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Formulation and *In-Vitro* Evaluation of Intra Pocket Drug Delivery Device Containing Gatifloxacin for Periodontitis

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ABSTRACT

Dental implant is a pharmaceutical device in the form of strip with very small loading and size of 0.25 sq cm. For site-specific one-time continuous delivery of Gatifloxacin an antimicrobial compound with excellent activity against anaerobic micro-organism in the treatment of periodontal disease was prepared by solvent casting technique using ethyl cellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose K4M and Eudragit RL-100 with dibutylphthalate as plasticizer. The physicochemical parameters like thickness, weight variation, content uniformity and release characteristics were evaluated The drug release was initially high on day one to achieve immediate therapeutic level of drug in pocket, followed by marked fall in release by day two, and progressive moderate release profile to maintain therapeutic level following anomalous transport mechanism. Formulation F6 released 97.34% of drug at the end of 144 h and was considered as best formulation. *In vitro* antibacterial activity was carried out on *Streptococcus mutans* .

Keywords: Dental Implant, Gatifloxacin, In vitro, physicochemical, antimicrobial, streptococcus.

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INTRODUCTION

Periodontal disease can and does occur in all groups, ethnicities, races, genders and socioeconomic levels. Periodontitis is a disease associated with periodontium in which irreversible step of loss of attachment occurs.[1] Periodontitis develops in specific site; it does not occur in everyone with uncontrolled dental plaque, on all the teeth of susceptible or on all the surfaces of these teeth. Supportive periodontal therapy such as scaling, root planning and surgeries are common methods of treatment. Reoccurrence is not uncommon, even in well-maintained root planning and daily oral hygiene practice. The risk of systemic side effects and development of bacterial resistance can also be important disadvantage of using systemic antibiotics. Topical applications with mouth washes, dentifrices and gels follow an exponential concentration profile, while blood and crevicular fluid levels remains at zero and fail to penetrate deep into periodontal pockets. Consequently, the high flow rate of GCF will cause a fast evacuation of the already released drug from the pocket to the mouth, thereby depleting the concentration of the drug in the pocket. Therefore, the rate of release should be higher at the initial stage of release, to achieve an immediate therapeutic level of drug in the pocket. The next stage should maintain therapeutic level, and moderate release profile is required. The average depth of a pocket is between 6 and 8 mm; therefore, the therapeutic drug device cannot be large. The present study was aimed to formulate site-specific controlled release dental implants for the treatment of periodontal diseases. The various pharmaceutical parameters like compatibility, stability, drug release characteristics and *in vitro* antibacterial activity on *streptococcus mutans* of dental implants are evaluated. In addition, the patients' acceptability for the delivery device was reported.[2]

MATERIALS AND METHODS

Ethyl cellulose, hydroxypropyl cellulose Hydroxypropylmethylcellulose [K_4M] , Eudragit RL-100 was obtained Zydus Cadila Health Care Limited, Kudaim Goa and dibutylphthalate were obtained from Loba Chemicals, Mumbai. Gatifloxacin was gift sample from and FDC Limited, Verna Goa and chloroform was procured from Ranbaxy Fine Chemicals Ltd.

Preparation of dental implants:

Method used for the preparation of dental implants was solvent casting technique[3] using chloroform and dichloromethane (1:1) mixture. A total of six formulations were designed, (Table1) shows composition of cast films for each dental implant. Dental implants were prepared by dissolving ethyl cellulose and copolymer HPC or HPMC [K_4 M] alone and in combination in

chloroform and dichloromethane (1:1) mixture, dibutylphthalate (50% v/w of that of polymer) as a plasticizer using magnetic stirrer in a closed beaker to get different concentrations of each polymer. Consequently, polymer ethyl cellulose and copolymer Eudragit RL 100 were dissolved in chloroform alone, containing dibutylphthalate 50% and 15% v/w of that of polymer respectively as a plasticizer. Into this, Gatifloxacin of required concentration was added. After complete mixing 10 ml solution was poured in a clean Petri dish (Anumbra® area 60.8 sq cm approximately) placed on a horizontal plane. The solvent was allowed to evaporate slowly by inverting a glass funnel with a cotton plug closed in the stem of the funnel on Petri dish at 24° for 24 h. After complete evaporation of solvent, cast films were obtained. Cast films were then cut into pieces of 0.5×0.5 cm and wrapped in an aluminum foil and stored in desiccators at relative humility at room temperature in a dark place until further use. Each film contained 1 mg of drug.

