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RESEARCH ARTICLE

# Formulation design and *in vitro* characterization of clotrimazole dental implant for periodontal diseases

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**Abstract:**
Clotrimazole is an imidazole derivative novel broad spectrum antimicrobial agent extensively used topically for fungal infection. The purpose of this study is to design and develop *in situ* implants containing clotrimazole that could be used in the treatment of periodontal diseases by direct periodontal intrapocket administration. The dental film of clotrimazole was prepared by solvent casting technique using hydroxyl propyl methyl cellulose, ethyl cellulose, hydroxyl propyl cellulose and chitosan in different concentrations as biodegradable rate controlling polymers. The drug polymers interaction was studied by Fourier Transform Infrared Spectroscopy (FTIR) and study suggesting no interaction between drug and polymers. The implants were characterized for weight variation, thickness, surface pH, tensile strength, folding endurance, moisture content, viscosity, drug content uniformity, *in vitro* drug release, mass balance, drug release kinetic, stability and *in vitro* antibacterial activity studies. Mean weight data showed that the different films were uniform. Minimum thickness was obtained with film containing chitosan. Almost all dental film formulations having satisfactory tensile strength. Good physicochemical properties were shown by the films. *In vitro* drug release data indicate that the films showed an initial burst release followed by sustained release of the drug(s). *In vitro* drug release rate for selected dental implant formulation (F6, containing 4.5 % w/w of chitosan) was found to sustain clotrimazole over 10 h obeying zero order kinetic. The stability study did not show any significant changes. The study suggesting the film containing chitosan is a potential drug delivery device for the topical treatment of periodontal diseases.

**Keywords:** Clotrimazole, periodontitis, dental implant, antimicrobial activity, *Streptococcus mutans.*

## Introduction

Periodontal disease includes 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 and periodontal pocket formation. This pocket provides an ideal environment for the growth and proliferation of anaerobic pathogenic bacteria[2].

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[4,5]. The use of drug delivery systems based on biodegradable polymers that can achieve a sustained release of the drug over days had been used in the treatment of periodontal diseases[6,7], with the advantages of self-elimination, avoiding the need to

remove the polymer system from the site of implantation after its use[8,9].Clotrimazole is an imidazole agent which binds to phospholipids in the fungal cell membrane altering cell wall permeability[9,10]. It is practically soluble in dichloromethane, alcohol, acetone, chloroform and methyl alcohol. It is poorly absorbed orally, its protein binding is 90%, it metabolizes in the liver and the half life of the clotrimazole is 2 h[11]. On conventional use at large dose shows adverse effects like Nausea, vomiting, unpleasant mouth sensations, allergic reactions and purities[12].

Clotrimazole is available in the market as a conventional dosage forms such as tablets, powders, ointments and parenterals for the treatment of microbial infections but not suitable means for the treatment of infection locally. This prompted us to design and develop periodontal films containing clotrimazole with rate controlling polymers for local and systemic treatment of periodontal diseases.

## MATERIALS AND METHOD

Clotrimazole was obtained as gift sample from Micro Lab. Pvt. Ltd., Bangalore, India. Ethyl cellulose, Hydroxy Propyl Cellulose (HPC) and Hydroxy Propyl Methylcellulose (HPMC K4M) were obtained from Loba Chemie Pvt. Ltd., Mumbai., India. Chitosan (Deacetylated with viscosity of 8000 to 11000 cps) was procured from Central Institute of Fisheries Technology, Kochi, India. All other chemicals used were of Analytical grade and procured from authorized dealer.

