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DESIGN AND DEVELOPMENT OF MUCOADHESIVE BUCCAL FILM BEARING ITRACONAZOLE

Yuvraj Singh Dangi*, Richa Solanki, Naina Dubey and Anamika Jain

Department of Pharmaceutics, Sagar Institute of Pharmaceutical Sciences, Sagar (M.P.) 470228.

***Corresponding Author: Yuvraj Singh Dangi**
Department of Pharmaceutics, Sagar Institute of Pharmaceutical Sciences, Sagar (M.P.) 470228.

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ABSTRACT
The purpose of this research was to develop and evaluate mucoadhesive films for buccal administration of itraconazole using film-forming and mucoashesive polymers. Buccal films of chitosan bearing Itraconazole were prepared by solvent casting technique. The films have been evaluated in terms of film weight, thickness, density, surface pH, FTIR, X-ray diffraction analysis, bioadhesion, swelling properties and in vitro drug release studies. It was found that film formulations of 2 cm^2 size having weight in the range of 204 ± 0.76 to 223 ± 2.09 mg and film thickness were in the range of 0.44 ± 0.11 to 0.57 ± 0.19 mm. Density of the films was found to be 0.102 to 0.126 g/ml. Drug content was found to be uniform in the range of 8.23 ± 0.07 to 8.73 ± 0.09 mg/cm^2 for formulation A_1 to A_4. Maximum bioadhesion force was recorded for HPMCbuccal films (A_2) i.e. 0.57 ± 0.47 as compared to other films. In vitro residence time was in range of 1.7 ± 0.12 to 7.65 ± 0.15 h. The drug release studies show that formulations follow non-fickian diffusion. These mucoadhesive formulations could offer many advantages in comparison to traditional treatments.

INTRODUCTION
The biomedical literature abounds with reports of macromolecules essential for fungal survival, growth, virulenceor cellular morphogenesis that have been proposed as potential targets for novel antifungal agents. The arrival of whole-genome sequence data for pathogenic fungi, such as Candida albicans(Scherer, 2002) Aspergillusfumigatus (Denning et al, 2002) and Cryptococcus neoformans (Schein et al, 2002) as well as for non-pathogens, such as Saccharomyces cerevisiae (Goffeau et al, 1997) has paved the wayfor discovery of genes encoding candidate antifungal targets on a previously unprecedented scale (De Backer et al, 2002). However, the antifungal discovery process based on screening compounds against molecular targets, which dates back before the genomics era, has so far not resulted in a single new agent emerging into the clinic or even the development pipeline.

For many years, the only available antifungal for IFIs was amphotericin B deoxycholate (AmBD). With the introduction of triazoles at the beginning of 1990s, the pace of drug development accelerated. Amphotericin B (AMB) was incorporated in three lipid formulations, whilst the first-generation triazoles (fluconazole (FLC) and itraconazole (ITC)) changed the epidemiology of Candidainfections and offered new treatment options. In the last 6 years, two new triazoles and three new echinocandins were approved for use. Many of these antifungals have proven to be less toxic and in some cases more effective than AmBD(Ostrosky-Zeichner et al, 2003).

The buccal mucosa, along with other mucosal tissues, has been investigated as a potential site for controlled delivery of macromolecular therapeutic agents, such as peptides, proteins and polysaccharides (de Vries et al, 1991; Ho et al, 1992; Squier, 1991) because of its accessibility and low enzymatic activity compared to the gastro-intestinal tract. Another interesting advantage is its tolerance (in comparison with the nasal mucosa and skin) to potential sensitizers.

Itraconazole is a potent broad-spectrum triazole antifungal drug with activity against histoplasmosis, blastomycosis and onychomycosis (Grant and Clissold, 1989; De Beule and Van Gestel, 2001). This compound was the first orally available drug with activity against Candida species such as Candida albicans, parapsilosis, tropicalis, glabrataand krusie and against Aspergillus species such as Aspergillusflavus and fumigatus. Candida sp. And Aspergillus sp. are the two most common human fungal pathogens (Jain and Sehgal, 2001). According to the biopharmaceuticals classificationsystem, itraconazole is an extreme example ofa class II compound meaning that its oral bioavailabilityis determined by dissolution rate in theGI tract (Amidon et al., 1995; Dressman et al., 1998, 2001).

In recent years, the development of mucoadhesive systems as potential delivery systems for controlled release of drugs has attracted significant interest (Park and Robinson, 1984; Peppasand Buri, 1985). However, buccal films are preferable over adhesive tablets in terms of flexibility and comfort. In addition, they can circumvent the relatively short residence time of oral gels on the mucosa, which are easily washed and removed by saliva. Moreover, buccal films are also suitable for protecting wound surfaces, thus reducing pain and increasing the treatment effectiveness (Peh and Wong, 1999).

Many researchers have tried to deliver a drug topically through the buccal cavity including antimicrobials (Senel et al. 2000; Jones et al. 2000), topical corticosteroids (Shin et al. 2000), and polypeptides (Langoth et al. 2000). But to our knowledge no one has tried to deliver an antifertility drug through buccal mucosa using natural polysaccharides so far. No controlled release formulation of Itraconazole is available in market at present. As Itraconazole also offers bioavailability issues presenting a drug in systemic circulation in a controlled manner may result in decrease in bioequivalence issues of highly variable drugs such as Itraconazole.

