



FORMULATION DESIGN, DEVELOPMENT AND EVALUATION OF METRONIDAZOLE PERIODONTAL FILM IN THE MANAGEMENT OF DENTAL INFECTIONS

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ABSTRACT

Objective: The study aims to design, develop, and evaluate a metronidazole-loaded periodontal film for targeted delivery in the management of dental infections, specifically periodontitis. **Background:** Periodontitis is a prevalent dental condition characterized by inflammation and infection of the supporting structures of the teeth. Localized drug delivery systems, such as periodontal films, offer targeted therapy, enhancing drug efficacy and reducing systemic side effects. **Methods:** Metronidazole, a potent antimicrobial agent, was selected for its efficacy against anaerobic bacteria commonly found in periodontal infections. The films were formulated using biodegradable polymers, such as chitosan and hydroxypropyl methylcellulose (HPMC), to ensure controlled drug release. Various formulations were prepared by solvent casting method. The physicochemical properties of the films, including thickness, weight uniformity, folding endurance, and drug content, were evaluated. In vitro drug release studies were conducted using a simulated periodontal fluid (SPF) to assess the release profile of metronidazole from the films. The antibacterial efficacy of the films was tested against common periodontal pathogens using agar diffusion method. **Results:** The formulated films demonstrated satisfactory physicochemical properties, with uniform thickness and weight, high folding endurance, and consistent drug content. In vitro release studies indicated a controlled and sustained release of metronidazole over a period of 7 days. The optimized formulation showed a release pattern fitting the Korsmeyer-Peppas model, indicating anomalous (non-Fickian) diffusion. Antibacterial studies confirmed significant inhibition zones against *Porphyromonas gingivalis* and *Prevotella intermedia*, demonstrating the efficacy of the films in combating periodontal pathogens. **Conclusion:** The metronidazole-loaded periodontal films developed in this study exhibit promising potential as a localized drug delivery system for the effective management of periodontitis. The films provide controlled and sustained release of the drug, ensuring prolonged therapeutic action at the site of infection, which could enhance patient compliance and treatment outcomes.

KEYWORDS: Metronidazole, Periodontal Film, Dental Infections, Local Drug Delivery, Periodontitis, Biodegradable Polymers, Controlled Release.

INTRODUCTION

Dental caries is a preventable disease that causes oral pain and tooth loss, affecting people of all ages. It is caused by an ecological imbalance between tooth minerals and oral biofilms, leading to demineralization and remineralization of teeth. Primary caries can develop on various tooth surfaces, such as approximal, pit, fissure, enamel, and secondary caries. The prevalence varies with age, gender, socioeconomic position, race, geographical location, eating choices, and oral hygiene practices. Dental caries affects approximately 2.43 billion people (36% of the global population) in their permanent teeth and 620 million people (9% of the

population) in baby teeth. The disease is most widespread in Latin American countries, the Middle East, and South Asia, with China having the lowest prevalence. Treatment aims to retain dental structures and avoid additional tooth damage. Noncavitated lesions can be stopped and remineralized with dietary adjustments, while cavitated lesions are more difficult to remineralize, especially if dentin is involved. Restorative dentistry involves using dental restorative material to restore the function, integrity, and morphology of missing tooth structure. Control and prevention include daily brushing and flossing, limiting snacking, reducing sugary beverage consumption, and chewing gum with xylitol.

Calcium and fluoride are recommended to prevent tooth caries by attaching to the hydroxyapatite crystals in enamel, making it more resistant to demineralization and decay.

in house specification and the results are listed in table 1. The drug source is identified and found complying with the specifications.

METHODOLOGY

Physico Chemical Characterization of Metronidazole

Metronidazole pure drug was obtained as gift sample from Pharmafabrikon (P) Ltd., Madurai was tested as per

Table 1: Characterization of Metronidazole.

S.No	Test	Specification	Results
1	Description	White to pale-yellow crystalline powder	White crystalline powder
2	Solubility	The solubility of metronidazole at 20°C (g/100 ml) is: Water (1.0); ethanol (0.5); ether (<0.05); and chloroform (<0.05). It is soluble in dilute acids.	Complies
3	IR Spectra	The Infrared spectrum of the should match with the standard spectrum	Complies

Chemical Compatibility Study by FTIR

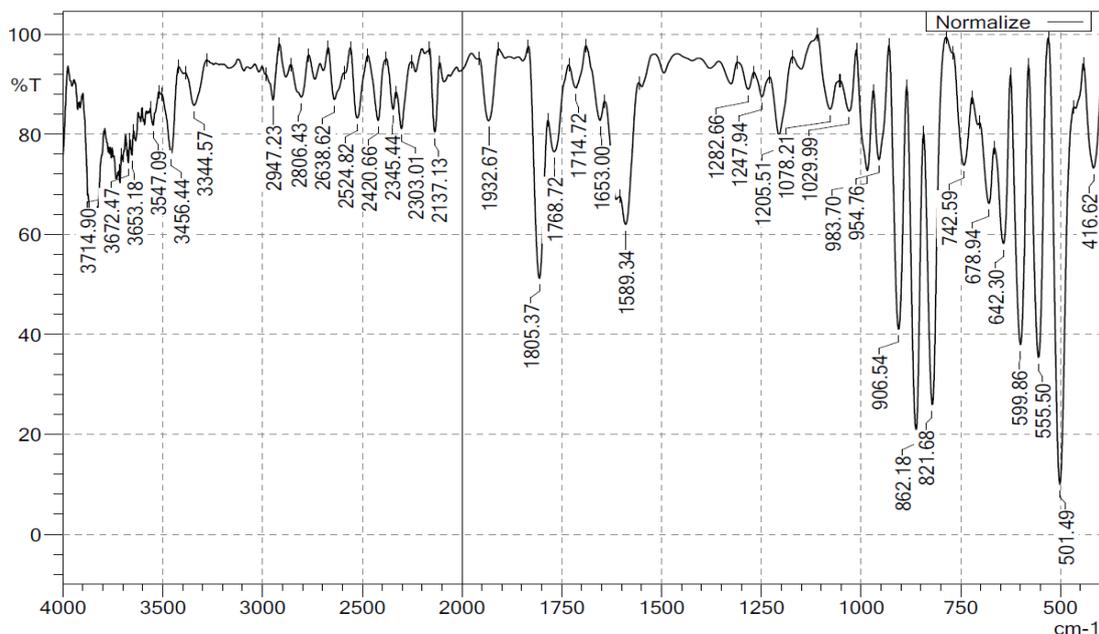
The samples for FTIR Spectral analysis were shown in figure 1 to 5. The physical mixture of drug and polymer was characterized by FTIR spectral analysis and DSC for any physical as well as chemical alterations of drug characteristics.

metronidazole were found to be unaltered in the drug polymer physical mixture.