### Preparation of dental film containing clotrimazole

Periodontal films were prepared by solvent casting technique[13,14], in which ethyl cellulose, HPMC K4M, HPC (Alone or in combination) and chitosan (Alone) was taken as biodegradable polymer. All polymers except chitosan were dissolved completely in chloroform and dichloromethane (1:1) solvent system, using dibutyl phthalate plasticizer. Clotrimazole was added in to the polymeric solution and mixed homogenously using magnetic stirrer in a closed beaker. After complete mixing of drug and polymer, 10 ml of the clear solution was poured into the clean petridish (Anumbra® area 60.8 square cm approximately) placed in horizontal plane. The solvent was allowed to evaporate slowly by inverting a glass funnel with a cotton plug closed into the stem of the funnel on petridish at room temperature for 24 hours. After complete evaporation of solvent, cast films were obtained, cut into pieces of 7×2 mm, wrapped in an aluminum foil and stored in a $CaCl_2$ desiccator at room temperature in a dark place for further evaluation studies.

### *Preparation of clotrimazole loaded chitosan films with cross linking*

Chitosan was soaked in acetic acid (1% v/v in water) for 24 h to get a clear solution, which was later filtered through a muslin cloth to remove undissolved polymer (chitin). Clotrimazole was added in to polymeric solution and vortexed (Talboys, Standard Vortex Mixer) for 15 min, to dissolve the drug in the chitosan solution. The viscous dispersion was kept aside for 30 min for complete expulsion of air bubbles. Films were cast by pouring the dispersion into the clean petridish (Anumbra® area 60.8 square cm approximately) placed in horizontal plane and allowed to dry at room temperature for 24 h[15]. The dry films were cut into strips of 7 × 2 mm, wrapped in aluminum foil and stored in a calcium chloride desiccator at room temperature for further processing. The films were cross linked by exposure to glutaraldehyde vapor in a chromatography chamber. The chamber was previously saturated with the vapour of 2% v/v glutaraldehyde for 24 h. The films were exposed to the vapour for 2 and 4 h respectively, and then dried[16]. The dried films were wrapped in aluminium foil and stored in a calcium chloride desiccator for further study.

### Drug polymer compatibility study by FTIR

The FT-IR (Shimadzu IR spectrophotometer, model 840, Japan) was used for these IR analyses in the frequency range between 4000 and 600 $cm^{-1}$ and at 1 $cm^{-1}$ resolution[17]. The samples of pure drug clotrimazole, polymers and drug polymer implant formulations were prepared separately by palletization technique in KBr using IR press. The IR peaks of pure pioglitazone were analyzed and were compared with the peaks (Spectra) obtained from FTIR spectra of polymers and drug polymer implant formulations.

### Characterization of clotrimazole dental implants

#### *Uniformity of weight of the films*

Twenty films of same size (7×2 $mm^2$) were weighed on an electronic digital balance (Sartorius Electronic balance, BT-2245, Calcutta, West Bangle, India). Average weight and weight variation were calculated[15]. All the experimental units were studied in triplicate, mean and standard deviation were calculated.

#### *Thickness uniformity of the films*

Thickness of the polymeric film (1×1 $cm^2$) was measured by using a film thickness tester (Model Mitutoyo 4026F, Tokyo, Japan) at different areas of the film (n = 3) was determined and the mean and standard deviation were calculated[18].

#### *Film surface pH*

Periodontal films were left to swell for 1 hour on the surface of the agar plate. The surface pH was measured by means of pH paper placed on the surface of the swollen film. All the experimental units were studied in triplicate, mean and standard deviation were calculated[19].

***Tensile strength measurement of films***

Tensile strength is the maximum stress applied to a point at which the film breaks. The mechanical properties of film were determined by Instron Universal Tensile Strength Measurement Instrument (Model 2046, Instron Pvt. Ltd., Japan) with a 5 Kg load cell. Film free from air bubble and physical imperfection was held between two clamps separated by a distance of 3 cm. Film was pulled by upper clamp at a rate of 100 mm/min, tensile strength and percentage elongation were measured in triplicate when film broke from the following equations[20],

Tensile strength = Breaking Force (N)/ Initial cross sectional area of film ($mm^2$) …… (1)

Elongation (%) = (Increase in length/ original length) × 100 ……. (2)

***Folding endurance***

This parameter was determined in triplicate by repeatedly folding a small strip of film 2 × 2 $cm^2$ at same place till film broke and standard deviation was calculated[16].

