IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

SUBGINGIVAL CONTROLLED-RELEASE GEL SYSTEM USING CURRENT POYSTERIC SYSTEM FOR TREATMENT OF CHRONIC ADULT PERIODONTITIS

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ABSTRACT

The present study was aimed to develop and optimize *in situ* gel for the treatment of periodontal disease. Temperature-sensitive *in situ* gel containing doxycycline hyclate (DH) was formulated by cold method using different polymers Methylcellulose-4000 cps and Polaxamer-407). A total of 12 different formulations were developed with varying concentrations of MC and PM keeping the DH (5%) and BKC (0.01%) as constant.

Vehicle in situ gels were prepared by dissolving weighed amounts of MC (4%, 5% and 6% w/v) into cold water under constant stirring and allowing refrigeration at least 24 hour for proper swelling. To formulate doxycycline bearing*in situ* gel required concentration of polymers (Methylcellulose-4000 cps and Poloxamer-407) was selected on the basis of gelation temperature, gelation time and syringeability study of the formulation. It was concluded that concentration of Methyl cellulose-4000 cps and Polaxamer-407, 5% w/v and 25% w/v respectively have given good syringeability, gelation time and gelation temperature (near physiological temperature).

So F5 and F11 were selected for preparation and characterization of in situ gel with various parameters. Formulation F11 was selected for further optimization processes. At 5-10 ℃ and stirring speed 600 rpm solubility of PM (25%w/v), MC (5% w/v) and mixture solution was good. Which occurred due to absence of lumps formation at 5-10℃, as temperature increased lumps formation increased. Hence formulation F111 was selected for the final preparation of doxycycline hyclate bearing *in situ* gel after the optimization of formulation and process variables of various formulations.

Doxycycline hyclate bearing *in situ* gel was characterized on the basis of physical appearance, average pH (at 25℃ and 37℃), average drug content, average viscosity (at 25℃ and 37℃) and drug release studies, and it was found that the appearance of optimized formulation was clear yellowish translucent liquids, which was due to the yellow color of the drug.

Average pH of the optimized formulation was found to be 6.82 at 25°C and 6.82 at 37℃, which were closer to the periodontitis pH and the formulation is safe to be used on periodontal pocket. Average drug content was found to be 96.93%. Average viscosity was found to be 7505 at 25°C and 14936 at 37°C.
Keywords: Periodontal, Doxycycline Hyclate, Syringeability, Polaxamer-407, Methylcellulose-4000.

INTRODUCTION

1.1 PERIODONTITIS

Periodontitis is an inflammation of the supporting tissue surrounding teeth caused by anaerobic bacteria. In the diseased state, supporting collagen of the periodontium is destroyed and the alveolar bones begin to resorb. The epithelium of the gingiva migrates along the tooth surface forming `periodontal pockets' that provide an ideal environment for the growth and proliferation of microbes. More severe stages of the disease lead to the loosening and ultimately loss of teeth **(Vyas *et al.*, 2000)**. Prominent amongst microbes are Bacteroides several species: *Bacteroides intermedius* and *Bacteroides gingivalis*; fusiform organisms: *Actinobacillusactinomycetemcomitans*, *Wolinella recta* and Eikenella several species; and various bacilli and cocci; spirochetes; amoebas and trichomonads **(Jain *et al.*, 2008)**. In the treatment of periodontitis and gingivitis, antibiotics such as doxycycline, tetracycline and metronidazole have been used **(Mundargi *et al.*, 2007)**. The tetracyclines (tetracycline hydrochloride, doxycycline, minocycline) are broad spectrum antibiotics that affect anaerobes and facultative organisms. They are bacteriostatic for many pathogens at concentrations found in the gingival crevicular fluid after systemic administration (3- 6μg/ml). However, local delivery of these agents provides high concentrations that are bactericidal. Local application of tetracyclines has been associated with minimal adverse side effects **(Greenstein and Polson, 1998)**. The combination of amoxicillin and metronidazole used short-term along with scaling and root planning especially in patients with a periodontal pocket depth of 6 mm or more has been clinically significant. The same combination has also been shown to be useful in the management of aggressive periodontitis. Azithromycin has been also shown to improve the clinical outcomes when used as an adjunct to scaling and root planning. In addition, azithromycin has been shown to be very effective in the management of cyclosporine-induced gingival overgrowth **(Kumar, 2019)**.

1.1.1 SIGNS AND SYMPTOMS

- Bad breath
- Foul taste in mouth
- Red and swollen gums
- Pain when chewing
- Build-up of plaque/ tartar on teeth

- Changes in position of teeth (loose teeth)
- Bleeding of gums during brushing

1.1.2 TYPES OF PERIODONTITIS

Chronic periodontitis (slow to moderate rates of disease progression)

Chronic periodontitis affects adults and corresponds to the amount of local factors, mainly plaque/calculus. It tends to progress slowly with periods of exacerbation. Systemic diseases, such as diabetes, and environmental risk factors, such as smoking, impact the severity of chronic periodontitis. **Figure 1**

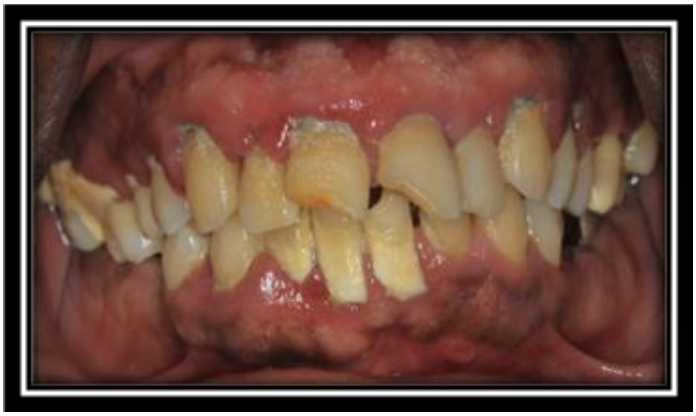


Figure 1: Chronic Periodontitis

Aggressive periodontitis (rapid rate of disease progression)

Aggressive periodontitis affects younger individuals (<25 years of age) with familial aggregation and the striking feature being rapid destruction of attachment and bone with little or no microbial deposits **(Kumar, 2019). Fig. 1.2**

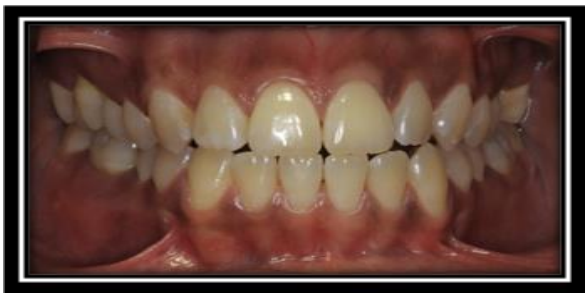


Figure 2: Aggressive Periodontitis

Risk factors

Certain factors increase the risk for periodontal disease:

✓ Smoking

✓ Diabetes

✓ Poor oral hygiene

- ✓ Stress
- ✓ Heredity
- ✓ Crooked teeth
- ✓ Underlying immuno-deficiencies—e.g., AIDS
- ✓ Fillings that have become defective
- ✓ Taking medications that cause dry mouth
- ✓ Bridges that no longer fit properly
- ✓ Female hormonal changes, such as with pregnancy or the use of oral contraceptives

1.1.3 PATHOPHYSIOLOGY

Periodontitis is as an infectious disease. Most of the forms of gingivitis and periodontitis are caused primarily by bacteria that colonize the gingival crevice and attach to intraperiodontal pockets. The omnipresence of many varieties of oral microorganisms growing as a film (bacterial biofilm) of plaquefor the most part on the non-self-cleansing areas of the teeth below the cervical convexityhas been recognized. Biofilms originate either from the normal gingival sulcus in case of marginal periodontitis, or from the gingival pocket in advanced periodontal disease. All reveal microorganisms of many different types. The composition of bacterial plaque associated with gingival health differs from that of plaque associated with the different periodontal diseases. In general, gram negative, facultative, anaerobic microorganisms are the principal bacteria associated with the periodontal diseases. Prominent among these are Bacteroids species, such as *Bacteroids gingivalis* and *Bacteroids intermedius,* Fusiform organisms, *Actinobacillus actinomycetemcomitans, Wolinella recta,* Eikenella species, various cocci and bacilli, sprirochetes and, in advanced periodonitis, amoebas and trichomonads. The normal oral flora is vast, however, making it impossible to prove conclusively that a particular type of microorganism is responsible for the pathogenesis of a specific periodontal disease. The flora is typically characterized by a predominance of gram-negative anaerobic rods. In juvenile periodontitis, gram-negative anaerobic rods increase in the areas of the deep pockets. A similar increase also occurs in the percent count of *Actinobacillus actinomycetemcomitans* and *Capnocytophaga sputigena*.

The periodontal pocket

A periodontal pocket is a pathologically dependent gingival sulcus and is one of the important clinical features of periodontal disease. Progressive pocket formation leads to destruction of the supporting periodontal tissues and loosening or exfoliation of the teeth. Microorganisms and their products that produce pathological tissue lead to the deepening of the gingival sulcus and create periodontal pockets. Pocket formation starts as an inflammatory process in the connective tissue wall of the gingival sulcus due to bacterial plaque **(Fig. 1.3).** Changes involved in the transition from the normal gingival sulcus to the pathological periodontal pocket are

associated with different proportions of bacterial cells in dental plaque. The cellular and fluid inflammatory exudes cause degeneration of the surrounding connective tissue, including gingival fibres. Two hypotheses have been proposed regarding the mechanism of collagen fibre loss from the local immune responses. Collagenase and other lysosomal enzymes from polymorphonuclearleucocytes and macrophages become extracellular and destroy gingival fibres or fibroblastsphagocytose collagen fibres by extending cytoplasmic processes to the ligament-cementum interface.

Leukocytes and oedema from the inflamed connective tissue infiltrate the epithelium lining in the pocket, resulting in varying degrees of degeneration and necrosis.

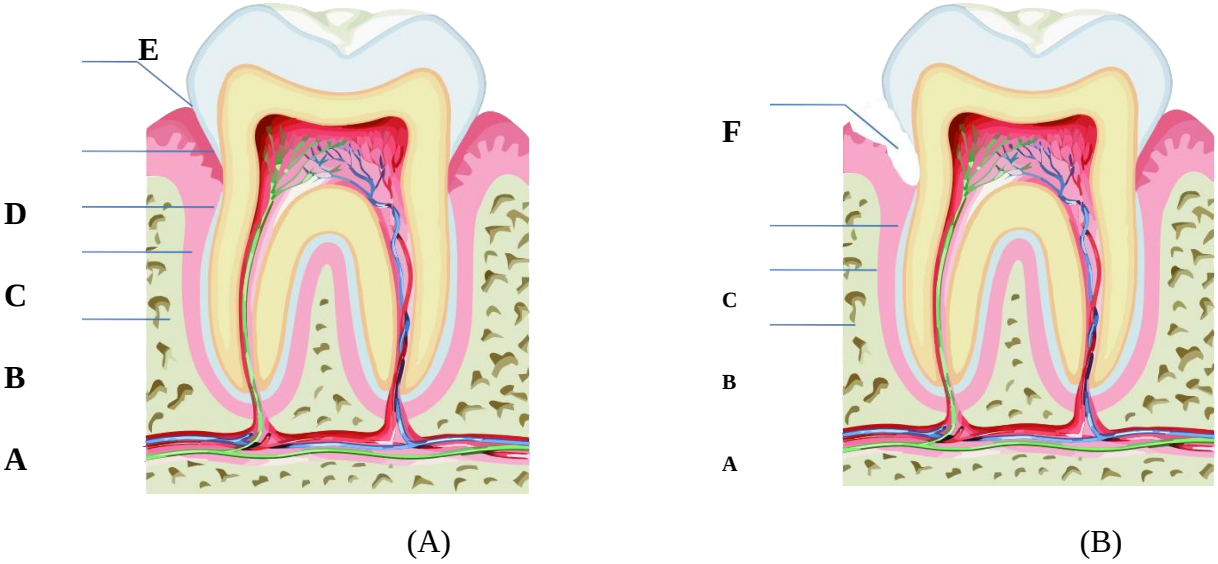


Figure 3: Diagrammatic presentation of pocket formation.(A) healthy periodontium, (B) periodontal pocket.A . Alveolar bone, B .periodontal ligaments, C .cementum, D. cementum enamel junction, E . sulcus,and F . periodontal pocket (Vyas *et al.*,2000).

