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DESIGN AND RELEASE STUDY OF DENTAL IMPLANTS CONTAINING METRONIDAZOLE API FOR PERIODONTAL DISEASE

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ABSTRACT

A controlled drug delivery dental implant designed for the treatment of periodontal disease with the aim of site-specific delivery of metronidazole, which has excellent activity against anaerobic microorganisms. The calibration curve for metronidazole was developed in pH 6.6 phosphate buffer at 287.6 nm in the range of 2 to 14 µg/ml. Metronidazole Dental implants were prepared by solvent casting technique using polymer ethyl cellulose acetate in two different concentrations with three Plasticizers in Acetone alone and in combination as chloroform: Acetone (1:1) solvent with Dibutylphthalate, PEG- 600 and in combination as plasticizers. No interaction between Metronidazole and polymers are found and is shown by FT-IR and UV spectroscopic methods. The dental implants were evaluated for their thickness uniformity, folding endurance, weight uniformity, content uniformity, tensile strength, surface pH and *vitro release pattern*. *In vitro* release from implants was fit to different equations and by kinetic models to reveal release kinetics. Kinetic models were studied for zero order, first-order equations and Hixson-Crowell and Higuchi models. Controlled release of drug is found in all batches of dental implants. A short-term stability study shows that drug content decreased in various films and was ranging from 0.8% to 3.02%.

KEYWORDS: Periodontal disease; Dental implant; Metronidazole; Cellulose acetate; Controlled release; In-vitro release.

INTRODUCTION

Periodontal diseases are one of the common microbial infections which affect 35% of adult population in the world.[1] Periodontal diseases are of two types- gingivitis and periodontitis. Gingivitis is a common and reversible problem which is associated with the limited inflammation of the gums. It is characterized by swelling and bleeding of the gum during brushing and nearly half of the adult population suffers from this disease. It is observed if regular brushing is discontinued and plaque is allowed to accumulate, the gingivitis will appear and untreated gingivitis can progress to chronic condition called **periodontitis**.

This involves general inflammation of the periodontal tissue that starts from the accumulation of sub gingival plaque and results in major damage to the soft tissue and bone. When it is not treated it results in loss of supporting structure of the tooth through resorption of alveolar bone and loss of periodontal ligaments.[1] Further destruction can finally results in loss of the tooth. Periodontal disease is a term that encompasses several pathological conditions affecting the tooth supporting structures. Periodontal disease includes conditions such as chronic periodontitis, aggressive periodontitis, systemic disease associated periodontitis and necrotizing periodontitis.[1] These conditions are characterized by destruction of the periodontal ligament, resorption of the alveolar bone and the migration of the junctional epithelium along with the tooth surface. The clinical signs of periodontitis are changes in the morphology of gingival tissues, bleeding upon probing as well as **periodontal pocket formation**.[4] This pocket provides an ideal environment for the growth and proliferation of anaerobic pathogenic bacteria.[2] High doses of antibiotics cause side effects such as gastrointestinal disorders, development of resistant bacteria and suprainfection. Systemic therapy has low benefit to high-risk ratio (Gordon & Walker, 1993). With advances in understanding of the etiology and pathogenesis of periodontal disease, attention has been focused on local drug delivery systems.[5] These include both sustained and controlled release polymeric systems which when inserted into periodontal pocket, release antimicrobial agents above minimum inhibitory concentration for a sustained period of time. Thus intra-pocket devices have high benefit to low risk ratio.[2]

Cellulose acetate is one of the most popular and well characterized polymeric materials for use in controlled drug delivery. It is biocompatible and produces little or no local and systemic toxicity on administration. *In the present study our objective was to formulate intra pocket dental implants* which could be easily placed into the periodontal pocket and be

capable of delivering therapeutic concentrations of Metronidazole for prolonged period of time at a much lower dose, hence obviating untoward side effects. Antimicrobial activity of films containing metronidazole with ethyl cellulose was also investigated against periodontal pathogens commonly found in periodontal infections.[2] These include *Bacteroidesmelaninogen icus, Bacteroides oralis, Bacteroides fragilis, Peptostreptococcus assachrolyticus, Peptostreptococcus* species, *Eubacterium limosum, Propioniobacterium acne, Staphylococcus aureus* and *Escherichia coli*.[6-9] Conventional therapy, based on scaling, surgery and the use of antibiotics or antimicrobials has been proposed.[3] But due to bacterial resistance and toxic side effects of the administered antibiotics local delivery system are designed to maintain the antibiotic, in the gingival crevicular fluid at a concentration higher than that achieved by systemic administration.[4] Reported to be more effective in the treatment of periodontitis was chosen for the present study.

Metronidazole is available in the market as a conventional dosage forms such as tablets, capsules and parenterals for the treatment of bacterial infections but not suitable means for the treatment of infection locally. Hence it was a challenge to develop periodontal implants containing Metronidazole with rate controlling polymers, which has a prolonged action and shows the antibacterial activity directly at the site of infection without loss of dosage. Considering the above discussions it was decided to develop local controlled drug delivery dental implant system containing Metronidazole.

MATERIALS AND METHODS

Materials

Metronidazole was obtained as gift samples from Sun Pharmaceuticals Ltd., Ahmadabad, India. Ethyl cellulose was obtained from Codex Laboratories Pvt. Ltd. Bhadurgarh Haryana. Boric acid, sodium hydroxide, dichloromethane, methanol were purchased from S.D. fines. Ltd. (Mumbai, India). Potassium chloride, disodium hydroxide orthophosphate, acetone and diethyl phthalate were obtained from Super chems. (P) Ltd. (Tilak Bazaar Delhi, India). All other materials used were of analytical reagent grade. Shimadzu UV/Visible spectrophotometer, 1601 model with spectral bandwidth of 2nm and wavelength accuracy of 0.5 nm was used for spectrophotometric analysis.

FABRICATION OF DENTAL IMPLANT BY DISPERSION METHOD

To determine the optimum combination of polymer, plasticizer and solvent placebo implant were evaluated on the basis of homogeneity, flexibility, stickiness and smoothness. The

implants, which exhibited all the characteristics, were loaded with the drug and were taken up for further studies. So in the present work cellulose acetate was investigated as implant forming polymer for periodontal use with metranidazole as drug. Cellulose acetate due to its film forming property and non-toxic and non-irritant to living tissue has been selected as a polymer and will be an ideal material for preparing implant embedding different drugs for local treatment of periodontal diseases. So it was thought of interest to formulate and evaluate cellulose acetate implant containing Metronidazol. The effect of nature of solvents (Acetone and Acetone + Chloroform) on the film property and on in-vitro release was investigated. The Polyethyleneglycole-600 (PEG-600). with Dibutylpthalate (DBP) and Dibutylpthalate with propylene glycol (PG) were used as plasticizers and their effect on in-vitro release rate was investigated. The concentration of polymer studied was 5 and 10% w/v and the drug concentrations investigated were 30% and 40% of the dry weight of polymer. Acetone and acetone+ chloroform mixture were used as solvent systems and PEG-600, DBP with PEG-600 and DBP with PG as plasticizer systems. The implants in each solvent, with two concentration of drug and with three plasticizer system were prepared and investigated for their physical properties and for in-vitro drug release.