***Moisture content (Loss) study***

The film of known weight and of predetermined size 2 × 2 $cm^2$ was placed in desiccator containing anhydrous Calcium chloride, after three days film was reweighed and moisture loss was calculated by using following equation[14],

Moisture loss (%) = ($W_i$ - $W_f$/ $W_i$) × 100 ……. (3)

Where, $W_i$ is initial and $W_f$ is final weight of film. Study is done in triplicate for each film formulation.

**Viscosity measurement**

Aqueous solutions containing both polymers and plasticizer were prepared in the same concentration as that of films. Viscosity was measured at 20 rpm at room temperature using Digital Brookfield viscometer[13] (Model DV-II, Brookfield Engineering Laboratories, Mumbai, India) attached to the helipath spindle number 18. The recorded values were mean of three determinations.

***Drug content uniformity study***

Strip of 7×2 $mm^2$ size was taken from different areas of the film and placed in to a 10ml volumetric flask containing 10 ml of ethyl alcohol and kept aside till the film is completely dissolved. From the volumetric flask, 1ml of solution was withdrawn and diluted up to 10 ml with phosphate buffer pH 6.6. The absorbance of the solution was measured at $\lambda_{max}$ 264 nm using UV-Visible spectrophotometer (Shimadzu UV spectrophotometer, model 1700, Japan) against the polymeric solution without drug serve as blank. In case of HPMC K4M and HPC films, combination of water and alcohol was used as solvent system to dissolve the films[21]**.** All the experimental units were studied in triplicate.

***In vitro drug release study***

Static dissolution model was adopted as the films will remain immobile in the periodontal pocket[21]. Sets of three films of known weight and dimension were kept in 1ml simulated gingival fluid i.e. phosphate buffer of pH 6.6, in three different test tubes. The tubes were sealed and kept at 37 ± 0.5 °C for 10 days. In interval of one day, the buffer was drained off and replaced with fresh phosphate buffer of pH 6.6. The concentration of drug in solution sample in each day was measured using UV-Visible spectrophotometer at $\lambda_{max}$ 264 nm. All the experimental units were studied in triplicate.

***Mass balance study***

Following the *in vitro* drug release study, the test films were further analyzed for the drug content left in the strip. Each strip was dissolved in 1% v/v acetic acid and suitably diluted. The amount of drug released in to the dissolution medium plus residual drug content in the film were accounted and compared with the actual drug content[16].

***Drug release kinetic study***

In order to study the exact mechanism of drug release from the tablets, drug release data was analyzed according to zero order[22], First order[22], Hixson-Crowell eqution[22] and Higuchi square root[23]. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test.

***Accelerated stability study***

The stability of the entire drug loaded polymer films (Selected formulation F6) of size 7 × 2 $mm^2$ were weighed in 3 sets (12 strips in each set). The films were wrapped individually in aluminium foil, also in butter paper and placed in petridishes. These containers were stored at temperature (25±2), (37±2) and (45±2) °C for a period of 3 months. All the polymeric films were observed for any physical changes such as colour, appearance, flexibility or texture, pH, viscosity and the drug content (Spectrophotometrically) was estimated at interval of 1 week[24].

***In vitro antifungal activity***

*In vitro* antifungal activity was performed on all film formulations by placing the film, cut in to 0.5 × 0.5 $cm^2$, on agar plates seeded with the oral fungus *Candida albicans*. After 48 h of incubation at 37°C, the films were transferred to freshly seeded agar plates and incubated for an additional 48 h. This procedure was repeated until no inhibition of fungal growth was detected on the agar plate. The growth inhibition zone on the agar plate was measured using 'Hi Antibiotic Zone Scale'[25].

***Statistical analysis***

Statistical data analyses were performed using the mean, standard deviation, standard error of mean and one way ANOVA at 5 % level of significance $p < 0.05$[26,27].