The physical parameters of drug as well as excipients concluded that there was no change in peaks of admixture compared with drug which indicates that the drug and excipients are compatible.

From the result it was concluded that there was no interface of functional groups as principle peak

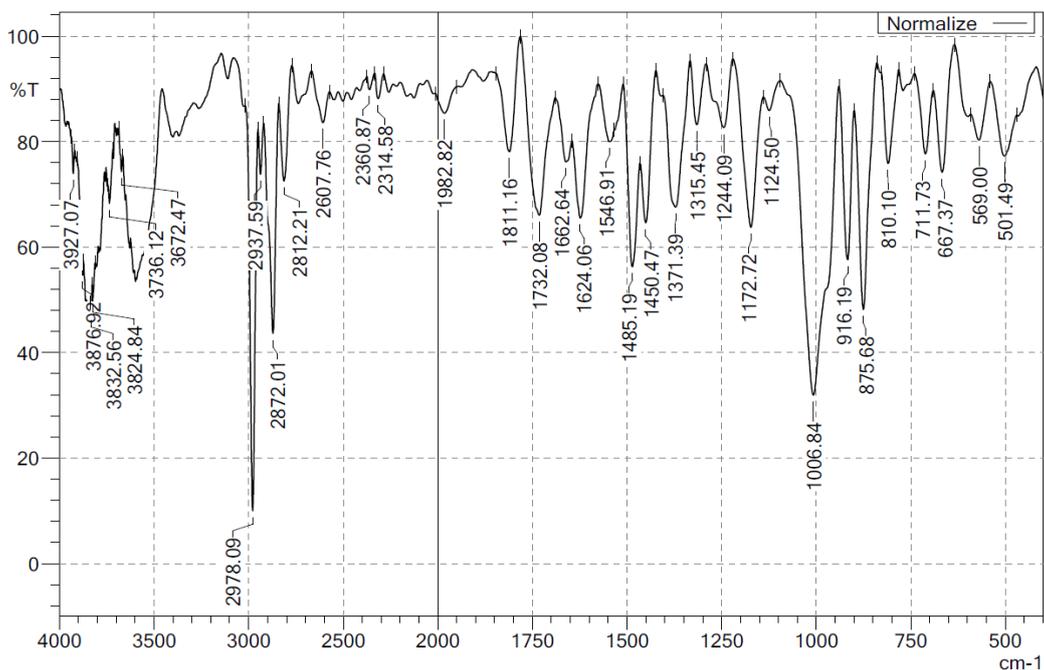
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Figure 1: FTIR of Metronidazole.

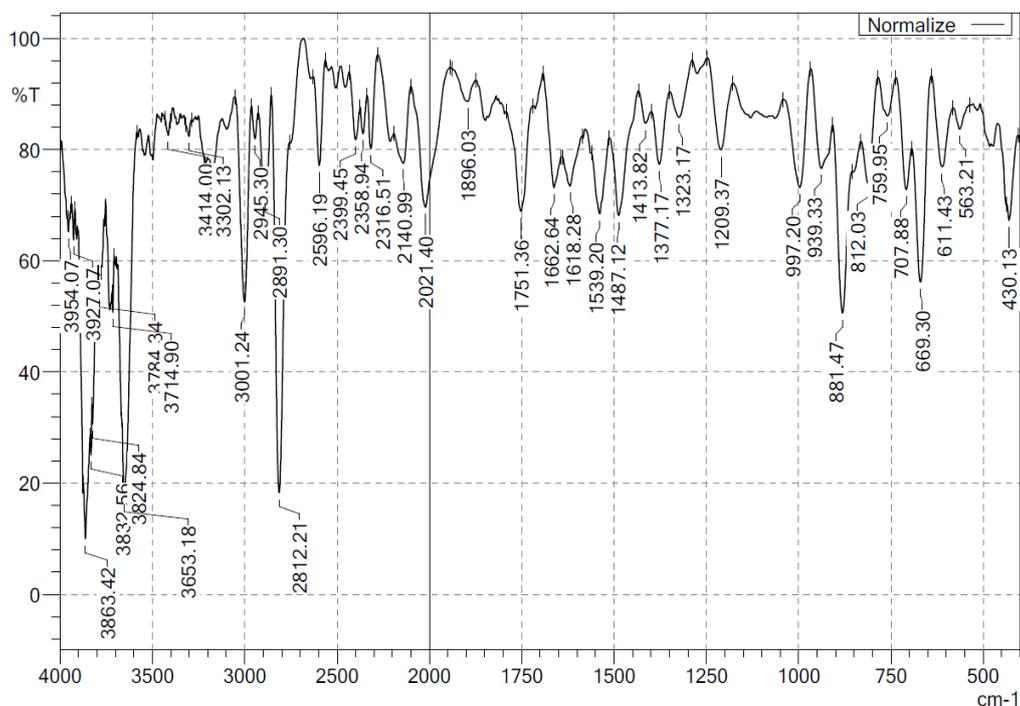
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Figure: 2 FTIR of Ethylcellulose.

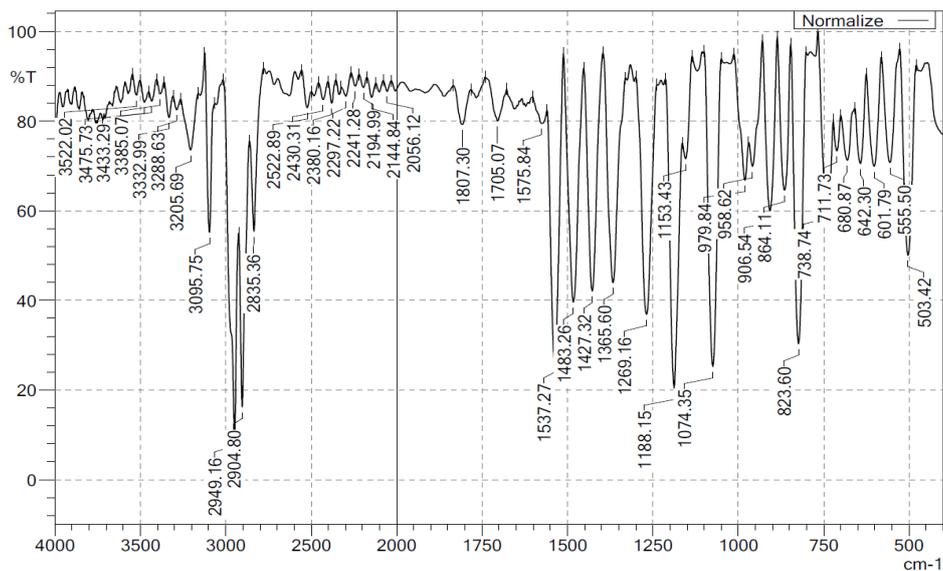
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Figure 3: FTIR of HPMC.

