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FORMULATION AND EVALUATION OF THE PERIODONTAL GEL FOR PEDIATRIC USE WITH AN OBJECTIVE OF INCREASING RESIDENCE TIME OF DRUG

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ABSTRACT

Periodontal gel formulations are currently a novel idea of delivering drugs to patients, yet achieve sustained release of drug for the desired period. Different delivery systems based on polymers have been developed, which are able to increase the residence time of the formulation at absorption site of drugs. The most important approach for periodontal treatment is the control of inflammation. Pain management has always been an important part of dental care. Therefore, the main categories of drugs to be used in such conditions are analgesics and anti-inflammatory agents. Also from the point of view that periodontal diseases have their origin in some form of infection. So it is worthwhile to use one of the antibiotics also. Considering the gelling property of Carbopol 940 is used as a gelling agent, an attempt was made to develop gel system for dental use. Chitosan was incorporated for sustained release action. Metronidazole, Clindamycin, Doxycycline and Serratiopeptidase, as antibiotics and anti-inflammatory agents were the choice of drugs.

KEYWORDS: Periodontal gel, dental care, analgesics and anti-inflammatory, Carbopol 940, sustained release, Metronidazole, antibiotics.

INTRODUCTION

The word periodontal literally means “around the tooth”. Periodontal disease broadly defines several diseases associated with the periodontium. In periodontal disease there is a formation

of periodontal pocket which is pathologically deepened sulcus. In normal sulcus, the space between the teeth and gums is generally between 1 and 3mm but in periodontitis the depth of pocket usually exceeds 5mm. Bacteria grow rapidly within periodontal pocket resulting in periodontal abscess.

Dental caries also known as tooth decay or a cavity is an infection, bacterial in origin that causes demineralization and destruction of the hard tissues usually by production of acid due to bacterial fermentation of food debris accumulated on the tooth surface. Local delivery of drugs to the tissues of oral cavity has a number of applications including in the treatment of periodontal diseases like periodontal abscess, chronic periodontitis, periapical granuloma, dental caries, and root caries. Dental caries is one of the most common preventable diseases which is recognized as the primary cause of oral pain and tooth loss. It is a major public health oral disease which hinders the achievement and maintenance of oral health in all age groups.[1]

WHO claimed that poor oral health may have a profound effect on general health as well as quality of life, and several oral diseases are related to chronic diseases. Dental caries refers to the localised destruction of susceptible dental hard tissues by acidic by-products from the bacterial fermentation of dietary carbohydrates. It is a chronic disease that progresses slowly in most of the people which results from an ecological imbalance in the equilibrium between tooth minerals and oral biofilms (plaque).

The biofilm is characterized by microbial activity, resulting in fluctuations in plaque pH. This is a result of both bacterial acid production and buffering action from saliva and the surrounding tooth structure. The tooth surface is therefore in a dynamic equilibrium with its surrounding environment. As the pH falls below a critical value, the demineralisation of enamel, dentine or cementum occurs, while a gain of mineral (remineralisation) occurs as the pH increases. The process of demineralisation and remineralisation takes place frequently during the day. Over time, this process leads to either caries lesions or the repair and reversal of a lesion.

Primary caries can occur on different tooth surfaces. On an approximal surface, the lesion starts and forms beneath the contact area between teeth. Caries on an occlusal surface is also a localised phenomenon in pit and fissure. On both occlusal and approximal surfaces, enamel caries is a three-dimensional subsurface demineralisation that spreads along the enamel

prisms. Secondary caries is a lesion located at the margin of a dental restoration. It represents a caries lesion adjacent to the margin and there may be signs of demineralisation (wall lesions) along the cavity wall which could be a consequence of micro leakage. However, clinical and microbiological studies indicate that this leakage does not lead to active demineralisation beneath the restoration.

Caries may be characterized by the experience of pain, problem with eating, chewing, smiling and communication due to missing, discolored or damaged teeth. The microbial community of caries is diverse and contains many facultatively and obligately-anaerobic bacteria belonging to the genera Actinomyces, Bifidobacterium, Eubacterium, Lactobacillus, Parvimonas and Rothia. It can also be caused by other bacteria, including members of the mitis, anginosus and salivarius groups of streptococci, Propionibacterium, Enterococcus faecalis, Scardovi, Prevotella, Selenomonas, Dialister, Fusobacterium, Pseudoramibacter, Veillonella, Atopobium, Granulicatella, Leptotrichia and Thiomonas. Bacteroides, Prevotella, and Porphyromonas species are prevalent on mucosal surfaces and reach very high concentrations in dental plaque, gingival crevices and tonsillar crypts.[2]

At present, the distribution and severity of dental caries vary in different parts of the world and within the same country or region. It is affecting 60-90% of school children and the vast majority of adults. It is also a most prevalent oral disease in several Asian and Latin American countries. The prevalence pattern of dental caries varies with age, sex, socio economic status, race, geographical location, food habits and oral hygiene practices. Nowadays, as a consequence of high prevalence of dental caries, the treatment need is increased. However, treatment cost for dental diseases is normally high. In the United States annual treatment costs are estimated to be at least $ 4.5 billion.[3]

1. Types of dental caries

Different types of caries are found which are as follows.

Table: 1.1 Types of dental caries.

Types of caries	Description
Incipient caries /Primary caries	Decay at a location that has not experienced previous decay.
Recurrent caries /Secondary caries	Appears at a location with a previous history of caries and is frequently found on the margins of fillings and other dental restorations.
Arrested caries	A lesion on a tooth that was previously demineralized but was remineralized before causing a cavitation.

1.1 Early childhood caries

Early childhood caries (ECC) is a pattern of decay found in young children with their deciduous teeth. The teeth most likely affected are the maxillary anterior teeth, but all teeth can be affected. This type of caries comes as a result of allowing children to fall asleep with sweetened liquids in their bottles or feeding children sweetened liquids multiple times during the day. The risk for ECC also may be determined by pre-existing developmental defects of the enamel called hypoplasia. Hypoplasia predisposes teeth to early colonization by Streptococcus mutans and malnutrition.

ECC exhibits a characteristic pattern related to the emergence sequence of the teeth and the tongue position during feeding**.** The lower teeth are protected from exposure to ingested liquids by the tongue during feeding and by the pooling of saliva and so usually are not affected. The incisors are the first upper teeth to emerge and are most affected by ECC. Depending on how long the caries process is active, the upper first primary molars are often involved, followed by the upper second molars and canines, and in severe cases, the lower teeth.[4]

1.2 Rampant caries

Rampant caries are severe decay on multiple surfaces of many teeth. It may be seen in individuals with xerostomia, poor oral hygiene, stimulant use due to drug-induced dry mouth and large sugar intake. If rampant caries is a result of previous radiation to the head and neck then it is called as radiation-induced caries. Problems can also be caused by the self-destruction of roots and whole tooth resorption when new teeth erupt.[5]

Classification of dental caries

Caries can be classified by rate of progression, affected hard tissues and location. These forms of classification can be used to characterize a particular case of tooth decay in order to more accurately represent the condition to others and also indicate the severity of tooth destruction.[6]

Table 1.2: Classification of dental caries.

Sr. no.	On the basis of	Classification	Description
1	Rate of progression	Acute	Signifies a quickly developing condition
		Chronic	Signifies an extended time to developing condition
2	Affected hard tissue	Enamel	Early in its development and may affect only enamel
		Dentinal	The extent of decay reaches the deeper level of dentin
		Cementum	The decay on roots of teeth
3	Location	Class I	Pit and fissure caries (anterior or posterior teeth)
		Class II	Approximal surfaces of posterior teeth
		Class III	Approximal surfaces of anterior teeth without incisal edge involvement
		Class IV	Approximal surfaces of anterior teeth with incisal edge involvement
		Class V	Gingival/cervical surfaces on the lingual or facial aspect (anterior or posterior)
		Class VI	Incisal edge of anterior teeth or cusp heights of posterior teeth.

1.4 Caries-promoting factors

Table 1.3: Caries promoting factors.

Sr. No.	Primary risk factors	Reasons
1	Saliva	(1) Ability of minor salivary glands to produce saliva (2) Consistency of unstimulated (resting) saliva (3) pH of unstimulated saliva (4) Stimulated salivary flow rate (5) Buffering capacity of stimulated saliva
2	Diet	(6) Number of sugar exposures per day (7) Number of acid exposures per day
3	Flouride	(8) Past and current exposure
4	Oral Biofilm	(9) Differential staining (10) Composition (11) Activity
5	Modifying Factor	(12) Past and current dental status (13) Past and current medical status (14) Compliance with oral hygiene and dietary advice (15) Lifestyle (16) Socioeconomic status (17) Tooth location

1.5 Pathogenesis of dental caries

The classic description of the cause of dental caries includes three factors: host, bacteria and diet. Dental caries occurs when a susceptible tooth surface is colonized with cariogenic bacteria and dietary source of sucrose or refined sugar is present. Bacterial pathogen produce lactic acid from fermentation of carbohydrates and this acid dissolves the hydroxyapatite crystal structure of the tooth which causes caries.

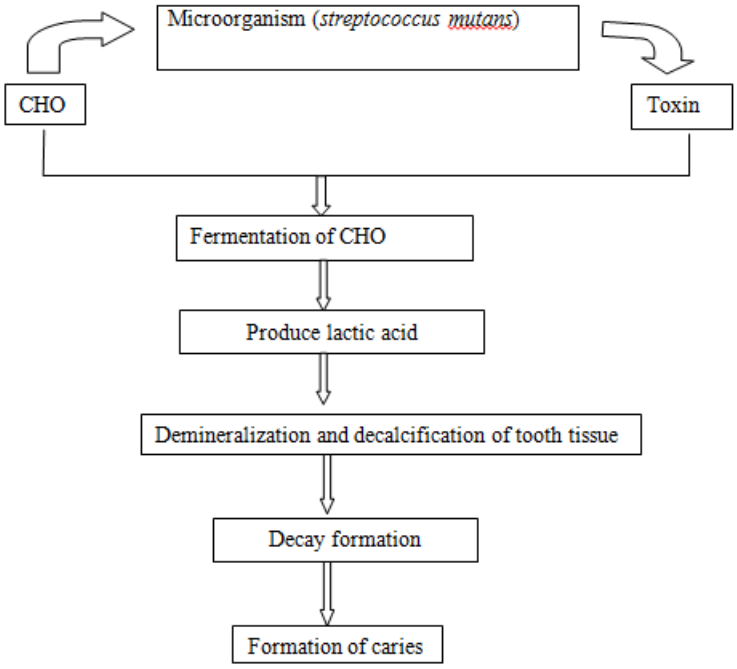


Fig. 1.1: Pathogenesis of dental caries.

1.6 Stages of dental caries

1.6.1 White spot stage

The acid produced by bacteria and yeast in dental plaque dissolve the mineral matrix of teeth. In the earliest stage, dental caries appears as a chalky white spot on the tooth.

At this stage, the surface is intact, and the subsurface lesion is reversible. White spot resulting from incipient caries can be difficult to distinguish from developmental hypocalcification. Further, white spot changes to black staining stage.[7]

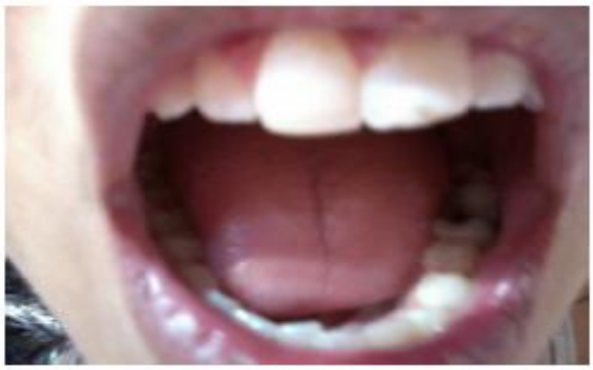


Fig. 1.2: Black staining stage.

1.6.2 Cavity stage

If mineral continues to be lost because of acid challenge, the surface is eventually broken or "cavitated" and the lesion cannot be reversed. If the lesion progresses, large areas of tooth can be lost. Active cavitated lesions are usually golden brown. Long standing lesions are darker, sometimes nearly black. Depth of the color is not a good indicator of the severity of the lesions because arrested decay is often the darkest.[7]

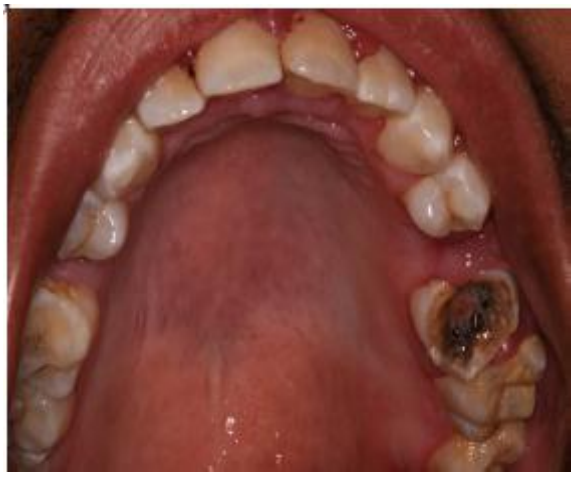


Fig. 1.3: Cavity stage.

1.7 Signs and symptoms of dental caries

The signs and symptoms of cavities vary, depending on their extent and location. When a cavity is just beginning, it may not have any symptoms at all. As the decay gets larger, it may cause signs and symptoms such as:

- Toothache and mild to sharp pain when eating or drinking something sweet, hot or cold called tooth sensitivity.[8]
- Visible holes or pits in teeth.[9]
- Brown, black or white staining on any surface of a tooth
- Bad breath and foul tastes.[10]
- Fever, chills, abscess, and trismus.

1.8 Complications

- Cavernous sinus thrombosis and Ludwig angina can be life- threatening[11]
- Toothache, pulpitis, tooth loss and dental discoloration.

1.9 Treatment

The goal of treatment is to preserve tooth structures and prevent further destruction of the tooth**.** Most importantly, whether the carious lesion is cavitated or noncavitated dictates the management.

Noncavitated lesions

Can be arrested and remineralisation can occur with extensive changes to the diet i.e, reduction in frequency of refined sugars.[12] It can be treated with non- operative method by tooth remineralization.

Tooth remineralization

Tooth remineralization is a process in which minerals are returned to the molecular structure of the tooth itself. Destroyed tooth structure does not fully regenerate, although remineralization of very small carious lesions may occur if dental hygiene is kept at optimal level such as tooth brushing twice per day with fluoride toothpaste and flossing, and regular application of topical fluoride. Such management of a carious lesion is termed "non-operative treatment".[13]

Cavitated lesion,

Especially if dentin is involved, remineralization is much more difficult and a dental restoration is usually indicated. Such management of a carious lesion is termed "operative treatment".

Dental restoration

A dental restoration or dental filling is a process in which dental restorative material (including dental amalgam, composite resin, porcelain, and gold) is used to restore the function, integrity and morphology of missing tooth structure. Composite resin and porcelain can be made to match the color of a patient's natural teeth and are more frequently used. Local anesthetics, nitrous oxide ("laughing gas"), or other prescription medications may be required in some cases to relieve pain during or following treatment or to relieve anxiety during treatment.[14]

Tooth extraction

The removal of the decayed tooth is performed if the tooth is too far destroyed from the decay process to effectively restore the tooth.[15]

Other measures

Dental sealants

A sealant is a thin plastic-like coating applied to the chewing surfaces of the molars to prevent food from being trapped inside pits and fissures.[16]

1.10 Prevention and control

Oral hygiene

Personal hygiene care consists of proper brushing and flossing daily. Proper brushing and flossing is to remove and prevent the formation of plaque or dental biofilm. Professional hygiene care consists of regular dental examinations and professional prophylaxis (cleaning).