## RESULTS AND DISCUSSION

FT-IR spectrum of clotrimazole alone and in combination with polymers was studied. FT-IR spectrum of the clotrimazole and the drug-polymer mixture have characteristic bands at 1723 $cm^{-1}$ (carbonyl group), 2935 $cm^{-1}$ (aromatic C-H stretching), and 3275.5 $cm^{-1}$ (O-H group of carboxyl moiety) indicating that clotrimazole is not involved in any chemical reactions with the polymers used (Fig 1). In the present study, periodontal films of clotrimazole were formulated using the polymer matrix of Ethyl cellulose and the effect HPMC K4M, HPC and chitosan as rate-controlling polymers.

The prepared implants were found to be good in color, clear, translucent and flexible in nature. The solvent casting method developed to prepare the films was reproducible. The results of the physicochemical evaluations are presented in Table 2. The films of all the batches were found to be of uniform weight, ranging from 4.57±0.2 to 4.68 ± 0.1 mg. the thickness of films was ranges from 0.49±0.2 to 0.85±0.4. The surface pH of all the films was found to be neutral and hence no periodontal pocket irritation is expected. The viscosities of the solutions were ranging from 4.9±0.9 to 16.6±0.2 cps for films F1 to F6. Viscosity of the film F1 solution was more when compared to other films, could be due to complete solubility of polymers in Chloroform and Dichloromethane (1:1) mixture. Folding endurance of the films was > 100 times indicate that the formulations have good film properties. Content uniformity studies of the films shows that the drug was uniformly dispersed and recovery was possible to the tune of 94.7 to 99.6 % for formulations F1 to F6 (Table 2). The tensile strength of all drug-loaded films was studied (Table 2). The effective cross linking was observed on addition of hydroxyl propyl methyl cellulose as a copolymer with ethyl cellulose, which also shows higher tensile strength when compared to all other formulations. The tensile strengths of films were in the order of F3 > F6 > F4 > F5 > F2 > F1. *In vitro* release studies of Chlotrimazole was carried out in pH 6.6 phosphate buffer for 10 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 Chlotrimazole (MIC ≤ 2 µg/ml). *In vitro* release studies shows that the drug release was more sustained in case of film F6 followed by F4 > F2 > F1 > F5 > F3. The regression values of films F1, F2, F4 and F6 are higher with zero order and therefore the release kinetics followed zero order from all the films signifying that the release rate of drug are independent of initial concentration of drug. The release data of Chlotrimazole films (F1 to F6) were given in Table 2 and Fig 2. Hixson Crowell cube root law and Higuchi's model were applied to test the release mechanism. The $R^2$ values are higher for Higuchi's model compared to Hixson Crowell cube root law for all the films except film F5. Hence Chlotrimazole release from all the films followed diffusion rate controlled mechanism. Data are as shown in Table 3. *In vitro* antibacterial activity was performed as mentioned in the methodology on *Candida albicans*. The zone of inhibition of the prepared formulations was found to be effectively higher in 48 h. The study indicates that the formulated polymeric films containing Chlotrimazole retained their antifungal activity. Ageing studies performed on all prepared periodontal films. It was found that in the stability study, the drug loss is less (Table 4), though the films were stored for three months. The films were also observed for their appearance and texture. These properties like viscosity and pH did not change in films during the period of study, signifying that film formulation F6 would be stable during storage period.

## CONCLUSION

*In vitro* characterization studies revealed that Clotrimazole can be incorporated in a slow release device for the treatment of periodontitis. Formulation F6 has achieved the targets of present study as prolonged zero order release and reduction in frequency of administration, thus improve patient compliance. Hence Clotrimazole dental implant using chitosan would be use for successful management of periodontitis diseases. Further, detailed investigation is required to establish *in vivo* efficiency of these films.

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**Table 1. Formulation design of clotrimazole dental implants containing biodegradable polymers.**

| Ingredients (%) | Formulation code | | | | | |
|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 |
| Ethyl Cellulose 10 cps | 10 | -- | 9.5 | 0.5 | -- | -- |
| HPMC K4M | -- | 10 | 0.5 | -- | -- | -- |
| HPC | -- | -- | -- | 9.5 | 10 | -- |
| Chitosan | -- | -- | -- | -- | -- | 4.5 |
| Dibutyl phthalate* | 50 | 50 | 50 | 50 | 50 | 50 |
| Clotrimazole* (%) | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |

* indicates percentage of total polymer weight. HPC - Hydroxy Propyl Cellulose.

