CHAPTER 11

Summary and Conclusion

11.1 SUMMARY

11.1.1 Introduction

There has been increasing attention paid in recent years to the oral/ dental inflammations and oral/ dental infectious diseases, in particular, infectious periodontal diseases. Periodontal disease is one of the world's most prevalent chronic diseases, which results in tooth loss. Periodontal disease has been considered as a possible risk factor in other systemic diseases such as cardiovascular disease, including coronary heart disease and stroke and pre-term low birth weight infants. Periodontal diseases are the localized inflammatory response due to the infection of a periodontal pocket arising from the accumulation of sub-gingival plaque. Untreated periodontitis results in the loss of the supporting structures of the tooth through resorption of alveolar bone and loss of periodontal ligament attachment. Clinically, as the disease progresses, the periodontal pocket, which is somewhat deeper than the sulcus of a healthy tooth, gets deeper with further destruction of the tooth's supporting structures, often resulting in tooth loss. In addition, there are numerous inflammatory mediators of bacterial and host origin that contribute to local periodontal destruction and become part of the pathogenesis of the disease. The current non surgical standard of therapy is scaling and root planning (SRP), a mechanical procedure to remove sub-gingival calculus and plaque. SRP has the advantage of being a localized treatment but does not always eliminate the pathogenic bacteria due to their presence within the periodontal tissues, or in the case of deeper pockets, their inaccessibility to the instrumentation. As the periodontal disease is associated with bacteria, treatment by specifically targeted antibiotic therapy 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 longer time periods. Systemic antibiotic therapy over a longer time period also raises the risk of undesirable side effects such as nausea, diarrhea, fever, abdominal pain and pseudo-membranous colitis. The local delivery of antibiotics therapy to periodontal pockets has the benefit of administering more drugs at the target site while minimizing exposure of the total body to the drug. The lack of drug retention in the periodontal pocket is probably the major reason for these mixed results. Local delivery of antibiotics therapy by sustained release delivery systems has been an active area of pharmaceutical development and clinical

research. 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. In addition, a local delivery device should have a high patient acceptance and a method of application acceptable to the periodontist's practice. Current research is aimed at the development of drug delivery system with maximum therapeutic benefit for safe and effective management of the diseases.

The aim of the present study was to develop targeted local delivery system for antibiotics therapy. Viscous solutions are also reported to increase residence time of drug in the periodontal cavity. Therefore application of in-situ gelling solutions of low molecular weight triblock copolymers 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, to obtain suitable gel strength and mucoadhesive property resulting in enhancement of the residential time in the periodontal cavity were obtained.

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 increases the treatment effectiveness.

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.

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 present investigation involves the preparation, characterization and in vivo evaluation of thermoreversible periodontal gel, mucoadhesive periodontal strips, microspheres and liposomal gel containing minocycline hydrochloride and clindamycin phosphate.

11.1.2 Preparation of Thermoreversible Periodontal Gel Containing Minocycline Hydrochloride/ Clindamycin Phosphate

Thermoreversible periodontal gels containing pluronic F127 and mucoadhesive polymers containing minocycline hydrochloride (1%)/ clindamycin phosphate (1%) were prepared by adopting the cold method technique. Individual drug was added to half the volume of water to be taken and maintained at 10°C in a beaker until a clear solution was obtained followed by addition of PEG 1000. In case of minocycline hydrochloride formulations sodium meta-bisulphite was added as an antioxidant. After formation of a clear solution, weighed amount of mucoadhesive polymer (Polycarbophil, HPMC, HEC, PVP, Carbopol 934P, PVA, and Polyacrylic acid), was added and dispersed prior to the addition of pluronic F127 and kept overnight at 4°C until a clear transparent solution was obtained. The pH of all the formulations was adjusted to 5.5-6.5 using 0.5% NaOH. Weight was adjusted with distilled water.