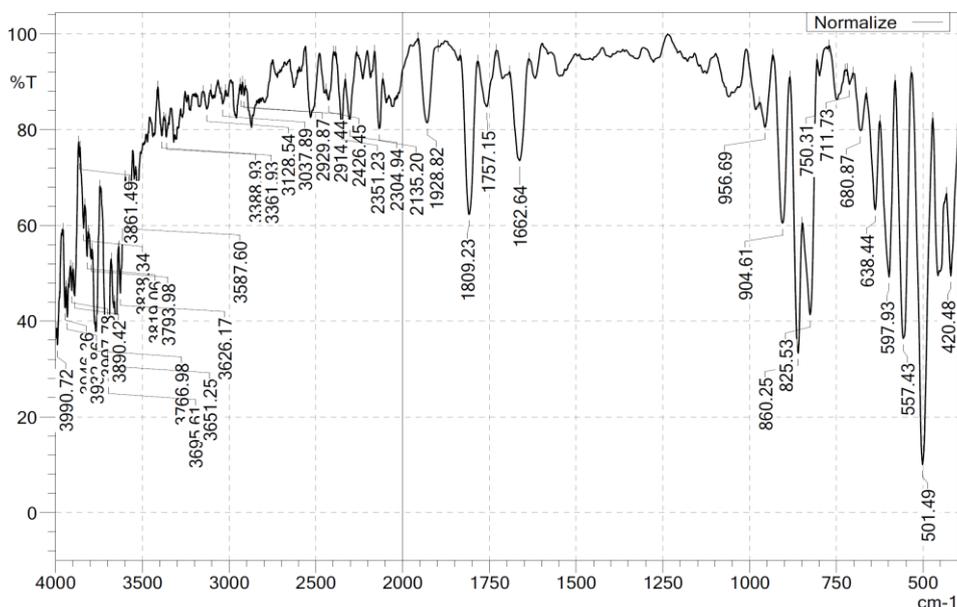
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Figure 4: FTIR of Metronidazole + Ethyl Cellulose.

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Figure 5: FTIR of Metronidazole + HPMC.

FTIR shows no alteration in peaks so there is no interaction between the drug-drug and drug-excipients.

Calibration Curve of Metronidazole

The calibration curve of Metronidazole in phosphate buffer pH 7.4 is given in Table 2 and Fig6.

Table 2: Calibration Curve of Metronidazole at λ_{max} 277 nm.

Concentration ($\mu\text{g/mL}$)	Absorbance at λ_{max} 277 nm
2	0.109
4	0.161
6	0.215
8	0.262
10	0.319

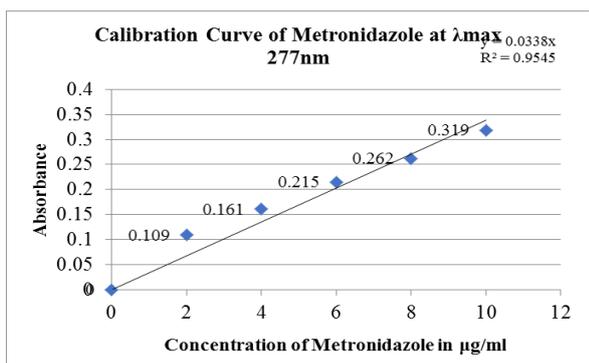


Figure 6: Calibration curve of Metronidazole at λ_{max} 277nm.

It was found that the solution of Metronidazole show linearity ($R^2 = 0.954$) in absorbance at concentrations of 5 -25 ($\mu\text{g/mL}$) and obey Beer Lambert Law.

Formulation of Metronidazole Peridental Film

The proposed formulation of Metronidazole Peridental Film was designed by using Design expert 10 using PEG, HPMC, PVP and Eudragit RS 100 as variable and the formulation design is given in the figure 7 and table.3.

Table 3: Formulation Design of Metronidazole Peridental Film.

Composition	F1	F2	F3	F4	F5	F6	F7	F8
Metronidazole(mg)	100	100	100	100	100	100	100	100
Ethyl cellulose (mg)	500	500	500	500	500	500	500	500
Poly vinyl pyrrolidone K30 (% w/w of EC)	10	20	10	-	-	20	-	-
Hydroxy propyl methyl cellulose K4M (% w/w of EC)	-	-	10	10	20	20	-	-
Eudragit RS-100 (% w/w of EC)	-	-	10	-	-	20	10	20
Poly ethylene glycol: Dibutyl phthalate	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1
Chloroform: Dichloromethane	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1
Alcohol(ml)	4	4	4	4	4	4	4	4

Preparation of Metronidazole Peridental Film

Periodontal films were prepared by solvent casting technique. Glass moulds were used for casting of the films. Formulations were designed as showed in the Table-5, in which Ethylcellulose was taken as the main non-biodegradable polymer in combination with different co-polymers of different concentrations for each cast films. Films were prepared by dissolving Ethylcellulose and copolymers (PVP K-30, HPMC K4M, and Eudrajit RS-100) in chloroform, dichloromethane & alcohol (1:1:0.5) solution, using dibutyl phthalate and PEG-400 as plasticizers. Metronidazole was added to the polymeric solution and mixed homogenously using magnetic stirrer in a closed beaker. After complete mixing, it kept for 15 minutes in closed condition to

remove the entrapped air bubbles. 10 ml of the solution was poured into the clean leveled glass moulds of 9 cm². 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, wrapped in an aluminum foil and stored in a desicator at room temperature in a dark place for further evaluation studies.^[59]

Formulation of Peridental Film Loaded with Metronidazole

The formulated Peridental Film Loaded with Metronidazole is shown in the figure 15.