Evaluation of polymeric dental implants:

The compactibility studies were conducted by using IR Spectroscopy of drug alone, and individual polymer along with drug. Various physicochemical properties such as size, thickness, content uniformity, weight variation, folding endurance, tensile strength and percentage moisture loss was determined on prepared implants. Thickness of three strips was measured using micrometer screw gauge.[4] Individual weights of ten strips were noted on an electronic single pan balance. Percentage moisture loss[5] was determined by keeping the implant in a desiccator containing anhydrous calcium chloride. After three days, the implants were taken out and re-weighed; the percentage moisture loss was calculated using formula (Initial wt - Final wt/Initial wt) ×100.

Folding endurance of the film was determined by repeatedly folding a small strip of film of 2×2 cm size at the same place till it broke. Tensile strength of the films was noted on an Instron apparatus[4] using a film strips of 4×1 cm Content uniformity[5] was noted by dissolving three implants individually in 10 ml dichloromethane and chloroform. This was extracted with two successive quantities, each of 10 ml of isotonic phosphate buffered saline pH 7.2, in a separating funnel. The aqueous phases were separated and absorbance was determined at 290 nm for Gatifloxacin after suitable dilution using Shimadzu UV/Vis spectrophotometer. The extract of implants without drug was served as blank. Values are recorded in Table 1.

***In vitro* drug release studies[7]:**

In vitro release was performed by taking five implants with the drug (separate formulations) in a vial containing 1 ml of phosphate buffered. One milliliter of the phosphate buffer was

withdrawn from 1st to 6th d and immediately replaced with 1 ml fresh phosphate buffer. The drug content was estimated by measuring the absorbance after suitable dilutions at 290 nm. *In vitro* antibacterial activity[8,9] was performed on all formulations by placing the film, cut into 0.5×0.5 sq cm, on chocolate agar plates seeded with the oral bacteria *Streptococcus mutans* . After 48 h of incubation at 37°C, the films were transferred onto freshly seeded agar plates for an additional 48 h for incubation. This procedure was repeated until no inhibition of bacterial growth was detected on the agar plate. The growth inhibition area on the agar plate was measured.

Table 1: Formulation composition

Ingredient	Composition					
	F1	F2	F3	F4	F5	F6
Ethyl Cellulose	9	8	8	-	-	9
Hydroxy Propyl cellouse	-	1	-	9	-	-
Hydroxy Propyl methyl celluose	-	-	0.25	-	2.5	-
Eudragits RL	-	-	-	-	-	0.25
Dibutyl phthalate (%w/w)	50	50	50	50	50	50
Gatifloxacin	2.5	2.5	2.5	2.5	2.5	2.5

RESULTS AND DISSUSSION

The physicochemical evaluation data presented in Table 2 indicates that the thickness of the dental film varies from 0.238±0.008 to 0.275±0.042 µm except formulation F5, which has 0.142±0.040 µm because of low concentration of HPMC K_4 M. All the formulations exhibited uniform thickness with low standard deviation values, ensuring the uniformity of the films prepared by solvent casting method. All the formulations were found to contain almost uniform quantity of drug as per content uniformity studies, indicating reproducibility of the technique. For all the formulations, the percentage moisture loss varied between 8.90±1.29 and 14.34 ±1.47. Formulation F5 showed maximum amount of moisture loss because of HPMC K_4 M undergoing moisture loss in dry condition. Formulation F1 showed minimum percentage moisture loss because of hydrophobic ethyl cellulose. All the formulations exhibited more than 200 folding endurance, and tensile strength varied from 216.8 to 565.4 kg/sq cm.

In vitro release studies performed using phosphate buffer pH 6.6 showed an initial burst release, (Figure 1), which is expected to kill most of the periodontal organism, followed by controlled release for about 6 d, sufficient to inhibit the growth of the micro-organisms.

