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Formulation of mucoadhesive mouth paint for oral infections

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Abstract

Oral mucoadhesive mouth paint preparation was designed and prepared for the treatment of oral candidiasis, where prolonged drug release at the infected area is essential. Fluconazole, a recent triazole derivative having antifungal activity is chosen as the desired drug in this study to formulate mucoadhesive mouth paint. Oral candidiasis is a common infection in debilated patients, AIDS patients and in persons who administer immunosuppressive drugs. Mouth paints containing 1% fluconazole with hydrophilic polymer HPMC was prepared and compared with mouth paint prepared without the addition of hydrophilic polymer. The prepared mouth paint formulation was subjected to various evaluation parameters like pH determination, drug content, rheological behavior, mucoadhesive studies, spreadability and IR spectral analysis. *In vitro* drug release studies were carried out at salivary pH 6.4 using cellophane membrane as barrier. Stability studies were carried out at different temperature conditions like ambient temp (R. T.), 8 ± 1oC, 45 ± 2oC at 75% ± 5% R. H. (accelerated temperature) 3 months and analyzed at different time intervals for drug content, physical appearance, pH, mucoadhesive strength and spreadability and the prepared formulation was found to be stable. Antimicrobial studies were carried out to ascertain the antifungal activity of prepared mucoadhesive formulation against the pure drug. The test organism Candida albicans was a clinical isolate obtained from a diseased patient suffering from oral Candidiasis. *In vitro* antifungal activity was evaluated using standard Agar cup-plate method by zone inhibitions studies. Formulations, containing HPMC showed good zone inhibition. *In vivo* oral mucosal skin irritancy tests were carried out using mucoadhesive formulation on lab experimental animals (Rabbits and Guinea-pigs) and on healthy human volunteers. No edema, erythema, inflammation or redness in the mucosal cavity of both animals and human volunteers were observed.

Keywords: Fluconazole; HPMC; Rheology; Mouth paint

1. Introduction

Oral candidiasis is predominantly caused by candida albicans; the yeast most frequently encountered human fungal pathogen; responsible for a wide range of superficial infections[1;2;3] include AIDS; diabetes mellitus; pregnancy; stress & depression; anemia and use of oral contraceptives; chemotherapy; antibiotics and steroids.[4] Topical antifungal are usually the drug of choice for localized candidiasis. Drug delivery to mouth includes mouth washes; ointments; gels and chewing gums. However; these suffer a common disadvantage in that they all have relatively short residence times and therefore fail to maintain therapeutic concentration for long enough to affect the microbial population. Mucoadhesive drug delivery systems prolong the residence time of the dosage form at the site of application or absorption and facilitate

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an intimate contact of the dosage form with the underline absorption surface and thus contribute to improved and better therapeutic performance of the drug.[5;6;7]

In current research work; attempt is being made to prolong residence time and increase patient compliance by preparing hydroxy propyl methyl cellulose (HPMC) based mucoadhesive mouth paint of Fluconazole; a recent triazole antifungal agent which is less lipophillic and more hydrophilic when compared to other azole antifungal agents.[8]

2. Material and methods

2.1. Materials

Drug Fluconazole was gift sample from Glenmark Pharmaceuticals Ltd., Nashik. HPMC procured from S.D. Fine Chemicals, Ltd., Mumbai cellophane membrane obtained from local market. All other chemicals used were of analytical grade.

2.2. Plan of Experimental Work

Two mouth paint formulations of fluconazole were prepared, containing with and without mucoadhesive polymer hydroxy propyl methyl cellulose following conventional method of mixing the ingredients (Table-1).

Table 1 Formula used to prepare mouth paints

Sl. No.	Ingredients	CMP*	MMP*
1.	Fluconazole	1.0 gm.	1.0 gm.
2.	Sodium citrate	1.0 gm.	1.0 gm.
3.	HPMC	-	2.0 gm.
4.	Glycerol	60 ml.	60 ml.
5.	Alcohol	10 ml.	10 ml.
6.	Water up to	100 ml.	100 ml.

CMP-Conventional mouth paint; MMP-Mucoadhesive mouth paint

2.3. Preparation of Mucoadhesive Mouth Paint

- HPMC, Sodium citrate and purified water mixed thoroughly and hydrated (for 24 hours).
- Fluconazole was dissolved in 10ml. of ethanol and added to above hydrated base.
- Then add glycerol to the above mixture on stirring to get a homogenous dispersion of drug. Another formulation was prepared without the addition of HPMC a mucoadhesive polymer.