INVESTIGATIONS, RESULT AND DISCUSSION

Itraconazole based mucoadhesive films were designed and developed to release the drug in a controlled manner thus minimizing variability among the subjects since Itraconazole is a highly variable drug. The optimization of formulations was carried out in order to select best of the formulation meeting the necessary requirements. It was found that films A_1 to A_4 of 2 cm^2 size were having weight in the range of 204 ± 0.76 to 223 ± 2.09 mg and film thickness was in the range of 0.44 ± 0.11 to 0.57 ± 0.19 mm. Density of films was in range of 0.102 to 0.126 g/ml. The surface pH of the film was found to be in the range of 6.12 to 7.01 for all the formulations. The pH observed was close to the physiological pH of buccal mucosa indicating that no irritation will be caused after its application. The buccal films were observed under scanning electron microscope to see its surface morphology after keeping the films in dissolution media for 0, 30, 60, 90 and 120 min. It was observed that initially the films were intact where afterwards cavities were formed due to erosion of the polymer.

Differential Scanning Calorimetry studies were also carried out to determine the thermal behavior of the pure drug, and along with different excipients to check the compatibility of drug with rest of excipients. The thermogram of the pure drug, Itraconazole, is given in Fig. In the thermogram a sharp endothermic peak at 167.65°C is obtained which is a characteristic peak of Itraconazole. DSC of Itraconazole along with polymer (Chitosan) revealed that their has been no considerable change in peak value which was found to be 167.47°C as compared to167.65°C for pure drug. This indicates compatibility of drug with polymer. DSC studies of another polymer (HPMC) used for preparation of buccal films indicates further no change in peak of drug which was found to be 167 75°C indicating compatibility of drug with polymer.

Fourier Transform Infrared Spectra were acquired to draw information on the molecular state of Itraconazole and mixture of Itraconazole and Chitosan and HPMC. Chitosan is an amino glucose characterized by a small proportion of amide groups via an amide linkage with acetic acid. In FTIR spectrum chitosan exhibited a broad peak at 3431 cm^{-1}, which is assigned to the N-H and hydrogen bonded O-H stretch vibrational frequencies, while a sharp peak at 3610 cm^{-1} is that of free O-H bond stretch of glucopyranose units. Further, in the C-H stretch region of FTIR spectrum, the higher intensity peak at 2923 cm^{-1} is assigned to the asymmetric and the lower intensity peak at 2857 cm^{-1} is assigned to the symmetric modes of CH_2. The peaks at 1550 and 1599 cm^{-1} were assigned to strong N-H bending vibrations of secondary amide, which usually occur in the range of 1640 to 1550 cm^{-1} as strong band. The characteristic IR peak of ITCZ alone over the frequency range 500 – 4000 cm^{-1} occurred at 3439, 3126 and 3069 cm^{-1} due to the absorption of NH2 groups, 2964 cm^{-1} resulted fromCH2 stretching frequency band and a sharp peak occurred at 1698 cm^{-1} due to C=O stretching vibration. The peaks observed at 1609 cm^{-1} and 1429 cm^{-1} may be assigned to the C=N and C-N bonds, respectively. The characteristic peaks occurred at 1510 and 1451 cm^{-1} owed to C-H deformation. These values are comparable to those reported for ITCZ by Nesseem. The IR region from 600 – 1400 cm^{-1} which is called the fingerprint, usually contains a large number of unassigned vibrations characteristic of the molecule. The IR spectra of the physical mixtures of ITCZ with HPMC and Chitosan did not show any significant differences in the characteristic bands of the respective spectra of the pure components and the functional groups still showing their characteristic bands indicating that there is no complex formation. FTIR spectra of HPMC gave the characteristic peaks at about 1643, 1109 and 1033 cm^{-1} vibration region. According to Henrikson et al. (1996), chitosan is a promising bioadhesive material at neutral or slightly alkaline pH, which is found to be advantageous for adsorption on the mucosal surface. It was suggested that, at this pH, chitosan exhibits numerous amine and hydroxyl groups that may increase the interaction of polymer with the negative mucin. The rheological interaction between chitosan and mucin, and/or hydrophilic additives and mucin produces strong force of attraction between polymer and mucus membrane and in turn influences mucoadhesive property of the films.

Assessment of the swelling behavior was done by measuring radial swelling. The degree of swelling was found to be higher for medicated patches compared to plain patches. It is observed that film A_3 is exhibiting maximum percent swelling (79.07 ± 0.68%) while film A_1 is showing only 37.18 ± 0.35% swelling. The percent

enhancement in percent swelling could be due to the presence of HPMC and gelatin in higher concentration i.e., 2 and 3%, respectively in formulation A_3. The water soluble hydrophilic additive dissolves rapidly introducing porosity. The void volume is thus expected to be occupied by the external solvent diffusing into the film and thereby accelerating the dissolution of the gel. Further there was a little reduction in swellability (66.21 ± 0.25%) for film A_4, this could be due to crosslinking of the polymer with glutaraldehyde. Percent radial swelling in different formulations was found to be in order of $A_1 < A_4 < A_2 < A_3$. Drug content was found to be uniform in the range of 8.23 ± 0.07 to 8.73 ± 0.09 mg/cm2 for formulation A_1 to A_4.