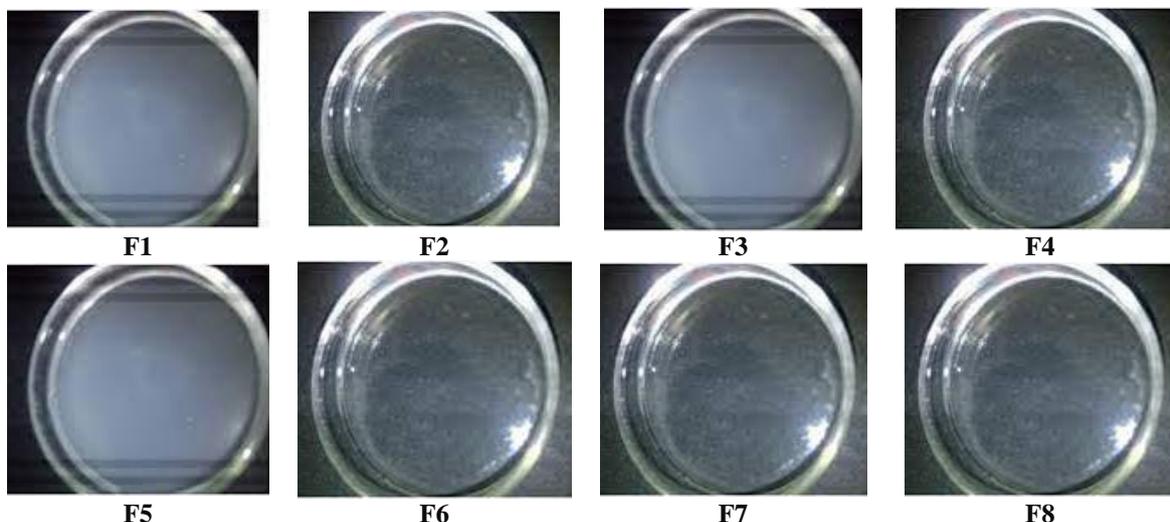




Figure 7: Peridontal Film Loaded with Metranidazole.

Evaluation of Peridontal Film with Metronidazole

The physico-chemical properties of metronidazole Peridontal films were evaluated.

Thickness of the Films

The thickness of each film was measured at 6 different points and the average thickness with Standard deviation was calculated. The thickness of various films is given in table 4. The data of films thickness indicated that there was no much difference in the thickness within the formulations.

Table 4: Thickness of Metronidazole Peridontal Films.

S.No	Formulation	Thickness Uniformity (mm)
1	F1	0.068 ± 0.004
2	F2	0.094 ± 0.009
3	F3	0.069 ± 0.006
4	F4	0.072 ± 0.007
5	F5	0.113 ± 0.004
6	F6	0.126 ± 0.002
7	F7	0.085 ± 0.004
8	F8	0.116 ± 0.002

Weight uniformity test

Drug loaded films (7*2 mm) were tested for uniformity of weight and the results of weight uniformity were given in table 10. Lesser standard deviation values indicated that the films were uniform in weight.

Table 5: Weight uniformity of Metronidazole Peridontal Films.

S.No	Formulation	Weight Uniformity(mg)
1	F1	0.75 ± 0.050
2	F2	0.87 ± 0.170
3	F3	1.07 ± 0.210
4	F4	0.99 ± 0.129
5	F5	2.16 ± 0.102
6	F6	2.18 ± 0.204
7	F7	0.95 ± 0.210
8	F8	1.56 ± 0.211

Percentage moisture loss

Moisture loss studies were conducted on all the formulations and reported in table 11. It was observed that formulation F3 showed maximum amount of moisture loss because of more concentration of PVP K30 undergoing moisture loss in dry condition.

Formulation F8 showed minimum percentage moisture loss because of hydrophobic eudragit RS 100.

Table 6: Percentage moisture loss of Metronidazole Peridontal Films.

S.No	Formulation	Percentage moisture loss
1	F1	14.01 ± 0.040
2	F2	13.20 ± 0.037
3	F3	14.10 ± 0.061
4	F4	12.60 ± 0.031
5	F5	8.30 ± 0.042
6	F6	9.04 ± 0.065
7	F7	10.30 ± 0.051
8	F8	7.50 ± 0.022

Folding endurance

The folding endurance value for all films was > 200; it indicates that all formulations had ideal film properties.

Table 7: Folding Endurance of Metronidazole Peridontal Films.

S.No	Formulation	Folding Endurance
1	F1	221.4 ± 0.12
2	F2	218.0 ± 0.23
3	F3	249.7 ± 0.18
4	F4	231.5 ± 0.44
5	F5	230.6 ± 0.28
6	F6	252.0 ± 0.12
7	F7	254.0 ± 0.32
8	F8	281.0 ± 0.26

Surface pH

Surface pH of all the formulations was determined as described in the methodology chapter. All the formulations were found to have pH between 6 –7. This reveals that the prepared films would not alter the pH of the gingival fluid in the periodontal pocket and therefore may not cause any irritation.

Table 8: Surface pH of Metronidazole Peridontal Films.

S.No	Formulation	Surface pH
1	F1	6.12 ± 0.01
2	F2	6.29 ± 0.02
3	F3	6.00 ± 0.02
4	F4	6.51 ± 0.13
5	F5	6.48 ± 0.07
6	F6	6.70 ± 0.12
7	F7	6.90 ± 0.04
8	F8	6.70 ± 0.11

Swelling index

Swelling index of all films was calculated and it was in the range of 17.3%-33.8%. Formulations containing eudragit RS 100 shows maximum swelling property than the other films. As the concentration of polymer increased, swelling index also increased. Swelling is very essential before the drug is released from the dosage form.

Table 9: Swelling index of Metronidazole Peridental Films.

S.No	Formulation	Swelling index (%)
1	F1	17.3±0.43
2	F2	20.6±0.21
3	F3	22.1±0.32
4	F4	18.2±0.16
5	F5	23.2±0.19
6	F6	26.4±0.53
7	F7	28.6±0.33
8	F8	30.7±0.25

Drug content uniformity

The percentage drug content in various formulations ranged from 71.78 % to 88.33% of metronidazole given in table 15. It was observed from the drug content data

that there was no significant difference in the uniformity of the drug content. However, when compared with the theoretical drug content the estimated drug content was slightly less; it may be due to the drug loss during fabrication of the films.

Table 10: Drug content uniformity of Metronidazole Peridental Films.

S.No	Formulation	Drug content uniformity (%)
1	F1	78.32 ± 0.12
2	F2	81.31 ± 0.25
3	F3	71.78 ± 0.43
4	F4	77.06 ± 0.18
5	F5	83.44 ± 0.78
6	F6	79.14 ± 0.66
7	F7	75.40 ± 0.40
8	F8	88.33 ± 0.63

Mass balance study

This shows how much amount of drug remains in the film after 2 days of release. The values obtained from the *in vitro* release studies and mass balance studies indicated that the drug content did not that much differs from the experimental drug content.

Table 11: Mass balance study of Metronidazole Peridental Films.

S.No	Formulation	Mass balance
1	F1	17.28±0.24
2	F2	9.82±0.21
3	F3	12.14±0.54
4	F4	13.09±0.32
5	F5	6.49±0.23
6	F6	4.10±0.22
7	F7	8.21±0.31
8	F8	6.01±0.28

In vitro drug release studies

The *In vitro drug release studies* showed % drug release 61.74% to 99.14% of Metronidazole up to 2 days. The

release time profile of various Peridental films is shown in fig. 16.

