FABRICATION AND CHARACTERIZATION OF PERIDONTAL FILM CONTAINING CIPROFLOXACIN WITH CLOVE OIL FOR THE TREATMENT OF PERIDONTITIS

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Abstract

Periodontal disease primarily affects the structures supporting teeth, notably the gums, which are susceptible to bacterial infection. This disease is a leading cause of adult tooth loss, potentially resulting in long-term disability. Periodontal disease progresses through three stages: gingivitis, periodontitis, and advanced periodontitis. Gingivitis, the initial and reversible stage, precedes periodontitis, which leads to degradation of soft tissues and supporting bone, ultimately loosening the teeth. If untreated, the final stage, advanced periodontitis, results in severe bone and periodontal tissue loss, often necessitating tooth extraction.

Symptoms include red or swollen gums, bleeding during brushing or flossing, loose teeth, foul breath, and oral discomfort. Causative agents are gram-negative, anaerobic bacteria such as Actinobacillus, Porphyromonas gingivalis, Treponema vincentii, and Eubacterium species (nodatum, timidum, brachy). Treatments involve antibiotics like doxycycline and clindamycin. Although ciprofloxacin shows limited efficacy against anaerobes, it has demonstrated moderate antibacterial activity against periodontitis.

For targeted therapy, biodegradable and bioadhesive films have been developed to deliver antibiotics and natural agents synergistically. A novel periodontal film is proposed that activates upon placement in the periodontal pocket. Compared to conventional oral drug delivery, film-based systems offer deeper penetration and improved therapeutic effects, particularly when incorporating curcumin and clove oil. These films are designed to enhance healing while minimizing clinical limitations. In-vitro drug release studies, drug content analysis, and optimized formulations suggest this periodontal film delivery system holds promise as an effective treatment modality.

Keywords: *Periodontal Disease, Periodontitis, Bioadhesive Film, Localized Drug Delivery, Anaerobic Bacteria, Curcumin, Clove Oil.*

Introduction

Periodontal Disease

Worldwide, periodontal diseases are regarded as most important local issue now a days. The most well-known enduring disorders affecting human health are linked to dental diseases. Periodontal diseases are commonly observed in people of all racial and ethnic backgrounds. They are seen to be one of the major challenges facing everyone's health.

The word periodontal actually means around the tooth in context. A persistent bacterial infection of the bones and gums around the teeth is called periodontal disease (also known as gum sickness, periodontal disease, or pyorrhea).**[1]**

The tissues surrounding the tooth are referred to as perio, which means around and not tooth. With periodontitis, microbes attack the tiny, delicate tendons that keep teeth firmly in place, slither into the outer layers of the tooth root, and then infiltrate the actual bone. When this happens, the gummy tissues are invaded by the microorganisms, which then frequently spread throughout the body. In reality, societies have found oral tiny creatures in the blood vessel plaques, veins, and coronary corridors.**[2]**

Etiology Of Periodontitis

A tight collar of strong gingiva surrounds the tooth's neck. The gingival margin is 1 mm broad and surrounds the teeth completely. The gingival border encloses the gingival sulcus or cleft's outer mass, which is clinically “0 to 2 mm” in diameter both inside and out. The periodontal tendon attaches the tooth to its attachment and protects it from a variety of everyday stresses. Although the gingival sulcus is mostly free of bacteria, there are a few gram-positive species that consume oxygen that are both equal to Only until the environment and dietary supplement structure are ideal for their requirements can periodontal microbes begin to develop. Once the disease has taken root, the periodontal microenvironment experiences significant changes. The gingival crevicular liquid (GCF) stream is fairly little in solid gingival sulci, but under unhealthy circumstances, it can build up to 3.5 ml/day or more as fiery exudates.**[3]**

