

DEVELOPMENT AND IN VITRO/ IN VIVO EVALUTION OF TRIPOLYMERS BASED CLOTRIMAZOLE IN SITU GEL FOR ORAL THRUSH

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ABSTRACT

The scope of the present study was to develop a prolonged the delivery of the active drug in the oral cavity using a suitable carrier such as *in situ* gels which can effectively deliver the drug for an extended duration of time hence not only reduce the systemic side effects but also improve the therapeutic efficacy, patient compliance. System containing *in situ* gels composed of HPMC, carbopol 934 and sodium alginate, loaded with drug clotrimazole, have been prepared and characterized by rheological measurements, pH, spreadability, gel strength, mucoadhesive force, FTIR, DSC and mice model studies. The viscosity of the solution was found to be in the range (356 to 729 cps), whereas for the *in situ* gels it was up to 12840 cps. The maximum gel strength and mucoadhesion was found to be up to (110 seconds) and (132.48 dynes/cm²) respectively. The *in vivo* animal studies for oral thrush which was conducted in mice revealed that the oral fungal burden at the end of 7th day was NOD (no organism detected) for the animals treated with the *in situ* system in comparison with that of control (log₁₀ 4.411 ± 0.54 CFU/ml) un treated animals. The developed *in situ* system is effective in terms of eradication of oral thrush.

Keywords: oral thrush, clotrimazole, mice model, sodium alginate, Carbomer 934, Hydroxyl propyl cellulose, *in situ* gel.

Introduction

Candida species are everywhere, human fungal pathogens capable of initiating a variety of recurring superficial diseases especially in the oral and vaginal mucosa¹⁻². In the late 1950s there was a progressively increasing number of reports on superficial *Candida* infections associated with the administration of broad-spectrum antibiotics such as tetracycline³⁻⁴. In subsequent years, the broad use of steroids, immunosuppressive agents in organ transplant recipients⁵⁻⁶, myeloablative radiation therapy⁷⁻⁹, and antineoplastics in patients with hematologic malignancies¹⁰⁻¹² contributed to the increasing morbidity associated with *Candida*. More lately, mucosal *Candida* infections have received profuse attention due to the advent of the human immunodeficiency virus (HIV) infection. For instance, it is now known that up to 90% of HIV-infected individuals suffer from oropharyngeal candidiasis¹³. This condition is a key mark in staging HIV disease and was once included as a marker in disease classification¹⁴. Curiously, HIV-infected patients appear to be more liable than immunocompetent individuals to oropharyngeal but not vaginal or disseminated candidiasis¹⁵⁻¹⁶. The other common risk factors for oral candidiasis are age (mainly the very young and the very old), denture prostheses, smoking, diabetes mellitus, iron and vitamin deficiencies¹⁷, and salivary gland hypofunction¹⁸. The most important agents that are currently used for oropharyngeal candidiasis belong to the polyenes group like nystatin and amphotericin B, the imidazoles group like clotrimazole, econazole, ketoconazole and miconazole or the triazoles groups like fluconazole, itraconazole¹⁹. Amphotericin B is less broadly used for this purpose due to its treatment-limiting adverse effects such as nephrotoxicity²⁰. Clotrimazole (1-o-chloro- α , α -diphenylbenzyl) imidazole is a synthetic imidazole, having a broad spectrum of fungicidal activity, being effective against both dermatophytes and yeast-like fungi. Several options are available for the treatment of

oropharyngeal thrush includes the systemic administration and topical solution. Topical agents, such as nystatin and clotrimazole troches, are generally used as first-line therapy, but they are inconvenient for patients to administer, multiple daily doses are usually required, less concentration of drug at the site of infection, dilution of drug in oral cavity, rapid elimination of topically applied drugs due to the flushing action of saliva, less effective than systemic therapies²¹⁻²³ and systemic adverse effects. Objective of the present study was to prolong the delivery of the active drug in the oral cavity using a suitable carrier such as *in situ* gels which can effectively deliver the drug for an extended duration of time hence not only reduce the systemic side effects but also improve the therapeutic efficacy, patient compliance. The *in situ* systems are made of biodegradable products, which can be injected via a syringe into the infected area and once injected; solidify to form a semisolid depot²⁴.