2.4. Characterization of prepared mouth paints

2.4.1. Drug Content Evaluation

Drug content was determined by dissolving 2.5 gms. Of mouth paint in methanol. After suitable dilution absorbance was recorded by using UV-Spectrophotometer at λ_{max} 261nm. (Table-2)

Table 2 Physico-chemical parameters of prepared mouth paints

Sl. No.	Parameters	CMP	MMP
1.	Drug Content	100.60%	97.56%
2.	pH	6.90±0.043	7.13±0.007
3.	Spreadability	10.14±0.034	9.30±0.004
4.	Mucoadhesive strength	11.00±0.480	38.0±0.200

Each reading is a mean of three replicates; each sample of 1 gm. Paint contains 10mg. of drug

2.4.2. Determination of pH: [9]

2.5 gm of prepared mouth paint was accurately weighed & dispersed in 25.0ml of purified water (diluted to 10 times), the pH of the dispersion was measured using Digital pH meter.

2.4.3. Spreadability

For the determination of spreadability, 3 gm. of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000 gm. Weight for 5 mins. Weight (50gm) was added to the pan. The time required to displace upper plate over the lower plate for a distance of 10 cms is noted (Table-2)

2.4.4. Rheological Studies: [10,11,12]

The viscosity of various formulated Fluconazole Mouth paints were measured by Brook field Viscometer (LVDV-III ultra-programmable Rheometer) using spindle CP-52 at varying speed & shear rates from 10, 15, 20, 25 & 30 rpm between 20-60 Sec^{-1} at room temperature to examine the hysteresis of the rheogram. (Table-3)

Table 3 Viscosity data of prepared Conventional Mouth Paint and Mucoadhesive Mouth Paint formulations

CMP					MMP				
Viscosity (CPS)	**Speed (rpm)**	**Shear Rate (Sec^{-1})**	**Temp. (°C)**	**Time Intervals (Sec.)**	**Viscosity (CPS)**	**Speed (rpm)**	**Shear Rate (Sec^{-1})**	**Temp. (°C)**	**Time Intervals (Sec.)**
721.40	10	20	28.60	30	1071.40	10	20	28.60	30
633.50	15	30	28.70	30	720.50	15	30	28.70	30
470.50	20	40	28.60	30	545.00	20	40	28.60	30
357.80	25	50	28.60	30	463.70	25	50	28.60	30
304.30	30	60	28.70	30	363.40	30	60	28.70	30

2.4.5. Mucoadhesive Strength determination [13,14]

A sample of 50gms of mouth paint was placed in a 100ml- graduated cylinder for measuring mucoadhesive strength was allowed to penetrate in the sample. The time (sec) the apparatus took to sink 5cms down through the sample. (Table-2)

2.4.6. In vitro drug diffusion studies[15]

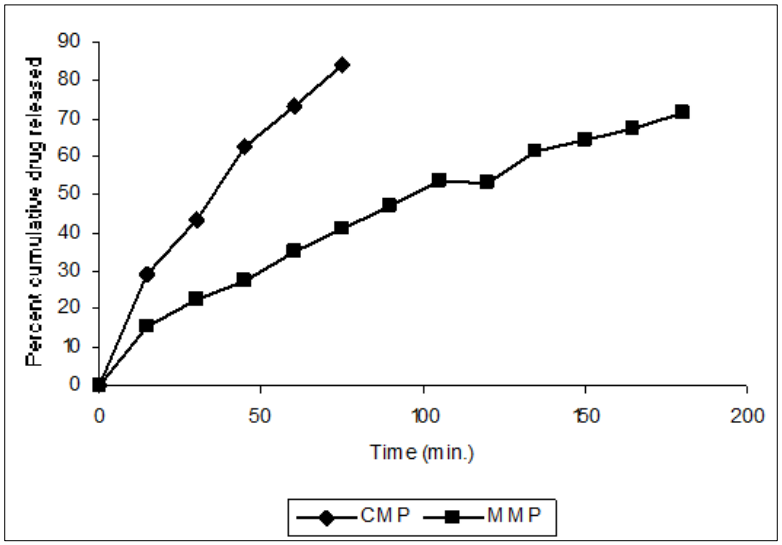

Figure 1 *In vitro* drug release of prepared Conventional Mouth Paint and Mucoadhesive Mouth Paint formulations

