CHAPTER 1

Introduction

1.1 INTRODUCTION

Human kind's effort to confront disease dates back to early civilization. Substances taken from nature were tested and used to treat dysfunctions of physiological life processes, pain and discomfort. With the advancement of science, the active ingredients of these materials, the drugs, were identified, isolated and in many cases their mechanism of action are elucidated. New drug candidates are tested even today in the quest to add increasingly effective tools against diseases.

Drug characteristics differ dramatically, even those aimed to treat the same symptoms; chemical composition, size, hydrophilicity and potency identify molecules whose function may be specific or highly complex. Drug activity, a result of molecular interaction(s) in certain cells, is therefore easily induced which is necessary for the drug to reach at the site of action following administration (oral, intravenous, local, transdermal, etc.) at sufficient concentrations. The scientific field dealing with this issue is known as drug delivery and essentially with the aim to deliver the drug at the right place, at the right concentration for the right period of time. When this is impossible by simply selecting an appropriate administration route, or when such administration causes patient discomfort, strategies based on the association of the drug with a carrier (a drug delivery system – DDS) are an alternative (Ferrari 2005, Allen et al., 2004). Additional motivations for such approaches include the reduction of required resources for therapy, accomplished by an increase of the drug's therapeutic index and the prevention of frequent, unpleasant or expensive treatments. Drug delivery systems, ranging from implantable electronic devices to single polymer chains, are required to be compatible with processes in the body (biocompatibility) as well as with the drug to be delivered. The drug delivery system mainly alters the bio-distribution and pharmacokinetics of the associated drug i.e. the time-dependent percentage of the administered dose in different organs of the body. Furthermore, obstacles arising from low drug solubility, degradation (environmental or enzymatic), fast clearance rates, non-specific toxicity, inability to cross biological barriers, etc. may be addressed by various suitable drug delivery systems (Allen et al., 2004).

Overall, the challenge of increasing the therapeutic effect of drugs, with a concurrent minimization of side effects, can be tackled through proper design and engineering of the novel drug delivery system, in a case-to-case manner (Ferrari 2005, Kostarelos 2003).

Periodontal disease is a collective term for a number of pathological conditions characterized by inflammation and degeneration of the gums (gingiva), supporting bone (alveolar bone), periodontal ligament and cementum. Periodontitis is an inflammation of the supporting tissue surrounding teeth caused by anaerobic bacteria and in the diseased state, supporting collagen of the periodontium is destroyed and the alveolar bones begin to resorb. The epithelium of the gingiva migrates along the tooth surface forming "periodontal pockets" that provide an ideal environment for the growth and proliferation of microbes (Slots, 1979). This pocket can extend from 4 mm to 12 mm and can harbor, depending on its depth and extent, from 10^7 to almost 10^9 bacterial cells (Socransky et al., 1991). More severe stages of the disease lead to the loosening and ultimately loss of teeth. The importance of bacteria in the etiology of periodontitis has been clearly established and the treatment is directed towards controlling the bacterial flora in the periodontal pocket (Baker et al., 1985).

A figure representing the healthy gum and diseased gum is given in figure 1.01. As periodontal disease is associated with bacteria, treatment by antibiotics which are specifically active against gram negative or gram positive or both appears to be appropriate. However, the systemic route of antibiotic administration may not be ideal because of the concern over the development of bacterial resistance that may be induced over long periods of time (Slots et al., 1996, Loesche, 1996). Systemic antibiotic therapy over a long period of time also raises the risk of undesirable side effects such as nausea, diarrhea, fever, abdominal pain and pseudo-membranous colitis. The local delivery of antibiotic therapy to periodontal pockets has the benefit of putting more drugs at the target site while minimizing exposure of the total body to the drug (Rams et al., 1996). Local delivery of antibiotics by sustained release delivery systems has been an active area of pharmaceutical development and clinical research since the early pioneering papers of Goodson (Goodson et al., 1979) and Lindhe (Lindhe et al., 1979). The attractiveness of treating periodontal disease by the sustained release of antibiotics in the periodontal pocket is based on the prospects of maintaining effective high levels of drug in the gingival crevicular fluid (GCF) for a sustained period of time to produce the desirable clinical benefits of attachment level gain, pocket depth reduction and bleeding on probing reduction.