11.1.3 Preparation of Mucoadhesive Periodontal Strips Containing Minocycline Hydrochloride/ Clindamycin Phosphate

The plain placebo strips were first prepared by the coating technique, using 5.0, 7.5, 10.0% w/v PVA and 1.0, 1.25, 1.50 % w/v HPMC. In all cases, 2.5% (w/v) propylene glycol was added as plasticizer. Adequate amount of PVA/ HPMC was dissolved in warm water at 40°C to 50°C, and then propylene glycol was added under stirring after cooling and the final

volume was adjusted with distilled water. The viscosity of the prepared gels was measured. The gels were left overnight at room temperature till clear; bubble free gels were obtained and then coated on a 30 mm polyester strip and allowed to dry in an oven maintained at 40°C till complete drying.

The drug loaded periodontal mucoadhesive strips were prepared using the method mentioned as above. The drug solution was added to the polymer solution containing propylene glycol with continuous stirring. After formation of a clear gel the volume was adjusted with distilled water. The dried periodontal strips were visually observed for any imperfections or air bubbles. The drug coated periodontal strips were cut into strips of $1cm^2$ diameter, so that each strip contains 2 mg of the drug. To improve the elasticity, film forming property and mucoadhesive property of the periodontal strips, carbopol 934P was added. Carbopol 934P was first dissolved in a little quantity of whole amount of distilled water, and then added to the polymeric solution and the periodontal strips were prepared as described above.

11.1.4 Preparation of Periodontal Microspheres Containing Minocycline Hydrochloride/ Clindamycin Phosphate

Periodontal microspheres were prepared by multiple emulsion solvent diffusion method. In this method, the organic internal phase contains designed amount of ethyl cellulose (2.50 % w/v, 5.00 % w/v, 7.50 % w/v, 10.00 % w/v, 12.50 % w/v, 15.00 % w/v) in 20 ml dichloromethane. To the organic phase 5 ml aqueous solution of minocycline hydrochloride/ clindamycin phosphate (10.00 % w/v) was emulsified using span 80 as the emulsifying agent to prepare the primary emulsion. The primary emulsion was gradually added drop wise into a glass beaker containing 500 ml saturated drug solution in distilled water containing 1.00% (w/v) of poly vinyl alcohol (PVA) as emulsifying agent. The mixture was stirred at 500 rpm for 12 h, at room temperature to remove dichloromethane from the beaker. The formed microspheres were separated by centrifugation and washed with distilled water till it completely removed the excess PVA and unentrapped minocycline hydrochloride/ clindamycin phosphate. The obtained microspheres were lyophilized to obtain the final product.

11.1.5 Preparation of Mucoadhesive Liposomal Periodontal Gel Containing Minocycline Hydrochloride/ Clindamycin Phosphate

Unilamellar vesicles (ULVs) of minocycline hydrochloride/ clindamycin phosphate were prepared by the thin lipid film hydration technique. Briefly, required amount of soya phosphatidyl choline (soya PC), suitable amount of cholesterol (varying molar ratio of 1:0.2, 1:0.4, 1:0.6) were dissolved in a mixture of chloroform and methanol (ratio 2:1 by volume) in a 250ml round bottom flask. The flask was rotated in the rotary flask evaporator at 120 ± 10 rpm for required time period in a thermostatically controlled water bath at 45°C under vacuum (600mm of mercury). The thin dry lipid film formed was hydrated using suitable amount of distilled water in which the required quantity of the drug (100mg) was dissolved to meet the required strength and the flask was rotated once again for complete hydration of the prepared thin film liposomes.

Minocycline hydrochloride/ clindamycin phosphate loaded liposome were loaded to the gel formulation. Carbopol 934P was used as the gel forming polymer. 0.5% Carbopol 934P was soaked in the distilled water for 1 hour. After hydration of the polymer, the dispersion was neutralized using 0.5 % w/v sodium hydroxide. To the gel formulation calculated amount of the drug loaded liposomal suspension was added to get 1% w/w liposomal periodontal gel formulation.