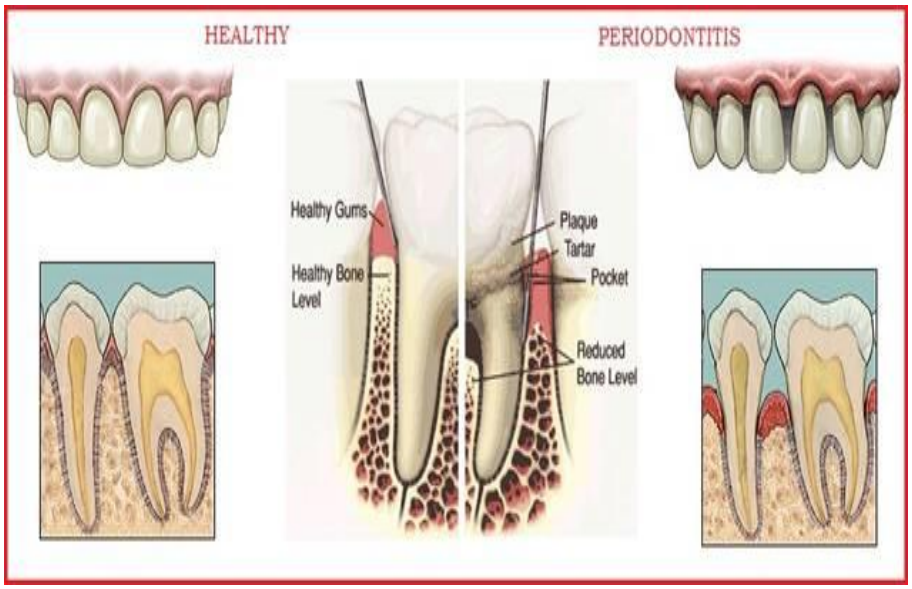
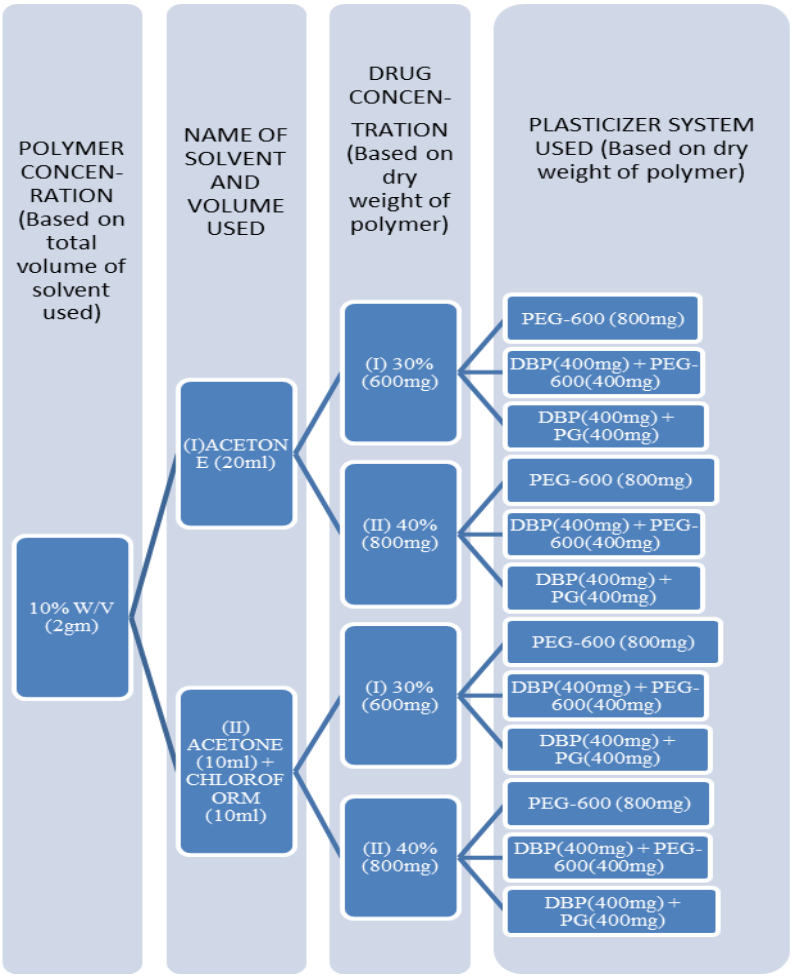


Diagram representaion of healthy and Teeth with Periodontitis. No-1.



Flow diagram no.1.

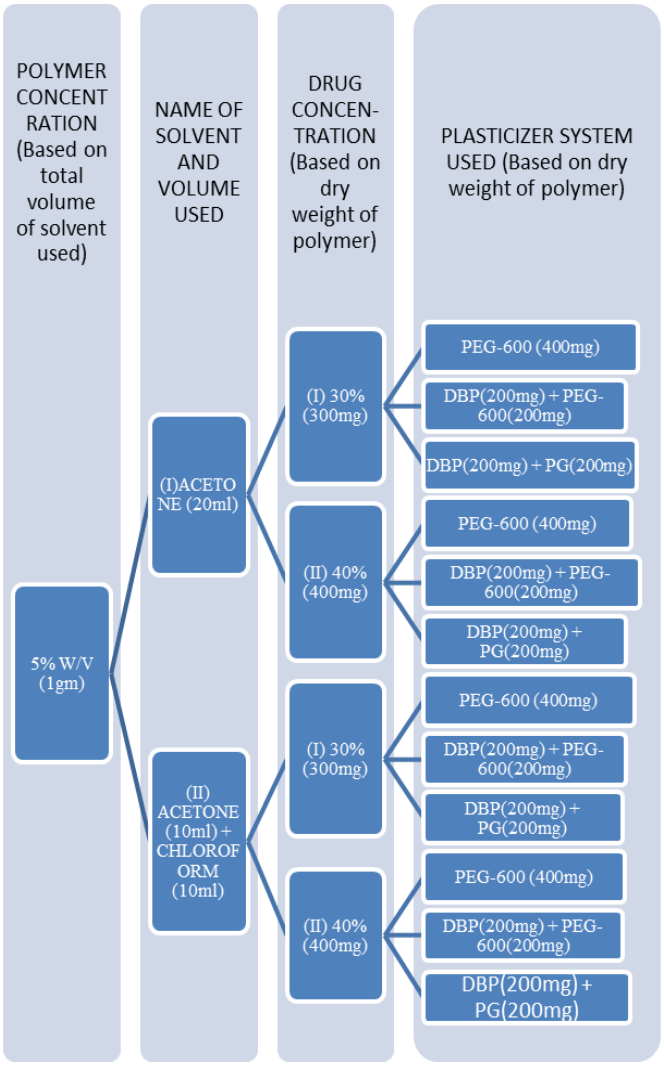
scheme of work for 10% w/v cellulose acetate Implants impregnated with metronidazole prepared in two solvent system and three plasticizer systems for peridontol use.

keywords

DBP- Dibutypthalate.