The permeation apparatus designed as described by Chowdary et. al., & Fites et. al. was employed to study the release of fluconazole from the formulation. Phosphate buffer saline of pH 6.4 using prehydrated Cellophane membrane as barrier. 1 gm. (10mg drug) of prepared mouth paint was taken in donor cell. Samples from receptor compartment (5ml) withdrawn at the interval of 15 min over a period of 180min & assayed for fluconazole at λmax 261nm. The volume with drawn at each time (5ml) was replaced with drug free receptor fluid (PBS of pH 6.4). (Table-4 & Figure-1)

Table 4 *In vitro* drug release of prepared Conventional Mouth Paint and Mucoadhesive Mouth Paint formulations

Sl. No.	CMP			MMP		
	Time (min)	% Cumulative drug released	% Cumulative drug remaining	Time (min)	% Cumulative drug released	% Cumulative drug remaining
1.	0	0.0	0.0	0	0.0	0.0
2.	15	29.12	70.88	15	15.5	84.5
3.	30	43.47	56.53	30	22.57	77.43
4.	45	62.83	37.17	45	27.49	72.51
5.	60	73.39	26.61	60	35.42	64.58
6.	75	84.14	15.86	75	41.40	58.6
7.		-	-	90	46.98	53.02
8.		-	-	105	53.55	46.45
9.		-	-	120	53.06	41.94
10.		-	-	135	61.175	38.82
11.		-	-	150	64.52	35.48
12.		-	-	165	67.28	32.72
13.		-	-	180	71.41	28.59

2.4.7. Infrared spectral analysis[16]

The studies were carried out using IR method with the help of Perkin-Elmer model 983 spectrometer to determine drug excipient interaction. (Figure-2)

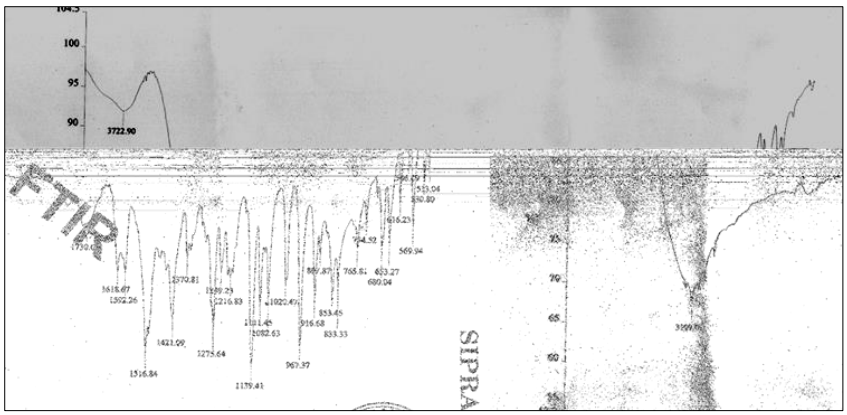

Figure 2 Comparative Study of I.R. Spectra of Pure Drug (Fluconazole) with MMP formulation containing fluconazole with other excipients

2.4.8. Anti-microbial studies[17,18,19,20]

The prepared mouth paints were evaluated for *In vitro* antifungal activity using standard Agar cup-plate method. The test organism Candida albicans was a clinical isolate obtain from a diseased patient suffering from oral candidiasis from our M.R. Medical College & General Hospital. Gulbarga under the guidance of department staff. The microorganism was collected by sweeping cotton-swab on the tongue of patient and stored this swab in peptone water. Nutrient Agar medium was used for the culture and maintenance of isolated microorganism.