The development of periodontal infection is firstly accompanied by an increase in the amount of gingival bacteria, followed by a change in the synthesis of the micro flora. Sub-gingival bacteria' direct harmful effects and the host's incendiary reaction's damaging consequences both injure the periodontium. During periodontitis, collagenase and other bacterial proteins logically attack the periodontium's connective tissues and tendons, and the obsessively formed sulcus results in the construction of a periodontal pocket that often measures more than 5 mm in depth. The germs that cause sickness are still in this compartment.**[4]**

Classification of gum periodontal disease

Seven main classes of periodontal diseases were listed in the categorization system for periodontal infections and disorders, of which 2–6 are deemed serious because the damage is essentially permanent. The seven categories are as follows.**[5]**

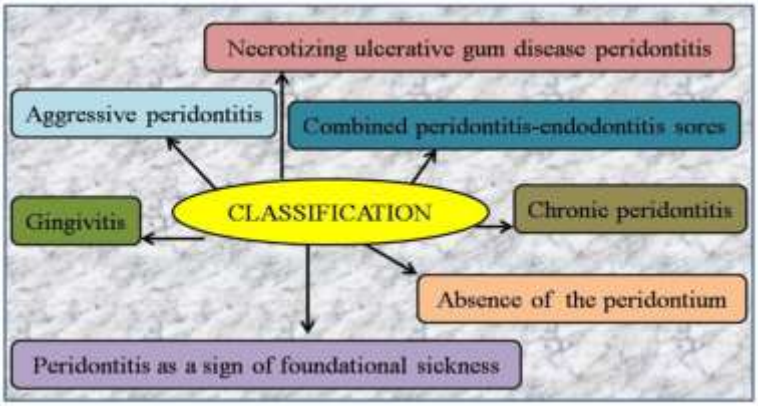

Fig. 1 Classification of gum periodontal disease

Microorganisms Associated With Periodontal Infections.[6]

The oral cavity is colonized by in excess of 400 types of vigorous and anaerobic microbes. Anaerobic microscopic organisms dwarf their oxygen consuming partners by a proportion of 10:1 to 100:1. These creatures possess the teeth, the gingival hole, the mucous films, the dorsum of the tongue and spit.

Dental contaminations can happen in various manners

1. Via the presentation of microbes of additional oral root,
2. Through an adjustment yet to be determined of the native greenery,
3. With the passage of microbes into the typically clean indispensable mash of the Tooth.

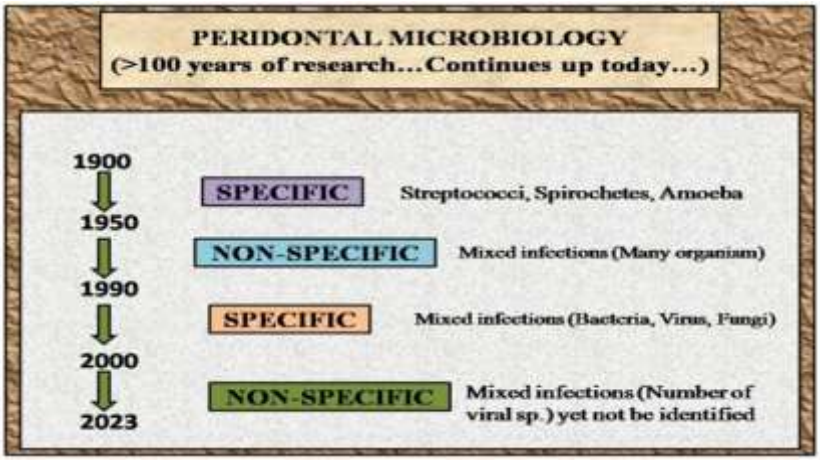

Fig. 2 Microorganisms Associated With Periodontal Infections

Present microbiological treatment of periodontitis requires either the mechanical brushing of the teeth with foundational anti-microbial or a confined conveyance framework with anti-infection. An assortment of issues emerge from the utilization of foundational anti-infection agents, for example, bacterial protection from managed anti- microbial and unfriendly

or harmful results. Huge dosages should be taken to accomplish sufficient focuses in the gingival reticular liquid of the periodontal pockets; this carries with it the related symptoms of anti-infection agents and issues in regards to anti-toxin obstruction. **[7]**

