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Research Article

DESIGN AND *IN VITRO* EVALUATION OF BUCCOADHESIVE TABLET OF ITRACONAZOLE

Abdul Sayeed[1*], Faizan Sayeed[1] and Md. Hamed Mohiuddin[2]

[1]MESCO College of Pharmacy, Hyderabad, Andhra Pradesh, India.
[2]Anwar Ul Uloom College of pharmacy Hyderabad, Andhra Pradesh, India.

ABSTRACT

The aim of this work was to develop a tablet for the buccal delivery of the poorly water-soluble drug itraconazole which is a broad spectrum triazole derivative useful in treatment of oropharyngeal candidiasis, for that an attempt was made to solubilizing itraconazole by complexation with β- CD and then delivery via buccal mucosa. HPMC K4M and carbopol 934P were selected as mucoadhesive polymers while ethyl cellulose, as backing material. The complexation was studied by phase solubility method which indicates the formation of complex with 1:1 stoichiometry. Modification of the release for a poorly water-soluble drug, itraconazole, from hydrophilic matrices using β-cyclodextrin complexation was evaluated. The buccoadhesive tablets for the delivery of itraconazole were prepared by direct compression of HPMC K4M and Carbopol 934P. The Surface pH of all formulations was found to be within ±1 units of neutral pH hence these formulations should not cause any irritation in buccal cavity. Carbopol showed superior bioadhesion properties compared to HPMC. The *in vitro* release results demonstrated that drug is released by non-Fickian diffusion mechanism with first order kinetics. The feasibility of buccal administration of itraconazole was assessed by permeation experiments on sheep excised mucosa. Our results demonstrate that, as there was increase in drug release rate from the tablets in solution as well as an increase in the amount of itraconazole permeated through sheep buccal mucosa. This system turns to be of great potential as buccal drug delivery system.

Keywords: Itraconaole; β- Cyclodextrin; Optimization; HPMC; Carbopol; Buccal delivery.

INTRODUCTION

There are various routes of drug administration meant for different pharmaceutical dosage forms like parental, topical and oral route. Among these the later one is the most preferred and convenient route for drug administration. This route however has certain demerits like drug inactivation by the hepatic first pass effect, degradation of drugs by gastro-intestinal tract enzyme. These factors affect the drug absorption and hence cause the poor bioavailability of active drugs which may lead to the formation of therapeutically inactive drug molecule. Advances in emerging trends in pharmaceutical sciences has designed different approaches to avoid first pass metabolism, buccal route seems to be more convenient and beneficial. Buccal mucosa is a potential site for the delivery of drugs to the systemic circulation. A drug administered through the buccal mucosa enters directly in the systemic circulation, thereby minimizing the first-pass hepatic metabolism and adverse gastro-intestinal effect. Buccal cavity possess ideal characteristics for drug absorption and hence it acts as an excellent site for the absorption of drugs.[1]

Mucoadhesive drug delivery systems

These may be defined as drug delivery systems, which utilize the property of bioadhesion of

certain water soluble polymers which become adhesive on hydration and hence can be used for targeting of drug to particular regions of body for extended periods of time. Hence buccal drug delivery systems are generally based on bioadhesive polymers which once hydrated adhere to the buccal mucosa and withstand salivation, tongue movements and swallowing for a significant period of time.

Buccal tissue is richly supplied with perfused blood capillaries hence this route has certain advantages such as; avoidance of irritation of the gastrointestinal membrane, relative permeability due to rich blood supply, reduced risk of overdose, non-invasive administration, ease of convenience and self-medication, improved patient compliance, higher bioavailability allowing lower doses, avoidance of liver or gastrointestinal metabolism, feasibility of beneficial adjunct product to existing product and reduced risk of infectious disease transmission leading to the acceptance of buccal delivery as an alternative dosage form.

The buccoadhesive drug delivery systems have been developed basically to increase the retention of drug in the oral cavity. The route provides intimate contact between a dosage forms and absorbing tissue thereby resulting in high drug concentration in a local area and hence continuous release of drug from the medication towards medium from where it is constantly removed. Such dosage forms are very much useful in the treatment of fungal diseases including oral candidiasis.