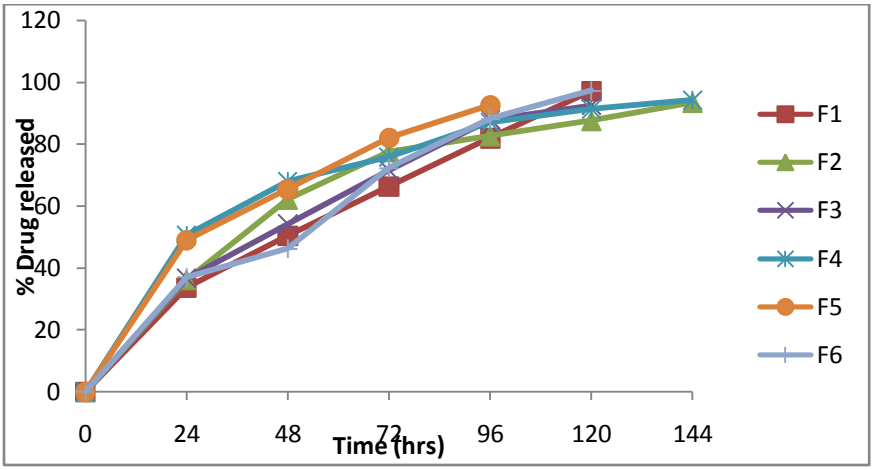

Figure 1: In vitro release of Gatifloxacin in pH 6.6 phosphate buffer

In case of formulation F1, F2, F3, & F6, 97.13%, 93.41%, 92.49% and 97.34% of the drug was released at the end of 6 days respectively. Formulation F4, 94.30% of drug was released at the end of 5 days. Formulation F5, 92.64% of drug released at the end of 4 days. Dental films made of HPMC (F3) and ethyl cellulose (F1) are better than others to the extent of release was maintained for about six days. Higher drug release from strip F1, F2, F3, & F6 is due to the formation of more pores and channels in the strips matrix due to its higher EC content.

As HPMC K_4 M and HPC polymer act as resorbable carriers, they dissolved readily during *in vitro* drug release as well as *in vitro* antibacterial activity after 72 and 96 h respectively. Table 3 shows cumulative amount of drug released and 'r' value is coefficient of correlation and 'n' value is higuchi an diffusion. The Peppas model was found to be linear with the correlation coefficient values of 0.98, 0.98, 0.98, 0.96, 0.97 and 0.99 for formulation F1, F2, F3, F4, F5 and F6, respectively. The "n" value of the various mathematical model fittings suggests that all the films exhibit anomalous transport, as shown in Table 3. *In vitro* antibacterial activity demonstrated significant antibacterial profile of all the formulations, as shown in Table 4 and Figure 2. In order to see whether the drug release is by diffusion, swelling or erosion mechanism, correlation values of various mathematical models were taken and compared as shown in Table - 3. R^2 values indicated that the regression values are higher with zero order release kinetics compared to First order. Initially there was busting release in the first day and shown zero order from 2^{nd} day, Therefore all the films follow zero order release kinetics that R^2 values are higher for Higuchi's model compared to Hixon - Crowell for all the films. Hence release of Gatifloxacin from all the films followed diffusion rate controlled mechanism. According to Korsmeyer-Peppas model, a 'n' value of slope between 0.5 and 1 indicates an anomalous behavior (Non-Fickian). So, it indicates all films are follows Non-Fickian diffusion (anomalous behavior).

Table 2: Physiochemical parameter of formulation F1 – F6

Formulation	Thickness in mm (n=5)	Weight in mg (n=10)	Drug content in % (n=3)	Tensile Strength (kg/sq cm)	*% Moisture Loss
F1	0.246±0.004	4.14±0.026	93.34±2.24	278.2	8.90±1.29
F2	0.275±0.042	4.18±0.042	89.47±3.71	464.2	9.46±0.93
F3	0.238±0.008	4.10±0.008	83.84±2.24	216.8	13.24±1.69
F4	0.274±0.016	5.16±0.48	86.66±0.75	255.7	10.74±0.69
F5	0.142±0.040	2.69±0.62	76.10±5.20	431.9	14.34 ±1.47
F6	0.246±0.071	4.24±0.071	81.33±2.21	565.4	9.66±0.82