To determine the previously mentioned weaknesses of conventional arrangements, we have attempted to form and test periodontal films with the objective of upgrading tolerant consistence, restorative viability, diminished dosing recurrence and site-explicit attachment and biodegradable polymeric frameworks and to get controlled arrival of the medication at the site of activity to diminish the results and to expand the bioavailability of the medication at the site of activity.**[8]**

Periodontal contaminations might be alluded to as blended diseases that contain a mix of gram-negative and gram-positive vigorous, microaerophilic, and anaerobic diet assumes a significant part in periodontal illness movement. It is notable that helpless sustenance builds the weakness to contamination and that dietary status is antagonistically influenced by disease.**[9]**

It was proposed that by changing the harmony between great microscopic organisms and terrible microorganisms, probiotics may help decrease dental caries, gum disease, periodontitis, oral yeast contaminations, and halitosis. With regards to sustenance and dental issues, the vast majority consider sugar most importantly.**[10]**

TYPES OF PERIODONTITIS

Mild Periodontitis

Gingiva has a surface that is red and sparkly and swollen and fragile or springy. Separation of the gums from the teeth creates pockets that plaque can collect in. The microorganisms in plaque release toxins that irritate the gums. Patients with gum disease will have pockets deeper than 3 millimeters.**[11]**

Fig. 3 Gingivitis

Moderate Periodontitis

Capnocytophaga spp., and Actinobacillus actionomycetemcomitns. The microscopic organisms increase in the periodontal pocket between the underlying foundations of the influenced teeth and delicate tissues. As more bone and tissue is removed, more profound pockets form. Because of the poisons, the body physically turns on itself, ripping apart the tissues and bone that hold the teeth and killing them. A pocket depth of up to 5 mm and the beginning stages of bone loss are hallmarks of periodontitis.**[12]**

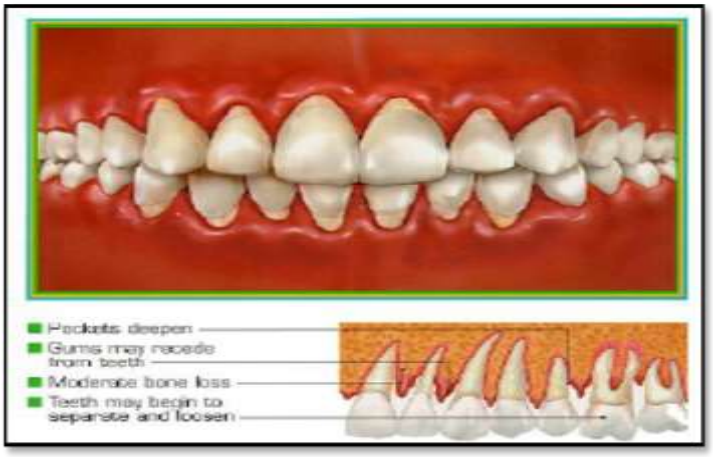

Fig. 4 Moderate Periodontitis

Advanced Periodontitis

This is an extreme case of periodontitis. Symptoms include puffy, bleeding gums, more extensive bone loss, gum recession, and a pocket depth of 6 mm or more, making treatment more challenging.Due to the extensive loss of bone and tissue, teeth may become unattached. Helplessness in the face of further decimation may lead to a shortage of teeth.**[13]**