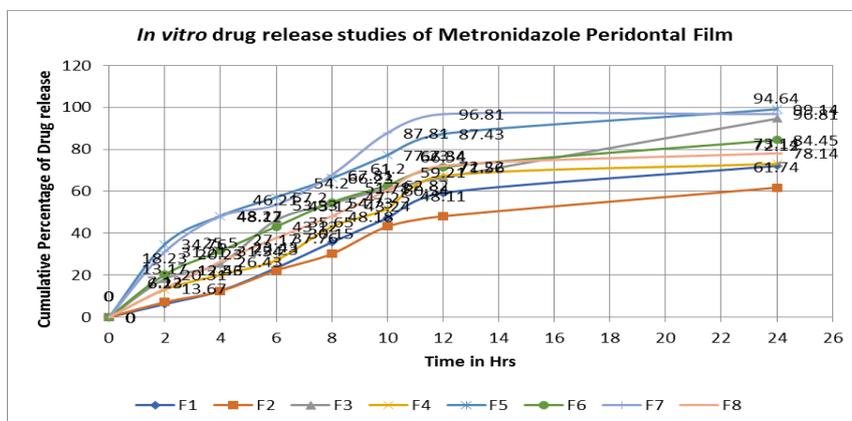


Figure 8: In vitro Release of Metronidazole in Peridental Film.

Optimization by 2³ Factorial Design

On the basis of defined constraints for each independent variable, the Design Expert® Software version 10

automatically generated the optimized formulation. The experiments were performed and the responses were obtained.

Table 12: Result of Independent Variable and Dependent Variable according to 2³Factorial.

Run	Factor 1 Poly vinyl pyrrolidine K30 (%w/w of EC)	Factor 2 Hydroxy propyl methyl cellulose K4M (%w/w of EC)	Factor 3 Eudragit RS-100 (%w/w of EC)	Response 1 Time taken for 50 % drug release (minutes)	Response 2 % Drug release at 24 hours
1	0	10	0	754	70.32
2	0	20	0	1260	60.14
3	10	0	0	540	97.64
4	20	0	0	570	74.14
5	0	0	10	252	98.14
6	0	0	20	390	87.45
7	10	10	10	223	73.53
8	20	20	20	258	98.11

Time taken for 50% drug release

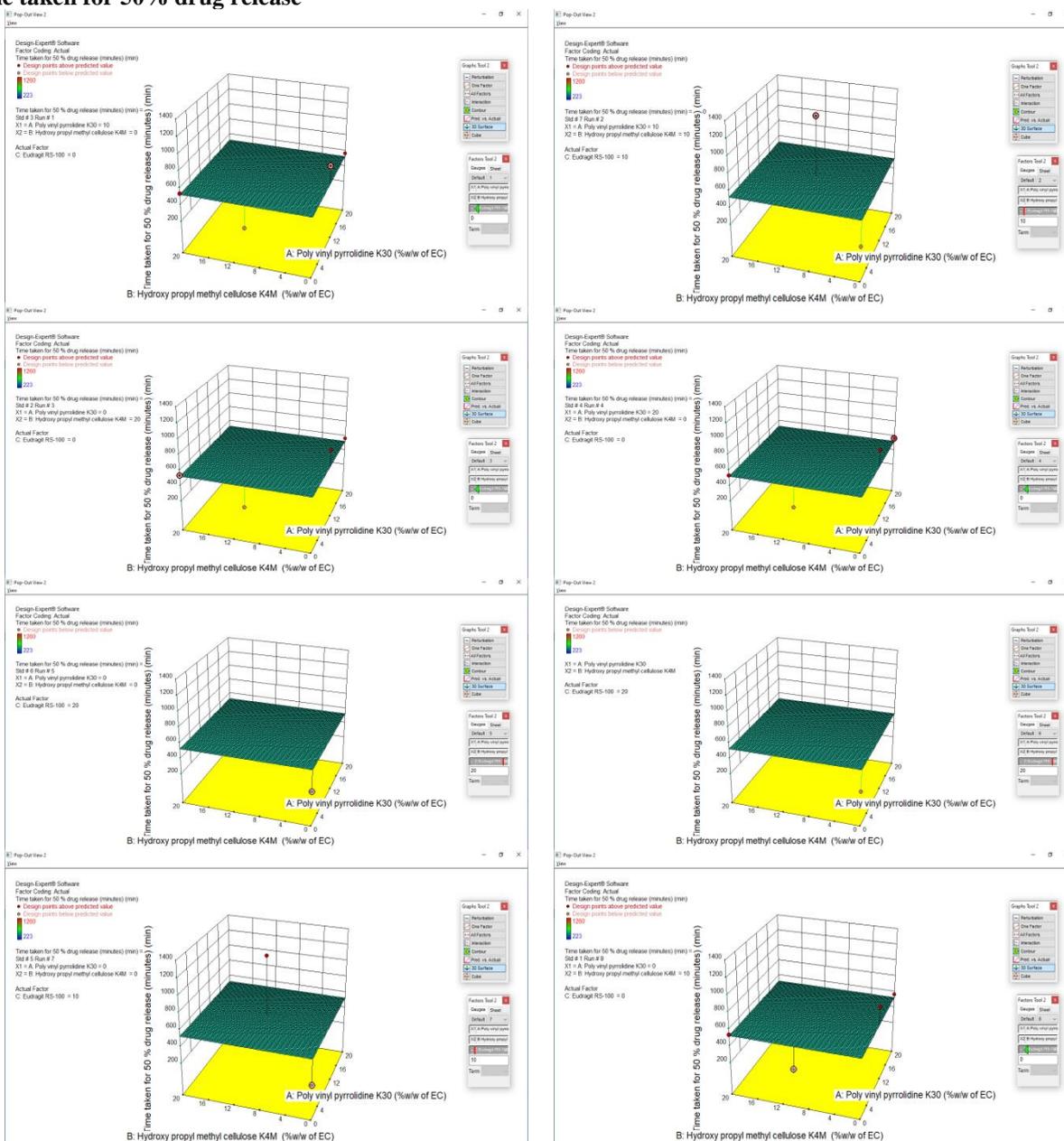


Figure 9: Effect of Polymer Poly vinyl pyrrolidine K30 (%w/w of EC) Hydroxy propyl methyl cellulose K4M (%w/w of EC) Eudragit RS-100 (%w/w of EC) on time taken for 50% drug release presented by response surface plots.

The Fig illustrates, when the amount of HPMC increase, time taken for 50 percent drug release increased.

