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Dental Implants of Cefuroxime axetil for the treatment of Periodontitis: A Technical Report

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ABSTRACT

Dental implant for the treatment of periodontitis was developed for site specific delivery of Cefuroxime axetil a broad spectrum antibiotic. Cefuroxime axetil implants were prepared by solvent casting technique using ethyl cellulose and other co-polymers (HPMC-K4M or Eudragit RL100) in chloroform: dichloromethane (1:1) solvent with glycerol as plasticizers. Drug excipient compatibility was studied using FTIR and DSC. The films were evaluated for their thickness uniformity, folding endurance, weight uniformity, content uniformity, surface pH, in-vitro drug release and in-vitro antibacterial activity. In-vitro drug release was subjected to curve fitting using different equations and kinetic models to reveal release kinetics. The implants made from EC and HPMC-K4M batch W2 containing EC(400 mg), HPMC-K4M (100mg) and glycerol (0.4 ml) showed best result in respect to physical properties, %drug content (98.44 %) and %drug release in 6 days (95.96%). The implants made from EC and Eudragit RL -100, batch W8 containing EC (500 mg), Eudragit RL -100 (100mg) showed best result with respect to physical properties, %drug content (98.00%) and percent drug release in 7 days (96.78%). The in vitro drug release data showed that implants shows initially burst release followed by prolonged release. In vitro antibacterial activity was studied on S. aureus and E. coli organisms. The zone of inhibition for all the batches were found to be effectively higher in 48 hrs and then declined. W2 and W8 formulations showed better antibacterial effects with higher zones of inhibition. Stability studies revealed that the drug remained intact and stable in the periodontal implants during storage.

Key words: Cefuroxime axetil, dental implant, drug excipient compatibility, *in-vitro* drug release, antibacterial activity, stability.

INTRODUCTION

Periodontitis are the group of conditions, which affect the supportive structures of the teeth[1]. Periodontitis is categorized depending on disease conditions such as chronic periodontitis aggressive periodontitis, disease-related periodontitis and acute necrotizing periodontal disease[2]. The development of periodontitis involve breakdown of the periodontal tissues, probably due to both direct effect of bacteria on the tissue and also the associated inflammatory response and the formation of the periodontal pocket between the surface of the tooth and the soft tissues. The periodontal pocket provides diverse environment for the colonization of micro-organism. The bacteria accumulate in the periodontal pocket that develops between the roots of affected teeth and soft tissues[3]. If the disease is allowed to progress, increased tooth mobility and possibly tooth loss may result.

Periodontal diseases are treated by antibiotics given by systemic route or by the local delivery system. Antibiotics are usually given to supplement the beneficial effects of scaling and root canaling, a common treatment for periodontal disease. Systemic administration has been useful in treating periodontal pockets, but repeated and long term use of systemic drugs is fraught with potential danger including resistant strains and superimposed infections. These drawbacks can be markedly reduced if antimicrobial agent to be used is applied locally. Concentration of drug in tissues can be enhanced by incorporating the active agent into controlled release delivery system and placing them directly in to periodontal pocket[4]. A local drug delivery system delivering the therapeutic agent at sufficient levels inside the pocket and at the same time minimizing the side effects associated with systemic drug administration.

Cefuroxime axetil is a semisynthetic, broad-spectrum cephalosporin antibiotic[5] presently it is available commercially in the form of oral tablets and capsules. In this study periodontal implants of cefuroxime axetil with rate controlling polymers were developed with an aim to prolong the antibacterial activity directly at the site of infection.

MATERIALS AND METHODS

Material

Cefuroxime axetil was obtained as gift sample from Macleods Pharmaceuticals Pvt. Ltd., Mumbai, India. Ethylcellulose and Hydroxy Propyl Methylcellulose (HPMC K4M) were obtained from Loba Chemie Pvt. Ltd., Mumbai. Eudragit RL- 100 was obtained from Evonik Degussa India Pvt Ltd. Mumbai. Other materials used in the study were of analytical grade.

Methods

Drug Excipient Compatibility:

FTIR analysis

Physical mixture comprising of drug and polymers in a ratio of 1:1 were dispensed in a 2 ml vial. The sample was stirred using the whisk and shaker systems and stored at 60ºC for 6 days to accelerate the interactions between drug and excipients[6].