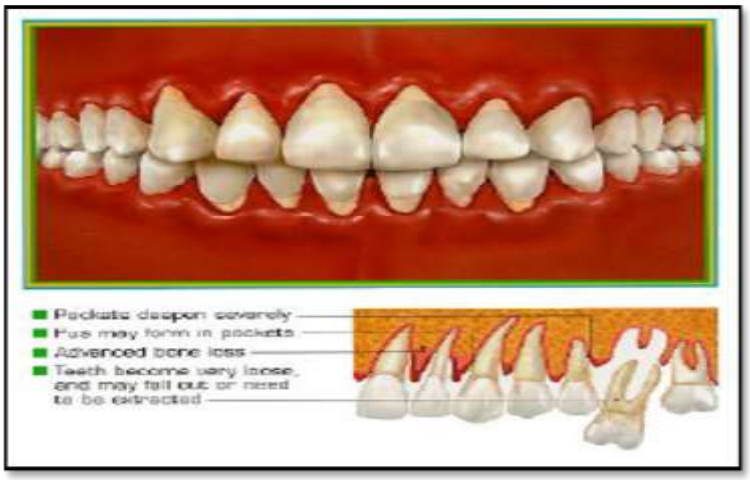

Fig. 5 Advanced Periodontitis

Refractory Periodontitis

This stage can frequently result in tooth loss since a considerable amount of bone is destroyed as a result of the extravagant annihilation of the bone and tissue that support the teeth.**[14]**

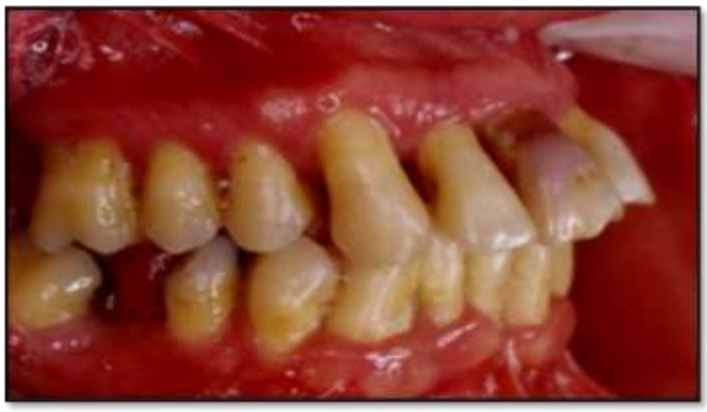

Fig. 6 Refractory Periodontitis

ANTIBIOTICS

Anti-toxins are a fundamental dynamic specialist normally utilized in the treatment of numerous bacterial protozoan and anaerobic diseases. A blend of oral and skin treatment is utilized for cases which persevere after oral treatment and where safe trichomonads are suspected. From gastrointestinal lot anti-infection agents drugs are promptly retained. In the liver, it is used. This is discharged in the pee, prevalently as forms and metabolites, and less significantly in the excrement. Biliary discharge might be fundamental when they are eliminated and its metabolites, as well.**[15]**

In the administration of H pylori, metronidazole is likewise empowered. Related to different prescriptions, duodenal ulcers.

To target nearby problems at the mucosal surface, bioadhesive dose structures have been utilized to diminish the generally speaking required measurement and to lessen the results that the fundamental organization of the medications can cause. As the glue part, bioadhesive plans use polymers. These polymers are additionally water solvent and they pull in water from the mucosal surface when utilized in a dry structure, and this exchange of water prompts a solid communication. At the point when hydrated with water, these polymers frequently structure gooey layers, expanding the maintenance time on the mucosal surfaces and adding to cement connections.**[16]**

Dental Drug Delivery System:

Different medication conveyance framework for treating periodontitis: [17]

a) Fibers

b) Film
c) Injectable frameworks
d) Films
e) Microparticle framework

Fibers

String-shaped devices called strands are placed radially into the pockets using a tool and confirmed with cyano-acrylate cement to provide the controlled release of the captured medicament into the periodontal pocket. The arrival of the antibiotic medication from cellulose acetic acid derivation filaments as happened by dispersion system is fast with roughly 95% of the medication delivered in the initial two hours and, in this manner, a solitary use of these strands doesn't give a viable medication fixation to long periods. Depression of periodontal microbes, decrease of seeping on testing, decline in examining pocket profundities and expansion in testing connection levels.**[18]**