MATERIALS AND METHOD

Clotrimazole was obtained from (Fourts India, Chennai); HPMC, carbopol 934 and sodium alginate was purchased from Merk, Mumbai. DMSO and propyl paraben was supplied by *Fine Chemicals (India)*. Methyl prednisolone was obtained from Neiss Labs Ltd, India; *Candida albican* came from bio genic laboratories, Hubli, Karnataka, and gentamycin sulphate injection came from M/S Pharmaceutical and Industrial Laboratories, India. All other chemicals were of reagent grade.

METHODS

Formulations were prepared with various ratio of polymers was soaked in sufficient quantity of deionised water and kept overnight for swelling and propyl paraben solution was added to the above polymeric mixture. Finally an appropriate amount of Clotrimazole was solubilized in suitable solvent with continuous stirring until uniform solution was obtained. (Formulations contain 10 mmol of calcium chloride and 0.1 w/v sodium citrate). Finally a small amount of triethanolamine was added to adjust pH 7. The detailed composition of prepared formulation is depicted in Table 1.

Table 1. Composition of ion induced formulation

| S. NO | Ingredient w/v % | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 | F13 | F14 | F15 |
|-------|---------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 | Clotrimazole | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| 2 | HPMC | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.2 | 0.3 | 0.4 | 0 | 0 | 0 | 0.2 | 0.3 | 0.4 | 0.5 |
| 3 | Carbopol 934 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 | 0.4 | 0.3 | 0.2 | 0.1 |
| 4 | Sod.alginate | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0 | 0 | 0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| 5 | propyl paraben | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| 6 | DMSO | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s |
| 7 | Triethanol amine | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s |
| 8 | Deionized water upto (ml) | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |

EXPERIMENTAL SECTION

Gelling capacity

The gelling capacity of the prepared formulation is determined by placing a drop of the formulation in a vial containing 2.0 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling is noted²⁵⁻²⁶.

Determination of Mucoadhesive Force

The experimental technique used for determining the mucoadhesive force has been derived from a previously published method²⁷⁻²⁸. The mucoadhesive force of the formulations was determined as follows; a section of membrane was cut from the chicken and instantly fixed with mucosal side out onto each glass vial (E) using rubber band. The vial with chicken mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan (A). Gel was added onto the mucosa of first vial. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then, the switch (C) of the infusion apparatus was opened to make the water drop into the glass vial (B) with a constant flow rate of (5 ml/min) as shown in Figure 1. The weight of the water in the glass vial (B) kept increasing until the gel and the mucosal tissue were detached. Mucoadhesive force, the detachment stress (dynes/cm^2), was determined from the minimal weights that detached the gel. The chicken membrane pieces were changed for each measurement. All measurements were performed in triplicate ($n = 3$).



(A) Modified Balance (B) Glass Reservoir (C) Infusion Device (D) Membrane (E) Vial
(B) Height Adjustment Pan.

Figure 1. Assembly of mucoadhesive force measuring device

Measurement of Gel Strength

A sample of 50 gm of gel was placed in a 100 ml graduated cylinder and gelled in a thermostat at 37 °C. The apparatus for measuring gel strength (weighing 27 gm) was allowed to penetrate in gel. The gel strength, which means the viscosity of the gels at physiological stimuli was

determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel²⁷.

Spreadability

The spreadability of the gel was determined using the following technique: 0.5g gel was placed within a circle of 1 cm diameter pre marked on a glass plate over which a second glass plate was placed. A weight of 1000 g was allowed to rest on the upper glass plate for 5 minutes. The increase in the diameter due to spreading of the gels was noted. The calculation of spreadability is as follows;

$$S = ML/T$$

Where M = Weight tide to the Upper Slide (g)

L = Length moved on the glass slide (cm)

T = Time taken

Viscosity Studies

The rheological studies were carried out using Brookfield programmable DVII+ Model pro II type (USA). The viscosity of *in situ* gel and the solution were determined at different angular velocities and average of two reading was used to calculate the viscosity.

