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Research Article

Design and Characterization of Mucoadhesive Buccal Patch Containing Antifungal Agent for Oral Candidiasis

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Abstract

Buccal bioadhesive films, releasing topical drugs in the oral cavity at a slow and predetermined rate, provide distinct advantages over traditional dosage forms for treatment of oral candidiasis. The study was to formulate and evaluate buccal patches containing an antifungal agent (miconazole nitrate), using different ratio of hydroxy propyl methyl cellulose, carbopol 934, hydroxy ethyl cellulose, hydroxy propyl cellulose and polyvinyl pyrrolidone. Nine different film formulations containing 20 mg of miconazole nitrate had been prepared by a solvent casting evaporation technique and characterized in terms of weight uniformity, film thickness, surface pH, swelling capacity, in *vitro* drug release and *in vitro* microbiological effectiveness against *Candida albicans* in comparison to a reference gel. Convenient bioadhesion, acceptable elasticity, swelling and surface pH were obtained. Patches exhibited sustained release over a period of 7 h and the addition of polyvinyl pyrrolidone (PVP) generally enhanced the release rate. Thickness of the prepared films ranged from 0.421 to 0.54 mm and the film weight ranged from 65.1 to 73.21mg, where the surface pH values of all films were in the range of 6.2□ 6.52 which is favorable for oral mucosa. The drug release mechanism was found to follow non- Fickian and Higuchi matrix diffusion. Furthermore, the antifungal activity of the selected film was significantly superior to the reference miconazole gel.

1. INTRODUCTION

The reason for the large interest in the local treatment of the oral cavity diseases is a result of their being amongst the most prevalent in humankind. Many oral diseases are chronic and, hence require chronic treatment regimens. In addition, most of the oral diseases can be treated locally, without the need for ingestion and systemic distribution of drugs. Formulations that prolong the drug release in the mouth offer great advantages in preventing and treating local diseases. The buccal mucosa is a feasible site for application of sustained or controlled-release delivery systems which could maintain a steady release of drug. For efficient and prolonged release of drugs, these delivery systems have to be in close contact with the mucosal membrane, resulting in high concentration in a local area, prolonged residence time at the absorption site, and hence high drug flux through the absorptive mucosa. Drug-loaded polymeric matrices have been used for the controlled buccal delivery of antimicrobial, antibiotic, local anesthetic, antifungal and antiviral drugs[1]. Bioadhesive patches are systems that may range from simple erodible and nonerodible adhesive films to more sophisticated systems, which can be designed to provide either unidirectional or multidirectional release of the drug. Buccal patches are highly flexible and thus much more readily tolerated by the patients than tablets. Patches also ensure more accurate dosing of the drug compared to gels and ointments. Candidiasis in the oral cavity is an opportunistic infectious condition caused by a ubiquitous, saprophytic fungus of the genus *Candida*, the most common of which is *Candida albicans*. Chronic antimycotic therapy in high doses is undesirable for treatment of oral infections due to potential side effects. Therefore, to minimize these adverse effects and the ominous risk of drug resistance, topical therapy should be considered the first□line candidate for the treatment of oral and pharyngeal candidiasis. Miconazole (MC) is one of the first line broad□spectrum antifungal agents that has been extensively used for the prophylaxis and treatment of oral and vaginal candidiasis. Oral candidal infections require prolonged therapy with antifungal agents and hence it may be advantageous to deliver these drugs in a sustained manner. The main aim of the present study was to develop buccal mucoadhesive patch to ensure satisfactory miconazole level in the mouth for prolonged periods. Antifungal activity of buccal patch against *Candida albicans* is performed and evaluated by comparing the zone of inhibition produced by the buccal patch and marketed gel[2, 3].

2. MATERIALS AND METHODS

Miconazole nitrate was the gift sample from Yarrow Chemicals, Mumbai. HPMC was procured from CDH, Pvt. Ltd., Mumbai, HPC, PVP, Carbopol 934 were obtained from Himedia Lab Pvt. Ltd., Mumbai, Sabaoraud dextrose HiVeg broth and Sabaoraud dextrose agar were purchased from Himedia Lab Pvt. Ltd., Mumbai. *Candida albicans* ATCC 90028 was obtained from A B Shetty Memorial Institute of Dental Sciences, Deralakatte. Other chemicals used were of analytical grade.