The drug release from various prepared buccal films was studied using the USP dissolution apparatus. Chitosan containing patches produced sustained release in formulation A_2. The minimum drug release was observed from the system (A_4) containing 5% w/v HPMC where only 7.3% itraconazole was released in the first hour and slowly progressed to 74.34% after 8 hr. The subsequent increase in diffusional path length and low attrition may be responsible for the distinct low release profile. The release profile of the drug from different formulations exhibits that the drug release from these films is following non-fickian diffusion as the value of diffusion release exponent (η) is in the range of 0.7 to 0.8. The value of (η) was calculated from the slope of the log % cumulative drug release vs. log time. The reason for the non-fickian diffusion of the drug from the buccal film could be due to the formation of solvent filled pore in the matrix and erosion of the polymeric matrix.

Value of the in vitro residence time as shown in Table 1 is different for all formulations. Chitosan films (A_1-A_4) remained attached to the membrane during the time of study (10 h) without erosion. However the addition of HPMC and gelatin to films A_3 and A_4 caused dislodgement within 4.15 and 1.7 h respectively, without erosion. The presence of Itraconazole, a water insoluble drug may have affected the residence time of the film. The in vitro residence time of the buccal film on the mucosal membrane was observed and it was noted that formulation A_2 remained on the mucosal membrane longer (7.65 ± 0.15 h) than formulation A_1 (4.36± 0.81 h). It could be due to the presence of higher concentration of chitosan polymer (2% in A_2), while the product A_4 exhibited minimum residence (1.70± 0.12 h) because the chitosan in this polymeric film was cross linked (Table). The water-soluble hydrophilic additive such as HPMC dissolves rapidly introducing porosity. The addition of certain amounts of the hydrophilic polymer HPMC increased surface wettability and consequently water penetration within the matrix. Additional shortening in the residence time was observed when a higher percentage of HPMC was added to the patches. The increase in water-soluble content promotes faster dissolution of the patch (Korsmeyer et al. 1983). No correlation was found between the bioadhesion force and the residence time of the polymers. It seems that highly bioadhesive polymers do not necessarily reside longer on the mucosal surface. Surface charge density and chain flexibility are considered to be prerequisites for bioadhesion, whereas the residence time is primarily dependent on the dissolution rate of the polymer. However, as regards the in vivo residence time data (Table), none of the polymers detached from the oral mucosa over the study period, which indicated that the bioadhesion values of all polymers were satisfactory to retain the film on the buccal mucosa. Film mucoadhesion time varied form 1.3 to 5.7 h. Formulation A_3 showed the highest degree of adhesion time whereas the film from formulation A_4 showed lowest mucoadhesion time. The difference may be due to use of HPMC which favors hydration and the outward diffusion of the drug from film matrix. Another important factor to be considered is the kind of film forming polymer used for the film preparation and the homogeneity of the polymer solution mixtures. Chitosan polymer are water soluble, HPMC is also water soluble and these characteristics influenced miscibility with the mucoadhesive polymer, the uniformity of the film as well as permeability to water. Drug being water insoluble needed to be dissolved in small quantity of methanol and dichloromethane first in a ratio of 1:1. Mucoadhesion increases with increase in HPMC content although the effect is not drastic. Mucoadhesion force values were between 0.19and 0.57N. F_2 formulation exhibits highest mucoadhesion force as compared to other formulations and the reason may be attributed to higher percentage of chitosan along with HPMC, which acts as a co adjuvant by increasing the mucoadhesive properties of chitosan. Bioadhesion was found to be in the order of $A_2>A_1>A_3>A_4$. The results were in concordant with results of Nafee et al. (2003) who observed decreased bioadhesion on increasing HPMC concentration while studying the mucoadhesivemiconazole containing chitosan patches. According to Henriksen et al. (1996), chitosan is a promising bioadhesive material at neutral or slightly alkaline pH, which is found to be advantageous for adsorption on themucosal surface. It was suggested that, at this pH, chitosan exhibits numerous amine and hydroxyl groups that may increase the interaction of polymer with the negative mucin. The rheological interaction between chitosan and mucin, and/or hydrophillic additives and mucin produces strong force of attraction between polymer and mucus membrane and in turn influences mucoadhesive property of the films. The cross linking reduces the number of free amino groups for the binding to mucus membrane and it also influences the rheological interaction of polymer and mucin. Assessment of the swelling behavior was done by measuring radial swelling. In the case of buccal films intended for local therapy, the contact area should be as large as possible, a requirement that must be balanced with patient compliance; excessive increase in patch diameter might cause discomfort and/or dislodgement of the swollen film.

Table 1: Characteristics of buccal films of optimized formulation.