Film

An undeniably more generally utilized type of intra-pocket conveyance gadget has been looking like film, arranged either by dissolvable projecting or direct processing. In order to embed larger films into the site of action, they can either be put inside the cavity; onto the cheek mucosa or gingival surface, or they can be chopped or punctured into appropriate sizes. Films are lattice delivery systems, in which drugs are incorporated into polymeric itself and then distributed across the film or grid before being absorbed by the body. Several polymer films have been developed to facilitate the gradual introduction of corrective substances. Researchers found that drug release from insoluble films occurs by diffusion, while drug release from solvents occurs via transporter dissolution during in vitro studies. The benefits of such a gadget incorporate simplicity of inclusion, measurements that affirm well with the components of the pocket and least torment on addition.

Injectable System

Injectable frameworks are especially alluring for the conveyance of anti-infection specialists into the periodontal pocket. The process can be effectively and quickly completed, with-out torment, by utilizing a needle. Accordingly, the expense of the treatment is extensively diminished contrasted with gadgets that need time to be put and made sure about. Also, an injectable conveyance framework ought to have the option to fill the pocket, consequently arriving at a huge extent of microorganisms. These frameworks permit simple utilization of restorative specialist utilizing a needle. They are likewise cost saving.**[19]**

Gel

Gel is applied sublingually with the assistance of gruff cannula and needle. The gel is just imperceptibly emotional in diminishing the anaerobic bacterial check. Privately applied

controlled delivery film may mostly balance the negative impact of smoking on periodontal recuperating. The wellbeing profile, longer-term maintenance, antimicrobial action recommends that antibiotic medication containing copolymer films addresses a protected and successful bio-erodible treatment for periodontitis protected and viable bio erodible treatment for periodontitis. **[20]**

Microparticle system

Polymers with a specific origin, modified common substances, and designed polymers all make up the microparticle-based system. They could be characterized as a chip, be crucial to a dental glue detailing, or be infused into the periodontal cavity in any case. **[21]**

Preparation Methods of Periodontal Film: [22]

Solvent casting technique

The films were cast using glass moulds. To get varied concentrations of polymeric solutions, polymer dissolves in solvent. The needed concentration of medication was added to these solutions. The once surface it had been well mixed. By placing it on the mould at 37ºC for 24 hours, the evaporation process of the solvent was slowly done. The film was acquired once the solvent had completely evaporated. To manage the drying rate, an inverted funnel was kept on the mould at all times. The desiccator was used to keep the prepared films, which were lined with butter paper.**[23]**

Semisolid casting method

The initial step in this process is to make a solution of a aqueous soluble film forming polymer. The resultant solution is then mixed with an acid-insoluble polymer solution. After that, a small quantity of plasticizer is added to get a film mass. Finally, heat- controlled drums are used to cast the film mass into the films or ribbon. The film is between 0.015 and 0.05 inches thick. The acid insoluble polymers should be used in a 1:4 ratio with the film forming polymer. **[24]**

Hot melt extrusion

The present process, managed by temperature and directional velocity, begins with the preparation of the mass. Next, the periodontal film was coated and dried in a drying chamber, where the environment is once again regulated in terms of temperature, airflow, and line speed. After that, the films go through a series of punches, pouches, and seals.**[25]**

Solid dispersion extrusion

In this method, immiscible components are combined with pharmaceuticals and then extruded to generate solid dispersion. The solid dispersions are then designed into films with the help of dies.**[26]**

DRUG PROFILE

Table 1: Drug profile of Ciprofloxacin

Ciprofloxacin	
Chemica Name	1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3- carboxylic acid
Molecular Formula	C17H18FN3O3
Molecular Weight	331.34g/mole
Melting point	318.32˚C
Volume of distribution	3.2 L/kg
Protein binding	30%
Metabolism	Liver
Bioavailability	70%
Half-life	8 hours
Excretion	Urine
Route	IV , Mouth topical
Solubility	It is insoluble in water, although it dissolves in diluted acetic acid and is mildly soluble in dehydrated alcohol and dichloromethane.