DSC analysis

Differential scanning calorimetry (DSC) analysis was performed for pure drug, Ethycellulose (EC) along with EudragitRL-100 or HPMCK4M physical mixtures using a DSC, Shimadzu TA 60WS, instrument. 1:1 physical mixture of drug and excipientS were mixed thoroughly for 5 min in mortar. The materials were then stored at 40±1°C, 75% relative humidity for 4 weeks. Each sample was accurately weighed (~1-3 mg) in an aluminum pan, crimped, and hermetically sealed, while an empty pan of the same type was used as a reference. The system was calibrated with high purity sample of indium. The samples were scanned at the heating rate of 20^0C/min over a temperature range of 100 to 300^0C under the nitrogen atmosphere[7].

Preparation of implants containing Cefuroxime axetil

Periodontal implants were prepared by solvent casting technique. Borosilicate glass moulds (10 sq. cm) were used for casting of the implants. Formulations were designed using EVOP method, varying amount of ethylcellulose was used in combination with different co-polymers. Films were prepared by dissolving ethylcellulose with co-polymers (Eudragit RL-100 and HPMC K4M,) in chloroform and dichloromethane (1:1) solution, using glycerol as plasticizer (Table-1). Cefuroxime axetil was added in to the polymeric solution and mixed homogenously using magnetic stirrer in a closed beaker. After complete mixing 10 ml of the solution was poured into the clean Borosilicate glass moulds. The solvent was allowed to evaporate slowly by inverting a glass funnel with a cotton plug closed into the stem of the funnel at room temperature for 24 hours. After complete evaporation of solvent, cast films were obtained, which were then cut into pieces of 0.5 X 0.5mm, wrapped in an aluminum foil and stored in a desiccator at 25°C ±2°C temperature in a dark place for further evaluation.

Table 1- Batches of medicated implants

Sr. No	Batches	Drug (mg)	EC (mg)	HPMC (mg)	Eudragit RL-100 (mg)	Glycerol (ml)
1	W1	10	300	100	-----	0.4
2	W2	10	400	100	-----	0.4
3	W3	10	500	100	-----	0.4
4	W4	10	600	100	-----	0.4
5	W5	10	700	100	-----	0.4
6	W6	10	300	-----	100	0.4
7	W7	10	400	-----	100	0.4
8	W8	10	500	-----	100	0.4
9	W9	10	600	-----	100	0.4
10	W10	10	700	-----	100	0.4

Evaluation of polymeric dental implants

The implants were evaluated for the parameters mentioned below, as the case applied respectively.

Thickness

The thickness of the implant was measured by micrometer screw gauge (Acculab®) with least count (L.C.) of 0.01mm. An average of five values determined at 5 different points on the film was calculated[8].

Weight variation
Uniformity in the weight the implant was determined. Five implants of $1cm^2$ each were weighed on an electronic balance and the mean weight was recorded[8].

Appearance
The Implants were visually inspected for any change in colour and physical form or appearance[9]

Flatness
Three centimeter longitudinal strips were cut out from each film, one from the centre and two from either side. The length of each strip was measured and the variation in length if any due to non-uniformity in flatness was measured by determining percent constriction, 0% constriction was considered equivalent to 100% flatness[10].

$$\% \text{ constriction} = \frac{L_1 - L_2}{L_2} \times 100$$

Where, L_1 =initial length, L_2= final length of each strip.

Surface pH
Implants were left to swell for 1 hour 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 dish to solidify at room temperature. The surface pH was measured in triplicate by means of pH paper placed on the surface of the swollen film[8].

Folding endurance
The folding endurance is expressed as the number of folds (number of times the film is folded at the same place, either to break the specimen or to develop visible cracks).This test is important to check the ability of the sample to withstand folding. This also gives an indication of brittleness. The specimen was folded in the center, between the 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 center of film. The total folding operations were named as folding endurance value[9].

Percentage moisture loss
Implants were kept in a desiccator containing anhydrous calcium chloride for three days. After three days, the implants were taken out and re-weighed; the percentage moisture loss was calculated using following equation[9]

$$\text{Percentage moisture loss} = \frac{\text{Initial wt-Final wt}}{\text{Initial wt}} \times 100$$

Drug content
Drug content uniformity in implants was determined. $1cm^2$ implant was placed in volumetric flask containing 10 ml of ethanol; the flask was vigorously shaken to extract the drug from the implant[8]. 1 ml of resulting solution was taken and diluted to 100 ml with phosphate buffer pH 6.8. The absorbance of the solution was measured spectroscopically at 281 nm. The polymeric

solution without drug served as blank. In case of HPMC films a mixture of ethanol-dichloromethane were used. The drug content was studied in triplicate and the mean reported.