Diffusion across the chicken cheek mucosa

Chicken cheek mucosa²⁹ was isolated from a healthy chicken which was obtained from the local slaughter house and was cleaned to remove blood cells. It was stored in normal saline with few drops of gentamycin sulphate injection, to avoid bacterial growth. The diffusion medium used was phosphate buffer (2.38 g Na₂HPO₄, 0.19 g KH₂PO₄ and 8 g NaCl in 1000 ml of distilled water adjusted to pH 7.4). The oral diffusion cell was designed as per the dimension given. The diffusion cells were placed on the magnetic stirrers. The outlet of the reservoir maintained at 37 ± 0.5°C and was connected to water jacket of diffusion cell using rubber latex tubes. The receptor compartment was filled with fluid. Then the prepared chicken cheek mucosa was mounted on the cell carefully so as to avoid the entrapment of air bubble under the mucosa. Intimate contact of mucosa was ensured with receptor fluid by placing it tightly with clamp. The speed of the stirring was kept constant throughout the experiment with the help of micropipette. Aliquots of samples were withdrawn at time intervals of one hour from sampling port of receptor compartment and same volume was replaced with receptor fluid solution in order to maintain sink condition. The samples were withdrawn and drug content was determined as per the above procedure.

Content Uniformity

The formed gel (1g) was completely crushed with the help of glass road followed by vigorous shaking until the formed gel gets completely dispersed to give clear solution³⁰. Final volume was adjusted to 100 ml with simulated saliva pH 7.4. Obtained solution was filtered through 0.45 micron filter membrane and the drug concentration was determined by UV Visible spectrophotometer at 260 nm.

Mice Model

New research on treatment for oral candidiasis requires an appropriate animal model. Although numerous animal models for this condition have been developed, researchers have lacked a convenient and reproducible technique for preparing adequate numbers of animals for statistical analysis³¹⁻³⁴. Healthy albino mice weighing 25 to 27 g were used throughout these experiments. Animals were individually housed and provided food and water. To establish oral infection of *C.albicans* in these animals, the host defense mechanism was suppressed by several methods. Methyl prednisolone, an immunosuppressant, and chlorpromazine, a tranquilizer, were administered to lowers the immune functions of the mice. The chlorpromazine tranquilizer that

lowers leukocyte functions also suppresses saliva secretion and facilitates the colonization of *C.albicans* perhaps due to prolonged sedative periods³⁵. Methyl prenisolone (4 mg/kg) was injected into the mice subcutaneously at the time tetracycline hydrochloride (0.5 mg/liter) was provided in drinking water. Twenty-four hours after initiation of tetracycline administration, chlorpromazine was soaked in a *C.albicans* solution and placed in the oral cavity of each mouse, for two days after the inoculation, the inflammatory response of the tongue primarily. The inflammation peaked five days after the inoculation, and then subsided thereafter. The number of viable *C.albicans* cells peaked three days after inoculation. In mice with oral thrush, curd -like patches (in which numerous mycelia of *C.albicans* cells grow) surrounded by redness were widely visible on the tongue surface. A histopathological study using PAS staining showed that *C.albicans* with hyphae was attached in layers to the oral epithelia on the dorsum of the tongue. These observations suggested that these murine model exhibited symptoms quit similar to human oral thrush and finding indicate that the murine model prepared by the procedure described above is a suitable animal model for oral candidiasis³².

Antifungal treatment

Treatment groups (four mice each) included clotrimazole (1 % or 10 mg/ml), in pluronic 188 *in situ gel*, and control (pluronic 188 *in situ gel* alone). Starting at day 3 after inoculation, mice were treated orally (25 μ l, b.i.d.) for 7 consecutive days.

Measurement of in vivo antifungal activity

Therapeutic response to clotrimazole was measured by daily oral mucosal quantitative cultures and determination of viable count. On the sixth day the animals were sacrificed and their tongues were dissected. Serial cross-sections of the tissues were obtained and stained with periodic acid-Schiff (PAS) for fungal visualization.

RESULT AND DISCUSSION

Physicochemical properties such as viscosity, pH, appearance and clarity test were performed and the results are recorded in Table 2. The viscosity of polymer solutions and gels of various formulations was determined at various shear rates. As the shear rate is increased, the viscosity of prepared *in situ gel* decreased as shown in Figure 2. In general increase in ratio of polymer concentration to drug caused an increase in viscosity. The aforementioned statement reflect to the formulation F15 having the maximum concentration of HPMC, carbopol 934 and sodium alginate (0.5:0.1:0.5) showed maximum viscosity in solution forms as well as in gels forms. Due higher concentration of polymer in formulation F15 is suitable for *in situ gel* formulation.