Minocycline Hydrochloride, a member of the tetracycline class of antibiotics, has a broad spectrum of activity with bacteriostatic nature, which exerts anti-microbial activity by inhibiting protein synthesis (Stratton, 2005). In vitro susceptibility testing has shown that the organisms *porphyromonas gingivalis, prevotella intermedia, fusobacterium nucleatum, eikenella corrodens* and *actinobacillus actinomycetemcomitans,* which are associated with periodontal disease, are susceptible to minocycline hydrochloride at a concentration of ≤ 8 μg/ml (Slots et al., 1990).

Clindamycin Phosphate, a semi-synthetic antibiotic, belonging to the group of Lincosamides can be produced by 7(S)-chloro-substitution of the 7(R)-hydroxyl group of the parent antibiotic Lincomycin. It has a broad spectrum of activity with bacteriostatic nature and exerts anti-microbial activity by inhibiting protein synthesis (Stratton, 2005).

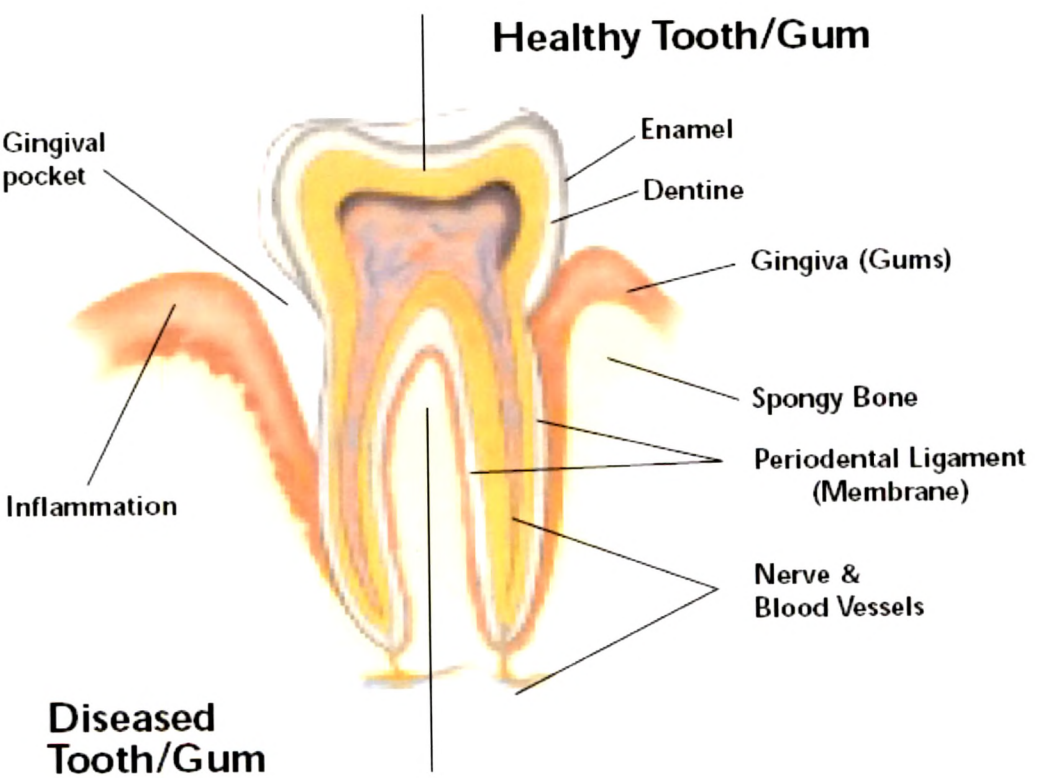

Figure 1.01: Represents the healthy gum versus diseased gum

Minocycline hydrochloride is available in oral dosages forms such as capsules, powder, extended release, gel, etc. Likewise clindamycin phosphate is available in capsule dosage forms for the treatment of periodontal infectious diseases. Although oral administration of a drug is considered as most convenient, this dosage form possesses particular problems when administering antibiotics to patients suffering from periodontal infectious diseases. Because of the chronic nature of the periodontal pathogens, treatment options that rapidly and effectively minimize the pathogenesis of the periodontal cavity are beneficial.