Table 5 Antimicrobial Studies Data

Formulation Code	Zone inhibition (mm) after 36 hrs.			
	Zone-1	Zone-2	Zone-3	Mean
MMP	13.0	18.0	18.0	16.33±2.88
Pure Drug	25.0	26.0	26.0	25.6±0.577

Each reading is a mean of 3 replicates; All above formulation contain 1% fluconazole

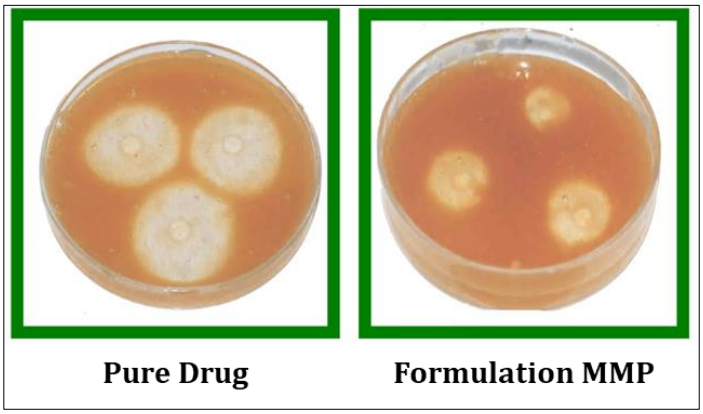

Figure 3 Photographs of Anti-microbiaL studies showing the comparative zone inhibition of PUre Drug as against Drug in Formulations

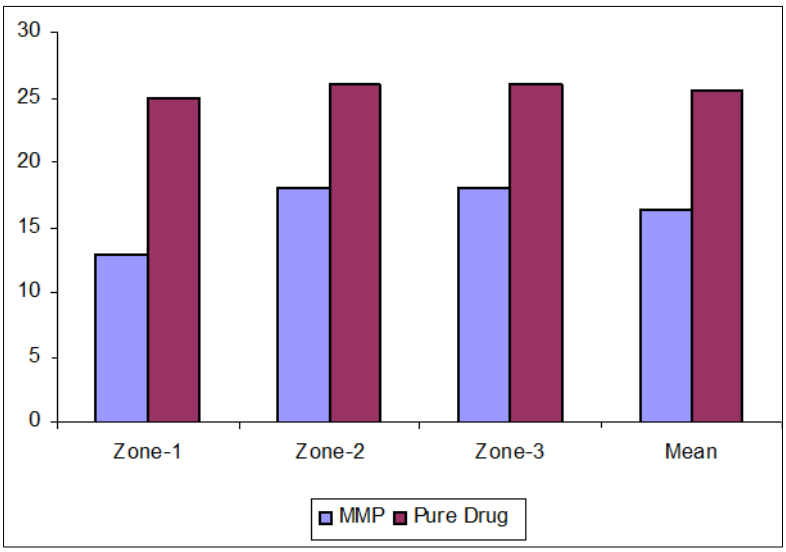

Figure 4 Histogram showing comparative zone inhibitions of Pure Drug and Drug in MMP formulation

A layer of peptone agar (2ml) seeded with test microorganism was allowed to solidify in Petri dishes by incubating at 37^{0}C for 24hrs. Cups (bore) were made on the solidified agar layer with the help of a sterile cork borer of 5mm diameter. Then approximately 2gm of mucoadhesive mouth paint formulations with drug and pure drug were then poured into the cups and incubated at 37^{0}C for 24-36hrs to observe the extent of zone inhibitions formed in different Petri plates. (Table-5 & Figure-3&4).

2.4.9. Stability studies

The prepared mucoadhesive mouth paint formulations were stored at different temperature condition like ambient temperature, 8±1^{0}C (refrigerator temperature), 45 ± 2^{0}C at 75% ± 5% R.H (condition of accelerated stability testing) for a span of three months & analyzed for drug content, physical appearance, pH spreadability and mucoadhesive strength.

2.4.10. In Vivo studies for oral mucosal skin irritation studies:

The experimental work plan for oral mucosal skin irritation studies was undertaken in: - Laboratory experimental Animals[21,22], and Healthy Human Volunteers.