11.1.6 Characterization of the Thermoreversible Gels

Gelation temperature was evaluated by visual inspection and by rheological method using a thermostatically controlled Brookfield Programmable Rheometer (Brookfield LVDV III+) fitted with CP-52 spindle. The gelling temperature was determined graphically as the inflection point on the curve of the apparent viscosity (mPas) as a function of the temperature (°C). Each preparation was tested trice to control the repeatability.

Viscosity of plain pluronic F127 gel and gels containing different concentration of mucoadhesive polymer were measured using the Brookfield's LVDV III+ model. The viscosity at various temperatures was recorded using CP-52 spindle.

Gel strength of the formulations was measured using the Universal Testing Machine (UTM), with slight modification. Gel hardness was measured at room temperature using a 6 mm diameter probe, on the UTM (Model, LF Plus, Lloyd Instruments, U.K.). The compression test was done with a 150 N weight beam, utilized with a cross head and chart speed of 50 mm/min and 20 mm/min respectively, with a recovery period of 60s between the end of the first compression and start of the second compression.

Mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from the oral mucosal tissue. A section of mucosal tissue, cut from the cheek mucous membrane of sheep, was washed thoroughly with distilled water followed by isotonic phosphate buffer pH 6.75 and kept in PBS pH 6.75 to remove all soluble components. It was secured with mucosal side out on to a glass plate using cyanoacrylate adhesives. The surface area of each exposed mucosal membrane was kept 2.0 cm^2. About 200 mg of gel formulations were added onto the mucosa and spread uniformly. The test was conducted at room temperature using a 6mm diameter probe, on the UTM (Model LF plus, Lloyd Instruments, U.K.). The probe was brought into contact with the mucosal surface. A preload of 20 g was placed over the gel surface for 2 min as initial pressure, and then the probe was moved upward at a speed of 3.00 mm/min until the probe is detached from the mucosal membrane.

Syringeability of the formulations was measured using the Universal Testing Machine (Model, LF Plus, Lloyd Instruments, U.K.) at room temperature using a 6 mm diameter probe, by filling the sample in a syringe. Before filling the syringe, the opening of the syringe was sealed. Samples were filled in a 3ml glass syringe up to 2ml mark from the back of the syringe and stoppered by the help of forceps. The sample syringe was placed in a holder for holding the syringe. The probe was attached to the load shell of the UTM. The compression test was done with a 150 N weight beam, utilized with a cross head and chart speed of 2.66 mm/min up to 40 mm.

The optimized thermoreversible gel formulations exhibited Newtonian behavior at 4°C, which converted to gel at 37°C. The presence of mucoadhesive polymers lowered the gelation temperature of thermoreversible gels and the extent of lowering was found to be

concentration dependent, which might be partly due to the increased viscosity due to uncoiling of polymer chain on exposure to water leading to polymer swelling and elastic gel formation.

11.1.7 Characterization of the Mucoadhesive Periodontal Strip

Assessment of mass uniformity was done in 10 different randomly selected periodontal mucoadhesive strips from each batch and thickness of the periodontal strips was measured at 10 different randomly selected spots of different periodontal strips using a micrometer (Digimatic micrometer, Mitutoyo, Tokyo, Japan) from each batch. A modification of the ASTM test No-D570-59T was used for testing of moisture absorption/ loss of water from periodontal strips (1.8 × 1.8 cm).

The surface pH was measured by means of pH indicator paper after strips were allowed to swell in an agar plate. A model LVDV-II Brookfield viscometer attached to a helipath spindle no. 4 was used to measure the viscosity at 20 rpm at room temperature.