Excipients Profile

Table 2: Excipients Profile

Clove oil [28]	
Molecular formula	C10H12O2
State	Liquid
Density	1.05 g/ml at 25˚C
Solubility	Insoluble in water
Flash point	>230˚F
Boiling point	251˚ C
Colour	Yellow to transparent
Refractive index	N20/D 1.532
Stability	Probably flammable
Occurrence	Obtained from bud of Eugenia caryophyllata
Discription	Clove oil's antimicrobial and analgesic characteristics make it a popular toothache remedy. Antiviral and antioxidant effects are also present. Eugenol, is the main chemical constituent of clove. Chemical components like eugenol, eugenyl acetate are present in clove.

Chitosan[29]	
Synonyms	2-Amino-2-deoxy-(1,4)-b-D-glucopyranan
Chemical name	Poly-b-(1,4)-2-Amino-2-deoxy-D-glucose
Acidity/alkalinity	pH = 4.0–6.0 (1% w/v aqueous solution)
Density	1.35–1.40 g/cm^3
Molecular weight	10,000 and 1,000,000
Particle size distribution	<30 mm
Organoleptic properties	Odorless
Solubility	Soluble in acetic acid solution , Sparingly soluble in water; practically insoluble in ethanol (95%)
Moisture content	The amount of water absorbed by chitosan from the air is determined by the original moisture content, as well as the temperature and relative humidity.

Ethyl Cellulose [30]	
Synonyms	Surelease Cellulose ethyl
Chemical name	$C_{20}H_{38}O_{11}$

Functional category	Food additives such as those used in tableting, as well as binders, fillers, and colorants,
Colour	Powder, free-flowing, white to pale tan
Odour/ taste	Odorless and tasteless powder
State	Slightly hygroscopic powder
Melting point	241-255 °C
Density	1.08-1.19
Solubility	Water, glycerol, and propane-1, 2-diol are practically insoluble, however certain organic solvents are soluble in different quantities.

RESULT AND DISCUSION

Organoleptic Properties

Organoleptic tests, such as those for nature, colour, and aroma, were all conducted by visual evaluation and compared to the API standard that is used in pharmacopoeia for drug distinctive criteria.

PRE-FORMULATION STUDY

Solubility studies of ciprofloxacin

The solubility was evaluated semi-quantitatively by adding the solvent to a volumetric flask containing a measured amount of the solute. As the system is aggressively agitated, any particles of undissolved solute are visually scrutinized. Solubility is determined by the concentration of the solute in relation to the solvent. In order to test the solubility of the antibiotic, three 100 mL conical flasks were filled with 25 mL of distilled water, phosphate buffer solution pH 6.8, and ethanol, and 15 mg of each drug was added to each flask.

Determination of partition co-efficient of Ciprofloxacin

Ciprofloxacin (10 mg) was agitated in a 100 ml separating funnel containing 10 ml of a 1:5 mixture of n-octanol and phosphate buffer solution with a pH of 6.8 for 30 minutes. It lasted for a whole 60 minutes. The amount of medication dissolved in both the water and oil layers was determined using a UV-Vis spectrophotometer. The formula was used to determine the medication's partition coefficient. **[31]**

Partition co-efficient = Conc. of API in organic phase / Conc. of API in aqueous phase

Drug excipients compatibility studies (FTIR)

API and pure excipients were weighed and their combination with drug excipient samples. Each vial received 10 liters of ultra-pure water after being mixed using a glass capillary that was left in the vial once the mixture was complete. Four weeks in a hot air oven at 50 degrees Celsius were spent precisely sealing each vial. Organoleptic characteristics, such as color and texture, were evaluated at the beginning and end of the first, second, third, and fourth weeks to detect chemical instability, The disappearance or weakening of some absorption bands, together with the appearance of additional bands, provides undeniable evidence of interactions. **[27]**

UV Spectrophotometric Study

1. Preparation of Stock Solution

- A standard stock solution of ciprofloxacin was prepared by accurately weighing 10 mg of ciprofloxacin.
- The weighed ciprofloxacin was then dissolved in 10 mL of phosphate buffer solution with a pH of 6.8.
- This resulted in a stock solution with a concentration of 1 mg/mL.