PEG- 600- Polyethylene glycol-600.

PG- Propylene glycol.



Flow Diagram-1.

scheme of work for 5% w/v cellulose acetate Implants impregnated with metronidazole prepared in two solvent system and three plasticizer systems for peridontol use *Flow diagram no. 1***.** Gives the formulae for selected drug loaded Implants. The implants were subjected to *in vitro* release studies. The Implants were initially dried at a temperature of -10 to -5^{O}C for 8 to 10 hours and at 25^{O}C for further 20 to 24 hours. After drying, the films were cut into slabs of 10 mm size.

METHODS

Drug polymer compatibility

Pure drug Metronidazole and polymers were subjected to FT-I.R studies alone and in combinations. 3 mg of pure drug/combination of drug-polymer were triturated with 97 mg of potassium bromide in a smooth mortar. The mixtures were placed in the sample holder and were analyzed by FT-IR to study the interference of polymers with the drug.

Preparation of cast film containing Metronidazole

Periodontal films were prepared by solvent casting technique. Glass moulds were used for casting of the films. Formulations were designed as shown in the Flow diagram-1 in which cellulose Acetate was taken as the main no biodegradable polymer in combination with different plasticizer for cast films In two solvent system. In the present work a quantity of 1gm (5% w/v) and 2gm (10% w/v) of cellulose acetate was dissolved in the respective two solvents systems, two concentrations of metronidazole 30% and 40% w/v based on dry weight of the polymer were investigated The solvent polymer solution along with drug and other additives was poured in a Teflon coated well cleaned petridishes (4 inch in diameter) which was previously accurately leveled on the surface with the help of leveling meter.

EVALUATION OF FILM CHARACTERSTICS

In these studies Implants were evaluated on the basic of their density, burst strength, tensile strength, water vapour permeability, drug constant uniformity and in- vitro drug release etc. Kanig and Godman pointed out the advantage of properties of the films and their variables.

In the present study, cellulose acetate implants studies have been done under the following heading.

1. *Physical properties of film* – weight, density, area, thickness and volume of films.
2. *Mechanical properties of film* – Burst strength of films.
3. *Water – absorption capacity of films*.
4. *Drug content uniformity.*
5. *In-vitro drug release study.*
6. *Periodontal films were evaluated for physical characteristics as follows.*

Weight Variation

Table 1: gives the average weight, range and maximum variation from the average weight of the films.

Table 1: Weight variation of intra pocket implants.

Average Weight (mg) (n = 6)	± SD	Range (mg)	Maximum Variation from average (%)
15.50	0.329	14.80-15.50	2.12

Thickness and density

The thickness of the cellulose acetate Implants were measured at 10 different points with the help of **"Peacock upright dial gauge"** their average value was recorded. The standard deviation (S.D.) and co-efficient of variance (C.V.) in the thickness were computed from the mean value. The density of the film was calculated by the formula.

D=M/V ……..1.

Where,

D= Density of the films in gm/cc.

M= Mass of the films in gms.

V= Volume of the films calculated as area × thickness of the film.

MECHANICAL PROPERTIES OF CAST FILMS

Polymeric films possess.[24] mechanical and rheological properties which are comparable to the viscosity properties of liquids. These film properties relate to the characteristics of films such as flexural strength, peel strength flexibility and physical stresses. Hence, it is very essential to estimate the mechanical strength of the cellulose acetate film impregnated with Metronidazole for periodontal use. The important mechanical property evaluated on cellulose acetate cast films were,

1. Bursting strength.
2. Tensile strength.

Burst strength of the films

The burst strength test is designated to give an indication of the cast films (Implants) toughness when expressed to a piercing force and varies with polymer to polymer.

The equipment used for this purpose was Ubique- power operated burst, strength tester. The cellulose acetate cast films to be tested was gripped between two annular clamps over a flexible diaphragm. Hydraulic pressure was expended by this diaphragm against the film and caused the letter to bulge. The pressure was increased until the film ruptured and the pressure at which the film ruptured called as the "burst strength" was recorded.

TENSILE STRENGTH

After burst strength, tensile strength of cellulose acetate implant was determined and it is also an important mechanical property of films. The tensile strength uses the constant increase of

the load and determines the stress strain relationship. The test also gives an idea of an extent to which a film can be elongated without rupture. For the estimation of the tensile strength Ueshima tensile strength testing equipment was used. Cellulose acetate implants were cut into dumbbell shape of which the centre portion gives **2 cm^2 (2 cm length and 2 cm width) area**. The cast implant was clamped to the bracket attached to the force gauge and to the grip attached to the test stand. The grip holding the ends of the sample were separated till the sample braked. The load reading applied to the cast film was then recorded as the tensile strength in kg/cm^2.

WATER – ABSORPTION CAPACITY STUDIES

All polymer membranes possess the ability to transmit liquids, gases and vapours, a property termed "permeation".[23] This property is an important parameter in determining the potential or actual usefulness of polymeric materials in much pharmaceutical application. The rate of permeation through films in highly dependent upon the barrier's nature. Polymeric film forming materials with low moisture permeability are said to possess five main characteristics as given below.

1. A saturated or nearly saturated carbon chain.
2. A minimum of chain branching.
3. A high degree of lateral symmetry.
4. A fair degree of longitudinal symmetry.
5. A very high proportion of relatively small, non-hydrophilic substitutes.

According to Lebovitz, a polymeric material must fulfill two conditions to be a good barrier. The structure must interfere with ease of the diffusion process. And the polymer must not possess chain structures similar to the permanent molecules. The amount of water vapour transmitted across a polymer membrane is influenced by several variables. Film thicknesses various methods have been proposed for measuring water absorption capacity of polymer films. In the present work a very simple method to evaluate the water absorption capacity was adopted. An accurately weighted amount of **Implants** without drug of $1cm^2$ was cut and it was placed in a 50ml of water and kept for 8 hours and 16 hours at room temperature. At the end of the respective immersion periods the strips were removed from the water and them gently wiped out (without squeezing) from the adhering moisture and then weighted. The difference in weight after the respective immersion period was expressed as percent water absorption capacity.

Table no: 2: Mean thickness of 10 determination of different batches of cellulose acetate implants embedding metronidazole.