**Table 2. Physicochemical characterization of various clotrimazole dental implant formulations.**

| Evaluation parameters | Formulation code | | | | | |
|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 |
| Weight (mg) (X±SD) | 4.63±0.1 | 4.57±0.2 | 4.68±0.1 | 4.58±0.3 | 4.60±0.2 | 4.61±0.1 |
| Thickness (mm) (X±SD) | 0.69±0.3 | 0.85±0.4 | 0.84±0.4 | 0.71±0.3 | 0.75±0.2 | 0.49±0.2 |
| pH (X±SD) | 6.9±0.12 | 7.1±0.09 | 6.8±0.08 | 6.9±0.10 | 6.7±0.11 | 7.2±0.12 |
| Tensile strength ($Kg/mm^2$) (X±SD) | 1.3±0.65 | 1.5±0.53 | 1.9±0.93 | 1.6±0.87 | 1.5±0.76 | 1.7±0.44 |
| Elongation (%) (X±SD) | 6.2±0.17 | 5.8±0.12 | 4.6±0.18 | 4.7±0.11 | 6.3±0.10 | 3.3±0.16 |
| Folding endurance (X±SD) | 150±0.4 | 112±0.6 | 184±0.4 | 123±0.5 | 132±0.8 | 105±0.7 |
| Moisture loss (%) (X±SD) | 8.7±0.48 | 11.6±0.5 | 8.4±0.41 | 11.0±0.4 | 9.3±0.51 | 3.5±0.48 |
| Viscosity (cps) (X±SD) | 16.6±0.2 | 4.9±0.9 | 10.6±0.8 | 11.4±0.3 | 10.8±0.7 | 8.4±0.5 |
| Drug content (%) (X±SD) | 98.3±0.8 | 97.2±0.6 | 94.7±0.6 | 96.1±0.7 | 97.4±0.9 | 99.6±0.5 |
| % Cumulative drug release,10 days study (X±SD) | 97.1±1.2 | 96.3±1.4 | 99.4±1.5 | 95.2±1.7 | 98.5±1.1 | 88.2±1.6 |
| ANOVA | | | | | | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 10.93 | 5 | 2.186 | 0.27065 | 0.02913 | 4.38737 |
| Within Groups | 48.46 | 6 | 8.07666 | | | |
| Total | 59.39 | 11 | | | | |

Each value is expressed as mean ± standard deviation (n = 3). Standard error of mean is less than 0.985. Data are found to be

significant (*F value* < *F crit*) by testing through one way ANOVA at 5 % level of significance ($p < 0.05$ that is $p = 0.02913$).

**Table 3. *In vitro* drug release kinetic study of various clotrimazole dental implant formulations.**

| Formulations | Zero order kinetics | First order kinetics | Higuchi kinetics | Hixon Crowell equation |
|---|---|---|---|---|
| | Regression coefficient ($R^2$) | | | |
| F1 | 0.8695 | 0.9848 | 0.9859 | 0.9666 |
| F2 | 0.8669 | 0.9908 | 0.9871 | 0.9806 |
| F3 | 0.9980 | 0.9781 | 0.9843 | 0.9802 |
| F4 | 0.8715 | 0.9918 | 0.9879 | 0.9824 |
| F5 | 0.8695 | 0.9848 | 0.9859 | 0.9666 |
| F6 | 0.9045 | 0.8593 | 0.9905 | 0.9842 |