***In vitro* drug release**

Static dissolution method reported in the literature was adopted[8]. Implants of known weight and dimensions (0.5 cm^2) were placed separately into vials containing 1 ml of pH 6.8 phosphate buffer. The vials were kept at 37 ºC for 24 hrs. The buffer was drained off and replaced with fresh 1 ml phosphate buffer of pH 6.8 after 24 hrs. The concentration of drug in the buffer was measured at 281 nm using UV spectrometry. The procedure was continued every 24hr for 6 to 7 days.

***In vitro* antibacterial activity**

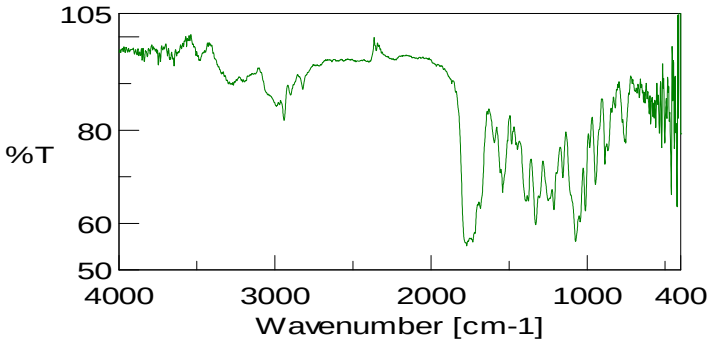
Nutrient agar was prepared and sterilized by autoclave under aseptic condition and the medium was transferred to sterile Petri plates. After the solidification of nutrient agar medium, they were inoculated with 0.1 ml of microorganism i.e. S.aureus and E.coli in separate Petri plates and implants (0.5 cm^2) were placed and the plates were incubated for 48 hrs at 37 ºC. The zone of inhibition observed after incubation was measured. The implants was replaced over fresh plates and subsequent zone of inhibitions were measured this procedure was continued for six days[11]. Drug solution 500 µg/ml was prepared and also subjected to *in-vitro* antibacterial studies as mentioned above.

Release kinetic studies

In order to understand the mechanism and kinetics of drug release, the data obtained from the *in-vitro* drug release studies were fitted in various release kinetic equations[13] such as zero order, first order, Hixon Crowell model, Higuchi matrix model and Peppas- Korsmeyer equation and the best fit model was determined using PCP disso V3 software.

Accelerated stability studies

The stability of the implants was studied at 40ºC± 5°C with RH 75%± 5%. The implants of size (0.5 cm^2) were weighed and wrapped in aluminum foil and placed in petri plates. These containers were stored for a period of three months. All the implants were observed for any physical changes, such as color, appearance, flexibility, or texture[12]. The drug content and *in vitro* drug release was estimated at an interval of each month.



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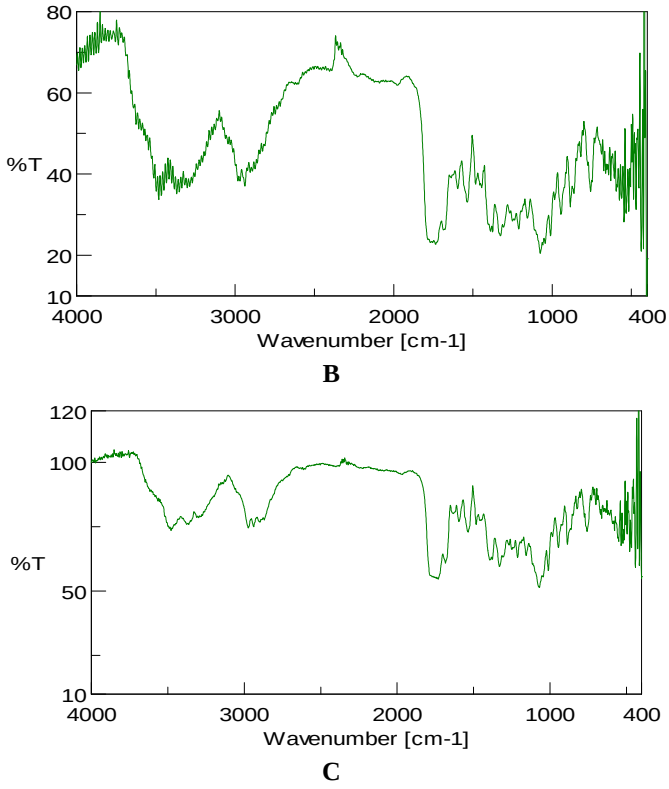