Dietary modification

Minimizing snacking is recommended, since snacking creates a continuous supply of nutrition for acid-creating bacteria in the mouth. Chewy and sticky foods (such as dried fruit or candy) tend to adhere to teeth longer, brushing the teeth after meals is recommended. For children, the ADA (Adenosine Deaminase) and the EAPD (European Association of Pediatric Dentistry) recommend limiting the frequency of consumption of drinks with sugar, and not giving baby bottles to infants during sleep. Chewing gum containing xylitol (a sugar alcohol) helps in reducing dental biofilm.[17]

Calcium and fluoride

Calcium is found in food such as milk and green vegetables, is often recommended to protect against dental caries. Fluoride helps prevent decay of a tooth by binding to the hydroxyapatite crystals in enamel. The incorporated calcium makes enamel more resistant to demineralization and, thus, resistant to decay. Topical fluoride include a fluoride toothpaste or mouthwash or varnish is now more highly recommended than systemic intake such as by tablets or drops to protect the surface of the teeth. After brushing with fluoride toothpaste, rinsing should be avoided. Flouride have pre-erruptive and post-erruptive effects on caries prevention.[18]

The main treatment option for a tooth cavity is to drill out the decay and put in a filling (restoration) made from various materials (e.g., composite resins, amalgam, porcelain).Extensive tooth decay may necessitate a crown, root canal treatment or even extraction of the tooth.[19]

Gel formulations are currently a novel idea of delivering drugs to patients, yet achieve sustained release of drug for the desired period. Different delivery systems based on polymers have been developed, which are able to increase the residence time of the formulation at absorption site of drugs.

The most important approach for periodontal treatment is the control of inflammation. Pain management has always been an important part of dental care. Therefore, the main categories of drugs to be used in such conditions are analgesics and anti-inflammatory agents. Also from the point of view that periodontal diseases have their origin in some form of infection. So it is worthwhile to use one of the antibiotics also.

2. Gel[20]

The word gel was coined by 19th-century Scottish chemist Thomas Graham by clipping from gelatine. A gel is a solid jelly-like material that can have properties ranging from soft and weak to hard and tough. Gels are defined as a substantially dilute cross-linked system, which exhibits no flow when in the steady-state. By weight, gels are mostly liquid, yet they behave like solids due to a three-dimensional cross-linked network within the liquid. It is the crosslinking within the fluid that gives a gel its structure (hardness) and contributes to the adhesive stick (tack). In this way gels are a dispersion of molecules of a liquid within a solid in which liquid particles are dispersed in the solid medium.

2.1 Types of gel

2.1.1 Hydrogels

A hydrogel is a network of polymer chains that are hydrophilic, sometimes found as a colloidal gel in which water is the dispersion medium. Hydrogels are highly absorbent (they can contain over 90% water) natural or synthetic polymeric networks. Hydrogels also possess a degree of flexibility very similar to natural tissue, due to their significant water content.

Common uses for hydrogels include:

- Environmentally sensitive hydrogels (also known as 'Smart Gels' or 'Intelligent Gels'). These hydrogels have the ability to sense changes of pH, temperature, or the concentration of metabolite and release their load as result of such a change.
- Sustained-release drug delivery systems
- Providing absorption, desloughing and debriding of necrotic and fibrotic tissue.
- Disposable diapers where they absorb urine, or in sanitary napkins

- Contact lenses (siliconehydrogels, polyacrylamides, polymacon)
- Rectal drug delivery and diagnosis.

2.1.2 Organogels

An organogel is a non-crystalline, non-glassy thermoreversible (thermoplastic) solid material composed of a liquid organic phase entrapped in a three-dimensionally cross-linked network. The liquid can be, for example, an organic solvent, mineral oil, or vegetable oil. The solubility and particle dimensions of the structure are important characteristics for the elastic properties and firmness of the organogel. Often, these systems are based on self-assembly of the structurant molecules. An example of formation of an undesired thermoreversible network is the occurrence of wax crystallization in petroleum. Organogels have potential for use in a number of applications, such as in pharmaceuticals, cosmetics, art conservation, and food.

2.1.3 Xerogels

A xerogel is a solid formed from a gel by drying with unhindered shrinkage. Xerogels usually retain high porosity (15–50%) and enormous surface area (150–900 m2/g), along with very small pore size (1–10 nm). When solvent removal occurs under supercritical conditions, the network does not shrink and a highly porous, low-density material known as an aerogel is produced. Heat treatment of a xerogel at elevated temperature produces viscous sintering (shrinkage of the xerogel due to a small amount of viscous flow) and effectively transforms the porous gel into a dense glass.

2.1.4 Nanocomposite hydrogels

Nanocomposite hydrogels are also known as hybrid hydrogels, can be defined as highly hydrated polymeric networks, either physically or covalently crosslinked with each other and/or with nanoparticles or nanostructures. Nanocomposite hydrogels can mimic native tissue properties, structure and microenvironment due to their hydrated and interconnected porous structure. A wide range of nanoparticles, such as carbon-based, polymeric, ceramic, and metallic nanomaterials can be incorporated within the hydrogel structure to obtain nanocomposites with tailored functionality. Nanocomposite hydrogels can be engineered to possess superior physical, chemical, electrical, and biological properties.[20]

MATERIALS AND METHODS

Chemicals and Materials

3.1 Drug Profile

3.1.1 Metronidazole:[33,34]

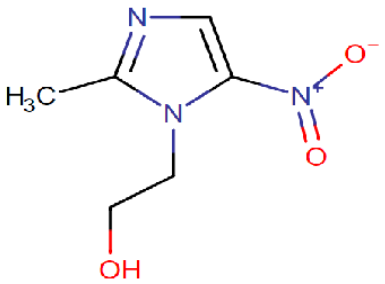
Drug Category : Antibiotics

Molecular Formula : $C_6H_{10}ClN_3O_3$

Chemical IUPAC Name : 2-(2-methyl-5-nitroimidazol-1- yl) ethanol; hydrochloride

Molecular weight : 207.614 gm/mol

Melting Point : 159-163°C



Structure : **Metronidazole**

Description: White to pale-yellow crystalline powder with a slight odor. Bitter and saline taste.

Solubility : solubility in various solvents at 25^0C.

Pharmacodynamics

Metronidazole, a synthetic antibacterial and antiprotozoal agent of the nitroimidazole class, is used against protozoa such as Trichomonas vaginalis, amebiasis, and giardiasis. Metronidazole is extremely effective against anaerobic bacterial infections and is also used to treat Crohn's disease, antibiotic-associated diarrhea, and rosacea.

Mechanism of action

Antibacterial (systemic); antiprotozoal—Microbicidal; active against most obligate anaerobic bacteria and protozoa by undergoing intracellular chemical reduction via mechanisms unique to anaerobic metabolism. Reduced metronidazole, which is cytotoxic but short-lived, interacts with DNA to cause a loss of helical structure, strand breakage, and resultant inhibition of nucleic acid synthesis and cell death.

3.1.2 Clindamycin hydrochloride[35]

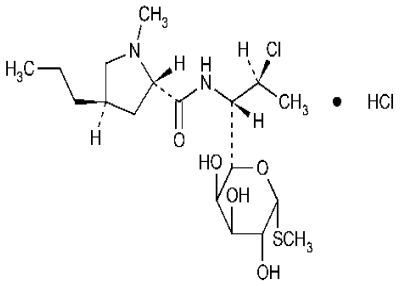
Drug Category: antibiotic

Molecular Formula : $C_{18}H_{34}ClN_2O_5S$

Chemical IUPAC Name : (2S,4R)-N-[(1S,2S)-2-chloro-1-[(2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-methylsulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide;hydrochloride

Molecular weight : 461.439 g/mol

Melting Point : 142.2-144.7^{0}C



Structure : **Clindamycin hydrochloride**

Description

Clindamycin hydrochloride is an antibacterial agent that is a semisynthetic analog of LINCOMYCIN.

Clindamycin Hydrochloride is the hydrochloride salt form of Clindamycin, a semi-synthetic, chlorinated broad spectrum antibiotic produced by chemical modification of lincomycin. It may be bacteriostatic or bactericidal depending on the organism and drug concentration.
It is a white crystalline powder.

Solubility

Freely soluble in water, dimethyl formamide and in methanol.

Pharmacodynamics

Clindamycin is an antibiotic, similar to and a derivative of lincomycin. Clindamycin can be used in topical or systemic treatment. It is effective as an anti-anaerobic antibiotic and antiprotozoal.

Mechanism of action

Systemic/vaginal Clindamycin inhibits protein synthesis of bacteria by binding to the 50S ribosomal subunits of the bacteria. Specifically, it binds primarily to the 23s RNA subunit.

Topical Clindamycin reduces free fatty acid concentrations on the skin and suppresses the growth of Propionibacterium acnes (Corynebacterium acnes), an anaerobe found in sebaceous glands and follicles.

3.1.3 DOXYCYCLINE HYCLATE[36]

Drug Category : Antibiotics

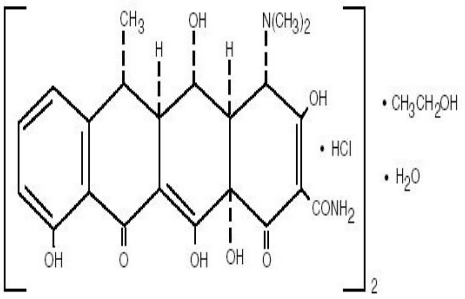
Molecular Formula : $C_{46}H_{58}C_{l2}N_4O_{18}$

Chemical IUPAC Name : (4S,4aR,5S,5aR,6R,12aR)-4-(dimethylamino)-1,5,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide;ethanol;hydrate;dihydrochloride

Molecular weight : 1025.88 g/mol

Melting Point : 206-209°C

Structure : **Doxycycline hyclate**



Description

It is yellow amorphous powder with slightly ethanolic odor. It is a synthetic tetracycline derivative with similar antimicrobial activity. Animal studies suggest that it may cause less tooth staining than other tetracyclines.

Solubility

-Very slightly soluble in water

-Sparingly soluble in alcohol

-Freely soluble in dil. Acid and alkali hydroxide solution

-Practically insoluble in chloroform and ether.

Pharmacodynamics

Doxycycline, a long-acting tetracycline derived from oxytetracycline, is used to inhibit bacterial protein synthesis and treat non-gonococcal urethritis and cervicitis, exacerbations of bronchitis in patients with COPD, and adult periodontitis.

Mechanisem of action

Doxycycline, like minocycline, is lipophilic and can pass through the lipid bilayer of bacteria. Doxycycline reversibly binds to the 30 S ribosomal subunits and possibly the 50S ribosomal subunit(s), blocking the binding of aminoacyl tRNA to the mRNA and inhibiting bacterial protein synthesis.

3.1.4 Serratiopeptidase

Drug Category : Anti-inflammatory

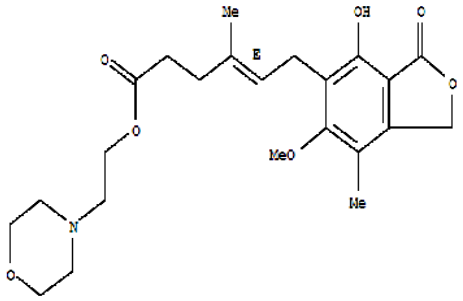
Molecular Formula : Ca_7Zn^{+16}

Chemical IUPAC Name : heptacalcium; zinc

Molecular weight : 345.926 g/mol

Melting Point : 163-168°C

Structure : **Serratiopeptidase**



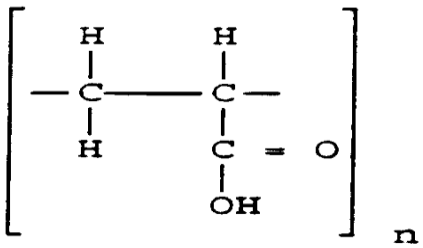
Pharmacology

Anti-inflammatory agents that are non-steroidal in nature. In addition to anti-inflammatory actions, they have analgesic, antipyretic, and platelet-inhibitory actions. They act by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase, which converts arachidonic acid to cyclic endoperoxides, precursors of prostaglandins. Inhibition of prostaglandin synthesis accounts for their analgesic, antipyretic, and platelet-inhibitory actions; other mechanisms may contribute to their anti-inflammatory effects.

3.2 Excipient profile

3.2.1 Carbopol 940p

Structure :



Synonyms: Poly acrylic acid, PAAc, Acrysol, Acumer, Alcosperse, Aquatreat, Carbomer, Sokalan

Chemical name: Carbomer 940

Molecular weight: 72.0626

Molecular formula: $(C_3H_4O_2)_n$

Description: Carbomers are white colored, fluffy, acidic, hygroscopic powder with slightly characteristic odour.

Solubility: Soluble in water and after neutralization soluble in ethanol (95%) and in glycerin.

Density (bulk and tapped): 1.76-2.08 g/cm^3 and 1.4 g/cm^3

Incompatibilities: Carbomers are incompatible with phenols, cationic polymers and strong acids, high levels of electrolytes.

Viscosity: Carbomers disperse in water to form acidic colloidal solutions of low viscosity which when neutralized produce highly viscous gels.

Packaging and storage: The powder should be stored in a well closed container in a cool and dry place.

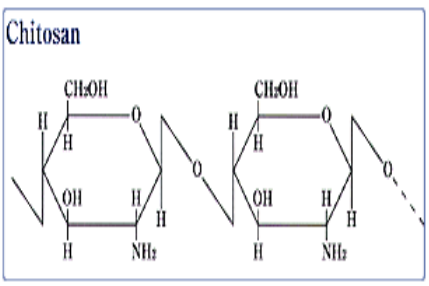
Stability

Carbomers are stable, though hygroscopic materials and can be heated at temperatures below 104^{0}C for upto 2 hours without affecting their thickening efficiency. Dry powder forms of carbomers do not support the growth of moulds and fungi but aqueous dispersions are very susceptible to microorganisms.

Applications: Emulsifying agent, suspending agent, tablet binder, viscosity enhancer.

3.2.2 Chitosan

Structural formula:



Nonproprietary names

BP: Chitosan hydrochloride

PhEur: Chitosan hydrochloridum

Synonyms: 2-Amino-2-deoxy-(1→4)-β-D-glucopyranan,Poly-(1,4-β-D-glucopyranosamine), Poly-(1→4)-β-D-glucosamine, Deacetylated chitin.

Chemical name: Poly-β-(1, 4)-2-Amino-2-D-glucose

Empirical Formula and Molecular Weight

Partial deacetylation of chitin results in teh production of chitosan, which is a polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine. Chitosan is the term applied to deacetylated chitins in various stages of deacetylation and depolymerisation and it is therefore not easily defined in terms of its exact chemical composition. A clear nomenclature with respect to the different degrees of N-deacetylation between chitin and chitosan has not been defined, and as such chitosan is not one chemical entity but varies i composition depending on the manufacturer. In essence, chitosan is chitin sufficiently deacetylated to form soluble amine salts. The degree of deacetylation necessary to obtain a soluble product must be greater than 80-85%. Chitosan is commercially available in several types and grades that vary in molecular weight by 10,000-1,000,000 and vary in degree of deacetylation and viscosity.

Functional category

- Coating agents
- Disintegrant
- Film-forming agent
- Mucoadhesive
- Tablet binder
- Viscosity-increasing agent

Pharmacopeial specification of chitosan

Table 3.2: Pharmacopeial specification of chitosan.

