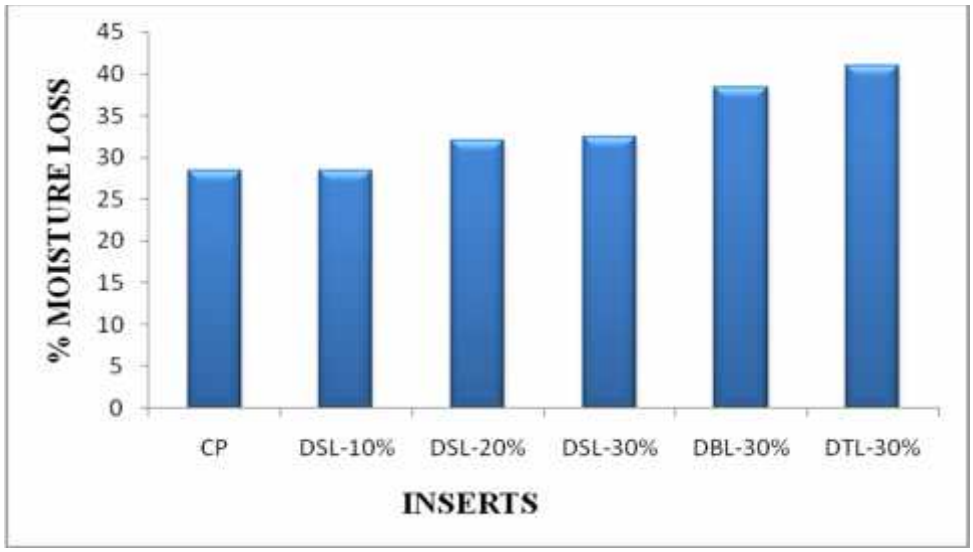
f) Percentage Moisture Absorption:

This test is of great significance as variation in moisture content causes a significant variation in mechanical properties of the inserts especially when inserts comprises of hygroscopic components. The capacity of the inserts to take up water is an important intrinsic parameter of the polymeric system in consideration to the release of drug. The data are given in the Table no. 6.4.

Table No.6.5: Data for Percentage Moisture Absorption of Doxycycline Inserts.

INSERT CODE	MOISTURE ADSORPTION (%) AM ± SD
CP	28.35± 16.02
DSL-10%	28.43±5.533
DSL-20%	32.048±20.18
DSL-30%	32.526±5.501
DBL-30%	38.393±11.366
DTL-30%	40.97 ±13.625

Figure No.6.10: Graph for Percentage Moisture Absorption of Doxycycline Inserts.



g) Water Uptake and Swelling Behavior:

Water uptake and swelling behavior studies were conducted on formulated inserts. Water uptake was found to be maximum with bilayer and trilayer inserts this may be due to the presences of more concentration of chitosan. The results are showed in the table no. After complete hydration, moderate swelling of the polymer were observed and there is a slight increase in the diameter of inserts.

Table No.6.6: Data for Swelling Index of Doxycycline Inserts.

INSERT CODE	WATER UPTAKE AND SWELLING BEHAVIOUR	
	INITIAL WEIGHT (mg) (0hr)	FINAL WEIGHT (mg) (2hrs)
CP	4.3	4.6
DSL-10	4.7	6.3
DSL-20	4.1	4.8
DSL-30	3.5	5.8
DBL-30	4.5	4.7
DTL-30	5.1	7.4

h) Tensile Strength :

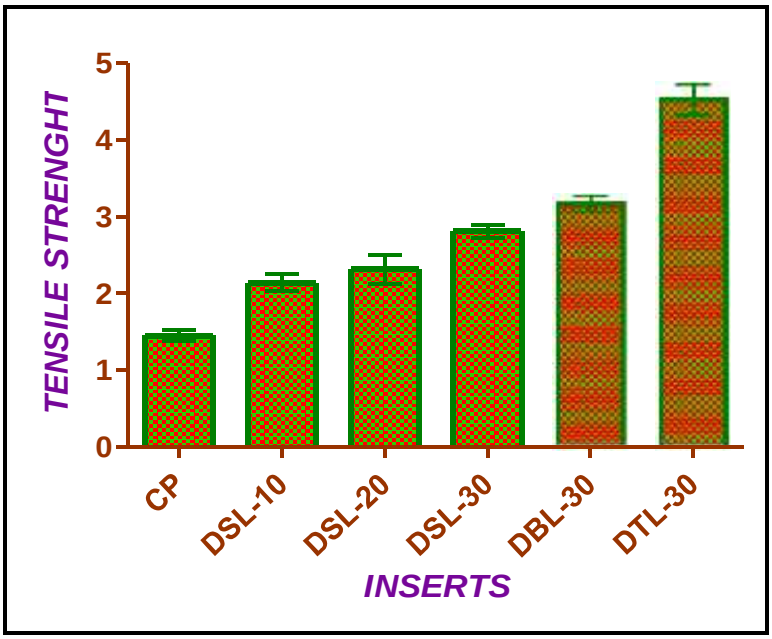
The tensile strength was determined using universal testing tensile machine for both drug loaded and unloaded inserts. The result are given in the table no.6.7. The tensile strength of drug loaded inserts was higher than dummy inserts. This is because of dissolved drug strengthen the bounding of polymer chain.

Table No.6.7: Data for Tensile Strength of Doxycycline Insert.

INSERTS CODE	TENSILE STRENGTH (Kg/sq.mm.) AM ± SD
CP	1.45± 0.054
DSL-10	2.140±0.110
DSL-20	2.318±0.185
DSL-30	2.810±0.060
DBL-30	3.170±0.102
DTL-30	4.533±0.200

*Each value was an average of three determinations.

Figure No.6.11: Graph for Tensile Strength of Doxycycline Inserts.



i) Drug Content Uniformity:

The drug content uniformity test is commonly employed for unit dose form in order to make sure about the uniform dispersion of drug in the inserts. The drugs content value of prepared inserts contains doxycycline hyclate were reported in the table no.6.8. The drug loading varied from 80-98 % depending on initial drug concentration and type of formulation.

Table No.6.8: Data of Drug Content Uniformity of Doxycycline Insert.

Inserts Code	Drug Content* (µg)	Theoretical Drug Loading (µg)	% Drug Loading
CS	----	----	---
DSL-10	107.810± 0.054	109.300	98.63
DSL-20	216.562± 0.089	218.250	99.00
DSL-30	274.062± 0.071	328.125	83.523
DBL-30	273.135± 0.064	328.125	83.241
DTL-30	272.279± 0.022	328.125	82.980

j) *In-vitro* Drug Release:

Since the pH of gingival fluid is between 6.5 to 6.8, phosphate 6.6 pH was used on stimulated gingival fluid for dissolution studies. Since, the inserts remain immobile in the periodontal pocket as static dissolution model was adopted in this work.

Release of DCL from Chitosan Inserts:

The release data of doxycycline hyclate are given in the table no.6.9, 6.10, and 6.11. The release studies were conducted for 8 days for single layer which containing different concentration of drug. The release time profiles from these inserts were shown in figures. From the results it was found that there was rapid initial release of drug on day 1 and there was a marked reduction in release from day 3 onwards and the release was controlled and extended up to 8 days.

The cumulative percentage drug release was 96.83%, 91.009 % and 92.210 % from inserts containing 10%, 20% and 30% of drug from single layer respectively.

A Persuval of figure no.6.12. indicated that initial rapid release must be due to burst release effect. Burst release is due to elution of drug from the outer surface and cut

edges of the matrix. Once the burst was completed (2days), drug was more sustained up to 8 days. Based on the results single layer inserts of 30% drug concentration was chosen for bilayer and trilayer studies respectively. These studies became essential in order to control the burst effect and release for more number of days.

Release of Doxycycline Hyclate from Bilayer and Trilayer:

The release data of bilayer and trilayer 30% DCL are given in the table no.6.12 and 6.13. The release time profile from bilayer and trilayer are showed in figure no.6.12. The bilayer and trilayer inserts showed a decreased initial burst release by more than 40%. The drug release was controlled and extended up to 13 days in case of bilayer where as 17 days in case of trilayer respectively.

The cumulative % release of drug from single layer insert was about 90-95% on the 8^{th} day. However the release of drug from bilayer and trilayer insert was about 90% on 13^{th} and 17^{th} day. Hence, it might be informed that developing bilayer and trilayer helped in sustaining the release for more number of days for local long term treatment for periodontitis.

Table No.6.9: Static Dissolution-Time Profile for Chitosan Inserts Containing Doxycyline-10%.

Time (days)	Absorbance*	Dilution factor	Conc. of drug released (µg) AM ± SD	Cumulative release (µg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.405	5	61.562	61.562	57.1023	42.897
02	0.135	5	19.375	80.937	75.073	24.927
03	0.074	5	9.843	90.780	84.136	15.864
04	0.052	5	6.406	97.186	90.1456	94.059
05	0.025	5	2.187	99.373	92.1741	7.8257
06	0.024	5	2.031	101.404	94.0580	5.942
07	0.027	5	2.343	103.747	96.231	3.769
08	0.018	3	0.656	104.403	96.839	3.161

- Each value is an average of three replication, Medium - Phosphate buffer 6.6 pH.
- Static dissolution conditions are: Temperature=37° C

Table No.6.10: Static Dissolution-Time Profile for Chitosan Inserts Containing Doxycyline-20%.

Time (days)	Absorbance*	Dilution factor	Conc. of drug released (μg) AM ± SD	Cumulative release (μg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.350	10	105.937	105.937	48.917	51.083
02	0.417	5	63.437	169.374	78.2103	21.789
03	0.103	5	14.375	183.749	84.848	15.152
04	0.042	5	4.843	188.592	87.0845	12.916
05	0.031	5	3.125	191.717	88.527	11.473
06	0.028	5	2.656	194.373	89.7539	10.247
07	0.030	3	1.781	196.154	90.576	9.424
08	0.021	3	0.937	197.091	91.009	8.991

- Each value is an average of three replication*
- Static dissolution conditions are: Temperature=37° C
- Medium = Phosphate buffer, pH 6.6 (l ml).

Table No.6.11: Static Dissolution-Time Profile for Chitosan Inserts Containing Doxycyline-30%.

Time (days)	Absorbance	Dilution factor	Conc. of drug released (μg) AM ± SD	Cumulative release (μg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.499	10	152.50	152.50	55.644	44.356
02	0.453	5	69.062	221.562	80.843	19.157
03	0.125	5	17.812	239.374	87.343	12.657
04	0.058	5	7.343	246.717	90.022	9.978
05	0.025	5	2.187	248.904	90.820	9.180
06	0.022	5	1.718	250.622	91.447	8.553
07	0.019	5	1.25	251.872	91.903	8.097
08	0.020	3	0.843	252.715	92.210	7.790

- Each value is an average of three replication*
- Static dissolution conditions are: Temperature=37° C
- Medium = Phosphate buffer, pH 6.6 (l ml).

Table No.6.12: Static Dissolution-Time Profile for Chitosan Bilayer Inserts Containing Doxycyline-30%.

Time (days)	Absorbance*	Dilution factor	Conc. of drug released (μg) AM ± SD	Cumulative release (μg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.469	10	143.125	143.125	52.400	47.600
02	0.365	5	55.312	198.437	72.651	27.349
03	0.166	5	24.218	222.655	81.518	18.482
04	0.068	5	8.906	231.561	84.778	15.222
05	0.039	5	4.375	235.936	86.380	13.620
06	0.030	5	2.968	238.904	87.467	12.533
07	0.025	5	2.187	241.091	88.268	11.732
08	0.033	3	2.062	243.153	89.023	10.977
09	0.030	3	1.781	244.934	89.675	10.325
10	0.029	3	1.6875	246.621	90.292	9.708
11	0.023	3	1.125	247.746	90.704	9.296
12	0.020	3	0.843	248.589	91.013	8.987
13	0.018	3	0.656	249.245	91.253	8.747

- Each value is an average of three replication*
- Static dissolution conditions are: Temperature = 37° C
- Medium = Phosphate buffer, pH 6.6 (1 ml).

Table No.6.13: Static Dissolution-Time Profile for Chitosan Tri Layer Inserts Containing Doxycyline-30%.

Time (days)	Absorbance*	Dilution factor	Conc. of drug released (μg) AM ± SD	Cumulative release (μg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.363	10	110.00	110.00	40.399	59.601
02	0.375	5	56.875	166.875	61.288	38.717
03	0.165	5	24.062	190.937	70.125	29.874
04	0.130	5	18.593	209.53	76.954	23.045
05	0.069	5	9.0625	218.592	80.282	19.717
06	0.043	5	5.000	223.592	82.118	17.881
07	0.035	5	3.750	227.342	83.495	16.504
08	0.027	5	2.500	229.842	84.414	15.585
09	0.025	5	2.187	232.029	85.217	14.783
10	0.023	5	1.875	233.904	85.906	14.093
11	0.027	3	1.500	235.404	86.456	13.543
12	0.025	3	1.312	236.716	86.938	13.061
13	0.023	3	1.125	237.841	87.351	12.648
14	0.021	3	0.937	238.778	87.696	12.303
15	0.019	3	0.750	239.483	87.955	12.044
16	0.017	3	0.562	240.045	87.996	12.003
17	0.015	3	0.375	240.420	88.299	11.700

- Each value is an average of three replication*
- Static dissolution conditions are: Temperature = 37° C,
- Medium = Phosphate buffer, pH 6.6 (1 ml).

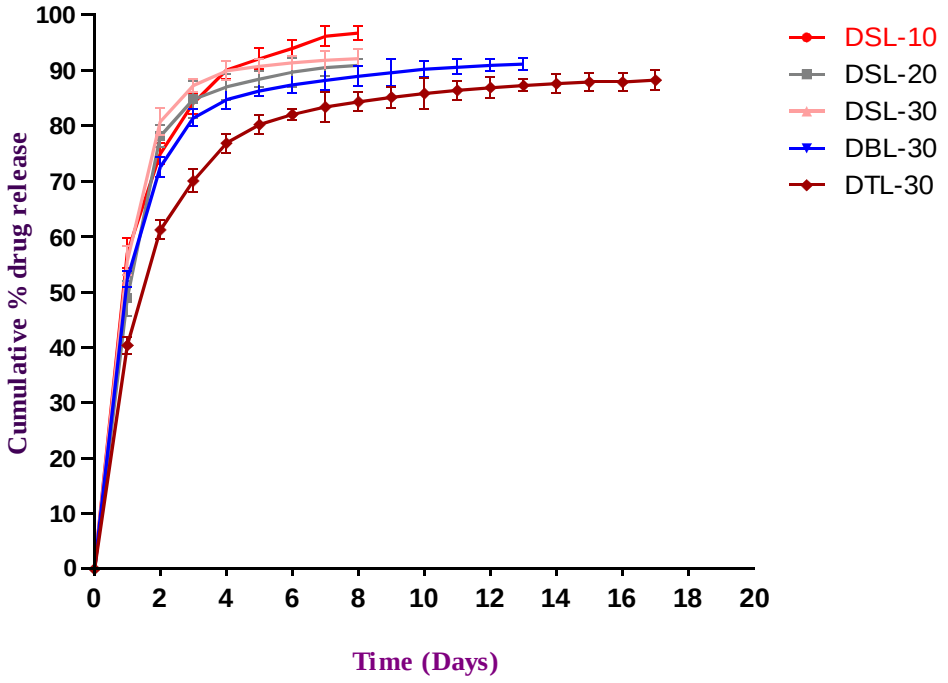


Figure No.6.12: Cumulative Percentage Release of Doxycycline Hyclate from Chitosan Inserts.

6. RELEASE KINETICS:

The release data of doxycycline hyclate was proposed to understand the linear relationship. The data was proposed for reggration analysis.

Release Mechanism:

Since the drug loaded chitosan inserts were intact during static dissolution studies, dissolution rate control release was ruled out. Hence data was fit according to higuchi ficks modle.

A Persuval of figure indicated that linear relationship was observed from 3rd to 8th day in case of single layer and 3rd to 13th day and 17th day in case of bilayer and trilarer respectively. The apperances of straight line indicates the release of drug from prepared inserts were diffusion rate control.

DEPENDENT-MODEL METHOD (KINETIC MODELING)

The results obtaining *in vitro* release studies were plotted in different models of data

Treatment as follows:

- ZERO ORDER
- FIRST ORDER
- HIGUCHI EQUATION
- KORSEMEYER PEPPAS EQUATION

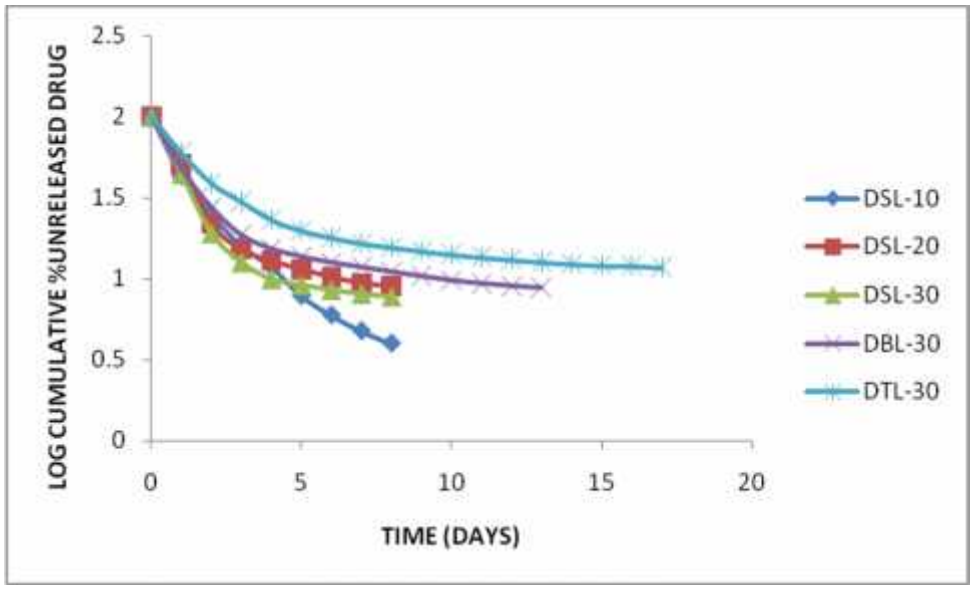
FIRST ORDER:-

Log of % Cumulative Drug Release VS. Time

Best Fit line for first order equation for chitosan inserts containing doxycycline.

Formulations are (DSL-10%, DSL-20%, DSL- 30%, DBL-30% and DTL-30%).

Figure No.6.13: First Order Equation for Chitosan Inserts Containing Doxycycline hyclate



HIGUCHI EQUATION:-

% Cumulative Drug Release Vs. Square Root of Time

Best Fit line for Higuchi equation for chitosan inserts containing doxycycline. Formulations are (DSL-10%, DSL-20%, DSL- 30%, DBL-30% and DTL-30%)

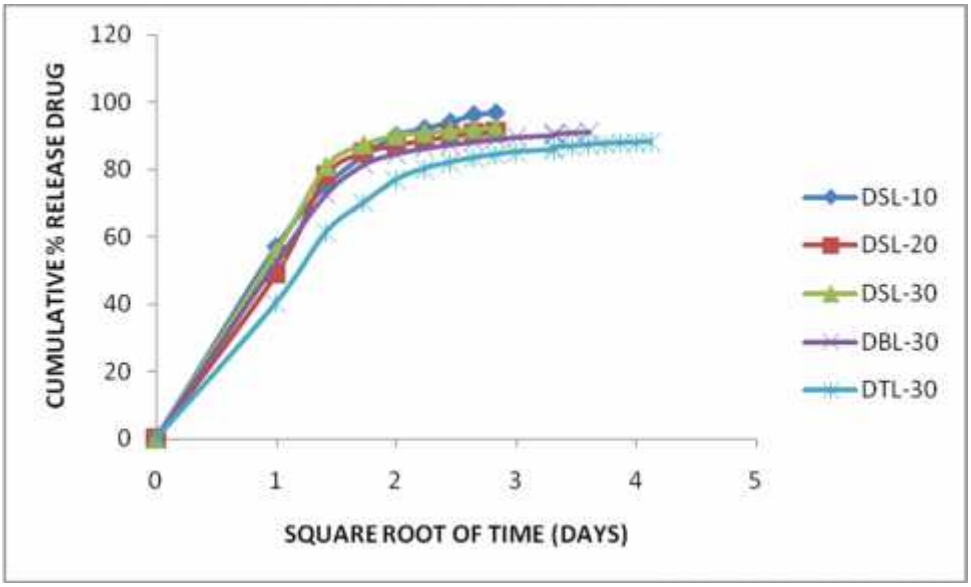


Figure No.6.14: Higuchi Equation for Chitosan Inserts Containing Doxycycline hyclate

KORSEMEYER PEPPAS EQUATION:-

Log of % Cumulative Drug Release Vs. Log Of Time

Best Fit line for Korsemeyer Peppas equation for chitosan inserts containing doxycycline. Formulations are (DSL-10%, DSL-20%, DSL- 30%,DBL-30% and DTL-30%) .