The folding endurance of the drug loaded mucoadhesive periodontal strips was determined by repeatedly folding one strip at the same place till it broke or folded up to 300 times at the same place without breaking gave the value of the folding endurance

Mechanical properties of the drug loaded mucoadhesive periodontal strips were evaluated using universal testing machine (UTM) equipped with a 10 kg load cell (LF Plus, Lloyd Instruments, UK). Periodontal strips prepared in dimension of 100×50 mm were checked to be free from air bubbles or any physical imperfections. The mucoadhesive strips were held between two clamps positioned at a distance of 5cm. A piece of paper was attached on the surface of the clamp via a double-sided tape to prevent the drug loaded mucoadhesive periodontal strip from being cut by the groove of the clamp. During measurement, the periodontal strips were pulled by the top clamp at a rate of 1.00 mm/s to a distance of 10 cm before returning to the starting point. The force and elongation were measured when the periodontal strip broke. Results from periodontal strip samples, which broke at and not between the clamps were not included in the calculation.

$$\text{Tensile strength } (kgmm^{-2}) = \frac{\text{Force at break (kg)}}{\text{Initial cross sectional area of the sample } (mm^2)}$$

$$\text{Elastic Modulus } (kgmm^{-2}) = \frac{\text{Force at corresponding strain (kg)}}{\text{Cross-sectional area } (mm^2)} \times \frac{1}{\text{Corresponding strain}}$$

$$\text{Elongation at break } (\%\ mm^{-2}) = \frac{\text{Increase in length (mm)}}{\text{Original length (mm)}} \times \frac{100}{\text{Cross-Sectional area } (mm^2)}$$

$$\text{Strain} = \frac{\text{Tensile strength}}{\text{Elastic modulus}}$$

The swelling studies of mucoadhesive periodontal strips were conducted using two media, namely, distilled water and PBS pH 6.75. Each periodontal strip sample (surface area 5 cm^2) was weighed and placed in a pre-weighed stainless steel wire mesh with sieve opening of approximately 500 μm. The mesh containing the periodontal strip samples was then submerged into 25 ml of the medium contained in a glass container. Increase in weight of the periodontal strip was determined at preset time intervals until a constant weight was observed.

The optimized formulations showed good mechanical and mucoadhesive property. Formulations were found to be more flexible and uniform in drug distribution and thickness. Addition of carbopol 934P increases the swelling index of the periodontal strips, which may be due to the rapid uptake of water molecule by the mucoadhesive polymer resulting in increase in the degree of hydration until a point where over hydration leads to an abrupt drop in adhesion strength due to the disentanglement at the polymer/ tissue interface.

11.1.8 Characterization of the Mucoadhesive Periodontal Microspheres

Particle size analysis was performed on minocycline hydrochloride/ clindamycin phosphate loaded periodontal microsphere formulations by Malvern Mastersizer (Malvern Instruments, Mastersizer 2000, U.K.). For morphology and surface characteristics, prepared microspheres

were coated with gold palladium under an argon atmosphere at room temperature and then the surface morphology of the microspheres was studied by scanning electron microscopy (SEM) using a JEOL JXA 840A (USA). The zeta potential, representative of particle charge, was measured by electrophoresis method, using a Malvern Zetasizer nanoZS apparatus. Porosity parameters of periodontal microspheres such as intrusion extrusion isotherms, pore size distribution, total pore surface area, average pore diameters, shape and morphology of the pores, bulk and apparent density were determined by using mercury intrusion porosimetry (AutoPore IV 9500, Micromeritics, USA).

Syringeability of the periodontal microsphere formulations was measured using the Universal Testing Machine (UTM) at room temperature with the 6 mm diameter probe (Model, LF Plus, Lloyd Instruments, U.K) by filling the sample in a syringe.

From the SEM photographs, the drug loaded periodontal microspheres were found to be spherical and uniform throughout with no free drug under visual observation, which enabled their easy injection into periodontal pockets. Porosity of the microspheres, measured by mercury displacement method, indicated that as the drug: polymer ratio increased, the average pore diameter decreased.

11.1.9 Characterization of the Periodontal Liposomes

Morphology and lamellarity of the conventional liposomes were determined using Olympus BX40 microscope and TEM photographs.

The mean particle size of the prepared liposomes was obtained by using Malvern Zetasizer nanoZS (Malvern Instruments, Zeta sizer, Nano Series, Nano ZS, Model No. ZEN 3600).