2. Preparation of Working Solution

- To create a working solution for spectroscopy, 1 mL of the stock solution was transferred into a 10 mL volumetric flask.
- The volume was adjusted by adding phosphate buffer solution with a pH of 6.8 to reach a final concentration of 100 µg/mL.

3. Preparation of Calibration Curve

- From the working solution, a series of standard solutions were prepared with concentrations of 2, 4, 6, 8, and 10 µg/mL.
- Each standard solution was pipetted into separate cuvettes.
- The absorbance of these standard solutions was measured using a UV-Vis Spectrophotometer at a wavelength of maximum absorption, which was determined to be 274 nm.
- A calibration curve was constructed by plotting the absorbance values against the respective concentrations of ciprofloxacin in the standard solutions

Method of preparation of periodontal film

The films were made using glass moulds. To create different concentrations of polymeric solutions, polymers were dissolved in solvent and plasticizer in a beaker using a magnetic stirrer. The precise amount of medicine was infused into these solutions. The mixture was completely blended before being carefully poured into a horizontal glass mould. An inverted glass funnel with a cotton plug in the stem was placed on the mould and left at room temperature for one day to allow the solvent to gradually evaporate. Once the solvent had fully evaporated, the cast film was collected. An inverted funnel was always left on the mould to control the drying pace.The desiccator was used to keep the prepared films, which were placed on a butter paper **[32] ,[33]**

Table 3: Formulation design for periodontal film by 3^2 factorial design

formulation code	Code		Actual value	
	X1	X2	X1 Ethyl cellulose	X2 Chitosa n
F1	λ	λ	100	300
F2	λ	β	100	250
F3	λ	ϒ	100	200
F4	β	λ	150	300
F5	β	β	150	250
F6	β	ϒ	150	200
F7	ϒ	λ	200	300

F8	**ϒ**	**β**	**200**	**250**
F9	**ϒ**	**ϒ**	**200**	**200**

For X1: 100 mg (**λ**), 150 mg (**β**), 200 mg (**ϒ**) and for X2: 300mg (**λ**), 250mg (**β**), 200 mg(**ϒ**)

Table 4: Formulation Table for Periodontal Film

Formulation Code	Ethyl Cellulose (X1)	Chitosan (X2)	Ciprofloxacin (mg)	Clove Oil (ml)
F1	**λ (100 mg**	**λ (300 mg**	**100**	**0.5**
F2	**λ (100 mg**	**β (250 mg)**	**100**	**0.5**
F3	**λ (100 mg**	**ϒ (200 mg)**	**100**	**0.5**
F4	**β (150 mg)**	**λ (300 mg)**	**100**	**0.5**
F5	**β (150 mg)**	**β (250 mg)**	**100**	**0.5**
F6	**β (150 mg)**	**ϒ (200 mg)**	**100**	**0.5**
F7	**ϒ (200 mg)**	**λ (300 mg)**	**100**	**0.5**
F8	**ϒ (200 mg)**	**β (250 mg)**	**100**	**0.5**
F9	**ϒ (200 mg)**	**ϒ (200 mg)**	**100**	**0.5**

EVALUATION OF FILM

1. Thickness uniformity

Each periodontal film was measured measured for thickness using a screw gauge at six different locations across the film, and the average was computed.

2. Uniformity of weight

Periodontal film was cut into 1 cm squares and sampled from several locations. The variation in film weight was calculated.**[34]**

Percent Drug Content Study

The drug content has always been one of the most crucial factors for any dose form. The overall amount of medication in the formulation shall not vary from the marked quantity within the established criteria.