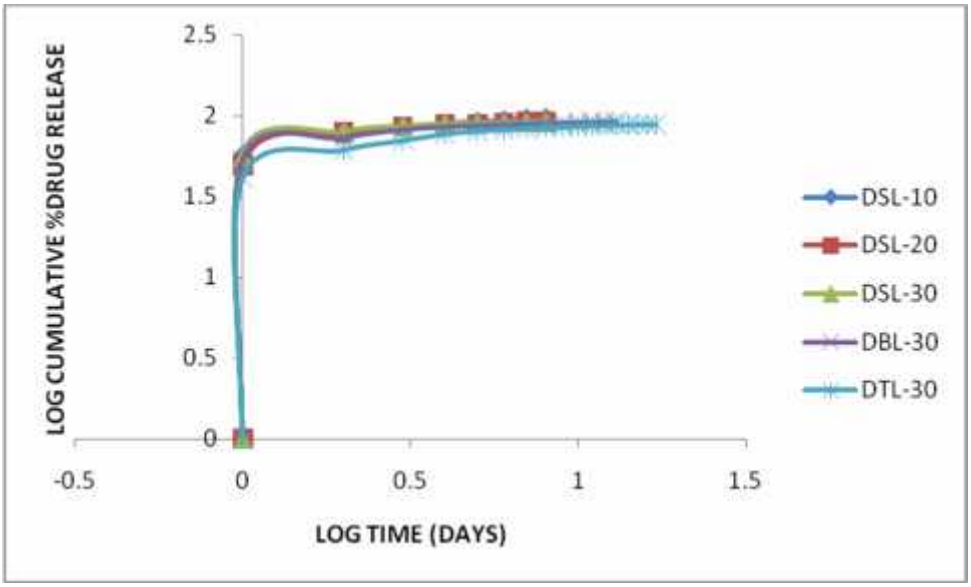


Figure No.6.15: Korsemeyer Peppas Equation For Chitosan Inserts Containing doxycycline hyclate.

The results obtaining by *In-vitro* release studies were plotted in different kinetic models as follows.

Table No.6.14: Data of Kinetic modeling of Doxycycline Inserts.

INSERT CODE	ZERO ORDER	FIRST ORDER	HUGUCHI MATRIX	KORSEMEYER PEPPAS	
	R^2	R^2	R^2	R^2	N
DSL-10	0.559	0.952	0.887	0.415	0.937
DSL-20	0.596	0.806	0.847	0.422	0785
DSL-30	0.559	0.766	0.823	0.402	0.789
DBL-30	0.479	0.743	0.738	0.388	0.811
DTL-30	0.513	0.750	0.764	0.421	0.822

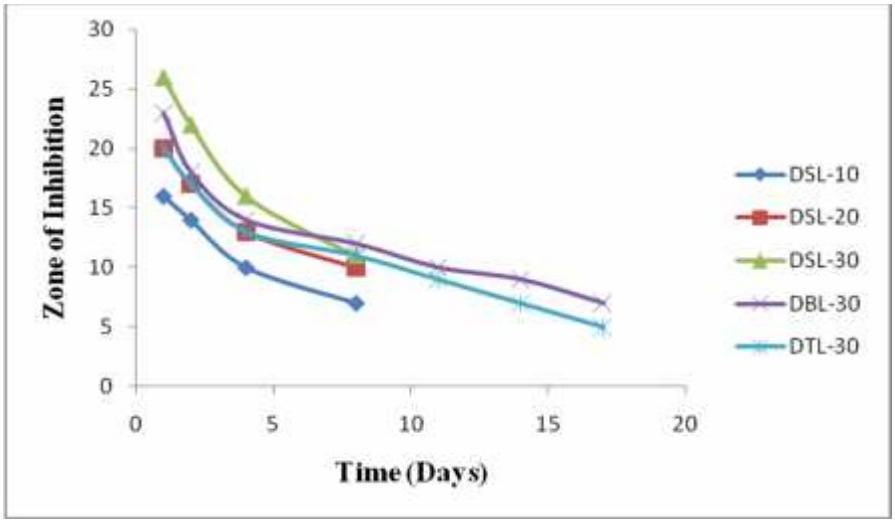
k) *In-Vitro* Antibacterial Activity:

Table no. 6.15. Shows more *in-vitro* antibacterial activity against *S. mutans* was found on day1 with all single layer inserts. Whereas with bilayer and trilayer less activity was observed on day 1. From the study it was conformed that bilayer and trilayer inserts exhibited antibacterial activity for a long period in comparison to single layer. This may be due to sustained release of drug from bilayer and trilayer inserts. The zone of inhibition values of DCL shown in figure no.6.16.

Table No.6.15: *In vitro* antibacterial activity data of Doxycycline hyclate.

Days	Diameter of inhibition of growth area of *S.mutans* (mm)				
	DSL-10	**DSL-20**	**DsL-30**	**DBL-30**	**DTL-30**
1	16	20	26	23	20
2	14	17	22	18	17
4	10	13	16	14	13
8	7	10	11	12	11
11	-	-	-	10	9
14	-	-	-	9	7
17	-	-	-	7	5

Figure No.6.16: Zone of inhibition of Doxycycline Hyclate.



L) Stability Study:

All the polymeric inserts were obsersved for physical change, color, appearance and drug content. Stability data at different temperature and humidity condition revelaed on physical appearances and uniformity in drug content. The drug remained intact and stable in the polymer on storage for 3 months. The results obtained from these methods and the discussion of the obtained results is given in the following tables.

Table No.6.16: Data for Physical appearance of Drug Stability Study.

TIME IN DAYS	DSL-10	DSL-20	DSL-30	DBL-30	DTL-30
0	No change				
30	No change				
60	No change				
90	Yellow to brown				

Table No.6.17: Stability Studies for Drug Content at various temperatures for Chitosan Inserts Containing Doxycycline Hyclate.

Insert code	Initial drug conc. (µg)	30 Days	60 Days	90 Days
		A.F	A.F	A.F
DSL10	107.810	107.800	107.783	107.700
DSL-20	216.562	216.560	216.534	216.487
DSL-30	274.062	274.058	274.022	273.987
DBL-30	273.135	273.130	273.102	273.029
DTL-30	272.279	273.274	273.250	273.147

Each value is an average of 3 determinations.

A.F = Packed in aluminum foil.

The inserts were also observed for their appearance, texture and drug content uniformity. These properties did not change in all the inserts during the period of study. Hence, prepared formulations have a good stability.

9.SUMMMARY

Periodontitis is the disease, which is caused by specific microorganism in periodontal pockets and led to an increased interest in and usage of antibacterial agent in periodontal therapy.

The problems of administering drugs systemically, such as adverse reaction, have focused attention on ways of applying the drug directly to its target site.

The use of drugs to treat plaque associated periodontal diseases is an attractive prospect, since conventional treatment can be demanding on both patient and operator. So there is much interest in developing alternative strategies.

Number of drug delivery system has been investigated for administration of antibacterial / antimicrobial agents into periodontal pocket for local action in the treatment of periodontitis.

Device placed into periodontal pocket can produce a local drug concentration 100 times higher than achievable systemically and importantly reduces the total patient dose by over 400 fold.

For developing the local drug delivery system, as it was proved antimicrobial agent is effective against wide range of oral pathogens.

Mucoadhasive dosage form utilizes the mechanism of bioadhesion and produces an intimate contact with the biological membrane. Hence, chitosan was selected as a biodegradable polymer for the development of drug delivery device which stays for longer time at the site of action

The inserts of chitosan polymer (biodegradable) 2% w/v were prepared by solvent casting method using 1% v/v acetic acid as a casting solvent. In the present work chitosan inserts containing doxycycline hyclate were prepared in three different

percentages (10%, 20% and 30%) to the weight of polymer .Bilayer and trilayer inserts also were prepared for preventing initial burst release.

Microscopical feature revealed that the drug was dissolved in the polymer matrix rather than dispersining. The average weight of single layer inserts range from 0.8 to 2.2 mg and average weight of bilayer and trilayer ranges from 2.9 to 3.9 mg. The thickness of the insets ranges from 0.103±0.015 mm to 0.456±0.0030 mm. There was no significant difference in the thickness among the different inserts since the drug was dissolved in the polymer matrix. The tensile strength of the inserts range from 1.45 ± 0.054 kg to 4.533 ± 0.200 kg. Tensile strength is minimum for plain inserts and maximum for inserts containing 30% drug. The data obtained revealed that incorporation of drug increases the physical strength of the inserts, however this may not cause any problem during their clinical application. The folding endurance studies showed that plain inserts exhibited maximum folding endurance followed by drug loaded inserts respectively.

In vitro dissolution rate studies were carried out by static dissolution method for inserts for a period of 8, 13 and 17 days respectively. This showed a burst release initially followed by a progressive fall in the release of the drug. The inserts DSL-10, DSL-20 and DSL- 30 showed 96.839%, 91.009 % and 92.210% respectively at the end of 8 days. The bilayer insert (DBL-30) showed 91.253% at the end of 13. The trilayer insert (DTL-30) showed 88.299 % respectively at the end of 17days of static dissolution period. Initial burst effect was reduced once the chitosan strips were formulated in multilayer form and also release of drug was extended and controlled up to 17 days. The plots of the cumulative amount of drug release per unit surface area against sq. root of time, confirms to Higuchi's diffusion model i.e., the release kinetics of doxycycline from chitosan strips followed first order. The initial burst

release is essential to achieve high concentration of drug in gingival sulcus. This is a primary objective of the periodontal therapy.

10.CONCLUSIONS

From the obtained results, it can be concluded that

- Novel multilayer inserts of doxycycline hyclate can be formulated by solvent casting method technique.
- Evaluation parameters like thickness, tensile strength and folding endurance indicates that inserts were mechanically stable in all the inserts formulations.
- Percentage weight variation and drug content uniformity found to be uniform in all the formulations.
- The FT-IR and DSC spectra revealed that, there was no interaction between polymer and drug. Hence, Polymer used was compatible with the drug.
- *In-vitro* drug release showed an abrupt release in the first day in single layer whereas this initial burst release was controlled by formulating in to bilayer and trilayer which was controlled the release uniformity in a specified period of time.
- *In-vitro* antibacterial activity showed good correlation with respective *in-vitro* drug release. Release kinetics from all the inserts follows diffusion rate controlled mechanism.
- All the inserts were found to be stable over the storage period of 90 days and condition tested.
- From this study it can be concluded that bilayer and trilayer were developed which can be delivered drug up to 13 and 17 days respectively.
- **Future scope:-**

- Addressing the final multilayer formulation needs considerable amount of study.
- *In-vivo* evaluation study of multilayer inserts of DCL and establishment of *in-vitro* and *in-vivo* correlation.

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DESIGN AND DEVELOPMENT OF NOVEL MULTILAYER CHITOSAN INSERTS CONTAINING DOXYCYCLINE HYCLATE

A dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI- 600 032.

In partial fulfillment of the requirements for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted By

Reg No: 26118151

DEPARTMENT OF PHARMACEUTICS

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1.INTRODUCTION

Periodontal diseases are recognized as the major public health problem throughout the world. Daily oral hygiene plays a vital role in maintaining healthy teeth and gums. Periodontal disease can do occur in all age groups, ethnicities, races, genders and socioeconomic levels.

The term “Periodontal Disease” broadly defines several diseases associated with the periodontium. Changes in the microflora, histopathological variations, clinical symptoms, and the location of the inflammation help to further delineate periodontal disease. Gingivitis, the moderate stage of the disease, caused by an accumulation of supragingival plaque is characterized by swelling, light bleeding and redness of marginal gingiva. Gingivitis is associated with a change in microflora, shifting from a grampositive anaerobic flora to a more gram-negative flora. Periodontitis a more severe stage of periodontal disease, results in the resorption of the alveolar bone and detachment of the periodontal ligaments supporting the tooth.

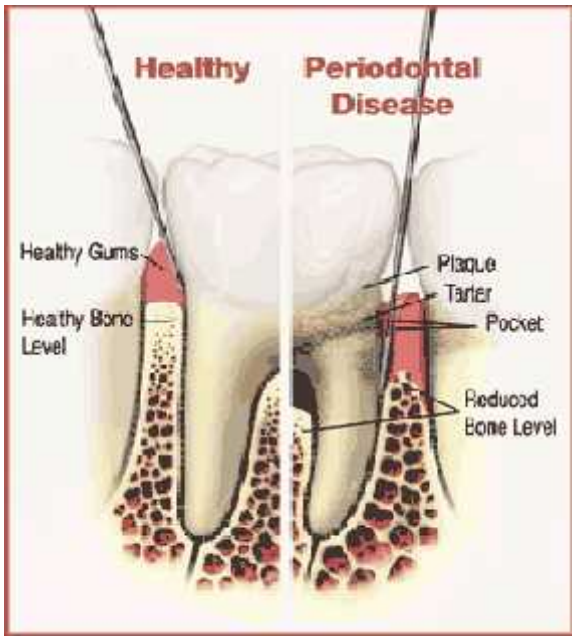


Figure No.1.1: Comparision of Healthy Periodontum/Gums and Periodontal Disease.

One of the clinical features of the periodontal disease is the formation of periodontal pocket, which is pathologically deepened sulcus. In normal sulcus, the gap between the gingiva and the tooth is normally between 1 and 3 mm deep. However, during periodontitis, the depth of pocket usually exceeds 5mm[1].

Causes of Periodontal Diseases:

The main cause of periodontal disease is bacteria plaque, a sticky, colorless film that constantly forms on teeth. However, factors like smoking/ tobacco use, genetics, pregnancy and puberty, stress, medication, clenching or grinding teeth, diabetes and poor nutrition also lead to periodontal diseases. Plaque (more plaque formation) Gingival inflammation Pocket formation Periodontal pathogens grow only where atmosphere and nutrient composition are strictly conductive to their requirements and the disease once established, causes major changes in the periodontal microenvironment. The gingival crevicular fluid (GCF) flow occurs at extremely low levels in healthy gingival sulci but increases enormously to 3.5ml/day or more. The most commonly grown anaerobic pathogenic bacteria are ***Actinobacillus actinomycetencomitans, Bacteroides gingivalis, Bacteroides melaninogenicus sub species intermedius, Porphyromonas gingivalis and Prevotella intermedia.*** Clinical signs such as bluish red thickened marginal gingiva, bluish red vertical zone from the gingival margin to the oral mucosa, gingival bleeding and localized pain are suggestive of the presence of periodontal pockets [2, 3].

Overview of the Pathogenesis of Periodontal Disease:

The normal healthy gingiva is characterized by its pink color, its firm consistency and free from histological evidence of inflammation, but this ideal condition is very rarely seen in microscopic tissue sections. This is because most human gingival tissues are, no matter how clinically healthy in appearance, slightly inflamed due to constant

presence of microbial plaque. Even in healthy state, the gingiva has a leukocyte infiltrate that is predominantly comprised of neutrophils or polymorphonuclear leukocytes. These leukocytes rephagocytose whose primary purpose is to kill bacteria in the gingival crevicular area or the gingival pocket. Neutrophils are recruited to the periodontal pocket or the gingival crevice because of attracting molecules, released by bacteria, called chemtactic peptides. Furthermore, as bacteria damage the epithelial cells, they cause epithelial cells to release molecules termed cytokines that further attract leukocytes to the crevice. The neutrophilscan phagocytose and digest bacteria within crevice and therefore remove these bacteria from the pocket. If the neutrophil becomes overloaded with bacteria, it degranulates or explodes. This causes tissue damage from toxic enzymes that are released from the neutrophils. Therefore, neutrophils can be viewed as being both helpful and potentially harmful. Depending on the individual, progression from gingivitis to periodontitis requires varying amounts of time. But in normal situation, more than 6 months may be needed for the lesion of gingivitis to change to periodontitis [4, 5, 6].

Microbiology of Periodontal Disease:

About 300 or 400 bacterial species are found in the human subgingival plaque samples. Of that number, possibly 10-20 species may play a role in the pathogenesis of destructive periodontal disease. These bacterial species must be able to colonize in order to survive and damage the periodontal tissues. To colonize subgingival sites, microbes must be able to,

1) Attach to periodontal tissues

2) Multiply

3) Compete with other microbes in their habitat and

4) Defend themselves from host defense mechanisms.

The gingival sulcus area is a lush place for microbial growth, but in order to colonize a subgingival site, a bacterial species must overcome a number of host-derived obstacles. If a species successfully enters underlying connective tissue, it faces a multitude of host immune cells including B and T lymphocytes, neutrophils, macrophages and lymphocytes. To be successful, bacteria must have the ability to evade all these host response mechanisms. The microbes involved in periodontal disease are largely gram-negative anaerobic bacilli with some anaerobic cocci and a large quantity of anaerobic spirochetes [4, 8, 9].

Histopathological Changes:

The major pathological features of periodontitis are the accumulation of an inflammatory infiltrate in the tissue adjacent to the periodontal pocket, breakdown of connective tissue fibers anchoring the root to gingival connective tissue and alveolar bone, apical migration of the epithelial attachment or junctional epithelium, and resorption of the marginal portion of the alveolar bone resulting in eventual tooth loss. Page & Schroeder developed a system to categorize the clinical and histopathological stages of periodontal disease and defined four histopathological stages of periodontal inflammatory changes.

- Health – Pristine Condition
- Initial lesion – Clinically healthy
- Early lesion – Early gingivitis
- Established lesion – Chronic gingivitis
- Advanced lesion – Chronic periodontitis

Following histopathological description by Kinane and Lindhe is based on model Proposed by Page & Schroeder is summarized in table below [4,7,10]

Table No.1.1: Histopathological Description

	Clinical condition	Histopathological condition
1.	Pristine gingiva	Histological perfection
2.	Normal healthy gingiva	Initial lesion
3.	Early gingivitis	Early lesion
4.	Established gingivitis	Established lesion with no bone loss nor apical epithelial migration (Plasma cell density between 10% and 30% of leukocyte infiltrate.
5.	Periodontitis	Established lesion with bone loss and apical epithelial migration from the cement enamel junction (Plasma cell density >50%).

The initial lesion appears within 4 days of plaque accumulation, characterized by an acute inflammatory response. The major features consist of an increased flow of crevicular fluid; inflammatory infiltrate occupies 5% to 10% of the gingival connective tissue below the epithelium and loss of collagen.

After approximately 7 days of plaque accumulation, an inflammatory infiltrate of mononuclear leukocytes develops at the site of initial lesion as it progresses to the early lesion. At this stage, the infiltrate occupies about 15% of the gingival connective tissue, with collagen destruction in the infiltrated area reaching 60-70%. Clinically the inflammatory changes are visible and present as edema and erythema.

After 2 to 3 weeks of plaque accumulation, the early lesion evolves into established

lesion, characterized by further increase in the size, predominance of plasma cells and lymphocytes at the periphery of lesion.

Features of advanced lesion include periodontal pocket formation, surface ulceration and suppuration, destruction of alveolar bone and periodontal ligament, tooth periodontitis is marked by the change in T-cell to B-cell predominance.

Mobility and drifting, and eventually tooth loss. The advanced lesion characterized by the same feature present in the established gingival lesion.

TREATMENT APPROACHES

Conventional Periodontal therapy:

The purpose of periodontal treatment is to cure the inflamed tissue, reduce the number of pathogenic bacteria and eliminate the depth of the diseased pockets and to stop bone resorption. The conventional methods of pocket elimination are more or less mechanical and are aimed at removal of supra and mechanical plaque and degenerated and necrotic tissue lining the gingival wall of periodontal pockets through scaling, root planning and curettage[11].

The mechanical debridement alone often leaves behind significant number of pathogens due to possible instrumentation or ability of microorganism to penetrate into deeper tissues. Inaccessibility and recolonization of pathogens can occur after scaling and root planning. With oral hygiene, a pathogenic subgingival microbial may reestablish within 42 - 60 days after a single periodontal debridement session[13]. Some deep periodontal pockets experience putative pathogen recolonization by 120 - 240 days despite multiple sessions of subgingival instrumentation and meticulous supra gingival plaque control[12].

Antibiotic Therapy:

The use of antibiotics in the treatment of periodontal diseases helps to reduce or eliminate bacteria that cannot be removed by scaling and root planning. Chemotherapeutic agents can be administered systemically or locally. Tetracycline's, imidazole derivatives, fluoroquinolones etc., are the most favored antibiotics.

