Research Article

Design and Characterization of *Aloe emodin* Dental implants for the Treatment of Dental caries

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ABSTRACT

Dental implants are strip like pharmaceutical dosage forms having about 0.25 sq. cm size with a small drug loading capacity. Periodontal pockets act as a natural reservoir filled with gingival crevicular fluid for the controlled release delivery of antimicrobial drugs directly. Aloe emodin is a poly phenolic phytoconstituent with good antimicrobial activity against *streptococcus mutans* which is proved by our MIC studies. Dental implants of aloe emodin were formulated by solvent casting technique using biodegradable polymers ethyl cellulose (EC), hydroxy propyl cellulose (HPC), hydroxy propyl methyl cellulose (HPMC) and eudragit RL 100 along with dibutyl phthalate as plasticizer for site specific continuous delivery of the drug. The dental implants were then evaluated for physicochemical properties such as appearance, thickness of the film, uniformity of weight, percentage moisture loss, folding endurance, tensile strength, surface pH, drug content and compatibility studies by FTIR spectroscopy. The results of weight uniformity and content uniformity were found to be uniform for all the formulations. In *in vitro* drug release studies, F6 showed better release compared to other formulations as the extent of release was maintained for 11 days. *In vitro* antibacterial activity of formulation was carried out on *streptococcus mutans* had an inhibitory effect after incubation.

Keywords: Dental implants, Aloe emodin, Antibacterial, *Streptococcus mutans,* Sustain release.

INTRODUCTION

Two major dental diseases in the world are dental caries and periodontal disease, both are caused by various bacteria in the oral cavity. Dental carries is a common oral disease that usually develops secondary to the formation of plaque biofilms on the tooth surface. The causative agents of dental caries are gram-positive bacteria such as *streptococcus mutans*, *streptococcus sobrinus*, *lactobacillus* spp and some non-mutans *streptococci* while periodontitis is an inflammatory response to the over growth of anaerobic organisms such as *treponema denticola* and *porphyromonas gingivalis* in the subgingiva and if unchecked, results in the destruction of the bone and soft tissues supporting the tooth, which results in tooth loss.[1, 2]

Dental caries is cavity formation in the enamel and dentine of the tooth. The main cause of dental caries is acid formation from the bacteria which dissolves the hard tissues (enamel, dentin and cementum) of the teeth.[3] The acid is produced by the bacteria when they break down food debris or sugar on the tooth surface. The adherence of food to the teeth and acid creation by the bacteria that makes up the dental plaques leads to the development of a cariogenic (causing decay) biofilm around the teeth.[4] The bacteria in the biofilm produce acid in the presence of fermentable carbohydrates such as sucrose, fructose and glucose.[5, 6] Dental caries can occur on any surface of a tooth that is exposed to the oral cavity, but not the structures that are retained within the bone.[7]

The earliest sign of a new carious lesion is the appearance of a chalky white spot on the surface of the tooth, indicating an area of demineralization of enamel. This is referred to as a white spot lesion, an incipient carious lesion or a microcavity. As the lesion continues to demineralize, it can turn brown but will eventually turn into a cavitation. Before the cavity forms, the process is reversible, but once a cavity forms, the lost tooth structure cannot be regenerated.[8, 9]

To prevent tooth decay and periodontal diseases, fluoride compounds and antibiotics such as penicillin, erythromycin, tetracycline or synthetic antibacterial agents such as chlorhexidine has also been used. But the excess use of fluorine causes the hardening of cartilage and the use of synthetic antibacterial agents cause various side effects. Medicinal plants have been used for thousands of years in folk medicine for maintaining oral hygiene. Most of these herbs are alkaline with high antibacterial activity. Hence these herbs help to maintain acid-alkaline balance of the saliva, decrease plaque/calculus formation and are less prone to periodontal diseases. It is also observed that the microorganisms found in inflamed gums are resistant to antibiotics but not to antibacterial plant extracts like neem. Among the various currently available herbal agents, aloe vera has been proved better in the treatment of periodontal disease, due to the presence of anthraquinones like aloe emodin, which possess antimicrobial and anti-inflammatory effect against resistant microorganisms found in oral pulp space.[10-12] Aloe emodin (1,3,8-trihydroxyanthraquinone) is an anthraquinone, exudate from the aloe plant. The molecular structure of aloe emodin is given in Figure 1. Molecular formula: $C_{15}H_{10}O_5$, Molar mass: 270.24 g/mol, melting point: 223 to 224 °C. It has antibacterial action