2.1 Formulation of Miconazole Nitrate Mucoadhesive Buccal Patch

Buccal patches were prepared by solvent casting method. Polymer combination was dissolved in distilled water with continuous stirring using magnetic stirrer. Polyethylene glycol 400 was added as plasticizer. The drug miconazole nitrate was dispersed in polymer mixture and the final volume was adjusted with distilled water. The beaker was covered with aluminium foil and the solution was allowed to stand overnight to remove air bubbles. Then the solutions was poured into the glass moulds of diameter 5× 5 cm^2 and allowed to dry in hot air oven maintained at 40°C till a flexible

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film was formed. The dried films were cut into size of 1.5×1.5 cm^2 diameter so that each film contained about 20 mg of miconazole nitrate. The composition of various formulations is given in table 1.

Table 1: Composition of various formulations

Ingredients	Formulation Code								
	FA1	FA2	FA3	FB1	FB2	FB3	FC1	FC2	FC3
Miconazole Nitrate (mg)	222	222	222	222	222	222	222	222	222
HPMC (mg)	--	--	--	--	--	--	550	525	500
Carbopol 934 (mg)	--	--	--	--	--	--	50	75	100
HPC (mg)	--	--	--	550	525	500	--	--	--
PVPK30 (mg)	50	75	100	50	75	100	--	--	--
HEC (mg)	550	525	500	--	--	--	--	--	--
Polyethylene glycol 400 (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Water (ml)	10	10	10	10	10	10	10	10	10

2.2 Characterization of Mucoadhesive Buccal Patch

2.2.1 FTIR studies

FT-IR spectra matching approach was used for detection of any possible chemical interaction between the drug and polymers. The individual sample of drug and polymer powder and the three different drug: polymer combination films were prepared and mixed with suitable quantity of potassium bromide. About 50 mg of this mixture was compressed to form a transparent pellet using a hydraulic press at 15 tons pressure. It was scanned between 4000 - 600cm^{-1} in an Alpha T Bruker FTIR spectrophotometer.

2.2.2 Uniformity of weight, film thickness, folding endurance and surface pH of the films

The individual weight of 10 samples of each formulation was determined and the average weight was calculated[4]. The thickness of 10 films of each formulation was determined using micrometer screw gauge and average thickness was determined[5]. Folding endurance test indicates the ability of the films to sustain mechanical handling as well as pliability during use in the oral cavity. The folding endurance 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[6]. The surface pH of the films was determined in order to investigate the possibility of any side effects due to change in pH *in-vivo*, since an acidic or alkaline pH may cause irritation to the buccal mucosa. The film to be tested was placed in Petri dish and was moistened with 0.5 ml of distilled water and kept for 30 sec. The pH was noted after bringing the electrode of pH meter in contact with the surface of the formulation and allowing equilibrating for 1 min[4].

2.2.3 Swelling index

The patch of size 1.5×1.5 cm^2 was weighed and placed in a preweighed stainless steel wire sieve of approximately 800 µm mesh. The mesh containing the sample was then submerged into 15 ml of simulated fluid of pH 6.8 contained in a porcelain dish. After definite time intervals, the stainless steel mesh was removed; excess moisture was removed by carefully wiping with absorbent tissue and reweighed. Increase in weight of the film was determined at each time interval until a constant weight was observed. The degree of swelling was calculated using the formula,

$$Swelling\ Index\ (S.I.) = \frac{W_t - W_0}{W_0}$$

Where, w_t is the weight of the patch at time t and w_0 is the weight of the patch at the time zero[7].

2.2.4 Measurement of tensile strength

The instrument which was designed in our laboratory as per literature was used for the measurement of tensile strength. The strips were clamped at the static end and were attached to the movable rod on railing with the help of a clip. The weights were gradually added to the pan to increase the pull force till the film was cut. The elongation was determined simultaneously by noting the distance travelled by the pointer, before break of the film, on the graph paper. The weight required to break the film was noted as the break force. The tensile strength was calculated using Allen's formula:

$$Tensile\ strength = \frac{Break\ force}{a \times b} \times \frac{1 + \Delta L}{L}$$

Where, a, b, L are the width, thickness and length of the patches. ΔL is the elongation at break.

$$\%\ Elongation\ at\ break = \frac{Increase\ in\ length}{Original\ length} \times 100$$