Figure 5 Skin irritation test of oral muscosa in rabbits

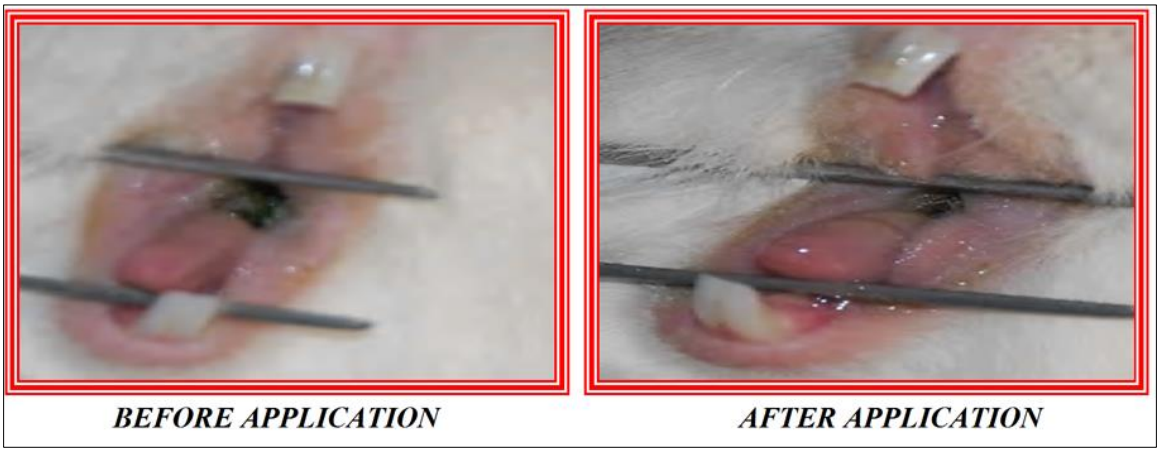

Figure 6 Skin irritation test of oral muscosa in guinea pigs

One gram of the prepared 1% Fluconazole mucoadhesive mouth paint was applied to animal's oral cavity to test oral skin irritancy effect, in 6 Rabbits and 6 Guinea-pigs. The rabbits were weighing 2.0 to 2.5 kgs and Guinea-pigs 500 to 800 gms. 3 times in 72 hours to each animal and the duration was approximately 24 hours between each application. Irritancy assessment was made by visually examining animals' oral cavity with the focus & magnifying lens to notice any changes in tissues after each application. Then comparison of the photographs of control as against the photographs

of oral cavity which have undergone application of mouth paint. Control photographs are the one taken prior to the first application i.e, at zero hr. of the 72 hrs. Study time. The oral mucosal skin irritancy study on animals was thus evaluated for any changes like sensitization, oral edema, erythema, redness, inflammation or acute ulcers. (Figure-5 & 6).