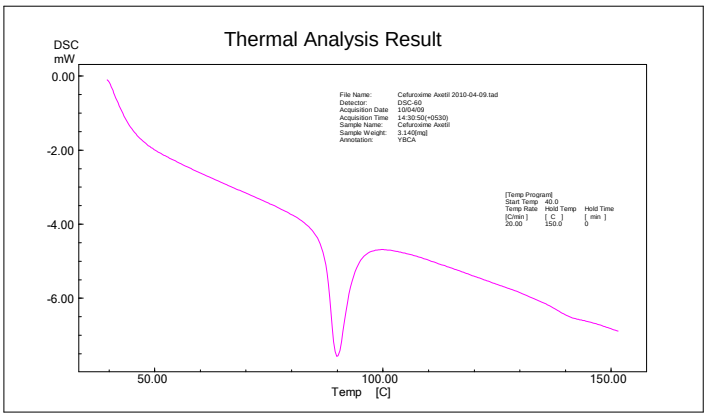
Fig 1. (A). FTIR spectrum of cefuroxime axetil (CA),
(B). FTIR spectrum of CA+EC+HPMC
(C). FTIR spectrum of CA+EC+Eudragit RL100.

RESULTS AND DISCUSSION

The FTIR spectra of individual compound and their physical mixtures indicate that no chemical interaction only a physical interaction takes place between them. These observations are based on the fact that all the characteristic peaks of CA remained unaltered (Fig 1(A), (B)&(C)).

Differences between the DSC thermogram of the pure drug and the blends were noted and may be attributed to the sample geometry effects and reduction in purity caused due to effect of mixing of components (Fig 2.(A), (B)&(C)).

The prepared dental implants were translucent and smooth surfaced with good tensile tensile strength. The procedure developed to prepare implants was reproducible. All the batches exhibited uniform thickness with minimum standard deviation (±0.007 to ±0.11) Weight variation of batches W1 to W10 was in the range of 4.2 to 5.8 mg with standard deviation within 1.0. An acidic or alkaline formulation causes irritation to the periodontal pocket[8] and hence this parameter assumes significance while developing local delivery system.



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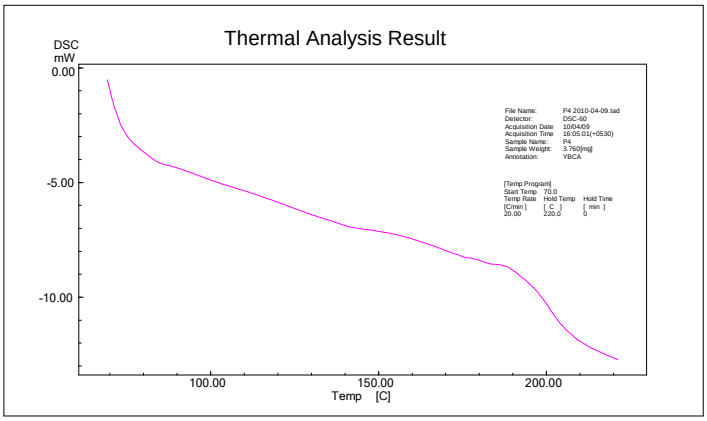

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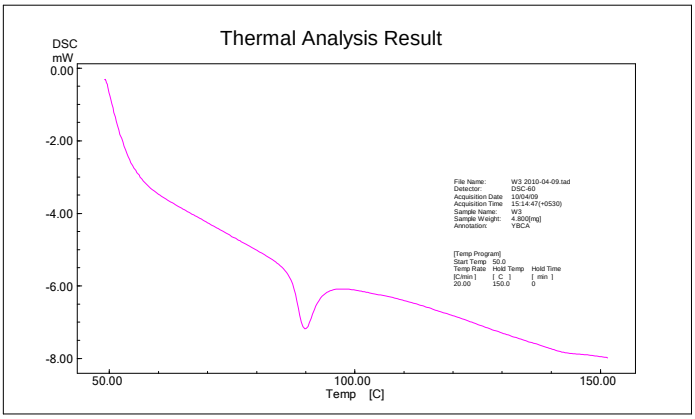

C

Fig 2. (A) DSC thermogram of cefuroxime axetil(CA)
(B) DSC thermogram of CA+EC+HPMC
(C) DSC thermogram of CA+EC+Eudragit RL 100.