Oropharyngeal candidiasis is the most common oral manifestation of HIV infection, reflecting progressive immunodeficiency. Oral Candida infections are observed in more than 90% of HIV-positive patients at some time during their disease, particularly in advanced immunosuppressant. The most common causative pathogen is Candida Albican. Itraconazole is a broad spectrum triazole derivative of antifungal agent developed for treatment of human mycotic infections and plays an essential role in the antifungal chemotherapy. It is weak base with poor water solubility. It required acidic environment for dissolution. The bioavability of oral Itraconazole is reduced due to extensive hepatic metabolism also in patients with AIDS, largely as a result of gastric hypochlorhydria.

Hence due to its low bioavailability and elimination half - life it needs to be administered twice daily. Therefore, it is selected as suitable drug for the design of mucoadhesive buccal tablet with a view of improve its oral bioavailability and patient compliance.

The aim of this study was to prepare a new buccoadhesive tablet formulation of Itraconazole against candida albicans. However, the design of buccal systems for poorly water-soluble drugs is a challenging issue. Lipophilic drugs, although being well absorbed through oral epithelia, exhibit too low fluxes due to a low chemical potential gradient, which is the driving force for transport.

In present work, an attempt has been made to formulate buccoadhesive tablet of itraconazole involving complexation of itraconazole with β-cyclodextrin and preparation of tablets using hydrophilic polymers like hydroxypropyl methylcellulose and carbopol.

MATERIALS AND METHODS

Itraconazole was obtained as gift sample from Glenmark Pharmaceuticals Ltd., Goa.β-cyclodextrin received from SA Pharmachem Pvt. Ltd., Mumbai. Hydroxypropyl methylcellulose 4 cps (HMPC 4 cps), Carbopol 934p,talc and magnesium stearate were procured from SD Fine Chem., Mumbai.

METHODS

Preparation of inclusion complexes of Itraconazole with β-cyclodextrin in 1:1 molar ratio by Physical mixing, co precipitation method[2]

In this method, physical mixtures were prepared by grinding known amounts of itraconazole and β-CD in a mortar with pestle. In the co-precipitation method known amounts of β-CD and itraconazole were dissolved in deionized water and methanol, respectively. Both solutions were heated to 65^0C and mixed together. The final solution was continuously mixed at 65^0C to remove organic solvent after which the mixture was cooled to 5^0C and the crystals were separated by filtration through 0.45µm membrane filters. The product was dried and kept in desicator overnight to remove traces of solvents.

Formulation of Itraconazole buccoadhesive tablets

Preparation: In this work, direct compression method has been employed to prepare buccal tablet with HPMC K4M and Carbopol 934P as polymers. For one tablet accurately weighed 32 mg “Itraconazole inclusion complex powder”

which is equivalent to 10 mg of Itraconazole was used in the formulation.

Procedure: All the ingredients were accurately weighed and passed through mesh # 60. In order to mix all ingredients thoroughly Itraconazole inclusion complex powder, polymers, lactose DC were blended geometrically in mortar and pestle for 10 minutes then talc, magnesium stearate were mixed one by one for 1-2 min.

The powder blends of various proportions were evaluated for angle of repose, Carr's compressibility index and compressed into tablets of diameter 8mm on Clit pilot press 10 Station machine. Using stainless steel flat surface dies and punches by maintaining individual tablet weight constant at 150 mg. The compression force was maintained in such way that the hardness of resulting tablets ranged between 4 – 5 Kg/cm^2.

The Ethyl cellulose solution (10%w/v in ethanol) was cast on the prepared tablets from three sides as an impermeable backing layer which was aimed to provide unidirectional drug release.[3]

The compositions of the prepared formulations are as specified in the tables-1 and 2.

Evaluation of Itraconazole Buccoadhesive tablets[5, 6]

Uniformity of weight: The weight (mg) of each of 20 individual tablets, selected randomly from each formulation was determined by dusting each tablet off and placing it in an electronic balance. The weight data from the tablets were analyzed for sample mean and percent deviation.

Hardness test: The hardness of three randomly selected tablets from each formulation was determined using Monsanto hardness tester by placing each tablet diagonally between the two plungers and applying pressure until the tablet broke down into two parts completely and the reading on the scale was noted down in Kg/cm^2.

Thickness: The thickness of three randomly selected tablets from each formulation was determined in mm using a vernier caliper (Pico India).