2.2.5 Measurment of bioadhesive strength

In vitro bioadhesive test of the prepared films was examined using chicken pouch as a model mucosal membrane. The tissue was obtained from chicken after slaughter, removed from its contents and surface fats and stored frozen in Kreb's buffer solution. It was thawed to room temperature before study. The apparatus comprised of a two arm balance, one side of which contains glass plates and the other side contained a container. One of the two glass plates was attached permanently to the base of the stage, and the other was attached to the arm of the balance by a thick a strong thread. Fresh chicken buccal mucosa was glued to the upper side of the lower plate and another was glued to the lower side of the upper plate by using cyanoacrylate adhesive. The patch was placed on the chicken cheek mucosa glued to the upper side of the lower plate. Then, the upper plate was placed over the lower plate and 50g preload force (or contact pressure) was applied for 5 min (preload time). After removal of the preload force, the water was kept in a bottle at some height and was siphoned into the container at a rate till the plates were detached from each other. The weight of the water (g) required for the detachment of the glass plates was considered as the mucoadhesion force of the applied patch. The mucodhesive strength is calculated by following formula

Detachment stress = m.g/A

Where 'm' is the weight of water infused at detachment, 'g' the acceleration due to gravity considered as 980 cm/s^2, and A the area of tissue exposed (cm^2)[8].

2.2.6 In Vitro Drug Release Studies

In vitro drug release study was carried out using USP dissolution apparatus, type II. The dissolution medium consisted of 200 ml of simulated salivary buffer (2% SLS) of pH 6.8. The release was performed at 37 ± 0.5 °C, at 50 rpm. One side of the buccal patch was attached to a glass disk with instant adhesive (cyanoacrylate). The disc was put in the bottom of the dissolution vessel so that the patch remained on the upper side of the disk. Samples (5 ml) were withdrawn at pre-determined time intervals and replaced with fresh medium. The samples were filtered through 0.45 mm Whatmann filter paper and were assayed spectrophotometrically at 273 nm[9].

2.3 Stability Studies

The stability of all the formulations was carried out at different temperaturesas per ICH guidelines. The selected formulations were wrapped in aluminum foil and placed in stability chamber. A stability study for the present work was carried out at 2 ±3 °C, 25±2 °C and 40±2 °C for period of 2 months and evaluated for their physical appearance and drug content at specified intervals of time.

2.4 Evaluation of antimicrobial activity

Antimicrobial evaluation is mainly done to check the efficacy of the prepared formulations against the microorganism *Candida albicans*. In the present study, comparison is done between prepared dosage form and marketed gel, to determine whether the prepared formulation is as efficacious as the marketed gel or more effective. The method used was agar diffusion method. Organism used for the study was *Candida albicans* ATCC 90028 and subculturing was done on Sabauroud Dextrose Agar. Selected formulations FA3, FB3, FC1 and marketed gel have been used for antimicrobial evaluation. The study was performed by placing 0.01 g of the reference marketed gel which contains 0.2 mg of miconazole on the sabouraud dextrose agar previously cultured with *Candida albicans*. A 0.0225 cm^2 of buccal film formulation containing drug and placebo were placed on the same media. Once all the materials were placed on the agar surface, the lid was replaced, the plates were invert and the samples were incubated at 37 °C for 24 h. The zones of growth inhibition were measured as a mean ± SD, n=6 [10, 11, 12]. Statistical analysis was performed. Each experiment was repeated six times and the results were expressed as mean ± standard deviation. Statistical significances were compared between the standard (marketed gel) and formulated buccal patches and analyzed by the Independent samples T-test using SPSS version 16.

3. RESULTS AND DISCUSSION

3.1 Drug-polymer Compatibility

IR studies were carried out for pure drugs, HPMC, HPC, carbopol, PVP K-30 and the formulated films to know the interaction between drug and the polymers. Specific FTIR spectra of miconazole powder was noticed in the range of 3174cm^{-1} due to imidazole C-N stretch, 3059cm^{-1} due to aromatic CH group, 2890 due to aliphatic -CH group, 1547cm^{-1} due to C=C aromatic, 1516cm^{-1} due to C=C aromatic, 1477cm^{-1} due to C-H bending, 1320cm^{-1} due to C-N stretching, 1083cm^{-1} C-C stretch, 712cm^{-1} due to C-H bending. From the spectra, it was observed that there was no significant change in the original peak of the drug and the polymers when compared with spectra of formulated films and this indicates that there was no interaction between drug and polymer.