and effective against the microorganisms which causes the periodontal diseases.

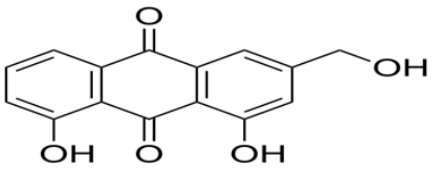

Figure 1: Structure of aloe emodin

Bhat G *et al.,* conducted clinical study to check the effectiveness of aloe vera when used as medicament in the periodontal pocket. A total number of 15 subjects were evaluated for clinical parameters like plaque index, gingival index, probing pocket depth at baseline, followed by scaling and root planing (SRP). Test site comprised of SRP followed by intra-pocket placement of Aloe vera gel, which was compared with the control site in which only SRP was done, and clinical parameters were compared between the two sites at one month and three months from baseline. Results exhibited encouraging findings in clinical parameters of the role of aloe vera gel as a drug for local delivery. They concluded that subgingival administration of Aloe vera gel results in improvement of periodontal condition.[13]

In conventional mode of drug administration the drug do not reaches target area in sufficient concentration, this problem can be overcome by administering the drug directly into the intended site of action with lesser dose. Sustained drug delivery systems are able to provide very precise control over drug release for prolonged period of time eliminating the need for frequent dosing and minimizing side effects, thereby increasing the patient compliance and comfort. A site-specific system called dental implants aims at delivering the active constituent at sufficient levels inside the periodontal pockets and at the same time minimizing the side effects associated with systemic drug administration.[14]

Dental Films, a widely used form of intra-pocket delivery device has been in the shape of film, prepared either by solvent casting or direct milling. Bigger films either could be applied within the cavity onto the cheek mucosa or gingival surface or could be cut or punched into appropriate sizes so as to be inserted into the site of action. Films are matrix delivery systems in which drugs are distributed throughout the polymer and release occurs by drug diffusion and/or matrix dissolution or erosion. This dosage form has several advantageous physical properties for intra-pocket use. The dimensions and shape of the films can be easily controlled according to the dimensions of the pocket to be treated. It can be rapidly inserted into the base of the pocket with minimal discomfort to the patient. If the thickness of the film does not exceed 0.4 mm, and it has sufficient adhesiveness, it will remain submerged without any noticeable interference with the patient's oral hygiene habits.

In contrast to the non-degradable systems discussed above, the films made up of degradable polymers erode or dissolve in the gingival crevice so that removal after treatment is not required. Prolonged concentration of tetracycline in GCF could be maintained for at least ten days by incorporating the drug in glutaraldehyde cross-linked at elocollagen. Application of these films resulted in a significant improvement in clinical parameters.

Udupa N and Karunakar B has designed and evaluated norfloxacin dental implants using solvent casting method. Attempt was made to formulate Norfloxacin as targeted sustained release dental implant which can be directly placed near the site of action in periodontitis and also to study various pharmaceutical parameters like physicochemical, stability and drug release characteristics.[15] Mastiholimath VS *et al.,* has formulated and evaluated ornidazole implants for periodontitis have been reported. Ornidazole has an excellent activity against anaerobic microorganism was prepared by solvent casting technique. The physicochemical parameters were evaluated. *In vitro* antibacterial activity was carried out on *streptococcus mutans*.[16]

MATERIALS AND METHODS

Aloe emodin was purchased from Sigma-Aldrich, Mumbai, India. Ethyl cellulose (EC), hydroxy propyl cellulose (HPC), hydroxy propyl methyl cellulose (HPMC), eudragit RL 100 and dibutyl phthalate were purchased from Loba Chemicals, Mumbai. Chloroform and dichloromethane were purchased from Hi Media Laboratory Pvt. Ltd, Mumbai, India. All chemicals/reagents were used of analytical grade.