Friability test: This was determined by weighing 10 tablets after dusting, placing them in the friabilator and rotating the plastic cylinder vertically at 25 rpm for 4 min. After dusting, the total remaining weight of the tablets was recorded and the percent friability was calculated.

Uniformity of drug content: 5 tablets were powdered in a glass mortar and the powder equivalent to 50 mg of drug was placed in a stoppered 100 ml conical flask. The drug was extracted with 40 ml methanol with vigorous shaking on a mechanical gyratory shaker (100 rpm) for 1 hour. Then heated on water bath with occasional shaking for 30 minutes and filtered into 50 ml volumetric flask through cotton wool and filtrate was made up to the mark by passing more methanol through filter, further appropriate dilution were made and absorbance was measured at 265 nm against blank (methanol).

Mucoadhesion strength[7-10]: The apparatus used for testing bioadhesion was assembled in the laboratory (fig.1). Mucoadhesion strength of the tablet was measured on a modified physical balance employing the method described by Gupta et al using bovine cheek pouch as model mucosal membrane.

A double beam physical balance was taken, the left pan was removed. To left arm of balance a thick thread of suitable length was hanged. To the bottom side of thread a glass stopper with uniform surface was tied. A clean glass mortar was placed below hanging glass stopper. In this mortar was placed a clean 500 ml glass beaker, within which was placed another glass beaker of 50 ml capacity in inverted position and weighted with 50 gm to prevent floating. The temperature control system involves placing thermometer in 500 ml beaker and intermittently adding hot water in outer mortar filled with water. The balance was so adjusted that right hand-side was exactly 5 gm heavier than the left.

Method

The balance adjusted as described above was used for the study. The bovine cheek pouch, excised and washed was tied tightly with mucosal side upward using thread over the base of inverted 50 ml glass beaker. This beaker suitably weighted was lowered into 500 ml beaker, which was then filled with isotonic phosphate buffer (pH 6.8) kept at 37^o C such that the buffer reaches the surface of mucosal membrane and keeps it moist. This was then kept below left hand side of balance. The buccal tablet was then stuck to glass stopper through its backing membrane using an adhesive (Feviquick). The 5gm on right hand

side is removed, this causes application of 5 gm of pressure on buccal tablet overlying moist mucosa. The balance was kept in this position for 3 minutes and then slowly weights were increased on the right pan, till tablet separates from mucosal membrane. The total weight on right pan minus 5 gm gives the force required to separate tablet from mucosa. This gives bioadhesive strength in grams. The mean value of three trials was taken for each set of formulations. After each measurement, the tissue was gently and thoroughly washed with isotonic phosphate buffer and left for 5 minutes before reading a new tablet of same formulation to get reproducible multiple results for the formulation (**Fig. 1**). The time for tablet to detach from the sheep buccal mucosa was recorded as the Mucoadhesion time Observed Bioadhesion times for different formulation were given in Table- 3-4.

Table 1: Preliminary Trial Formulations

Ingredients (mg)	T_1	T_2	T_3	T_4	T_5	T_6	T_7
ITRACONAZOLE inclusion complex powder	32	32	32	32	32	32	32
HPMC K4M	10	20	-	-	30	20	10
Carbopol 934P	-	-	10	20	10	20	30
Lactose DC	106	96	106	96	76	76	76
Magnesium stearate	1	1	1	1	1	1	1
Talc	1	1	1	1	1	1	1

Table 2: Final Formulations table

Ingredients(mg)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	C1	C2
ITRACONAZOLE inclusion complex powder	32	32	32	32	32	32	32	32	32	32	32
HPMC K4M	10	10	10	20	20	20	30	30	30	15	25
Carbopol 934P	5	10	15	5	10	15	5	10	15	7.5	12.5
Lactose DC	98.5	93.5	88.5	88.5	83.5	78.5	78.5	73.5	68.5	91	76
Magnesium stearate (2%)	3	3	3	3	3	3	3	3	3	3	3
Talc (1%)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

Fig. 1: Bioadhesion Testing Apparatus

Tablet hydration study[11]

The tablet hydration studies were carried out in Petri dishes with pH 6.8 phosphate buffer. Periodically, the tablets were withdrawn from the beaker and weighed on electronic balance after removal of surface water by light blotting with a lab tissue.