Composition/ Characteristics	Formulations			
	A1	A2	A3	A4
Itraconazole (g)	1	1	1	1
Chitosan (% w/w)	1	2	1	1
Glutaraldehyde (0.2% w/v) ml	0	0	0	2
Polyvinyl pyrrolidone K – 30 (%w/v)	0	1	2	5
Gelatin (% w/v)	0	1	3	3
Film thickness* (mm)	0.44± 0.11	0.51 ± 0.36	0.52 ± 0.30	0.57 ± 0.19
Density (g/ml)	0.126	0.120	0.115	0.102
Film mass (mg)	219 ± 0.54	223 ± 2.09	204 ± 0.76	215 ± 1.14
Surface pH	6.94	6.86	7.01	6.12
Bioadhesion time (h)	1.3	5.7	3.2	4.4
Bioadhesion force* (N)	0.19 ± 0.15	0.57 ± 0.47	0.33 ± 0.20	0.42 ± 0.64
Residence time* (h)	4.36 ± 0.81	7.65 ± 0.15	4.15 ± 0.36	1.7 ± 0.12
Drug content uniformity (mg/cm^2)	8.46 ± 0.12	8.73 ± 0.09	8.54 ± 0.10	3.23± 0.07

*Mean Values ± SE; n = 6 for patch thickness and n = 10 for film mass.

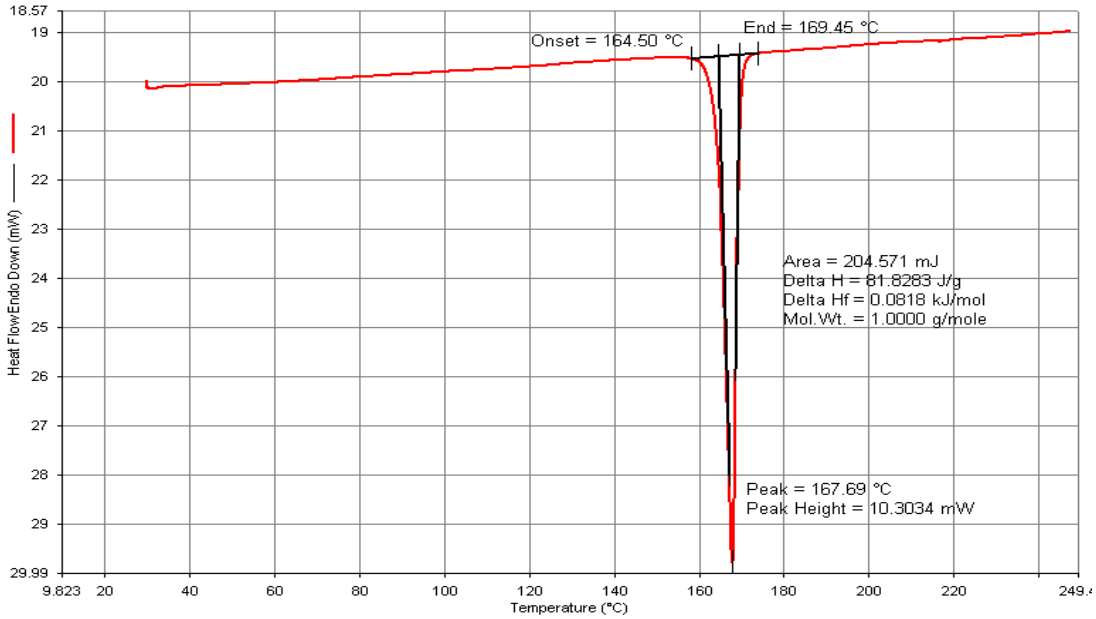

Figure 1: DSC spectra of Itraconazole.

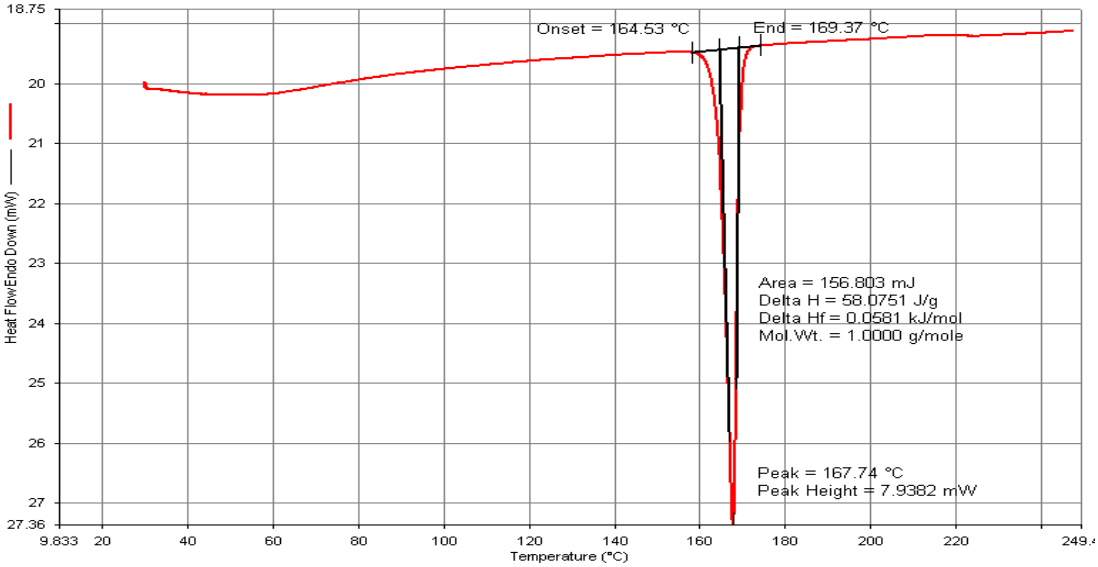

Figure 2: DSC spectra of physical mixture of Itraconazole and chitosan.