Preformulation studies of aloe emodin

Preformulation study relates to pharmaceutical and analytical investigation carried out proceeding and supporting formulation development efforts of the dosage form of the drug substance. Preformulation studies yield basic knowledge necessary to develop suitable formulation. It gives information needed to define the nature of the drug substance and provide frame work for the drug combination with pharmaceutical excipients in the dosage form. Hence, the preformulation studies like determination of organoleptic characteristics, melting point, λ_{max} and standard calibration curve were performed on the obtained sample of drug. The drug-polymer compatibility studies were conducted by using FTIR Spectroscopy of pure drug, physical mixtures of drug and polymers.[17-20]

Minimum inhibitory concentration (MIC) of Aloe emodin

The antimicrobial activity at different concentrations of the aloe emodin on *streptococcus mutans* was determined by employing the agar diffusion method. Culture strains of *streptococcus mutans* were maintained on Muller hinton agar plates. The agar plate was prepared in sterile glass petri dish, seeded with innocula and kept overnight under anaerobic condition. The agar plate

contains 4 wells of 3.0 mm in diameter and 2 mm deep, wells were cut out on the seeded plate using sterile cork borer and each of the well was filled with 30µL of the aloe emodin dilution of varying concentrations (0.1 mg/ml, 0.125 mg/ml, 0.250 mg/ml, 0.500 mg/ml, 0.750 mg/ml and 1.0 mg/ml). The plate was incubated at 37 °C for 72 hrs. The sensitivity of the tested pathogenic organisms to aloe emodin was shown by zones of inhibition after incubation. The zones of inhibition were measured using a plastic ruler. For each concentration, the zone of inhibition was measured three times and the mean was recorded.[21]

Formulation of dental implants

Periodontal dental implants were prepared by solvent casting technique. Glass moulds were used for casting films. Total six formulations were designed are given in Table 2 which shows composition of cast films for each dental implant. Dental implants were prepared by taking polymer ethyl cellulose (EC) and co polymers hydroxy propyl cellulose (HPC) or hydroxy propyl methyl cellulose (HPMC) alone or in combination and dissolved in mixture of chloroform and dichloromethane (1:1) in a beaker. Dibutyl phthalate (50% v/w of that of polymer) as plasticizer is added to above solution in beaker kept on magnetic stirrer. For another formulation polymer EC and copolymer eudragit RL 100 were dissolved in solvent mixture, containing diabutyl phthalate 50% v/w of that of polymer as a plasticizer. Required concentration of drug is added to these and stirred thoroughly. After complete mixing 10 ml of solution was poured in a cleaned glass mould of 14 cm^2 placed on a horizontal plane in order to obtain uniform thickness. The solvent was allowed to evaporate slowly by inverting a glass funnel over glass mould at room temperature for 24 hrs, in order to prevent the formation of bubbles due to rapid evaporation of chloroform and dicloromethane. After complete evaporation of solvent, cast film was obtained. Percentage of drug and plasticizer was based on weight of polymer. Cast films were then cut into pieces of 0.5×0.5 cm and wrapped in an aluminium foil and stored in desiccator until further use. Each film contained 1 mg of drug.[22,23]

Table 1: Composition of dental implants.