Drug release at 12 hours

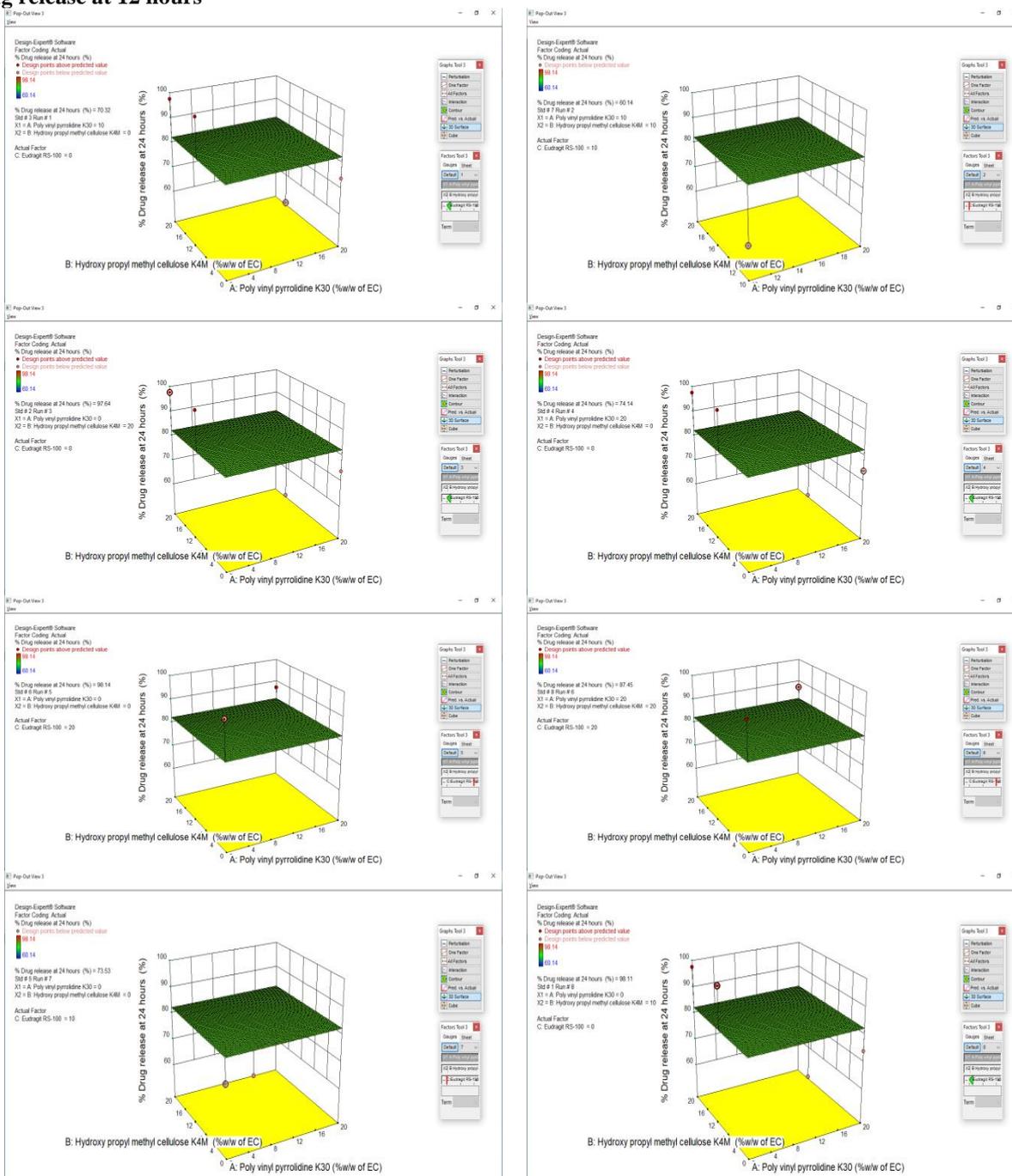


Figure 10: Effect of Polymer Poly vinyl pyrrolidone K30 (%w/w of EC) Hydroxy propyl methyl cellulose K4M (%w/w of EC) Eudragit RS-100 (%w/w of EC) on drug release at 12 hours presented by response surface plots.

The Fig illustrates, when the amount of Polymer Eudragit RS-100 increase, time taken for drug release at 24 hours release increased.

Point prediction

The Metronidazole Peridontal film was formulated and responses were measured. The software generated the optimized formulation and predicts the response based on the constraint. Then batch was formulated based on the suggested formulation and responses were observed. The observed values of responses were compared to the predicted values of the response and % error was

calculated to validate the method. The observed value of Y1 and Y2 were in a close agreement to the predicted one. By this the validity of optimization procedure was proven. The point prediction has been shown in the Table 18.

Desirability of optimum formulation was 0.932. When desirability value is between 0.8 and 1, the formulation quality is regarded to be acceptable and excellent. When this value is <0.53, the formulation quality is regarded as poor.

Table 13: Optimum Formulation Derived by Factorial Design.

Factor	Poly vinyl pyrrolidone K30 (%w/w of EC)	HPMC K4M (%w/w of EC)	Eudragit RS-100 (%w/w of EC)	Desirability
Optimum formulation	14.22	12.11	15.24	0.932

Table 14: Point Prediction for Metronidazole Peridental Film.

Point Prediction	Time taken for 50% drug release	Drug release at 24 hours (min)
Predicted	530.9	82.43
Observed	542.3	84.25
% error	2.15%	2.20%

% error = (observed value-predicted value)/predicted value x 100

Table 15: In-Vitro release of Optimized Metronidazole Peridental Film Formulation.

Time	Optimized Formulation
0 hour	0
2 hour	21.43
4 hour	32.14
6 hour	39.44
8 hour	47.36
10 hour	59.11
12 hour	67.64
24 hour	81.11

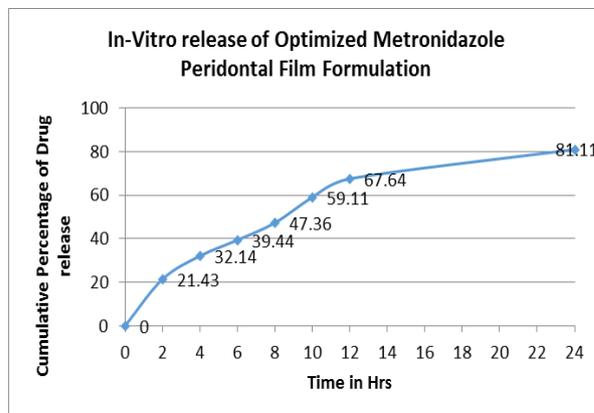


Figure 11: In-Vitro release of Metronidazole in Optimized Metronidazole Peridental Film Formulation.

In-Vitro Kinetics Study of Metronidazole Peridental Film

The values obtained from *in vitro* dissolution of Metronidazole Dental Film was fitted in various kinetics models. The results are given in Table 21 and Figure 20 to 24.

Table 16: In vitro Release Kinetics of Metronidazole in Peridental Film.