Microbiology of periodontal disease

Periodontaldisease is now considered to be a group of diseasesor infections. Each disease is associated with adifferent group of microorganisms. The resultingclinical signs and symptoms can be similar orunique. The mechanisms by which subgingivalbacteria may contribute to the pathogenesis ofperiodontal disease are varied **(Figure 4)**. The periodonto- pathogens possess numerous factors thatpermit them to directly damage the periodontiumor to indirectly trigger a pathologic host response.

Explains the possible pathogenic mechanisms **(Vyas *et al.*, 2000)**.

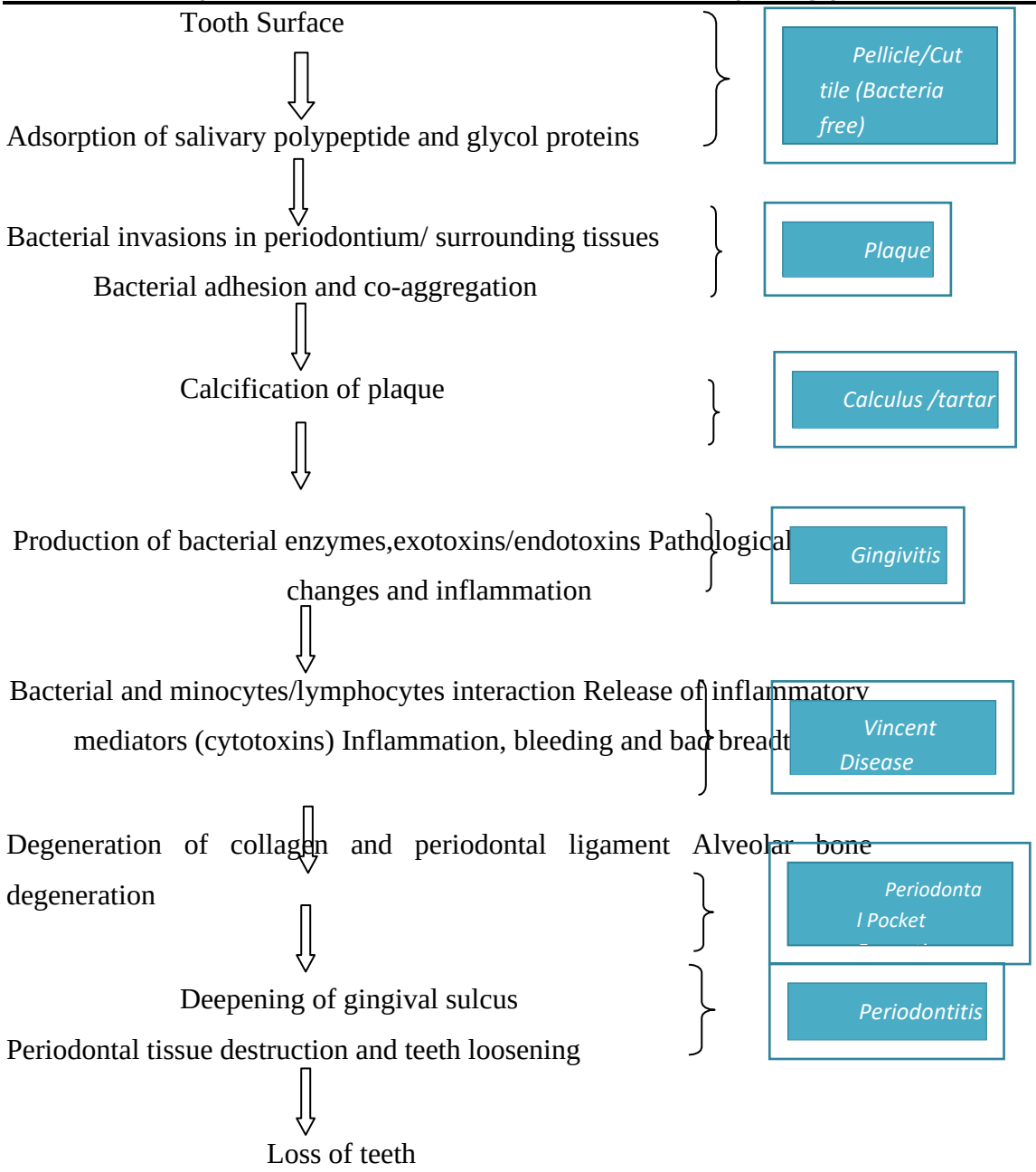
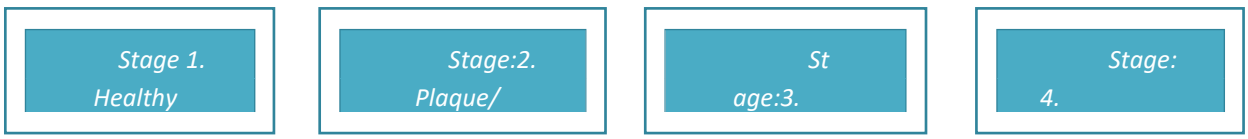


Figure 4: Flow chart representing pathogenesis of periodontal diseases. Formation ofbacterial plaque; calcification of plaque; pathological and immunologicalmanifestations resulting in gingivitis and periodontitis(Jain *et al.*, 2008).



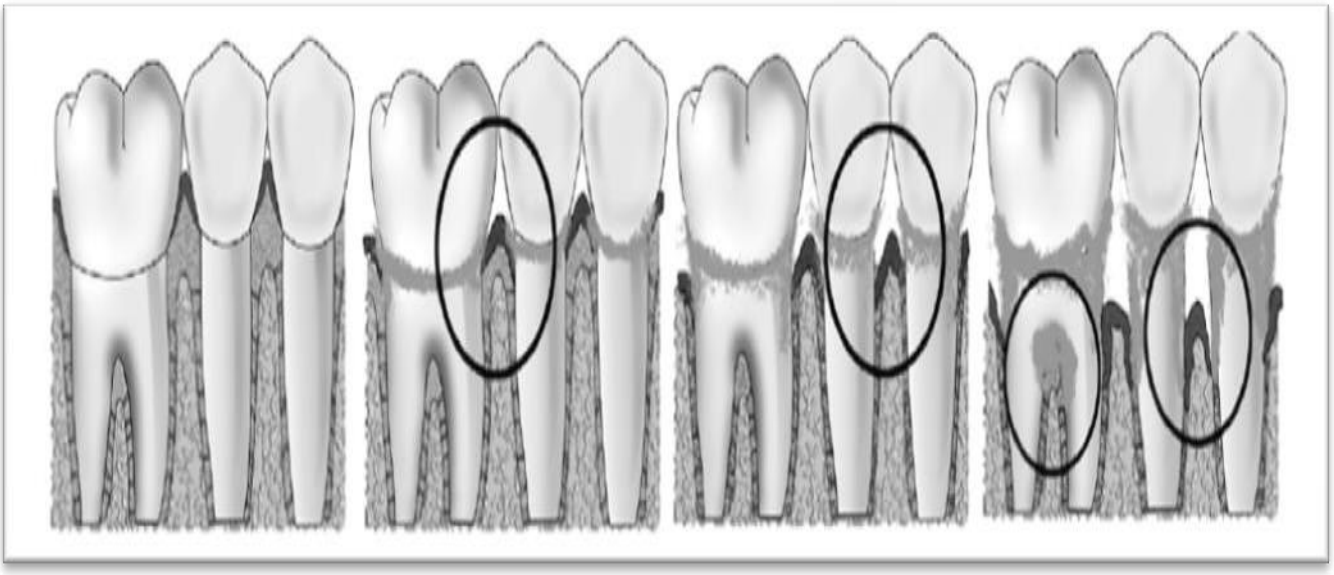


Figure 5: Diagrammatic representation of changes involved in the transition from healthy gingivae to the pathological periodontitis. Stage 1: healthy gum tissue (gingiva); Stage 2: plaque formation due to bacterial invasion; Stage 3: bacterial toxins irritate gums and trigger host-mediated responses that lead to gingivitis; Stage 4: destruction of gingiva and bone that support the tooth leading to periodontitis (Jain *et al.*,2008).

1.2 PERIDONTIUM

The periodontium is the supporting structure of a tooth, helping to attach the tooth to surrounding tissues and to allow sensations of touch and pressure. The word comes from the Greek terms *peri* meaning "around" and *odons*, meaning "tooth." Literally taken, it means that which is "around the tooth". The periodontium consists of four principal components:- in **Figure 6**

- Gingiva or the gum
- Cementum, covering the root of the tooth
- Alveolar bone
- Periodontal ligament

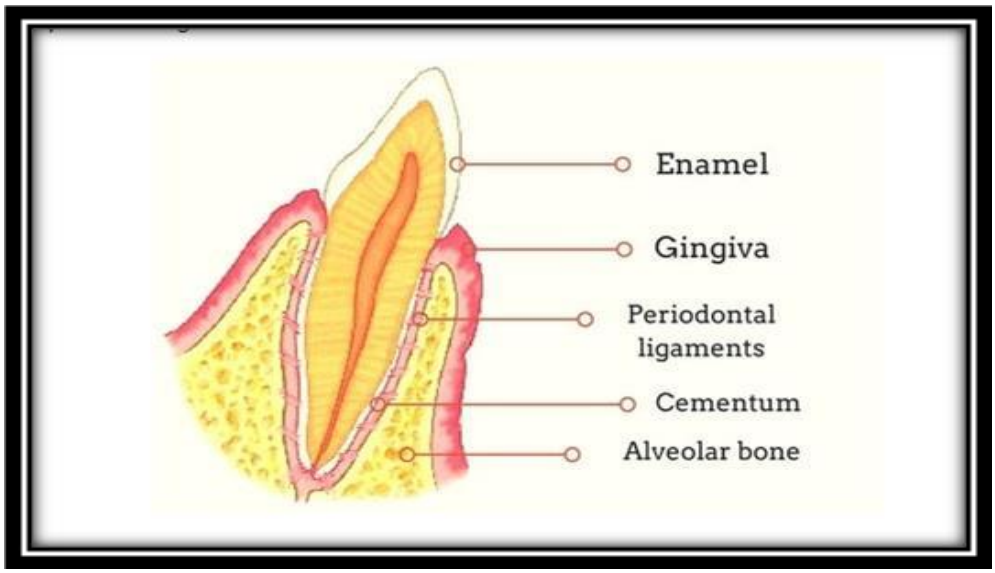


Figure 6: Components of periodontium

1.2.1 GINGIVA OR THE GUM

Gingiva is a soft tissue that overlays the jaw bone and surrounds the teeth providing a seal around them. Healthy gingival tissue is tightly bound to the underlying bone creating an effective barrier for periodontal insults to deeper tissues.

Healthy gingiva is usually coral pink but may contain melanin pigmentation. Healthy gingiva has a smooth "arcuate" appearance around each tooth, a firm texture that is resistant to movement and no reaction like bleeding to normal disturbance such as brushing or periodontal probing.

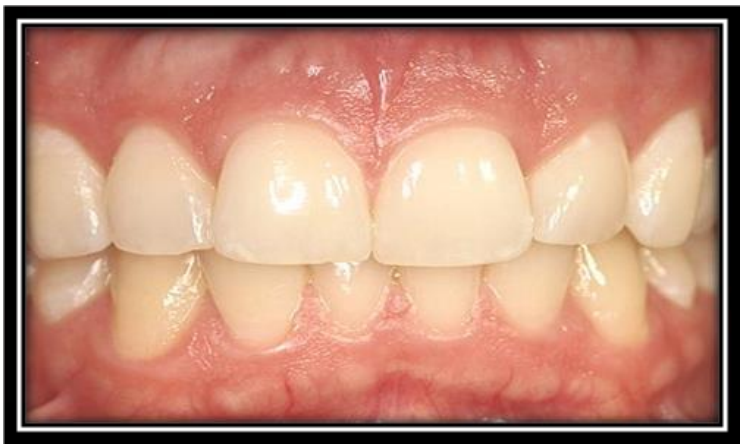


Figure 7: Healthy gingiva

1.2.2 CEMENTUM

Cementum is a specialized calcified substance covering the root of a tooth. It is the part of the periodontium that attaches the teeth to the alveolar bone by anchoring the periodontal ligament.Cementum is formed continuously throughout life because a new layer of cementum is deposited to keep the attachment intact as the superficial layer of cementum ages. It has a light yellow color and the highest fluoride content of all mineralized tissues.