Hence a means of directly inserting the drug locally into the periodontal cavity would be appropriate for the administration of antibiotics. The intra periodontal route may be a viable alternative to overcome some of the limitations. Intra periodontal administration appears to be an attractive alternative by avoiding gastro-intestinal degradation, the hepatic first pass effect and allowing convenient and simple administration along a direct route to the periodontal cavity. However, problems associated with periodontal delivery posses lower retention time of the formulation in the periodontal cavity resulting in lower bioavailability of the medicament due to the movement of the oral cavity. Hence to meet the various drawbacks of other delivery systems and facilitate the effective therapy for the treatment of periodontal diseases, there exists the need for the development of intra periodontal drug delivery systems that would increase residential time in the periodontal cavity associated with increased drug absorption thereby improved bioavailability. Very little research has been done on the development of intra periodontal formulation of antibiotics, and hardly any report is available pertaining to increasing residence time of minocycline hydrochloride and clindamycin phosphate in the periodontal cavity and thereby enhancing its absorption in the periodontal cavity.

The major factor limiting the bioavailability of the periodontal administration of antibiotics is low residence time and rapid elimination of the formulation from the periodontal cavity. To overcome these problems and to facilitate periodontal absorption of drugs; two main approaches have been used; by using a non-biodegradable drug carrier system and by using a bio-adhesive system, in liquid formulation, in powders, and in matrixes that increases the mucoadhesive property. Over the past few decades, the use of mucoadhesive polymers as a nontoxic alternative to enhance the delivery system has been investigated. Mucoadhesive drug delivery systems improve drug absorption by various mechanisms (Hochman et al., 1994). However, mucoadhesive drug delivery systems

absorb water from their site of deposition, leading to swelling and adhesion onto the mucus thereby swing down the mucociliary clearance. Mucoadhesion also causes a transient widening of epithelial tight junctions (Hochman et al., 1994) and thus increases residential time within the periodontal cavity resulting in improved drug absorption.

Mucoadhesive polymers have been used to improve the bioavailability of several drugs. The anionic polymers like; poly acrylic acid, carbomer, polycarbophil, cationic polymers such as; hydroxy propyl methyl cellulose, hydroxy ethyl cellulose, ethyl cellulose, hydroxy propyl cellulose, etc. are very widely used in attempts to formulate mucoadhesive drug delivery systems for application to various mucosal sites. These mucoadhesive polymers facilitate adhesion to the mucosa and increases the retention time of the formulation in the mucosa resulting in enhanced absorption of the active ingredients through the membrane and hence increases the bioavailability of the drug as compared to drug delivery systems administered without mucoadhesive polymers.

Viscous solutions are also reported to increase residential time in the periodontal cavity. Therefore application of in-situ gelling solutions of low molecular weight tri-block co-polymers of poly (ethylene oxide) and poly (propylene oxide) exhibiting thermoreversible properties have been proposed which remain liquid below the body temperature and converts to gel at body temperature. By modulating the gelation temperature of different pluronic F127 solutions, liquid bases for the periodontal formulation which form a gel in the periodontal cavity at body temperature with suitable gel strength resulting in enhancement of the residential time in the periodontal cavity may be formulated. Thermoreversible gels, widely used in various routes such as rectal, vaginal, ophthalmic have been evaluated (Gariepy at al., 2004). Poloxamers like pluronic F127 are also reported to exhibit mucoadhesive potential. Furthermore, in order to fortify the adhesion of formulation onto the mucosal surface, mucoadhesive polymers such as polycarbophil, hydroxy-propyl-cellulose and poly vinyl pyrrolidone have been added to the in-situ gelling systems (Chu et al., 1991). Effect of addition of mucoadhesive polymer on rheological behavior, mucoadhesive strength, gel strength, syringeability and in vitro permeation across mucosal membrane has not been studied. In the past no attempt has been done to study effect of pluronic F127 or its combination with mucoadhesive polymers on permeation of antibiotics across the mucosal membrane. Moreover, combination of polymeric surfactant pluronic F127 as thermoreversible polymer and

anionic polymer such as polyacrylic acid, polycarbophil, carbopol 934P as mucoadhesive polymer has never been explored as potential periodontal drug delivery systems with numerous advantages.