Antimicrobials used to treat dental infections can be divided into two main categories, i.e., broad spectrum and narrow spectrum. Narrow-spectrum antimicrobials include penicillin, amoxicillin, cephalexin, macrolides and tetracyclines. These drugs are having a limited antimicrobial efficacy (Table1.2), as they are not effective against aerobic and anaerobic beta lactamase producers, as well as other specific organisms[14]

Systemic periodontal antimicrobial therapy is based on the premise that specific microorganism cause destructive periodontal disease and that the antimicrobial agent in the periodontal pocket can exceed the concentration necessary to kill the pathogens. Systemic antibiotics can reach microorganisms at the base of deep periodontal pockets and furcation areas via serum and may also affect organisms residing within gingival epithelial and connective tissue. With systemic antibiotic therapy there is a considerable variability in the therapeutic activity due to factors like poor absorption in the gastrointestinal tract, first pass metabolism, systemic distribution, bacterial sensitivity and resistance[15].

Some studies also report poor results due to the fact that the active product does not reach in adequate concentration at the site of action, as it is not retained locally for sufficient period of several thousand folds before it reaches the site of action

necessitating ingestion of large doses.

The increased toxic effects of these elevated dose levels make systemic administration unacceptable due to low benefit to risk ratio. Repeated long term use of systemic antibiotics is fraught with potential danger including resistant strains and super infections[17].

These draw backs can be markedly reduced if antimicrobial agent to be used locally. Because of the smaller dosage used and topical chemotherapy is much safer than systemic chemotherapy in avoiding the side effects of antibacterial agents[16]

Local Drug Delivery:

Local applications (as mouth rinse, gels, tooth paste etc,) control only supra gingival microbial plaque or periodontal disease involving pocket formation and also requires high initial concentrations and multiple applications in order to provide sustained effectiveness[18.]

Local application of antibiotics has been achieved either by subgingival irrigation or by incorporating the drug into different devices for insertion into periodontal pockets. Many drugs like chlorhexidine, tetracycline are tried as mouth rinses in the treatment of periodontal diseases. In-spite of its superior effects, chlorhexidine does not reach the periodontal pocket when administered as mouth rinse. Subgingival irrigation of antimicrobial involves local drug delivery but not controlled release.

Local drug delivery devices are of two types. In the first type, the drug delivery system is designed to deliver agent locally in the periodontal pocket but without any mechanism to retain therapeutic levels for a prolonged period of time. Such device generally exhibits exponential increase and decrease in drug concentration at the

site[19].

Second type is the controlled release local drug delivery devices which may secure antimicrobial effect for a prolonged period of time at the diseased site, than that can be achieved by systemic or local topical applications and also by passes the systemic complications[20].

The controlled release delivery of antimicrobials directly into periodontal pocket has received greatest interest and appears to hold some promise in periodontal therapy. These delivery systems are produced by immobilizing antibiotic and antimicrobial agents with a carrier substance to provide controlled local release.

Local antimicrobial therapy in periodontitis involve direct placement of antimicrobial agents into subgingival sites minimizing the impact of the agents on non oral body sites. Local antimicrobial agents may be personally applied as a part of home care oral hygiene regimens and/or professionally applied as part of clinic based treatment procedures. Local antimicrobial therapy in periodontitis may be further classified as providing either non-sustained or sustained subgingival drug delivery. Non-sustained subgingival drug delivery provides high pocket concentrations of the antimicrobial agent over an extended time period within periodontal pockets. Controlled drug release can be provided with subgingival irrigation of an agent intrinsically substantive for both tooth surfaces or pocket placement of commercial antimicrobial fibers, gel or films. The potential application of these new concepts to periodontology and the treatment of periodontal infections was championed and developed into a viable concept primarily by Dr. J. Max Goodson[24].

Table 1.2:- Susceptibility of infective microorganisms to the major antimicrobials.

	Pen Ciln e	Oxacilln	Amoxicillin	Cef/1	Macolides	Clindamycin	Metronidazole	Tetracycline	Levofloxacin	Gatifloxacin
Aerobic bacteria										
Streptococc-us Group A	+	+	+	+	+	+	0	A±	+	+
Streptococc-us spp	+	+	+	+	+	+	0	+	+	+
Staphylococ-cus spp	0	+	+	+	+	+	0	A±	A±	+
Capnocytop-haa spp	+	+	+	A±	A±	+	0	+	+	+
Eikenella spp	+	0	+	0	A±	0	0	+	+	+
Anaerobic bacteria										
Peptostrepto-coccus spp	+	+	+	+	+	+	A±	A±	A±	+
Actinomyce-s spp	+	+	+	+	+	+	+	A±	+	+
Prevotella spp	+	A±	+	0	0	+	+	A±	A±	A±
Porphyromo-ns spp	A ±	A±	+	0	0	+	+	A±	0	A±
Fusobaceriu-m spp	A ±	A±	+	0	A±	+	+	A±	0	A±
Bacteoride	A ±	A±	+	0	0	+	+	A±	0	A±

A ±: Higher activity towards organisms

+: Moderate activity

0: No activity

Cef/1 – first generation cephalosporins

LOCAL ANTIMICROBIAL AGENT POCKET DELIVERY

Advantages of Local Controlled Release Devices:

- It is useful in controlling and monitoring the desired drug levels in the site.
- It allows local modification of tissue permeability inhibit protease activity or decrease immunogenic response.
- It is a useful means of delivery of drug to the oral cavity that is not absorbed into the gastro intestinal system (e.g. chlorhexidine).
- It bypasses hepatic first pass metabolism, therapy offering a greater bioavailability and reduction in dosage[21].
- The drug escapes the acidic environment of the stomach.
- Therapeutic serum concentrations of the drug can be achieved more rapidly
- Sustained release drugs offer a onetime application has an advantage over repeated application.

Disadvantages:

- There is difficulty in placing therapeutic concentrations of antimicrobial agent into deeper parts of periodontal pockets and frication lesions.
- Personal application of antimicrobial agent by patient's lack of adequate manual dexterity, limited understanding of periodontal anatomy and poor compliance and performance with recommended procedures.
- The task of professionally applying local antimicrobial agent in periodontitis patients with numerous advanced lesions distributed throughout their mouth is time consuming and labor intensive.

- Antimicrobial agents locally applied into periodontal pockets do not markedly affect periodontal pathogens residing within adjacent gingival connective tissues and on extra-pocket oral surfaces, which increases the risk of later reinfection and disease recurrence in treated areas.

Success of any drug system designed to target periodontal infection depends upon its ability to deliver the anti-microbial agent to the base of pocket at a bacteriostatic or bactericidal concentration. It must also facilitate retention of medicament long enough to ensure an efficacious result. Commonly used techniques[22] to administer antimicrobial with regard to attaining these criteria are compared in Table 1. 3.

TableNo.1.3: Comparison of Drug Delivery Systems for Management of Periodontitis.

	Mouth Rinse	Subgingival Irrigation	Systemic delivery	Controlled delivery
Reaches site of disease and activity	Poor	Good	Good	Good
Adequate drug concentration	Good	Good	Fair	Good
Adequate duration of therapy	Poor	Poor	Fair	Good

Local antimicrobial therapy in periodontitis involves direct placement of an antimicrobial agent(s) in to subgingival sites, minimizing the impact of the agent(s) on non oral body sites.

Types Of Local Antimicrobial Agent Therapy In Periodontal Diseases Include.

1. Personally applied (in-patient home self care)

- Non-sustained subgingival drug delivery (home oral irrigation)
- Sustained subgingival drug delivery (Not developed to date)

2. Professionally applied (in dental office)

- Non sustained subgingival drug delivery (Professional pocket irrigation)
- Sustained subgingival drug delivery (Controlled release devices)

Controlled drug delivery systems are designed to deliver the drug slowly for prolonged periods for sustaining drug action. These dosage forms are commonly referred as sustained-release, controlled-release, timed-release and slow release. These technologies assure therapeutic concentrations of the antimicrobial agents in the subgingival area, at least for 3 days following a single application. Most controlled release devices for periodontal application are polymer based, with diffusion of drugs across a rate controlling membrane.

CONTROLLED RELEASE LOCAL DELIVERY DEVICES

These devices employ the controlled release technologies to assure therapeutic concentrations of the antimicrobial in the sub gingival area for at least 3 days following a single application.

Different Types of Controlled Release Local Delivery Devices Fall Into Following Groups

Reservoir devices (membrane diffusion systems)

This includes dialysis tubing 3-5 mm long, 0.2 mm wide containing a core of drug

solution. This is left in the pocket for a week. Reservoir devices that lack rate control include hollow fibers, gels and dialysis tubing. These systems tend to release chemotherapeutic agents very quickly and only marginally qualify as sustained release devices. The problems associated with these devices are, irritation of the pocket, premature loss from the pocket and rapid drug release[23].

Monolithic devices

Bio absorbable, biodegradable materials can be left in situ. This eliminates the risk of disturbing a site after therapy. The controlled release local delivery devices (monolithic) are usually polymers (films/fibers) containing homogeneously dissolved or dispersed drug.

In these devices, the drug is dispersed in a solid polymer matrix, Examples include acrylic strips and ethylene vinyl acetate (EVA) fibers. Acrylic strips are typically 0-2 mm thick. Treatment is carried out over 2 - 4 weeks, with a replacement of new strips inserted each week. Drug release occurs over a period of 10 - 14 days. Strips tend to be lost from the pocket. This can be avoided by applying periodontal dressing. Other monolithic devices include strips made of ethyl cellulose (EC), poly ethylene glycol (PEG), Hydroxy propyl methyl cellulose (HPMC) and cross linked collagen films[23].

The two basic types are:

1. Fixed monolithic devices: Where the polymer maintains its integrity as the drug is lost.
2. Erodible monolithic devices: Which breaks up as the drug is released.

If the drug is present as dispersion, the release will be proportional to the square root of time and if the drug is present only dissolved in the constant release rates, then decay exponentially. It is possible to obtain more constant release rates by increasing

the concentration of drug towards the center of the monolithic core to produce a laminated device. The various polymers used for the monolithic device are ethylene vinyl acetate co-polymer, ethyl cellulose, methyl methacrylate, poly ethylene-2 hydroxy ethyl methylacrylate, poly (ortho esters), atellocollagen etc.

The device should be designed for rapid insertion and to minimize the pain and discomfort to the patient. The monolithic devices can be prepared conveniently by using simple polymer fabrication techniques.

Melt fabrication Technique

Films can be produced by extrusion in to thin film. Another process known as calendaring, where the polymer is squeezed between heated rollers to form film[23].

Solution casting

The polymer is dissolved in a suitable solvent to form a viscous solution, which is then spread on flat non-adhesive surface and the solvent is allowed to evaporate. The resultant film is peeled from the surface.

Polymerization In situ

A liquid polymer or pre polymerized inside a suitable mould. The release from monolithic devices depends on diffusion of drug through matrix. By manipulating the system, selecting the ideal polymer, adjusting the cross-linking, fillers, plasticizers and by using co-polymers, release of some low molecular drug can be achieved.For an antimicrobial agent to be successful the pathogen must be known, it must be susceptible to the drug. It should not readily develop resistance for an adequate period of time. Also the drug should have little or no side effects [24].

MECHANISMS OF DRUG RELEASE

The release of drug from a localized drug delivery system can be achieved by the following mechanisms.

1. Pure diffusion
2. Chemical reactions
3. Counter-current diffusion
4. Externally imposed controls

Controlled release polymeric systems can be classified on the basis of the mechanism controlling the release of the incorporated drug.

DIFFUSION CONTROLLED SYSTEMS

a) Reservoir systems

In these systems, core of the drug is surrounded by a swollen or non swollen polymer film and diffusion of the drug through the polymer is the rate limiting step. Reservoir devices are also of two types. Reservoir without a rate controlling system. Reservoirs that lack rate control include hollow fibers, gels and dialysis tubing. Reservoirs with rate controlling systems include erodible polymeric matrices, polymer membranes, monolithic matrices and coated particles[25].

b) Matrix systems

In these systems, the drug in uniformly distributed throughout a solid polymer. The drug diffusion through the polymer matrix is the rate limiting step.

CHEMICALLY CONTROLLED SYSTEMS

a) Bioerodable System:

In these systems, a drug is uniformly distributed throughout the polymer, and the drug is released by diffusion as the polymer phase decreases with time. As the polymer surrounding the drug resorbed, the drug escapes.

b) Pendant Chain System:

In these systems, the drug is chemically bound to a polymer back bone and drug release occurs via hydrolytic or enzymatic cleavage.

2.NEED FOR THE STUDY

In conventional mode of administration many drugs do not reach target areas in the body in sufficient concentration because of premature inactivation and excretion. This problem can be overcome by administering the drug directly to the intended site of action with lesser dose. In recent years, novel drug delivery systems are becoming popular as pharmaceutical technologists today are able to provide the drug delivery systems with very precise control over drug release for a prolonged period of time eliminating the need for frequent dosing and minimizing side effects, thereby increasing patient compliance and comfort.

The most common dental infections include dental carries, dental alveolar infections, gingivitis, periodontitis, deep facial face infections and osteomyelities. If untreated, dental infections spread and contribute to polymicrobial infections at other sites. Among the above mentioned dental infections periodontitis, a common cause of tooth lose is one of the chronic infections of oral cavity, primarily caused by gram positive, gram negative and anaerobic bacteria that reside in sublingual area.

The systemic administration of drugs for treating dental conditions can reach the systemic circulation via serum and hence the microorganisms at the depth of the periodontal sites and also the possible organisms residing within gingiva and capable of eliminating pathogens not only from periodontal lesion but from oral cavity as well.

The greatest disadvantage of systemic administration of antimicrobials for periodontitis is that, the drug is diluted several thousand folds before it reaches the site and exposes the rest of the body to potential side effects. In some patients, loss of

attachments develops very rapidly and continuously in spite of proper therapy.

A site specific system aims at delivering the therapeutic agent at sufficient levels inside the pocket and at the same time minimizing the side effects associated with systemic drug

3.AIMS&OBJECTIVES

The purpose of the present investigation is to develop the polymeric inserts (chitosan) containing different concentration of the anti-bacterial agent Doxycycline for local controlled drug delivery into periodontal pockets. Apart from this, an investigational study is planned to prolong the drug release for more number of days by formulating in to multilayer (polymer) inserts.

Specific objectives of the present investigation are as follows,

1. To develop a chitosan single layer with different concentration of doxycycline hyclate (10%, 20% and 30%).
2. To develop a chitosan multilayer inserts containing doxycycline hyclate.
3. Evaluation of prepared inserts for physical characteristics such as

- ✓ Thickness and Weight Variation
- ✓ Folding Endurance
- ✓ Percentage Moisture Loss
- ✓ Percentage Moisture Absorption
- ✓ Drug Content Uniformity
- ✓ Tensile Strength
- ✓ Differential Scanning Calorimetry
- ✓ Fourier Transfer – Infrared Spectroscopy(FT-IR)
- ✓ Water Uptake and Swelling Index

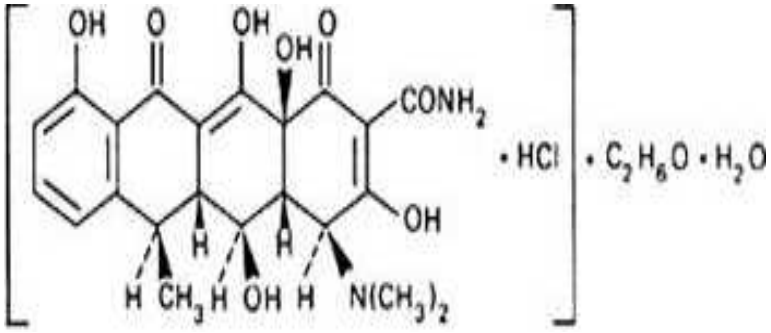
4. To study static dissolution pattern of the drug dosage forms.
5. *In-vitro* antibacterial activity.
6. Stability studies at different temperature as per ICH guidelines.

4. DRUG PROFILE

DOXYCYLINE HYCLATE:

Chemical Name:-(4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)1,4,4a,5,5a,6, 11,12aoctahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacene carboxamide monohydrochloride, compound with ethyl alcohol (2:1), mono-hydrate; [4*S*-(4α,4aα,5α,5aα,6α, 12aα)]-4-(dimethylamino)1,4,4a,5,5a,6,11,12a-octahydro-3,5,10, 12, 12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacene carboxamide monohydrochloride[26].

- **Structure:-**



- **Molecular formula :-** (C22H24N2O8 · HCl)2 · C2H6O ·H2O
- **Molecular weight :-** 444.435 g/mol
- **Melting point :-** 201-202 ^{0}C
- **Physical form :-** yellow crystalline powder
- **Solubility :-** Freely soluble in water acetic acid, 6.6 pH buffer and acetic acid
- **Pharmacology:**

Tetracyclines are readily absorbed and are bound to plasma proteins in varying degree. They are concentrated by the liver in the bile, and excreted in the urine and

feces at high concentrations and in a biologically active form. Doxycycline is virtually completely absorbed after oral administration.Following a 200 mg dose, normal adult volunteers averaged peak serum levels of 2.6mcg/ml of doxycycline at 2 hours decreasing to 1. 45 mcg/ml at 24 hours.

Excretion of doxycycline by the kidney is about 40%/72 hours in individuals with normal function (creatinine clearance about 75 ml/min). This percentage excretion may fall as low as 15%/72 hours in individuals with severe renal insufficiency (creatinine clearance below 10mL/min). Studies have shown no significant difference in serum half-life of Doxycycline (range 18-22 hours) in individuals with normal and severely impaired renal function.

Hemodialysis does not alter serum half-life. Results of animal studies indicate that tetracycline cross the placenta and are found in fetal tissues[27].

- **Mechanism of action:**

Doxycycline, like minocycline, is lipophilic and can pass through the lipid bilayer of bacteria. Doxycycline reversibly binds to the 30 S ribosomal subunits and possibly the 50S ribosomal subunit(s), blocking the binding of aminoacyl tRNA to the mRNA and inhibiting bacterial protein synthesis. Doxycycline prevents the normal function of the apicoplast of Plasmodium falciparum, a malaria causing organism[27].

- **Pharmacokinetics:**

1	Absorption	Complete, no interference by food
2	Protein binding	High
3	Bioavailability	90-95 %
4	Plasma half life	18-22 hrs
5	Metabolism	Hepatic
6	Excretion	Urine, feces

- **Elimination: Half-life :-** 18-22 hours

- **Drug interactions:**

If used in conjunction with the anesthetic methoxyflurane there can be severe or fatal kidney damage. May interact with anticoagulants and its effectiveness is lowered by over the counter antacids and bismuth subsalicylate, barbiturates, the anticonvulsants carbamazepine and phenytoin. Tetracycline blocks the action of bactericidal antibiotics such as penicillin and should not be given in combination with such antibiotics.

- **Contraindications:**

Doxycycline and other tetracyclines must not be given to women during the last half of pregnancy or to children under the age of eight. It can affect development of the skeleton in the fetus and can cause discoloration and malformation of the teeth. Any demonstrated sensitivity to tetracycline contraindicates use. Some people may show increased sensitivity to sunlight[28].

- **Side effects:**

Losses of appetite, nausea, vomiting and diarrhea have been observed. Hypersensitivity, anaphylaxis, and exacerbation of immune disorders have been seen. Hemolytic anemia and other disturbances of the white cell population of the blood are known[28].

5.POLYMER PROFILE

CHITOSAN

- **Synonyms :-** 2-Amino-2-deoxy-(1,4)-β-D-glucopyranan; deacetylated chitin; deacetylchitin; β-1,4-poly-Dglucosamine; poly-D-glucosamine; poly-(1,4-β-D-glucopyranosamine).
- **Chemical Name and CAS Registry Number :-** Poly-β-(1,4)-2-Amino-2-deoxy-D-glucose [9012-76-4]
- **Molecular weight :-** 10000-1000000
- **Origin of Chitosan**

One reason why chitosan has become of interest is undoubtedly because it can be obtained from natural sources that are abundant and renewable. Chitosan is prepared from chitin, the polymer second most abundant in nature after cellulose (Roberts, 1992). Chitin is the primary structural component of the outer skeletons of crustaceans, and of many other species such as molluscs, insects and fungi. The role played by chitin is similar to the roles played by cellulose in plants and collagen in higher animals. It is a reinforcing material, which occurs in three polymorphic forms, and chitin. Where hardness in needed chitin is found, where flexibility is required and chitin occurs. Chitin is inert in aqueous environment. This property limits the use of chitin as such. Chitosan is prepared from chitin to obtain a more reactive polymer[29].

Physical Properties

- Particle size < 30nm
- Density 1.35 – 1.49 g/cc
- Solubility: Insoluble in water but soluble in acids.