Appearance	White or creamy-white powder
Odour	Odorless
Matter insoluble in water	≤0.5%
pH (1% w/v solution)	4.0-6.0
Viscosity	+
Degree of deacetylation	66-99.8%
Moisture content	More than 10%
Ash value	More than 2%
Loss on drying	Less than 10%
Glass transition temperature	203^0C

3.2.3 Propylene glycol

Synonyms **:** 1, 2-Dihydroxypropane; E1520; 2-hydroxypropanul; methyl ethylene glycul; methyl glycul; propane-1,2diul.

Description **:** Propylene glycol is a clear, colorless, viscous, practically odorless liquid with a sweet, slightly acrid taste similar to that of glycerin.

Physical state **:** Viscous liquid.

Solubility **:**

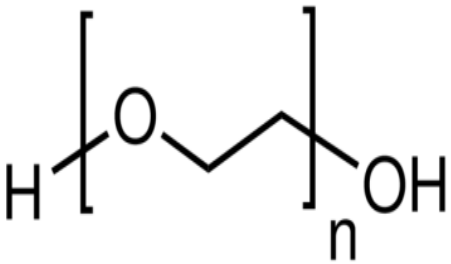
Table 3.3: Solubility of Propylene glycol.

Soluble	Acetone, chloroform, ethanol (95%), glycerine, water and ether.
Insoluble	Light mineral oil or fixed oils, essential oils.

Specification

Viscosity	58.1mPas (58.1cP) at 20°C
Specific gravity	1.035 – 1.040

3.2.4 PEG 200



Structure:

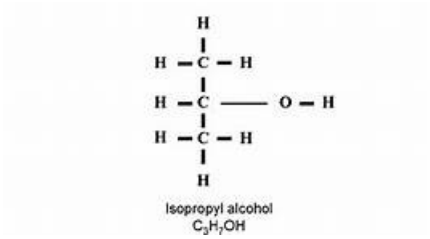
Synonym **:** Polyglycol, Polyethylene oxide, Polyoxy ethylene, PEG 200

Chemical formula : $HO\ (C_2H_4O)_nH$

Molecular weight : 190 - 210 g/mol
Boiling point : >150 °C
Melting point : −55-−40 °C (lit.)
Density : 1.124 g/cm3 (20 °C)
Appearance : clear, viscous, colourless or almost colourless hygroscopic liquid
pH value : 5 - 7 (100 g/l, H_2O, 20 °C)
Viscosity (20 °C) : 53.6 - 59.8 mm²/s
Vapor pressure : <0.01 hPa (20 °C)
Solubility : 70 g/l soluble

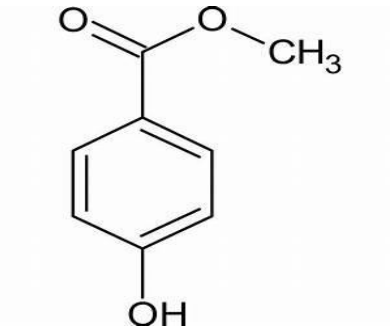
3.2.5 Isopropyl alcohol

Structure:



IUPAC name : Propan-2-ol[21]
Other names : 2 propanol, Isopropnol, sec-propyl alcohol, dimethyl carbinol
Chemical formula : C_3H_8O
Molar mass : 60.10 $g.mol^{-1}$
Appearance : Colourless liquid
Density : 0.786 g/cm^3 (20^0C)
Melting point : -89°C (-128 °F; 184 K)
Boiling point : 82.6 °C (180.7 °F; 355.8 K)
Solubility in water : Miscible with water
Solubility : Miscible with benzene, chloroform, ethanol, ether, glycerine and soluble in\acetone
Viscosity : 2.86 cP at 15 °C
1.96 cP at 25 °C
1.77 cP at 30 °C

3.2.6 Methyl paraben

Structure: 

IUPAC name : Methyl 4-hydroxybenzoate

Other names : Methyl paraben, Methyl p-hydroxybenzoate, Methyl parahydroxybenzoate.

Chemical formula : $C_8H_8O_3$

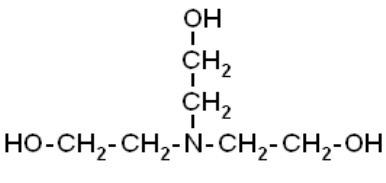
Molar mass : 152.15 $g.mol^{-1}$

Appearance : Colorless crystals or white crystalline powder

Melting point : 125.2 °C

Solubility : Slightly soluble in water; very soluble in ethanol, ether, acetone; soluble in trifluoroacetic acid

3.2.7 Triethanolamine

Structure: 

triethanolamine (TEA)

IUPAC name : 2,2',2''-Nitrilotri(ethan-1-ol)

Other names : 2,2',2''-Nitrilotriethanol, Tris (2-hydroxyethyl) amine, Triethylolamine, Trolamine, TEA

Chemical formula : $C_6H_{15}NO_3$

Molar mass : $C_6H_{15}NO_3$

Appearance : Colorless liquid

Odor : Ammonical

Density : 1.124 g ml^{-1}

Melting point : 21.60 ºC

Boiling point : 335.40 ºC

Solubility in water : 149 g L^{-1}

4. Experimental

4.1 Materials

Table 4.1: List of materials used and their suppliers.

Sr. no.	Drug / Excipient	Brand/Supplier
1	Metronidazole	Zim Laboratories, Nagpur
2	Clindamycin hydrochloride	Alkem Laboratories, Mumbai
3	Doxycycline hyclate	Alkem Laboratories, Mumbai
4	Serratiopeptidase	MP Biomedicals, LLC
5	Triethanolamine	Merck Specialities Pvt. Ltd, Mumbai.
6	Carbopol 940p	Akhil Healthcare Private Limited, Sayajigunji, Vadodara, Gujarat.
7	Chitosan	HiMedia Laboratories, Mumbai.
8	Disodium Hydrogen Phosphate	Fisher Scientific India, Pvt. Ltd, Mumbai.
9	Methyl paraben	Sigma Aldrich, Mumbai.
10	Hydrochloric acid	Merck Specialities Pvt. Ltd, Mumbai.
11	Methanol	Finar Pvt. Ltd
12	Propylene glycol	Merck Specialities Pvt. Ltd, Mumbai.
13	Pottassium dihydrogen phosphate	HiMedia Laboratories, Mumbai.
14	Sodium hydroxide	Merck Specialities Pvt. Ltd, Mumbai.
15	Polyethylene glycol-200	Merck Specialities Pvt. Ltd, Mumbai.
16	Isopropyl alcohol	Sigma-Aldrich Corporation & Biotechnological company.
17	Glacial acetic acid	Fisher Scientific India, Pvt. Ltd, Mumbai.

4.2 Instruments used

Table 4.2 List of instrument used.

Sr. no.	Equipment/Apparatus	Company Name/Model
1.	Digital Balance	SHIMADZU AUX220
2.	Digital pH meter	ELICO
3.	Double Beam UV – Spectrophotometer	JASCO UV Spectrophotometer Model No. V-630
4.	Modified Franz Diffusion Cell	Alka Scientific, Nagpur
5.	Heating mentle	REMI 1RML
6.	Homogenizer	REMI Equipment Pvt. Ltd, India
7.	Water bath	Hicon
8.	Magnetic stirrer	REMI Equipment Pvt. Ltd, India
9.	Mechanical stirrer	REMI Equipment Pvt. Ltd, India
10.	FTIR	Shimadzu 1800
11.	Ultrasonicator	PCI Mumbai
12.	Stability chamber	Thermolab Scientific Equipment Pvt. Ltd.
13.	Viscometer	BROOKFIELD DV-E VISCOMETER

4.3. Drug characterization

4.3.1. Metronidazole

4.3.1.1 Organoleptic properties

The drug sample was tested for its colour, odour and appearance.

4.3.1.2 Determination of Melting Point[37]

The melting point of Metronidazole was determined using capillary method and checked, whether it complies with the reported one or not.

4.3.1.3 Solubility of Metronidazole[38]

Solubility of Metronidazole was determined in distilled water, methanol, ethanol and dil. acetic acid. Solubility studies were performed by taking excess amount of API in different apparatus containing the solvents. The mixture was shaken at regular intervals. The solutions were filtered and analyzed spectrophotometrically at 320nm.

4.3.1.4 Infrared Spectrophotometric Analysis[39]

Metronidazole was dried in hot air oven at 50°C for 2 hr. The drug was prepared by mixing thoroughly with potassium bromide. This physical mixture was compressed under pressure of 10 Ton/Nm^2 and converted in a circular disc. This disc was then placed in the scanning slot of Fourier Transform Infra-Red (FT-IR) Spectrophotometer and scanned at the range from 400 to 4000 Cm^{-1} to obtain the IR of Metronidazole. FTIR Spectrum of Metronidazole Nitrate compared with reference spectrum.

4.3.1.5. UV-Visible spectrophotometric analysis

The calibration curve of the Metronidazole was plotted in phosphate buffer pH 6.8

i) Standard stock solution

Accurately weighed 1mg of Metronidazole was carefully transferred to 10 ml volumetric flask. The volume was made using Phosphate buffer pH 6.8 (100µg/ml). From the above solution 2 ml were pipette out and diluted upto 10 ml (20µg/ml). Scanning of the above 20µg/ml prepared solution was done in the range of the 400-200 nm, to determine the λmax.

Table 5.3: Stock solution concentration of metronidazole.

Drug	Solvent	Stock solution
Metronidazole	Phosphate buffer pH 6.8	µg/ml

ii) Working stock solution

From the above prepared standard stock solution 1ml, 2ml, 3ml, 4ml, 5ml, were pipette out and diluted up to 10ml with Phosphate buffer pH 6.8 in separate volumetric flask to get solution in the range of 10µg/ml to 50µg/ml respectively. The absorbance of all the solutions (10µg/ml to 50µg/ml) was measured at 320nm using UV spectrophotometer. The calibration curve was plotted by considering absorbance on Y-axis and concentration on X-axis.

4.3.1.6 Differential Scanning Calorimetry (DSC)[40]

DSC analysis of Metronidazole was performed on a DSC-25 Mettler Toldo system equipped with refrigerated cooling system. Sample was weighed accurately (3-5 mg) in aluminum pans and heated at a predefined rate of 10ºC/min over the temperature range from 20 to 300ºC in nitrogen atmosphere. Nitrogen gas was introduced at 2 bars and flow rate of 20 mL min^{-1}. On the other side of calorimeter, an empty crimped aluminium pan was placed as a reference standard. The scans were recorded and plots between flow of heat and temperature (ºC) were obtained.

4.3.2. Clindamycin hydrochloride

4.3.2.1 Organoleptic properties

The drug sample was tested for its colour, odour and appearance.

4.3.2.2 Determination of melting point[37]

The melting point of Clindamycin hydrochloride was determined using capillary method and checked, whether it complies with the reported one or not.

4.3.2.3 Solubility of Clindamycin hydrochloride[38]

Solubility of Clindamycin hydrochloride was determined in distilled water, methanol, ethanol and dil. acetic acid. Solubility studies were performed by taking excess amount of API in different apparatus containing the solvents. The mixture was shaken at regular intervals. The solutions were filtered and analyzed spectrophotometrically at 205 nm.

4.3.2.4 Infrared spectrophotometric analysis[39]

Clindamycin hydrochloride was dried in hot air oven at 50°C for 2 hr. The drug was prepared by mixing thoroughly with potassium bromide. This physical mixture was compressed under pressure of 10 Ton/Nm^2 and converted in a circular disc. This disc was then placed in the scanning slot of Fourier Transform Infra-Red (FT-IR) Spectrophotometer and scanned at the

range from 400 to 4000 Cm^{-1} to obtain the IR of Clindamycin hydrochloride. FTIR Spectrum of Clindamycin hydrochloride compared with reference spectrum.

4.3.2.5 UV Scanning of clindamycin hydrochloride

The calibration curve of the Clindamycin hydrochloride was plotted in phosphate buffer pH 6.8.

i) Standard stock solution

Accurately weighed 1mg of Clindamycin hydrochloride was carefully transferred to 10 ml volumetric flask. The volume was made using Phosphate buffer pH 6.8 (100µg/ml). From the above solution 2 ml were pipette out and diluted upto 10 ml (20µg/ml). Scanning of the above 20µg/ml prepared solution was done in the range of the 400-200 nm, to determine the λmax.

Table 4.4: Stock solution concentration of clindamycin.

Drug	Solvent	Stock solution
Clindamycin hydrochloride	Phosphate buffer pH 6.8	100µg/ml

ii) Working stock solution

From the above prepared standard stock solution 1ml, 2ml, 3ml, 4ml, 5ml, 6ml were pipette out and diluted up to 10ml with Phosphate buffer pH 6.8 in separate volumetric flask to get solution in the range of 10µg/ml to 60µg/ml respectively. The absorbance of all the solutions (10µg/ml to 60µg/ml) was measured at 205 nm using UV spectrophotometer. The calibration curve was plotted by considering absorbance on Y-axis and concentration on X-axis.

4.3.2.6 Differential scanning calorimetry (DSC)[40]

DSC analysis of Clindamycin hydrochloride was performed on a DSC-25 Mettler Toldo system equipped with refrigerated cooling system. Sample was weighed accurately (3-5 mg) in aluminum pan and heated at a predefined rate of 10ºC/min over the temperature range from 20 to 300ºC in nitrogen atmosphere. Nitrogen gas was introduced at 2 bars and flow rate of 20 mL min^{-1}. On the other side of calorimeter, an empty crimped aluminium pan was placed as a reference standard. The scans were recorded and plots between flow of heat and temperature (ºC) were obtained.

4.3.3. Doxycycline hyclate

4.3.3.1 Organoleptic properties

The drug sample was tested for its colour, odour and appearance.

4.3.3.2 Determination of Melting Point[37]

The melting point of Doxycycline hyclate was determined using capillary method and checked, whether it complies with the reported one or not.

4.3.3.3 Solubility of doxycycline hyclate[38]

Solubility of Doxycycline hyclate was determined in distilled water, methanol, ethanol and dil. acetic acid. Solubility studies were performed by taking excess amount of API in different apparatus containing the solvents. The mixture was shaken at regular intervals. The solutions were filtered and analyzed spectrophotometrically at 273 nm.

4.3.3.4 Infrared spectrophotometric analysis[39]

Doxycycline hyclate was dried in hot air oven at 50°C for 2 hr. The drug was prepared by mixing thoroughly with potassium bromide. This physical mixture was compressed under pressure of 10 Ton/Nm^2 and converted in a circular disc. This disc was then placed in the scanning slot of Fourier Transform Infra-Red (FT-IR) Spectrophotometer and scanned at the range from 400 to 4000 Cm^{-1} to obtain the IR of Doxycycline hyclate. FTIR Spectrum of Doxycycline hyclate compared with reference spectrum.

4.3.3.5 UV Scanning of doxycycline hyclate

The calibration curve of the Doxycycline hyclate was plotted in phosphate buffer pH 6.8

i) Standard stock solution

Accurately weighed 1mg of Doxycycline hyclate was carefully transferred to 10 ml volumetric flask. The volume was made using Phosphate buffer pH 6.8 (100µg/ml). From the above solution 2 ml were pipette out and diluted upto 10 ml (20µg/ml). Scanning of the above 20µg/ml prepared solution was done in the range of the 400-200 nm, to determine the λmax.

Table 4.5: Stock solution concentration of doxycycline hyclate.