Ingredients	Composition (%)					
	F1	F2	F3	F4	F5	F6
Aloe emodin	2.5	2.5	2.5	2.5	2.5	2.5
Ethyl Cellulose (EC)	9	8	8	-	-	8
Hydroxy propyl cellulose (HPC)	-	1	-	9	-	-
Hydroxy propyl methyl cellulose (HPMC)	-	-	0.25	-	0.25	-
Eudragit RL 100	-	-	-	-	-	0.25
Di butyl phthalate (%v/w)*	50	50	50	50	50	50

*Based on dry powder weight.

In each formulations 10 ml of chloroform:dichloromethane (1:1) mixture was used as solvent.

Evaluation of Dental Implants

Various physicochemical properties of the formulated films such as appearance, thickness of the film, uniformity of weight, percentage moisture loss, folding endurance, tensile strength, surface pH, drug content and *in vitro* drug release were determined. All the evaluation tests were done in triplicates (n=3) and the standard deviations of parameters were computed from their mean value.

Film thickness of strips was measured using micrometer screw gauge. The weight variation test was carried out by cutting patches from different places of same formulation and their individual weights were determined by using the digital balance. The mean value was calculated. To determine the percentage moisture loss, implants were weighed and kept in a desiccator containing anhydrous calcium chloride. After three days, implants were taken out and reweighed. The percentage moisture loss was calculated by using following formula.

$$\% \, Moisture \, loss = \frac{Initial \, weight - Final \, weight}{Initial \, weight} \, X \, 100$$

The folding endurance of the film was determined by repeatedly folding a small strip of film (2x2 cm) in the center, between fingers and the thumb and then opened. This was termed as one folding. The process was repeated till the film showed breakage or cracks in the center of film. The number of times the film could be folded at the same place without breaking gave the value of folding endurance. Tensile strength of the films was determined by using universal strength testing machine. To determine surface pH dental implants were left to swell for 1 hr on the surface of the agar plate, prepared by dissolving 2 % (w/v) agar in warmed double distilled water with constant stirring and poured into the petri plate to solidify at room temperature. The surface pH was measured by pen pH meter placed on the surface of the swollen film. To determine the drug content of the prepared implants, one implant containing aloe emodin was dissolved in 10ml of dicloromethane and chloroform (1:1). This was extracted with two successive

quantities each of 10 ml phosphate buffer pH 6.6 in a separating funnel. The aqueous phases were separated and 2 ml was pipetted out which was made upto 10ml with phosphate buffer in a 10 ml standard flask. Absorbance of the solution was then determined at 254 nm using UV Visible spectrophotometer. The extract of implant without drug was served as a blank.[24]

In vitro drug release

The pH of gingival fluid lies between 6.5 – 6.8, phosphate buffer pH 6.6 was used as simulated gingival fluid. Also, since the film should be immobile in the periodontal pocket, a static dissolution model was adopted for the dissolution studies. Films of known weight and dimension (14 mm^2) were placed separately in small sealed test tubes containing 1.0 ml of phosphate buffer (pH 6.6) and kept at 37 ± 0.5 °C for 24 hrs. The buffer was then drained off and replaced with a fresh 1.0 ml of buffer. The concentration of drug was determined by UV/Visible spectrophotometer at 254 nm the procedure was continued for 5 consecutive days for the films of different polymers respectively.

Kinetic studies

In order to describe the kinetics of the release process of drug in the different formulations, models were fitted to the dissolution data of optimized formulations using linear regression analysis. In zero order model, to study the release kinetics, data obtained from *in vitro* drug release studies were plotted as cumulative amount of drug released versus time. In first order model, the data obtained were plotted as log cumulative percentage of drug remaining vs. time. In Higuchi model, the data obtained were plotted as cumulative percentage drug release versus square root of time. In Korsmeyer-Peppas model, the data obtained were plotted as log cumulative percentage drug release versus log time.