1.2.3 ALVEOLAR BONE

The alveolar bone is the bone of the jaw that contains the tooth sockets on bones that hold teeth.The alveolar process contains a region of compact bone called the lamina durathat is attached to the cementum of the roots by the periodontal ligaments.Like any other bone in the human body, alveolar bone is modified throughout life; under the effect of various external factors, it may suffer processes of bone resorption or bone formation.

1.2.4 PERIODONTAL LIGAMENT

The periodontal ligament is a specialized connective tissue that attaches the cementum of a tooth to the alveolar bone. They are a network of elastic fibres that help support the tooth inside the alveolar bone socket.The functions of the periodontal ligaments include attachment of the tooth to the bone, support for the tooth, formation, and resorption of bone during tooth movement, sensation, and eruption **(Palumbo,2011)**.

1.3 TREATMENT OF PERRIODONTITIS

1.3.1 MEDICATION

In the treatment of gingivitis and periodontitis antibiotics such as tetracycline, doxycycline and metronidazole have been used **(Mundargi *et al.*, 2007).** Anaerobes and facultative organisms are affected by broad spectrum antibiotics such as tetracyclines (doxycycline, minocycline and tetracycline hydrochloride). After systemic administration at concentration 3-6µg/ml in gingival crevicular fluid it was found to be bacteristatic. High concentration of these agents achieved by local delivery and that are bacteriocidal and has been related with the minimum side effects **(Greenstein and Polson, 1998).**

Amoxicillin and metronidazole combination has been used in aggressive periodontitis management. In the management of gingival overgrowth induced by cyclosporine azithromycin was found to be very effective **(Kumar, 2019)**. Diclofenac potassium and etoricoxib both drugs have ability to decrease alveolar bone loss **(Moro et al., 2019).** It was found that using laser and chlorhexidine impart anti-inflammatory and anti-microbial effect that leads to reduction in bacterial count and increases healing **(Bansal et al., 2019).**Combination of 0.4 percent moxifloxacin with SRP (Scaling and Root Planning) proves significant benefits in various stages of chronic periodontitis on local application **(Herrera et al., 2012).**

1.3.2 SCALING AND ROOT PLANNING

SRP is also known as non-surgical, conventional therapy for periodontal disease. Removal of calculus and dental plaque achieved by deep cleaning, resulting smoothing and planning of exposed surfaces of the peridontium roots. Some agents like microorganisms, toxins are impregnated on the dentine and cementum causes inflammation. To prevent inflammation, which occur due to impregnated dentine and cementum were removed.

1.3.3 FLAP SURGERY

It is a surgery technique with intact blood supply, any kind of tissue lifted from donor site to recipient site and moved. This is different from graft, which has not intact blood supply.

1.3.4 BONE AND TISSUE GRAFT

To repair the bone fractures a surgical procedure used which are bone grafting, it replaces bones which are missing.

1.3.5 DENTIN GRAFT

Dentin bone, made from extracted teeth, Dentin comprises more than 85% of tooth structure, and the enamel consists of HA mineral and comprises 10% of tooth structure. Dentin is similar to bone in its chemical composition, by volume 70-75% is HA mineral and 20% organic matrix, mostly fibrous type I collagen. Dentin, like bone, may release growth and differentiating factors while being resorbed by osteoclasts **(Murata *et al.*, 2011).**

1.3.6 SIGNIFICANCE OF INTRA-POCKET DRUG DELIVERY SYSTEM

The use of systemic antibiotic for the treatment of periodontitis has shown some beneficial effect however, in recent years systemic antibiotics are only recommended for the treatment of rapidly progressing or refractory periodontitis. Multiple systemic doses of antibiotics have shown several drawbacks including:inadequate antibiotic concentration at the site of the periodontal pocket , a rapid decline of the plasma antibiotic concentration to subtherapeutic level, development of microbial resistance and high peak-plasma antibiotic concentrations,which may be associated with side effects. These obvious disadvantages have evoked an interest in the development ofnovel intra-pocket drug delivery systems for the treatment ofperiodontal diseases.

The periodontal pocket provides a natural reservoir, which is easily accessible for the insertion of a delivery device. The GCF provides a leaching medium for the release of a drug fromthe dosage form and for its distribution throughout the pocket. These features, together with the fact that the periodontal diseases are localized tothe immediate environment of the pocket, make the periodontal pocket a natural site for treatment with local delivery systems. The diagrammatic representation of application of various intra- pocket delivery devices in the periodontal pocket is shown in **Figure 8**

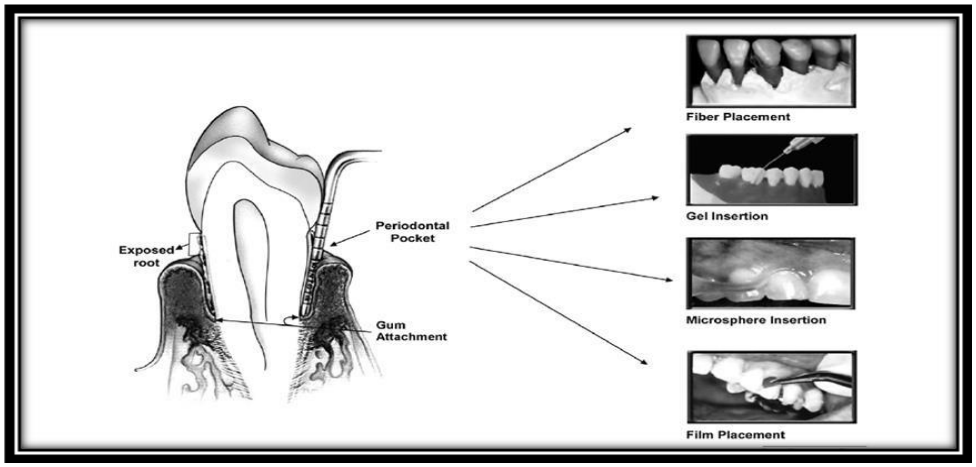


Figure 8: Diagrammatic representation of application of various intra-pocket delivery devices. Fibre packed under gum; gel injected into periodontal pocket; microspheres expelled under gum; and film placement under gum (Jain *et al.*, 2008).

Intra-pocket drug delivery systems are highly desirable due tothe potentially lower incidence of undesirable side effects, improved efficacy and enhanced patient compliance. The attractiveness of treating periodontal diseases by the intra-pocket drug delivery systems is Intra-pocket drug delivery systems are highly desirable due tothe potentially lower incidence of based on the of maintaining effective high levels of drug in the GCF for a prolonged period of time to produce the desirable clinical benefits.For these systems, the delivery vehicles can be of natural originor semi-synthetic or synthetic nature. Recent developments inpolymer prspects sciences have disclosed biocompatible and biodegradable synthetic polymers, which can be modified to meet pharmacological and biological requirements (Jain *et al.*,2008).

1.4 DIAGNOSIS

Diagnosis of periodontal disease has been done by following investigations are given as:

A. **Radiograph**

1. Periapical radiograph, Bitewing radiographs, Panoramic X-ray or combination of all these is used to diagnose the prognosis of patients.

2. Radiograph provides detailed information about patient's tooth condition. The degree of bone loss and depth of periodontal pocket can be assessed by using Radiograph and also pattern and amount of bone loss.

B. **Vitality test**

1. Electric Pulp tester or Thermal stimuli is used to diagnose the pulp vitality of tooth.

C. **Other tests**

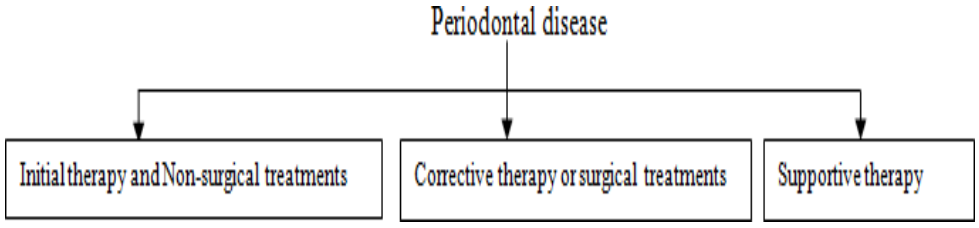
1. Full hematological screening.

2. Blood glucose level test.

3. INR or microbial plaque sampling

1.5 TREATMENT AND MANAGEMENT

Treatment plan for periodontal disease are divided into three phases as follows:



1.5.1 Initial therapy

This therapy is given at initial stage of gingivitis to control the microbial plaque formation and identify any modifiable

Types of radiograph and their parameters

1.5.2 Radiograph types	Parameters
Periapical radiograph	• Long cone parallel technique.
	• Good clarity of images as compared to horizontal radiograph.
	• Time consuming process.
Horizontal bitewings radiograph	• Use for caries detection.

	• Alveolar crest can be visualized.
	• Provides good quality of image for bone loss.
Vertical bitewing radiograph	• Shows 90° angle bitewing film image.
	• Better quality of image for extensive bone loss
Panoramic radiograph	• All teeth seen in one image or film.
	• Newer machine generated for good quality of images.
	• Details are much fine as compared to intraoral radiographs.

1.6 MANAGEMENT

Periodontal disease has capacity to control the progression of disease and inhibit the growth of microorganisms. However, the success of therapy for periodontal disease depends upon appropriate management with proper treatments. [61] The management of periodontal disease consists of removal of supra-gingival and sub- gingival dental plaque followed by healing in tooth loss. [62] In general it takes around 3 months of treatment interval to control the chronicity of periodontal disease. Maintenance period has been customized depends upon severity of disease. Supportive therapy aims long term maintenance of disease, so proper measures are taken to improve the compliances of management by patients to control the disease progression.

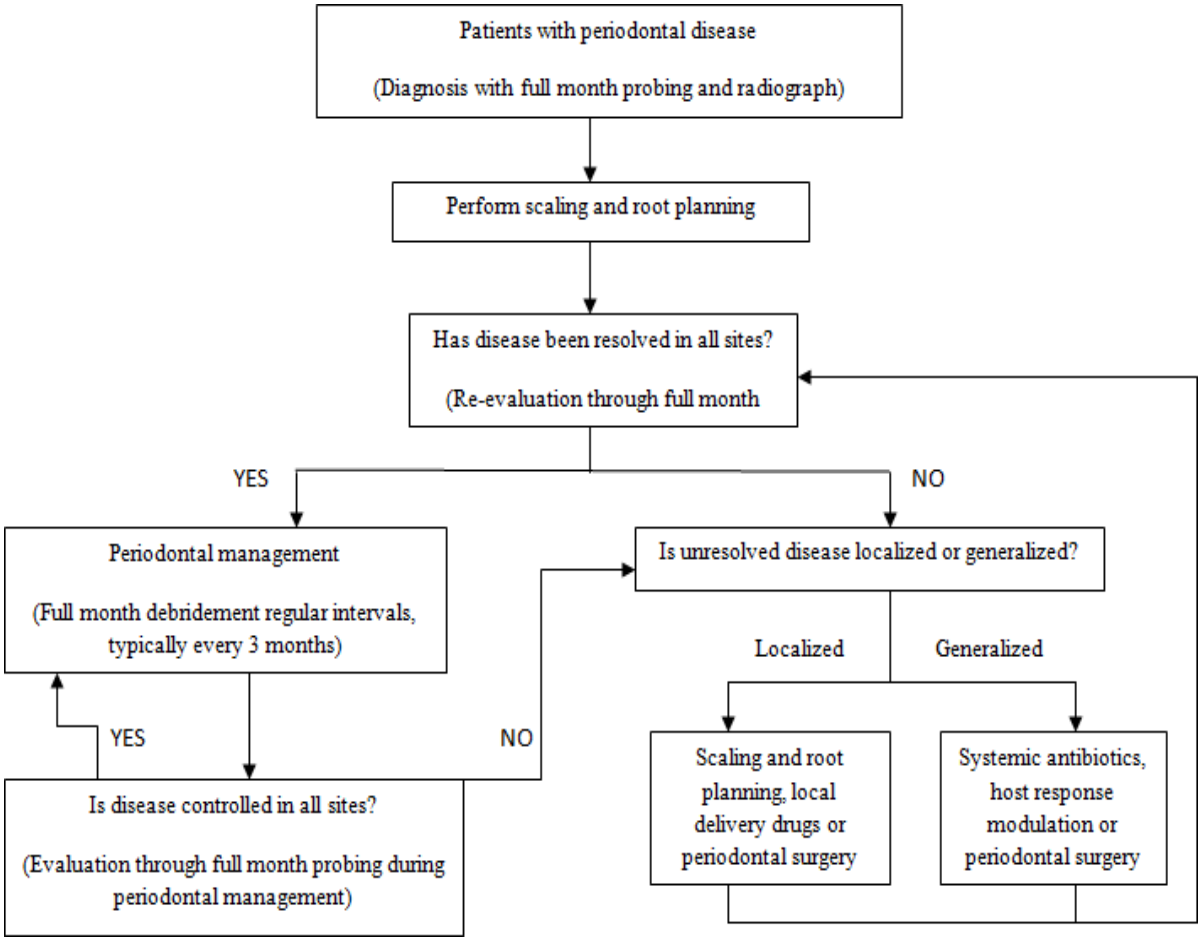


Figure 9 : Decision making for management of periodontitis

1.7 DRUG DELIVERY DEVICES

1.7.1 FIBRES

Fibres or thread-like devices are reservoir-type systems, placed circumferentially into the pockets with an applicator and secured with cyanoacrylate adhesive for the sustained release of the entrapped drug into the periodontal pocket.