Drug	Solvent	Stock solution
Doxycycline hyclate	Phosphate buffer pH 6.8	100µg/ml

ii) Working stock solution

From the above prepared standard stock solution 1ml, 2ml, 3ml, 4ml, 5ml, were pipette out and diluted up to 10ml with Phosphate buffer pH 6.8 in separate volumetric flask to get solution in the range of 10µg/ml to 50µg/ml respectively. The absorbance of all the solutions

(10µg/ml to 50µg/ml) was measured at 273 nm using UV spectrophotometer. The calibration curve was plotted by considering absorbance on Y-axis and concentration on X-axis.

4.3.3.7 Differential Scanning Calorimetry (DSC)[40]

DSC analysis of Doxycycline hyclate was performed on a DSC-25 Mettler Toldo system equipped with refrigerated cooling system. Sample was weighed accurately (3-5 mg) in aluminum pan and heated at a predefined rate of 10ºC/min over the temperature range from 20 to 300ºC in nitrogen atmosphere. Nitrogen gas was introduced at 2 bars and flow rate of 20 mL min^{-1}. On the other side of calorimeter, an empty crimped aluminium pan was placed as a reference standard. The scans were recorded and plots between flow of heat and temperature (ºC) were obtained.

4.3.4. Serratiopeptidase

4.3.4.1 Organoleptic properties

The drug sample was tested for its colour, odour and appearance.

4.3.4.2 Determination of melting point[37]

The melting point of Serratiopeptidase was determined using capillary method and checked, whether it complies with the reported one or not.

4.3.4.3 Solubility of Serratiopeptidase[38]

Solubility of Serratiopeptidase was determined in distilled water, simulated fluid pH 6.8, methanol, ethanol, acetic acid. Solubility studies were performed by taking excess amount of API in different apparatus containing the solvents. The mixture was shaken at regular intervals. The solutions were filtered and analyzed spectrophotometrically at 205 nm.

4.3.4.4 Infrared Spectrophotometric analysis[39]

Serratiopeptidase was dried in hot air oven at 50°C for 2 hr. The drug was prepared by mixing thoroughly with potassium bromide. This physical mixture was compressed under pressure of 10 Ton/Nm^2 and converted in a circular disc. This disc was then placed in the scanning slot of Fourier Transform Infra-Red (FT-IR) Spectrophotometer and scanned at the range from 400 to 4000 Cm^{-1} to obtain the IR of Serratiopeptidase. FTIR Spectrum of erratiopeptidase compared with reference spectrum.

4.3.4.5 UV Scanning of Serratiopeptidase

The calibration curve of the Serratiopeptidase was plotted in phosphate buffer pH 6.8.

i) Standard stock solution

Accurately weighed 1mg of Serratiopeptidase was carefully transferred to 10 ml volumetric flask. The volume was made using Phosphate buffer pH 6.8 (100µg/ml). From the above solution 2 ml were pipette out and diluted upto 10 ml (20µg/ml). Scanning of the above 20µg/ml prepared solution was done in the range of the 400-200 nm, to determine the λmax.

Table 4.6: Stock solution concentration of serratiopeptidase.

Drug	Solvent	Stock solution
Serratiopeptidase	Phosphate buffer pH 6.8	100µg/ml

ii) Working stock solution

From the above prepared standard stock solution 1ml, 2ml, 3ml, 4ml, 5ml, were pipette out and diluted up to 10ml with Phosphate buffer pH 6.8 in separate volumetric flask to get solution in the range of 10µg/ml to 50µg/ml respectively. The absorbance of all the solutions (10µg/ml to 50µg/ml) was measured at 205 nm using UV spectrophotometer. The calibration curve was plotted by considering absorbance on Y-axis and concentration on X-axis.

4.3.4.6 Differential scanning calorimetry (DSC)[40]

DSC analysis of Serratiopeptidase was performed on a DSC-25 Mettler Toldo system equipped with refrigerated cooling system. Sample was weighed accurately (3-5 mg) in aluminum pan and heated at a predefined rate of 10ºC/min over the temperature range from 20 to 300ºC in nitrogen atmosphere. Nitrogen gas was introduced at 2 bars and flow rate of 20 mL min^{-1}. On the other side of calorimeter, an empty crimped aluminium pan was placed as a reference standard. The scans were recorded and plots between flow of heat and temperature (ºC) were obtained.

4.3.5 Study of physical interaction between drugs by Fourier Transform Infrared Spectroscopy (FTIR) study

Metronidazole, Clindamycin hydrochloride, Doxycycline hyclate and Serratiopeptidase were dried in hot air oven at 50°C for 2 hr. The drugs were prepared by mixing thoroughly with potassium bromide. This physical mixture was compressed under pressure of 10 Ton/nm^2 and converted in a circular disc. This disc was then placed in the scanning slot of Fourier ansform Infra-Red (FT-IR) spectrophotometer and scanned at range from 400 to 4000 cm^1 to obtain the FTIR.

4.3.6 Study of physical interaction between drugs by Differential Scanning Colorimetry (DSC) study

DSC analysis of Metronidazole, clindamycin hydrochloride, Doxycycline hyclate and Serratiopeptidase were performed on a DSC-25 Mettler Toldo system equipped with refrigerated cooling system. Samples were weighed accurately (3-5 mg) in aluminum pans and heated at a predefined rate of 10ºC/min over the temperature range from 20 to 300ºC in nitrogen atmosphere. Nitrogen gas was introduced at 2 bars and flow rate of 20 mL min-1. On the other side of calorimeter, an empty crimped aluminium pan was placed as a reference standard. The scans were recorded and plots between flow of heat and temperature (ºC) were obtained.

4.4 Formulation of gel

1. Chitosan was dissolved in 2% acetic acid solution.
2. Carbopol 940 was accurately weighed and dissolved slowly in distilled water with continuous stirring on mechanical stirrer.
3. Chitosan solution was added in Carbopol solution with continuous stirring.
4. To this solution PEG 200, propylene glycol & isopropyl alcohol was added.
5. Then all four drugs (Metronidazole, Clindamycin, Doxycycline and Serratiopeptidase) were added with continuous stirring.
6. At last methyl paraben was added as a preservative.
7. The gel pH was adjusted by added triethanolamine until pH 6.8 was reached. Then a clear slightly tacky gel was formed.

Table no. 4.7: Formulation design of gel.

Ingredients	Formulation code (%w/v)					
	F1	F2	F3	F4	F5	F6
Metronidazole	5	5	5	5	5	5
Clindamycin hydrochloride	5	5	5	5	5	5
Doxycycline hyclate	1	1	1	1	1	1
Serratiopeptidase	0.5	0.5	0.5	0.5	0.5	0.5
Carbopol 940	3	3	3	3	3	3
Chitosan	0.1	0.2	0.5	0.6	0.8	1
Isopropyl alcohol	10	10	10	10	10	10
Polyethylene glycol 200	10	10	10	10	10	10
Methyl paraben	0.15	0.15	0.15	0.15	0.15	0.15
Distilled water	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100

4.5 Evaluation of prepared gel

4.5.1. Physical appearance

The physical appearance of the formulation was checked visually[41].

4.5.2. Determination of pH

The pH of gel was determined using digital pH meter by dipping the glass electrode completely into the gel system.

4.5.3. Determination of viscosity

Viscosities of formulated gels were determined using Brookfield viscometer, spindle no. 64. Viscosity was measured at 50 to 100 rpm at room temperature.

4.5.4. Drug content in gel

About 1 g (weighed by transfer method) of gel was dissolve in pH 6.8 phosphate buffer. It was filtered through a whatman filter paper. The sample was analyzed by UV spectrophotometer using Shimadzu UV 1700 spectrophotometer, using phosphate buffer (pH 6.8) as the blank and the concentration of drug in each sample was determined from standard curve.[42]

$$Drug\ content = \frac{Concentration \times Total\ formulation \times Dilution\ factor}{1000}$$

$$\%\ Drug\ content = \frac{Drug\ content}{Total\ drug} \times 100$$

4.5.5 Syringeability study

For drug delivery into the periodontal pocket, injectable systems are useful. The use of injectable systems is easy and rapid. Syringeability of gel formulations was evaluated through 22 G needle.[43]

4.5.6. In-Vitro drug release

Modified Franz diffusion cell volume of 3 cm^2 was used to evaluate drug release characteristics. A dialysis membrane (0.65 µm) was used in donor compartment. The receptor phase (pH 6.8 phosphate buffer) was continuously stirred and kept at a temperature of 37 ± 2^0C during experiments. 1 g of gel formulation was placed in the donor compartment. 1 ml of sample was withdrawn from the receiver compartment in 10 ml of volumetric flask and volume was made up using phosphate buffer (pH 6.8) solution and same amount of fresh

solution (1 ml) was added to receiver compartment to keep the volume constant. Samples were withdrawn from the receiver compartment in two hours interval. The samples were analyzed at a suitable wavelength (Metronidazole-320, Clindamycin hydrochloride-205, Doxycycline hyclate-273, Serratiopeptidase-205) using Shimadzu UV 1700 spectrophotometer, taking phosphate buffer (pH 6.8) as the blank and the concentration of drug in each sample was determine from standard calibration curve as given in fig. 6.3, 6.7, 6.11 and 6.15).

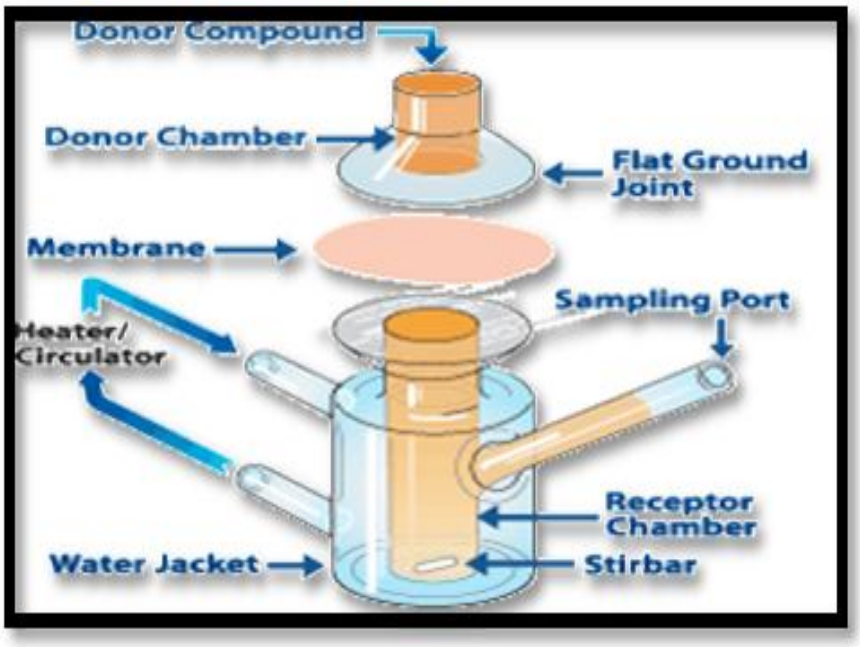


Fig. 4.1: Franz diffusion cell with dialysis membrane mounted between compartments.

4.5.7. Collection of goat mucosal membrane

The goat cheek mucosa was collected from a nearby local slaughter house within 20 min after the goat was sacrificed. A section of mucosal tissue was cut from the goat cheek mucous membrane and was thoroughly washed with distilled water followed by isotonic phosphate buffer pH 6.8 then soaked in phosphate buffer saline pH 6.8 to remove all the soluble components.[44]

4.5.8. Ex-Vivo drug permeation study

Ex vivo permeation study was conducted using a Modified Franz diffusion cell. The receptor phase (containing pH 6.8 phosphate buffer) was continuously stirred and kept at a temperature of 37 ± 2^{0}C during experiments. The freshly excised cheek mucosal membrane was mounted on the donor compartment. 1 g of gel was placed on the donor compartment of

cheek mucosal membrane. 1 ml of the sample was withdrawn from the receiver compartment in 10 ml of volumetric flask and volume was made up using phosphate buffer (pH 6.8) solution and replenished with an equal amount of phosphate buffer solution (1 ml) was added to receiver compartment to keep the volume constant.

Each experiment was run in 6 independent cells and each sample was withdrawn from the receiver compartment in two hour interval. Absorbance of the samples was measured by UV spectrophotometer using Shimadzu UV 1700 spectrophotometer, taking phosphate buffer (pH 6.8) as the blank. The amount of drug permeated was calculated from the calibration curve fig. 6.3, 6.7, 6.11 and 6.15). The mean cumulative percentage of drug permeated was plotted against time. Permeation area was 3 sq. cm.

Fig. 4.2: Franz diffusion cell apparatus with mucosal membrane mounted between compartments.

4.5.9 Stability study of optimized batch

Stability studies were carried out on gel formulation according to ICH guidelines. A sufficient quantity of gel in glass bottles was stored in stability chamber at 25°C/60%RH±5% and 45°C/65%RH±5% and samples were withdrawn at 0 and 30 days. The physical stability of gel was observed and pH of formulation and viscosity were measured.

5. RESULTS AND DISCUSSION

5.1 Drug characterization

5.1.1 Metronidazole

5.1.1.1 Organoleptic properties

It is white, odourless, crystalline powder.

5.1.1.2 Melting Point

The melting point of the Metronidazole was found to be 159 to163°C, which complies with melting point reported in United States Pharmacopoeia 2011.

5.1.1.3 Solubility of Metronidazole

Table 5.1: Solubility of metronidazole

Sr. no.	Media	Solubility
1	Water	10mg/ml
2	Methanol	<0.5mg/ml
3	Ethanol	5mg/ml
4	Acetic acid	Soluble

5.1.1.4 Fourier transform infrared spectrophotometric analysis (FTIR)

All the prominent and primary peaks were observed in FTIR spectrum of Metronidazole (Fig. 6.1) and compared with the reference spectrum as per United State Pharmacopoeia 2011.

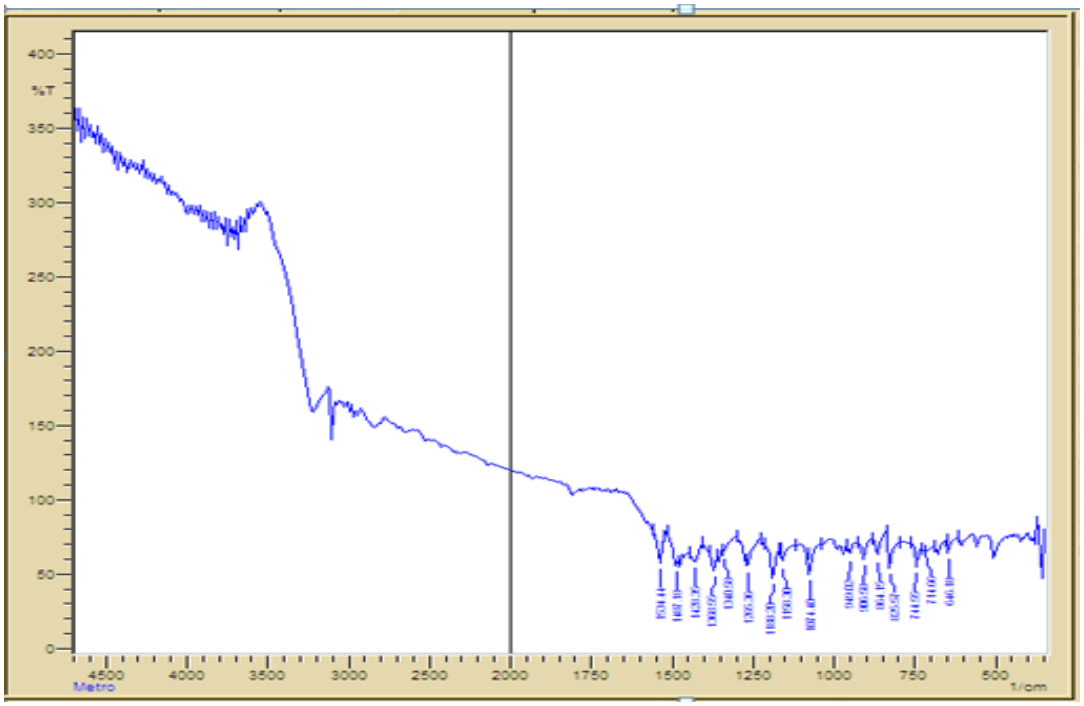


Fig 5.1: FTIR spectrum of Metronidazole.