The entrapped volume of the liposomes was measured after centrifuging at 20000 rpm at 4°C for 30 min. The supernatant fluid was collected and analyzed for their quantification at 246nm and 210 nm respectively for minocycline hydrochloride and clindamycin phosphate against blank using spectrophotometer. The noted drug content was the mean of three readings.

From the above study the optimized mucoadhesive periodontal formulations meant to be administered locally into the periodontal cavity are given in table no. 11.01.

11.1.10 In-Vitro Release, Permeation Studies and Permeation Kinetics Studies of Minocycline Hydrochloride/ Clindamycin Phosphate from Periodontal Formulations

The in vitro release study of gel formulations was performed by using sigma dialysis bag (MWCO 3500 and diameter 2.4 cm), which was filled with 500 mg of formulation. The bags were individually immersed in a beaker containing 25 ml of receiver phosphate buffer solution pH 6.75. The temperature was maintained at 37 ± 1°C and the receptor medium were constantly stirred at 100 rpm to maintain the sink condition. At appropriate time intervals, samples were withdrawn from the receiver solution and an equal volume of pre-warmed buffer was replaced and the samples were assayed spectroscopically after appropriate dilution to quantitate the drug release through the membrane.

In vitro permeation studies were done by using cheek mucosal tissues prepared from fresh sheep cheek mucosal membrane as described earlier and was fixed onto the Franz Diffusion cell. The 500 mg of gel was spread uniformly on to the mucosa previously fixed in between the donor and the receptor compartment of Franz Diffusion cell. The receptor compartment contained phosphate buffer, pH 6.75. The temperature of the elution medium was thermostatically controlled at 37±1°C by a surrounding water jacket and the medium was stirred with a bar magnet at 500 rpm, using a magnetic stirrer. Aliquots withdrawn at predetermined intervals over a predetermined time period. Samples were spectroscopically estimated to quantitate the drug permeated through the membrane.

Table No. 11.01: Composition of various optimized periodontal formulations

Compositions in % w/w	Formulation Code							
	MGF02	CGF54	MSF27	CSF27	MMF01	CMF01	ML14	CL14
	Periodontal Gel		Periodontal Strip		Periodontal Microsphere		Periodontal Liposomal Gel	
MnHCl	1.00	-	1.00	-	1.00	-	1.00	-
$ClPO_4$	-	1.00	-	1.00	-	1.00	-	1.00
Pluronic F127	20.00	20.00	-	-	-	-	-	-
Polycarbophil	0.20	-	-	-	-	-	-	-
Carbopol 934P	-	0.20	0.50	0.50	-	-	0.50	0.50
Sodium metabisulphite	0.50	-	0.50	-	0.50	-	0.50	-
PEG 1000	15.00	15.00	-	-	-	-	-	-
0.5% NaOH	2.00ml	2.00ml	-	-	-	-	2.00ml	2.00ml
Purified water	qs	qs	qs	qs	-	-	qs	qs
PVA	-	-	10.00	10.00	-	-	-	-
Drug: Ethyl cellulose	-	-	-	-	1:1	1:1	-	-
Soya PC: Cholesterol: Drug	-	-	-	-	-	-	1:0.4:0.1	1:0.4:0.1

The steady state permeation flux was determined from the slope of the linear portion of the cumulative amount permeated (Q) versus time (t) plot. The lag time (t_L) was determined by extrapolating the linear portion of Q versus t curve to the abscissa. The permeability coefficient (P) was calculated using the relation derived from fick's first law of diffusion. Diffusion coefficient was calculated using the relation derived from fick's second law of diffusion.

In vitro release and permeation study of periodontal thermoreversible gel formulations showed a sustain release of the drug for a period of 8 hours compared to plain drugs. The highest release of the minocycline hydrochloride occurs from MGF02 (20%w/w pluronic F127 along with 0.2% polycarbophil). Similarly in case of clindamycin phosphate the highest release was found from CGF54 (20 % w/w pluronic F127 along with 0.2 % w/w carbopol 934P). A preliminary study shows that MGF02, and CGF54 possess comparatively least viscosity than other formulations. As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF02, and CGF54, hence possess more available waters to diffuse consequently showing more diffusion through the membrane.