1.7.2 STRIPS

Strips are thin and elongated matrix bands in which drugs are distributed throughout the polymer. Generally, strips are madeup of flexible polymers having a position securing mechanism and accommodate a wide range of interproximal spacing.

1.7.3 FILMS

A far more widely used form of intra-pocket delivery device has been in the shape of film, prepared either by solvent casting ordirect milling. Bigger films either could be applied within thecavity onto the cheek mucosa or gingival surface or could becut or punched into appropriate sizes so as to be inserted intothe site of action. Films are matrix delivery systems in which drugsare distributed throughout the polymer and release occurs by drug diffusion and matrix dissolution or erosion. This dosage form has several advantageous physical properties for intra-pocket use. The dimensions and shape of the films can be easily controlled according to the dimensions of the pocket to be treated.Itcan be rapidly inserted into the base of the pocket with minimal discomfort to the patient. If the thickness of the film does not exceed 400 mm, and it has sufficient adhesiveness, it will remain submerged without any noticeable interference with the patient'soral hygiene habits. Films that release drugs by diffusion alone are prepared using water-insoluble non-degradable polymers, whereas those that release by diffusion and matrix erosion or dissolution use soluble or biodegradable polymers.

1.7.4 INJECTABLE GELS

Together with the solid devices, semisolid formulations also receive reasonable attention for the localized delivery of antibiotics. Semisolid or gel formulations can indeed have some advantages. In spite of the relatively faster release of the incorporated drug, gels can be more easily prepared and administered. Moreover, they possess a higher biocompatibility and bioadhesivity, allowing adhesion to the mucosa in the dental pocket and finally, they can be rapidly eliminated through normal catabolic pathways, decreasing the risk of irritative or allergic host reactions at the application site. Bioadhesive semisolid, polymeric system can be utilised as an important intra-pocket delivery vehiclebecause it can easily pass through a cannula into a periodontal pocket where it solidifies *in situ* to deliver the therapeutic agent fora prolonged period. These systems exhibit a pseudo-plastic flow and thermoresponsive behavior, existing as a liquid at room temperature and gel at 34–37 8C. Micro-particulate system on-biodegradable as well as biodegradable materials have been investigated for the preparation of microspheres. These materials include the polymers of natural origin, modified natural substances and synthetic polymers. They could preferably be formulated as achip or could be part of a dental paste formulation, or otherwise bedirectly injected into the periodontal cavity.

1.7.5 NANOPARTICULATE SYSTEM

Modern drug delivery systems are designed for targeted controlledslow drug release. Up to now polymer or microparticle-based hydrogels have been applied in dentistry, which can affect therate of release because of their structure. Recently, intensive research is being performed all over the world to improve the effectiveness of delivery systems. The nanoparticulate system provides several advantages as compared with microspheres, microparticles and emulsion-based delivery systems, includinghigh dispersibility in an aqueous medium, controlled release rateand increased stability. Nanoparticles, owing to their small size penetrate regions that may be inaccessible to other delivery systems, such as the periodontal pocket areas below the gum line. These systems reduce the frequency of administration and further provide a uniform distribution of the active agent over an extended period of time.

1.7.6 VESICULAR SYSTEM

Vesicular liposomal systems are designed to mimic the bio-membranes in terms of structure and bio-behavior, and hence are investigated intensively for targeting periodontal biofilms **(Jain *et al.*, 2008)**.

1.8 DRUG PROFILE

DRUG NAME: Doxycycline hyclate

DESCRIPTION: Doxycycline hyclate is the hyclate salt form of doxycycline, a synthetic, broad spectrum tetracycline antibiotic exhibiting antimicrobial activity. Doxycycline hyclate binds reversibly to the 30S ribosomal subunit, thereby blocking the binding of aminoacyl-tRNA to the mRNA-ribosome complex. This leads to an inhibition of protein synthesis. In addition, this agent has exhibited inhibition of collagenase activity.

CHEMICAL STRUCTURE:

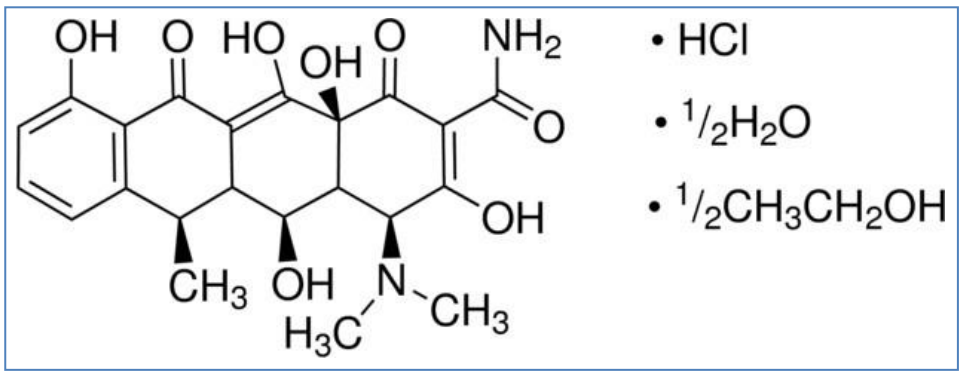


Figure 10: Chemical structure of Doxycycline

CHEMICAL NAME: [4S(4aR,5S,5aR,6R,12aS)]-4-(dimethylamino)- 1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl- 1,11deoxonaphthacene-2-carboxamide monohydrochloride, compound with ethyl alcohol (2:1), monohydrate.

SYNONYM: Doxycycline hydrochloride hemiethanolate hemihydrate

MOLECULAR FORMULA: C22H24N2O8.HCL.0.5H2O.0.5C2H8O

MOLECULAR WEIGHT: 512.94g/mol

STATE: Powder

COLOR: Yellow to yellowish green

MODE OF ACTION:

The main mechanism of action of doxycycline hyclate is on protein synthesis. Doxycycline hyclate passes directly through the lipid bilayer of the bacterial cell wall and an energy dependent active transport system pumps the drug through the inner cytoplasmic membrane. Once inside the cell doxycycline hyclate inhibits protein synthesis by binding to 30S ribosomes and prevents the addition of amino acids to the growing peptide chain **(Fig. 2.1)**. Doxycycline hyclate will impair protein synthesis in mammalian cells at very high concentrations but these cells lack the active transport system found in bacteria.

ANTIBIOTIC ACTIVITY SPECTRUM: Gram negative, gram positive, mycoplasma, parasites.

STORAGE TEMPERATURE: Store at controlled room temperature below 25°C

MELTING POINT: 180- 201 °C

SOLUBILITY: Soluble in water (50 mg/ml), yielding a clear, yellow-green solution. Mild warming may be required to fully dissolve the material. This product is also reported to be soluble in methanol, sparingly soluble in ethanol, and insoluble in chloroform or ether.

DOSE: Adults - The usual dose of oral doxycycline is 200 mg on the first day of treatment followed by a maintenance dose of 100 mg/day. The maintenance dose may be administered as a single dose or as 50 mg every 12 hours. In the management of more severe infections 100 mg every 12 hours is recommended.

Children above eight years of age- The recommended dosage schedule for children weighing 100 pounds or less is 2 mg/lb of body weight divided into two doses on the first day of treatment, followed by 1 mg/lb of body weight given as a single daily dose or divided into two doses on subsequent days. For more severe infections up to 2 mg/lb of body weight may be used. For children over 100 lb, the usual adult dose should be used.

BIOAVAILABILITY: Following a 200 mg dose, normal adult volunteers averaged peak serum levels of 2.6 mcg/mL of doxycycline hyclate at 2 hours decreasing to 1.45 mcg/mL at 24 hours.

HALF-LIFE: 16 to 22 hours.

PLASMA PROTEIN BINDING: Between 82 and 93%

PHARMACOKINETIC PROPERTIES:

ABSORPTION: Doxycycline hyclate is almost completely absorbed and is not subject to presystemic metabolism, the mean bioavailability being approximately 93%. Absorption is rapid and the peak serum concentration occurs after 2 to 4 hours. Almost the entire product is absorbed in the upper part of the digestive tract. Absorption is not modified by administration with meals, and milk has little effect.

DISTRIBUTION: Tissue distribution is good and doxycycline hyclate a strong affinity for renal and lung tissue. The volume of distribution for doxycycline ranges from 0.9- 1.8 lkg/1.

BIOTRANSFORMATION: No significant metabolism occurs.

ELIMINATION: Doxycycline hyclate is cleared intact by renal and biliary mechanisms. The antibiotic is concentrated in the bile about 40% of the administered dose is eliminated in 3 days in active form in the urine and about 32% in the fecal.

Urinary concentrations are roughly 10 times higher than plasma concentrations at the same time. In the presence of impaired renal function, urinary elimination decreases, fecal elimination increases and the half-life remains unchanged. The half-life is not affected by haemodialysis.

PHARMACODYNAMIC PROPERTIES: The tetracyclines, including doxycycline, are mainly bacteriostatic and are thought to exert antimicrobial effects by the inhibition of protein synthesis. Bacteriostatic antibiotics suppress the growth of bacteria, or keep them in the stationary phase of growth. The tetracyclines, including doxycycline, have a similar antimicrobial spectrum of activity against a variety of gram positive and gram-negative microorganisms, treating numerous infectious diseases. Cross- resistance of these microorganisms to tetracyclines is a common occurrence. Doxycycline shows favorable inta-cellular penetration, with bacteriostatic activity on a wide range of bacteria. Doxycycline has antiparasitic effects. In addition to the above effects, this drug has demonstrated anti- inflammatory actions, which may help to manage inflammatory conditions such as rosacea.

ADVERSE REACTIONS:

GASTROINTESTINAL: Anorexia, nausea, vomiting, diarrhea, glossitis, dysphagia, enterocolitis, and inflammatory lesions (with monilial overgrowth) in the anogenital region. Hepatotoxicity has been reported rarely. These reactions have been caused by both the oral and parenteral administration of tetracyclines. Rare instances of esophagitis and esophageal ulcerations have been reported in patients receiving capsule and tablet forms of drugs in the tetracycline class. Most of these patients took medications immediately before going to bed.

SKIN: Maculopapular and erythematous rashes. Exfoliative dermatitis has been reported but is uncommon. Photosensitivity is discussed above.

HYPERSENSITIVITY REACTIONS: Urticaria, angioneurotic edema, anaphylaxis, anaphylactoid purpura, serum sickness, pericarditis, and exacerbation of systemic lupus erythematosus.

BLOOD: Hemolytic anemia, thrombocytopenia, neutropenia, and eosinophilia have been reported.

OTHERS: Bulging fontanels in infants and benign intracranial hypertension in adults. When given over prolonged periods, tetracyclines have been reported to produce brown-black microscopic discoloration of thyroid glands. No abnormalities of thyroid function are known to occur

1.9 EXCIPIENT PROFILE: METHYLCELLULOSE

Methylcellulose is a compound used as a bulk forming laxative and is not an approved medication.