Table 5.2: Details of FTIR study of Metronidazole

Sr. no.	Reported Frequencies (cm^{-1})	Observed frequencies (cm^{-1})	Assignment
1	1350-1480	1368.55, 1428.35	-C-H alkane bending
2	1080-1360	1158.30, 1340.58	C-N amine stretch
3	1515-1560 & 1345-1385	1534.44	N-O nitro stretch

The reported frequencies were found to be concurrent with a reference spectrum of Metronidazole. The IR spectrum of Metronidazole exhibits main bands near or at wave number ($cm^{-1)}$ 1368.55, 1428.35, 1158.30, 1340.58, 1534.44 which was concurrent to reported frequencies.

5.1.1.5 UV-Visible spectrophotometric analysis

a) UV Spectroscopy

The maximum absorption value of pure drug, Metronidazole was found at 320 nm wavelength in phosphate buffer pH 6.8. Therefore 320 nm was recorded as λmax of the pure drug Metronidazole. The observed λmax value of drug was found to complied with the specification of Indian pharmacopoeia. Hence the drug was considered to be pure. The UV spectrum of Metronidazole is shown in Figure 5.2.

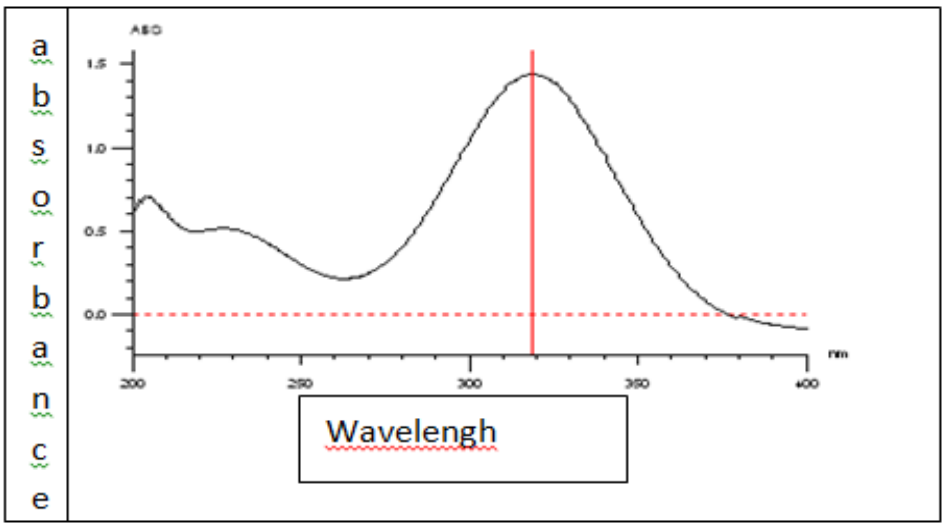


Figure 5.2: U.V Spectrum of Metronidazole in phosphate buffer pH 6.8.

b) Calibration curve of Metronidazole in phosphate buffer pH 6.8

A solution of 100 µg/ml of Metronidazole was scanned in the range of 400 to 200 nm. The drug exhibited the λmax at 320 nm and showed reproducibility. From the standard curve of Metronidazole in phosphate buffer pH 6.8 it was observed that the Metronidazole obeys

Beers-Lambert's law in the range 10-50µg/ml in the medium as shown in table 5.3 and figure 5.3.

Table 6.3: Calibration of metronidazole.

Sr. No.	Conc. (µg/ml)	Absorbance
0	0	0
1	10	0.5763
2	20	0.888
3	30	1.28
4	40	1.7045
5	50	2.0555

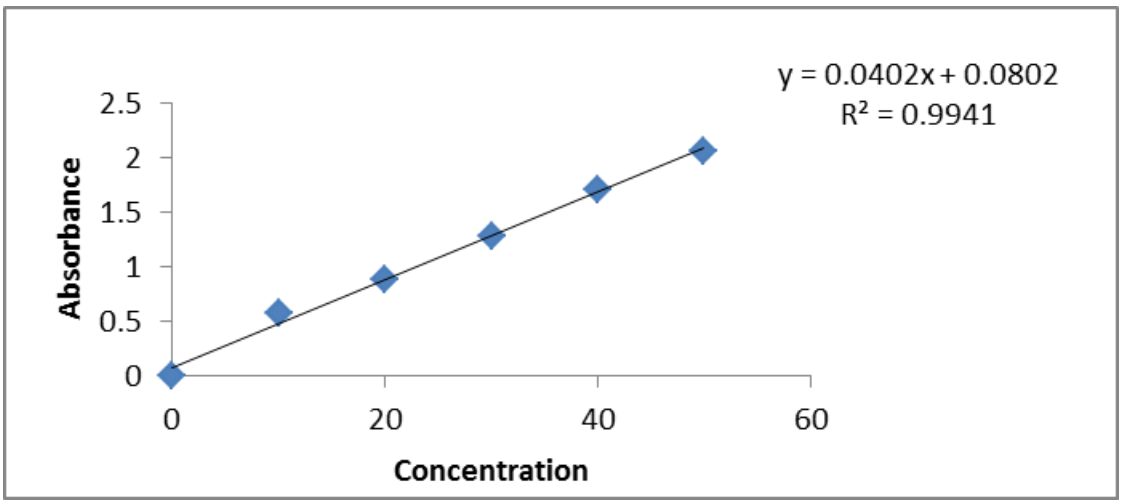


Fig. 5.3: Calibration curve of metronidazole in phosphate buffer pH 6.8.

5.1.1.6 Differential scanning calorimetry (DSC)

DSC thermogram of Metronidazole showed melting endothermic peak at 162.68°C which complies with melting point of Metronidazole.

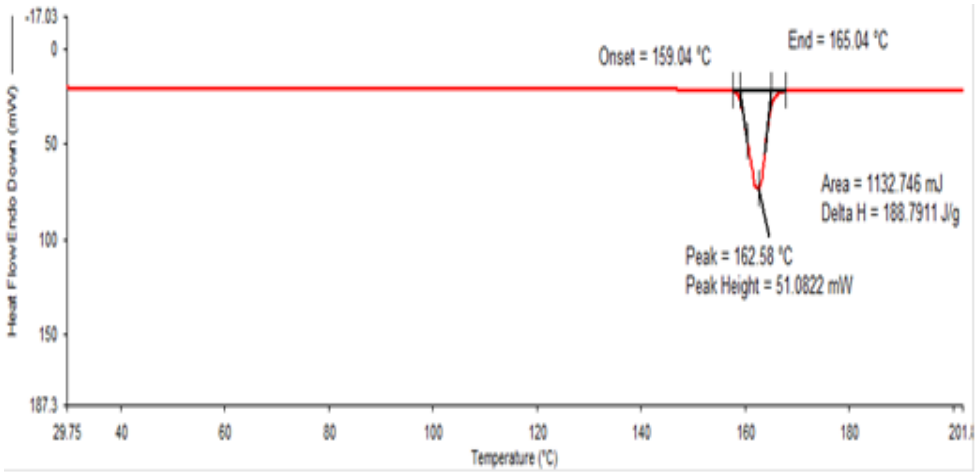


Fig. 5.4: DSC Thermogram of pure metronidazole.

5.1.2 Clindamycin hydrochloride

5.1.2.1 Organoleptic Properties

It is white, odourless, crystalline powder.

5.1.2.2 Melting Point

The melting point of the Clindamycin hydrochloride was found to be 142 to147°C, which complies with melting point reported in Indian Pharmacopoeia.

5.1.2.3 Solubility of Clindamycin hydrochloride

Table 5.4: Solubility of clindamycin hydrochloride.

Sr. no.	Media	Solubility
1	Water	50 mg/ml
2	Methanol	20 mg/ml
3	Dil. Acetic acid	Freely soluble

5.1.2.4 Infrared spectrophotometric analysis

All the prominent and primary peaks were observed in FTIR spectrum of Clindamycin hydrochloride (Fig. 6.5) and compared with the reference spectrum as per United State Pharmacopoeia 2011.

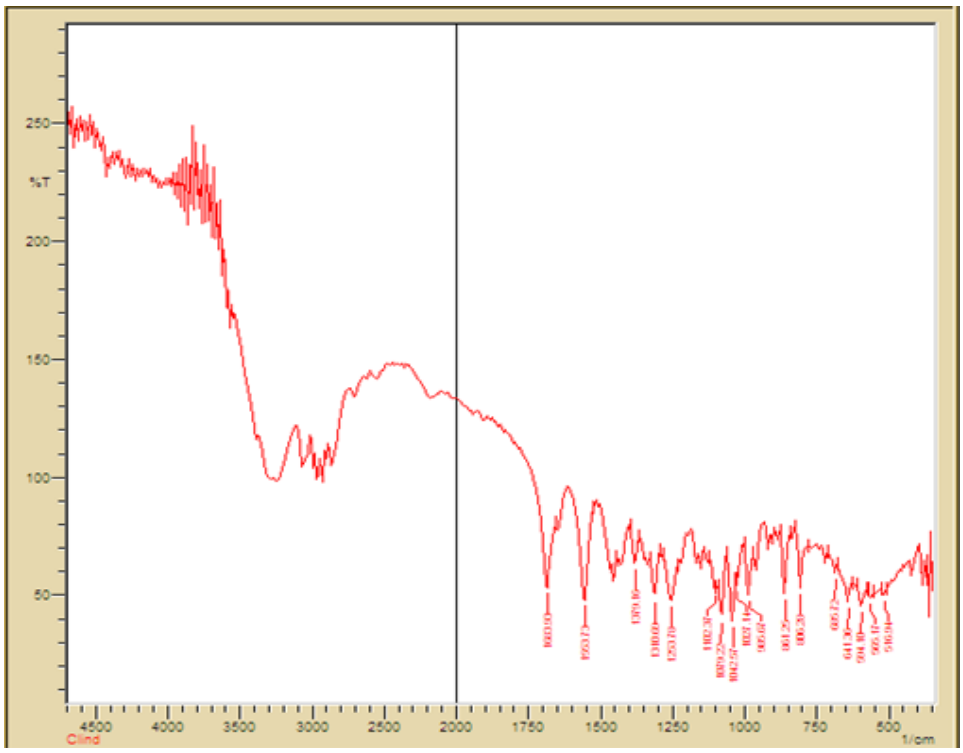


Fig. 5.5: FTIR spectrum of clindamycin hydrochloride.

Table 5.5: Details of FTIR study of Clindamycin hydrochloride.

Sr. no.	Reported Frequencies (cm^{-1})	Observed frequencies (cm^{-1})	Assignment
1	1350-1480	1379.16	-C-H alkane bending
2	600-800	641.36, 685.72	C-Cl alkyl halide Strech
3	1080-1360	1102.37, 1310.89	C-N amine Strech
4	1670-1820	1683.93	C=O carbonyl stretch

The reported frequencies were found to be concurrent with a reference spectrum of Clindamycin hydrochloride. The IR spectrum of Clindamycin hydrochloride exhibits main bands near or at wave number ($cm^{-1)}$ 1379.16, 641.36, 685.72, 1102.37, 1310.89, 1683.93 which was concurrent to reported frequencies.

5.1.2.5 UV-Visible spectrophotometric analysis

a) UV Spectroscopy

The maximum absorption value of pure drug, Clindamycin hydrochloride was found at 205 nm wavelength in phosphate buffer pH 6.8. Therefore 205 nm was recorded as λmax of the pure drug Clindamycin. The observed λmax value of drug was found to complied with the specification of Indian pharmacopoeia. Hence the drug was considered to be pure. The UV spectrum of Clindamycin is shown in Figure 5.6.

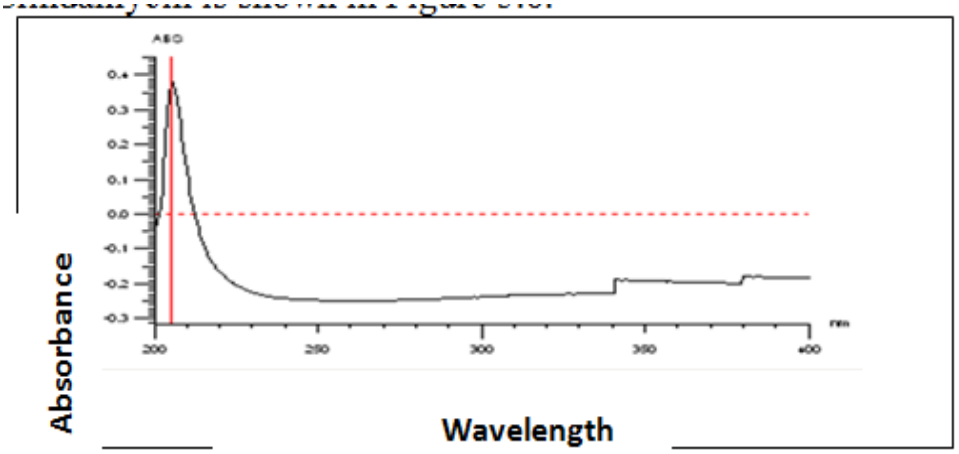


Fig. 5.6: U.V Spectrum of Clindamycin in phosphate buffer pH 6.8.

b) Calibration curve of Clindamycin in phosphate buffer pH 6.8

A solution of 100 µg/ml of Clindamycin hydrochloride was scanned in the range of 400 to 200 nm. The drug exhibited the λmax at 205 nm and showed reproducibility. From the standard curve of Clindamycin hydrochloride in phosphate buffer pH 6.8 it was observed that the Clindamycin hydrochloride obeys Beers-Lambert's law in the range 10-60µg/ml in the medium as shown in table 6.6 and figure 5.7.

Table 5.6: Calibration of clindamycin hydrochloride.

Sr. no.	Conc. (µg/ml)	Absorbance
0	0	0
1	10	0.137
2	20	0.394
3	30	0.477
4	40	0.690
5	50	0.801
6	60	1.010

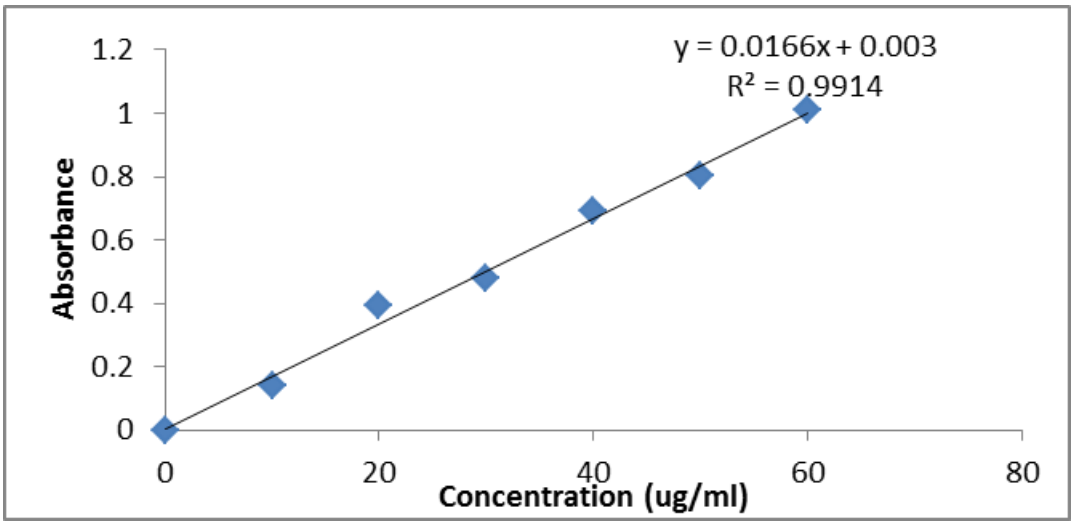


Fig. 5.7: Calibration curve of Clindamycin in phosphate buffer pH 6.8.