3.2 Uniformity of weight, film thickness, folding endurance and surface pH

The values of thickness, weight, pH and folding endurance was given in Table 2. It was observed that weight of the entire film sample in each formulation was uniform. Between the formulations, the weight increased with increased content of polymers used ranging from 65.1-73.21mg. The thickness of all film samples were uniform in each formulation and ranged between 0.421-0.54 mm. The films with increased polymer content showed a slight increase in thickness. It was found that all the formulations showed good folding endurance greater than 300. Since all the formulations were prepared by hydrophilic polymers showed good flexibility. The surface pH of each film was between 6.2 to 6.52 indicating the films may have less potential to irritate the buccal mucosa.

3.3 Bioadhesive force

The values of bioadhesive force of prepared films are given in Table 2. It was found that among the formulations FA1, FB1 and FC1, those with more amounts of mucoadhesive polymers such as HEC, HPC, carbopol and HPMC combination showed more bioadhesive force compared to other formulations of the combination. As the amount of mucoadhesive polymers increased there was an increase in the bioadhesive force. The HPMC, HPC, HEC and carbopol 934 particles were higher in quantity and so provided greater surface area for contact with the mucus membrane. The films had higher buccoadhesive strength because the moisture absorbed may just be the maximum required to produce maximum buccoadhesive interaction in the swollen films. As a result, buccoadhesive strength was enhanced since the films contained higher amount of HPMC, HEC, carbopol 934.

3.4 Tensile strength measurement

A weak and soft polymer is characterized by a low tensile strength and elongation at break; a hard and brittle polymer shows a moderate tensile strength and elongation at break; a soft and tough polymer showed a high tensile strength and elongation at break. The values of tensile strength are given in Table 2. The results showed that for the formulations FA1, FB1 and FC1, the tensile strength and percentage elongation increased with the increase in the percentage of mucoadhesive polymers. In the case of FA3 and FB3 formulations, the inclusion of PVP decreased the tensile strength and exhibited lesser elasticity when compared to other formulations.

3.5 Swelling Index

The rate and the extent of film hydration and swelling affect the film adhesion and consequently the drug release from the film. The rate of swelling affects the duration of adhesion with faster swelling resulting in adhesion of shorter duration. Formulation containing more amounts of hydrophilic polymers FA1, FB1, FC3 showed higher swelling index values in the range of 2.12, 2.025 and 2.43. Formulation containing more amounts of PVP and HPMC i.e. FA3, FB3 and FC1 showed lesser swelling index values in the range of 1.81, 1.75 and 1.925. As the swelling index decreased, rate of drug release increased. The swelling index values of FA2, FB2 and FC2 ranges from 1.9, 1.85 and 1.96 respectively. The swelling index time ranged from 20-25 minutes. As the swelling index increased, the drug diffusional path length increased thereby drug release into medium is delayed. This depends mainly on the type of polymers used.

Table 2: Uniformity of weight, thickness, folding endurance and surface pH of Mucoadhesive patch

Formulation Code	Weight (mg)*	Thickness (mm)*	Surface pH #	Folding endurance	Detachment Stress (dynes/cm^2)	Tensile Strength Kg/ mm^2	% Elongation
FA1	69.4 ±0.51	0.48±0.008	6.41 ±0.01	> 300	3321.66 ± 0.57	0.3726±0.005	31.2 ± 0.72
FA2	68.53±0.3	0.44±0.007	6.33 ±0.01	> 300	12008.15 ± 0.79	0.3348±0.005	23.46±0.55
FA3	67.5 ±0.26	0.42±0.007	6.28 ±0.01	> 300	11241.75 ± 0.66	0.2377±0.001	17.3 ± 0.61
FB1	67.4 ±0.46	0.46±0.006	6.52±0.03	> 300	12910.32 ± 0.58	0.3323±0.005	27.46 ± 0.5
FB2	66.5 ±0.53	0.44±0.007	6.43±0.02	> 300	11969.07 ± 0.90	0.2884±0.005	21.16±0.76
FB3	65.1 ±0.43	0.42±0.007	6.30 ±0.15	> 300	10831.08 ± 0.88	0.2329±0.004	14.5 ± 0.52
FC1	73.2 ±0.44	0.54 0.008	6.46±0.02	> 300	13335.49 ± 0.50	0.5375±0.007	57.16±0.76
FC2	71.1 ±0.38	0.52±0.008	6.35±0.01	> 300	13805.68 ± 0.58	0.4179±0.007	47.16±0.70
FC3	70.1 ±0.30	0.49±0.007	6.27±0.05	> 300	14298.87 ± 0.81	0.3246±0.006	30.8 ± 0.54