SOLVENT	Drug concentration 40%w/w dry Weight of Polymer						
	POLYMER CONCENTRATION 5% w/v				10% w/v		
	NATURE OF PLASTICIZER	MEAN (mm)	S.D. (mm)	CV%	MEAN (mm)	S.D. (mm)	CV%
ACETONE	PEG-600	0.22	0.022	35.45	0.47	0.17	36.09
	DBP + PEG-600	0.32	0.033	10.31	0.49	0.073	14.89
	DBP + PG	0.23	0.034	14.78	0.5	0.108	21.6
ACETONE + CHLOROFORM MIXTURE	PEG-600	0.25	0.041	77.44	0.49	0.073	14.89
	DBP +PEG-600	0.23	0.041	17.44	0.44	0.059	13.25
	DBP + PG	0.22	0.078	35.45	0.4	0.055	13.58

Table no 3:

SOLVENT	Drug concentration 30%w/w dry Weight of Polymer						
ACETONE	POLYMER CONCENTRATION	5% w/v			10% w/v		
	NATURE OF PLASTICIZER	MEAN (mm)	S.D. (mm)	CV%	MEAN (mm)	S.D. (mm)	CV%
	PEG-600	0.16	0.039	35.45	0.49	0.073	14.89
	DBP + PEG-600	0.24	O64	26.12	0.45	0.078	35.45
	DBP + PG	0.27	0.05	18.5	0.51	0.11	21.35
ACETONE + CHLOROFORM MIXTURE	PEG-600	0.22	0.078	35.45	0.45	0.078	17.14
	DBP +PEG-600	0.26	0.061	23.46	0.45	0.07	15.55
	DBP + PG	0.2	0.028	14	0.43	0.11	25.58

Table no. 4a: Physical parameters of metronidazole (40% w/w) cast films of cellulose acetate (5% w/v) prepared in two solvent system and three plasticize.

POLYMER CONCENRATION 5%				DRUG CONCENRATION 40%		
Nature of Plasticizer SOLVENT	ACETONE			ACETONE + CHLOROFORM		
	PEG-600 DBP+DBP			PEG-600 DBP + DBP		
DENSITY (gm/cc)	4.687	2.551	2.314	2.272	2,884	4,008
BURST SNGTH(kg/cm²)	3.2	3.3	4.2	3.4	3.7	4.6
TINSILESTRENGTH (Kg/cm^2)	13.7	15.51	16.06	14.2	16.4	23.52
AREA (cm²)	0.2	0.2	0.2	0.2	0.2	0.2
VOLUME (cm³)	0.0032	0.0049	0.0054	0.0044	0.0052	0.004

Mean thickness of 10 determinations of different batches of cellulose acetate implants embedding metronidazole.

Table no. 4: % water absorption capacity of cellulose acetate cast film of area 1cm² with three plasticizer after 8 hours and 16 hours without drug.

TIME	IN HOURS	8	HOURS	16	HOURS
NATURE OF SOLVENT	NATURE OF PLASTICIZER		POLYMER	PERCENTAGE	
		5% W/V	10% W/V	5% W/V	10% W/V
	PEG-600	6.00%	4.95%	9.93%	8.57%
ACETONE	DBP + PEG-600	9.86%	3.92%	11.31%	7.05%
	DBP + PG	11.55%	5.97%	13.44%	9.07%
	PEG-600	5.60%	3.59%	7.76%	4.77%
ACETONE + CHLOROFORM	DBP + PEG-600	6.52%	4.74%	7.12%	3.05%
	DBP + PG	9.09%	4.78%	11.09%	6.40%

Table no. 5: Physical parameters of metronidazole (40% w/w) Implants of cellulose acetate (10% w/v) prepared in two solvent system and three plasticizer.

POLYMER CONCENTRATION	10% W/V			DRUG	CONCENTRATION	30% W/W
NATURE OF SOLVENT		ACETONE			ACETONE + CHLOROFORM	
NATURE OF PLASTICIZER	PEG-600	DBP + PEG-600	DBP + PG	PEG-600	DBP + PEG-600	DBP + PG
DENSITY (gm/cc)	1.479	2.009	2.301	1.477	2.565	2.432
BURST STRENGTH (kg/cm²)	4.8	5.2	4.4	6.7	6.8	5.7
TENSILESTRENGTH (kg/cm²)	30.4	35.4	45.3	32.6	38.6	50.71
AREA (cm²)	0.2	0.2	0.2	0.2	0.2	0.2
VOLUME (cm³)	0.0098	0.00102	0.001	0.0088	0.0076	0.0074

Table no: 6 physical parameters of metronidazole (40% w/w) Implants of cellulose acetate.

POLYMER CONCENTRATION	10% W/V		DRUG CONCENTRATION		40%W/W	
NATURE OF SOLVENT			ACETONE + CHLOROFORM			
NATURE OF PLASTICIZER	PEG-600	DBP + PEG-600	DBP + PG	PEG-600	DBP + PEG-600	DBP + PG
DENSITY (gm/cc)	1.617	2.307	1.747	2.307	2.500	1.627
BURST STRENGTH (kg/cm²)	4.4	4.8	4.6	5.1	4.5	4.8
TINSILE STRENGTH (kg/cm²)	29.31	40.2	48.6	38.4	42.3	49.07
AREA (cm²)	0.2	0.2	0.2	0.2	0.2	0.2
VOLUME (cm³)	0.001	0.0091	0.00103	0.0078	0.009	0.0086

EVALUATION OF DRUG CONTANT UNIFORMITY: PREPRATION OF STANDARD CURVE

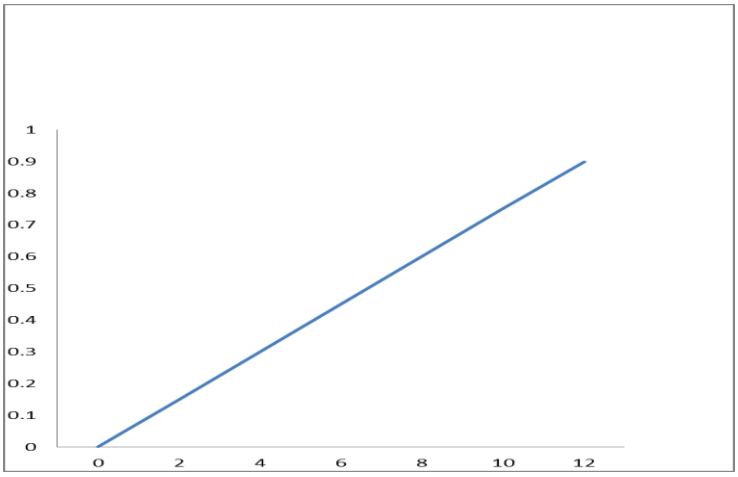
A quantity of 100mg of metronidazole was accurately weighted and transfers to 100ml volumetric flask and completely dissolved in artificial simulated gingival fluid of pH 6.6 and volume made upto100ml. 10ml of this is then diluted to 100ml with the simulated gingival fluid of pH 6.6 to get final concentration of 100µg/ml. This solution was termed as stock solution. From this aliquots of 2ml, 4ml, 6ml, 8ml, 10ml and 12ml were taken and diluted to 100ml with simulated gingival fluid of pH 6.6 to get 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml and the absorbance of resultant solution was measured at 320.5 nm (λ_{max}) using Shimadzu Uv-1201, Spectrophotometer against blank. The standard graph was obtained by plotting absorbance versus concentration in µg/ml. The beer lamberts law seems to obeyed at a concentration of 2µg/ml to 10µg/ml.

ARTIFICIAL GINGIVAL FLUID

Artificial gingival fluid (saliva) of pH 6.6 was prepared according to USP XXI113. From 10% w/v cellulose acetate Implants the strips of 10mm in length, 2mm in breadth and 0.5 mm in thickness were cut and from 5% w/v cellulose acetate dental Implants of 10mm in length, 2mm in breadth and 0.25mm.

