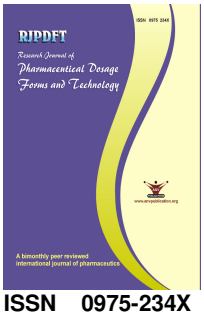

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Research Article

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Formulation and *In Vitro* Evaluation of Periodontal Films Containing Metronidazole

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ABSTRACT
Local delivery devices are designed to deliver the drug locally into periodontal pocket. Metronidazole is a nitroimidazole used to treat protozoal infections. For local delivery, metronidazole films were prepared by solvent casting technique using ethyl cellulose, hydroxy propyl methylcellulose and eudragit RL-100 with dibutylphthalate and polyethylene glycol 400 as plasticizers. FTIR and UV spectroscopic methods revealed no interaction between metronidazole and polymers. The films were evaluated for their thickness uniformity, folding endurance, weight uniformity, content uniformity, tensile strength, and surface pH. Data of *in-vitro* release from films were fit to different equations and kinetic models to explain release kinetics. Hixon-Crowell, Higuchi, and Korsmeyer-Peppas models were used to fit the *in-vitro* release data. Formulation F_6 released 94.18% of drug at the end of 120 h, was considered as best formulation. Short-term stability study revealed that drug content decreased in various films was ranging from 1.361 to 2.209%.

KEYWORDS: Metronidazole; periodontal pocket; periodontal films; local delivery; *in-vitro* release.

INTRODUCTION
Periodontitis is a disease associated with periodontium in which irreversible step of loss of attachment of teeth occurs.[1] The effective use of antimicrobial agent for the treatment of periodontal disease requires an adequate drug concentration at the site of action and a means to maintain that level for a sufficient duration to allow the agent to act. Topical applications like mouthwashes, dentifrices and gels have been successfully tried in controlling the microbial plaque. Topical agents follows an exponential concentration profile while blood and crevicular fluid levels remains at zero, initial salivary concentration reach levels 20 to 50 times bactericidal levels, following expectoration, salivary drug level rapidly fall to approximately one tenth of their initial concentration. Topical agent fail to penetrate deep into periodontal pockets, hence their effectiveness is limited to supragingival areas. So to overcome all these limitations various controlled drug delivery systems, administrating therapeutic levels of antibacterial agents directly into periodontal pocket have been tested as a way to minimize total body dosage and resulting side effects and to maintain therapeutic drug levels in the gingival crevicular fluid.[2,3]

Metronidazole is a nitroimidazole, used to treat protozoal infections. It is bactericidal to anaerobic organisms and is believed to disrupt bacterial DNA synthesis. Metronidazole is not the drug of choice for treating *Actinobacillus actinomycetemcomitans* infections. However, it is effective against *Actinobacillus actinomycetemcomitans* when used in combination with other antibiotics.[4,5] Metronidazole is also effective against anaerobes such as *Porphyromonas gingivalis* and *Prevotella intermedia*.[6] The dose recommended for the treatment of anaerobic infection is 500 mg oral; i.v every 6-8 hours for seven days. Systemic treatment for periodontitis by the oral route using oral tablets requires a total of about 5250 to 7000 mg of metronidazole. However, non-systemic treatment for periodontitis with the slow release composition uses only about 2.4 mg per periodontal pocket.

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As patients with the most advanced cases of periodontitis may have about 20 diseased pockets, and if the metronidazole films are placed in each of these pockets, then the total dosage of metronidazole would be about 50 mg. This total dosage is less than 1% of the total systemic (oral) dose and reflects a substantial safety factor over systemic treatment with oral metronidazole. A preferred dosage for a periodontal pocket, is one in which each pocket receives about two mg of metronidazole, preferably in a slow release form that releases from 0.01 to 0.2 mg per day for each day that the polymeric device remains in the periodontal pocket.[7]

MATERIALS AND METHODS:

Materials:

Metronidazole was obtained as gift sample (Zydus cadila health care limited Ahmadabad, India), Ethyl cellulose was obtained from KAPL (Bangalore, India), 47 cps hydroxy propyl methylcellulose (HPMC) was obtained from Rolex Chemical Industries (Mumbai, India), and Eudragit RL-100 was obtained from Rohm-pharm, Germany. Polyethylene glycol (PEG)-400, dibutyl pthalate was purchased from Loba Chemie, Mumbai. Chloroform was purchased from Ranbaxy fine chemicals Ltd, Acetone was purchased from S.D. Fine Chemicals Ltd., Mumbai; Ethanol was purchased from Anilex Enterprises Inc., USA. Distilled water was prepared in the laboratory using all glass distillation apparatus. Other materials used in the study were of analytical grade.