Since the pluronic F127 gels are viscous isotropic liquid crystals containing micelles, it was hypothesized that the drug release follows diffusion through the extra micellar water channels of the gel matrix. Presence of polycarbophil and carbopol 934P results in very rapid dissolution of the drug due to swelling and dissolution of mucoadhesive polymer. However, presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micellar structures. Addition of the mucoadhesive polymer increases the drug permeation compared to the plain pluronic F127 formulations, which may be due to increase in concentrations of ionized carboxyl group to a level required to cause conformational changes in the polymer chain.

Similarly basing on the data obtained from various characterization studies of mucoadhesive periodontal strip formulations the formulations prepared with 10% PVA along with 0.5%w/w carbopol 934P (MSF27 and CSF27) were found to be the best with good mechanical and mucoadhesive property. In vitro release and permeation study showed a sustain release of the drug over a period of 24 hours. The investigation of permeation kinetics data showed that diffusion is the mechanism of drug release which followed higuchi order of release model.
The actual drug content of the optimized periodontal microsphere formulations (MMF01 and CMF01) decreased on increasing the amount of polymer concentration. SEM photographs of the periodontal formulations conformed to be spherical and uniform throughout with no free drug. The study of kinetics parameters resulted in the release of drug following higuchi order of release. In vitro permeation data of drug loaded periodontal microspheres showed that the polymer concentration reversibly controls the lag time, permeability coefficient and diffusion coefficient; this may be because of increase of the path of the drug molecule.

The liposome formulations prepared with various proportions of soya phosphatidyl choline: cholesterol: drug with varying hydration time and volume were optimized basing on particle shape and size, particle size distribution, zeta potential and encapsulation efficiency. The molar ratio of soya phosphatidyl choline: cholesterol: drug was optimized at 1:0.4:0.1 with 1hr of hydration time and 2 ml of hydration volume.

The amount of drug loaded liposome formulation to be incorporated into the conventional carbopol 934P (0.5%) gel was calculated to get 1% w/w. The close examination of the

photomicrographs and TEM of the prepared liposome indicates that the majority of the prepared liposome were spherical and unilamellar in nature.

The in vitro release data of drug loaded periodontal liposomal gel indicated that the increase in the concentration of cholesterol along with increase in hydration volume increases the drug release significantly. An initial burst of release was caused by the presence of free drug at the surface of the periodontal liposomal gel which was followed by a release rate approaching higuchi order. In vitro permeation data of minocycline hydrochloride/ clindamycin phosphate loaded periodontal liposomal gel formulations showed that as the polymer concentration increases, lag time, permeability coefficient and diffusion coefficient decreases, which may be because of the increase of the diffusional path of the drug molecule.

11.1.11 Stability Study of the Prepared Periodontal Formulations

All the optimized batches were subjected to the stability studies. Formulation was stored in tightly closed amber colored vials at room temperature and at 4°C for six months at the conditions according to ICH guidelines i.e. 2-8°C with ambient humidity and 30±2°C/ 60±5% RH.

At predetermined interval of time, samples were removed and studied for various physico-chemical parameters such as drug content, pH, gelling temperature and visual changes for thermoreversible periodontal gels. Similarly drug content, mucoadhesive force, mechanical property, etc. were evaluated for periodontal strips. The characteristics like; drug content and encapsulation efficiency, etc. were determined for periodontal microspheres. For liposomal formulations the actual drug content and particle size distribution were evaluated.

Results of the stability studies indicated that the all the periodontal formulations were stable after 6 months.