EVALUATION OF DRUG CONTANT UNIFORMITY PREPRATION OF STANDARD CURVE

A quantity of 100mg of metronidazole was accurately weighted and transfers to 100ml volumetric flask and completely dissolved in artificial simulated gingival fluid of pH 6.6 and volume made upto100ml. 10ml of this is then diluted to 100ml with the simulated gingival fluid of pH 6.6 to get final concentration of 100µg/ml. This solution was termed as stock solution. From this aliquots of 2ml, 4ml, 6ml, 8ml, 10ml and 12ml were taken and diluted to 100ml with simulated gingival fluid of pH 6.6 to get 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml and the absorbance of resultant solution was measured at 320.5 nm (λ_{max}) using Shimadzu Uv-1201, Spectrophotometer against blank. The standard graph was obtained by plotting absorbance versus concentration in µg/ml.



Standard graph for spectrophotometric estimation of metronidazole.

The beer lamberts law seems to obeyed at a concentration of 2µg/ml to 10µg/ml. From 10% w/v cellulose acetate films the strips of 10mm in length, 2mm in breadth and 0.5 mm in thickness were cut and from 5% w/v cellulose acetate Implants of 10mm in length, 2mm in breadth and 0.25mm average thickness were cut. All the Implants were weighed accurately to 20mg of 10% w/v of cellulose acetate and 15mg of 5% w/v of cellulose acetate. All Implants were placed in 100ml of artificial gingival fluid of pH 6.6 and kept for 12 hours at room temperature. After 12 hours, the buffer pH 6.6 slightly warmed and 10ml aliquots were transferred to 50ml volumetric flask and made up to the volume with the artificial gingival fluid of pH 6.6. The optical density of the resultant solution was recorded with Shimadzo – spectrophotometer at 320.5mm. the determination were made in five replicates and mean drug content, standard deviation (S.D.) and coefficient of variance were calculated.

Table no. 7: Content uniformity of cellulose acetate (5% w/v) Implant imprennated with 30% w/w metronidazole with different plasticizers.

SOLVENT	**DRUG CONCENTRATION**	**30%**	**W/W**	**DRY**	**WEIGHT**	**OF**	**POLYMER**
	POLYMER CONCENTRATION		**5% w/v**			**10% w/v**	
	NATURE OF PLASTICIZER	**MEAN (mm)**	**S.D. (mm)**	**CV%**	**MEAN (mm)**	**S.D. (mm)**	**CV%**
	PEG-600	0.16	0.039	35.45	0.49	0.073	14.89
ACETONE	DBP + PEG-600	0.24	.O64	26.12	0.45	0.078	35.45
	DBP + PG	0.27	0.05	18.5	0.51	0.11	21.35
	PEG-600	0.22	0.078	35.45	0.45	0.078	17.14
ACETONE + CHLOROFORM MIXTURE	DBP +PEG-600	0.26	0.061	23.46	0.45	0.07	15.55

TABLE NO. 8: Content uniformity of cellulose acetate (5% w/v) Implants imprennated with 40% w/w metronidazole with different plasticizers.

SOLVENT	DRUG CONCENTRATION	40%W/W	DRY	WEIGHT	OF	POLYMER	
	POLYMER CONCENTRATION		5% w/v			10% w/v	
	NATURE OF PLASTICIZER	MEAN (mm)	S.D. (mm)	CV%	MEAN (mm)	S.D. (mm)	CV%
	PEG-600	0.22	0.022	35.45	0.47	0.17	36.09
ACETONE	DBP + PEG-600	0.32	0.033	10.31	0.49	0.073	14.89
	DBP + PG	0.23	0.034	14.78	0.5	0.108	21.6
	PEG-600		0.041	77.44	0.49	0.073	14.89
ACETONE + CHLOROFORM MIXTURE	DBP +PEG-600	0.23	0.041	17.44	0.44	0.059	13.25
	DBP + PG	0.22	0.078	35.45	0.4	0.055	13.58

IN-VITRO RELEASE STUDIES

To stimulate the actual physiological conditions prevailing in the oral cavity an in-vitro dissolution test was designed and used in the present work. From each batch of 10% w/v cellulose acetate Implants of the dimension 10mm in length and 2mm in breadth and average thickness up to 0.5 mm in five replicates were cut. Similarly from the 5 of w/v cellulose acetate Implants of the dimensions 10mm in length, 2mm in breadth with average thickness 0.25mm were cut in five replicates, all Implants of cellulose acetate in five replicates were tied with a thread to a small (3mm in breadth) glass piece. A quantity of 5ml of buffer of pH 6.6 (simulated gingival fluid of pH 6.6) was placed into10ml vials and in these vials the glass pieces with tied film strips were immersed. The vials containing the strips were then placed in incubator at $37^0 \pm 1^0$c temperature and kept for 5 days. At the end of each day a quantity of 0.25ml were withdrawn accurately by using a 0.5ml pipette and replaced with equivalent amount of fresh dissolution fluid. The aliquots withdrawn were suitably diluted to 50ml with simulated gingival fluid of pH 6.6 and was analysed by reading the absorbance at 320.5nm using a Shimadzu Uv-spectrophotometer against blank.

The release rate obtained are tabulated and graphed according to three mode of data treatment.

1. Percent cumulative drug diffused versus time in days.
2. Percent cumulative drug diffused versus square root of time in days.

3. Log percent cumulative drug diffused versus log time in days.
4. Initially the results obtained from the Implants of cellulose acetate (5% w/v) with 30% w/w drug prepared in acetone as solvent with three different plasticizers will be discussed and are shown in table no. 11 & 12.

TABLE NO.9: In-vitro diffusion of metranidazole 30% w/w from Implants (0.2cm²) of cellulose acetate 5% w/w prepared in acetone with PEG-600 SYSTEM.

TIME (DAYS) (t)	CUMMULATIVE DRUG DIFFUSED (mg)	PERCENT CUMULATIVE DRUG DIFFUSED	LOG PERCENT CUMULATIVE DRUG DIFFUSED	LOG 't'	√t
1	0.44	21.15	1.325	0	1
2	0.631	30.33	1.481	0.301	1.414
3	0.731	35.11	1.545	0.477	1.732
4	0.939	45.04	1.653	0.602	2
5	1.004	28.26	1.683	0.698	2.236

Weight of the Implant=15mg and contains 2.082mg of active drug.

Table no. 10: in vitro diffusion of metranidazole 30% w/w from Implants (0.2cm²) of cellulose acetate 5% w/w prepared in acetone with 'DBP + PEG-600' AND 'DBP + PG' system.

NATURE OF PLASTICIZER								
DBP + PEG-600					DBP + PROPYLENE GLYCOL			
time in days (t)	log 't'	√t	cummulative drug diffused (mg)	percent cumulative drug diffused	log percent cumulative drug diffused	cummulative drug diffused (mg)	percent cumulative drug diffused	log percent cumulative drug diffused
1	0	1	0.554	26.76	1.427	0.746	35.22	1.546
2	0.301	1.414	0.66	31.88	1.503	0.784	37	1.568
3	0.477	1.732	0.803	38.79	1.588	1.004	47.4	1.675
4	0.602	2	0.927	44.78	1.651	1.109	52.36	1.718
5	0.698	2.236	1.043	50.38	1.702	1.195	56.42	1.751

weight of film strip =15mg. amount of drug in Implant = 2.07mg & 2.118mg.