Mucoadhesive periodontal strip type of dosage forms can be used for localized effect against periodontal diseases (Banga and Chien, 1988). The advantages of such therapy include localization of the formulation in the inflammatory periodontal cavity by reducing the drug dose. Recently, various bio-adhesive mucosal dosage forms including adhesive tablets, gels and strips have been developed (Lee and Park Robinson 2000). However, the mucoadhesive periodontal strips are preferred over adhesive tablets in terms of flexibility and comfort. In addition, they can circumvent the relatively short residence time of oral periodontal gels on the mucosa, which are easily washed and removed by saliva. Moreover mucoadhesive periodontal strips are also suitable for protecting wound surfaces, thus reducing pain and increasing the treatment effectiveness (Peh and Wong, 1999).

Microspheres may be defined as the solid spherical particles ranging from 1-100 μm in size and containing drug dispersed within it either in the solution or in the crystalline form. Mucoadhesive microspheres which are bioadhesive in nature possessing desired release property may also provide benefit for prolong and sustain release effect of antibiotics by increasing the residential time in the periodontal cavity. The ideal particle size for periodontal route of administration is 10-100 μm which promotes them to adhere to the periodontal cavity, consequently increasing the contact time (Wolff et al., 1999, Goodson, 2003).

Liposome is another attractive system for targeted drug delivery due to its composition from natural biological lipids and also structural resemblance to cell membranes which suggests metabolic compatibility. Liposomes are the phospholipid vesicles or microscopic particles composed of a lipid bilayer membrane. Their nature of encapsulating an aqueous phase within the lipid bilayer suggests the possibility of both hydrophilic and lipophilic drugs to be encapsulated and delivered easily. The major advantages of using liposomes as target drug delivery systems are;

- Better therapeutic index
- Biodegradability

- Low toxicity
- Low immunogenicity
- Ability to trap drugs without the necessity of covalent bonding
- Potential for high drug loading capacity
- Ease of preparation in various dosage forms
- Ability of sustained and controlled release of drug

However, the relatively fast size dependant clearance, more tendencies towards the localization in tissues of the mononuclear phagocyte system (MPS) with limited distribution in non-MPS tissues, less stability and scale up difficulties had restricted liposome to be used as a successful drug delivery system in earlier stages of their discovery.

The aim of the present work is to make an attempt to develop and evaluate novel intra periodontal delivery systems containing minocycline hydrochloride and clindamycin phosphate using thermoreversible polymer pluronic F127 and mucoadhesive polymers such as poly acrylic acid, polycarbophil, carbopol 934P, hydroxyl ethyl cellulose and hydroxy propyl methyl cellulose. The formulations were evaluated for their rheological characteristics, mucoadhesive force, gel strength, syringeability and in vitro permeation across sheep cheek mucosal membrane.

Attempts are made in the thesis to develop and evaluate novel mucoadhesive periodontal strip systems containing effective amount of minocycline hydrochloride and clindamycin phosphate along with mucoadhesive polymers HPMC and PVA, which has the property of increasing the residential time in the periodontal cavity and increasing the absorption of minocycline hydrochloride and clindamycin phosphate across the periodontal cavity to the localized target sites. This local delivery of minocycline hydrochloride and clindamycin phosphate will not only result in rapid onset of action but also provides increased residence time in the periodontal cavity. Although there are previous reports, where strips are used for the drug delivery through the oral mucous membranes, investigations has not been carried out for the periodontal delivery of minocycline hydrochloride and clindamycin phosphate for the treatment of infectious periodontal diseases. Hence an attempt is also made to optimize mucoadhesive strip as a means of local delivery system of minocycline hydrochloride and clindamycin phosphate for the treatment of periodontal diseases.