Evaluation of *in vitro* Antimicrobial Activity

The antimicrobial activity of dental implants on *streptococcus mutans* was determined by agar diffusion method. Culture strains of *streptococcus mutans* were maintained on Muller hinton agar plates. The agar plates were prepared in sterile glass petri dishes, seeded with innocula and kept overnight under anaerobic conditions. After solidification of agar plates, implants of each batch were placed in these plates. The implants containing drug were allowed to diffuse into the medium and the plates were incubated at 37 °C for 72 hrs. The sensitivity of the tested pathogenic organisms to various implants was shown by zones of inhibition after incubation. The zones of inhibition were measured using a plastic ruler. For each batch of implants the zone of inhibition was measured three times and the mean was recorded.[21,25]

RESULTS AND DISCUSSION

Preformulation studies

The preformulations studies on drug aloe emodin revealed that the purchased drug was pure with 223 ºC melting point and showed absorption maximum at 254 nm which matches the standard reported values. The standard calibration curve was obtained with slope value y = 0.0341 and regression value R^2 = 0.9915.

The IR spectra of the drug and polymer combinations were compared with the standard spectrum of pure aloe emodin and the characteristic peaks associated with specific functional groups and bonds of the molecule and their presence/ absence were noted. The overlay of the IR spectra of aloe emodin and physical mixture of drug and polymers is shown in the Figure 2. The prominent peaks associated with functional groups like O-H (phenols) at 3305.56 cm-1 , C-H (alkanes) at 2932.18cm^{-1}, C=C (aromatic ring) at 1575.86cm^{-1} , C–O–C (polysaccharides) at 1142cm^{-1} were analyzed. The range of peak values were found to be the same indicates that there were no interaction of aloe emodin with different polymers confirming the stability of the drug in the formulations.

Minimum inhibitory concentration of aloe emodin was found at the concentration of 0.25mg/ml and the diameter of the zone was found to be 12 mm shown in Figure 3.

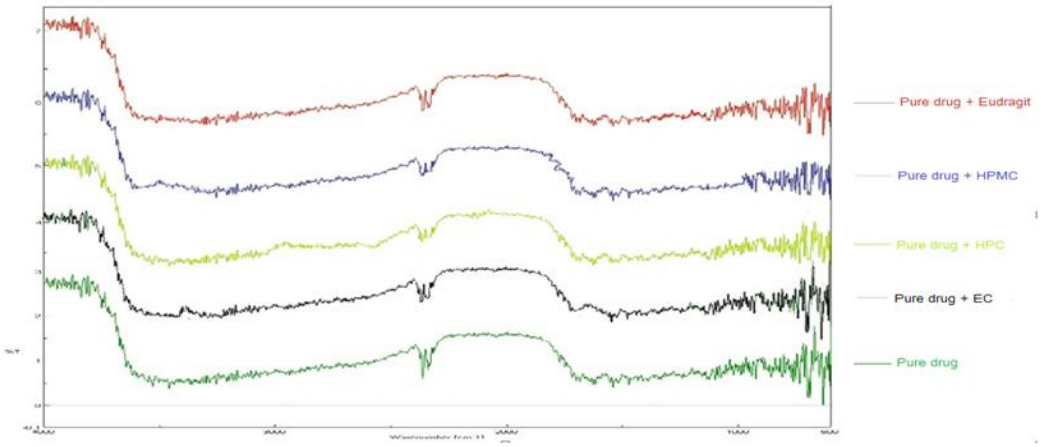

Figure 2: FT-IR spectra of pure aloe emodin and physical mixture of drug and polymers.

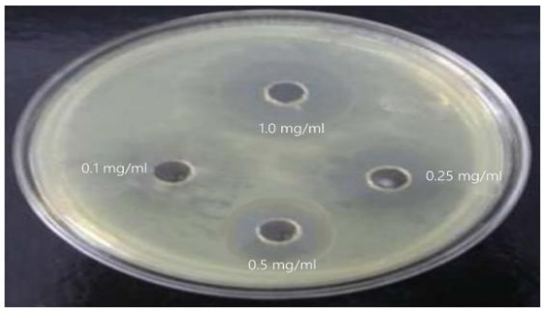

Figure 3: Zone of inhibition of various concentrations of Aloe emodin.