**Table 4. Accelerated stability study of optimized clotrimazole dental implant formulation (F6) in different storage conditions as per ICH guidelines.**

| **Temperature** (°C) | **Parameters** | **Period of studies in week** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1st day | 2nd | 4th | 6th | 8th | 10th | 12th |
| 25±2 | Drug content (%) | 99.6 | 99.6 | 99.5 | 99.3 | 99.2 | 99.2 | 99.1 |
| | pH | 7.2 | 7.2 | 7.2 | 7.1 | 7.1 | 7.0 | 6.9 |
| | Viscosity (cps) | 8.4 | 8.4 | 8.4 | 8.2 | 8.2 | 8.1 | 8.1 |
| 37±2 | Drug content (%) | 99.6 | 99.4 | 99.4 | 99.2 | 99.2 | 99.0 | 99.8 |
| | pH | 7.2 | 7.2 | 7.0 | 7.0 | 6.9 | 6.8 | 6.7 |
| | Viscosity (cps) | 8.4 | 8.4 | 8.4 | 8.2 | 8.2 | 8.1 | 8.1 |
| 45±2 | Drug content (%) | 99.6 | 99.4 | 99.3 | 99.1 | 98.9 | 98.7 | 98.7 |
| | pH | 7.2 | 7.1 | 7.0 | 6.9 | 6.9 | 6.6 | 6.6 |
| | Viscosity (cps) | 8.4 | 8.3 | 8.1 | 8.0 | 7.9 | 7.8 | 7.8 |

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 1.793015 | 6 | 0.298835 | 0.0142 | 0.044012 | 2.2655 |
| Within Groups | 117710.4 | 56 | 2101.971 | | | |
| Total | 117712.2 | 62 | | | | |

Data are found to be significant (*F value* < *F crit*) by testing through one way ANOVA at 5 % level of significance ($p < 0.05$ that is $p = 0.044012$).