Formulation	% Drug release	Zero order kinetics	First order Log % drug remaining	Higuchi Square root of time	Pepas Log % of time	Hixon Cube root of drug release
Optimized	81.11	0.926	0.974	0.993	0.974	0.957

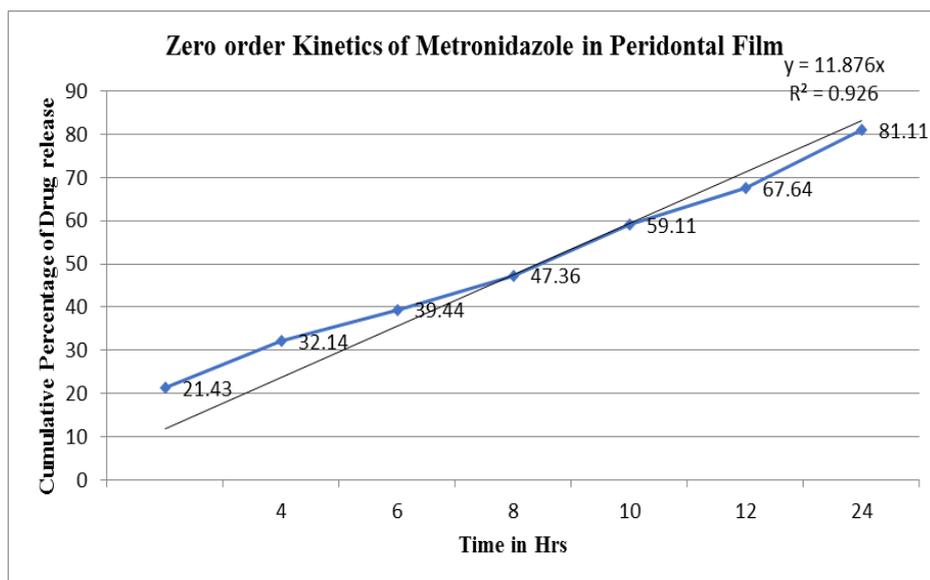


Figure 12: Zero order Kinetics of Metronidazole in Peridental Film.

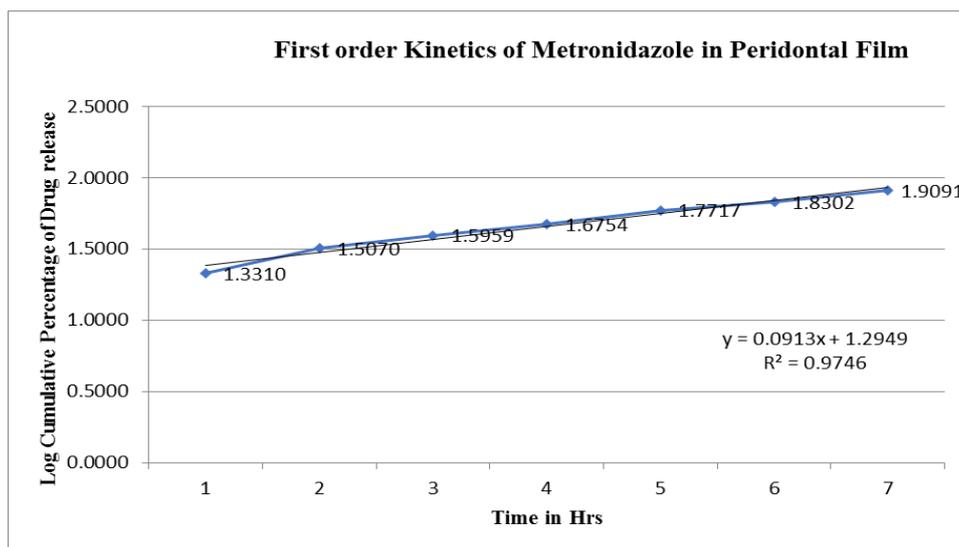


Figure 13: First order Kinetics of Metronidazole in Peridontal Film.

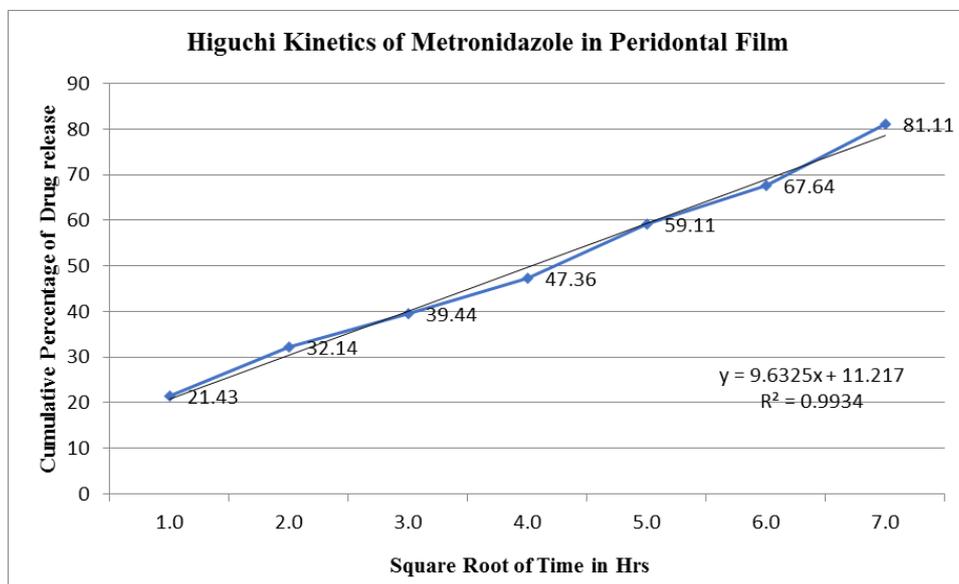


Figure 14: Higuchi Kinetics of Metronidazole in Peridontal Film.

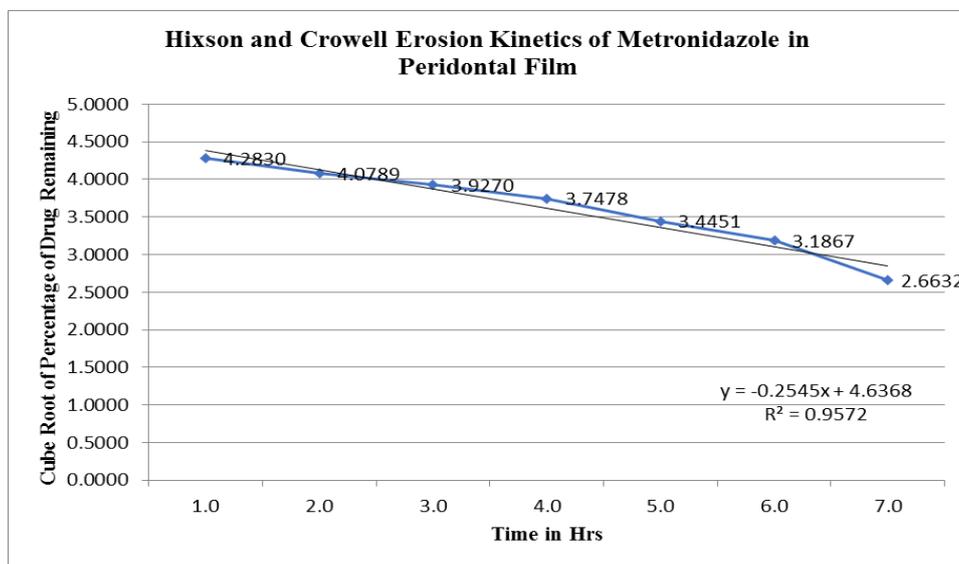


Figure 15: Hixson and Crowell Kinetics of Metronidazole in Peridontal Film.