11.1.12 Antimicrobial Activity of the Periodontal Formulations

All the optimized periodontal formulations were evaluated for their antimicrobial activity against Staphylococcus aureus ATCC 29213 (gram positive) and Escherichia coli ATCC 25922 (gram negative) organisms and were shown to possess in vitro antimicrobial activity

against the same. Minocycline hydrochloride loaded periodontal formulations were evaluated for the antimicrobial activity at a concentration of 1μg/ml and clindamycin phosphate loaded periodontal formulations were evaluated for the antimicrobial formulations at a concentration of 2μg/ml. Formulations were also evaluated for the antimicrobial activity at each of the points of drug release, which were resulted to posses the diameter of zone of inhibition, which indicates the release of minocycline hydrochloride and clindamycin phosphate from the periodontal formulations were above the MIC.

11.1.13 Radiolabelling, In Vivo Blood Kinetics and Gamma Scintigraphy Study of the Periodontal Formulations

The activity study of the radio labeled minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations with reduced 99mTc were carried out.

All the periodontal formulations were labeled with ^{99m}Tc by direct labeling method. Briefly 1ml of ^{99m}Tc (2mci/ml) was mixed with 100μl of stannous chloride solution in 10% acetic acid (2mg/ml) for all the periodontal formulations. The pH was adjusted to 5.5-6 using 0.5 M sodium bicarbonate solutions. To this mixture, 1ml of minocycline hydrochloride/ clindamycin phosphate solution (1mg/ml) or the minocycline hydrochloride/ clindamycin phosphate loaded formulations (1%w/w strength) was added. The radio chemical purity of labeled complex was estimated by ascending thin layer chromatography using 100% acetone or 0.9% sodium chloride as developing solvent.

The binding affinity of the labeled complex was conformed by transchelation using DTPA. The stability and strength of the binding of ^{99m}Tc with all the periodontal formulations was examined by challenging with various concentrations of DTPA. The labeling efficiency was determined using ITLC-SG strip using normal saline as mobile phase, which allowed the separation of free pertechnetate and DTPA complex (R_f = 0.8-1.0) from the 99mTc-drug/ formulation complex.

Healthy New Zealand albino rabbits, of either sex, were administered with the radio labeled minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations by locally injecting into the periodontal cavity of each rabbit of known weight. At different time

intervals blood samples were withdrawn from the marginal dorsal ear vein of the rabbit and the radioactivity was measured in the well type gamma ray counter (Gamma ray spectrometer, Type GRS23C; Electronics corporation of India Ltd., Mumbai) calibrated for ^{99m} Tc Energy. An amount equal to 7% of the body weight was considered to represent the amount of whole blood in the body and data were expressed as the percentage of dose administered at each time interval.

The gamma scintigraphy study of minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations were carried out using the abscess bearing Sprague- Dawley rats weighing about 250-400gm after administration of ^{99m} Tc labeled complex formulations locally into the periodontium. The rat was fixed on a board and imaging was performed using a Single Photon Emission Computerized Tomography (SPECT., Lc 75-005, Diacam, Siemens, USA) gamma camera. The gamma scintigraphy imaging photographs of the rats administered with radio labeled minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations were determined.

11.2 CONCLUSION

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. In addition, a local delivery device should have a high patient acceptance and a method of application acceptable to the periodontist's practice.

Thermoreversible periodontal gel formulations were prepared and optimized based on various physico-chemical, mechanical and biological properties. The optimized thermoreversible formulations were resulted to follow zero order release kinetics of both the drugs for a period of 8 hours compared to the plain drug. Moreover periodontal thermoreversible gel is comfortable due to its non-irritant, biodegradable nature and may be preferred over other dosages forms in terms of easy application and capability to protect the inflamed surface.

The periodontal strips formulated using PVA and HPMC as strip forming polymer along with mucoadhesive polymer carbopol 934P for the delivery of both the drugs to the periodontal cavity for the treatment of dental infectious diseases were screened for their physico-chemical and mechanical characteristics. In vitro release and permeation showed a sustain release of the drug over a period of 24 hours compared to plain drugs which followed the diffusion mechanism for drug release with higuchi order of release model. Periodontal strip formulation is found to be comfortable as non-irritant, biodegradable and may be preferred over other dosages forms in terms of elasticity, flexibility and capability to protect the inflamed surface.