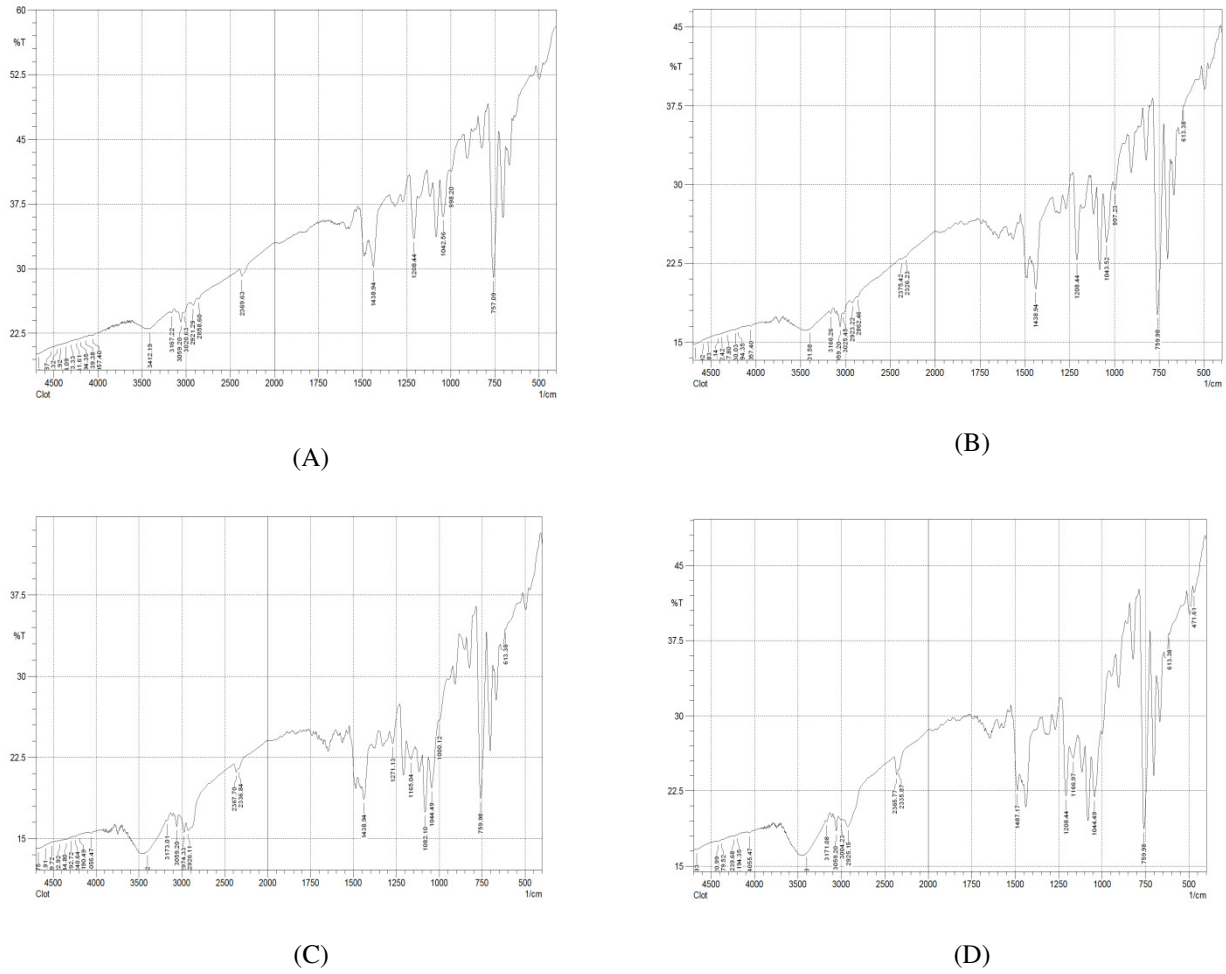


**Fig 1. FTIR spectra of pure drug (A), drug + chitosan (B), drug + ethyl cellulose (C) and drug + HPMC K4M + HPC (D) implant formulations at resolution of 1 $cm^{-1}$.**

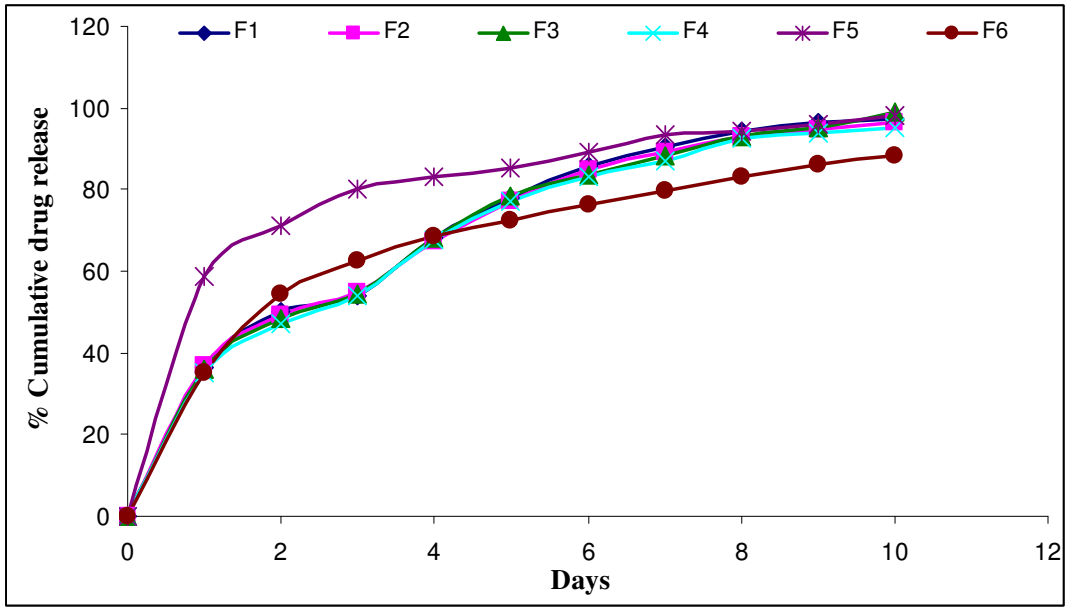


**Fig 2. *In vitro* drug release profile of clotrimazole dental implant formulations in phosphate buffer pH 6.6.**

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