The sampling times of hydration studies were 0.5, 1, 2, 4, 6, 8, 12 and 24 hours. The rate of hydration was calculated according to the model describing the absorption of liquid into polymeric matrices via diffusion. SI after 6 hours was shown in Table- 3-4.

Tablet surface pH evaluation[12]

The surface pH of the tablets was determined in order to investigate the possibility of any side effect, in vivo. As an acidic or alkaline pH may cause irritation to the buccal mucosa, it was our attempt to keep surface pH as close to neutral as possible.

The tablets were placed in glass tubes and allowed to swell in contact with 1 ml of distilled water for 2 hours. The surface pH was noted by bringing glass micro electrode near the surface of tablet and allowing it to equilibrate for 1 min. Thereafter surface pH measurements were recorded and shown in Table- 3-4.

***In vitro* Dissolution studies**[13]

In vitro dissolution studies of buccal tablets of Itraconazole were carried out in USP TDT 06P tablet dissolution test apparatus-II (Electrolab), employing a paddle stirrer at 50 rpm using 900ml of pH 6.8 Phosphate buffer + 0.5% w/v SLS solution at 37 ±0.5°C as dissolution medium. One tablet was used in each test. The tablets were supposed to release drug from one side only; therefore an impermeable backing membrane side of tablet was fixed to a 2×2 cm glass slide with a solution of cyanoacrylate adhesive. Then it was placed in dissolution apparatus.

At predetermined time intervals 5ml of the samples were withdrawn by means of a syringe fitted with a pre filter. The volume withdrawn at each interval was replaced with same quantity of fresh dissolution medium maintained at 37±0.5°C. The samples were analyzed for drug release by measuring the absorbance at 265 nm using UV-Visible spectrophotometer after suitable dilutions. The result of in-vitro drug release study was shown in Table No: 6-7 and Figure No: 3 - 6.

***In-vitro* Drug Permeation studies**[13, 15]

In vitro permeation of Itraconazole from Buccal tablets through the excised Sheep buccal mucosal membrane was studied using modified Franz diffusion cell.

Diffusion cell: The diffusion studies were done to get an idea of permeation of drug through barrier from the membrane system. These In-vitro studies are also done for Buccal DDS development. Usually, two types of diffusion cells are used as horizontal and vertical. The Franz and Keshary Chien (K-C) type of diffusion cells are of horizontal type of cells. Diffusion cells generally comprise two compartments, one containing the active component (donor compartment) and the other containing receptor solution (receptor compartment), separated by barrier i.e. Sheep buccal mucosal membrane.

The cell consisted of sampling port and temperature maintaining jacket. The outlet and inlet was connected with latex tube so the jacket had stagnant water inside and heat was provided by hot plate. The stainless steel pin was used to stirr the receptor solution using magnetic stirrer. The Sheep buccal mucosa was placed on receptor compartment and both compartments held tight by clamps.

Tissue isolation: Fresh Sheep buccal mucosa was obtained from a local slaughter house and used within 2 hours of slaughter. The tissue was stored in phosphate buffer pH 6.8 at 4°C after collection. The epithelium was separated from the underlying connective tissue with a surgical technique and the delipidized membrane was allowed to equilibrate for approximately one hour in receptor buffer to regain lost elasticity.