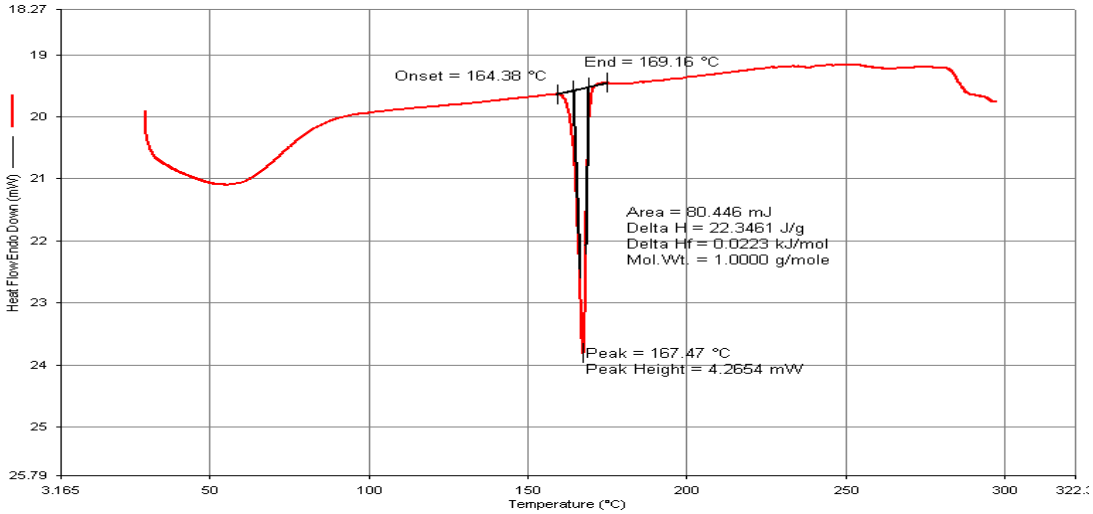

Figure 3: DSC spectra of physical mixture of Itraconazole and HPMC.

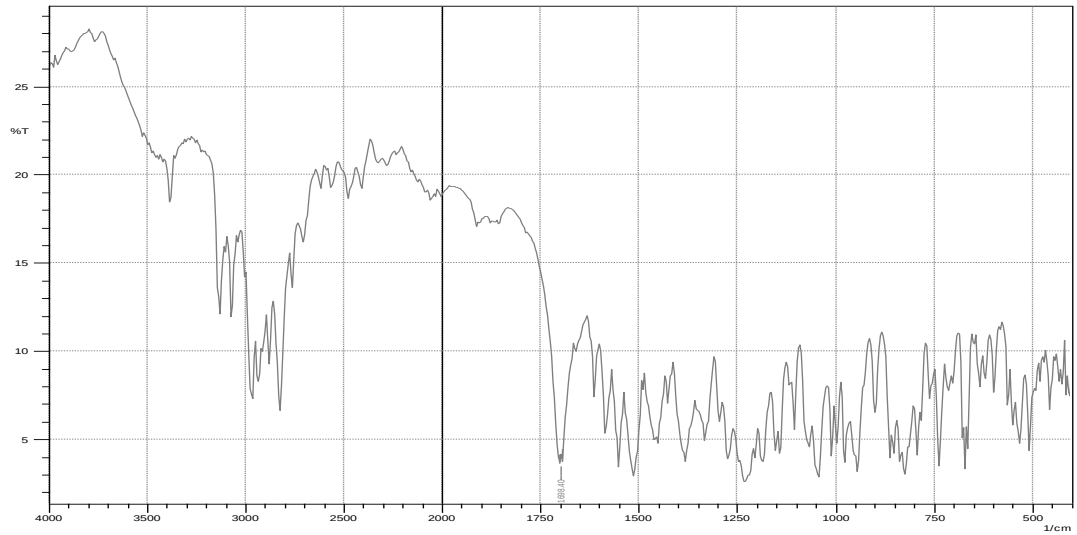

Figure 4: FT-IR spectra of Itraconazole.