Table 3: Model fitting of the Release profiles Using Five Different Models

Formulation Code	Mathematical Models (Kinetics)					
	Zero order	First order	Higuchi Matrix	Hixon Crowell	Peppas	'n' value
F1	0.94	0.86	0.98	0.95	0.98	0.96
F2	0.98	0.85	0.99	0.96	0.98	0.97
F3	0.97	0.92	0.99	0.96	0.98	0.96
F4	0.89	0.84	0.97	0.97	0.96	0.97
F5	0.98	0.90	0.99	0.95	0.97	0.99
F6	0.95	0.92	0.97	0.96	0.99	0.96

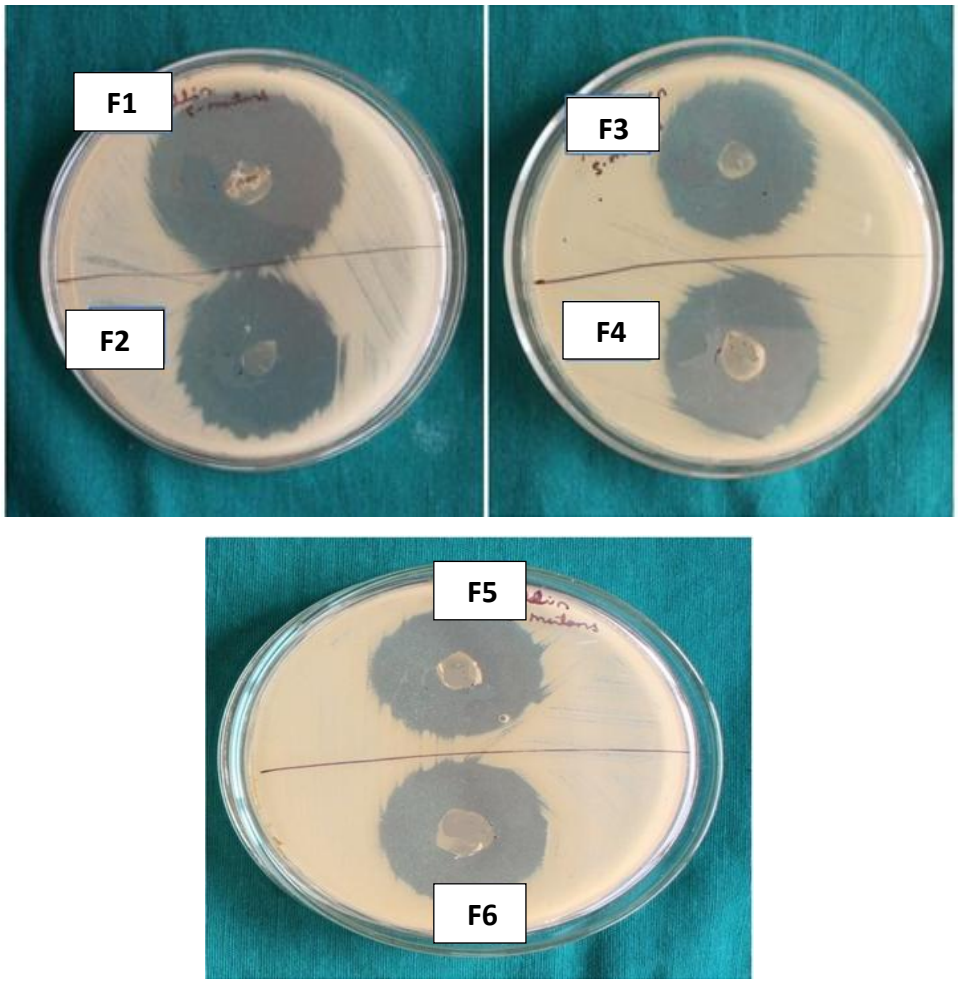

Figure 2: Zone of inhibition of films F1 to F6 against *S.mutans* on first day

Table 4: Antibacterial activity of films against *S. mutans*

Formulation	Zone of inhibition per mm/48 hrs		
	2 days	4 days	6 days
F1	16	12	08
F2	18	15	10
F3	19	16	11
F4	12	08	--
F5	13	09	--
F6	15	12	07