The results showed an amount of 21.15%, 26.76%, 35.22% of the drug was found to be released from the Implants of cellulose acetate (5% w/v) prepared in acetone with PEG-600, DBP + PEG-600and DBP + PG systems respectively, the corresponding amount of 48.26%, 50.38% and 56.48% were found to be diffused at the end of 5th day in the above mentioned system with same additives. The results indicated that the plasticizer were found to be

effective in retarding the drug diffusion in the order of effectiveness PEG-600 > DBP + PEG-600>DBP + PG in acetone system. The results further showed that around 30% of the drug was released quickly within the 1st day and the remaining drug was found to be diffused at a relatively slower rate during next four days of the diffusion study. This trend can be considered as desirable for antimicrobial action of the embedded drug during the initial period of treatment during 18-24 hours. duing which period more drug will be required to reduced the biobarden of the infected tissues. Since the minimum inhibitory concentration of metronidazole is 8µg/litre for all type of infective anaerobic bacteria normally causing gingivitis. The result showed that throughout the 5- days the concentration of the drug present was well above the minimum inhibitory concentration of the drug so it.

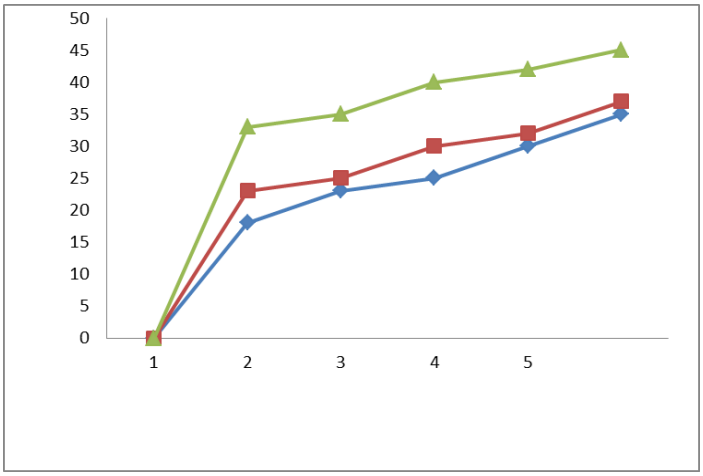


Figure: 1 In-vitro diffusion of metronidazole from cellulose acetate (5% w/v) implant prepared in acetone and in presence of three plasticizers and 30% w/w drug from phosphate buffer of ph-6.6.

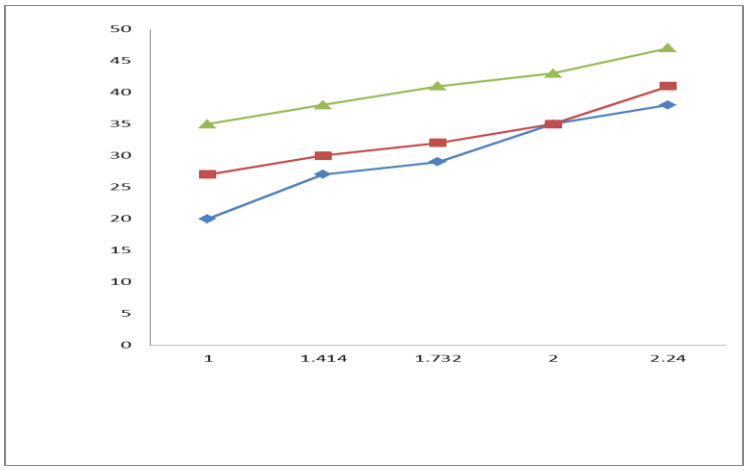


Figure: 2 HIGUCHI'S plots showing the diffusion of metronidazole (30% w/w) from cellulose acetate implant (5% w/v) prepared in acetone as solvent in presence of three plasticizers.

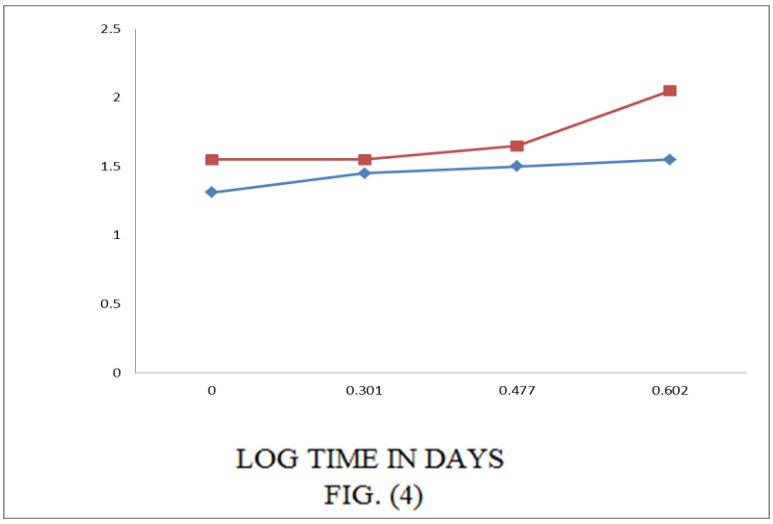


Figure: 3 DOUBLE LOG plots for cellulose acetate (5% w/w) Implant of metronidazole (30% w/w) prepared in acetone with three plasticizers system in phosphate buffer of ph-6.6.

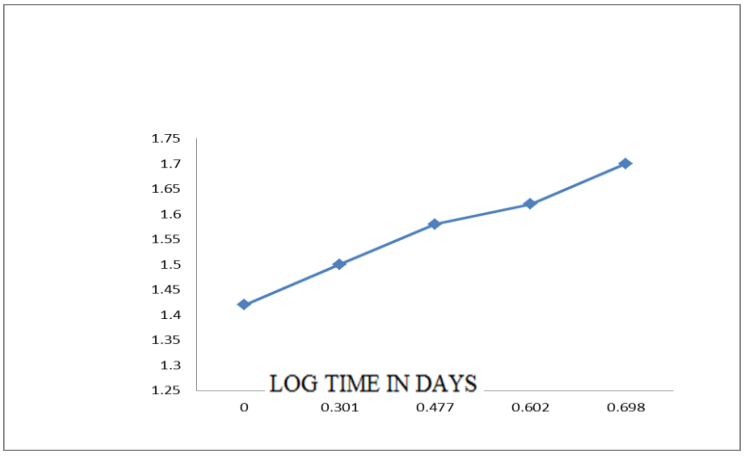


Figure: 4 **DOUBLE LOG plots for cellulose acetate (5% w/w) Implant of metronidazole (30% w/w) prepared in acetone with three plasticizers system in phosphate buffer of ph-6.6.**

The result obtained from the Implants of cellulose acetate (5%w/v) with 30% w/w metronidazole prepared in acetone + chloroform mixture as solvent in presence of three plasticizer system will be discussed. The results obtained in in-vitro drug diffusion are tabulated in table no. 10 the data has been graphed according to three modes of data treatment.