- **pH (1% w/v solution) :** 4.0–6.0

❖ **Solubility:**

Sparingly soluble in water; practically insoluble in ethanol (95%), other organic solvents, neutral or alkali solutions at pH above approximately 6.5.

❖ **Description:**

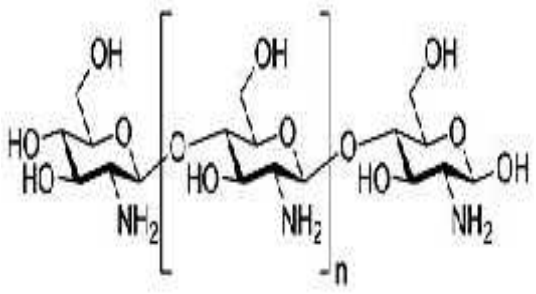
Chitosan occurs as odorless, white or creamy-white powder or flakes. Fiber formation is quite common during precipitation and the chitosan may look 'cottonlike'.

❖ **Functional Category:**

Coating agent, disintegrate, film-forming agent, mucoadhesive, tablet binder, viscosity increasing agent.

❖ **Structure:**

Chitosan is a linear polysaccharide consisting of ß (1-4)-linked 2-amino- 2-deoxy-D-glucose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (N-acetylglucosamine) units. The structure of chitosan is very similar to that of cellulose (made up of ß (1-4)-linked D-glucose units), in which there are hydroxyl groups at C2 positions of the glucose rings[30].



❖ **Mechanism of action:**

Chitosan formulations can easily be prepared using conventional tableting or granulating methods. The simplest way of achieving slow release of drugs by means of chitosan is to employ it as a gel-forming excipient in matrix-type formulations.

Retardant effects of chitosan on drug release were noticed as early as the 1980s. Chitosan was found to decrease rates of release of drugs from tablets during dissolution tests at acidic and slightly acidic pH levels (Kawashima et al., 1985; Acartürk, 1989). However, Akbua (1993a), who carried out studies at higher pH levels (pH 7.4), reported that chitosan exhibited no slow-release properties[29, 30].

❖ **Features:**

The Chitosan (CS) is known to be non-toxic and odorless. Chitosan has received considerable attention as a possible pharmaceutical excipient in recent decades Chitosan is an excellent polymer to be used for buccal delivery because it has muco/bioadhesive properties and can act as an absorption enhancer. Chitosan gives prolonged release of the drug in the buccal cavity improving the antimicrobial activity of the drug. Chitosan periodontal film with drug incorporated have antimicrobial activity due to the chitosan. The buccal bilayered devices (bilaminated films,) using a mixture of drugs and chitosan, with or without anionic crosslinking polymers has promising potential for use in controlled delivery in the oral cavity[30].

❖ **Properties of Chitosan:**

Cationic Polyamine

Chitosan has highly charged density at pH < 6.5 i.e. one charge per glycosamine unit. The ionic character along with reactive functional group in chitosan has made it a suitable polymer for utilization in controlled release technology. The molecular weight of chitosan varies from 50 KDa to 2000 KDa[29].

Amenable to Chemical Modifications

Chitosan is linear polyamine where amino groups are readily available for chemical reaction and salt formation with acids. The possibility of amino groups suggests that

chitosan could be used to formulate therapeutic system where the drug release kinetics depends on erosion pathway of polymeric network due to hydrolysis of cross-linking bonds.

Flocculation and Mucoadhesive Properties

Chitosan formulations exhibit ease of delivery [30, 31] a good retention at the application site, and a controlled release of the drug. Chitosan is shown to have an antimicrobial activity against *P. gingivalis* and higher activity with high molecular weight chitosan.The combination of chitosan with chlorhexidin showed a significant higher activity, when compared to that of chlorhexidine alone, which would provide chlorhexidine application at lower concentrations thus avoiding its unwanted side effects. Chitosan films and gels seem to be promising delivery systems for local therapy of periodontal diseases with its bioadhesive property and antimicrobial activity [32].

Biological Properties

Its biological properties include non toxicity, biodegradability and biocompatibility.

N-acyl chitosan showed the best blood biocompatibility due to increase of surface hydrophilicity and induction in hydrophilic and hydrophobic properties at the surface.

CLINICAL APPLICATION

Wound Healing

Chitin and chitosan are effective wound healing accelerators, in both animal and human tests, the surgical adjuncts of regenerated chitin are physiologically compatible, bioabsorbable and effective wound healing accelerators. N-Carboxybutyl

chitosan was studied in wound healing in rabbits; where complete re-epithelialization was observed with all the epithelial layers represented on 30th day of treatment, an interesting role in tissue reconstruction. Chitosan has also been used to prepare bandages for wound healing.

The physiological compatibility of chitin with living tissues, combined with its ability to form readily sulfate esters which are non thrombogenic appears to make chitin a most promising candidate for prosthetic structural devices of any desired shape or sizes[31, 33].

Haemostatic Agent

Chitosan solution formed a coagulum in contact with whole blood, where the possible mechanism appeared to be a reaction between red cell membrane and the chitosan solution leading to cross linkage or re-polymerisation. Chitosan is an effective and adequate homeostatic agent even under the most severe conditions of anticoagulation. Introduction of uronic acid carboxyl groups increases the anticoagulant activity of sulfacted chitosan.

Application in Dentistry

Many advantages are attainable by use of chitosan in periodontology such as decrease subjective symptomatology, good homeostatic action, and delayed release of antibiotics, wound healing acceleration and better condition for asepsis. Chitosan has been used in 24 patients all of which recovered completely. No allergic reactions or infections took place. Chitosan could be used as transparent membrane or preferably as a thin powder soaked in antibiotic solution, it accelerated wound healing, promoted regular fibrin formation and favored the epithelialization.

6. REVIEW OF LITERATURE

REVIEW OF PAST STUDY DONE ON DOXCYCLINE DRUG

- **Gaurav Tiwari1 et al.,** release studies of metronidazole and doxycycline from polycaprolactone films prepared by solvent evaporation. metronidazole and doxycycline with different antibacterial spectra are proposed to be formulated as combination therapy to have a broader antibacterial therapy, which is effective against both aerobic and anaerobic periodontal microflora. Simultaneous use of metronidazole and doxycycline is effective against wide range of periodontal pathogens. Metronidazole and doxycycline release from polycaprolactone films was zero-order after an initial period. Significant burst effects were observed with metronidazole and doxycycline (5, 10% w/w) [34].
- **Mohammed M Al-Abdaly, et al.,** studied the effects of topical application of Atridox (Doxycycline gel) in management of chronic periodontitis. This study was carried out on 15 patients (aged 25-55) with chronic periodontitis. They were received scaling and root planning (SRP) alone in one side and SRP plus Atridox (Doxycycline gel) in other side. Each individual was subjected to the following measurements. Evaluation of the clinical parameters pre and post treatment to detect the outcome of the treatment modality and Denial plaque samples initially and at 3, 6, 9 and 12 months were obtained for microbiological evaluation. Atridox (Doxycycline gel) delivered locally into periodontal disease sites reduced all subgingival bacteria. Both treatment and modality led to a highly statistically significant reduction in microbiological counts as well as clinical parameters applied. No clinical relevant side effects were observed[35].
- **Thawatchai Phaechamud et al.,** studied the chitosan sponges loaded with Doxycycline hyclate and their antibacterial activities. The pore density of

chitosan sponge prepared with freeze drying technique was increased as the higher concentration of chitosan solution was used. The sponge prepared from 10% w/w of the chitosan solution and crosslinking with glutaraldehyde solution was utilized for loading with doxycycline hyclate. The drug release and sustainable antibacterial activity of fabricated sponge were assessed using dissolution test and agar diffusion test, respectively. Drug release from non-crosslinked sponge into phosphate buffer pH7.4 was slower than that from crosslinked sponge since the former could absorb the medium and form gel to retard the initial drug diffusion. Sustainable antibacterial activity of developed sponge was evident against *S. aureus* and *E. coli*. Results indicated that the *in vitro* release profile and antibacterial efficiency of doxycycline hyclate could be sustained using chitosan sponge[36].

- **N. Damodharan et al.,** developed delayed release doxycycline tablets for targeting to small intestine tablets were prepared by wet granulation method and enteric coating of tablets (conventional standard coating technique). Using pH depended polymers like Eudragit and HPMC Phthalate. Preformulation studies like angle of repose, bulk density, tapped density, porosity, Carr's index, Hausner's ratio were performed. Six batches (F1 to F6) were formulated and evaluated for hardness, friability, weight variation, drug content, disintegration and *in-vitro* dissolution. Among the six batches, batch F4 was showed 94% drug release and was considered as best formulation[37].
- **S.Shanmuganathan N et al.,** prepared Doxycycline loaded

chitosan microspheres were developed using a novel water-in-oil emulsion technique, involving oil phase ionic gelation. Microspheres were prepared by using 6% v/v of chitosan (3% w/v in acetic acid), soya oil–n-octanol oil mixture (1:2 v/v) as continuous phase and 5% span 80 as emulsifier. Doxycycline was entrapped by equilibrium swelling method with 8.4% total entrapment. The drug-loaded spheres were spherical with smooth surface morphology. The MTT assay showed that doxycycline-loaded microspheres were able to improve the percentage cell viability in comparison to the pure drug. In vitro release studies showed that a burst release of 42% in 6 h was achieved and maintained an equilibrium concentration of 72% in 24 h. Assessment of antibacterial activity showed that doxycycline was able to exhibit a minimum microbicidal concentration (MIC) of 16.5, 17.4, 11.2 and 98.3µg against ***Klebsiella pneumoniae*** **(ATCC15380),** ***Escherichiacoli*** **(ATCC25922),** ***Staphylo coccusaureus*** **(ATCC9144) and** ***Pseudomonas aeruginosa*** **(ATCC 25619),** respectively. Gelatin zymography studies revealed that it could inhibit MMP 2 and MMP9 at sub-antimicrobial concentration. The present investigation provides scope for using doxycycline-loaded chitosan microspheres for healing infected wounds[39].

- **MarcoAntonio Botelho etal.,** aim of this study was to test the efficacy of a locally applied 8.5% nanostructure doxycycline (DOX) gel in preventing alveolar bone loss in experimental periodontal disease (EPD) in rats by using the tapping mode atomic force microscopy (AFM). Material and method EPD was induced in 24 Wister rats. Animals were treated with the doxycycline gel topically, immediately after EPD induction, and 3 times a day during 11 days. Four groups

(n=6) were formed as follows: each group (animals not subjected to EPD nor treated); non-treated (NT) group (animals subjected to EPD, but not treated); vehicle gel (VG) group (animals subjected to EPD and treated with topical gel vehicle); and DOX group (test group) animals subjected to EPD and treated with the 8.5% DOX gel. In order to investigate topographical changes in histological sections, a novel simple method was used for sample preparation, by etching sections from paraffin-embedded specimens with xylol. Results: comparing the AFM images, several grooves were observed on the surface of the alveolar bone and other periodontal structures in the NT and VG groups, with significantly greater depths when compared to the DOX group[40].

- **Narayana Charyulu et al.,** developed strips of chitosan polymer 2% containing ciprofloxacin Hcl, norfloxacin and doxycycline by solution casting method using 1% acetic acid as casting solvent. Macroscopical features revealed that drugs were dissolved in the polymer. The DSC and UV scan studies confirmed the absence of any chemical interaction between the drugs and polymer. Short term stability studies were carried out at different temperature. The static dissolution showed a burst release effect initially followed by a progressive fall in the release. Mass balance studies did not deviate by more than 3%. It was observed from the above studies that the chitosan strips have an ability to sustain the release of drugs and more useful as slow release device for periodontal disease[41.]
- **Heba A. Gad et al.,** formulated *in situ* implants containing doxycycline hydrochloride and/or secnidazole that could be used in the treatment of periodontitis by direct periodontal intrapocket administration. Biodegradable polymers [poly (lactide) (PLA) and poly (lactide-co-glycolide) (PLGA)], each polymer in two concentrations 25%w/w, 35%w/w were used to formulate the

insitu implants. The rheological behavior, *in vitro* drug release and the antimicrobial activity of the prepared implants were evaluated. Increasing the concentration of each polymer increases the viscosity and decreases the percent of the drugs released after 24 h. PLA implants showed a slower drugs release rate an PLGA implants in which the implants composed of 25% PLGA showed the fastest drugs release. The in vitro drug release and antimicrobial activity results were compared with results of Atridox®. Results revealed that the pharmaceutical formulation based on 25% PLGA containing secnidazole and doxycycline hydrochloride has promising activity in treating periodontitis in comparison with Atridox[42].

PAST WORK DONE ON POLYMER (CHITOSAN)

- **Shankraiah et al.** were prepared Flexible films of sparfloxacin for periodontitis treatment for easy insertion into periodontal pockets. The optimum concentration of chitosan used for the preparation of strips was found to be 2% w/v, because at this concentration the strips were flexible and easily removable from the die. An optimum concentration of drug to be loaded was found to be 30% w/v to the polymer or less than that. For the present investigation, chitosan strips containing sparfloxacin with three different concentrations, i.e.10, 20, and 30% to the weight of the polymer, were prepared using the solvent casting method. All the formulations were found to contain almost uniform quantity of drug as per content uniformity studies, indicating reproducibility of the technique[43].
- **Dan Mei et al.,** The objective of this work was to assess and compare the absorption promoting effect of different molecular-weight chitosans, trimethyl chitosans and thiolated chitosans for intranasal absorption of 2,3,5,6-tetramethylpyrazine phosphate (TMPP). An in situ nasal perfusion technique in rats was utilized to test the rate and extent of TMPP absorption in situ. *In vivo* studies were carried out in rats and the pharmacokinetic parameters were calculated and compared with that of intravenous injection. All the chitosan derivatives investigated could enhance the intranasal absorption of TMPP significantly. However, thiolation could not improve the absorption-enhancing capacity of chitosan remarkably even when the thiolation ratio was as high as 152 lmol/g. In contrast, trimethylated chitosan exhibited stronger absorption-enhancing ability than the homopolymer chitosan. The permeation enhancing effect of chitosan increased with increasing molecular weight up to Mw 100 kDa. In vivo studies indicated that chitosan 100 kDa and TMC 50 kDa had comparable

absorption-enhancing effect but chitosan 100 kDa functioned for more than 120 min versus 90 min for TMC. A good correlation was found between the in situ absorption data and plasma concentration in vivo for the polymers investigated. This study demonstrated that both chitosan structural features and chitosan molecular weight play a key role on promoting the intranasal absorption of TMPP. Taking safety reason into account, chitosan 100 kDa is the most promising as an intranasal absorption enhancer.[44].

- **Barat R et al.,** chitosan based metronidazole inserts were fabricated by the casting method and characterized with respect to mass and thickness uniformity, metronidazole loading and *in vitro* metronidazole release kinetics. The fabricated inserts exhibited satisfactory physical characteristics. The mass of inserts was in the range of 5.63 ± 0.42 to 6.04±0.89 mg. The thickness ranged from 0.46 ±0.06 to 0.49 ±0.08 mm. Metronidazole loading was in the range of 0.98 ±0.09 to 1.07±0.07 mg except for batch CM3 with MZ loading of 2.01V± 0.08. The inserts exhibited an initial burst release at the end of 24 hr, irrespective of drug to polymer ratio plasticizer content or cross linked. However further drug release was sustained over the next 6 days, cross linking to 10% (m/m) of glutaraldehyde inhibited the burst release by~30 % and increased the mean dissolution time (MDT) from 0.67 to 8.59 days. The decrease in drug release was a result of reduced permeability of chitosan due to cross linking[45].
- **Senel S, IkicI G.et.al.,** developed the formulation containing chitosan for local delivery of chlorhexidine gluconate (chx) to the oral cavity. Gels at (1 or 2% concentration) or film forms of chitosan were prepared containing 0.1-0.2% chx (chlorhexidine gluconate) and there *in vitro* release properties were studied. The

antifungal activity of chitosan itself as well as their various formulations containing chlorhexidine gluconate (chx) was also examined. Release of chlorhexidine gluconate (chx) from gels was maintained for 3hr. A prolong release was observed with film formulation. No lag time was observed in release of chx from either gels or films. The highest antifungal activity was obtained with 2% chitosan gel containing 0.1% chlorhexidine gluconate (chx.)[46].

- **Amal Hassan El-Kamel et al**., developed a local, oral mucoadhesive metronidazole benzoate (MET) delivery system that can be applied and removed by the patient for the treatment of periodontal diseases. Mucoadhesive micromatricial chitosan poly(ε-caprolactone) (CH/PCL) films and chitosan films were prepared. Thermal behavior, morphology, and particle size measurements were used to evaluate the prepared films. The effect of different molar masses of CH and different ratios of medium M.wt.molar mass chitosan (MCH): PCL on water absorption, *in vitro* bioadhesion, mechanical properties, and *in vitro* drug release was examined[47].
- **Ritu B. Dixit et al.,** developed cefadroxil drug loaded biopolymeric films of chitosan-furfural schiff base were prepared by reacting chitosan with furfural in presence of acetic acid and perchloric acid respectively for the external use. Prepared films were evaluated for their strength, swelling index, thickness, drug content, uniformity, tensile strength, percent elongation, FTIR spectral analysis and SEM. The results of *in vitro* diffusion studies revealed that the films exhibited enhanced drug diffusion as compared to the films prepared using untreated chitosan. The films also demonstrated well to moderate antibacterial activities against selective gram positive and gram negative bacteria[48].

- **Mohammed Gulzar Ahmed et al,** developed chitosan strips containing Gatifloxacin (10%, 20% and 30% to the weight of polymer) were prepared by solution casting method using 1% v/v acetic acid in water. Further strips containing 30% gatifloxacin were cross-linked by exposing to the vapours of 2% v/v glutaraldehyde in water intended to extend the release. The prepared films were evaluated for their thickness, content uniformity, weight variation, tensile strength, hardness and *in-vitro* dissolution. The average weight and thickness of both the cross linked and uncross-linked strips were uniform. There was a reduction in the tensile strength and increase in hardness when the films were cross-linked. Static dissolution studies showed a burst release initially followed by a progressive fall in the release of the drug and extended up to 19 days once the strips were cross-linked. Release kinetics of gatifloxacin from chitosan strips followed the higuchi's diffusional model and also showed zero order release profile[49].

- **S.Sangeetha et al.,** Nanoparticle made up of biodegradable carrier such as chitosan have advantage of providing stearic interference in the systemic circulation. Cytarabine nanospheres were prepared by ionic gelation method with an objective of improving its intracellular targeting and thereby targeting the cancer cells. The average particle size determined through SEM was found to be 466.45 ± 5.32 nm. The average drug loading was found to be 62% for the batch loaded with 1.50 mg/ml of drug when compared to other batches. The cumulative percentage of drug release from all the drug loaded batches at 18 hours was found to be in the range of 86.73 % to 93.50%.Among the four batches of nanoparticles formulated the batch containing 1.50mg of drug/ml of polymer showed the highest of about 93.50% of release. Release of drug from the matrix was by non-

Fickian analomous diffusion mechanism. *Invivo* bio distribution studies showed that the Cytarabine in the form of nanoparticles was having a greater bio distribution when compared to free drug in different organs like liver, spleen, lungs and kidney[50].

- **K. Bansal et al.,** develop satranidazole-containing mucoadhesive gel for the treatment of periodontitis. Different mucoadhesive gels were prepared, using various gelling agents like sodium carboxymethylcellulose (SCMC), poloxamer 407, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl methyl cellulose, and the mucoadhesive polymer carbopol 934P. The selected formulations were studied for different mechanical properties, such as mucoadhesive strength, hardness, compressibility, adhesiveness, and cohesiveness through texture profile analyzer. *In vitro* satranidazole release from the prepared formulations was also determined and compared with marketed preparation of metronidazole. The formulation SC30 (Containing SCMC 3% w/v) showed maximum mucoadhesive strength (167.72 ± 3.76 g) and adhesiveness (−46.23 ± 0.34 Nmm), with low hardness (9.81 ± 0.04 N) and compressibility (40.05 ± 0.48 Nmm) and moderate cohesiveness (0.87 ± 0.01). SC30 formulation exhibited long-term release. Thus, SC30 gel was evaluated for its clinical effectiveness along with marketed metronidazole gel. At the end of the study (42 days of clinical studies), both formulations were found to significantly reduce the probing depth, plaque index, gingival index, calculus criteria, and bleeding index. However, the SC30 gel was more effective in reducing the above parameters than marketed metronidazole gel. This study confirmed the acceptability and effectiveness of satranidazole gel for treatment of

periodontitis.[51].

- **Mohammed Gulzar Ahmed et al.,** prepared Chitosan films containing tetracycline in three different concentrations were prepared by the solution casting method, using 1% v/v acetic acid solution for therapy of periodontal disease. The prepared films were evaluated for various properties. The stability studies did not show any significant changes. Static dissolution studies showed a burst release initially followed by a progressive fall in the release of the drug, and also showed extended release when cross-linking was attempted. The *in-vitro* release kinetics of tetracycline followed the zero order patterns[52].