This thesis includes the attempt of development and evaluation of novel microspheres containing required amount of minocycline hydrochloride and clindamycin phosphate using ethyl cellulose as mucoadhesive polymer which has got the inherent property of increasing the residential time. The local delivery of these microspheres containing minocycline hydrochloride and clindamycin phosphate results in rapid onset of action resulted due to increased residence time in the periodontal cavity. Although there are earlier reports of ethyl cellulose microspheres by solvent diffusion method, investigation has been made for the optimization of the process by thin film hydration method for the preparation of mucoadhesive microspheres containing minocycline hydrochloride and clindamycin phosphate.

However, to develop delivery systems containing minocycline hydrochloride and clindamycin phosphate with increased accumulation at the target site and decreased systemic delivery, it was conjectured that the liposomal delivery systems may act as suitable dosage forms. As sustained and targeted delivery not only would minimize interaction of drugs with other tissues of the body thereby reducing side effects commonly associated with this type of therapy but also would lead to significant dose reduction. Thus, overall increase in therapeutic efficacy would be achieved.

1.2 OBJECTIVE OF THE WORK

1. Preparation and characterization of pluronic F127 thermoreversible periodontal gel of minocycline hydrochloride and clindamycin phosphate along with mucoadhesive polymers; poly acrylic acid, polycarbophil, carbopol 934P, hydroxy propyl methyl cellulose and poly vinyl alcohol.
2. Formulation and evaluation of periodontal strips loaded with minocycline hydrochloride and clindamycin phosphate.
3. Formulation, characterization and evaluation of microspheres of minocycline hydrochloride and clindamycin phosphate containing ethyl cellulose as mucoadhesive polymer.
4. Preparation, characterization and evaluation of minocycline hydrochloride and clindamycin phosphate loaded liposomes incorporated within the conventional carbopol 934P gel.
5. In vitro release study of minocycline hydrochloride and clindamycin phosphate and their various periodontal formulations.
6. In vitro permeation evaluation of minocycline hydrochloride and clindamycin phosphate and their formulations.
7. In vitro evaluation of various minocycline hydrochloride and clindamycin phosphate loaded formulations for their antimicrobial efficacy.
8. Ex vivo evaluation of potential of formulations to increase transport of minocycline hydrochloride and clindamycin phosphate across sheep cheek mucosa to target site for improved intra periodontal therapy.
9. In vivo pharmacokinetics of the formulations by using radioisotopes in rabbits.
10. To evaluate potency of the formulations for their wound healing characteristic in rats by dental imaging by gamma scintigraphy.

1.3 REFERENCES

Allen TM and Cullis PR (2004). Drug Delivery Systems. Entering the Mainstream Science, 303, 1818-1822.

Baker PJ, Evans RT, Slots J, Genco RJ (1985). Susceptibility of human oral anaerobic bacteria to antibiotics suitable for topical use. Journal of Clinical Periodontology, 12, 201-204.

Banga AK and Chien YW (1988). Systemic delivery of therapeutic peptides and proteins. Int. J Pharm 48, 15-50.

Chu JS, Amidon GL, Weiner ND, Goldberg AH (1991). Mixture experimental design in the development of a mucoadhesive gel formulation. Pharm. Res., 8, 1401-1407.

Ferrari M (2005). Cancer nanotechnology: opportunities and challenges. Nature Reviews Cancer, 5, 161-171.

Gariépy ER, Leroux JC (2004). In situ-forming hydrogel-review of temperature sensitive systems. Eur. J. Pharmacy and Biopharm., 58, 409-426.

Goodson JM, Haffajee AD, Socransky SS (1979). Periodontal therapy by local delivery of tetracycline. J Clin Periodontol, 6, 83– 92.

Goodson JM (2003). Gingival crevice fluid flow. Periodontology 2000, 31, 43–54.

Hochman J, Artursson P (1994). Mechanism of absorption enhancement and tight junction regulation. J.Control. Rel., 29, 253-267.