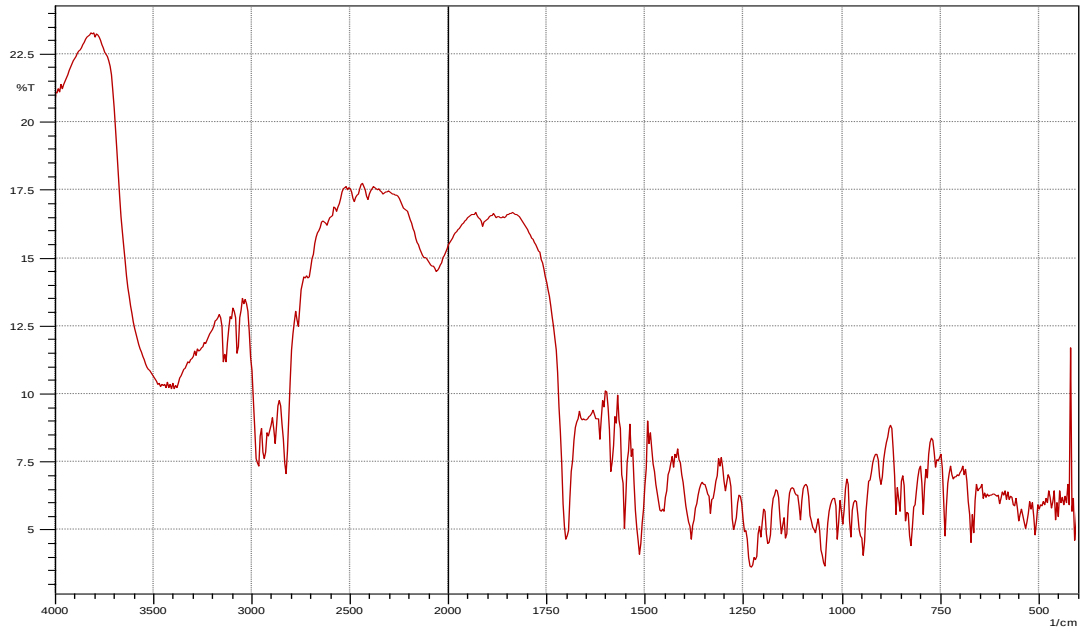

Figure 5: FT-IR spectra of physical mixture of Itraconazole and HPMC.

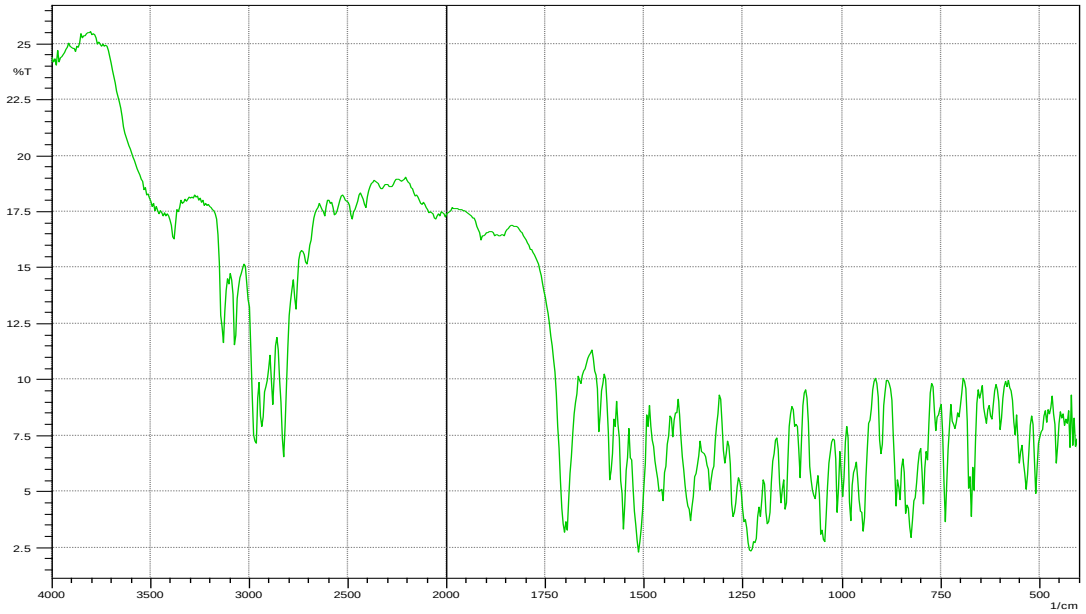

Figure 6: FT-IR spectra of physical mixture of Itraconazole and chitosan.

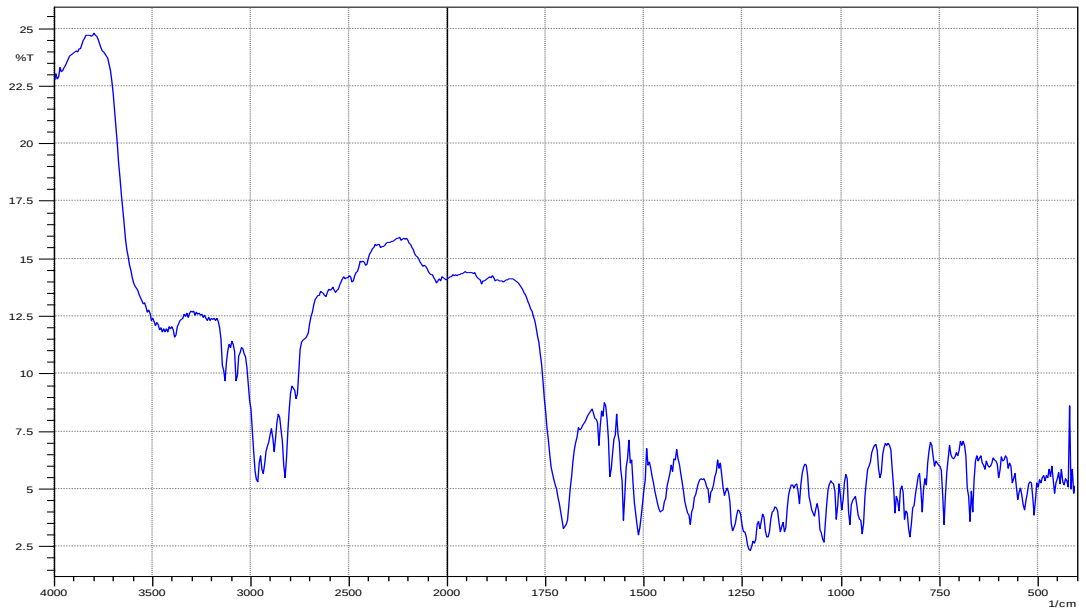

Figure 7. FT-IR spectra of final formulation.