3.6 In-vitro drug release studies

The percentage drug release of formulations FA, FB and FC was given in Figure 1, 2, 3 respectively. Among the formulations FA, FB and FC with more amount of HPMC, HEC, HPC and carbopol i.e. FA1, FB1 and FC1 showed slower release extended up to 8 h. In case of FC3, as carbopol had highest swelling rate and ability to hydrate more rapidly and burst release was observed. This is because at pH 6.6, carbopol is present in the ionized state and as a result the polymeric network gets loosened comparatively. The FC3 formulation had the ability to hydrate more rapidly than the other 2 formulations. The resulting drug diffusional path length for FC3 was therefore the longest. However, the drug dissolution rates showed that FC3 had the highest drug release rates. The fact that FC3 had the fastest swelling rates but did not yield slowest release rates could be explained by polymer relaxation/erosion. Erosion increased the drug release rates, thus compensating, to some extent for the high swelling capacity and the consequent slowing of drug diffusion by the increasing diffusional path length. The *in vitro* drug release was more sustained for the buccal patches which were composed with high proportion of HPMC and hence FC1 formulation showed highest drug release with 99.045%. In case of FA formulations containing HEC and HPC, relatively high swelling of HEC increased the gel layer thickness and consequently the diffusion path length which in turn may be the cause of the slower drug release from HEC patches. In the case of FA3 and FB3 formulations, it was observed that with the increased content of PVP, the rate and extent of drug release was more. This was due to wettability of PVP and penetration of water into the film matrices and hence increased diffusion of the drug.

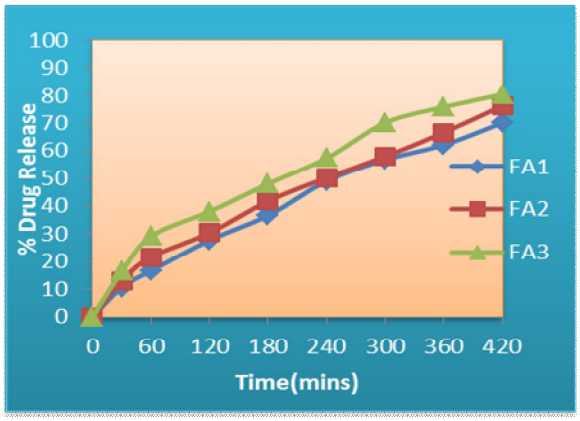


Figure 1: Drug release of FA formulations

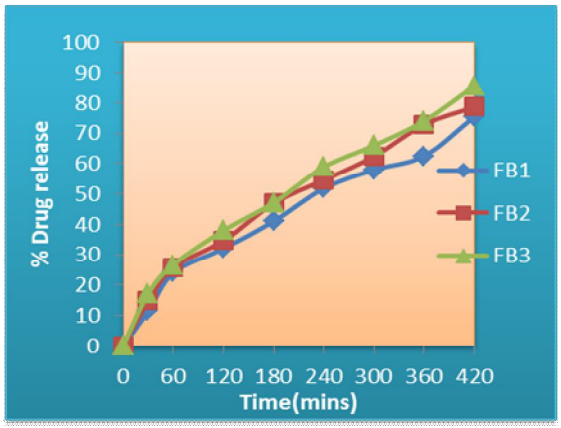


Figure 2: Drug release of FB formulations

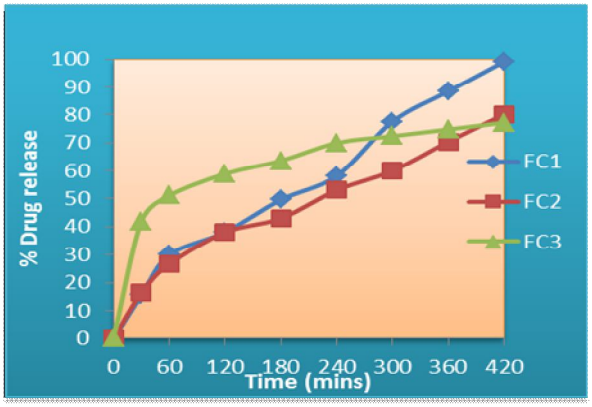


Figure 3: Drug release of FC formulations

A perusal of graphs, it was found that among FA formulations, FA3 showed highest drug release with 80.43% and FA1 showed least drug release of 70.09%. In case of FB formulations, FB3 showed highest drug release with 85.61% and FB1 showed least 75.49%. In case of FC formulations, FC1 had highest drug release of 99.045% and FC3 had least of 77.24%. From the results it is evident that the formulations FA3 and FB3 showed more release rates because of the presence of PVP. In case of FC1 formulations yielded highest drug release rates because it contained lesser amount of carbopol 934 in comparison to other formulations. The percentage drug release of FA2, FB2 and FC2 was found to be 76.34%, 78.68% and 80.2% respectively.