Methods:

Preparation of film containing metronidazole:

Periodontal films were prepared by solvent casting technique. Glass moulds were used for casting the films. Ethylcellulose, Eudragit RL-100 alone and in combination with hydroxy propyl methyl cellulose were dissolved in chloroform and alcohol mixture with dibutyl phthalate as a plasticizer in a beaker using magnetic stirrer to get different concentration of polymeric solutions. Into these solutions metronidazole of required concentration was added. After complete mixing, the solution was poured into a clean glass mould placed on a horizontal plane. The solvent was allowed to evaporate slowly by inverting a glass funnel with a cotton plug in the stem of the funnel was placed on the mould at room temperature for 24 h. After complete evaporation of solvent, cast film was obtained. Inverted funnel was continuously kept on the mould to control drying rate. The prepared cast films were lined with butter paper and stored in a dessicator. To accommodate different variables, batches of cast films were prepared. The compositions of films are given in Table 1.

Evaluation of the films:

Formulated films were subjected to the preliminary evaluation tests. Films with any imperfections, entrapped air, or differing in thickness, weight or content uniformity were excluded from further studies.

Thickness uniformity of the films: The thickness of each film was measured using screw guage (thickness tester) at different positions of the film and the average was calculated.

Uniformity of weight of the films: *Film (size of 1 cm^2) was taken from different areas of film. The weight variation of each film was calculated.*

Tensile strength of the films: Tensile strength of the films was determined with Universal strength testing machine. The sensitivity of the machine is 1 gram. It consists of two load cell grips; the lower one is fixed and the upper one is movable. The test film of specific size (4×1 cm^2) was fixed between these cell grips and force was gradually applied till the film breaks. The tensile strength of the film was taken directly from the dial reading in kilograms.

Drug content uniformity of films: Film (size of 1 cm^2) was taken from different areas of film and placed in a 10 ml volumetric flask; 10 ml of ethyl alcohol was added and kept aside till the film dissolve completely. From this solution, 1 ml was pipette out and diluted to 10 ml with double distilled water. The absorbance of the solution was measured at 320.4 nm. The polymer solution without drug serves as a blank. In case of HPMC film, combination of water and alcohol is used to dissolve the film.

Folding endurance: As described by Kevin et al., the folding endurance of the films was determined by repeatedly folding one film at the same place till it broke or folded up to 300 times, which is considered satisfactory to reveal good film properties. The film was folded number of times at the same place without breaking gave the value of the folding endurance.[8] This test was done on all the films for five times.

Surface pH: Periodontal films 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 under stirring and then pouring the solution into the petridish to gelling / solidify at room temperature. The surface pH was measured by means of pH paper placed on the surface of the swollen film. The mean of three readings was recorded.[9]

Viscosity: Aqueous solutions containing both polymer and plasticizer were prepared in the same concentration as that of films. Brookfield viscometer (LVDV-E model) attached to the helipath spindle number 18 was used. The viscosity was measured at 20 rpm at room temperature. The recorded values were the mean of five determinations.

In-vitro *drug release:* In-vitro drug release was performed by taking 1 cm^2 of periodontal film in a vial containing one ml of double distilled water. One ml of double distilled water was withdrawn from 1^{st} to 5^{th} day, every day and immediately replaced with one ml of fresh double distilled water.[10] The drug content was estimated by measuring the absorbance after suitable dilution at 320.4 nm.

Ageing: Optimized medicated films were subjected to stability testing. Films were placed in a glass beaker lined with aluminium foil and kept in a humidity chamber maintained at 40 $\pm$ 2°C and 75 $\pm$ 5% RH for 1 month. Changes in the appearance and drug content of the stored films were investigated after storage. The data presented were the mean of 3 determinations.[11]

Table 1: Composition of different formulations containing metronidazole

Ingredients	Film Code					
	F_1	F_2	F_3	F_4	F_5	F_6
Metronidazole (mg)	120	120	120	120	120	120
Ethyl cellulose (mg)	*	*	440	460	460	480
Eudragit RL 100 (mg)	460	440	*	*	20	*
HPMC (mg)	20	40	40	20	*	*
Dibutyl phthalate (ml)	*	*	0.2	0.2	0.15	0.15
PEG 400 (ml)	0.1	0.1	*	*	*	*
Alcohol (ml)	15	15	2	2	2	2
Chloroform (ml)	*	*	13	13	13	13

** No ingredient is added*

Table 2: Physicochemical characteristics of periodontal films containing metronidazole

FC	TN (mm)	WU (mg)	TS (kg)		CU loaded (%)	FE
			Dummy films	Drug films		
F_1	0.225	33.801	4.466	5.033	85.612	> 250
F_2	0.24	34.231	3.000	4.133	87.067	> 250
F_3	0.28	34.712	1.720	2.700	85.219	> 250
F_4	0.268	32.590	2.133	3.066	83.292	> 250
F_5	0.215	33.352	2.866	3.800	82.977	> 250
F_6	0.246	32.338	2.300	3.666	82.368	> 250

FC is film code (F_1, F_2, F_3, F_4, F_5, and F_6 are formulations). TN, WU, TS, CU, and FE are thickness, weight uniformity, tensile strength, content uniformity, and folding endurance, respectively. Each value is an average of three determinations.