Cumulative drug diffused versus time in days.

1. Percent cumulative drug diffused versus square root of time in days.
2. Log of Percent cumulative drug diffused versus square root or time in days.

The data obtained are plotted in fig. no. 5 to 9 and the kinetic values obtained from these plots are tabulated in table no. 10.

The result showed that an amount of 25.33%, 27.39% and 27.81% of the drug was released at the end of 1st day with three plasticizer systems and the corresponding amount of 53.08%, 50.04% and 50.44% of the drug was found to be released at the end of 6th day from the film prepared in acetone + chloroform mixture with plasticizer system PEG-600, DBP + PEG-600 and DBP + PG respectively. In these batches of Implants it was found that the plasticizers were effective in retarding the drug release in following order:

DBP + PG > DBP + PEG-600 > PEG-600.

The results further indicated that the solvent systems used were found to differ in their drug release pattern values are given billow for comparison.

Table 11: Cumulative percent drug diffused on 1st day and 6th day from 5% w/v cellulose acetate implants with different Plasticizers is as below.

Table SOLVENT SYSTEMS		Polymer concentration 5% w/v		Drug concentration 30% w/w
		Plasticizers used		
		PEG-600	DBP + PEG-600	DBP + PG
ACETONE	At the end of 1st day	21.75%	26.76%	35.22%
	At the end of 6th day	48.26%	50.38%	56.48%
ACETONE + CHLOROFORM	At the end of 1st day	25.33%	27.39%	27.81%
	At the end of 6th day	53.08%	50.04%	50.44%

RESULTS AND DISCUSSION

The above results indicated that cellulose acetate Implants prepared in acetone were found to be more permeable for the drug diffusion than films prepared in chloroform + acetone mixture. The reason can be attributed less polar nature of chloroform. In this system also the drug release was by diffusion as shown by linear graphs of the data, when plotted according to the Higuchi classical diffusion equation fig. 2. The diffusion constant obtained were found to be 22.313, 17.241 and 18.0938% mg $t^{1/2}$. For PEG-600, DBP + PEG-600 and DBP + PG respectively. These result showed that here in films prepared with chloroform + acetone

mixture the release rate of drug from films with PEG-600 was more than release rate of DBP + PG which in turn was more than Dibutylphalate +polyethyleneglycol-600. The slope values obtained from log plots (fig. 2) were found to be 0.398, 0.342 and 0.412 for PEG-600, DBP + PEG-600 and DBP + PG respectively. These values indicated that there were no much swelling occurred during of in-vitro drug release With the cast films of cellulose acetate (5% w/v) with 40% metronidazole prepared in acetone in presence of three plasticizers was studied for in-vitro release of drug in simulated gingival fluid of pH 6.6 at 37^0C. The results obtained in in-vitro release are tabulated in table no. with there respective kinetics values according to **Higuchi's diffusion equation** and for double log plots are tabulated in table no.10. The basic treatment was done in the same mode as mentioned with previous batches. The graphical representation of the basic data has been shown in fig.1 to 4. The results showed that an amount of 32.75%, 21.81% and 31.7% of the total drug present in Implant was diffused out at the end of 1^{st} day while 43.79%, 45.69% and 51.49% at the end of 6^{th} day For three plasticizer systems i.e PEG-600, DBP + PEG-600 and DBP + PG respectively. In this batch of cast film implants the plasticizer also exerted influence over the drug diffusion. PEG-600 retarded the drug release more than the DBP + PEG-600 while DBP + PG had least retardation effect in drug release. Thus the order of retardation of drug release in this batch found is

PEG-600 > DBP + PEG-600 > DBP + PG.

FT-IR spectrum of Metronidazole alone and in combination with polymers was studied. FT-IR spectrum of the Metronidazole and the drug-polymer mixture have characteristic bands at 2935 cm–1 (aromatic C-H stretching) and 3275.5 cm–1(O-H group of carboxyl moiety) indicating that Metronidazole is not involved in any chemical reactions with the polymers (Cellulose acetate) used. Further, the interference was also verified using UV spectrophotometric method. In the present study, periodontal Implants of Metronidazole were formulated using the polymer matrix of cellulose acetate rate-controlling polymers. The prepared Implants were translucent and smooth surfaced with good tensile strength. The procedure developed to prepare the Implants was reproducible. All the implants have uniform thickness throughout with the standard deviation of ± 0.00339 mm (n = 6). The implants of all the batches were found to be of uniform weight, ranging from 15.501± 0.00164 mg to 14.950 ± 0.00152 mg. (n = 6). The surface pH of all the Implants was found to be neutral and hence no periodontal pocket irritation is expected. Folding endurance of the implants was > 250 times indicate that the formulations have good film properties. Content uniformity

studies of the implants shows that the drug was uniformly dispersed and recovery was possible to the tune of 93.01 to 99.09% for all formulations The tensile strength of all drug-loaded Implants was studied (Table 2). *In vitro* release studies of Metronidazole was carried out in pH 6.6 phosphate buffer for 6 days which shows that there was an abrupt release observed in first three days, and there after the release of drug was found to be controlled. Average amount of drug release per day after fourth day is found to be above the minimum inhibitory concentration of Metronidazole (MIC $\leq$ 2 µg/ml). *In vitro* release studies shows that the drug release was more sustained. These result showed that here in films prepared with chloroform + acetone mixture the release rate of drug from films with PEG-600 was more than release rate of DBP + PG which in turn was more than Dibutylphalate +polyethyleneglycol-600. The slope values obtained from log plots (fig. 2) were found to be 0.398, 0.342 and 0.412 for PEG-600, DBP + PEG-600 and DBP + PG respectively. These values indicated that there were no much swelling occurred during of in-vitro drug release With the implants of cellulose acetate (5% w/v) with 40% metronidazole prepared in acetone in presence of three plasticizers was studied for in-vitro release of drug in simulated gingival fluid of pH 6.6 at 37^0C. Ageing studies performed on all prepared periodontal films. Decrease in the drug content from the implants ranged from 0.90 to 3.41%. It was found that the drug loss is less, though the cast films and Implants were stored for one month. The cast films and Implants were also observed for their appearance and texture. These properties did not change in films during the period of study.

CONCLUSION

Periodontal Implants containing Metronidazole were prepared. *In vitro* characterization studies revealed that Metronidazole can be incorporated in a slow release device for the treatment of periodontitis. Ageing studies shows that the drug remained intact and stable in the periodontal films during storage. Spectroscopic data shows there is no significant chemical interaction between the drug and polymers. Further, detailed investigation is required to establish *in vivo* efficiency of these films.