Brand Names: Citrucel

Generic Name: Methylcellulose

Background: Methyl cellulose polymer consisting of numerous linked glucose molecules used as a stabiliser, thickener and emulsifier for foodstuffs and cosmetics. The Degree of Substitution (DS) of a given form of methyl cellulose is defined as the average number of substituted hydroxyl groups per glucose with a theoretical maximum of 3, however more typical values are 1.3 2.6. Methyl cellulose is a hydrophilic white powder in pure form and dissolves in cold (but not in hot) water, forming a clear viscous solution or gel. It is available under a variety of trade names as a treatment for constipation. Like cellulose, it is not digestible, not toxic, and not allergenic

Type: Small Molecule

Groups: Approved

Synonyms: Cellulose methyl, cellulose methyl ether, Cellulose methylate, Methyl cellulose, methylated cellulose, Methylcellulose, Methylcellulosum, Metilcelulosa

External IDs : E461

PHARMACOLOGY

Indication

Solutions containing methyl cellulose are used as substitute for tears or saliva if the natural production of these fluids is disturbed. It is also used or constipation, diverticulosis, hemorrhoids and irritable bowel syndrome. Used in the manufacture of capsules in nutritional supplements. Its edible and nontoxic properties provide a vegetarian alternative to the use of gelatin. Reduce drug development failure rates, Build, train, & validate machine-learning models with evidence-based and structured datasets.

Associated Conditions: Constipation, Dry Eye Syndrome (DES)

Associated Therapies: Eye disinfection, Contraindications & Blackbox Warnings

Avoid life-threatening adverse drug events: Improve clinical decision support with information on contraindications & blackbox warnings, population restrictions, harmful risks, & more.

Pharmacodynamics

It increases the bulk in your stool, an effect that helps to cause movement of the intestines. It also works by increasing the amount of water in the stool, making the stool softer and easier to pass.

Mechanism of action

Methylcellulose absorbs water in the gastrointestinal lumen thereby increasing the bulk of the stool. This leads to distension and stimulation of peristalsis. The ability of methylcellulose to absorb water may contribute to its efficacy in the management of diarrhea by once again increasing the bulk and consistency of the stool.

Absorption

Cellulose derivatives considered in this report are virtually unabsorbed and little or no degradation of absorbed and little or no degradation of absorbable products occurs in the human digestive tract. In humans, virtually 100 percent of orally ingested methyl cellulose can be recovered in the feces withihn four days, indicating that absorption does not occur.

Volume of distribution: Accumulation in liver, spleen, lymph nodes, kidney, and vascular walls.

Protein binding: Not Available

Metabolism: Reported that when methylcellulose was given iv to dog and rabbit , aside from effect upon circulating blood, inability of body to degrade substance led to its retention & accumulation in liver, spleen, lymph nodes, kidney, and vascular walls.

Route of elimination: When swallowed they are not absorbed to any appreciable degree and appear unchanged in feces.

Half-life: 4.2 minutes

Clearance: Not Available

Adverse Effects: Improve decision support & research outcomes, With structured adverse effects data, including: blackbox warnings, adverse reactions, warning & precautions, & incidence rates.

Toxicity: Organism: Mouse Test type: LD50

Route : Intraperitoneal

Reported Dose: 275gm/kg (275000mg/kg)

Toxic Effect: Details of toxic effects not reported other than lethal dose value

Organism: Mouse Test type: LDLo

Route : Intravenous

Reported Dose: 1gm/kg (1000mg/kg)

Toxic Effect: Details of toxic effects not reported other than lethal dose value

2.1 LITERATURE REVIEW

Noah S et al., (2022) Periodontal diseases are disorder methods related to the periodontium, a term used to explain the supportive equipment surrounding a tooth, which incorporates the gingival tissue, alveolar bone, cementum, and periodontal ligament. Gingivitis is the mildest shape of periodontal disorder and may be determined in as much as 90% of the populace. It is a reactive condition this is reversible upon the development of oral hygiene. Periodontitis is when the periodontal condition has advanced past gingivitis right into a chronic, unfavourable, irreversible inflammatory ailment country. The micro organism then can penetrate deeper into the tissues and surrounding periodontium. This triggers a number reaction in an attempt to defend towards the invading micro organism. Periodontitis results in loss of attachment of the periodontium, which ultimately progresses to alveolar bone loss, probably resulting in loss of the affected teeth. This hobby describes the assessment and control of periodontal sicknesses and highlights the position of the interprofessional healthcare crew in figuring out and treating sufferers with these conditions.

Rahimi A et al., (2021) periodontitis is the 6th customary disorder amongst people and plainly there are not unusual danger elements between these sicknesses that are growing conversation among prevalence and treatment. The motive of this observe is to assess the articles that reviewed the connection between coronary heart sicknesses and periodontitis. Three databases, consisting of PubMed, Scopus, and Web of Science had been searched until November 2020. The search phrases "periodontal disease, periodontitis, oral health, cardiovascular sickness, atherosclerosis, myocardial infarction, hypertension, coronary heart sickness, angina pectoris, arterial fibrillation, arrhythmia, and peripheral artery disorder" have been utilized in aggregate to perceive the guides providing data. MI, HTN, atherosclerosis illnesses for coronary artery, IE, HF, AF, and PAD have been related to periodontitis. It seems that the treatment of periodontitis can also assist to enhance the state of mentioned coronary heart-related sicknesses. However, extra studies are needed to prove this relationship.

Preshow PM et al., (2020) Periodontitis and diabetes are complicated persistent sicknesses, related by a longtime bidirectional courting. Risk for periodontitis is extended to three instances in humans with diabetes as compared to individuals with out, and the level of glycaemic control is prime in determining threat. In people who do now not have diabetes, periodontitis is related to better glycated haemoglobin (HbA1c) and fasting blood glucose stages, and severe periodontitis is associated with increased danger of developing diabetes. In human beings with kind 2 diabetes, periodontitis is related to higher HbA1c tiers and worse diabetes headaches. Treatment of periodontitis in humans with diabetes has been shown to result in stepped forward glycaemic manipulate, with HbA1c discounts of three-4 mmol/mol (zero.3-0.4%) within the brief time period (three-four months) publish-remedy. Given that remedy of periodontitis consequences in clinically relevant discounts in HbA1c, the dental crew has an critical position in the management of sufferers with diabetes. Improved interprofessional working in terms of diabetes and periodontitis has been advocated by professional and clinical corporations, though practical and systemic barriers make this challenging.

Mann J et al., (2020) One of the most general diseases affecting human beings are those which have an effect on the oral hollow space. Several illnesses can have an effect on the mouth, inclusive of oral cancer, dental caries, Lichen Planus and, of direction, periodontal disease with its variants. This evaluate discusses gingivitis, periodontitis and peri-implantitis with a quick cognizance at the distinctive number one prevention aids, which includes mechanical, chemical and the brand new generation of potential merchandise to be used within the future dental marketplace, products which are prepared from herbal assets, the overall fashion worldwide. In this evaluation, not all new innovations may be referred to, but, the role of elderly garlic extract (AGE) might be defined further to a new toothpaste which originates from the Dead Sea and the Purecare dental which produces the Ozone (H3) for the prevention of periodontal ailment

Dubey P et al., (2020) Periodontal illnesses consists of a extensive variety of inflammatory situations which reasons degeneration of Periodontium and affects all supporting systems of teeth consisting of gingiva, periodontal ligament, cementum and alveolar bone and many others. Followed by tooth loss. WHO had reported approximately 10-15% of the sector population is affected by severe periodontal situation. It is complex infectious ailment resulting from aggressive microbial increase on tooth. The essential aim of this look at is to offer systemic update on periodontal sickness regarding its degrees, occurrence, pathophysiology, diagnosis, remedy and management. The pathophysiology of periodontal disease is associated with dental plaque, microbial biofilm formation and immunogenicity of the host cell. The severity of this sickness relies upon upon threat elements and chronological degrees. Prevention is attained via day by day protection of oral hygiene. Various surgical and non-surgical treatments are to be had to manipulate the formation of microbial biofilm. Daily maintenance and periodic management of this disease control worsening of condition and shows precise improvement in oral fitness.

Shah A et al., (2017) carriedout their paintings on periodontitis is gingivitis wherein inflammatory changes consisting of bleeding and swelling are restricted to marginal gingiva and surrounding connective tissue, with out the involvement of periodontal ligament. Periodontitis takes place when inflammatory modifications attain the periodontal ligament and alveolar bone in the long run main to enamel loss. The underlying motive of the disorder is the presence of a polymicrobial biofilm that forms as plaque on the tooth surface and the ensuing host response that this plaque induces. Inflammatory responses are initiated by some of late stage colonizers and the virulence elements they produce. Among those late level pathogens is P. Gingivalis, a Gram bad, asacchrolytic bacterium with a huge arsenal of virulence factors.

Kinane DF et al., (2018) Periodontal sicknesses contain a wide range of inflammatory conditions that have an effect on the supporting systems of the teeth (the gingiva, bone and periodontal ligament), that can result in teeth loss and contribute to systemic irritation. Chronic periodontitis predominantly impacts adults, however competitive periodontitis may additionally sometimes occur in kids. Periodontal disorder initiation and propagation is through a dysbiosis of the commensal oral microbiota (dental plaque), which then interacts with the immune defences of the host, leading to infection and disorder. This pathophysiological scenario persists thru bouts of hobby and quiescence, till the affected teeth is extracted or the microbial biofilm is therapeutically removed and the irritation subsides. The severity of the periodontal disorder depends on

environmental and host chance factors, both modifiable (for instance, smoking) and non-modifiable (for example, genetic susceptibility). Prevention is done with day by day self-carried out oral hygiene and professional removal of the microbial biofilm on a quarterly or bi annual basis. New remedy modalities that are actively explored encompass antimicrobial therapy, host modulation remedy, laser therapy and tissue engineering for tissue repair and regeneration.

Highfield J et al., (2009) Periodontal sicknesses have been identified and treated for at least 5000 years. Clinicians have recognized for decades that there are apparent variations inside the presentation of periodontal diseases and feature tried to classify these sicknesses. Systems of classifications of sickness have arisen allowing clinicians to increase systems which may be used to discover sicknesses on the subject of aetiology, pathogenesis and remedy. It lets in us to prepare effective remedy of our patients' diseases. Once a disease has been diagnosed and labeled, the aetiology of the circumstance and appropriate evidence-based totally treatment is usually recommended to the clinician. Common systems of class additionally permit effective verbal exchange between health care professionals using a commonplace language. Early attempts at class had been made on the basis of the clinical characteristics of the diseases or on theories in their aetiology.

Chakrabarty and Nath, 2018 This assessment article offers facts about several possible mechanisms that cause in situ gel formation: solvent alternate, UV-irradiation, ionic go-linkage, pH trade, and temperature modulation.

Gadad A.P et al.,(2017) Oxiconazole nitrate is every other topical extensive variety antifungal specialist used to treat shallow parasitic contaminations. It has a low watery dissolvability because of which various systems are applied to enhance its bioavailability. Emulgel has evolved as a standout among the most intriguing topical medication conveyance framework for hydrophobic medications like Oxiconazole as it has double discharge manipulate frameworks i.E. Emulsion and gel.

Usmania et al., (2017) Minoxidil is a first rate vasodialator (antihypertensive medicinal drug), i.E. Coordinate unwinding of arteriolar smooth muscle with little impact on venous capacitance. Minoxidil is the primary FDA affirmed topical answer with tested adequacy for the remedy of androgenic alopecia. Alopecia is portrayed through spherical or oval patches of non-scarring male sample baldness. It is depended on that it simply aims scalp male pattern baldness that is probably incomplete (transient or diligent) or end (alopecia totalis), but sometimes it might enhance to motive add up to body male sample baldness (alopecia universalis). Eumlgels are emulsion gels which incorporates haphazardly circulated oil microdroplets. They are emulsions each of oil-in-water or water-in-oil write, which are gelled by mixing with gelling expert.

Samala M.L and Sridevi G (2016) targeting the fundamental little bit of numerous polymers as gelling specialists in the listing of emulgels. The assume similarly achieves the unconventional technique for accessibility and examination parameters of emulgels**.**

KAUR J et al.,(2016) taken into consideration Optimum remedial results calls for valid remedy willpower in addition to possible conveyance of medicine. In the course of latest many years, controlled medication conveyance has grew to become out to be steadily important in the pharmaceutical commercial enterprise. The pharmacological response, i.E wanted helpful and undesired restorative impact of a medicinal drug relies upon grouping of medicine came to its web site of hobby and thusly is based upon dose frame. With everyday remedy conveyance frameworks, negative patient consistence is a noteworthy trouble saw in clinical exercise. Human skin is a directly available floor of drugs conveyance. The capability of utilising skin as an objective has been perceived but its furthest layer goes about as an obstruction to the entrance of materials permitting simply little debris to infiltrate over some undefined time frame. As of past due distinct tactics has been utilized to avoid the stratum corneum and to construct movement through the pores and skin movie utilizing diverse saturation upgrade strategies.