By adding 1 gm of the created film formulations to a 100 ml volumetric flask, the amount of medication in each formulation was measured. This volumetric flask received 50 mL of pH 6.8 phosphate buffer before being continuously shaken until the film was released. Or, to put it another way, they were scattered all over. 1 mL of the sample was taken after 2 hours of nonstop shaking and diluted with phosphate buffer 6.8. It had 1 ml taken out of it once more, and 10 ml

of phosphate buffer 6.8 had been added to the mixture before being spectrophotometrically tested for drug content at 274 nm. **[32]**

Tensile strength

The system's tensile strength was determined by its architecture. With a sharp blade, a 1 cm square of film was shaved off a glass plate. The basic parts of the apparatus were a horizontal wooden platform with a fixed scale and attachments for two clips carrying periodontal film. Both clips could be moved, but one was permanently attached. Weights were suspended from one end of the pulley, while the other was attached to a pivoting clip. The wooden platform was constructed such that it would not move during the experiment. The film was stretched and tested for its tensile strength by being dragged through a series of pulleys. The pulling power was increased by adding weights to the pan. Eventually, the film was broken.

Folding endurance

According to the folding endurance value, all periodontal film formulations displayed ideal periodontal film characteristics.**[34]**

Field Emission Scanning Electron Microscopy

The surface morphology of the nanofibers produced was examined using a field emission scanning electron microscope from the TESCAN facility at the Oniosomes, Chandigarh. **[32]**

Determination of *In vitro* Drug Release

Using a Franz Diffusion cell, a study on in vitro drug release was accomplished. The diffusion cell's donor and receptor compartments were separated by a synthetic cellophane membrane. The generated film was applied to the drug release membrane in a 1 cm^2, and phosphate buffer solution pH 6.8 was added to the receptor compartment of the diffusion cell.

Everything was mounted on a magnetic stirrer, and a magnetic bead was used to agitate the fluid in the receptor compartment at 50 RPM in order to maintain its 37°C temperature. 1 ml samples were taken at each time at intervals of 1, 2, 3, 4, 5, 6, 7 and 8 hours, and they were analyzed for drug release using UV at 274 nm for ciprofloxacin against a phosphate buffer solution pH 6.8 blank. A chart was made showing how much material was released from the image over time.**[35]**

DRUG RELEASE KINETICS OF FILM FORMULATION

The data was subjected to first request (log combined level of medication remaining versus time), Higuchi's (Cumulative level of medication delivered versus time), and Korsmeyer's (log aggregate level of medication delivered versus log time) conditions, as well as a zero-request

(Cumulative level of medication discharge versus time) design, in order to identify the component of medication discharge from these detailing. **[34]**

ANTIBACTERIAL STUDY

Staphylococcus aureus bacteria that were Gram positive used as the model microorganisms. Staphylococcus aureus was used as a test subject for the antibacterial capabilities of the film. By correcting the optical density of the bacterial suspension to a turbidity similar to spectrophotometric absorbance of 0.546 at 630 nm, the inoculum quantity of each strain was standardized to 0.1 mL for each formulation. Both the polymer's (chitosan+EC) antibacterial activity and the polymer's (HPMC+EC) combined antimicrobial activity with the drug (CIPROFLOXACIN) activity were measured in Cm squires. The antimicrobial activity of the polymer (HPMC+EC), API (CIPROFLOXACIN), and natural agent was assessed in Cm squires as well as the antibacterial activity of the polymer (HPMC+EC) with natural agents. The temperature throughout the 24-hour incubation period was 37ºC.

The inoculation plates were carefully arranged with all four formulations on them, and they were then incubated for 24 hours at 37°C. The zones of inhibition were identified using the clear region that was formed around each film sample. The outcomes demonstrate that the formulation is effective in limiting the bacterial population that causes the sickness.**[36]**

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