The surface pH of the prepared batches was in the range of 6 to7 which indicates that there is no risk of irritation. Folding endurance test ensures the tensile strength of the implant. Higher folding endurance of implants exhibit good physical and mechanical properties. The batches W1 to W3 and W6 toW8 showed folding endurance above 100 these implants have good physical

and mechanical properties. Implants from batches W4, W5, W9 and W10 showed less folding endurance as compared to the other batches because of the higher solid content in implants. % moisture loss for all the batches was observed in the range of 8.6% to 10.2%. With an increase in the EC concentration, percent moisture loss decreases this may be due to the hydrophobic nature of EC[9]. Percentage drug content of batches W1 to W10 was found to be in the range of 88.13 % to 98.44% with minimum standard deviation. (Table2a and 2b)

Table 2a - Evaluation of medicated implants for physical parameters

Sr. No.	Batches	Thickness (mm)± S.D	Weight variation (mg) ± S.D	Appearance	% flatness	% constriction
1	W1	0.332 ± 0.0083	4.638 ± 0.0526	+++	100.00	0.00
2	W2	0.352 ± 0.0083	4.412 ± 0.0549	+++	100.00	0.00
3	W3	0.372 ± 0.0130	4.920 ± 0.0494	+++	100.00	0.00
4	W4	0.420 ± 0.0070	5.320 ± 0.0484	++	96.66	3.33
5	W5	0.454 ± 0.011	5.716 ± 0.0634	++	93.33	6.66
6	W6	0.350 ± 0.11	4.360 ± 0.0254	+++	100.00	0.00
7	W7	0.364 ± 0.0158	4.738 ± 0.030	+++	100.00	0.00
8	W8	0.380 ± 0.0158	4.848 ± 0.031	+++	100.00	0.00
9	W9	0.446 ± 0.0151	5.454 ± 0.024	++	94.34	5.66
10	W10	0.478 ± 0.0130	5.612 ± 0.034	++	96.00	4.00

++ Corresponds to satisfactory uniform appearance, +++ Corresponds to good uniform appearance.

Table 2b - Evaluation of medicated implants for other parameters

Sr. No.	Batches	Surface pH	Folding endurance	%moisture Loss ± S.D	Mean % drug content ± S.D
1	W1	6-7	>100	10.2 ± 0.152	96.44 ± 0.654
2	W2	6-7	>100	9.8 ± 0.100	98.44 ± 0.260
3	W3	6-7	>100	9.73 ± 0.150	95.75 ± 0.397
4	W4	6-7	76	9.6 ± 0.260	91.50 ± 0.794
5	W5	6-7	70	9.2 ± 0.200	89.43 ± 0.789
6	W6	6-7	>100	9.63 ± 0.150	94.62 ± 0.980
7	W7	6-7	>100	9.33 ± 0.050	93.15 ± 0.654
8	W8	6-7	>100	9.16 ± 0.110	98.00 ± 0.397
9	W9	6-7	63	9.03 ± 0.115	94.62 ± 0.98
10	W10	6-7	59	8.6 ±0.200	88.13 ± 0.395

In vitro drug release studies of implants were carried out in pH 6.8 phosphate buffer. The percent drug release for the all batches varied from 81.39 to 96.78 %. From the study it was found that the drug release was more sustained i.e. for 7 days in case of the implants made from the EC and Eudragit RL-100 (W6 to W10). In case of the implants made from EC and HPMC (W1 to W5) drug release was sustained for 6 days .All the batches showed initial burst release and prolonged release in the later phase. In case of the implants made from the EC and HPMC faster drug release was observed from the batch W2 i.e. 95.96% in 144 hours or 6 days and amongst implants made from the EC and Eudragit RL-100 faster drug release was observed from the batch W8 i.e. 96.78% in 7 days.

Table 3 - *In vitro* drug release from batches prepared with EC and HPMC

Sr.No	Time (hours)	Cumulative % drug release for different batches				
		W1	W2	W3	W4	W5
1	24	23.34	29.42	21.25	17.77	17.25
2	48	41.47	46.96	39.12	33.82	32.32
3	72	56.98	62.62	54.51	49.05	46.88
4	96	69.90	75.04	67.11	62.31	59.66
5	120	80.76	85.95	77.79	74.65	71.41
6	144	90.68	95.96	87.71	84.61	81.39

Table 4 - *In vitro* drug release from batches prepared with EC and Eudragit RL-100.