Jain et al., 2008 Estimated the role of local drug shipping gadgets in the management of periodontal diseases which include fibers, strips, movies injectable gels, microparticulate device, nanoparticle machine and vesicular device.

Rifkin et al., 1993 In that regard, tetracyclins and their chemically-modified analogs have been shown to inhibit the hobby of the matrix metalloproteinase (MMP), collagenase.

Rajendran et al., 2017 States that the polaxamer 25% and methyl cellulose 5% fashioned a perfect thermosensitive injectable gel at 37°C for subgingival shipping of SMV and additionally show managed drug release in vitro.

Ruel-Gariepy et al., 2004 Methylcellulose solutions remodel into opaque gels between forty and 50 °C. This segment transition temperatures can be decreased by way of chemical or bodily changes. For instance, NaCl decreases the transition temperature of methylcellulose solutions to 32–34°C

Bhowmik et al., 2011 The gel temperature of one% w/v methylcellulose (MC) became 60°C. It was discovered that five–7% w/v sodium chloride (NaCl) was able to reducing the gel temperature below physiological temperature, i.E. 37°C.

Smith and Bradley, 1983 Studied the melancholy of freezing factor because of the addition of sweeteners. Sweeteners assayed in this newsletter had been monosaccharides – glucose, galactose and fructose; diasaccharides- lactose, maltose and sucrose. And the result became located that the monosaccharides have better affinity than disaccharides to lessen the freezing factor at equal attention**.**

He et al., 2018 This assessment summarizes periodontitis-associated biomarkers, conventional scientific strategies, and these days advanced point-of-care periodontitis checking out structures (LOC and paper-based structures) for diagnosing periodontitis.

Botelho et al., 2018 Suggested that topics with aggressive periodontitis have better salivary cortisol degrees than wholesome ones or sufferers with persistent periodontitis. Such salivary cortisol response distinction may additionally have a terrible impact at the periodontium, contributing to worsening the weight of competitive periodontitis disorder.

Gong et al., 2019 stated that the concentrated on ability of FA-F27-PCL nanoparticles with 10% molar content of folate group on the floor become higher than that with 50% and 91% molar content material of folate organization.

Smith and Bradley, 1983 Studied the despair of freezing point because of the addition of sweeteners. Sweeteners assayed in this article have been monosaccharides – glucose, galactose and fructose; diasaccharides- lactose, maltose and sucrose. And the result became observed that the monosaccharides have better affinity than disaccharides to lessen the freezing factor at same concentration.

RESEARCH ENVISAGED

Periodontitis is the inflammation of the surrounding tissues of teeth, caused by anaerobic gram-negative bacteria. This results in destruction of collagen of periodontium and resorption of alveolar bones.

Treatment of periodontitis is difficult with presently available therapeutic systems. These therapeutic systems possess limited availability at target site and easily wash out with saliva resulting in termination of drug action. Hence, there is a need to develop such system which can facilitate the controlled release of drug for longer duration and are persistent in nature. Therefore, it is envisaged to design thermoresponsive *in situ* gelling system for periodontitis with better and enhanced therapeutic efficacy during their treatment.

The proposed thermoresponsive in situ gelling system bearing doxycycline hyclate will definitely enhances the drug delivery to the periodontal pockets and provide better therapeutic efficacy for the periodontitis treatment

PLAN OF WORK

A. Literature survey and procurement of materials

B. Pre-formulation studies

- Identification of drug
  - ✓ Physical appearance
  - ✓ Melting point
  - ✓ I.R. spectroscopy
  - ✓ UV spectroscopy
  - ✓ Solubility studies

- ✓ Partition coefficient
- ✓ Preparation of standard curve of drug
- ✓ Drug-excipients compatibility study

C. Preparation and optimization of the formulation

D. Characterization of formulation.

- ➢ Appearance
- ➢ pH
- ➢ Viscosity
- ➢ Syringibility study
- ➢ Gelling temperature
- ➢ Gelling time
- ➢ Total drug content

E. *In-vitro* drug release study

F. Stability studies

- ➢ Effect of storage temperature on appearance and clarity
- ➢ Effect of storage temperature on viscosity

G. Results and Discussion

H. Compilation of statistical analysis data and submission of thesis.

5. PREFORMULATION STUDIES OF DOXYCYCLINE HYCLATE

In order to prepare and characterize pharmaceutical dosage form having therapeutic moiety, pre-formulation studies are done to check the physicochemical, natural properties of the drug that can play the major role in the development of formulation.

PREFORMULATION STUDIES

5.1 DRUG IDENTIFICATION TESTS

5.1.1 Physical appearance

5.1.2 Light absorption test for doxycycline

5.1.3 Melting point

5.1.4 IR spectroscopic analysis

5.1.5 DSC of doxycycline

5.2 SOLUBILITY PROFILE

5.3 UV SPECTROSCOPIC ANALYSIS

5.3.1 Determination of absorbance maxima

5.3.2 Standard curve of DH in PBS pH 6.8

5.4 PARTITION COEFFICIENT STUDIES

5.5 DRUG-POLYMER COMPATIBILITY STUDY USING DSC

5.6 RESULTS & DISCUSSION

5.7 IDENTIFICATION TESTS

5.8 PHYSICAL PROPRTIES

Doxycycline Hyclate (DH) was found to have yellow, ethanolic odor, crystalline powder and compared with the reported information in official monographs **(I.P 2010).**

5.9 LIGHT ABSORPTION TEST FOR DOXYCYCLINE

Absorbance of a 0.001% solution in a mixture of 99 Volume of methanol and 1 volume of 1M HCl, measured within 1 hour of preparing the solution and absorbance should come between the ranges of 0.300-0.335 at λ 349nm.

5.10 (MP) MELTING POINT

MP of Doxycycline Hyclate determined through the MP apparatus (Superfit, India). For the MP determination drug filled and sealed in the capillary and placed in the MP apparatus and melting time was recorded.

Table 1: Physical appearance and melting point

Properties	Observations	Standard (I.P.,2010)
Color	Yellow	Yellow
Odor	Slightly ethanolic	Slightly ethanolic
Physical Appearance	Crystalline powder	Crystalline powder
Melting Point	175-195°C	180-201°C

5.11 IR spectroscopy

IR spectroscopy gives information about FG or nature about that particular compound. FTIR of the sample was performed using the FTIR instrument. (Bruker FTIR 8400S ALPHA) at ADINA institute of pharmacy, Sagar. Drug sample was dessicated for 24 hours before performing IR. IR spectra was interpreted, which matched with the IR spectrum as reported in the official monograph.

Table 2: FTIR data interpretation of Doxycycline Hyclate

Range(cm^{-1})	Frequency (cm^{-1}) Observed	Functional group
3400-3300	**3340.7047**	O-H stretching
3000	**2980.6580**	C-H stretching
2962	**2946.2699**	CH3 Asymmetric stretching
2872	**2854.8146**	CH3 Symmetric stretching
1715	**1677.5937**	C=O stretching
1640-1550	**1534.7224**	Amide N-H bending
1600-1450	**1482.8463**	Aromatic C=C stretching
1375	**1367.8125**	CH3 bending
1200	**1152.6182**	C-C Stretching
1260-1000	**1121.6979**	C-O Stretching
750	**784.0527**	C-Cl Stretching

5.12 DIFFERENTIAL SCANNING CALORIMETRY (DSC) OF DOXYCYCLINE

The DSC curve of Doxycycline was performed using NETZSCH STA 449 F1 Jupitor DSC instrument. 5mg of Doxycycline was weighed and taken in a standard aluminum pan, while the empty pan of was used as a reference standard. The heat cycle for sample was set from 0-250˚C with constant heating rate of 10˚C per minute using nitrogen gas as an inert gas. Calibration curve of temperature and heat flow was reported experiment.

5.13 SOLUBILITY PROFILE OF DOXYCYCLINE HYCLATE

The solubility of the drug was determined in different solvent by taking the 0.5 gm samples for the 24 hours. Solubility profile of DH shown in table 3.3.

Table 3: DH solubility in Solvents

Solvents	Solubility
PBS (pH 6.8)	++++
Distilled water	++++

5.14 UV SPECTROSCOPIC ANALYSIS

5.14.1 DETERMINATION OF ABSORBANCE MAXIMA IN PBS (PH 6.8)

10mg of DH was dissolved in PBS pH6.8 and volume adjusted upto 100ml. and different aliquots of concentration ranging from 0- 20 microgram/ml was prepared and checked its absorbance at λmax 273nm.

A. PBS (PH-6.8) preparation

11.45g of KHP04 and 28.80gm Na2HPo4 in 900mL of water and diluted upto 1000 ml with the same solvent.

B. SC of DH PBS (pH 6.8)

DH (10mg) was dissolved in PBS (pH 6.8). and aliquots of resulted solution was prepared This 0.2ml, 0.4ml, up to 2ml. The solutions were filtered and analyzed at λ max 273nm using

Table 4 : SC data of DH in PBS (pH 6.8)

S.NO.	CONC. (µg/ml)	ABS
1	**0**	0
2	2	0.072
3	4	0.151
4	6	0.220
5	8	0.290
6	10	0.359
7	12	0.435

8	14	0.481
9	16	0.571
10	18	0.637
11	20	0.722

5.15 (PC) PARTITION COEFFICIENT

Partition of drugs from oil phase n- octanol and aqueous phase water .

$$P_{O/W} = [\ C_{ORGANIC} / C_{AQUEOUS}\ ] \text{ equilibrium}$$

Table 5: PC of DH

Medium	Partition coefficient (P)
n-octanol :Water	**0.80**
n-octanol : PBS (6.8)	**0.63**

5.16 DRUG-POLYMERS COMPATIBILITY STUDY USING DSC

The DSC spectra of Doxycycline hyclate and excipients mixture were recorded. These different spectra were then compared with respective DSC spectra of drug samples for any interaction. The different spectra obtained were shown in **Fig. 3.5**

Fig. 3.5 DSC curve of Doxycycline Hyclate, Methylcellulose and Polaxamer 407

5.17 RESULTS AND DISCUSSION

Preformulation Study of DH studied using various tests. Identification of drugs by UV- spectrophotometric and FTIR methods along with the study of organoleptic properties of drugs. The Preformulation study was further carried out with the quantitative estimation of drug i.e. estimation of absorption maxima, calibration curve, melting point and solubility determination.

The supplied drug was found to be yellow crystalline powder with ethanolic odor. Physical appearance was same as reported by literature. The IR spectrum of the drug was interpreted for the structure of DH. The obtained IR spectra of drug indicated all prominent peaks (i.e.3340, 2980, 2946, 2854, 1677, 1534, 1482,1367,1152,1121 and 784 1/cm) indicates the presence of FG (OH stretching, CH stretching, CH_3 asymmetric stretching, CH_3 symmetric stretching, C=O stretching, amide N-H bending, aromatic C=C stretching, CH_3 bending, C-C stretching, C-O stretching and C-Cl stretching) present in DH. These findings indicated that the supplied samples of DH were pure in nature. (Table 3.2 and Fig.3.2). The solubility profile

of DH was determined in different solvents at room temperature and it was observed that drug was soluble in distilled water and PBS (6.8) and all the observation compliance with I.P 2010. The partition coefficient of DH was found to be 0.63 and 0.80 in PBS pH 6.8: n octanol (1:1) and DW: n octanol (1:1) which represents the hydrophilic nature of drug.

The Absorbance maximum of DH solution in PBS (pH-6.8) was measured by UV-visible spectrophotometer (Shimadzu 1601) and it was found to be 273 nm. The above maxima were found to be matched with the standard **(Phaechamud *et al.*, 2019)**.

The calibration curve was prepared in PBS using the UV- visible spectrophotometric method. The analysis of the calibration curves showed good linearity data in the curve as the correlation coefficient was found to be much closer to one. The result shows that the drug followed Lambert- Beer's law for DH. (Fig 3.3 and 3.4). Drug excipients compatibility was determined by DSC. It showed that there was no any shift or widening or narrowing of peaks being observed in either of the drug samples to be used for future work. It can be summarized that the drug were authentic and results were in accordance with the standards given in IP. Various analytical techniques confirmed the chemical identification of the supplied drugs.

5.18 OPTIMIZATION, FORMULATION AND CHARACTERIZATION

gel formulated through in-situ gel formation process at site of application. .*In situ gel* is widely used for the intra periodontal pockets delivery of drugs. They provide controlled and sustained release of drugs. This chapter comprises the preparation and characterization of *in situ* gel containing doxycycline hyclate for treating the periodontitis.