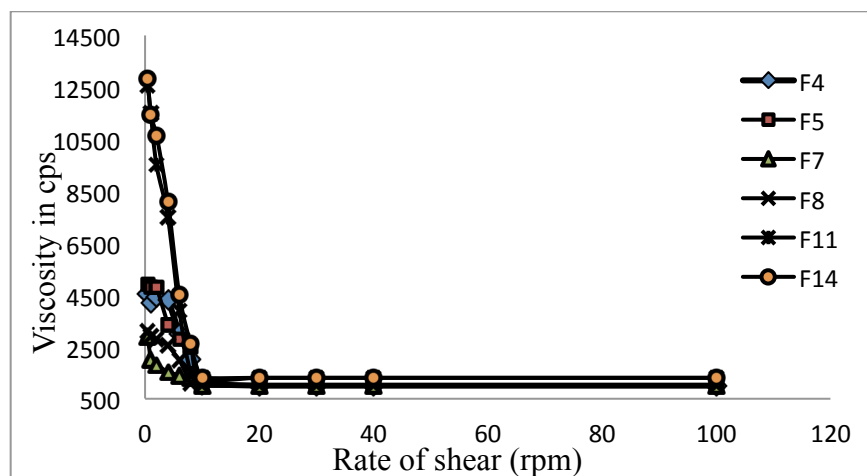


Figure 2. Showing the viscosity of profile of in situ gel F code formulations

The effect of Ca^{2+} ion on gelation capacity

The quantities of the chelating agents calcium chloride and sodium citrate should not contain free ionic calcium form in the formulation so as to ensure that they are in fluid state before administration, but sufficient Ca^{++} ions must be released when the complex is broken down (due to dissociation) in presence of simulated salivary fluid to cause gelation. The formulation prepared by ion induced method were fluid state at pH 6.9-7.0 with appropriate amount (mmole) of calcium chloride before administration in to the buccal cavity, rapidly undergoes gelation after administration by reacting with Ca^{2+} ion present in the saliva. Polymers (a. HPMC/ b. Sodium alginate/c. Carbopol 934) were utilized without compromising their gelation capacity and rheological properties. Gelation capacity and rheological properties of the *in situ* system may be achieved by the addition of combination of polymers a: c, a: b, a: b: c except b: c. from the results of rheological studies it was also found that combination of b: c does not achieved better sol to gel conversion at simulated saliva composition on the contrary the polymer combination of (a: b: c) shows better sol to gel conversion at simulated saliva composition. Determination of the optimum amounts of complex agents for sodium alginate solutions showed that only those containing 10 mmol calcium chloride in combination with 0.1% (w/v) sodium citrate were found to be satisfactory to cause gelation. Low level of cations present in the solution was sufficient to hold the molecular chains together and inhibit hydration. From the above result, the experiment was performed with formulations containing 10 mmol calcium chloride and 0.1% (w/v) sodium citrate. The two main prerequisites of *in situ* gels are viscosity and gelation capacity. All the optimized formulations were found to have good gelation capacity. The formulation F4 (0.4: 0.5) F5 (0.5: 0.5) containing (HPMC: SG), exhibited good gelation immediately after addition in to the simulated saliva fluid and remained for about 8 hours. The formulations F7, F8 showed moderate gelation capacity and remained for few hours. The *in situ* gel F9 and F10 could not show any gelation. The formulation F11(0.1/0.3/0.5) and F14 (0.4/0.2/0.5) (HPMC / Sodium alginate / Carbopol 934) were found to be best gelation capacity and remained for extended period of time as shown in Table 3.

Spreadability

The spreadability studies of formulations F11, F14 (30.79, 36.36 gm.cm/sec) was found to be more as compared to formulations F4, F5 (23.14, 23.7 gm.cm/sec). The formulations F7, F8 exhibited less spreadability (20.11, 21.34 gm.cm/sec), in comparison with F4 and F5 as shown in Table 3.