5.1.2.6 Differential scanning calorimetry (DSC)

DSC thermogram of Clindamycin hydrochloride showed melting endothermic peak at 68.38°C.

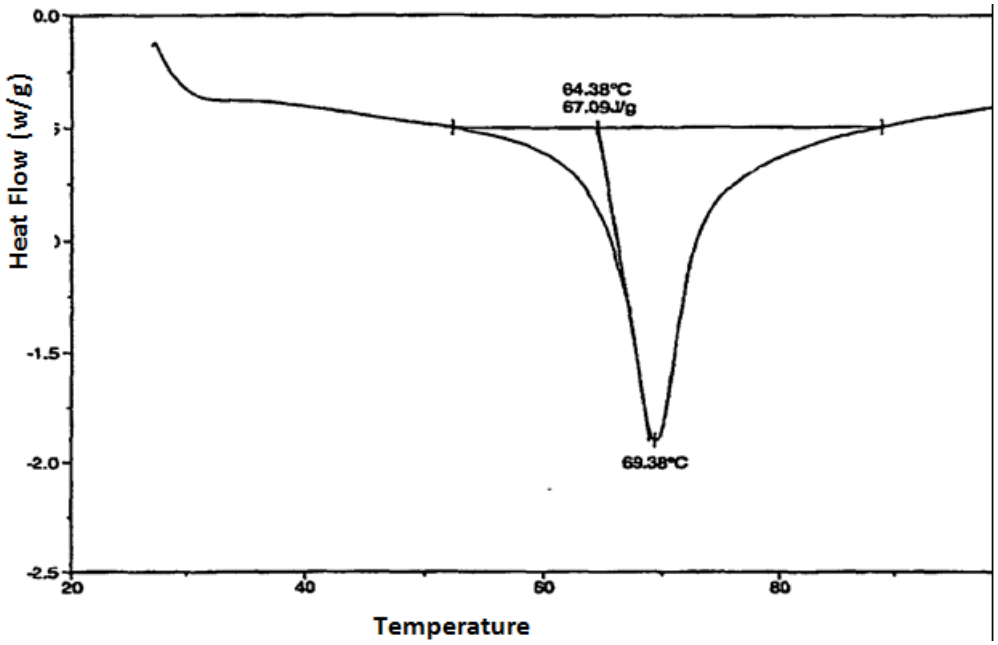


Fig. 5.8: DSC Thermogram of pure clindamycin hydrochloride.

5.1.3 Doxycycline hyclate

5.1.3.1 Organoleptic Properties

It is yellow crystalline powder with slightly ethanolic odor.

5.1.3.2 Melting Point

The melting point of the Doxycycline hyclate was found to be 203 to 205°C, which complies with melting point reported in Indian Pharmacopoeia**.**

5.1.3.3 Solubility of doxycycline hyclate

Table 5.7: Solubility of Doxycycline hyclate.

Sr no.	Media	Solubility
1	Water	50 mg/ml
2	Alcohol	Insoluble
3	Ethanol	Insoluble
4	Dil. Acetic acid	Freely soluble

5.1.3.4 Infrared spectrophotometric analysis

All the prominent and primary peaks were observed in FTIR spectrum of Doxycycline hyclate (Fig. 5.9) and compared with the reference spectrum as per United State Pharmacopoeia 2011.

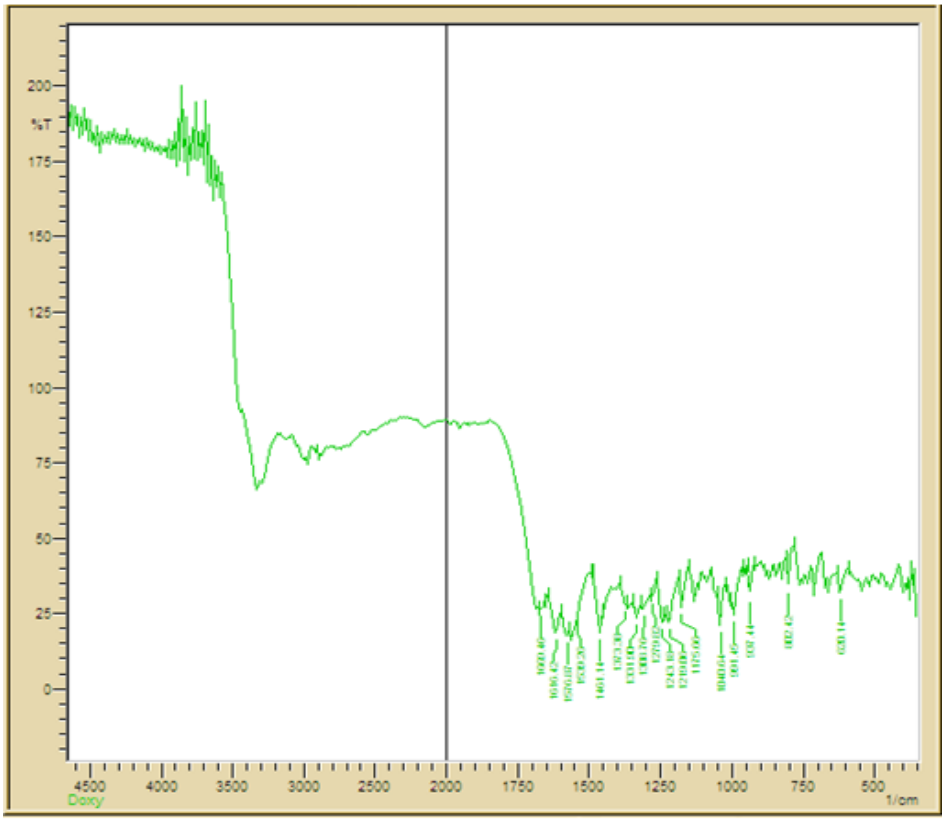


Fig. 5.9: FTIR Spectrum of Doxycycline hyclate

Table 5.8: Details of FTIR study of Doxycycline hyclate.

Sr. no.	Reported Frequencies(cm^{-1})	Observed frequencies (cm^{-1})	Assignment
1	1350-1480	1373.38, 1461.14	-C-H alkane bending
2	1400-1600	1461.14, 1578.87	C=C aromatic stretch
3	1080-1360	1175.66, 1331.90	C-N amine Strech
4	1670-1820	1689.48	C=O Carbonyl Strech
5	1550-1640	1578.87, 1618.42	N-H amide Bending

The reported frequencies were found to be concurrent with a reference spectrum of Doxycycline hyclate. The IR spectrum of Doxycycline hyclate exhibits main bands near or at wave number ($cm^{-1)}$ 1373.38, 1461.14, 1461.14, 1578.87, 1175.66, 1331.90, 1689.48, and 1578.87, 1618.42 which were concurrent to reported frequencies.

5.1.3.5 UV-Visible spectrophotometric analysis

a) UV Spectroscopy

The maximum absorption value of pure drug, Doxycycline hyclate was found at 273 nm wavelength in phosphate buffer pH 6.8.Therefore 273 nm was recorded as λmax of the pure drug Doxycycline hyclate. The observed λmax value of drug was found to complied with the

specification of Indian pharmacopoeia. Hence the drug was considered to be pure. The UV spectrum of Doxycycline was shown in Figure 5.10.

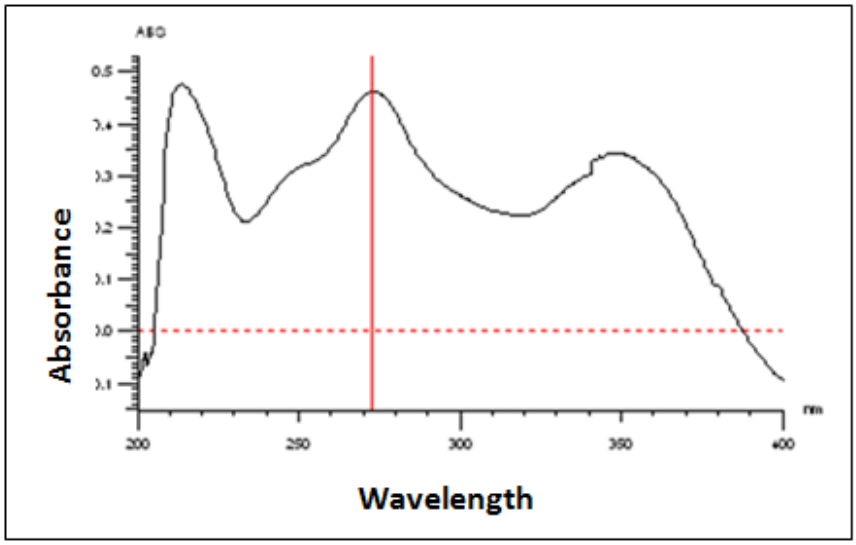


Figure 5.10: U.V Spectrum of Doxycycline hyclate in phosphate buffer pH 6.8.

b) Calibration curve of Doxycycline hyclate in phosphate buffer pH 6.8

A solution of 100 µg/ml of Doxycycline hyclate was scanned in the range of 400 to 200 nm. The drug exhibited the λmax at 273 nm and showed reproducibility. From the standard curve of Doxycycline hyclate in phosphate buffer pH 6.8 it was observed that the Doxycycline obeys Beers-Lambert's law in the range 10-50µg/ml in the medium as shown in table 5.9 and figure 5.11.

Table 5.9: Calibration of doxycycline hyclate.

Sr. no.	Conc. (µg/ml)	Absorbance
0	0	0
1	10	0.116
2	20	0.168
3	30	0.251
4	40	0.315
5	50	0.396

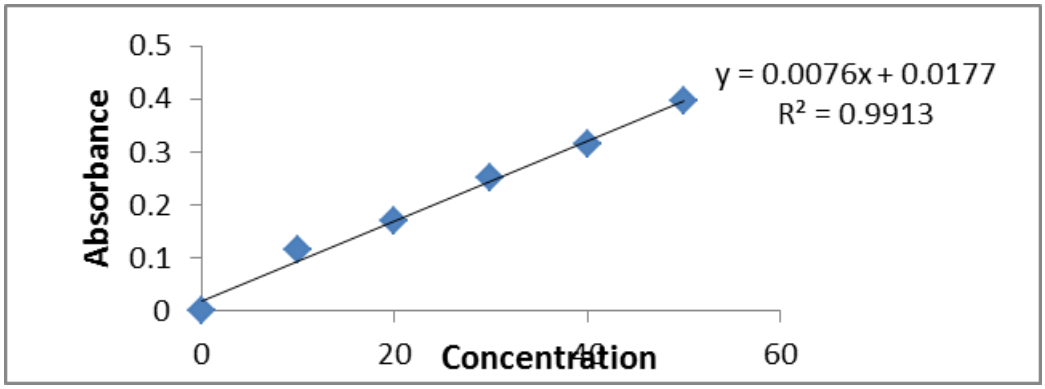


Fig. 5.11: Calibration curve of doxycycline hyclate in phosphate buffer pH 6.8.

5.1.3.6 Differential Scanning Calorimetry (DSC)

DSC thermogram of Doxycycline hyclate shows melting endothermic peak at 201.5°C which complies with melting point of Doxycycline hyclate.

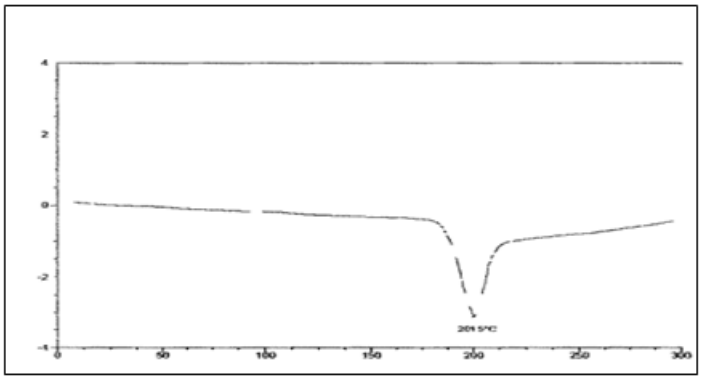


Fig. 5.12: DSC Thermogram of Pure Doxycycline hyclate.

5.1.4 Serratiopeptidase

5.1.4.1 Organoleptic Properties

It is white, odourless powder.

5.1.4.2 Melting Point

The melting point of the Serratiopeptidase was found to be 163-168°C, which complies with melting point reported in United States Pharmacopoeia 2011.

5.1.4.3 Solubility of metronidazole.

Table 5.10: Solubility of serratiopeptidase

Sr no.	Media	Solubility
1	Water	0.1 mg/ml
2	Methanol	0.1-1 mg/ml
3	Ethanol	0.1-1 mg/ml
4	Dil. Acetic acid	Freely soluble

5.1.4.4 Fourier transform infrared spectrophotometric analysis (FTIR)

All the prominent and primary peaks were observed in FTIR spectrum of Serratiopeptidase (Fig. 5.13) and compared with the reference spectrum as per United State Pharmacopoeia 2011.

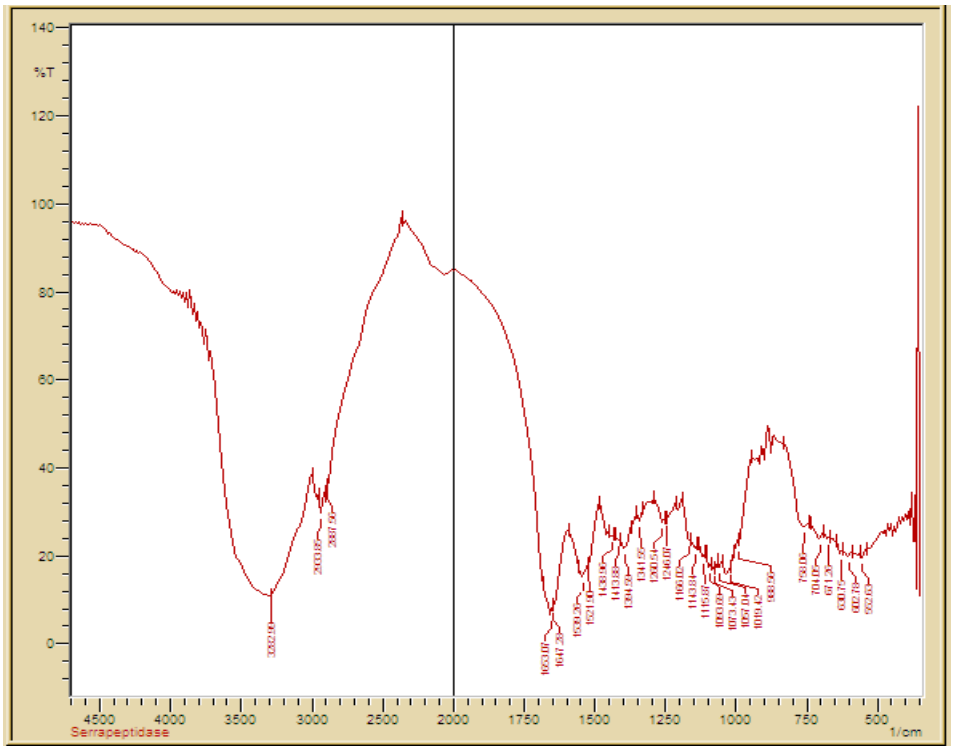


Fig. 5.13: FTIR Spectrum of Serratiopeptidase.