REVIEW STUDY DONE ON LOCAL DRUG DELIVERY SYSTEM

- **Shankraiah et al.,** developed a sustained release device containing ornidazole for insertion within periodontal pockets. Cast films of ethyl cellulose with dibutyl phthalate as plasticizer, containing ornidazole were prepared. The films were evaluated for thickness, folding endurance, weight variation, content uniformity, tensile strength, *in vitro* antibacterial activity and *in vitro* release study. The release data obtained were subjected for release kinetics study. The study revealed that drug release was found to be diffusion controlled with sustained release of ornidazole over a period of nine days within the periodontal pocket[53].

- **G.L.Prabhushankar et al.,** developed a levofloxacin dental films for periodontitis between levofloxacin and polymers.The films were evaluated for their thickness uniformity, folding endurance, weight uniformity, content uniformity, tensile strength, surface pH and *in vitro* antibacterial activity[54].

- **Mastiholimath V.S et al.,** site specific one time delivery of ornidazole an antimicrobial compound with excellent activity against anaerobic micro-organism in the treatement of periodontal disease was prepared by solvent casting technique using ethyl cellulose, hydroxyl propyl cellulose, hydroxypropylmethyl cellulose K4M and eudragit RL-100 With dibutyl phthalate is plasticizer.The physiochemical parameters like thickness, weight variation, content uniformity and release characteristics were evaluated. The drug release was initially high on day one to achieve immediate therapeutic level of drug in pocket, followed by marked fall in release by day two and progressive moderate release profile to maintain therapeutic level following anomalous transport release profile mechanism. Formulation V6 released 97.07 % of drug at the end of 120 hour and was considered as best formulation. *In vitro* antibacterial activity was carried

out on *Streptococcus Mutans*[55].

- **Hong Hua., Chi ping.et al.,** prepared multilayer membranes which were loaded with drug for guided tissue regeneration were prepared using an immersed precipitation phase inversion technique, single layer, bilyer and trilayer mechanism were fabricated with chitosan used as carrier and tinidazole as medicine model which was loaded on the membrane. The influence of layer on structure and properties of membrane were studied by SEM, UV spectrometer and mechanical test. Drug release properties of three types of layer membrane also investigated. The result showed that release rate could be shown in both bilayer and trilayer membrane (11 days and 14 days respectively) and trilayer membrane lastest the longest. After a process of rapid release, the concentration of tinidazole which was released by the membrane was maintained at an efficient dosage level compared with single layer and bilayer membranes, be found trilayer membrane could play a role in controlling low rate drug release especially at the early stage of release, and keep an efficient dosage at affected part for a long period of time. The loss of drug which loaded on membrane decreased from 84.6% for Single layer to 13.04% for trilayer the mechanical strength of three types of membrane were detected an showed that it could meet the requirements of clinical practice the membranes specially with trilayer could be more valuable in application[56].

- **Sujatha Muchalambet al.,** Specific one time continuous delivery of sparfloxacin an antimicrobial compound with excellent activity against anaerobic micro-organisms in the treatment of periodontal disease was prepared by using solvent casting technique using hydroxy propyl cellulose, hydroxy methyl cellulose,

eudragit RL-100 and ethyl cellulose with dibutyl phthalate as plasticizer. The physicochemical parameters like thickness, weight variation, content uniformity and release characteristics were evaluated. The drug release was initially high on day one to achieve immediate therapeutic level of drug in periodontal pocket followed by marked fall in release by day two with progressive moderate release profile to maintain therapeutic level following anamolous transport mechanism. Formulation F4 released 90.24% of drug at the end of 120 hr and was considered as best formulation. *In vitro* antibacterial activity was carried out on *Streptococcus mutans*[57].

- **K. Schwach-Abdellaoui et al.,** Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by groups of specific microorganisms. Aggressive forms of periodontitis can be localized or generalized. The concept that localized problem sites may be treated by local drug delivery appears attractive as the antimicrobial agent is delivered within periodontal pockets and the therapy is targeted on specific pathogenic microorganisms. Local delivery of antimicrobial agents using controlled release systems should be considered as adjunctive to mechanical debridement for the treatment of localized forms of periodontal destruction. This article reviews various types of delivery systems evaluated in practical periodontal therapy. Despite the large number of studies showing an enhanced effectiveness of local antibiotherapy, there are insufficient Comparative data to support any of the local delivery system[58].

- **Vineet Bhardwaj et al.,** Chitosan films of different concentrations containing ofloxacin were prepared by solvent casting method. Some of the drug-loaded films were crosslinked with 2% gluteraldehyde for 1,2 and3 hrs, respectively. The films were then evaluated for their physicochemical properties including

weight variation, thickness, moisture loss, tensile strength, elongation, *in vitro* anti bacterial activity and *in vitro* release. I*n vitro* drug release data indicate that the films showed an initial burst release followed by sustained release of the drug. The drug-loaded films that were not crosslinked had effect on for up to 9 days and the films which are crosslinked for different duration shows release depended on concentration of chitosan in different films (1%, 2%, 3%)[59].

- **Steinberg et al.,** developed a degradable sustained release device composed of a cross linked protein containing chlorhexidine. The in-vitro release profile of chlorhexidine (from the degradable films) was altered by the amount of chlorhexidine loaded, cross- linking density of the polymer and type of chlorhexidine salt. This work demonstrated the release of chlorhexidine and showed that the degradation of the matrix can be controlled by factors in the formulation[60].

- **Hitzig et al.,** observed 28 patients suffering from adult periodontitis for a span of 3 months. They treated the patients with 5% metronidazole in collagen device in periodontal pocket more than 5 mm, keeping mechanical root debridement as control. Analysis of data from 28 patients indicated that both debridement and metronidazole therapy decrease pocket depth, bleeding on probing and gingival index, but results were significantly better with metronidazole[61].

- **Kurien et al.,** evaluated the efficacy of ciprofloxacin and norfloxacin when used in a sustained release system in periodontal pockets. Sustained release inserts were prepared using ethyl cellulose (EC) and hydroxypropyl methyl cellulose as (HPMC) co-polymers along with polyvinyl pyrrolidine (PVP), drug and ßeta cyclodextrin complex. The rate of release of the drug was controlled and

prolonged for 14 days period. It was found that both norfloxacin and ciprofloxacin appeared to be equally effective in reducing periodontal pathogens and in controlling the inflammation compared with the placebo treated control sites[62].

7. MATERIAL AND METHODS

MATERIALS

Drug, Polymer and Chemicals:

S.No	Name of the Chemicals	Supplier Name
1.	Doxycycline hyclate	Micro labs, Banglore.
2.	Chitosan	Central Institute of Fisheries Technology , Metysapuri,Cochin.
3.	Acetic acid	S-difine Chemicals., Mumbai
4.	Potassium hydrogen ortho Phosphate	S-difine Chemicals., Mumbai
5.	Sodium hydroxide	S-difine Chemicals., Mumbai
6.	Glutaraldehyde	S-difine Chemicals., Mumbai
7.	Calcium chloride	S-difine Chemicals., Mumbai
8.	Anhydrous aluminum chloride	S-difine Chemicals., Mumbai
9.	Acetone	S-difine Chemicals., Mumbai

All the other chemicals used in this study are of analytical reagent grade (A.R. grade).

List of Instruments Used For Formulation and Evaluation

S.No	Instruments	Supplier
1.	UV/Visible Spectrophotometer 1700	Shimadzu
2.	Vortex mixer VM 301	Remi., Mumbai
3.	Digital pH meter	HANNA Instruments, Italy
4.	Electronic weighing Balance	Essaie-Teraoka Ltd., USA
5.	Tensile strength tester	Digital testing apparatus
6.	Digital screw gauge	Mitutoyo., Japan
7.	FT-IR	Shimadzu
8.	Levelled glass moulds	Designed in our laboratory

4. EXPERIMENTAL METHODS:

1. PREFORMULATION STUDIES

Preformulation testing is the first step in the rational development of dosage form of the drug. It can be defined as an investigation of physical and chemical properties of drug substances, alone and when combined with excipents. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be mass produced.

A thorough understanding of physicochemical properties may ultimately provide a rational for formulation design or support the need for molecular modification or nearly conform that there are more significant barriers to the compounds development. The goals of the program therefore are to establishment the necessary physicochemical characteristics of new drug substances

- Determine its kinetic release rate profile.
- To establish its compatibility with different excipents.

Hence, preformulation studies on obtained drug sample include physical tests and compatibility studies.

- **Melting Point determination:**

Melting point was determined by taking small amount of drug in a capillary tube whose one end was sealed by melting. The capillary tube was placed in the Digital melting point apparatus. The temperature was slowly increased with simultaneous observation of the sample. The temperature at which the drug starts melting was recorded as melting point. This process was performed three times for Doxycycline. The mean of three readings was recorded.

2. COMPATIBILITY STUDIES

Drug- Excipient interaction studies:

The successful formulation of a stable and effective solid dosage form depends on the careful selection of the excipients that are added to facilitate administration, promote the consistent release and bioavailability of the drug.

FTIR and DSC studies can be used to investigate and predict any physicochemical interactions between components in a formulation and can therefore be applied to the selection of suitable chemically compatible excipients.

a. Fourier transforms infrared (FTIR) Spectral studies:

FTIR spectra were taken on a Shimadzu instrument to investigate the possible chemical interactions between the drug and the blend matrix. FTIR spectra of plain doxycycline and physical mixture were scanned in the range between 4000 and 500 cm^{-1}. The spectra's are compared with the peak intensities found in a standard reference.

Sample preparation

Samples are taken in the ratio of 1:100, where 1 is the amount of sample taken and 100 was the amount of potassium bromide taken and triturated with due precaution not to contact with the moisture, which may interfere with the test. Samples were crushed with KBr to get the pellets by applying a pressure of 300 kg/cm^2. The sample along with blank (KBr), reference standard drug are placed in order on the disk provided. Peaks obtained in FTIR are compared to that of standard peaks for any significant change[64].

b. Differential scanning calorimetry (DSC):

The DSC measurements were performing on a DSC – 61000 (Seiko Instruments, Japan) differential scanning colorimeter with thermal analyzer. All accurately weighed samples (about 5 mg of doxycycline and chitosan) were placed in a sealed aluminum pans, before heating under nitrogen flow (20 ml/min) at a scanning rate of 20^0C per min from 100 to 300^0C. An empty aluminum pan was used as reference[65].

3. ANALYTICAL METHODS

a. PREPARATION OF REAGENTS

Preparation of 1% v/v acetic acid:

Accurately measured 1 ml of concentrated acetic acid was dissolved in 100 ml of distilled water.

Potassium dihydrogen orthophosphate 0.2M:

Dissolved 27.218 grams of potassium dihydrogen orthophosphate in distilled water and volume adjusted to produce 1000 ml.

Sodium hydroxide 0.2 M:

8.0 grams of NaOH was dissolved in distilled water and volume adjusted to produce 1000 ml[63].

Table no.4.1: Preparation of 6.6 pH phosphate buffer solution

pH	Potassium hydrogen phosphate solution 0.2M(ml)	NaOH 0.2M Solution (ml)	Adjusted (ml) to
6.6	50	16.4	200

b. Preparation of Standard Stock Solution:

Standard stock solution of doxycycline hyclate was prepared by dissolving accurately weighed 100mg of doxycycline hyclate in small quantity of 6.6 pH phosphate buffer in 100ml volumemetric flask. The volume was made up to 100ml by using 6.6 pH buffer to obtaine the solution of 1000µg/ml.

c. Determination of Analytical Wavelength:

Scanning of Doxycycline Hyclate by UV Spectrophotometer:

From the standard stock solution 4ml was pipetted out into 100ml of volumemetric flask. The volume was then made up to 100ml with 6.6 pH phosphate buffer. The resulting solution containing 40µg/ml was scanned between 200-400nm and scan is shown in Figure no.6.1.

Calibration Curve of Doxycycline Hyclate in 6.6 pH Phosphate Buffer:

From the standard stock solution of doxycycline hyclate (40µg/ml), Appropriate liquates were taken in to different volumetric flask and volume made up to 10ml with 6.6 pH phosphate buffer, so as to get solution with drug concentration of 4 to 24 µg/ml. The absorbencies of the drug solution were measured at 274nm.These procedure was performed six times to validate the calibration curve. The data are given in Table no.6.1. The calibration curve constructed is showed in Figure no.6.2.

4. PREPARATION OF INSERTS

Inserts were prepared by using antimicrobial agent Doxycycline hyclate and polymer chitosan by solvent casting method.

a. Preparation of drug loaded chitosan inserts:

The general flow diagram for the preparation of chitosan inserts was given in figure 4.1. Chitosan (2% w/v) was soaked in acetic acid (1% v/v in water) for 24 hours to get a clear solution. This dispersion was filtered through a muslin cloth to remove undissolved portion of the polymer (chitin). Required amount of the drug was added and vortexed for 1-2 hrs to dissolve the drug in chitosan solution. The viscous dispersion was kept aside for complete expulsion of air bubbles. The films were casted by pouring the drug-polymer dispersion into the center of leveled glass moulds and allowed to dry at room temperature (30°C) for 24 hours. After drying, films were cut into inserts of required size (7 × 2 mm). Inserts containing zero percent (placebo), 10%, 20% and 30% w/w of the drugs to the weight of polymer were prepared These were wrapped in aluminum foil and stored in desiccators until further use [56].

b. Preparation of bi-layer inserts:

15 ml 2% chitosan cast solution without drug was poured into mould and dry at room temperature to form the first layer of bi-layer membrane. 20 ml chitosan cast solution with 30% drug was cast on the first layer and it was dried to room temperature. Then the dried inserts were kept in desiccators for further study[56].

c. Preparation of tri-layer membrane:

15 ml of 2% chitosan cast solution without drug was cast into mould and then dried to room temperature to form the down layer of the tri-layer. 20 ml chitosan cast solution with 30% drug was cast on the down layer after it was dry to form the middle layer of the tri-layer. And then 15 ml of 2% cast solution without drug was cast onto the

previous membrane also after it had been dry again to form the upper layer of the tri-layer. Then dried inserts were kept in desiccators for further use.

Table No. 4.2: Composition of different periodontal inserts

Inserts	**Inserts code**	**% of drug loaded**
Plain Inert	CP	0
Single layer	DSL-10	10
Single layer	DSL-20	20
Single layer	DSL-30	30
Bilayer insert	DBL-30	30
Trilayer insert	DTL-30	30

CP = Chitosan polymer, DSL-10, 20, 30= Doxycycline single layer with 10%, 20% and 30%, DBL-30 =Doxycycline bilayer with 30% and DTL-30= Doxycycline trilayer with 30%.

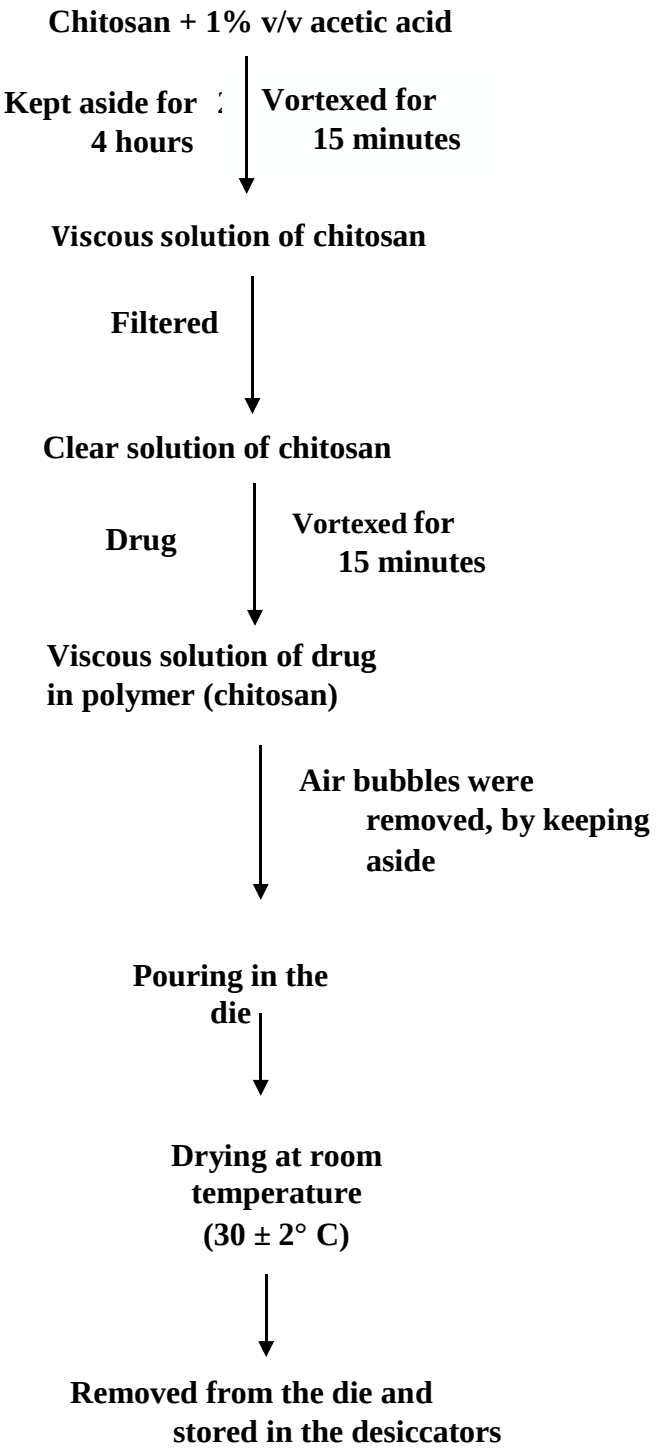


Figure No. 4.1: Protocol for the Preparation of Drug Loaded Chitosan Inserts

5. CHARACTERIZATION OF THE POLYMERIC INSERTS

All the prepared periodontal inserts were evaluated for various parameters like thickness, weight variation, folding endurance, tensile strength, drug content, *in vitro* release, swelling index, *in-vitro* anti microbial activity and stability studies.

a) Thickness Measurement:

The thickness of the polymer inserts (2x7 cm) was measured by using a digital screw gauge (Mitutoyo) at different areas of the inserts and the average was calculated[53].

b) Weight Variation:

The weight variation test was carried out by weighing 6 inserts cut from different places of same formulation and their individual weights were determined by using the digital balance. The mean valve was calculated. The standard deviations of weight variation were computed from the mean value[53].

c) Folding Endurance Studies:

Folding endurance of the inserts was determined by repeatedly folding the insert at the same place till it broke or folded. The number of times, the insert could be folded at the same place, without breaking, gave the value of folding endurance[53].

d) Tensile Strength Measurement:

Tensile strength of the inserts was determined by Universal strength testing machine. It consists of two load cell grips, the lower one is fixed and upper one is movable. The test inserts of specific size (2 × 2 cm) was fixed between these cell grips and force was gradually applied till the film breaks. The tensile strength of the inserts was taken

directly from the dial reading in kilograms Measurements were run in triplicate for each film[53].

Figure No. 4.2: Digital Tensile Strength Measurement Instrument.

e) Estimation of Drug Content:

The drug loaded chitosan insert of known weight (7 × 2 mm) were dissolved in small volume of 1% (v/v) acetic acid and drug solution was suitably diluted with 6.6 pH phosphate buffer and the absorbance was measured at 274nm[53].

f) Percentage Moisture Absorption:

The percentage moisture absorption test was carried out to check physical stability or integrity of the inserts. Periodontal inserts of known size were weighed and placed in a dissector containing 100ml of saturated solution of aluminum chloride and 79.5% humidity was maintained. After three days the inserts were taken out and reweighed. The percentage moisture absorption was calculated using the formula.

$$\% \text{ Moisture absorbance} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

g) Percentage Moisture Loss:

The percentage moisture loss test was carried out to check physical stability or integrity of the inserts. Periodontal inserts of known size were weighed and placed in a dissector containing 100ml of saturated Calcium chloride and 79.5% humidity was maintained. After three days the inserts were taken out and reweighed. The percentage moisture loss was calculated using the formula.

$$\% \text{ Moisture Loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

h) Determination of Water Uptake and Swelling Behavior:

Water uptake was determined gravimetrically. Drug loaded inserts were placed on a filter paper. The lower side of filter paper was immersed in a beaker containing 6.6 pH phosphate buffer, and incubated at 30 ^{0}C. Weight of each inserts was determined with digital weight balance at predetermined time points. The size changes of the inserts due to swelling investigated macroscopically.

i) *In-Vitro* Antibacterial activity:

In-vitro antibacterial activity was performed on all formulation by placing the insert (2x7) on agar plates seeded with oral bacteria *steptococuss mutans*. After 48 hrs of incubation at 37 ^{0}C, the inserts were transformed to freshly seeded agar plates and incubated for additional 48 hrs. This procedure was repeated until no inhibition of bacterial growth was detected on agar plate. The growth inhibition zone on the agar plate was measured.

j) *In-vitro* Release Studies:

Since the pH of the gingival fluid lies between 6.5 – 6.8, phosphate buffer pH 6.6 was used as simulated gingival fluid for the dissolution studies and the inserts remains immobile in the periodontal pocket, a static dissolution model was adopted.