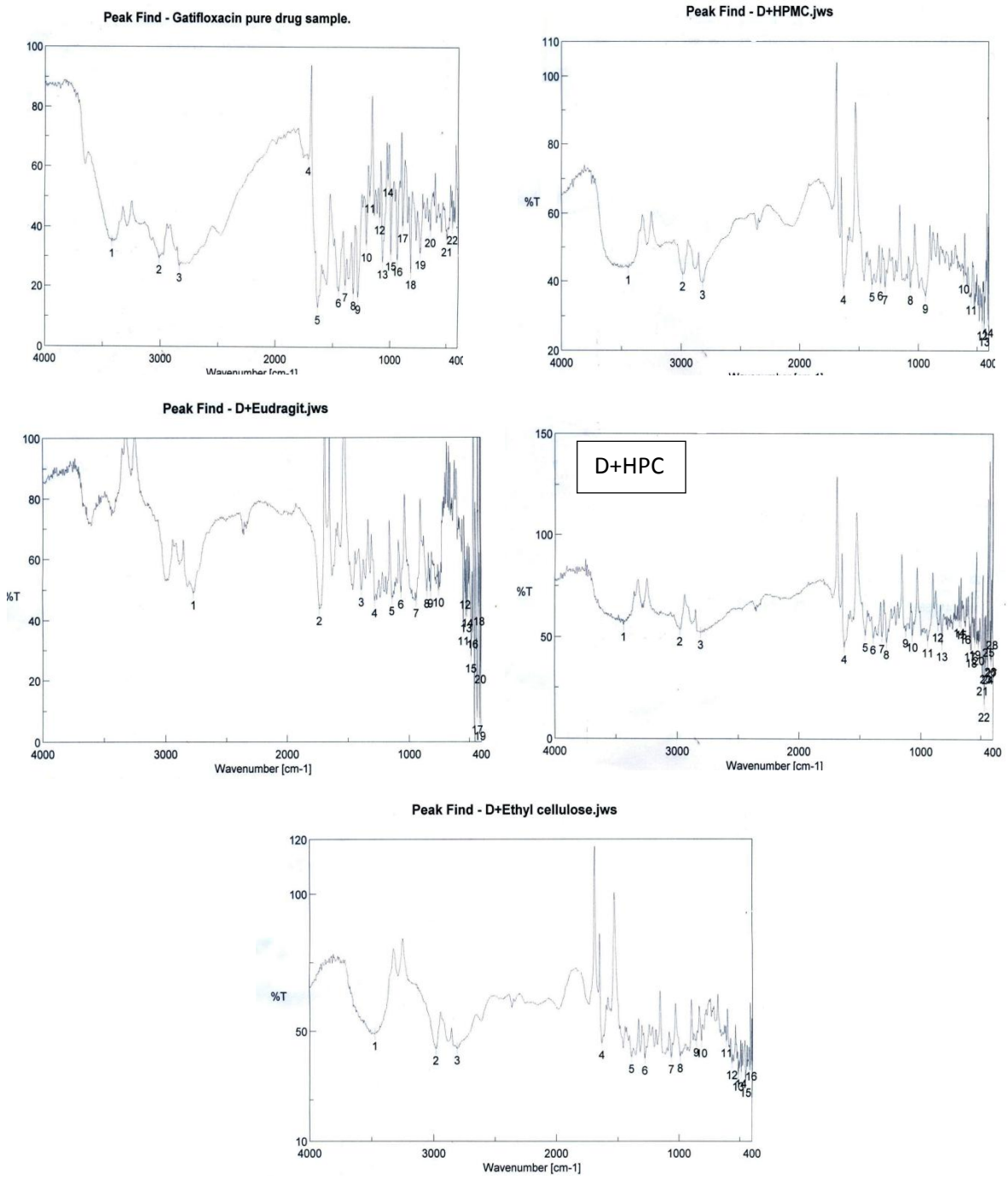

Figure 3: Compatibility study of Drug and polymer

CONCLUSION

Compatability study was done by using IR Spectroscopy of drug alone and in combination with the individual polymer (Figure 3). Stability studies were conducted on implants at room temperature and exposure to direct sunlight for one month. The drug content reduced markedly after exposing to humidity chamber. The stability of drug was improved by formulating them in polymer matrix.

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