RESULTS AND DISCUSSION:

Drug-polymer compatibility:

FT-IR spectra of metronidazole alone and its combination with polymers are shown in Figure 2. FT-IR spectra of the pure metronidazole and the drug-polymer mixture showed characteristic bands at 2520.51 cm^{-1}, 2135.78 cm^{-1}, 1803.12 cm^{-1}, and 1464.67 cm^{-1} due to functional groups, indicating the chemical stability of metronidazole in the chosen polymeric mixture. This also indicates that metronidazole is not involved in any chemical reactions with the polymer used. Further, the interference was also verified using UV-spectrophotometric method[12].

Evaluation of films:

The physicochemical properties of metronidazole periodontal films are presented in Table 2.

Thickness uniformity: All the films have uniform thickness throughout with the standard deviation of ± 0.018 µm (n = 3).

Weight uniformity: Drug loaded films (1 x 1 cm^2) were tested for uniformity of weight. The weight was found to be uniform in the prepared batches with standard deviation of ± 0.856 mg per film (n = 3)

Tensile strength: The tensile strength of drug-loaded films was higher than dummy films (Table 2). This is justified because dissolved metronidazole strengthened the bonding of polymer chains. The tensile strengths of films were in the order of $F_1 > F_2 > F_5 > F_6 > F_4 > F_3$.

Content uniformity: The results of content uniformity indicated that the drug was uniformly dispersed. Recovery was possible to the tune of 82.36 to 87.06 % for formulations F_1 to F_6 (Table 2). The drug content analysis of the prepared formulations had shown that the process employed to prepare the films in this study was capable of giving films with a uniform drug content and minimum batch variability.

Folding endurance: Films did not show any cracks even after folding for more than 250 times. Hence it was taken as the end point. Folding endurance did not vary when the comparison was made between dummy films and drug-loaded films (Table 2).

Table 3: *In-vitro* release profile of metronidazole films from F_1 to F_6

Time (h)	Percentage of drug release					
	F_1	F_2	F_3	F_4	F_5	F_6
24	86.430	89.858	71.330	66.242	67.783	55.23
48	93.675	96.757	90.690	82.450	80.154	77.54
72	97.770	98.909	95.760	92.150	91.486	90.20
96	-	-	96.818	93.245	93.135	92.50
120	-	-	-	-	94.314	94.18

Viscosity: The viscosities of the solutions were ranges from 12.10 to 33.70 cps for films F_1 to F_6. Viscosity of the solution of film F_6 was highest when compared to others, because of the presence of Ethyl cellulose.

***In vitro* release studies:** *In vitro* release studies performed using double distilled water showed an initial burst release (Figure 1), which is expected to kill most of the periodontal organisms, followed by controlled release, sufficient to inhibit the growth of the micro-organisms.

Periodontal film made of ethyl cellulose (F_6) is better than others because the extent of release was maintained for about 5 days. All the formulations showed initial burst release and controlled release in later phases, as shown in Figure 1. Higher drug release from films F_1, F_2, and F_3 showed 97.7%, 98.9%, and 96.8% respectively. The higher drug release from these films was possible because of the formation of more pores and channels due to presence of higher HPMC content. As HPMC act as resorbable carriers, it dissolved readily during *in vitro* drug release. The cumulative amounts of drug released from the films are shown in (Table 3).

Eudragit RL100 being an inert polymer, solvent penetration into the film was rate limiting factor for the release of the active principle. At the beginning of the process, the active substance at and near the surface of the film dissolves quickly. When the dissolution process advances, there is a greater resistance to the penetration of the solvent in the inside of the matrix film, due to the non-hydrophilicity of the polymer and the decreasing length of the solvent front. The drug, easily accessible by water immediately dissolves and diffuses from the interface between the film surface and surrounding media after which diffusion process slows down. *In-vitro* release studies showed that the drug release was more sustained in case of film F_6 followed by $F_5 > F_4 > F_3 > F_2 > F_1$. The regression values of films F_1 to F_6 are higher with first order and therefore the release kinetics followed first order from all films.

Hixon Crowell cube root law and Higuchi's models were applied to test the release mechanism. The R^2 values are higher for Higuchi's model compared to Hixon Crowell cube root law for all the films. Hence metronidazole release from all the films followed diffusion rate controlled mechanism. According to Korsmeyer-Peppas model, the release mechanism from films F_1 to F_4 followed case II transport ($n > 1$). However, films F_5 and F_6 followed Non-Fickian diffusion or anomalous behavior (a value of slope between 0.5 and 1).