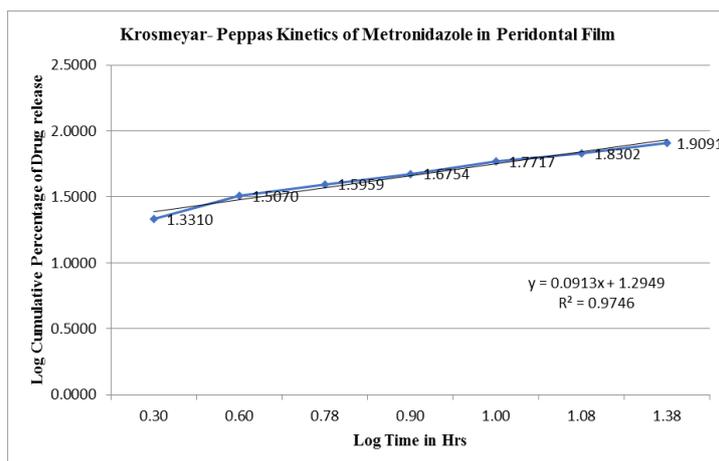


Figure 16: Krosmeyar- Peppas Kinetics of Metronidazole in Peridental Film.

Determination of drug release mechanism of Metronidazole Peridental Film

From the drug release kinetic study the results indicates that the *in-vitro* release of bilayer tablets is fitted with various models such as zero order kinectics, first order kinectics, hixoncrowell, krosmeyar- peppas.

- The models were evaluated based on the slope and regression (R^2) values, the respective R^2 values of models were given in the table 26.
- The *in-vitro* drug release of the optimized formulation is best fitted and found to follow Higuchi kinetics with a higher R^2 value of 0.993 of metronidazole

In vitro Antibacterial study

In vitro antibacterial activity was performed as mentioned in methodology on *S. aureus* and *E. coli*

organisms. The results of antibacterial activity were shown in table 22 and in fig. 25 & 26. The study indicates that the formulated polymeric film optimized Formulation retained their antibacterial activity and also it shows the same effect as the standard solution of Metrinidazole drug.

Table 17: Zone of Inhibition against *E.coli* and *S. aureus*

Organism	Sample	Zone of inhibition (mm)
<i>E. coli</i>	Pure drug	18
	Optimized Dental film	16
<i>S. aureus</i>	Pure drug	33
	Optimized Dental film	32

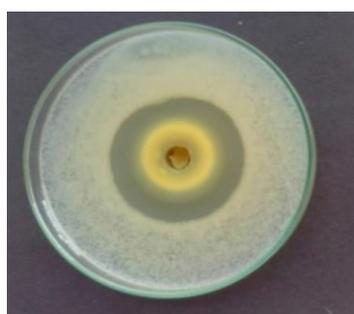


Optimized Formulation



Standard Drug Solution of Metronidazole

Figure 17: Antibacterial zone of *S. aureus*.



Optimized Formulation



Standard Drug Solution of Metronidazole

Figure: 18 Antibacterial zone of *E. coli*.

Study of Surface Morphology by using Scanning electron microscopy (SEM)

Scanning electron micrographs indicate that prior to drug release, the top surface of the films is smooth which exhibits pores as can be seen in fig. 27. These pores indicated that the optimized film had sufficient drug loading capacity. After the release of the drug, the surface became more rough and irregular as can be seen in Figure 27. The reason for this is the coagulation of polymers after the release of the drug from the pores.

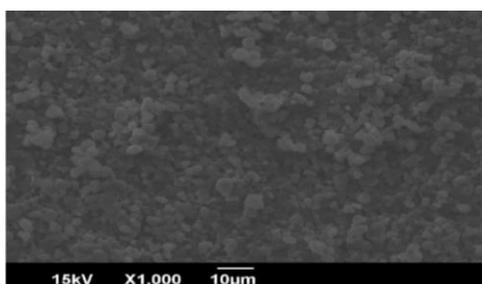


Figure 19: Scanning electron microscopy (SEM) Images of Optimized dental film after drug release study.

Stability studies

The selected formulation was subjected to stability testing. Changes in the appearance, surface pH, folding endurance, and drug content of the stored films were investigated at a period of 10 days and after 1 month. The folding endurance and drug content of the formulation were found to be decreasing but there was no much deviation from the original value. The results at different temperatures are shown in tables 18.

From the *stability studies* it was confirmed that the optimized film of metronidazole remained stable at various temperatures.

Table 18: Accelerated Stability study data of Peridontal Film at 45±2°C.

Time(days)	Physical appearance	Folding endurance	Surface pH	% Drug content
0	No changes	281±0.260	No change	85.08±0.270
10	No changes	270±0.003	No change	82.10±0.221
20	No changes	263±0.120	No change	80.023±0.112
30	No changes	253±0.321	No change	79.2±0.23

CONCLUSION

Metronidazole Peridontal films were prepared using solvent casting technique and ethyl cellulose as the main polymer and various copolymers in different ratios. FTIR studies confirmed the absence of chemical interaction between the drug and the polymers, and all films had uniform thickness and weight. The surface pH of the formulations was close to the mucosal pH, indicating negligible irritation to the mucosal membrane. Eudragit RS100 had a low percentage moisture loss due to its hydrophobic character. Design expert software was used to find the optimized formulation using the Factorial design, and drug content studies showed uniform and homogeneous distribution of the drug into the films. In vitro drug release studies showed slow and controlled release of the drug above the MIC level for 2 days. The optimized film confirmed good antibacterial activity against *E. coli* and *S. aureus*. Stability studies revealed that the optimized film was stable at accelerated temperatures. The drug release mechanism from the film followed Anomalous (non-Fickian) diffusion. The results confirm the benefits of using metronidazole for the treatment of periodontitis in the form of films made up of ethyl cellulose as the main non-biodegradable polymer and eudragit RS100 as the copolymer in an optimized concentration for controlling the rate of drug release above the MIC for 2 days.

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