A static dissolution method reported in the literature was adopted in this thesis. Sets of 3 inserts of known weight and dimension (7 × 2 mm) were placed separately into small test tubes containing 1.0 ml phosphate buffer, pH 6.6. The tubes were sealed and kept at 37°C ±1 for 24 hours. The buffer was then drained off and replaced with a fresh 1.0 ml phosphate buffer pH 6.6. The concentration of drug in the buffer was measured at 274nm .The procedure was continued for consecutive days[53].

6. DEPENDENT-MODEL METHOD (KINETIC MODELING):

The results obtaining *in vitro* release studies were plotted in different models of data treatment as follows:

ZERO ORDER KINETIC

It describes the system in which the drug release rate is **independent** of its concentration.

$$Qt = Qo + Ko\ t$$

Where,

Qt= Amount of drug dissolved in time t

Qo = Initial amount of drug in the solution, which is often zero and

Ko = zero order release constant.

If the zero order drug release kinetic is obeyed, then a plot of **Qt versus t** will give a straight line with a **slope of Ko** and an **intercept at zero**.

FIRST ORDER KINETIC:

It describes the drug release from the systems in which the release rate is concentration dependent.

$$\mathbf{Mt / M\infty = ktn}$$
$$\mathbf{Log\ [Mt / M\infty] = \ log\ k + n}$$

Where,

Qt = amount of drug released in time t.

Qo = initial amount of drug in the solution

k = first order release constant

If the first order drug release kinetic is obeyed, then a plot of **log (Qo- Qt) versus t** will be straight line with a **slope of kt / 2.303** and an **intercept at t=0 of log Qo.**

HIGUCHI MODEL:

It describes the fraction of drug release from a matrix is proportional to square root of time.

$$\mathbf{Log\ Qt = log\ Qo + kt / 2.303}$$

Where,

Mt and M∞ are cumulative amounts of drug release at time t and infinite time, and **kH** = Higuchi dissolution constant reflection formulation characteristics.

If the Higuchi model of drug release (i.e. Fickian diffusion) is obeyed, then a plot of Mt / M∞ versus $\mathbf{t^{1/2}}$ will be straight line with slope of kH.

KORSMEYER-PEPPAS MODEL (POWER LAW):

The power law describes the drug release from the polymeric system in which release deviates from Fickian diffusion, as expressed in following equation.

$$\mathbf{Mt / M\infty = kHt^{1/2}}$$

Where,

Mt and M∞ are cumulative amounts of drug release at time t and infinite time

(i.e. fraction of drug release at time t),

k = constant incorporating structural and geometrical characteristics of CR d

device,

n = diffusional release exponent indicative of the mechanism of drug release

for drug dissolution.

- To characterize the release mechanism, the dissolution data {Mt / M∞ <0.6} are evaluated.

- A plot of **log {Mt / M∞} versus log t** will be linear with **slope of n and intercept gives the value of log k**.
- Antilog of log k gives the value of k.
- Peepas used the **n value** in order to characterize different release mechanisms as shown in the table below,

Table No.4.3: Release Mechanism of Peppas

'n'	**MECHANISM**
0.5	Fickian diffusion
0.5 < n <1	Non- fickian diffusion
1	Class II transport

Stability Studies:

The stability of the drug loaded polymer inserts were studied at different temperatures using the reported procedure. The 3 inserts of size (7 × 2 mm) were weighed. The strips were wrapped in aluminum foil and placed in petridishes. These container was stored at ambient humid conditions, at room temperature (27 ± 2°C), oven temperature (40 ± 2°C**)** and in refrigerator (5 - 8°C) for a period of 3 months. The samples were analyzed for physical changes such as color and texture.

The drug content was estimated at an interval of 1 month using the procedures reported earlier in this thesis[53].

The results obtained from these methods and the discussion of the obtained results is given in the following chapter "Results and Discussion".

8.RESULT AND DISCUSSION

Periodontal inserts were prepared by solvent casting method and inserts were characterized for various parameters like thickness, weight variation, swelling index, moisture absorption, folding endurance, tensile strength, percentage moisture loss**,** drug content, stability study and *in-vitro* release studies. Table no.4.2 shows composition of various formulations of periodontal inserts with aimed at providing local long term release of Doxycycline (DCL).

1. PREFORMULATION STUDIES

The following preformulation studies were performed for drugs and polymer.

Determination of Melting Point:

Melting point was determined and it was found to be **202^0- 203^0C** $\pm 2^0$C (N =3). This value is very close to that of literature citation. Thus, indicating purity of the drug.

2. EXPERIMENTAL METHODS

a) Analytical Methods

Determination of λ max:

Absorption spectrum of doxycycline hyclate was obtained using phosphate buffer pH 6.6 by scanning the sample in UV spectrophotometer in the range of 200-400 nm. The absorption maxima were found to be at 274 nm and the spectra is shown in the figure no.6.1.

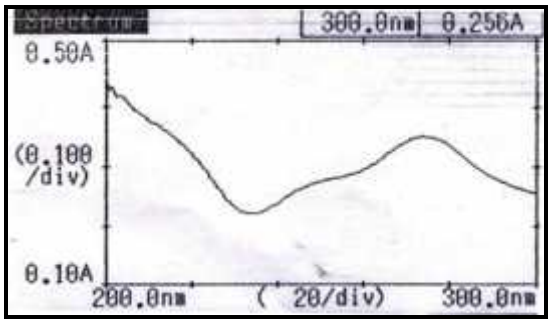


Figure No.6.1: Analytical Wavelength of Doxycycline Hyclate.

Calibration Curve of Doxycycline:

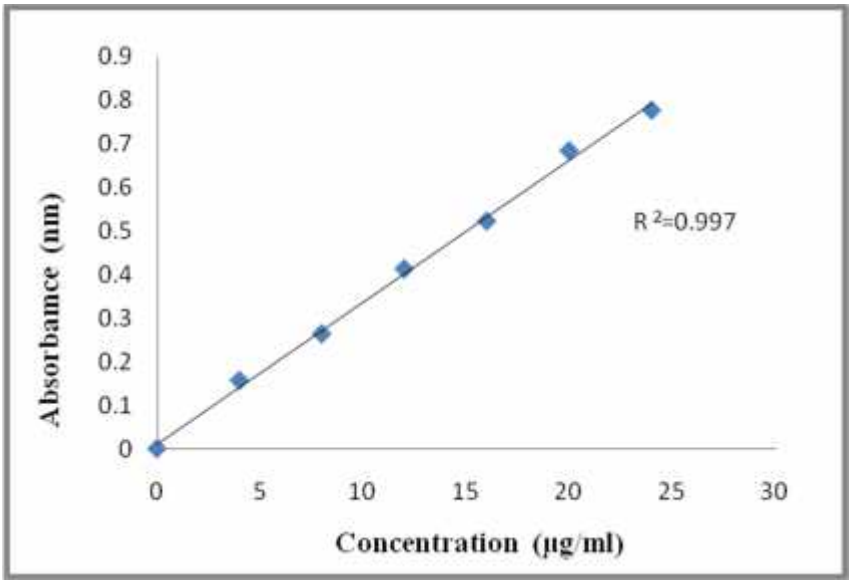
Table no.6.1 shows the absorbance reading of DCL. Standard solutions containing 4- 24 µg/ml in phosphate buffer pH 6.6. Figure no. 6.2 shows standard calibration curve for DCL with the correlation coefficient of 0.997. The drug content uniformity and *in vitro* drug release study are based on this calibration curve. The absorbance was measured at λ max of 274 nm.

Table No. 6.1.: Calibration Data of Doxycycline hyclate in 6.6 pH Phosphate Buffer.

SL.NO.	CONCENTRATION (µg/ml)	ABSORBANCE* AM ±SD
1	4	0.157 ± 0.002
2	8	0.264 ± 0.005
3	12	0.413 ± 0.006
4	16	0.523 ± 0.001
5	20	0.684± 0.028
6	24	0.778 ± 0.007

Each value is an average of six replications* R^2=0.997

Figure No.6.2: Calibration Curve of Doxycycline in pH 6.6 Phosphate Buffer.



3. DRUG EXCIPIENT COMPATIBILITY STUDIES

- **Fourier Transform Infrared Spectroscopy (FT-IR) studies:**

The preformulation studies between the drug and formulation component under the experimental conditions were done by using IR spectrum and was recorded on shimadzu FT-IR by preparing KBr disc. The entire characteristics peak obtained in the spectra's of the formulation correlate with peaks of drug spectrum. This indicates that the drug is compatible with all the formulation components. The spectra of all formulations were shown Figure no. from 6.3-6.5.

The results of IR spectral analysis showed the following major peaks for DCL, chitosan and their formulation components are tabulated in the table no.6.2.

Figure No.6.3: Fourier Transform Infrared Spectroscopy (FT-IR) of Doxycycline.

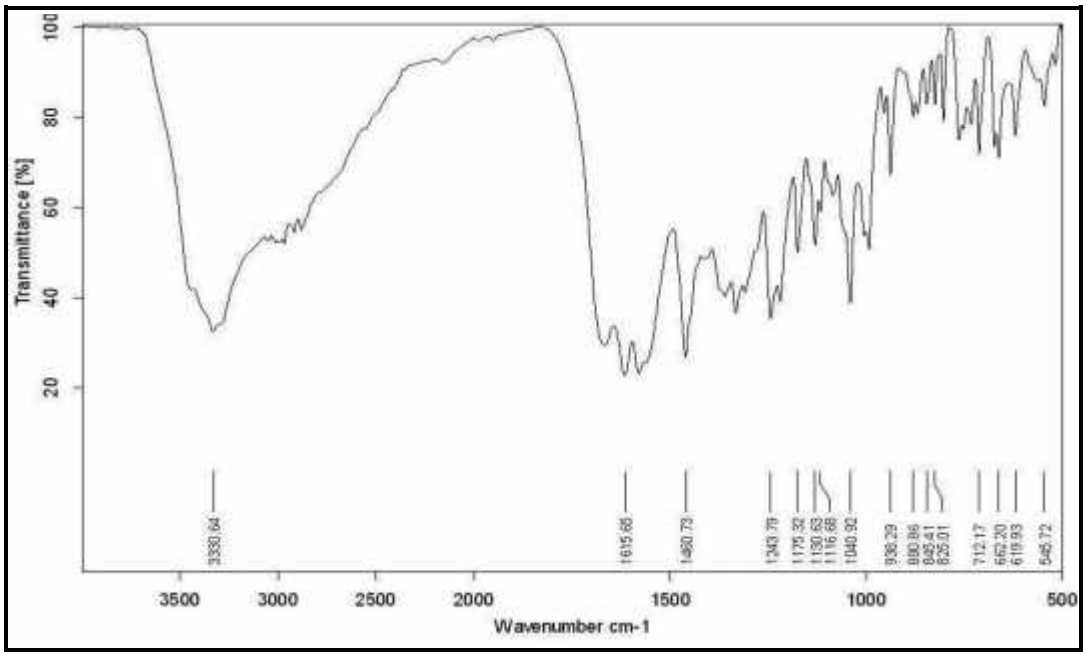


Figure No. 6.4: Fourier Transform Infrared Spectroscopy (FT-IR) Of Chitosan.

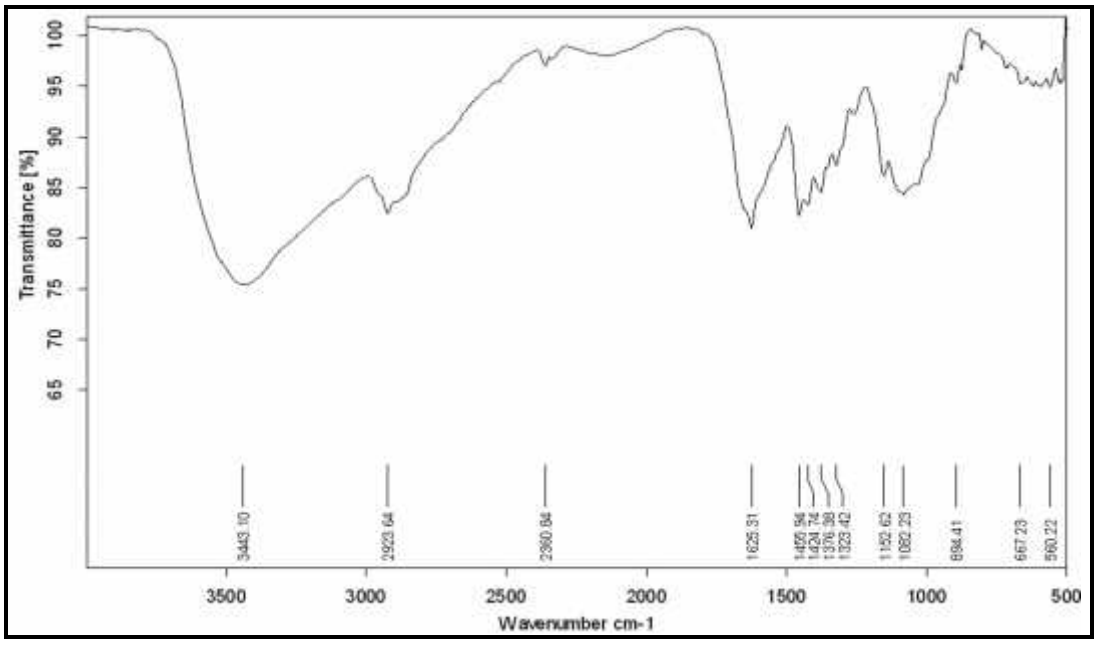
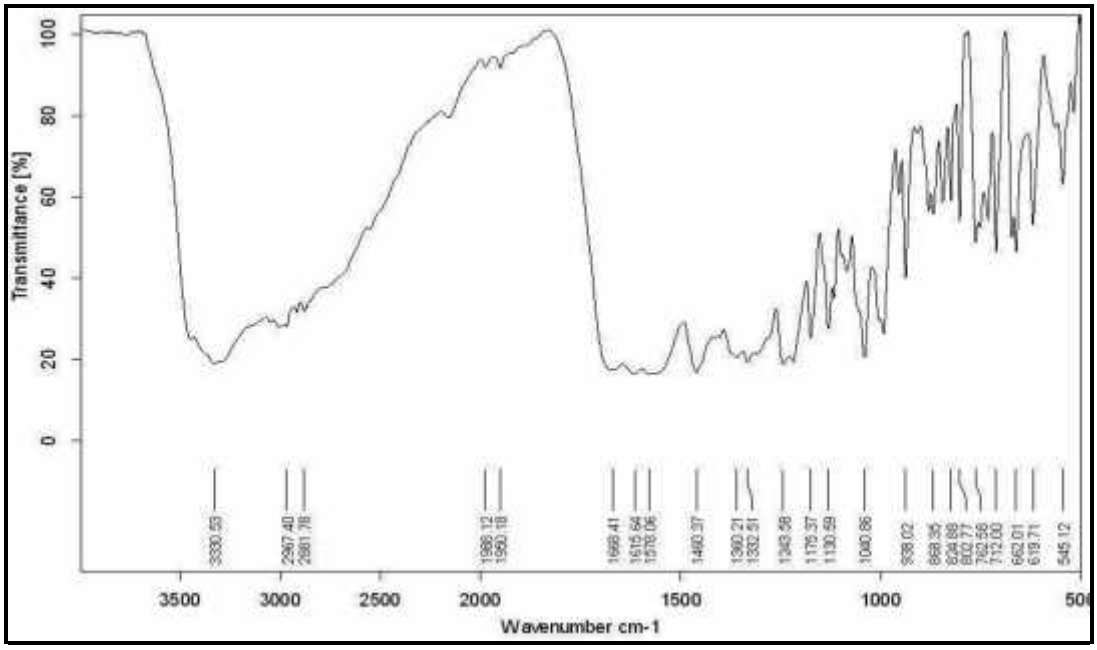


Figure No.6.5: Fourier Transform Infrared Spectroscopy (FT-IR) of formulation.



Fourier Transform Infrared Spectroscopy:-

The peaks of formulation were compared with the peaks of the drug and we found the maximum similarities. Finally we conclude that the drug retains its originality even after formulation, so the drug may not get disturbed in the formulation.

Table No.6.2: Data of the FT-IR Spectra of Pure Doxycycline(DCL) and Polymer Chitosan Peaks of Functional Group (Cm^{-1}).

FORMULATION	C=O Stretching cm^{-1}	Ar-CH Stretching cm^{-1}	-OH stretching cm^{-1}	N-H bending cm^{-1}	-OH bending cm^{-1}	C-N Stretching cm^{-1}	C-C-C O Stretching cm^{-1}
DCL	1615.65	2900	3330	1570	1460.73	1243.79	1175.32
DCL+ Chitosan	1615.64	2967	3330.33	1578.06	1460.37	1243.58	1175.37

- **Differential Scanning Calorimetry:**

DSC is useful in the investigation of solid state interactions. Thermograms are generated for pure drug and physical mixtures of drug along with other excipients. In the absence of any interaction, the thermogram of mixtures show endothermic peak corresponding to that of pure drug. In the event that interaction occurs, this is indicated in the thermogram of a mixture by the shift in the value of melting endotherm of the pure drug.

The DSC thermogram of pure DCL and its mixture with different excipients are shown in figure. Pure DCL showed a sharp endotherm at 219.78C corresponding to its melting point/transition temperature. There was no appreciable change in endotherm peaks of formulation compared to pure drug. This observation further supports the IR spectroscopy results, which indicated the absence of any interaction between drug and additives used in the preparation.

Figure No.6.6: Differential Scanning Calorimetric (DSC) Thermogram of Doxycycline Hyclate Drug.

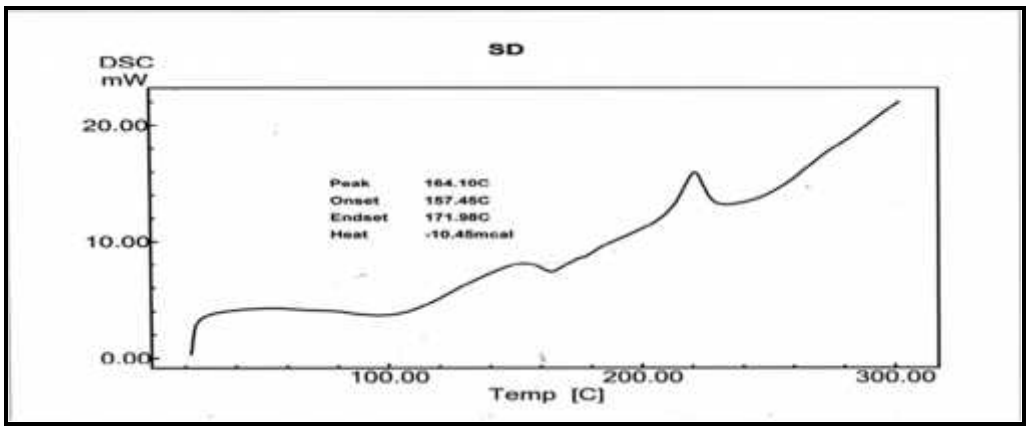


Figure No.6.7: Differential Scanning Calorimetric (DSC) Thermogram of Chitosan Polymer.