For the preparation of the *in situ* gel doxycycline hyclate was purchased from Swapnaroop Pharma Pvt. Ltd. Aurangabad (Maharashtra).

5.19 PREPARATION OF IN SITU GEL

5.19.1 MATERIALS

The drug (Doxycycline hyclate) was purchased from Swapnaroop Pharma Pvt. Ltd. Aurangabad (Maharashtra).PM-407, i.e. Polaxamer-407, MC-4000 cps (Methyl cellulose-4000 cps), Benzalkonium chloride (Preservative) were obtained from a chemical store of the Department of Pharmaceutical Sciences.

5.19.2 PREPARATION OF DOXYCYCLINE HYCLATE BEARING *IN SITU* GEL

weighted amount of drug DH (5% w/v) was uniformly dispersed in MC solution under a magnetic stirrer at 600 RPM for 20 minutes and allowed to refrigerate for 24 hour. Further medicated *in situ* gels were prepared using similar methods as vehicle *in situ* gels. The compositions of different batches are shown in **Table 4.1 and 4.2**.

5.20 OPTIMIZATION

Various variables which affected the preparation and properties of *in situ* gel like process variables, formulation variables were optimized and studied. The formulation variables include drug concentration and polymer concentrations were optimized on the basis of gelation time, gelation temperature and syringeability study.

- **Optimization of formulation variables**
- Optimization of poloxamer 407 concentration
- Optimization of methyl cellulose 4000 cps concentration
- **Optimization of process variables**
- Optimization of stirring temperature
- Optimization of stirring speed and time

5.21 OPTIMIZATION OF FORMULATION VARIABLES

Various formulation variables i.e., concentration of polymers (Polaxamer 407 and Methylcellulose 4000 cps) which affect the preparation and properties of in situ gel formulation were identified and studied. The optimization was carried out on the basis of gelation temperature, gelation time and syringeability study.

5.21.1 OPTIMIZATION OF POLOXAMER 407 CONCENTRATIONS

The in situ gel was prepared with different concentration of Polaxamer 407 (20%, 25%, 30% w/v) keeping methyl cellulose concentration constant. Optimization was done on the basis of good syringeability, temperature of gelation and time of formulation. The syringeability, temperature of gel formation and gelation time of optimized formulation is shown in **Table 6.**

TABLE 6: OPTIMIZATION OF POLOXAMER 407 CONCENTRATIONS BASED ON SYRINGEABILITY, GELATION TEMPERATURE AND GELATION TIME BY TAKING CONSTANT CONCENTRATION OF METHYL CELLULOSE 4000 CPS (4%, 5%, 6% W/V) AND DRUG (5% W/V).

S. N.	Conc. of methyl cellulose (% w/v)	Conc. of Poloxamer 407 (% w/v)	Conc. of preserv-ative (% w/v)	Gelation temp. (°C)	Gelation time (seconds)	Syringeability by 21 gauge needle
P1	4	20	0.01	41	206	Pass
P2	**4**	**25**	**0.01**	**38**	**68**	**Pass**
P3	4	30	0.01	35	24	Fail
P4	5	20	0.01	40	135	Pass
P5	**5**	**25**	**0.01**	**37**	**28**	**Pass**
P6	5	30	0.01	34	20	Fail
P7	**6**	**20**	**0.01**	**38**	**70**	**Pass**
P8	**6**	**25**	**0.01**	**36**	**24**	Fail
P9	6	30	0.01	33	18	Fail

5.21.2 OPTIMIZATION OF METHYL CELLULOSE 4000 CPS CONCENTRATION

The in situ gel formulations were prepared with varying concentrations of methyl cellulose 4000 cps (4%, 5%, 6% w/v) keeping poloxamer 407 concentration constant. Optimization was done on the basis of good syringeabilitytemperatute and time at which gel formation . The syringeability, temperature and time of gel formulation optimized is shown in **Table 7**

TABLE 7: OPTIMIZATION OF METHYL CELLULOSE 4000 CPS CONCENTRATIONS BASED ON SYRINGEABILITY, GELATION TEMPERATURE AND GELATION TIME BY TAKING CONSTANT CONCENTRATION OF POLOXAMER 407 25% W/V AND DRUG (5% W/V).

S. N.	Conc. of methyl cellulose (% w/v)	Conc. of Polaxamer 407 (% w/v)	Conc. of preserv-ative (% w/v)	Gelation temp. (°C)	Gelation time (Seconds)	Syringibility by 21 guage needle
F10	4	25	0.01	38	206	Pass
F11	**5**	**25**	**0.01**	**37**	**68**	**Pass**
F12	6	**25**	0.01	39	135	Pass

5.22 OPTIMIZATION OF PROCESS VARIABLES

5.22.1 OPTIMIZATION OF STIRRING TEMPERATURE DURING THE MIXING OF POLAXAMER 407, METHYL CELLULOSE AND MIXTURE SOLUTION (METHYL CELLULOSE, DRUG AND PRESERVATIVE)

Effect of different temperature (5-10°C, 10-15°C, 15-20°C and 20-25°C) on solubility of PM-407, MC-4000 cps and mixture solution (MC-4000 cps, drug and preservative) was studied by using the magnetic stirrer, at optimized RPM (600 RPM) as seen in **Table 8**.

TABLE 8: OPTIMIZATION OF STIRRING TEMPERATURE BASED ON SOLUBILITY OF PM-407, MC-4000 CPS AND MIXTURE SOLUTION (MC-4000 CPS, DRUG AND PRESERVATIVE)

				Effect On Stirring of

S. N.	Formulation code	Stirring Speed (RPM)	Temperature (°C)	Polaxamer 407 Solution (25% w/v)	Methyl Cellulose 4000 CPS (6% w/v)	Mixture solution
1	F111	600	5-10	Very Good	Good	Good
2	F112	600	10-15	Good	Poor	Poor
3	F113	600	15-20	Poor	Very poor	Very poor
4	F114	600	20-25	Very poor	Don't solubilize	Don't solubilize

5.22.2 OPTIMIZED PARAMETERS FOR *IN SITU* GEL PREPARATION

In situ gel with optimized parameters was prepared as shown in **Table given below**

TABLE 9: VARIABLES OPTIMIZED IN SITU GEL PREPARATION

S.N.	PARAMETER	OPTIMIZED VALUES
1	Polymer (Polaxamer-407) concentration	25% w/v
2	Polymer (Methyl cellulose-4000 cps) concentration	5% w/v
3	Drug (Doxycycline hyclate)	5% w/v
4	Preservative (Benzalkonium chloride)	0.01%
5	Stirring temperature	5-10°C
6	Stirring speed	600 rpm
7	Stirring time	10 minutes

5.23 CHARACTERIZATION TECHNIQUES

This section describes several techniques being used for the characterization of *IN- SITU* gel. Optimized *in situ* gel formulation was characterized for a variety of attributes.

4.4.1 pH of optimized formulation

4.4.2 Drug content of optimized formulation

4.4.3 Viscosity of optimized formulation

5.23.1 pH OF OPTIMIZED FORMULATION

The pH of *gel* was determined by pH meter at 25℃ and 37℃. The determination was carried out in n=3 and the avg. of three readings is recorded at both temperatures. *in situ* gel pH shown in.

TABLE 10: pH OF OPTIMIZED IN SITU GEL

S.N.	pH OF OPTIMIZED FORMULATION	
	AT 25℃	AT 37 ℃
1	6.88	6.75
2	6.79	6.89
3	6.80	6.84
AVERAGE pH	**6.82**	**6.82**

5.23.2 DRUG CONTENT OF OPTIMIZED FORMULATION

Table 11: PERCENTAGE DRUG CONTENT OF OPTIMIZED *IN SITU* GEL

S.N.	ABSORBANCE	PERCENTAGE DRUG CONTENT	AVERAGE PERCENTAGE DRUG CONTENT
1	0.177	97.8	**96.93**
2	0.174	96.2	
3	0.175	96.8	

5.23.3 VISCOSITY OF OPTIMIZED FORMULATION

The viscosity of formulated *in situ gel* was determined by brook-field viscometer at S-15 at 4±1℃, 37±1℃ and the determination was carried out in triplicate viscosity of *in situ* gel observed and shown in **Table 4.8**.

TABLE 12: VISCOSITY OF OPTIMIZED IN *SITU* GEL

S.N.	RPM	SPINDLE NO.	VISCOSITY OF OPTIMIZED FORMULATION IN CPS	
			AT 25±1°C	AT 37±1°C
1	100	4	7452	14768
2	100	4	7561	15162
3	100	4	7503	14879
AVERAGE VISCOSITY IN CPS			**7505**	**14936**

5.24 DRUG RELEASE STUDY IN VITRO

The drug release study was performed using dialysis method, dialysis membrane was soaked overnight in PBS pH 6.8. In this technique, 1 ml of formulated drug was placed in each bag, and buffered and sink condition was maintained. The cumulative percent of drug released was calculated using (Shimadzu UV Thermostat 8000, Double beam, Japan) at 273 nm **(Bansal *et al.*, 2018)**.

TABLE 13: PERCENT DRUG RELEASE PBS pH 6.8

S.N.	TIME (HOURS)	% CUMULATIVE DR IN PBS pH 6.8
1	0	0
2	1	19.24
3	2	26.35
4	3	34.16
5	4	39.96
6	5	47.16
7	6	54.27
8	7	60.89

9	8	68.19
10	9	74.87

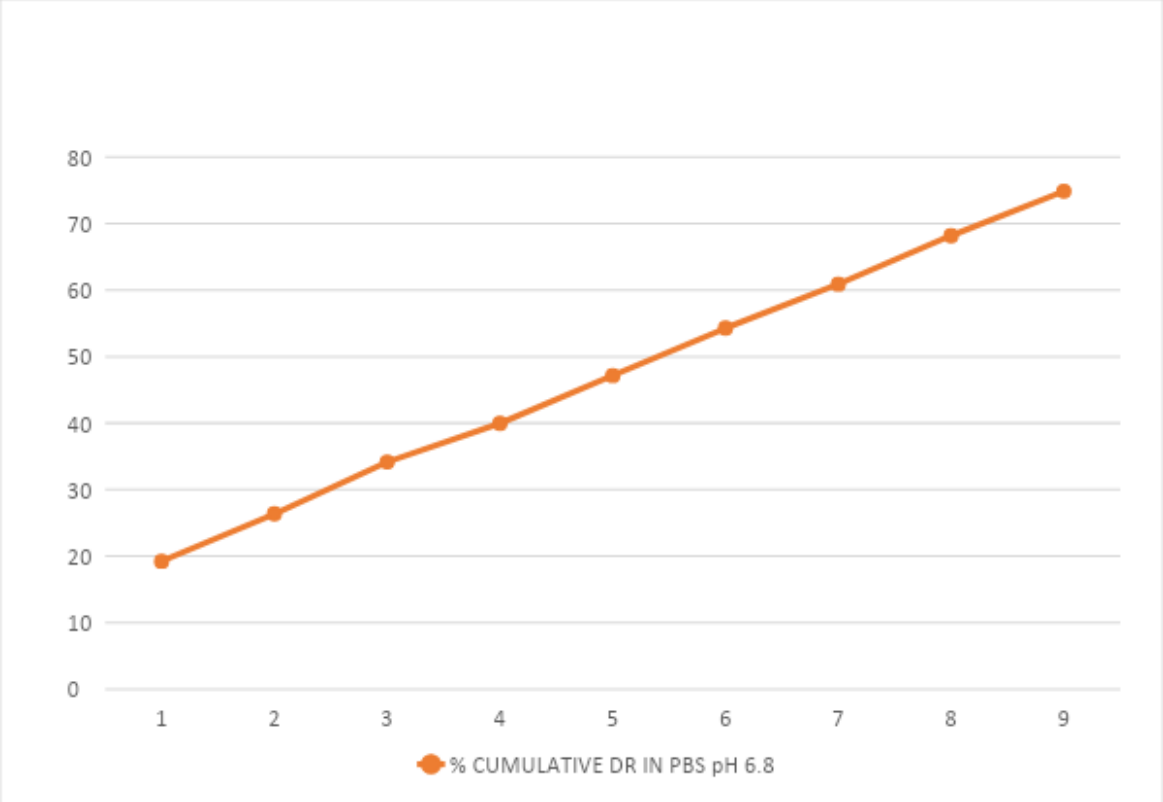


Figure 11: Plot of percentage drug release as a function of time in hrs of *in situ* gel formulation

5.25 RESULTS AND DISCUSSION

In situ gel was prepared by cold method using Preparation of doxycycline hyclate in situ gel was polymers (Methylcellulose-4000 cps and Polaxamer-407). Total twelve formulation was prepared using different concentrations of MC, DH (5%) and BKC (0.01%) as constant. Vehicle in situ gels were prepared by dissolving weighed amounts of MC (4%, 5% and 6% w/v) into cold water under constant stirring and allowing refrigeration at least 24 hour for proper swelling. To formulate doxycycline bearing *in situ* gel required concentration of polymers (Methylcellulose-4000 cps and Poloxamer-407) was selected on the basis of gelation temperature, gelation time and syringeability study of the formulation. It was found that concentration of Methyl cellulose-4000 cps and Polaxamer-407, 5% w/v and 25% w/v respectively have given good syringeability, gelation time and gelation temperature (near physiological temperature). According to these parameters it was concluded that Methylcellulose-4000 cps and Polaxamer-407, 5% w/v and 25% w/v respectively for *in situ* gel formulation was good formulation. So this concentration of polymers was selected for preparation and characterization of in situ gel with various parameters.