Evaluation of dental implants

The results of physicochemical evaluation of the dental implants are given in Table 2. The formulated dental implants were found to be pale orange coloured, thin, clear, smooth, uniform and flexible with desired thickness for aloe emodin delivery to treat dental carries. The thickness of the films was almost uniform in all formulations and it was found to vary between 0.36 ± 0.002 and 0.38 ± 0.006. The weight of films varied from 5.3 ± 0.001 and 5.6 ± 0.016. By taking the initial weight of the films and the weight after keeping for three days in the desiccator, the percentage moisture loss was calculated for F1 to F6 formulations and was found in between 9 ± 1.36 to 15 ± 0.83 %. All six formulations were subjected to folding endurance studies which showed no cracks after 200 fold and all the formulations maintained integrity after folding and showed that the folding endurance is in the range of 225 to 300. The tensile strength of the films varied from 1.16 kg/cm^2 to 1.40 kg/cm^2. The formulation F6 was having highest tensile strength (1.40 kg/cm^2) might be due to the presence of ethyl cellulose and Eudragit RL-100 which gives supported strength to the film. All the formulations showed the surface pH around 7 which confirms the prepared films will not alter the pH of the gingival fluid in the periodontal pocket. For various formulations content uniformity was found to vary between 89.57 ± 1.68 and 95.24± 1.15. The percentage cumulative drug retained and cumulative percent drug release by the each film in the *in vitro* release studies were based on the mean content of drug present in the respective films.

Figure 4 shows the curves of cumulative percentage drug released as a function of time in days for six formulations. F6 showed better release compare to other formulations as the extent of release was maintained for 11 days and it was more sustained compared to other formulations might be due to the effective cross linking between ethyl cellulose and eudragit which forms a mesh with minute pores for the release of the drug. Polymers used above in the formulations act as resorbable carriers, they will readily dissolve in the pockets within 8-9 days, but F6 containing ethyl cellulose and eudragit which dissolved after 11 days leaving no residue after respective period.

Models with highest regression co-efficient were judged to be the most appropriate model for the dissolution rate. Hence the data of various models revealed that all formulation coded F1 to F6 follows peppas exponential model (Figure 5) which gives a linear graph with the correlation coefficient values of F1 0.9963, F2 0.9979, F3 0.9957, F4 0.9979, F5 0.9963 and F6 0.9964. The 'n' values for F1 to F6 were F1 1.0490, F2 1.0925, F3 1.0650, F4 1.0919, F5 1.0310 and F6 1.0331 respectively. Formulations F1, F5, F6 and F7 had 'n' value equal to 1 which indicates the drug transport mechanism as Case-2 transport, suggested the drug released through the polymer by Zero-order kinetics, while in formulations F2, F3 and F4, the 'n' value was greater than 1, indicated the release of the drug by Super Case II transport which suggests the release mechanism is dominated by the erosion and swelling of the polymer approximates, Fickain diffusion mechanism.

In vitro antibacterial activity studies demonstrated significant antibacterial profile of all the formulations. Among the formulated films of aloe emodin, F6 showed better zone of inhibition compared to other formulations against *streptococcus mutans* after incubation period (Figure 6).

Table 2: Physical characteristics of film.

Formulation Code	Thickness (mm) (n=3)	Average weight (mg) (n=3)	% moisture loss (%) (n=3)	Folding endurance	Tensile strength (kg/cm^2) (n=3)	Surface pH (n=3)	Drug content (%) (n=3)
F1	0.37±0.007	5.4±0.008	07±1.18	225	1.16±0.13	7.1±0.1	91.31±1.27
F2	0.38±0.006	5.3±0.011	08±1.24	245	1.32±0.26	7.0±0.1	94.45±1.23
F3	0.37±0.008	5.6±0.015	15±0.81	234	1.18±0.14	7.0±0.1	89.57±1.68
F4	0.36±0.006	5.5±0.009	13±1.21	274	1.26±0.18	7.0±0.1	92.17±1.45
F5	0.36±0.002	5.3±0.008	14±1.37	237	1.37±0.26	6.9±0.1	91.68±2.78
F6	0.37±0.005	5.6±0.016	11±0.93	260	1.40±0.21	7.0±0.1	95.24±1.15