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REFERENCES

1. Chilton N.W and Trenton N.J., "Some Public health Aspects of Periodontam Disease," J. Am. Dent. Assoc., 1950; 40: 28-37.
2. Deasy P.B., Collins A.E.M., Maccarthy D. J and Russell R- J, Use of strips Containing Tetracycline Hydrochloride or metronidozole for the treatment of Advanced periodontal Diseases", J. Pharm, Pharmacol., 1989; 41: 694- 699.
3. Medlicott N.J., Jones D.S., Tucker I-G. and Holborow D, Preliminary Release studies of Chlohexidine (base and diacetate) from poly (Caprolactone) films prepared by solvent evaporation", Int. J. Pharma., 1992; 84: 85-89.
4. Hoag P.M. and Pawlak E.A. "Basic Etiology of periodontal Diseases", in "Essentials of periodontics", ed., Robert W.R. 4th ed., C.V. Mosby Company, Missouri, 1990; 19.
5. Macphee T. and Cowely G., "The pathology of periodontitis", in essentials of periodontology and periodontics", ed., Macphee T. and Cowely G., 3rd ed., Blackwell scientific publications, Edinburgh, 1981; 31.
6. Armitage G.C. Dickinson W.R., Jenderseck R.S., lavine S.M. and chambers D.W., "Relationship between the percentage of subgingival spirochetes and the severity of periodontal Disease." J. Periodontal, 1982; 10(53): 550.
7. Carranza F.A. "Gingival inflammation". in "Glickman's Clinical Periodontology", ed., John Dyson, The ed, W.B. Saunders company, philadelphia, 1990; 103-108.
8. Page R. and schroeder H., "Pathogenesis of inflammatory periodontal Disease" in, "Periodontal disease, 1976; 235 through Ref. 4.
9. Hoag P.M. and paolak E.A., "Gingivits", in Essentials of periodontics", ed., Robert W.R., 4th- ed., e.v. company missouri, 1990; 69-85.
10. Carranza F.A., "Gingival inflammation "in," Glickman's clinical periodontology", ed., John Dyson, 7th ed., W.B. Saunders Company, Philadelphia, 1990; 103-108.
11. Goodson S.M., Holborow B. Dunn R.L. and Dunham S., "Monolithic Tetracycline Containing Fiber for Controlled Delivery to Periodontal pockets ", J. P'eriodontol, 1985; 54: 265.
12. Gorden S.M., Walker C.13 Rnodson J.M. and Socransky S.S. "Concentration of Tetracycline in Human Gingival fluid after single Doses", J. Clin Periodontal., B, 1981; 117.
13. Lozden J., "The use of metronidazole in the treatment of acute ulcerative gingivitis. A double-blind controlled trial", Br. Dent. J., 1971; 130: 194.

14. Shinn D.H., "Metronidazole in acute ulcerative gingivitis", Lanect, 1962; 1: 1191. through Ref. 29.
15. Heijl H. and Lindhe J., "The effect of metronidazole on the development of plaque and gingivitis in the beagle dog", J. Clin, Periodontal, 1979; 6: 197.
16. Greenstein G., "Effects of subgingival Irrigation on Periodontal status", J. Periodontal., 1987; 58: 827.
17. Lindhe J., Liljenberg B., Adielson B. and Borjesson L. "Use of metronidazole as a probe in the study of Human periodontal disease", J. Chin. Periodontal., 1983; 10: 100.
18. Soh L.L., Necoman H.N. and strahan J. D., "Effects of subgingival chlorhexidine irrigation on periodontal inflammation", J. Clin. Periodontal., 1982; 66.
19. Baker P.J., Evans R.T., slots J. and Genco R.J. "Antibiotic Susceptibility of anaerobic bacteria from the oral cavity", J. Dent. Res., 1985; 65: 1233.
20. Ibid, "Susceptibility of human oral anaerobic bacteria to antibiotics suitable for topicl use", J. Clin. Periodontal., 1985; 12: 201.
21. Newman M.G., Hulem C., Colgate J. and Anselmoc. Antibacterial susceptibility of plaque bacteria", J. Dent. Res., 1979; 58: 122.
22. 'Wiliam R.C., "Tetracycline Treatment of Periodontal Disease in the Beagle Dog. I clinical and Radiographic course over 12 Months-Maximum Effect on rate of Alreolar Bone Loss", J. Periodontal., Res., 1981; 16: 659.
23. Donbrow M. and Friedman M., "Enhancement of Permeability of Ethyl Cellulose films for Drug penetration" J. Pharm. Pharmald., 1975; 27: 663-635.
24. Banker G.S, Gore. A.Y and Swarbrick J., "water-vapoor Transmission Properties of Free Polymer Films," J. Pharm. Plarmacol., 1966; 18: 458.
25. Korsmeyer, R.vl., Gruny. R. Doelkar, E. Bard. P. and Paper, M.A "Mechanism of Solute release from porous hydrophilic polymers," Int J. Pharm, 1983; 15: 23-25.
26. Dr. Manvi. F.V, Singh Gurdip & Vastrad J.S. "Controlled Release film strips containing Tetracyline Hydrochloride. For local treatment of Advanced Periodontal. Disease: invitro release study "A-30, 45th Indian Pharmaceulical Congress, Scientic Abstract, 1993; India, PP-B.
27. KARUNAKAR B, Sabu. k., Dr. Udupa N. "Design and Evalulation of Tinidazole Dental implants Int. Pharm. Abstracts, 31(14): July 30, 94 3107422.
28. Higuchi, T., "Mechanism of sustained Action Medication theoritical analysis of Rate of Release of Solid Drugs Dispersed in solid matrices, J. Pharm. Sci., 1963; 52: 1145-1149.

29. Peppas, N.A. Mathematical 011odeling of Diffusion Processes in Drug Delivery Polymeric Systems". In Stone, V.F. and Ball, L.A (ed), Controlled In Drug Bioavailability, Drug Product Design and Performance, witey, New york, 1984; 1: Ch. 7.
30. Sunil, Agarwal; Venkatesh, M and Udupa, N "Controlled drug delivery systems for periodontitis", The Pharm. Review, 2004; 61-82.
31. Pandit, JK "Targeted Devices for Periodontal Disease", Ed by N K Jain, "Controlled and Novel Drug Delivery", CBS Publishers and distributors, 2004; 2: 130.
32. Stabholz A, Soskolne WA, Friedman M, Sela MN. The use of sustained release delivery of chlorhexidine for the maintenance of periodontal pockets: 2-year clinical trial. Journal of periodontology, 1991; 62: 429-433.
33. Natalie et al, Delivery system for the administration of drugs to the periodontal pockets, Advanced drug delivery review, 1994; 13: 181-203.
34. D. Steinberg, M. Friedman, A. Soskolne, M.N. Sela, A new degradable controlled release device for treatment of periodontal disease. In vitro release study, J. Periodontol, 1990; 61: 393-398.
35. G.I. Maze et al. Response to intracrevicular controlled delivery of 25% tetracycline from poly (lactide: glycolide) film strips in SPT patients J. Clin. Periodontol, 1995; 22: 860–867.
36. K. Higashi, M. Matsushita, K. Morisaki, S.I. Hayashi, T. Mayumi, Local drug delivery systems for the treatment of periodontal disease, J. Pharmacobio. Dyn., 1991; 14: 72-81.