Sr. No.	Time (hours)	Cumulative % drug release for different batches				
		W6	W7	W8	W9	W10
1	24	17.63	17.77	19.32	17.61	15.68
2	48	33.46	34.65	37.02	32.71	30.29
3	72	48.44	49.69	52.21	46.54	43.53
4	96	61.11	62.76	65.13	59.13	55.54
5	120	71.79	74.01	77.36	69.32	65.56
6	144	81.65	83.92	87.80	79.19	75.28
7	168	89.76	92.04	96.78	87.29	83.35

The release mechanisms of cefuroxime axetil from various batches were studied the data was treated to the best linear fit model & it was found that all batches showed best fit model for Korsmeyer- peppas model

$$Q_t / Q_\infty = Kt^n$$

Where Q_t is the amount of drug dissolved in time t and n is diffusion coefficient which is indicative of transport mechanism, this model describes the fraction of drug release relates exponentially with respect to time[13].

The R values obtained for all the batches after curve fitting with Korsmeyer- peppas equation were in the range of 0.9974 to 0.9994. The n values were found to be between 0.5 and 1.0, the mechanism of transport was Anomalous transport[13]. k values were between 1.04 to 3.60 the highest k value was observed for W2 (3.61) which indicated a higher initial burst release from this formulations.

In-vitro antibacterial activity was performed on *S.aureus* and *E.coli* organisms. The zone of inhibition for all the batches were found to be higher in 48 hrs then the zone of inhibition observed subsequently between 48 to 96 hours and 96 to 144 hours respectively. Higher zone of inhibition in 48 hrs may be due to the initial burst release from the implants[9]. W2 showed highest zone of inhibition compared to other formulations (Table 5 & Table 6).

W2 and W8 were selected as the best batches as they showed good physical and mechanical characters, drug release and antibacterial activity.

These implants were subjected to stability studies. The implants were observed for physical and chemical parameters. Appearance did not change during the period of study, surface pH remained between 6 and 7 and folding endurance was observed to be more than 100. Drug content after the 3 months storage was within limits and there was no significant change. Drug release from W2 was 94.102 % after 6 days and from W8 it was 95.346% after 7 days when observed after the stability test period of 3 months, in comparison to initial drug release of 95.96 102 % after 6 days and 96.78 % after 7 days respectively. Thus the formulations were found to be stable.

Table 5- *In vitro* Antibacterial activity on S.aureus

Sr.No.	Batches	Zone of inhibition (mm)		
		48 hrs	96 hrs	144 hrs
1.	Drug solution	22	-	-
2.	W1	17	15	11
3.	W2	19	16	12
4.	W3	16	13	10
5.	W4	14	11	9
6.	W5	14	11	9
7.	W6	15	13	11
8.	W7	16	15	13
9.	W8	17	16	14
10.	W9	16	14	13
11.	W10	14	13	11

Table 6- *In vitro* Antibacterial activity on E.coli

Sr.No.	Batches	Zone of inhibition (mm)		
		48 hrs	96 hrs	144 hrs
1.	Drug solution	25	-	-
2.	W1	16	14	10
3.	W2	20	17	13
4.	W3	17	14	11
5.	W4	15	13	11
6.	W5	15	12	10
7.	W6	15	13	12
8.	W7	17	16	14
9.	W8	18	16	15
10.	W9	16	14	13
11.	W10	15	14	12

CONCLUSION

Periodontal implants containing Cefuroxime axetil were prepared. *In vitro* release studies revealed that Cefuroxime axetil can be incorporated in a sustained release device with initially burst release followed by prolonged release, for the treatment of periodontitis. FTIR data shows there is no significant chemical interaction between the drug and polymers. Stability studies shows that the drug remained intact and stable in the periodontal implants during storage. The dental implants prepared by solvent casting technique containing EC (500 mg), Eudragit RL-100 (100mg) and glycerol (0.4 ml) i.e W8 was the best formulation and found to be promising for

local delivery of Cefuroxime axetil for the treatment of periodontitis. The study need be continued for prospective investigations required to establish *in-vivo* efficiency of the implants.

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