Values are mean ± SEM (n=3)

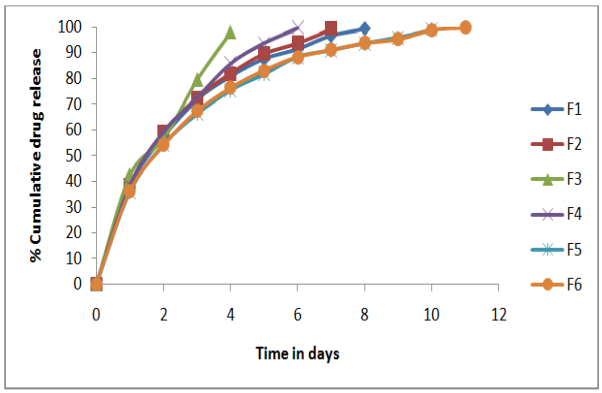

Figure 4: *In vitro* drug release profile of all formulations in phosphate buffer pH 6.6.

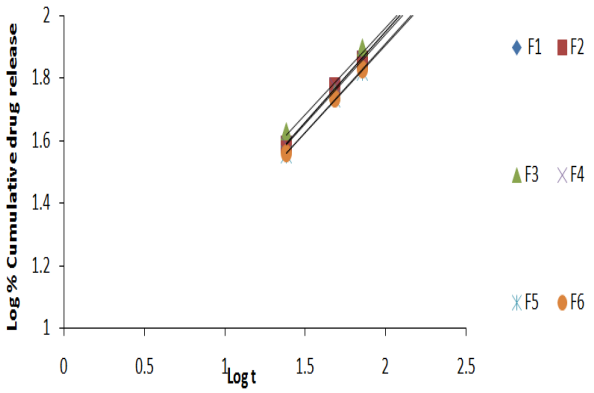

Figure 5: Plot of log % cumulative drug release v/s log of time (Kosermeyer-Peppas model).

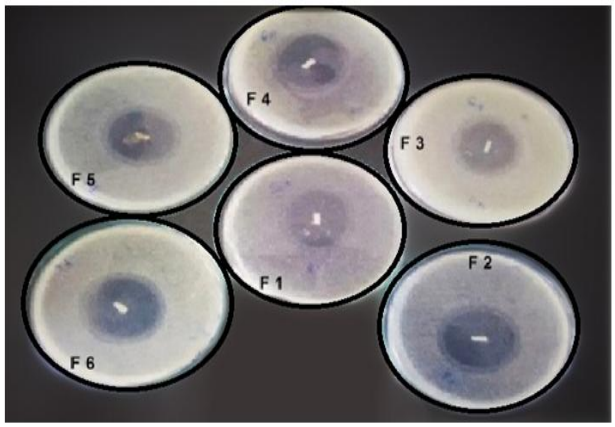

Figure 6: Zone of inhibition of dental implant formulations.

CONCLUSION

In this current work, dental implants of aloe emodin were successfully developed by solvent casting technique using degradable polymers which erode or dissolve in the gingival crevice. The formulated films exhibited acceptable physical characteristics with good flexibility, folding endurance, tensile strength. The formulated dental implants showed the sustained drug release up to 11 days after initial burst release. All the formulations showed bacteriostatic action against *streptococcus mutans* confirmed by zone of inhibition. The dental implants formulated by using polymer ethyl cellulose and co polymer eudragit showed the best results and found promising for sustained release-site specific delivery of the aloe emodin for the treatment of dental caries.

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