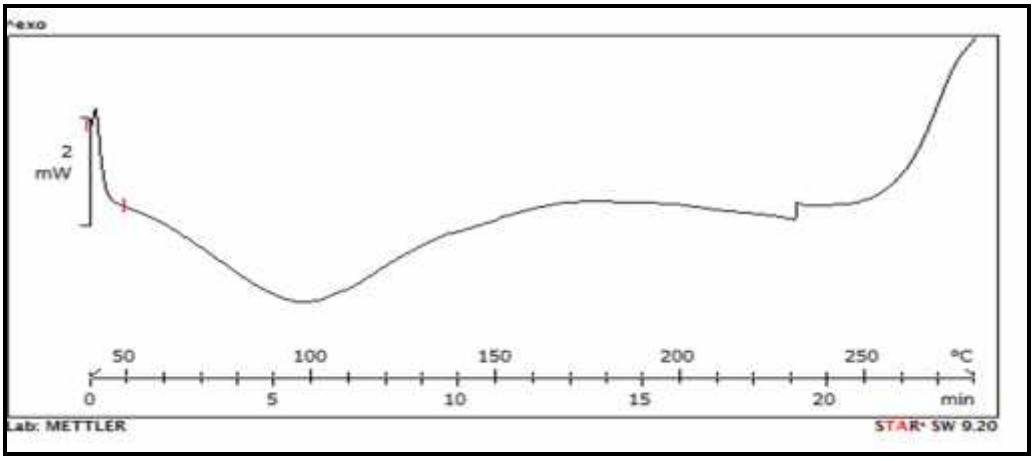
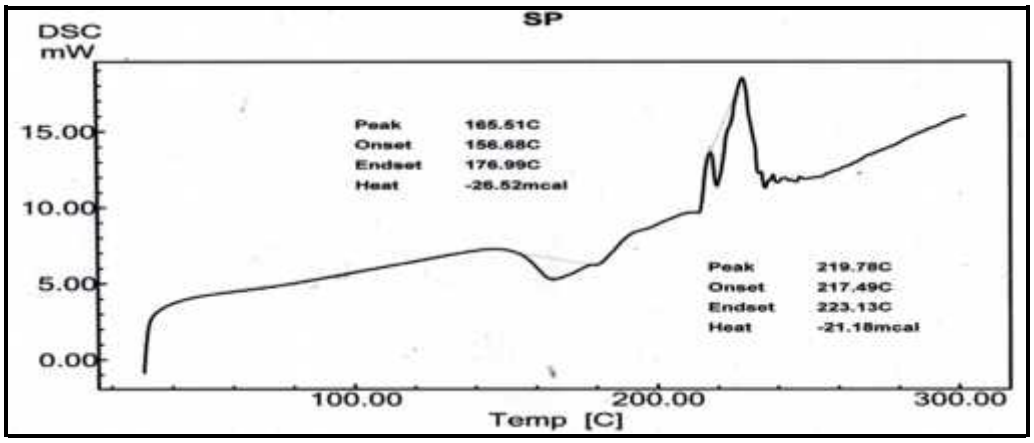


Figure No.6.8: Differential Scanning Calorimetric (DSC) Thermogram of Formulation.



4. Preparation of Drug Loaded Chitosan Inserts:

The optimum concentration of chitosan used for the preparation of inserts was found to be 2% w/w, because at this concentration the inserts were flexible and easily removable from die, at higher conc. of polymer, the polymer dispersion was highly viscous and difficult to filter using muslin cloth, inserts were brittle and difficult to remove from die.

The amount of drug added to polymer solution changes the inserts characteristics an optimum conc. of drug to be loaded was found to be less than 30% w/w to polymer, at higher drug conc. (i.e. >30%) the inserts were stiff and brittle. Plasticizer was not used because inserts obtained were flexible with plain chitosan. There was no visible sign of crystallization.

5. Evaluation of Physical Parameters of DCL Loaded Chitosan Inserts:

a) Physical appearance:

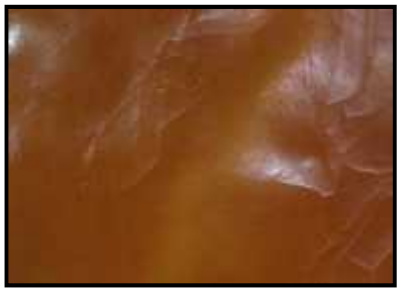
Both drug and polymer combination used for formulation of periodontal inserts showed good film properties and reproducibility. The fabricated inserts were thin, flexible, elastic, smooth and non transparent.

Photography of doxycycline hyclate was shown in the figure no.6.6.

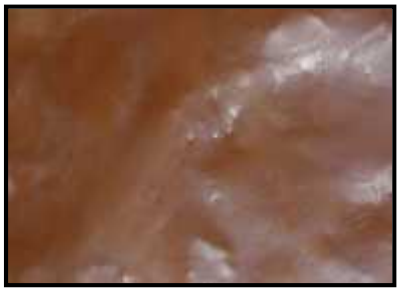
Figure No.6.8: Photograph Showing Doxycycline Hyclate Periodontal Inserts.

1) Plain Chitosan Insert

2) Single Layer With 10% of DCL

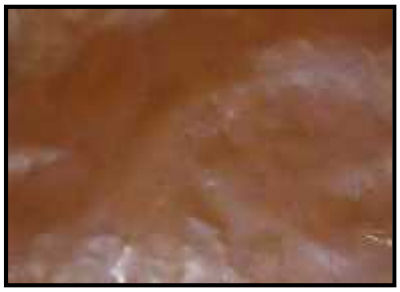


3) Single Layer With 20% of DCL

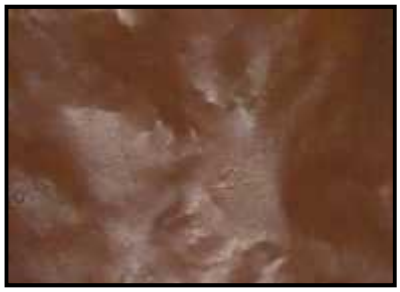


4) Single Layer With 30% of DCL

5) Bilayer Layer With 30% of DCL



6) Trilayer Layer With 30% of DCL



b) Thickness Measurement:

Thickness of each insert was measured at six different points and average thickness with standard deviation was calculated. The thickness of the various inserts as given in the table no.6.3. The data of inserts thickness indicates that there was no much difference in the thickness within the formulation. The thickness of the inserts was increased as increased in the drug concentration and more thickness was obtained in bilayer and trilayer respectively.

c) Weight variation test:

Drug loaded inserts (2X7) were test for uniformity of weight, the results of weight uniformity are given in the table no.6.3 with less standard deviation values which indicates that the inserts were uniform in weight. The data of weight uniformity indicates that as the concentration of the drug increases there is an increase in the weight of the insert. This is an agreement with uniformity of thickness of the film as shown in the table no.6.3.

d) Folding endurance:

Table no.6.3. Shows the value of folding endurance. There replication of each test was carried out. It was found that folding endurance of the inserts was decreased by increase in the drug concentration. Bilayer and trilayer inserts exhibits minimum folding endurance as compared to the other inserts. It is evident from the literature that the films having folding endurance having more than 100-150 is considered as optimum. In the present study the folding endurance was reduced in all inserts upon the increase in concentration of drug and also formulating into bilayer and trilayer inserts. However, the folding endurance of the bilayer and trilayer inserts containing 30% drug was also more than 150. Therefore the inserts of the study were having very good folding endurance and can be considered as optimum.

Table No. 6.3: Data of Physical Measurement values of Doxycycline Inserts.

Insert Code	Thickness (mm)AM ± SD	Folding Endurance	Weight Uniformity (mg)
CP	0.103±0.0157	340±2.581989	0.8±0.208167
DSL-10%	0.124 ±0.049	280±14.719	1.0± 0.208
DSL-20%	0.171 ±0.278	260.75±13.50	1.5 ±0.346
DSL-30%	0.197 ±0.024	201.75±19.431	2.2 ±0.057
DBL-30%	0.360 ±0.010	187±9.128	2.9 ±0.30
DTL-30%	0.456±0.0030	155.25±9.844	3.9 ±0.888

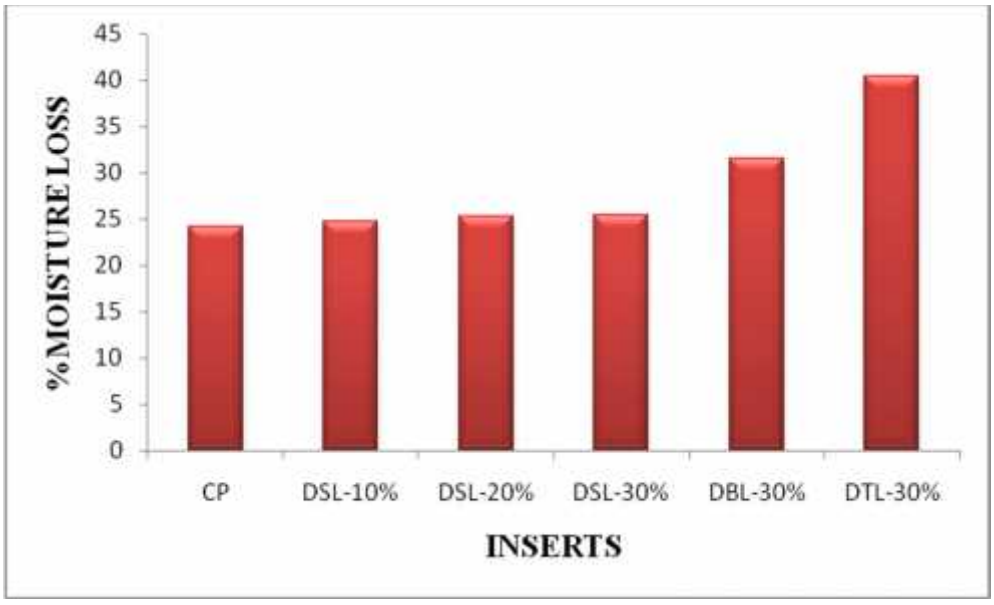
e) Percentage Moisture Loss:

This test is of great significance as variation in moisture content causes a significant variation in mechanical properties of the inserts especially when inserts comprises of hygroscopic components. Moisture loss studies were conducted on various formulations and reported in the Table no.6.3. It was observed that formulations containing maximum amount of drug showed maximum amount of moisture loss because of more concentration of drug which undergoing moisture loss in dry condition.

Table No.6.4: Data for Percentage Moisture Loss of Doxycycline Inserts.

INSERT CODE	MOISTURE LOSS (%) AM ± SD
CP	24.155±7.35
DSL-10%	24.77 ±12.365
DSL-20%	25.318±12.744
DSL-30%	25.37± 6.4593
DBL-30%	31.498±12.879
DTL-30%	40.43±11.737

Figure No.6.9: Graph for Percentage Moisture Loss of Doxycycline Inserts.



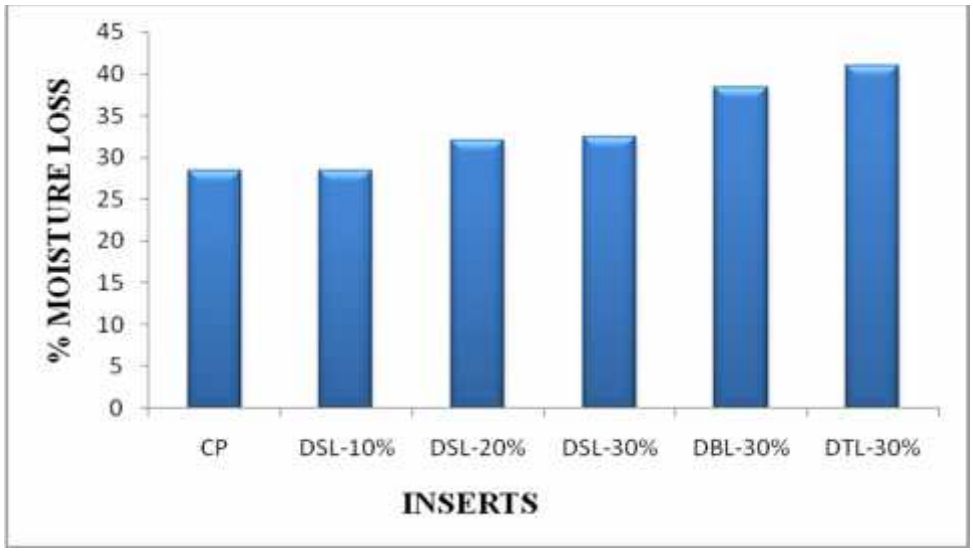
f) Percentage Moisture Absorption:

This test is of great significance as variation in moisture content causes a significant variation in mechanical properties of the inserts especially when inserts comprises of hygroscopic components. The capacity of the inserts to take up water is an important intrinsic parameter of the polymeric system in consideration to the release of drug. The data are given in the Table no. 6.4.

Table No.6.5: Data for Percentage Moisture Absorption of Doxycycline Inserts.

INSERT CODE	MOISTURE ADSORPTION (%) AM ± SD
CP	28.35± 16.02
DSL-10%	28.43±5.533
DSL-20%	32.048±20.18
DSL-30%	32.526±5.501
DBL-30%	38.393±11.366
DTL-30%	40.97 ±13.625

Figure No.6.10: Graph for Percentage Moisture Absorption of Doxycycline Inserts.



g) Water Uptake and Swelling Behavior:

Water uptake and swelling behavior studies were conducted on formulated inserts. Water uptake was found to be maximum with bilayer and trilayer inserts this may be due to the presences of more concentration of chitosan. The results are showed in the table no. After complete hydration, moderate swelling of the polymer were observed and there is a slight increase in the diameter of inserts.

Table No.6.6: Data for Swelling Index of Doxycycline Inserts.

INSERT CODE	WATER UPTAKE AND SWELLING BEHAVIOUR	
	INITIAL WEIGHT (mg) (0hr)	FINAL WEIGHT (mg) (2hrs)
CP	4.3	4.6
DSL-10	4.7	6.3
DSL-20	4.1	4.8
DSL-30	3.5	5.8
DBL-30	4.5	4.7
DTL-30	5.1	7.4

h) Tensile Strength :

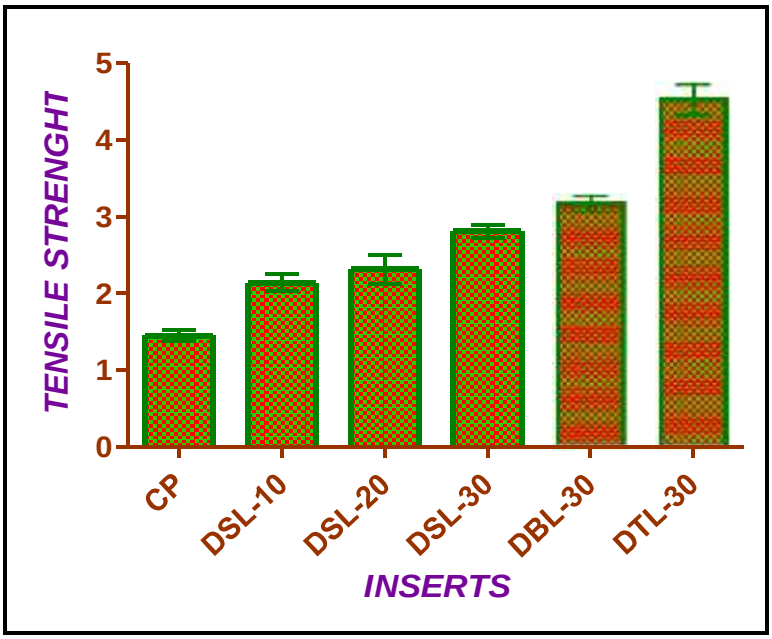
The tensile strength was determined using universal testing tensile machine for both drug loaded and unloaded inserts. The result are given in the table no.6.7. The tensile strength of drug loaded inserts was higher than dummy inserts. This is because of dissolved drug strengthen the bounding of polymer chain.

Table No.6.7: Data for Tensile Strength of Doxycycline Insert.

INSERTS CODE	TENSILE STRENGTH (Kg/sq.mm.) AM ± SD
CP	1.45± 0.054
DSL-10	2.140±0.110
DSL-20	2.318±0.185
DSL-30	2.810±0.060
DBL-30	3.170±0.102
DTL-30	4.533±0.200

*Each value was an average of three determinations.

Figure No.6.11: Graph for Tensile Strength of Doxycycline Inserts.



i) Drug Content Uniformity:

The drug content uniformity test is commonly employed for unit dose form in order to make sure about the uniform dispersion of drug in the inserts. The drugs content value of prepared inserts contains doxycycline hyclate were reported in the table no.6.8. The drug loading varied from 80-98 % depending on initial drug concentration and type of formulation.

Table No.6.8: Data of Drug Content Uniformity of Doxycycline Insert.

Inserts Code	Drug Content* (µg)	Theoretical Drug Loading (µg)	% Drug Loading
CS	----	----	---
DSL-10	107.810± 0.054	109.300	98.63
DSL-20	216.562± 0.089	218.250	99.00
DSL-30	274.062± 0.071	328.125	83.523
DBL-30	273.135± 0.064	328.125	83.241
DTL-30	272.279± 0.022	328.125	82.980

j) *In-vitro* Drug Release:

Since the pH of gingival fluid is between 6.5 to 6.8, phosphate 6.6 pH was used on stimulated gingival fluid for dissolution studies. Since, the inserts remain immobile in the periodontal pocket as static dissolution model was adopted in this work.

Release of DCL from Chitosan Inserts:

The release data of doxycycline hyclate are given in the table no.6.9, 6.10, and 6.11. The release studies were conducted for 8 days for single layer which containing different concentration of drug. The release time profiles from these inserts were shown in figures. From the results it was found that there was rapid initial release of drug on day 1 and there was a marked reduction in release from day 3 onwards and the release was controlled and extended up to 8 days.

The cumulative percentage drug release was 96.83%, 91.009 % and 92.210 % from inserts containing 10%, 20% and 30% of drug from single layer respectively.

A Persuval of figure no.6.12. indicated that initial rapid release must be due to burst release effect. Burst release is due to elution of drug from the outer surface and cut

edges of the matrix. Once the burst was completed (2days), drug was more sustained up to 8 days. Based on the results single layer inserts of 30% drug concentration was chosen for bilayer and trilayer studies respectively. These studies became essential in order to control the burst effect and release for more number of days.

Release of Doxycycline Hyclate from Bilayer and Trilayer:

The release data of bilayer and trilayer 30% DCL are given in the table no.6.12 and 6.13. The release time profile from bilayer and trilayer are showed in figure no.6.12. The bilayer and trilayer inserts showed a decreased initial burst release by more than 40%. The drug release was controlled and extended up to 13 days in case of bilayer where as 17 days in case of trilayer respectively.

The cumulative % release of drug from single layer insert was about 90-95% on the 8^{th} day. However the release of drug from bilayer and trilayer insert was about 90% on 13^{th} and 17^{th} day. Hence, it might be informed that developing bilayer and trilayer helped in sustaining the release for more number of days for local long term treatment for periodontitis.

Table No.6.9: Static Dissolution-Time Profile for Chitosan Inserts Containing Doxycyline-10%.

Time (days)	Absorbance*	Dilution factor	Conc. of drug released (µg) AM ± SD	Cumulative release (µg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.405	5	61.562	61.562	57.1023	42.897
02	0.135	5	19.375	80.937	75.073	24.927
03	0.074	5	9.843	90.780	84.136	15.864
04	0.052	5	6.406	97.186	90.1456	94.059
05	0.025	5	2.187	99.373	92.1741	7.8257
06	0.024	5	2.031	101.404	94.0580	5.942
07	0.027	5	2.343	103.747	96.231	3.769
08	0.018	3	0.656	104.403	96.839	3.161

- Each value is an average of three replication, Medium - Phosphate buffer 6.6 pH.
- Static dissolution conditions are: Temperature=37° C

Table No.6.10: Static Dissolution-Time Profile for Chitosan Inserts Containing Doxycyline-20%.

Time (days)	Absorbance*	Dilution factor	Conc. of drug released (μg) AM ± SD	Cumulative release (μg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.350	10	105.937	105.937	48.917	51.083
02	0.417	5	63.437	169.374	78.2103	21.789
03	0.103	5	14.375	183.749	84.848	15.152
04	0.042	5	4.843	188.592	87.0845	12.916
05	0.031	5	3.125	191.717	88.527	11.473
06	0.028	5	2.656	194.373	89.7539	10.247
07	0.030	3	1.781	196.154	90.576	9.424
08	0.021	3	0.937	197.091	91.009	8.991

- Each value is an average of three replication*
- Static dissolution conditions are: Temperature=37° C
- Medium = Phosphate buffer, pH 6.6 (l ml).

Table No.6.11: Static Dissolution-Time Profile for Chitosan Inserts Containing Doxycyline-30%.

Time (days)	Absorbance	Dilution factor	Conc. of drug released (μg) AM ± SD	Cumulative release (μg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.499	10	152.50	152.50	55.644	44.356
02	0.453	5	69.062	221.562	80.843	19.157
03	0.125	5	17.812	239.374	87.343	12.657
04	0.058	5	7.343	246.717	90.022	9.978
05	0.025	5	2.187	248.904	90.820	9.180
06	0.022	5	1.718	250.622	91.447	8.553
07	0.019	5	1.25	251.872	91.903	8.097
08	0.020	3	0.843	252.715	92.210	7.790

- Each value is an average of three replication*
- Static dissolution conditions are: Temperature=37° C
- Medium = Phosphate buffer, pH 6.6 (l ml).

Table No.6.12: Static Dissolution-Time Profile for Chitosan Bilayer Inserts Containing Doxycyline-30%.