Table 5.11: Details of FTIR study of serratiopeptidase.

Sr. no.	Reported Frequencies (cm^{-1})	Observed frequencies (cm^{-1})	Assignment
1	3200-3600	3282.99	O-H alcohol Streching
2	1050-1150	1057.04, 1143.43	C-O alcohol stretching
3	2850-3000	2887.56, 2933.85	C-H alkane stretching
4	1080-1360	1093.69, 1341.55	C-N amine stretching
5	1640-1690	1647.28, 1653.07	C=O amide stretching

The reported frequencies were found to be concurrent with a reference spectrum of Serratiopeptidase. The IR spectrum of Serratiopeptidase h exhibits main bands near or at wave number (cm^{-1}) 3282.99, 1057.04, 1143.43, 2887.56, 2933.85, 1093.69, 1341.55, 1647.28, 1653.07 which was concurrent to reported frequencies.

5.1.4.5 UV-Visible spectrophotometric analysis

a) Uv spectroscopy

The maximum absorption value of pure drug, Serratiopeptidase was found at 205 nm wavelength in phosphate buffer pH 6.8. Therefore 205 nm was recorded as λmax of the pure drug Serratiopeptidase. The observed λmax value of drug was found to complied with the specification of Indian pharmacopoeia. Hence the drug was considered to be pure. The UV spectrum of Serratiopeptidase was shown in Figure 5.14.

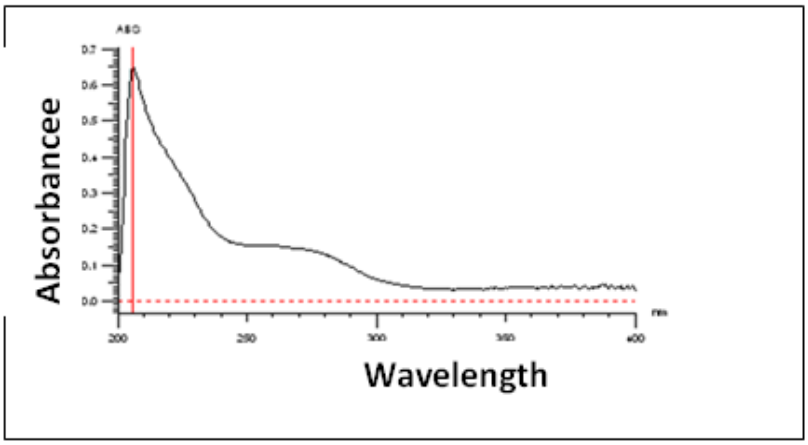


Figure 5.14: U.V Spectrum of serratiopeptidase in phosphate buffer pH 6.8.

b) Calibration curve of Metronidazole in phosphate buffer pH 6.

A solution of 100 µg/ml of Serratiopeptidase was scanned in the range of 400 to 200 nm. The drug exhibited the λmax at 320 nm and showed reproducibility. From the standard curve of Serratiopeptidase in phosphate buffer pH 6.8 it was observed that the Serratiopeptidase obeys Beers-Lambert's law in the range 10-50µg/ml in the medium as shown in table 5.12 and figure 5.15.

Table 5.12: Calibration of serratiopeptidase.

Sr. no.	Conc. (µg/ml)	Absorbance
0	0	0
1	10	0.200
2	20	0.370
3	30	0.599
4	40	0.785
5	50	0.900

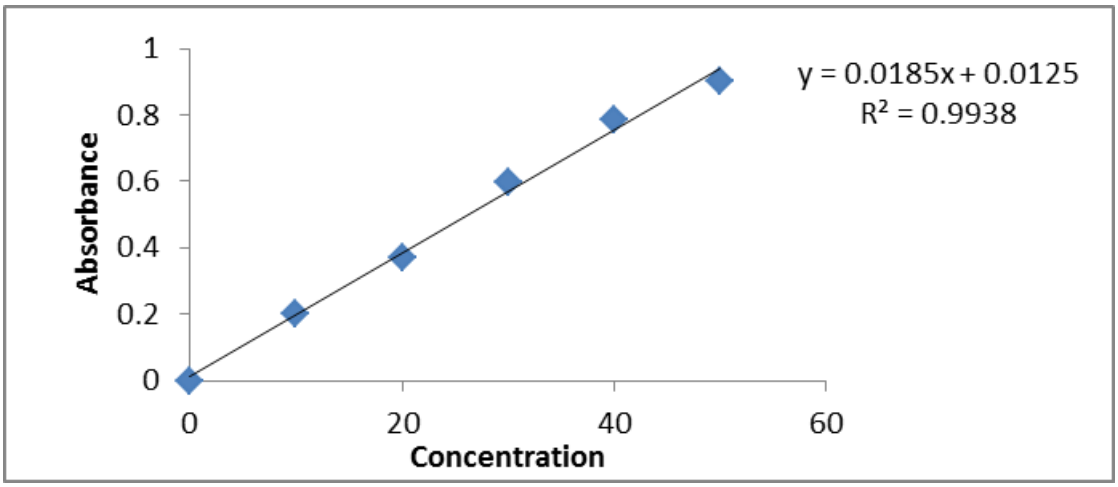


Fig. 5.15: Calibration curve of Serratiopeptidase in phosphate buffer pH 6.8.

5.1.4.6 Differential scanning calorimetry (DSC).

DSC thermogram of Serratiopeptidase showed melting endothermic peak at 170.35°C which complies with melting point of Serratiopeptidase.

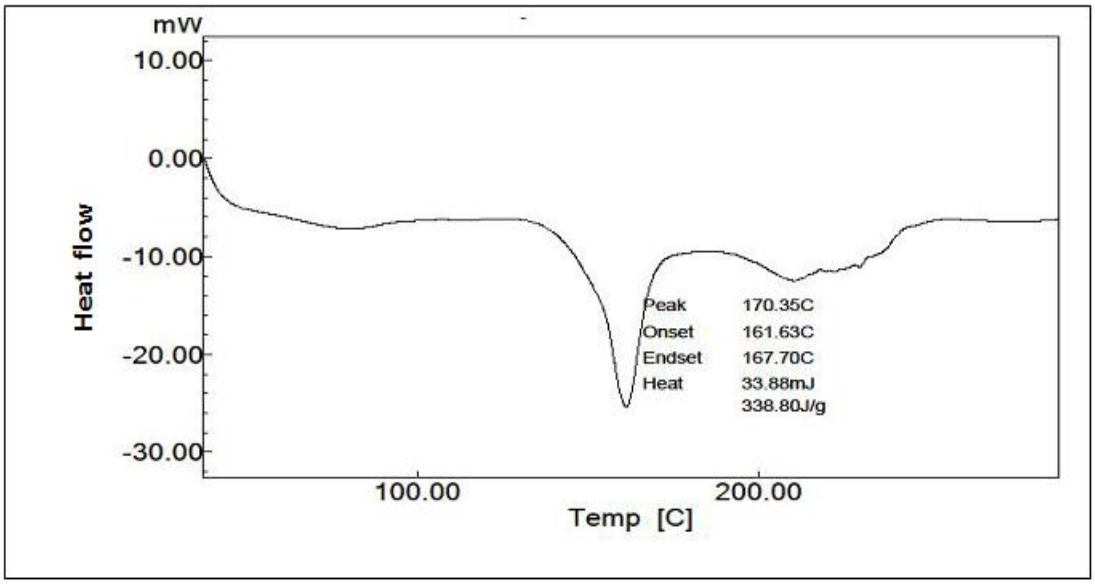


Fig. 5.16: DSC thermogram of pure serratiopeptidase.

5.1.5 Study of physical interaction between drugs by Fourier Transform Infrared Spectroscopy (FTIR) study.

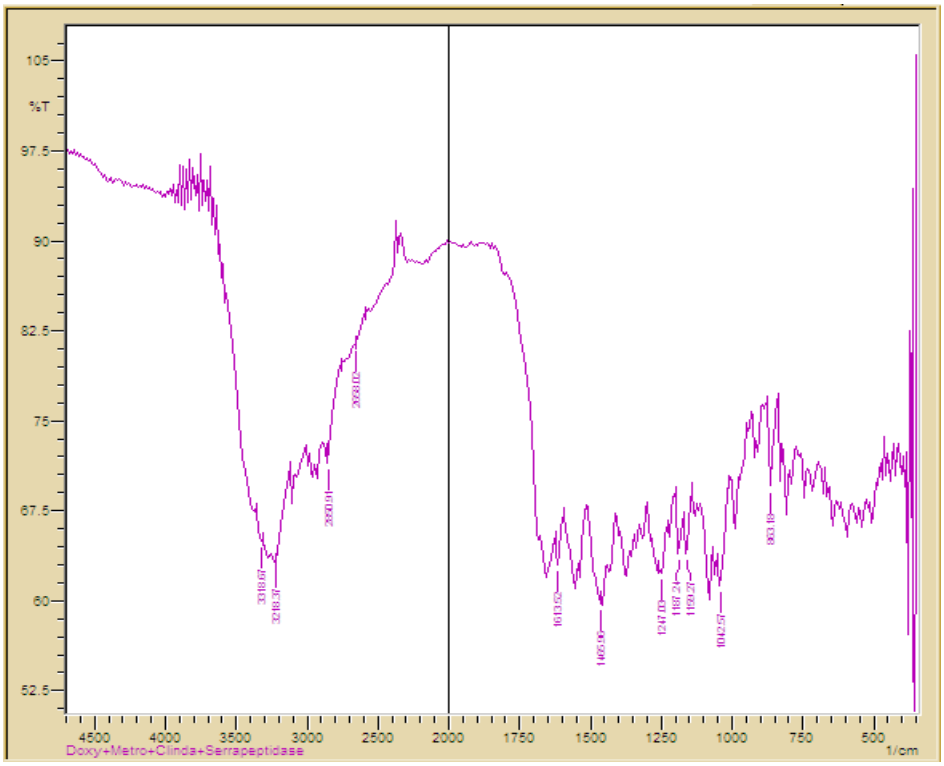


Fig. 5.17: FTIR Spectrum of physical mixture of four drugs (metronidazole, clindamycin, doxycycline and serratiopeptidase).

Table 5.13: Details of FTIR study physical mixture of Metronidazole, clindamycin hydrochloride, doxycycline hyclate and serratiopeptidase.

Sr. no.	Reported Frequencies (cm^{-1})	Observed frequencies (cm^{-1})	Assignment
1	2850-3000	2850.91	C-H alkane stretch
2	1350-1480	1465.96	-C-H alkane bending
3	1080-1360	1159.27, 1187.24, 1247.03	C-N amine stretch
4	1550-1640	1613.52	N-H amide bending
5	3200-3600	3218.37, 3318.67	O-H alcohol stretch

The infrared spectra of Metronidazole, Clindamycin hydrochloride, Doxycycline hyclate and Serratiopeptidase shows a characteristic peak of Metronidazole at 1465.96 cm^{-1} corresponding to the -C-H bending, at 1159.27 cm^{-1} corresponding to C-N stretching.

The characteristic peak of Doxycycline hyclate showed at 1187.24cm^{-1} corresponding to the C-N, at 1613.52 cm^{-1} corresponding to the N-H bending.

The characteristic peak of Clindamycin hydrochloride showed at 1247.03 cm^{-1} corresponding to the C-N stretching.

The characteristic peak of Serratiopeptidase at 2850.91 cm^{-1} corresponding to the C-H stretching and at 3218.37, 3318.67 cm^{-1} corresponding to O-H stretching.

By comparing the peaks of drug observed in drug spectrum, it was observed that no significant changes occurred in peak of Metronidazole, Clindamycin hydrochloride, Doxycycline hyclate and Serratiopeptidase. Hence it was concluded that all four drugs was compatible with each other.

5.1.6 Study of physical interaction between drugs by Differential Scanning Colorimetry (DSC) study

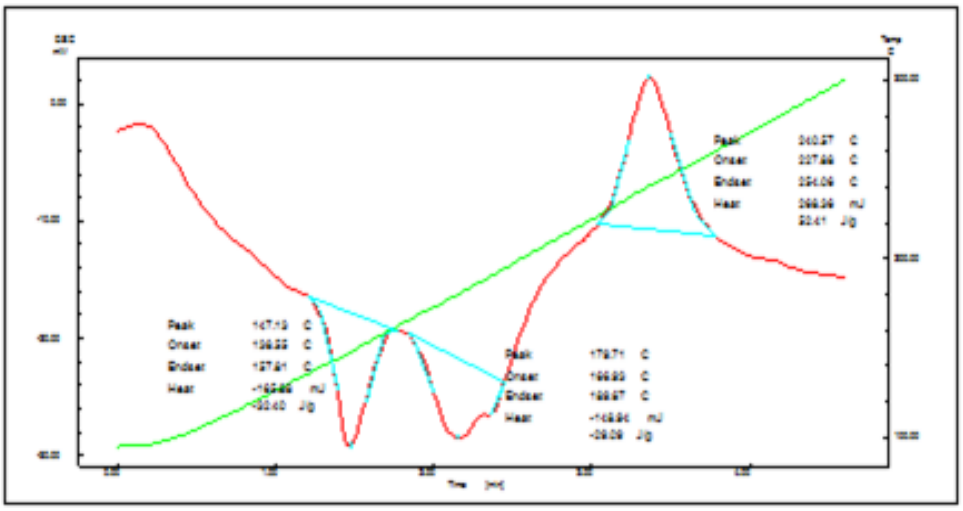


Fig. 5.18: DSC Thermogram of Physical interaction between drugs.

The physical mixture composed of Metronidazole, Clindamycin hydrochloride, Doxycycline hyclate and Serratiopeptidase, showed an endothermic peak of Clindamycin hydrochloride at 147.13^{0}C, exothermic peak of Doxycycline hyclate appeared at 240.57^{0}C, and endothermic peak of Serratiopeptidase appeared at 179.71^{0}C , s it may assume that all drugs would be melt and dissolve with each other when temperature was increase.

5.2 Formulation of gel

The gels were prepared by using Carbopol 940, Chitosan, PEG, PG, IPA, methyl paraben and combination of four drugs i.e. Metronidazole, Clindamycin hydrochloride, Doxycycline hyclate and Serratiopeptidase. The prepared gels were characterized for various physiochemical parameters such as physical appearance, pH, viscosity, drug content, syringeability, In vitro release and Ex vivo drug permeation study.

5.3 Evaluation of prepared gel

5.3.1. Physical appearance

All the prepared gelling systems ware evaluated for physical appearance and all the formulations were found to be transparent and clear.

Table 5.14: Physical appearance.

Sr. no.	Formulation code	Appearance	Clarity
1	F1	Transparent	Clear
2	F2	Transparent	Clear
3	F3	Transparent	Clear
4	F4	Transparent	Clear
5	F5	Transparent	Clear
6	F6	Transparent	Clear

5.3.2. Determination of pH

The pH value for the formulations were measured using digital pH meter shown in table 6 and found to be in the range of 6.13 ± 0.040 to 7.41 ± 0.005. The observation revealed that all the formulations were near to neutral pH.