Gel Strength

Gel strength of formulations F4 and F5 (74, 75 sec) was found less as compared to formulations F7, F8 (82, 85 sec). Formulations F11 and F14 (105,110 sec) exhibited good gel strength which may be due to increase in concentration of HPMC and Sodium alginate as shown in Table 3.

Mucoadhesive Force

The result of mucoadhesive force studies indicated that the formulation F4 and F5 was found to be more (98.23, 99.41 dynes/cm²) than that of formulations F7 and F8 (95.24, 98.56 dynes/cm²). Similarly mucoadhesive force of the formulations F11, F14 (128.98, 132.48 dynes/cm²) were showed higher values of than F4, F5, F7, F8 formulations. It may be due to the combination of three polymers in the formulation F14 and was the best optimized formulation and also the formulation containing alginic acid shown ability to gel in the oral cavity, due to its mucoadhesive properties as shown in Table 3.

Table 2.Physicochemical Evaluation of Various Clotrimazole Solution ($n=3$)

| Tests | F-4 | F5 | F7 | F8 | F11 | F14 |
|-------------------|-----|-----|-----|-----|-----|-----|
| visual appearance | *** | *** | *** | *** | *** | *** |
| clarity | *** | *** | *** | *** | *** | *** |
| pH | 7.0 | 6.9 | 7.0 | 6.9 | 7.0 | 7.0 |
| Viscosity(Cps) | 356 | 423 | 580 | 600 | 675 | 729 |

Table 3 .Characteristics of Various Clotrimazole Gel Formulations code F ($n = 3$).

| Formula code | Viscosity (Cps) | % drug content(w/w) | mucoadhesive force(dynes/cm ²) | Spread ability (gm.cm/sec) | Gel Strength seconds | Gelation Capacity | Gelation Time |
|--------------|-----------------|---------------------|--|----------------------------|----------------------|-------------------|---------------|
| F4 | 4524 | 95.6 | 98.23 | 23.14 | 74 | ** | 72 |
| F5 | 4920 | 95.7 | 99.41 | 23.77 | 75 | ** | 70 |
| F7 | 2875 | 96.7 | 95.24 | 20.11 | 82 | ** | 115 |
| F8 | 3087 | 96.5 | 98.56 | 21.34 | 85 | ** | 100 |
| F11 | 12580 | 95.3 | 128.98 | 30.79 | 105 | *** | 60 |
| F14 | 12840 | 95.5 | 132.48 | 36.36 | 110 | *** | 58 |

** Exhibited good gelation capacity *** Exhibited excellent gelation capacity *($n = 3 \pm SD$)

Gelation time

Gelation time defined as the time when the elasticity modulus became higher than the viscosity modulus. All optimized formulations showed gel-like rheologic properties at simulated Saliva. The gelation time of F4, F5 was observed at 72 sec. and 70 sec respectively also the formulations F11 and F14 showed gelation within 60 sec and 58 sec. Whereas the gelation time was found to be 105 sec and 110 sec in formulations F8 and F7 respectively as shown in **Table 3**.

In vitro Release Studies

The *in vitro* diffusion profile of clotrimazole from *in situ* gels from formulations F4, F5, F7, F8, F11 and F14 were conducted in diffusion medium (2.38 g Na₂HPO₄, 0.19g KH₂PO₄ and 8.0g Nacl in 1000 ml of distilled water adjusted to pH 7.4). The formulations F7 and F8 containing the two polymer ratio (0.3:0.2), (0.4:0.3) showed 70.3% and 75.8% respectively at 6 hours, Whereas formulations F4 and F5 released 55.7% and 52.4% respectively at 8 hours. But the formulations F10, F11 and F12 which contain combination of three polymers, the release rate was 72.4%, 68.29% and 69.3% respectively.

Drug Release Kinetics

The correlation coefficient 'r' of prepared formulation indicated that the drug release followed diffusion controlled mechanism from the *in situ* gel, as the value of 'r' for first order kinetics ranged from 0.893 to 0.997 and also found to be higher than that of zero order which ranged from 0.8905 to 0.9804 and Higuchi square root of time ranged from 0.881 to 0.978. All the formulations were subjected to PCP DISSO software analysis. Formulation F10, F11 and F12 exhibited good *in vitro* release kinetics with fickian type of diffusion mechanism. More over to comprehend the drug release mechanism the data were fitted in to korsmeyer -peppas exponential model where the 'n' values were in the range of 0.077 to 0.152. It was clearly

indicated that F code formulation were following predominantly first order and fickian diffusion mechanism of drug release as shown in Table 4.