Periodontal microspheres prepared by solvent diffusion method were seemed to be promising with being easy, reproducible, and has an advantage of avoiding solvent toxicity. Formulations were optimized basing on particle size, entrapment efficiency, zeta potential, porosity parameters, syringeability and mucoadhesive property. The microspheres prepared with 1:1 drug: ethyl cellulose ratio was found to be the best. The in vitro release data of optimized periodontal microspheres indicated an initial burst release caused due to the

presence of drug at the surface of the periodontal microspheres which followed a release rate approaching higuchi order.

A systemic attempt was made to make the antibiotics therapy more effective and safe by incorporating the minocycline hydrochloride/ clindamycin phosphate in liposomal formulation. The liposome formulations prepared with molar ratio of soya phosphatidyl choline: cholesterol: drug was optimized at 1:0.4:0.1 with 1hr of hydration time and 2 ml of hydration volume. The close examination of the photomicrographs and TEM of the prepared liposome indicated the spherical nature and unilamellarity of optimized liposomes. The in vitro release data of drug loaded periodontal liposomal gel indicated the release rate approaching higuchi order.

All the optimized periodontal formulations were subjected to the stability studies for a period of six months in tightly closed amber colored vials at room temperature and at 4°C at conditions according to ICH guidelines i.e. 2-8°C with ambient humidity and 30±2°C/ 60±5% RH and found to be stable.

From the antimicrobial activity study it can be concluded that the different periodontal formulations such as mucoadhesive thermoreversible gel, strips, microspheres and liposomes showed in vitro antimicrobial activity against Staphylococcus aureus ATCC 29213 and gram negative Escherichia coli ATCC 25922 which are some of the organisms responsible for the periodontal diseases. Minocycline hydrochloride loaded periodontal formulations were evaluated for the antimicrobial activity at a concentration of 1µg/ml whereas clindamycin phosphate loaded periodontal formulations were evaluated at 2µg/ml. Antimicrobial activity at each of the points of drug release, indicates the release of minocycline hydrochloride and clindamycin phosphate from the periodontal formulations were above the MIC. From the antimicrobial activity point of view, all the drug loaded periodontal formulations were found to be a promising delivery system against infectious periodontal diseases.

Radiolabeling of the minocycline hydrochloride and clindamycin phosphate and their periodontal formulations were carried out using ^{99m}Tc by direct labeling method successfully with high labeling efficiency with no incubation time period. The radio labeled complexes were found to be highly stable in both in vitro and in vivo conditions.

The blood kinetics studies of the periodontal formulations reveals that all the formulations are locally concentrating in the periodontal cavity. Among all the formulations thermoreversible gel formulations showed less drug concentration in blood as compared to other three delivery systems. From the results it may be anticipated that due to low concentration of drugs eliciting the antimicrobial activity locally, the drug associated side effects would be minimal.

Thus the present study demonstrates some new findings, which may be exploited in improving the therapeutic efficacy of the antibiotic therapy using thermoreversible gel, strips, microspheres and liposomal system. Other mucoadhesive polymers can be investigated for the preparation of thermoreversible gel and strips. Similarly other method of preparation of microspheres using different polymeric systems can be tried. Other methods of preparation for conventional and sterically stabilized liposomes need to be investigated further so that the drug lipid ratio can be increased to reduce the cost of the formulations.

Based on the above study, we believe that these systems have the potential to improve the delivery of the antibiotics to the periodontal cavity. Their effective use will not only reduce the dose of the antibiotics but also helps to retain in the localized tissues in the periodontal cavity. This study also revealed that thermoreversible gel delivery systems were found to be more effective for delivering minocycline hydrochloride and clindamycin phosphate in comparison to mucoadhesive strips as well as colloidal drug delivery systems such as microsphere and liposomes in gel form for the treatment of the periodontal diseases.

LIST OF PAPERS PUBLISHED

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