Gelation temperature, gelation time and syringeability study of all 12 formulations were recorded/determined. In which formulations F_1, F_2, F_4, F_7, F_{10} and F_{12} fails to attain gelation temperature, close to body temperature (41°C, 38°C, 40°C, 38°C, 38°C and 39°C respectively). These all formulations fails to attain gelation temperature close to body temperature due to lower concentration of PM-407 (which is helpful for gelation) except F_2 and F_{10} in which concentration of MC was low so viscosity of formulation was low (not appropriate

gelation). Formulations F_3, F_6, F_8 and F_9 had gelation temperature lower than body temperature (≤37°C) but failed the syringeability test (by 21 gauge syringe), due to high viscosity of polymeric solutions. Formulations F_5 and F11 passed all tests which have gelation temperature close to body temperature (37°C) and passed the syringeability test and gelation time also found within 60 seconds, gelation temperature close to body temperature was found due to appropriate concentration of PM-407. It was concluded that the formulation F_5 and F_{11} were optimized formulations which have concentration of MC-5% w/v and PM- 25% w/v, drug 5% w/v and preservative 0.01%w/v and it passed all parameters of optimization which were already mentioned above.

Optimization of process variables was determined through the heating on at temperature, stirring speed and stirring time. Optimization of stirring temperature during the mixing of PM-407, MC-4000 cps and mixture solution (MC, drug and preservative) was done at stirring speed 600 rpm. Optimization of stirring temperature was determined by stirring of PM (25% w/v), MC (5% w/v) and mixture solution of MC (5% w/v), drug (5% w/v), preservative (0.01% w/v) at optimized stirring speed (600 rpm) and various temperature range such as 5-10°C, 10-15°C, 15-20°C and 20-25°C. It was observed that at temperature 5-10°C and stirring speed 600 rpm solubility of PM (25%w/v), MC (5% w/v) and mixture solution was good **(Table 4.3)**. Which occurred due to absence of lumps formation at 5-10°C, as temperature increased lumps formation increased i.e., lumps formation is directly proportional to temperature.

Optimization of stirring speed and time to mix the mixture solution (MC-4000 cps, drug and preservative) in solution of PM-407 at 5-10 °C temperature was done at various stirring speeds such as 100, 200, 300, 400 and 500 rpm. It was observed that at 400 rpm different mixture solutions completely mixed in different concentrations of PM-407(20%, 25% and 30% w/v) solutions within 10 minutes and clear yellowish translucent liquid produced **(Table 4.4)**. At 100 and 200 rpm (low speed) magnetic beads don't drive/rotate due to higher concentration of polymer.

Hence formulation F_5 and F_{11} was selected for the final preparation of gel with optimized parameters of process variables such as stirring temperature (5-10°C), stirring speed (600 rpm) and stirring time (10 minutes).

Doxycycline hyclate bearing *in situ* gel was characterized on the basis of physical appearance, average pH (at 25°C and 37°C), average drug content, average viscosity (at 25°C and 37°C) and release studies, and it was found that the appearance of optimized formulation was clear yellowish translucent liquids, which was due to the yellow color of the drug. Average pH of the optimized formulation was found to be 6.82 at 25°C and 6.82 at 37°C, which were closer to the periodontitis pH of the formulation indicated that the formulation is safe to be used in periodontal pockets without irritation. Average drug content of formulation was found to be 96.93%. Avg viscosity was found to be 7505 at 25°C and 14936 at 37°C.

The *in-vitro* drug release profile of optimized *in situ* gel was determined through a dialysis tube in 8 hrs **(Table 4.9)** in PBS pH 6.8., %drug release cumulative was described in Fig.4.2. It is concluded that the drug release of *in situ* gel was slow due to diffusion controlled drug release and it was found to be 74.87%.

5.26 STABILITY STUDIES

Stability study was conducted with slight modifications. Briefly, optimized formulation was selected which was subjected to stability testing under storage conditions of 4±2℃ and at room temperature (25±2℃). The formulation was stored in small glass bottles.*In situ* gel stability was determined by appearance, pH, viscosity of optimized *in situ* gel at different time intervals ranging from 0-20 days. These parameters can be assessed by effect of temperature storage on viscosity and effect of storage on appearance repeatedly over time at varying storage conditions.

5.26.1 EFFECT OF STORAGE TEMPERATURE ON VISCOSITY OF IN SITU GEL BEARING DOXYCYCLINE HYCLATE

Optimized formulation of in situ gel subjected to stability testing at 4±2℃/75±5% and at room temperature 25±2℃/60±5%. Different Formulations were stored glass bottles at 4±2℃ and 25±2℃. Stability of in situ gel formulation is determined by the REMI stability chamber.

Stored formulations were evaluated after 0, 5, 10, 20 and 30 days for subsequent change in viscosity, pH and appearance of in situ gel at 4±2℃ and 25±2℃ in **Table 14 and 15.**

TABLE 14: EFFECT OF STORAGE TEMPERATURE ON VISCOSITY OF IN SITU GEL BEARING DOXYCYCLINE HYCLATE

S.NO.	TIME INTERVAL (DAYS)	VISCOSITY (CPS)	
		25±2℃	25±2℃
1	0		
2	5		
3	10		
4	20		
5	30		

5.26.2 EFFECT OF STORAGE TEMPERATURE ON APPEARANCE AND pH OF FORMULATION

After storing the formulations for a specified period of time of 5, 10, 20 and 30 days, the appearance and pH of the formulation was displayed in **Table 5.2**.

TABLE 15: EFFECT OF STORAGE TEMPERATURE ON APPEARANCE AND pH OF FORMULATION

S.NO.	TIME INTERVAL (DAYS)	EFFECT OF TEMPERATURE ON APPEARANCE AND pH (4±2°C)		EFFECT OF TEMPERATURE ON APPEARANCE AND pH (25±2°C)	
		APPEARANCE	pH	APPEARANCE	pH
1	0	Translucent yellow	5-6	Translucent yellow	5-6
2	5				
3	10				
4	20				
5	30				

6.1 RESULTS AND DISCUSSION

Stability study of doxycycline *in situ* gel was performed at different storage conditions such as refrigerator temperature (4±2°C) and RT (25±°C) for different time intervals (5, 10, 20 and 30 days) using REMI Stability Chamber. The stability was determined on the basis of change in viscosity, appearance and pH of prepared formulation. significant changes of appearance and clarity of the gel after storing at different storage conditions was not recorded.

The effect of different storage temperatures was also observed on appearance and pH. The appearance of doxycycline hyclate *in situ* gel formulation assessed by assuming clarity and grittiness. By the storage evaluation it was found that appearance, pH and clarity of doxycycline *in situ* gel formulation remained constant during one month storage conditions at 4±2°C and 25±2°C. It was a clear yellowish translucent liquid. That's why experimental results it can be better to store formulations at 4±2°C. Without significant drug loss. The stability testing data indicated that 4±2°C is the netter storage condition. May be suggested as storage temperature for formulation for their better stability.

7.1 SUMzARY

The specific aims of the present study were to prepare the stable formulation of doxycycline bearing thermoresponsive *in situ* gel for treatment of periodontitis. For this first, the Preformulation studies were performed. Preformulation studies showed that the drug supplied by Swapnroop Pharmaceutical, Aurangabaad (Maharashtra), was yellowish crystalline powder with ethanolic odor. Physical appearance of the drug such as reported to be the same in official and research literature. MP of the drug was found to be the same 175-195℃ for DH, as in I.P. 2010. The FT-IR of drug samples was found to be in concordance with the reference FT-IR which describes functional groups present in drug samples. Thermogram obtained for doxycycline hyclate was determined and it was found to be the same according to melting point of drug. The solubility profile of DH was determined in different solvents at room temperature and it was observed that drug was soluble in distilled water and PBS (6.8) and all the observation compliance with I.P 2010. The partition coefficient of DH was found to be 0.63 and 0.80 in PBS pH 6.8: n octanol (1:1) and DW: n octanol (1:1), which represents the hydrophilic nature of drug. The Absorbance maximum of DH solution in PBS (pH-6.8) was measured and it was found to be 273 nm. The above maxima were found to be matched with the standard **(Phaechamud *et al.*, 2019)**. The calibration curve was prepared using the UV visible spectrophotometric method. The analysis of the calibration curves showed good linearity data in the curve as the correlation coefficient was found to be much closer to one. Drug excipients compatibility was determined by DSC. It showed that there was no any shift or widening or narrowing of peaks being observed in either of the drug samples to be used for future work. Preparation of doxycycline hyclate in situ gel was carried out employing cold method, which involves the rapid diffusion of drug in the polymers (Methylcellulose-4000 cps and Polaxamer-407). A total of 12 different formulations were developed with varying concentrations of MC and PM keeping the DH (5%) and BKC (0.01%) as constant. Vehicle in situ gels were prepared by dissolving weighed amounts of MC (4%, 5% and 6% w/v) into cold water under constant stirring and allowing refrigeration at least 24 hour for proper swelling... To formulate doxycycline bearing *in situ* gel required concentration of polymers (Methylcellulose-4000 cps and Poloxamer-407) was selected on the basis of gelation temperature, gelation time and syringeability study of the formulation. It was concluded that concentration of Methyl cellulose-4000 cps and Polaxamer-407, 5% w/v and 25% w/v respectively have given good syringeability, gelation time and gelation temperature (near physiological temperature). According to these parameters it was concluded that Methylcellulose-4000 cps and Polaxamer-407, 5% w/v and 25% w/v respectively for *in situ* gel formulation was good formulation. So F_5 and F_{11} were selected for preparation and characterization of in situ gel with various parameters. Formulation F_{11} was selected for further optimization processes. At 5-10 ℃ and stirring speed 600 rpm solubility of PM (25%w/v), MC (5% w/v) and mixture solution was good. Which occurred due to absence of lumps formation at 5-10℃, as temperature increased lumps formation increased. Hence formulation F_{111} was selected for the final preparation of doxycycline hyclate bearing *in situ* gel after the optimization of formulation and process variables of various formulations.

Doxycycline hyclate bearing *in situ* gel was characterized on the basis of physical appearance, average pH (at 25℃ and 37℃), average drug content, average viscosity (at 25℃ and 37℃) and drug release studies, and it was found that the appearance of optimized formulation was clear yellowish translucent liquids, which was due to the yellow color of the drug. Average pH of the optimized formulation was found to be 6.82 at 25℃ and 6.82 at 37℃, which were closer to the periodontitis pH and the formulation is safe to be used on periodontal pocket. Average drug content was found to be 96.93%. Average viscosity was found to be 7505 at 25°C and 14936 at 37°C.

Drug release profile gel was determined through a dialysis tube in 8 hrs **(Table 4.9)** in phosphate buffer pH 6.8. % cumulative DR was described in Fig.4.2. It is clear that the drug release of *in situ* gel was slow due to diffusion controlled drug release and it was found to be 74.87%.

7.2 CONCLUSION

The present study describes the formulation and characterization of thermoresponsive *in-situ* gel formulation containing doxycycline hyclate(DH) for the management of periodontitis. PM-407 (25% w/v) and MC-4000 cps (5% w/v) and it is evaluated in terms of pH, drug content and viscosity studies. Compatibility study was done with the help of DSC. *In-vitro* drug release study through dialysis membrane was performed. The results of *drug* release studies proved that the formulation is releasing drug in controlled manner over time.

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