Kostarelos K (2003). Rational design and engineering of delivery systems for therapeutics: biomedical exercises in colloid and surface science. Adv. Colloid Interface Sci., 106,147-168.

Lee JW, Park Robinson JH (2000) Bioadhesive based dosage forms: the next generation. J Pharm Science 89, 850-866.

Lindhe J, Heijl L, Goodson JM, Socransky SS (1979). Local tetracycline delivery using hollow fiber devices in periodontal therapy. J Clin Periodontol, 6, 141–149.

Loesche WJ (1996). Antimicrobials in dentistry: with knowledge comes responsibility. J Dent Res, 75, 1432–1433.

Peh KK, Wong CF (1999) Polymeric films as vehicle for buccal delivery: swelling, mechanical, and bioadhesive properties. J Pharm Pharm Sci 2, 53-61.

Rams TE, Slots J (1996). Local delivery of antimicrobial agents in the periodontal pocket. Periodontology 2000, 10, 139–159.

Slots J, Rams TE (1990) Antibiotics in periodontal therapy: advantages and disadvantages. J Clin. Periodontol., 17, 479-493.

Slots J, Pallasch TJ (1996). Dentist's role in halting antimicrobial resistance. J Dent Res, 75, 1338–1341.

Socransky SS, Haffajee AD, Smith C, and Dibart S (1991). Relation of counts of microbial species to clinical status at the sampled site. J. Clin. Periodontol. 18, 766–775.

Stratton CW (2005). Molecular mechanism of action of antimicrobial agent: general principle and mechanism for selected classes of antibiotics. In: Lorian V. (Eds) Antibiotics in Laboratory Medicine, 5th edition, Williams and Wilkins, Baltimore, MD: 532-563.

Wolff MS, Kleingberg I. (1999). The effect of ammonium glycopyrrolate (Robinul)-induced xerostomia on oral mucosal wetness and flow of gingival crevicular fluid in humans. Arch. Oral Biol., 44, 97–102.

1.4 MATERIALS

Acetone- S. D. Fine chemicals, Mumbai, India.

Carbopol 934P- B.F. Goodrich (USA)

Chloroform- Qualigens (India)

Cholesterol - S. D. Fine chemicals, Mumbai, India.

Clindamycin phosphate- Bharat Parenterals (India)

Di-sodium hydrogen phosphate- S. D. Fine chemicals, Mumbai, India.

EDTA (ethylene diamine tetra acetate)- Himedia (India)

Ethyl-cellulose- The Dow Chemical Company, New Milford, USA

Formaldehyde- S. D. Fine chemicals, Mumbai, India.

HSPC (Hydrogenated soya phosphatidyl-choline)- Lipoid GmBH, Ludwigshafen, (Germany)

Hydroxy- propyl- methyl-celulose- S. D. Fine chemicals, Mumbai, India.

Hydroxy-ethyl-celulose- S. D. Fine chemicals, Mumbai, India.

Methanol- S. D. Fine chemicals, Mumbai, India.

Minocycline hydrochloride- Ipca Laboratories (India)

n-octanol- S. D. Fine chemicals, Mumbai, India.

Pluronic F127- Sigma Chemicals, St. Louis MO, U.S.A.

Poly-acrylic Acid - Noveon (USA)

Poly carbophil- Noveon (USA)

Polyethylene glycol 1000- The Dow Chemical Company, New Milford (USA)

Poly-vinyl-alcohol- Loba Chemi Pvt. Ltd (India)

Poly-vinyl-pyrolidone- S.D. Fine chemicals Ltd. (India)

Potassium dihydrogen phosphate- S.D. Fine chemicals Ltd. (India)

Propylene glycol- S. D. Fine chemicals, Mumbai, India.

Span 80 - S. D. Fine chemicals, Mumbai, India.

Sodium chloride- S.D. Fine chemicals Ltd. (India)

Sodium hydroxide- S.D. Fine chemicals Ltd. (India)

Sodium meta-bisulfite- S. D. Fine chemicals, Mumbai, India.