Table 4. Release kinetics of F Code formulations

| Order of process | F4 | F5 | F7 | F8 | F10 | F11 | F12 |
|------------------|-----------------------|--------|--------|--------|--------|--------|-------|
| Zero order | R ² 0.9804 | 0.949 | 0.8905 | 0.8984 | 0.9268 | 0.9223 | 0.906 |
| | M 6.906 | 6.2 | 11.28 | 11.59 | 9.558 | 8.965 | 9.136 |
| | C -9.311 | -10.55 | 0.457 | 1.914 | -1.991 | -0.936 | 0.45 |
| First order | R ² 0.928 | 0.893 | 0.536 | 0.997 | 0.952 | 0.937 | 0.927 |
| | M -0.038 | -0.039 | -0.053 | -0.088 | -0.077 | -0.068 | -0.07 |
| | C 2.027 | 2.052 | 1.873 | 1.923 | 1.994 | 1.979 | 1.966 |
| Higuchi | R ² 0.978 | 0.952 | 0.966 | 0.994 | 0.854 | 0.901 | 0.881 |
| | M 7.164 | 6.666 | 7.994 | 9.485 | 8.281 | 8.104 | 8.109 |
| | C -3.864 | -7 | 25.98 | 19.8 | 16.33 | 12.9 | 15.4 |
| Korsmeyer | R ² 0.928 | 0.974 | 0.801 | 0.858 | 0.731 | 0.769 | 0.747 |
| | M 0.136 | 0.152 | 0.086 | 0.077 | 0.101 | 0.093 | 0.09 |
| | C 0.741 | 0.55 | 1.396 | 1.457 | 1.19 | 1.216 | 1.259 |

DSC spectra:

The results of DSC thermogram indicated that clotrimazole has a sharp melting endothermic peak at 151°C. The DSC curve of the sodium alginate polymer showed a broad endothermic peak at 119.29°C and 257.73°C. The DSC curve of the HPMC showed a peak at 114.62°C. Combination of three polymers showed an endothermic peak at 114.62°C. Physical mixture of polymer and drug showed an endothermic peak at 112.99°C and 150.95°C. From the DSC studies it can be concluded that there was no significant change in the peak value in comparison with pure drug which revealed that the excipients are compatible with drug in formulations as shown in Figure 3.

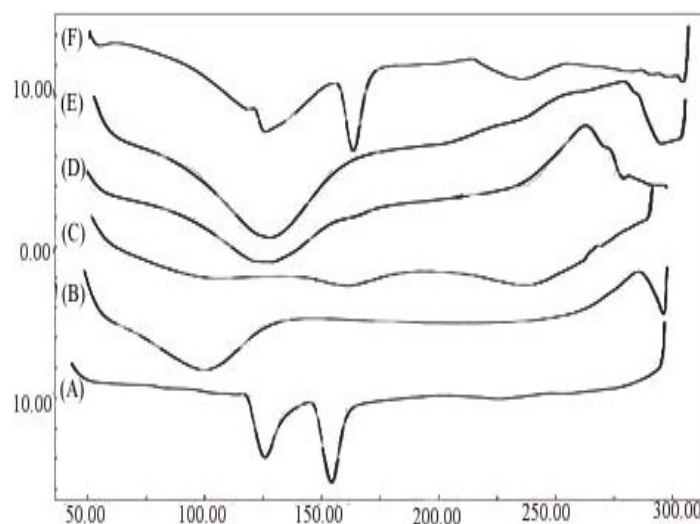


Figure 3. Showing the DSC Spectra

FTIR spectra

The FTIR studies showed that the significant peaks of clotrimazole are C-H stretching at 2920 cm^{-1} , C-Cl at 720 cm^{-1} , C-N cm^{-1} vibration Ar-tertiary at 1360 cm^{-1} and C-H bending at 1460 cm^{-1} and mixture of polymers showed eight significant peaks are C-H cm^{-1} stretching at 2920 cm^{-1} Carboxylate anion at 1720 C-Cl cm^{-1} stretching at 720 OH bending at 1100 C-N cm^{-1} vibration Ar-tertiary at 1360 C-N cm^{-1} vibration Ar-tertiary at 1460 cm^{-1} and Anhydride stretching or Ester at 1720 cm^{-1} . Finally the FTIR studies of mixture of polymers and drug does not show any significant change. This results in indicating that there is no any interaction between drug and selected polymers as shown in Figure 4.