Time (days)	Absorbance*	Dilution factor	Conc. of drug released (μg) AM ± SD	Cumulative release (μg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.469	10	143.125	143.125	52.400	47.600
02	0.365	5	55.312	198.437	72.651	27.349
03	0.166	5	24.218	222.655	81.518	18.482
04	0.068	5	8.906	231.561	84.778	15.222
05	0.039	5	4.375	235.936	86.380	13.620
06	0.030	5	2.968	238.904	87.467	12.533
07	0.025	5	2.187	241.091	88.268	11.732
08	0.033	3	2.062	243.153	89.023	10.977
09	0.030	3	1.781	244.934	89.675	10.325
10	0.029	3	1.6875	246.621	90.292	9.708
11	0.023	3	1.125	247.746	90.704	9.296
12	0.020	3	0.843	248.589	91.013	8.987
13	0.018	3	0.656	249.245	91.253	8.747

- Each value is an average of three replication*
- Static dissolution conditions are: Temperature = 37° C
- Medium = Phosphate buffer, pH 6.6 (1 ml).

Table No.6.13: Static Dissolution-Time Profile for Chitosan Tri Layer Inserts Containing Doxycyline-30%.

Time (days)	Absorbance*	Dilution factor	Conc. of drug released (μg) AM ± SD	Cumulative release (μg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.363	10	110.00	110.00	40.399	59.601
02	0.375	5	56.875	166.875	61.288	38.717
03	0.165	5	24.062	190.937	70.125	29.874
04	0.130	5	18.593	209.53	76.954	23.045
05	0.069	5	9.0625	218.592	80.282	19.717
06	0.043	5	5.000	223.592	82.118	17.881
07	0.035	5	3.750	227.342	83.495	16.504
08	0.027	5	2.500	229.842	84.414	15.585
09	0.025	5	2.187	232.029	85.217	14.783
10	0.023	5	1.875	233.904	85.906	14.093
11	0.027	3	1.500	235.404	86.456	13.543
12	0.025	3	1.312	236.716	86.938	13.061
13	0.023	3	1.125	237.841	87.351	12.648
14	0.021	3	0.937	238.778	87.696	12.303
15	0.019	3	0.750	239.483	87.955	12.044
16	0.017	3	0.562	240.045	87.996	12.003
17	0.015	3	0.375	240.420	88.299	11.700

- Each value is an average of three replication*
- Static dissolution conditions are: Temperature = 37° C,
- Medium = Phosphate buffer, pH 6.6 (1 ml).

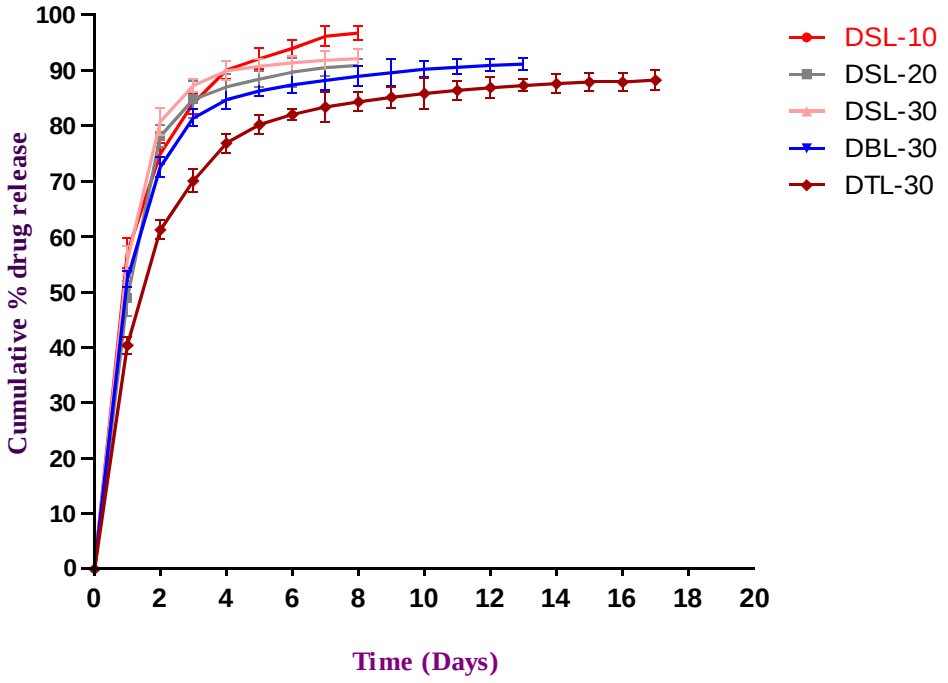


Figure No.6.12: Cumulative Percentage Release of Doxycycline Hyclate from Chitosan Inserts.

6. RELEASE KINETICS:

The release data of doxycycline hyclate was proposed to understand the linear relationship. The data was proposed for reggration analysis.

Release Mechanism:

Since the drug loaded chitosan inserts were intact during static dissolution studies, dissolution rate control release was ruled out. Hence data was fit according to higuchi ficks modle.

A Persuval of figure indicated that linear relationship was observed from 3rd to 8th day in case of single layer and 3rd to 13th day and 17th day in case of bilayer and trilarer respectively. The apperances of straight line indicates the release of drug from prepared inserts were diffusion rate control.

DEPENDENT-MODEL METHOD (KINETIC MODELING)

The results obtaining *in vitro* release studies were plotted in different models of data

Treatment as follows:

- ZERO ORDER
- FIRST ORDER
- HIGUCHI EQUATION
- KORSEMEYER PEPPAS EQUATION

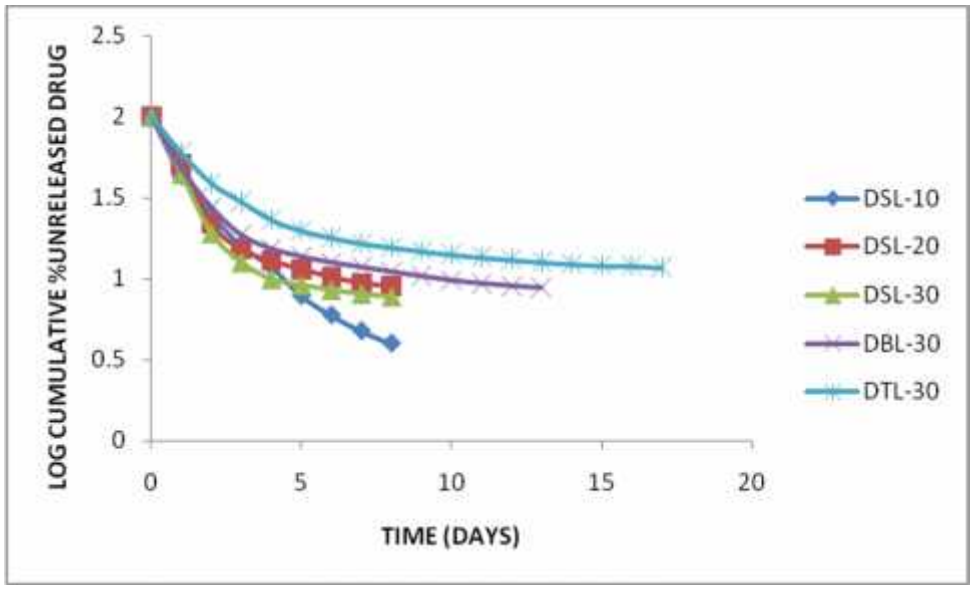
FIRST ORDER:-

Log of % Cumulative Drug Release VS. Time

Best Fit line for first order equation for chitosan inserts containing doxycycline.

Formulations are (DSL-10%, DSL-20%, DSL- 30%, DBL-30% and DTL-30%).

Figure No.6.13: First Order Equation for Chitosan Inserts Containing Doxycycline hyclate



HIGUCHI EQUATION:-

% Cumulative Drug Release Vs. Square Root of Time

Best Fit line for Higuchi equation for chitosan inserts containing doxycycline. Formulations are (DSL-10%, DSL-20%, DSL- 30%, DBL-30% and DTL-30%)

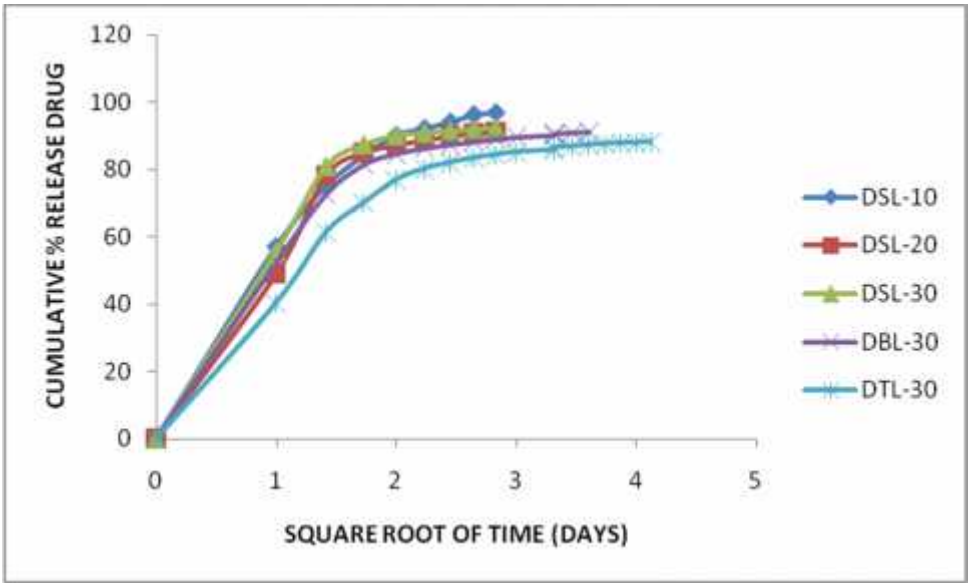


Figure No.6.14: Higuchi Equation for Chitosan Inserts Containing Doxycycline hyclate

KORSEMEYER PEPPAS EQUATION:-

Log of % Cumulative Drug Release Vs. Log Of Time

Best Fit line for Korsemeyer Peppas equation for chitosan inserts containing doxycycline. Formulations are (DSL-10%, DSL-20%, DSL- 30%,DBL-30% and DTL-30%) .

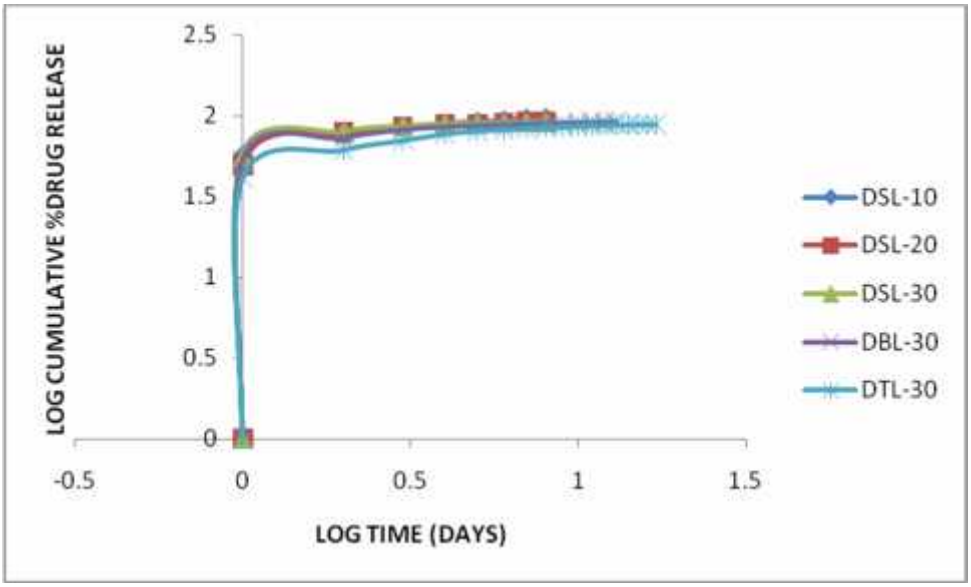


Figure No.6.15: Korsemeyer Peppas Equation For Chitosan Inserts Containing doxycycline hyclate.

The results obtaining by *In-vitro* release studies were plotted in different kinetic models as follows.

Table No.6.14: Data of Kinetic modeling of Doxycycline Inserts.

INSERT CODE	ZERO ORDER	FIRST ORDER	HUGUCHI MATRIX	KORSEMEYER PEPPAS	
	R^2	R^2	R^2	R^2	N
DSL-10	0.559	0.952	0.887	0.415	0.937
DSL-20	0.596	0.806	0.847	0.422	0785
DSL-30	0.559	0.766	0.823	0.402	0.789
DBL-30	0.479	0.743	0.738	0.388	0.811
DTL-30	0.513	0.750	0.764	0.421	0.822

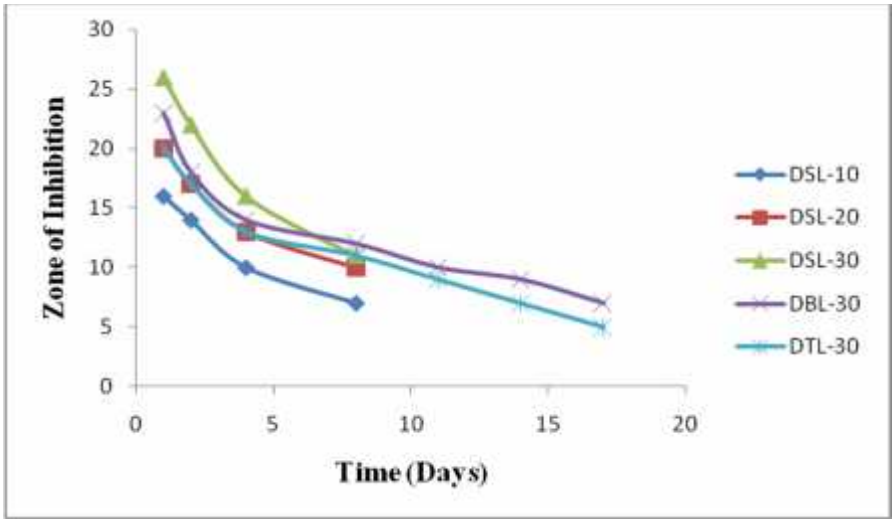
k) *In-Vitro* Antibacterial Activity:

Table no. 6.15. Shows more *in-vitro* antibacterial activity against *S. mutans* was found on day1 with all single layer inserts. Whereas with bilayer and trilayer less activity was observed on day 1. From the study it was conformed that bilayer and trilayer inserts exhibited antibacterial activity for a long period in comparison to single layer. This may be due to sustained release of drug from bilayer and trilayer inserts. The zone of inhibition values of DCL shown in figure no.6.16.

Table No.6.15: *In vitro* antibacterial activity data of Doxycycline hyclate.

Days	Diameter of inhibition of growth area of *S.mutans* (mm)				
	DSL-10	**DSL-20**	**DsL-30**	**DBL-30**	**DTL-30**
1	16	20	26	23	20
2	14	17	22	18	17
4	10	13	16	14	13
8	7	10	11	12	11
11	-	-	-	10	9
14	-	-	-	9	7
17	-	-	-	7	5

Figure No.6.16: Zone of inhibition of Doxycycline Hyclate.



L) Stability Study:

All the polymeric inserts were obsersved for physical change, color, appearance and drug content. Stability data at different temperature and humidity condition revelaed on physical appearances and uniformity in drug content. The drug remained intact and stable in the polymer on storage for 3 months. The results obtained from these methods and the discussion of the obtained results is given in the following tables.

Table No.6.16: Data for Physical appearance of Drug Stability Study.

TIME IN DAYS	DSL-10	DSL-20	DSL-30	DBL-30	DTL-30
0	No change				
30	No change				
60	No change				
90	Yellow to brown				

Table No.6.17: Stability Studies for Drug Content at various temperatures for Chitosan Inserts Containing Doxycycline Hyclate.

Insert code	Initial drug conc. (µg)	30 Days	60 Days	90 Days
		A.F	A.F	A.F
DSL10	107.810	107.800	107.783	107.700
DSL-20	216.562	216.560	216.534	216.487
DSL-30	274.062	274.058	274.022	273.987
DBL-30	273.135	273.130	273.102	273.029
DTL-30	272.279	273.274	273.250	273.147

Each value is an average of 3 determinations.

A.F = Packed in aluminum foil.

The inserts were also observed for their appearance, texture and drug content uniformity. These properties did not change in all the inserts during the period of study. Hence, prepared formulations have a good stability.

9.SUMMMARY

Periodontitis is the disease, which is caused by specific microorganism in periodontal pockets and led to an increased interest in and usage of antibacterial agent in periodontal therapy.

The problems of administering drugs systemically, such as adverse reaction, have focused attention on ways of applying the drug directly to its target site.

The use of drugs to treat plaque associated periodontal diseases is an attractive prospect, since conventional treatment can be demanding on both patient and operator. So there is much interest in developing alternative strategies.

Number of drug delivery system has been investigated for administration of antibacterial / antimicrobial agents into periodontal pocket for local action in the treatment of periodontitis.

Device placed into periodontal pocket can produce a local drug concentration 100 times higher than achievable systemically and importantly reduces the total patient dose by over 400 fold.

For developing the local drug delivery system, as it was proved antimicrobial agent is effective against wide range of oral pathogens.

Mucoadhasive dosage form utilizes the mechanism of bioadhesion and produces an intimate contact with the biological membrane. Hence, chitosan was selected as a biodegradable polymer for the development of drug delivery device which stays for longer time at the site of action

The inserts of chitosan polymer (biodegradable) 2% w/v were prepared by solvent casting method using 1% v/v acetic acid as a casting solvent. In the present work chitosan inserts containing doxycycline hyclate were prepared in three different

percentages (10%, 20% and 30%) to the weight of polymer .Bilayer and trilayer inserts also were prepared for preventing initial burst release.

Microscopical feature revealed that the drug was dissolved in the polymer matrix rather than dispersining. The average weight of single layer inserts range from 0.8 to 2.2 mg and average weight of bilayer and trilayer ranges from 2.9 to 3.9 mg. The thickness of the insets ranges from 0.103±0.015 mm to 0.456±0.0030 mm. There was no significant difference in the thickness among the different inserts since the drug was dissolved in the polymer matrix. The tensile strength of the inserts range from 1.45 ± 0.054 kg to 4.533 ± 0.200 kg. Tensile strength is minimum for plain inserts and maximum for inserts containing 30% drug. The data obtained revealed that incorporation of drug increases the physical strength of the inserts, however this may not cause any problem during their clinical application. The folding endurance studies showed that plain inserts exhibited maximum folding endurance followed by drug loaded inserts respectively.

In vitro dissolution rate studies were carried out by static dissolution method for inserts for a period of 8, 13 and 17 days respectively. This showed a burst release initially followed by a progressive fall in the release of the drug. The inserts DSL-10, DSL-20 and DSL- 30 showed 96.839%, 91.009 % and 92.210% respectively at the end of 8 days. The bilayer insert (DBL-30) showed 91.253% at the end of 13. The trilayer insert (DTL-30) showed 88.299 % respectively at the end of 17days of static dissolution period. Initial burst effect was reduced once the chitosan strips were formulated in multilayer form and also release of drug was extended and controlled up to 17 days. The plots of the cumulative amount of drug release per unit surface area against sq. root of time, confirms to Higuchi's diffusion model i.e., the release kinetics of doxycycline from chitosan strips followed first order. The initial burst

release is essential to achieve high concentration of drug in gingival sulcus. This is a primary objective of the periodontal therapy.

10.CONCLUSIONS

From the obtained results, it can be concluded that

- Novel multilayer inserts of doxycycline hyclate can be formulated by solvent casting method technique.
- Evaluation parameters like thickness, tensile strength and folding endurance indicates that inserts were mechanically stable in all the inserts formulations.
- Percentage weight variation and drug content uniformity found to be uniform in all the formulations.
- The FT-IR and DSC spectra revealed that, there was no interaction between polymer and drug. Hence, Polymer used was compatible with the drug.
- *In-vitro* drug release showed an abrupt release in the first day in single layer whereas this initial burst release was controlled by formulating in to bilayer and trilayer which was controlled the release uniformity in a specified period of time.
- *In-vitro* antibacterial activity showed good correlation with respective *in-vitro* drug release. Release kinetics from all the inserts follows diffusion rate controlled mechanism.
- All the inserts were found to be stable over the storage period of 90 days and condition tested.
- From this study it can be concluded that bilayer and trilayer were developed which can be delivered drug up to 13 and 17 days respectively.
- **Future scope:-**

- Addressing the final multilayer formulation needs considerable amount of study.
- *In-vivo* evaluation study of multilayer inserts of DCL and establishment of *in-vitro* and *in-vivo* correlation.

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