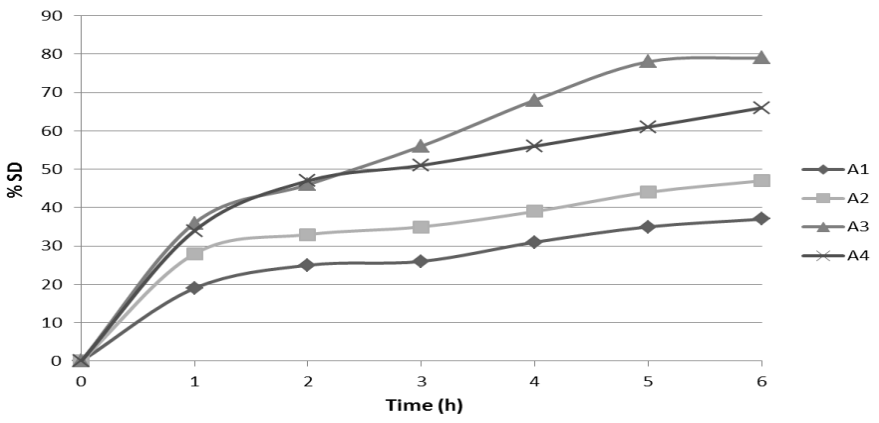

Figure 8: Degree of radial swelling for various formulations.

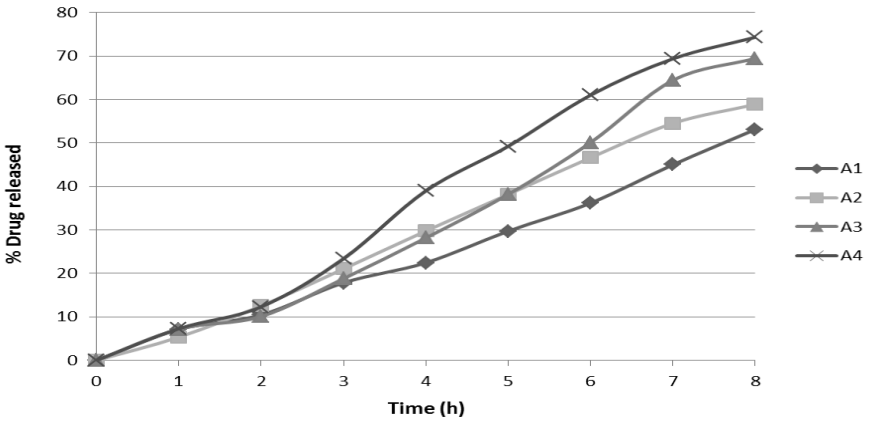

Figure 9: Drug release profiles for various formulations.

EXPERIMENTAL

Materials

Itraconazole was procured from M/s. Zydus Cadila Healthcare Ltd, Ahmedabad, Gujarat, India. Chitosan and Hydroxy propyl methyl cellulose were obtained as a gift samples from M/s. Torrent Pharmaceuticals, Gujarat, India. All other reagents were of Analytical Grade.

Methods

Buccal films bearing Itraconazole were prepared by solvent casting method employing aluminum foil cups (10 mm in diameter placed on glass surface) as substrate. Chitosan (1 g) was dissolved in 50 ml of 2% (v/v) acetic acid under constant stirring using a magnetic stirrer for 48 h. The resultant viscous solution was filtered through muslin cloth. The filtrate was left to stand until all air bubbles disappeared. Then, hydrophilic polymer (HPMC and gelatin) were dissolved in small volume of dichloromethane (2 ml) and this solution was added to chitosan solution with stirring. Glutaraldehyde was added drop wise to this solution with constant stirring. Itraconazole (1 g) was dissolved in minimum quantity of ethanol (5 ml) and mixed well with the above polymer solution. This polymer mixture (20 ml) was poured on the aluminum foil placed on plain glass surface. This whole assembly was then placed in the oven and dried the film at 30 ± 2°C. After drying, the films were carefully removed from the aluminum foil, checked for any imperfections or air bubbles and cut in to patches 2 cm2 (2 cm x 1 cm) size. The films were packed in aluminum foil and stored in glass container maintained at room temperature.

Characterization of buccal films

1. Film weight

Three buccal films of each formulation were taken, individually weighed and an average weight was determined.

2. Thickness

Thickness of buccal films was measured using a Screw gauge at different places of randomly selected films. The mean thickness of the buccal films was calculated.

3. Density

The density of the buccal films was calculated by using formula m/v where m and v are weight and volume of the films of size 2 cm^2. The volume of the film was calculated from its area multiplied by thickness.