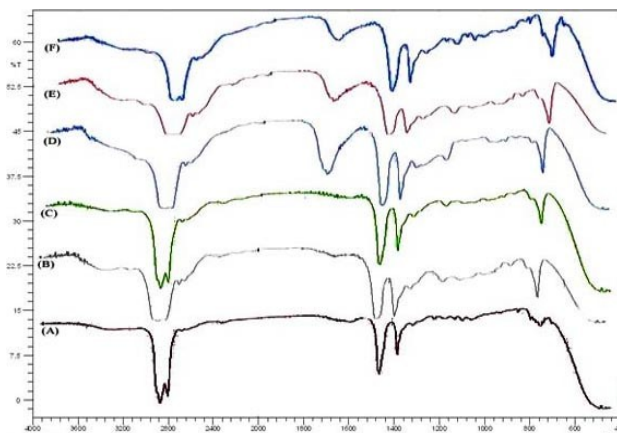


Figure 4. Showing the FTIR Spectra

Animal studies

The efficacy of antifungal agents for the treatment of oral thrush, we evaluated the efficacy of clotrimazole *in situ gel*. When clotrimazole system was administered at 10 mg/kg of body weight/dose starting on day 3, on day 4 the number of cfu in the oral tissues progressively decreased from ($4.674 \pm 0.23 \log_{10}$ cfu/ml) to NOD (no organism detected on day 7). In contrast, the oral fungal burden of the control mice remained constant on day 4 and 7 (\log_{10} cfu/ml 4.513 ± 0.41 and 4.411 ± 0.54). The animals treated with the *in situ* system are effective in terms of eradication of oral thrush as shown in Figure 5.

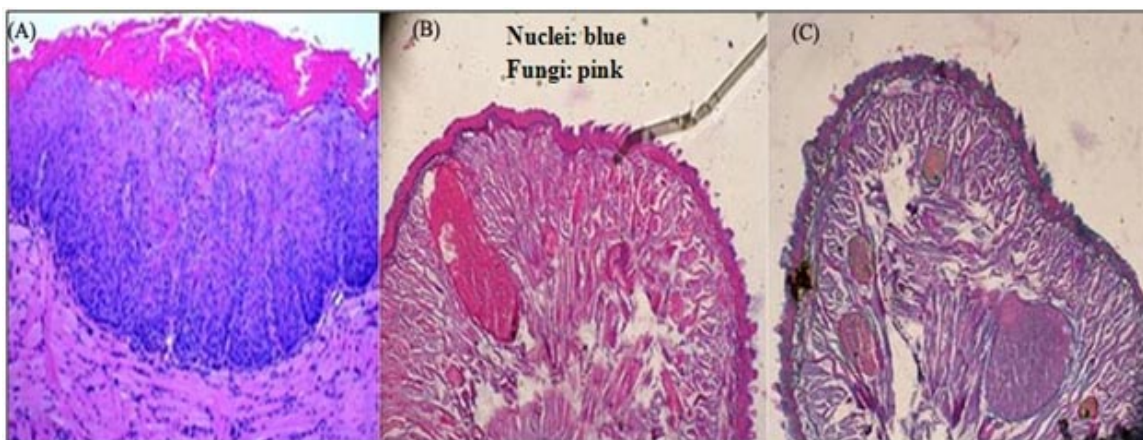


Figure 5. Histopathological analysis of the tongue (A) control -infected (B) Tissue of mice infected with *C. albicans* after 4th day (C) Tissue of mice treated with clotrimazole systems after 3rd day (PAS stain).

CONCLUSION

The ion induced in situ gels were prepared by taking the clotrimazole having compatibility between drug and excipients which were used in the formulae. The formulation made with carbopol 934, HPMC and SG with clotrimazole was found to have the entire suitable characteristic required for in situ gels for targeting and sustaining the drug release in the treatment of oral thrush.

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