3.7 Drug release kinetics

The *in vitro* drug release kinetic analysis is done by the software "PCP Dissolution Version 2.08". In order to determine the mechanism of drug release from the formulations, different models were examined which include zero order, first order, Higuchi's plot and Korsmeyer-peppas model. The release exponent (n) describing the mechanism of drug release from the matrices was calculated by regression analysis using the following equation.

$$Mt/ M\infty = Kt^n$$

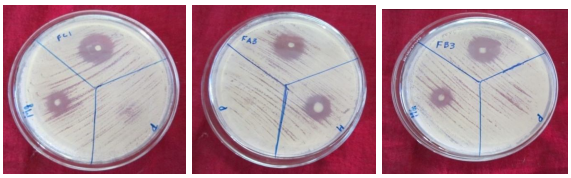
Where Mt/M∞ is the fraction of drug released (using values of Mt /M∞ within the range 0.10–0.60) at time t and K is a constant incorporating the structural and geometric characteristics of the release device. A value of n=0. 5 indicates case I (Fickian) diffusion, 0.5 < n < 1 indicates anomalous (non-Fickian) diffusion, and n =1 indicates case II transport (Zero order release), n >1 indicates super case II transport. The correlation coefficient values (R^2) indicates that the *in vitro* drug release data of all the formulations followed first order kinetics except for FC1 whose drug release followed zero order kinetics. The mechanism of drug release for all the formulation was best fit to Peppas model except FC3 which followed matrix diffusion process. The values of n were obtained by the linear regression of log (Mt/M∞) vs. log t and were between 0.5 to 1 indicating non - Fickian diffusion for all formulations except for FC3 where the value of n is 0.4129 indicating Fickian diffusion. Apart from that the R^2 values of Higuchi matrix model for most of the formulations were more than 0.93 indicating diffusion of drug from the swelled polymer. Hence it could be concluded that the mechanism of drug release from the films followed the matrix diffusion process.

3.8 Stability Studies

Stability testing was carried out for all formulations for a period of eight weeks. All the formulations are evaluated with respect to physical appearance, drug content, surface pH, swelling index and *in vitro* drug release. The formulations were taken out at and checked for any physical change, color, appearance, flexibility and drug content for 2 months. It was found that there is no significant change in drug content and all other aspects of the patches.

3.9 Antimicrobial Efficacy Studies

The antimicrobial activity of the selected formulations FA3, FB3, FC1, and marketed gel against *Candida albicans* ATCC 90028, strain is shown in Figure 4. In the present experiment, all values are expressed in mean zone of inhibition ± standard deviation; n=6. From the figure 4, 5, 6 it was evident that the formulation FA3, FB3 and FC1 showed higher zone of inhibition values of 12.16 ± 0.752, 13 ± 0.894 and 14.33 ± 0.8164 in comparison to marketed gel value of 10.5 ± 0.836. The P values for formulations FA3, FB3 and FA1 was **P< 0.005, **P< 0.001, ***P<0.001 respectively. From the P values, it can be found that the difference in zone of inhibition between formulations FA3, FB3 and marketed gel was highly significant and that between formulation FC1 and marketed gel is very highly significant. The formulations FA3, FB3 showed highly significant activity against *candida albicans* and FC1 showed very high significant activity against *candida albicans* when compared to the marketed gel. It indicates that the prepared buccal patches have significant activity than marketed gel against *candida albicans*



(a) (b) (c)

Figure 4: Zone of inhibitions of selected buccal patch and marketed gel against *Candida albicans* strain, where formulations are denoted as (a) FA3, (b) FB3, (c) FC1

4. CONCLUSION

In the present work, an attempt has been made to formulate buccal films of miconazole nitrate, using different ratios of hydroxy propyl methyl cellulose, carbopol 934, hydroxy ethyl cellulose, hydroxy propyl cellulose and poly vinyl pyrrolidone. Effects of these polymers on buccal films of miconazole nitrate were investigated. Furthermore, the antifungal activity of the selected films was significantly superior to the reference miconazole gel. Further work is recommended to support its efficacy claims by pharmacodynamic and pharmacokinetic studies in human beings.

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