4. Surface pH

Each film (2 cm^2) was left to swell for 2 h on the surface of an agar plate. Then surface pH was measured by mean of a pH paper placed on the surface of the swollen film. The color developed was compared with the standard color scale.

5. Surface morphology

The films were observed under scanning electron microscope before and after keeping the film in 0.1 N HCl and in phosphate buffer saline (pH 6.8) for 0, 30, 60, 90 and 120 min. The dried films were coated under argon atmosphere withgold-palladium to achieve a film of 10 nm thicknesses and then observed under a scanning electron microscope.

6. Fourier transform infrared spectrum analysis

The itraconazole loaded chitosan film and blank film was analyzed by FT-IR spectroscopy to confirm loading of the drug in the film. The polymer samples were crushed with KBr to make pellets. Spectra were taken on a FTIR Perkin Elmer (Pyrogon 1000) and scanned between 400–4000 cm^{-1}.

7. Drug content uniformity

Three films (2 cm^2 size each) of each formulation were weighed accurately and transferred into a separate 100 ml volumetric flask, containing 100 ml of 0.1N HCl (pH 1.0) containing methanol (10%v/v). The contents of the flask were stirred constantly for 24 h using a magnetic stirrer. The solution was filtered and analyzed for drug content at 247 nm with an UV-Spectrophotometer (Cintra 10, Japan). The average of three observations was recorded.

8. Bioadhesive time and force

The ex vivo mucoadhesion time was performed (n = 6) after application of the films on freshly cut porcine

buccal mucosa. The porcine buccal mucosa was fixed on the internal side of a beaker with cyanoacrylate glue. Each film was divided in portions of 2 cm^2 and cut, a side of each film was wetted with 50 ml of simulated saliva fluid (SSF) and was pasted to the porcine buccal mucosa by applying a light force with the finger tip for 20s. The beaker was filled with 800 ml of the simulated saliva fluid and was kept at 37°C. After 2 min, a 100 rpm stirring rate was applied to simulate the buccal cavity environment and film adhesion was monitored during 8 h (Han et al. 1999).

9. Swelling

Three films were tested for each formulation. The diameter of the original buccal films was determined. Three films of each formulation were allowed to swell on the surface of agar plate kept in an incubator maintained at 37°C. Measurement of the diameter of the swollen film was done at one hour intervals for 6 h. The percent radial swelling (SD %) was calculated using equation (I):

$$S_D\,(\%) = \frac{[D_t - D_0]}{D_0} \times 100 \ldots\ldots\ldots (I)$$

Where S_D (%) is the percent swelling obtained by the diameter method, D_t is the diameter of the swollen film after time t, D_0 is the initial diameter of film at time zero.

10. Drug release from buccal films

The USP dissolution apparatus (Type-1) was used throughout the study. A portion of 2 cm^2 (2 cm x 1 cm) of film was used. One film of each formulation was fixed to the central shaft using an acrylate adhesive. The dissolution medium consisted of 900 ml of 0.1N HCl containing 1% Sodium lauryl sulphate. The release study was carried out at 37 ± 0.5°C with a rotation speed of 50 rpm. The release study was performed for 8 h. After every hour, a 3 ml sample was withdrawn from each station and immediately replaced with fresh media. The withdrawn samples were filtered; 2 ml of the filtrate were diluted to 10 ml using HCl (pH 1.5). The samples were analyzed spectrophotometrically at 264 nm.

11. Residence time

The in vitro residence time was determined using a laboratory designed apparatus for analysis of in vitro residence time. The vessel of test apparatus was filled with 800 ml PBS (pH 6.8) maintained at 37 ± 2°C. The segment(3 cm) of porcine buccal mucosa was glued (using cyanoacrylate adhesive) to the surface of a glass slab vertically attached to the apparatus. Six mucoadhesive films of each formulation were hydrated from one surface usingPBS (pH 6.8) and then the hydrated surface was brought into contact with the mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down in such a way that the film was completelyimmersed in the buffer solution at the lowest point and at the highest point. The time required for complete erosion or detachment of the film from the mucosal surface was recorded. The in vitro residence time of differentformulations (Table) are 1 compared with plain patch. Four healthy human volunteers (2 males and 2 females, 25-30 years old) agreed to participate in the in vivo test study after signing informed consents. The study was conducted in accordance to the Declaration of Helsinki guidelines. The experiment was carried out with plain films only. The bioadhesive film was placed on the buccal mucosa by applying a light force between the cheek and the gingiva in the region of upper canine and gently pressed onto the mucosa for about 30s. The patch and the innerupper lip were carefully moistened with saliva to prevent film from sticking to the lip. The subjects were not allowed to eat or drink during the study (5h). They were asked to monitor the ease with which the systemwas retained on to the mucosa and note any tendency of detachment. The adhesion time was indicated by either complete erosion of the patch or failure of the adhesive bond. Any complaints and bad feeling were alsorecorded. Repeated application of the bioadhesive films by same volunteer was allowed after a two-day period.

12. Statistical analysis

Data are expressed as the mean ± standard deviation (SD) of the mean. The significance of differences was evaluated by analysis of variance (ANOVA) and differences were considered statistically significant at $P < 0.01$.

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