Fig. 1: *In-vitro* release profile of metronidazole from film F_1 to F_6

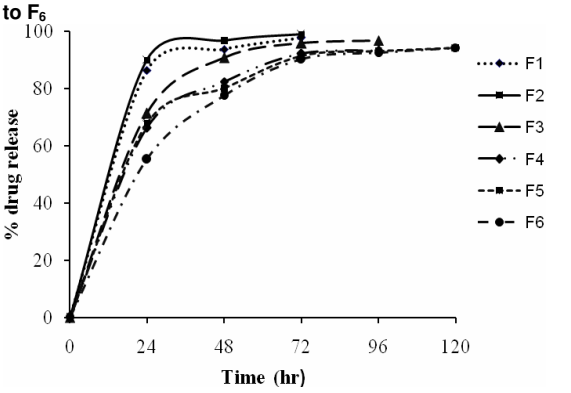
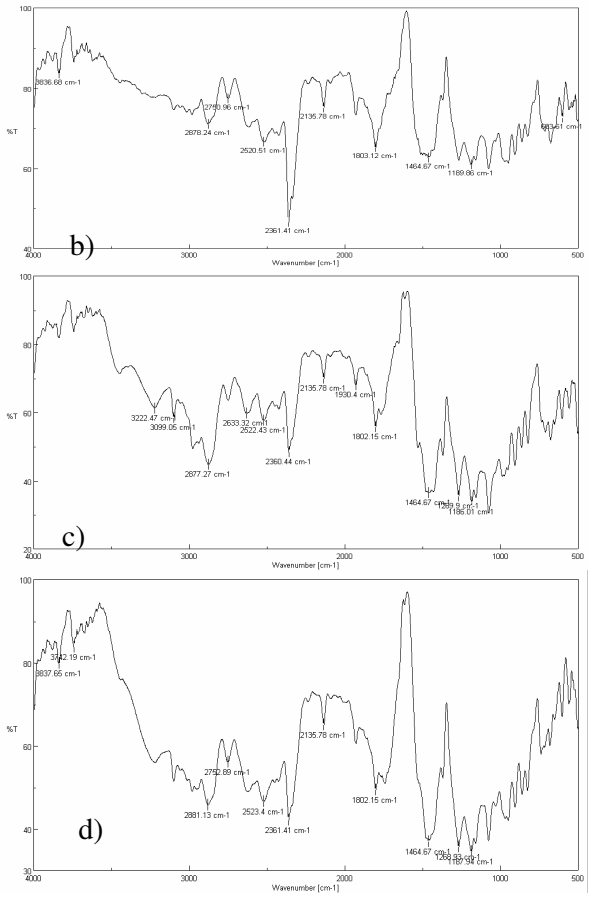
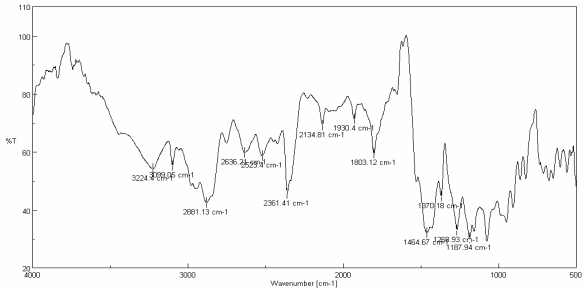


Fig. 2: a) IR spectrum of Metronidazole pure, b) IR spectrum of metronidazole and ethyl cellulose mixture, c) IR spectrum of metronidazole and eudragit RL-100 mixture, d) IR spectrum of metronidazole and HPMC 47 cps mixture





Ageing: Films that were placed in humidity chamber for short time stability studies were withdrawn every week and analysed for their drug content. Percentage drug present in the films were determined spectrophotometrically. Decrease in the drug content from the films ranged from 1.361to 2.209%. It was found that the drug loss is less though the films were stored for one month. The films were also observed for their appearance and texture. These properties did not change in films during the period of study. Periodontal films containing metronidazole using ethyl cellulose, HPMC, and eudragit RL100 polymers showed satisfactory characteristics without being drastically influenced by ageing.

CONCLUSION:

On the basis of *in vitro* characterization it was concluded that metronidazole could be incorporated in a slow release device for the treatment of periodontitis. Periodontal films consisting of ethyl cellulose, the bioadhesive polymer HPMC, and rate-controlling polymer of Eudragit RL100 with dibutyl pthalate and PEG-400 as plasticizers demonstrated sustained and controlled release of the drug. The drug remained intact and stable in the periodontal films during storage, with no significant chemical interaction between the drug and the excipients. Further, detailed investigation is required to establish *in-vivo* efficiency of these films.

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