Table 5.15: Determination of pH

Sr. no.	Formulation code	pH
1	F1	7.0
2	F2	6.8
3	F3	6.7
4	F4	6.4
5	F5	6.8
6	F6	6.9

5.3.3. Determination of viscosity

Viscosity is an expression of the resistance of a fluid to flow. Viscosity is an important parameter for Gel to be evaluated because this parameter is applicable to mixing of drug in a bulk of formulation and flow of material.

Table 5.16: Determination of viscosity.

Formulation	Viscosity (Spindle no. 64)			
	50(rpm)		100(rpm)	
	CP	%	CP	%
F1	11590	96.6	5982	99.7
F2	11600	99.2	5904	98.4
F3	11520	93.5	5934	98.9
F4	11030	91.9	5898	98.3

F5	11390	94.9	5989	98.5
F6	11710	99.7	5996	99.6

Viscosity of gels was determined by using Brookfield Viscometer. The viscosity values in dyne/cm^2 are shown in Table. No 6.16. The values were found to be 11590, 11400, 11520, 11030, 11390 and 11710 dynes/cm2 at 50 rpm and 5982, 5904, 5934, 5898, 5989, and 5996 dynes/cm2 at 100 rpm for formulations F1 to F6 respectively.

5.3.4. Drug content in gel

Table 5.17: Drug content of gel.

Formulation code	Drug	Drug content
F1	Metronidazole	44.44%
	Clindamycin	40.25%
	Doxycycline	57.71%
	Serratiopeptidase	90.55%
F2	Metronidazole	48.90%
	Clindamycin	46.51%
	Doxycycline	70.42%
	Serratiopeptidase	93.22%
F3	Metronidazole	46.26%
	Clindamycin	42.1%
	Doxycycline	68.28%
	Serratiopeptidase	91.55%
F4	Metronidazole	47.47%
	Clindamycin	40.28%
	Doxycycline	60.71%
	Serratiopeptidase	90.33%
F5	Metronidazole	45.61%
	Clindamycin	42.87%
	Doxycycline	67.57%
	Serratiopeptidase	86.66%
F6	Metronidazole	44.32%
	Clindamycin	41.97%
	Doxycycline	69.00%
	Serratiopeptidase	91.88%

Drug content uniformity in the drug delivery system is an important aspect that determines the performance of the system in vivo conditions. If the drug is not distributed uniformly throughout the formulation it could either lead to availability of subtherapeutic dose or toxic dose. Drug content uniformity was also performed to ensure minimum batch to batch variations. Table no. 6.17 show the values of drug content of formulated gels which were analyzed spectrophotometrically using Phosphate buffer 6.8. All the formulations exhibited

fairly uniform drug content. This is because of easy and single step preparation i.e. addition of drug to the polymer solution accounted for minimal or no drug loss.

5.3.5 Syringeability study

All the formulation easily and uniformly passed through needle no-22.

5.3.6 In-Vitro drug release

To determine the in vitro release of the chosen drugs, Metronidazole was alone evaluated as single drug for its in vitro release.

Table 5.18: In vitro release of metronidazole.

Sr no.	Time (hr)	Formulations					
		F1	F2	F3	F4	F5	F6
1	2	3.30%	8.95%	33.47%	33.41%	46.77%	4.99%
2	4	33.4%	11.58%	73.92%	71.98%	81.37%	6.54%
3	20	41.55%	21.92%	76.85%			8.49%
4	22	69.22%	23.08%	88.30%			10.82%
5	44		32.04%				11.50%
6	46		32.65%				18.38%
7	68		34.25%				19.73%
8	70		35.40%				20.58%
9	92		42.58%				23.64%
10	94		45.62%				24.35%
11	96		58.12%				

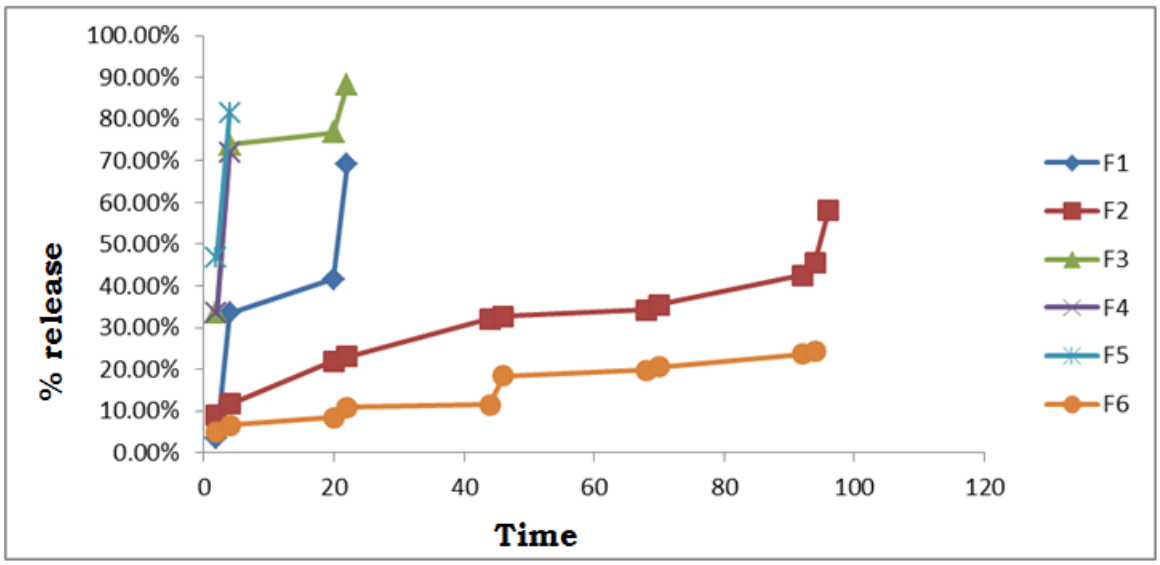


Fig 5.19: In vitro drug release profile of gel formulation containing metronidazole.

The release profile for the formulation F1-F6 (containing 0.1% to 1% of Chitosan) shows maximum sustained release in formulation F2.

Amongst the prepared formulation maximum sustained release was found in formulation F2 containing O.2% Chitosan. Formulation F2 showed maximum sustained release of 58.12% in 96 hr.

Table 5.19: In-vitro release of F2 formulation containing 0.2% Chitosan.

Sr no.	Time (hr)	% Release			
		Metronidazole	Clindamycin	Doxycycline	Serratiopeptidase
1	2	3.27%	4.25%	2.70%	2.46%
2	4	14.72%	6.76%	4.14%	4.99%
3	20	16.67%	7.10%	5.73%	5.70%
4	22	30.46%	11.69%	10.92%	8.84%
5	44	30.12%	12.02%	12.49%	9.45%
6	46	33.06%	23.67%	20.08%	18.35%
7	68	32.81%	23.10%	23.17%	20.81%
8	70	30.62%	31.59%	26.77%	24.62%
9	92	32.77%	32.86%	28.50%	26.76%

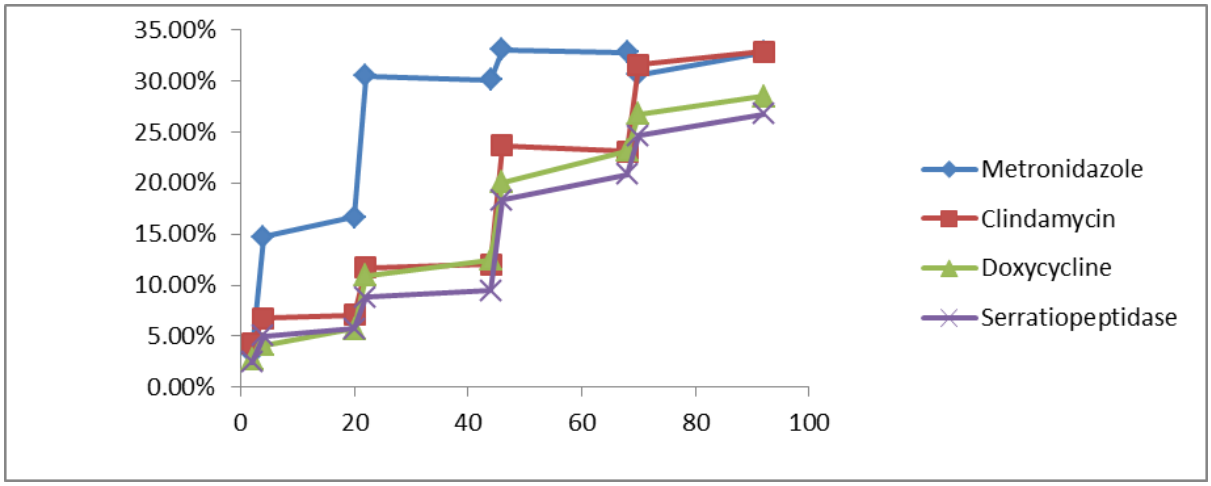


Fig. 5.20: In vitro drug release profile of gel formulation Containing 0.2% of Chitosan.

The in vitro drug release profile for the formulation F2 (containing 0.2% of Chitosan) shows maximum sustained release. Formulation F2 showed maximum release of Metronidazole 32.81% in 68 hr, Clindamycin 32.86%, Doxycycline 28.50% and Serratiopeptidase 26.76% all in 92 hr.

5.3.7 Ex-Vivo drug permeation study

Table 5.20: Ex vivo drug permeation study.

Sr no.	Time (hr)	% Release			
		Metronidazole	Clindamycin	Doxycycline	Serratiopeptidase
1	2	5.30%	1.14%	3.40%	3.6%
2	4	6.78%	1.26%	9.79%	9.10%
3	20	15.35%	2.92%	11.16%	12.60%
4	22	25.98%	4.17%	15.56%	16.14%
5	44	27.27%	15.96%	17.82%	17.12%
6	46	29.71%	20.63%	20.52%	20.02%
7	68	31.73%	32.05%	29.35%	26.69%

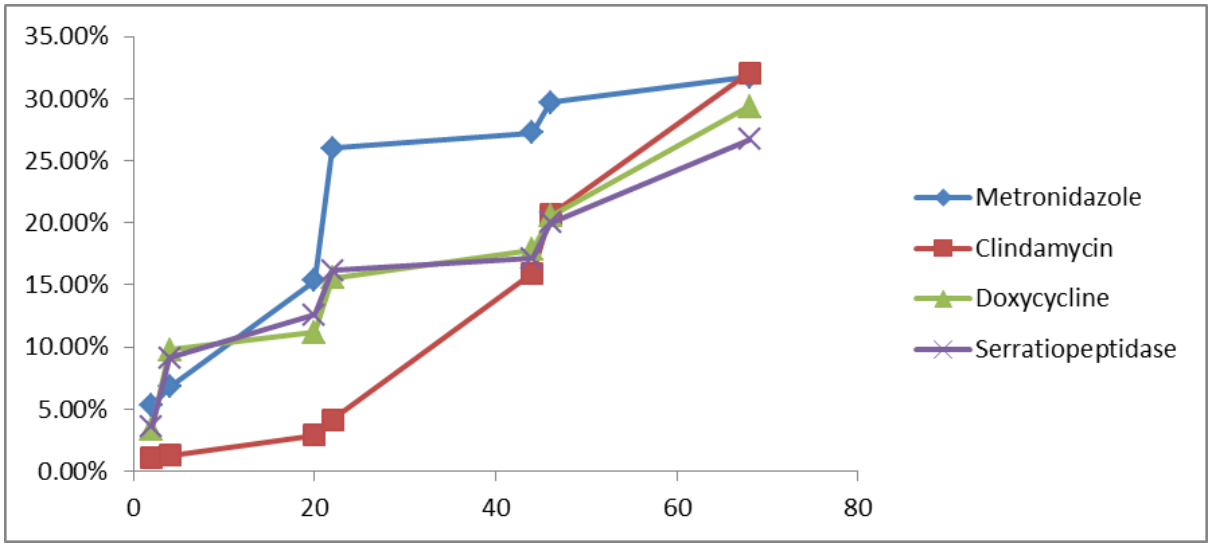


Fig 5.21: Ex vivo drug permeation study of gel formulation Containing 0.2% of Chitosan.

The Ex vivo drug permeation study for the formulation F2 (containing 0.2% of Chitosan) showed maximum sustained release action. Formulation F2 showed maximum release of Metronidazole 31.73% in 68 hr, Clindamycin 32.05% in 68 hr, Doxycycline 29.35% in 68 hr and Serratiopeptidase 26.69% in 68 hr.

5.3.8 Stability studies

Stability studies were carried out on gel formulation according to ICH guidelines. A sufficient quantity of gel in glass bottles was stored in stability chamber at 25°C/60%RH±5% and 45°C/65%RH±5% and samples were withdrawn at 0 and 30 days. The results indicated that there was no any significant change in physical appearance, pH and viscosity. The formulation was found to be stable with respect to its physical appearance, pH and viscosity.

Table 5.21: Stability study of optimized gel.

Sr. no.	Parameters	Initial		After 30 days	
1	Appearance	Transparent and clear		Transparent and clear	
2	pH	6.8		6.7	
3	Viscosity	50(rpm)	100(rpm)	50(rpm)	100(rpm)
		11600	5904	11559	5726

6. SUMMARY AND CONCLUSION

Periodontal gel formulations are currently a novel idea of delivering drugs to patients, yet achieve sustained release of drug for the desired period. Different delivery systems based on polymers have been developed, which are able to increase the residence time of the formulation at absorption site of drugs.

The most important approach for periodontal treatment is the control of inflammation. Pain management has always been an important part of dental care. Therefore, the main categories of drugs to be used in such conditions are analgesics and anti-inflammatory agents. Also from the point of view that periodontal diseases have their origin in some form of infection. So it is worthwhile to use one of the antibiotics also.

Considering the gelling property of Carbopol 940 is used as a gelling agent, an attempt was made to develop gel system for dental use. Chitosan was incorporated for sustained release action. Metronidazole, Clindamycin, Doxycycline and Serratiopeptidase, as antibiotics and anti-inflammatory agents were the choice of drugs.

Preformulation studies were carried out by determination of meting point, ultraviolet visible Spectrophotometric analysis, FTIR analysis, and DSC analysis. The FTIR study was performed to assess the drug compatibility. The studies revealed that, all drugs were satisfactorily compatible.

Six formulations (F1 to F6) were prepared by varying the concentration of sustained releasing polymer, i.e. Chitosan. The prepared formulations were evaluated for different parameters like pH, appearance, rheological studies, in vitro release, syringeability study, ex vivo drug permeation study.

The pH of all formulations was found to be between 6.7 to 7.0 and drug content were metronidazole-48.90%, Clindamycin-46.51%, Doxycyline-70.42% and Serratiopeptidase-

93.22% in optimized gel. In syringeability study all the formulations easily and uniformly passed through needle no-22.

The in vitro release data indicated that F2 showed maximum sustained release amongst all the formulations. The results of drug release indicate that F2 formulation (containing 0.2% Chitosan) exhibited maximum sustained release of the drugs over 92 hours.

The Ex vivo drug permeation study for the formulation F2 (containing 0.2% of Chitosan) showed maximum sustained release. Formulation F2 showed maximum release of Metronidazole 31.73%, Clindamycin 32.05%, Doxycycline 29.35% and Serratiopeptidase 26.69% in 68 hr.

Hence from the above results we can conclude that it is possible to formulate periodontal gel by using combination of Metronidazole, Clindamycin, Doxycycline and Serratiopeptidase.

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