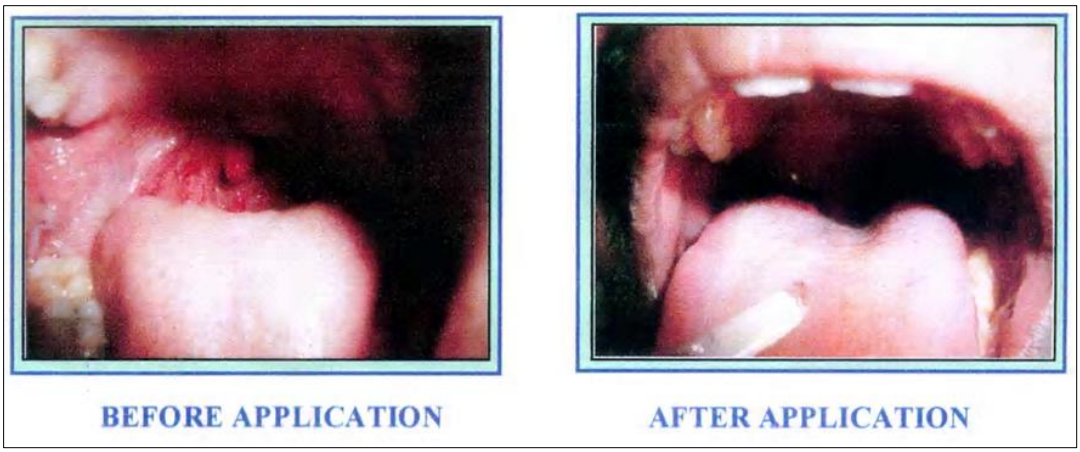

Figure 7 Skin irritation test of oral mucosa in human volunteers

2.4.11. Healthy Human Volunteers Studies

The oral mucosal skin irritation test was performed on six healthy male volunteers by applying 1gm mouth paint formulation (l0mg drug). The volunteers were of age group between 23-28 years and weighing 55 to 65 Kgs. All the volunteers were regular in their oral hygiene regime and usually brushed and gargled twice a day. The legal ethical committee approved the study and each subject gave written informed consent before indulging in the study. The volunteers were fasted without food and water for atleast 3hrs before each application. The volunteers abstained from taking any medicines/ alcoholic drink / chewing tobacco for over 30hrs at the start of test and during the entire 72hrs. Of study. During other times the regular food and water was served / allowed. The prepared mucoadhesive mouth paint formulation of 1gm for each application (l0mg drug) was applied with the aid of brush on the Dorsal part of tongue, hard and soft palate and buccal region of the mouth of volunteers for a period of 72hrs (3 days) study with a duration of approximately 24hrs per application. Volunteers were parted into 3 groups involving 2 volunteers in each group. The formulation was applied on each group of two volunteers for the study. The preparations were allowed to retain for overnight restricting the volunteers from taking any liquid / solid intake. Xs

2.4.12. Oral Mucosal Irritancy Assessment

It was performed primarily by examining each volunteer oral cavity barely with naked eyes using focus and magnifying lens to notice any changes in tissues after application of formulations. Then photographic imaging of oral cavity of human volunteers was taken out after subsequent application for 72hrs i.e., at completion of study period and these images were compared to determine the difference with the images taken at zero hr. of study i.e., prior to first application of formulation. Moreover, mucosa irritation was evaluated by questioning the human volunteers at regular interval of time about the feeling of irritancy, which appears to be highly subjective for the study. Finally, the oral mucosal skin irritancy was evaluated for any changes like oral erythema, inflammation, redness, hemorrhagic lesions or acute painful ulcers (canker sores).

3. Results and discussion

In the present piece of investigation, the mucoadhesive mouth paint preparations of Fluconazole can be designed using hydrophilic polymer like HPMC for the treatment of oral candidiasis. During our physico–chemical evaluation studies the formulation was found to have good spread ability and mucoadhesive strength. The drug content for MMP was 97.56%. In our present investigation of *In vitro* drug release studies, the mucoadhesive mouth paint showed optimum release of 71.41% in 3 hrs. as against conventional mouth paint formulation showed 84.14% in 75 minutes. The rheological behavior of both mucoadhesive mouth paint and conventional mouth paint were studied. Mucoadhesive mouth paint data shown shear thinning (pseudo plastic) behavior, where there is decrease in viscosity by increasing shear rate. This shear thinning behavior is a desirable property for topical preparations, as they should be thin during application and thick otherwise. The formulation mucoadhesive mouth paint showed good mucoadhesive strength (38 secs) when compared to conventional mouth paint formulation (11 secs), which measure the viscosity at physiological

temperature. During microbiological investigation against the causative organism collected from the patient of oral candidiasis, the hydrophilic polymer containing HPMC, formulation showed good zone inhibition when compared with pure drug. IR studies revealed that there is no drug excipients interaction. The undisturbed peaks of pure drug at 1644 cm^{-1} and 1421 cm^{-1} is due to C=C and C=N stretching vibrations and at 1212 cm^{-1} aromatic C-F stretching vibration confirm the undisturbed drug in formulation. In our present investigation of stability studies, the prepared formulation did not segregate, ferment or physically deteriorated during storage & use at different temperature conditions for a period of 3 months. The formulation did not undergo phase separation or gassing fermentation or otherwise deterioration aesthetically. *In vivo* studies were carried out to study the oral mucosal skin irritation on both laboratory experimental animals (Rabbits & Guinea–Pigs) in our animal house and on Healthy Human volunteers with the help of staff ENT Dept. of our medical college. The studies revealed that the usage of 1% Fluconazole mucoadhesive mouth paint formulation did not produce oral mucosal skin irritancy. On observation no edema, erythema, inflammation or redness seen in the mucosal cavity of both animals & Human volunteers indicating formulations high compliance with oral mucosal surface, thereby passing the test of compatibility studies. The present study revealed that the prepared Fluconazole mucoadhesive mouth paint formulation with more retentive time in oral cavity will be useful than conventional mouth paints, which have short retentive time.

4. Conclusion

The results of present study on mucoadhesive mouth paint designed for the treatment of oral candidiasis will be useful for drug industry to formulate localized drug delivery to benefit the patients suffering from oral candidiasis of all ages and sex.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

The legal ethical committee approved the study.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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