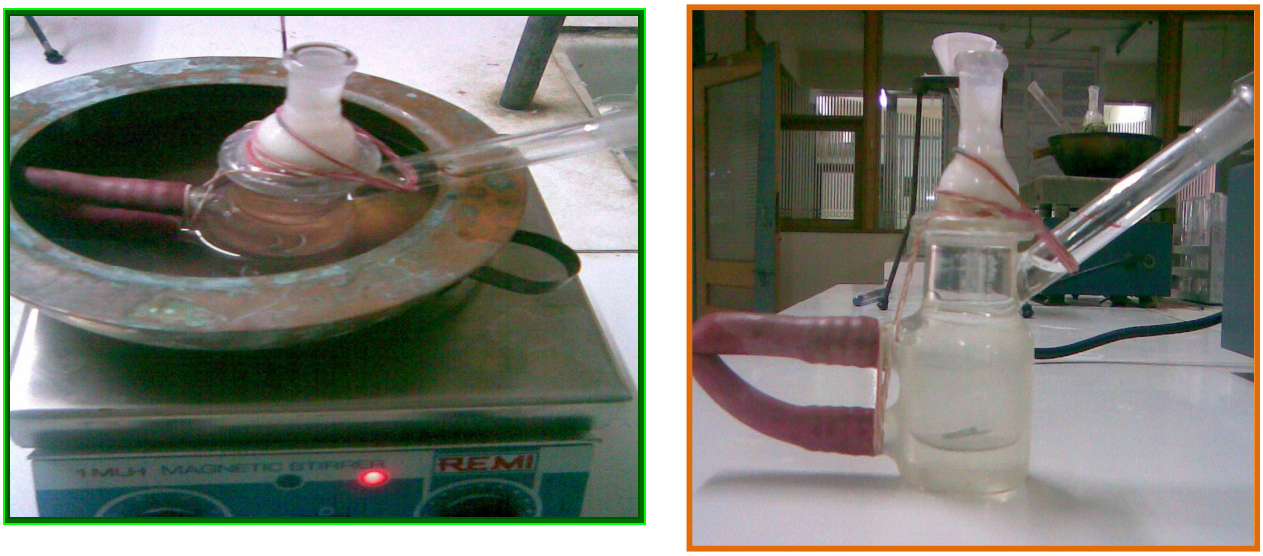

Fig. 2: Modified Franz diffusion cell used for Drug Permeation studies

***In-vitro* drug permeation through Sheep buccal mucosal membrane**

The Keshary- Chein cell with receptor side volume of 25 ml and diffusion area of 4.90 cm^2 was used. Fresh Sheep buccal mucosa was mounted between donor and receptor compartment of diffusion cell. The selected tablet containing 10 mg of Itraconazole (i.e. 32 mg of Itraconazole inclusion complex) was placed on the membrane to the donor side, and the compartment was clamped together in such a way that the membrane side will be in contact with receptor medium.

The donor compartment of diffusion cell was filled with 1 ml of pH 6.8 phosphate buffer. The receptor compartment (25 ml capacity) was filled with ethanol: propylene glycol: phosphate buffer pH 7.4 (40:15:45) as a media. And the hydrodynamics in the compartment was maintained by stirring with a bent stainless steel pin at uniform slow speed. The temperature was maintained at 37± 1^oC by placing the diffusion cell in a water bath over hot plate with magnetic stirrer.

For selected formulation, diffusion was carried out for 10 hours through Sheep buccal mucosa. 1 ml sample was withdrawn at predetermined time intervals from the receptor compartment and replaced with an equal volume of the above by ethanol: propylene glycol: phosphate buffer pH 7.4 (40:15:45) media to maintain sink condition. The amount of Itraconazole in the diffusion samples was estimated by the UV spectrophotometer at 265 nm. Resulted data is summarized in Table-8 and Figure- 7.

Stability studies

Stability studies were performed at a temperature of 40^0 C at 75 % RH, over a period of three months (90 days) on the promising buccal tablets of Itraconazole formulation F_1. Sufficient number of tablets (15) were packed in amber colored screw capped bottles and kept in stability chamber maintained at $40^0 \pm 1^0$C & 75 % RH. Samples were taken at monthly intervals for drug content estimation. At the end of three months period, dissolution test and drug content studies were performed to determine the drug release profiles and drug content. . Resulted data is summarized in Table-11

Drug- Excipient Interaction Study: FTIR spectra of the drug, promising formulations and polymers were obtained by potassium bromide pellet method using Perkin-Elmer FTIR series (model-1615) spectrophotometer in order to rule out drug-excipient interactions.

RESULTS AND DISSCUSSION

The aim of this work was to develop a tablet for the buccal delivery of the poorly water-soluble drug Itraconazole, for that Solubilization of Itraconazole by complexation with β-Cyclodextrin and then delivery via buccal mucosa using Buccal tablets of Itraconazole to release drug at mucosal site in unidirectional

pattern for extended period of time without wash out of drug by saliva. Hydroxypropyl methylcellulose K4M and carbopol 934P were selected as mucoadhesive polymers on the basis of their matrix forming properties and mucoadhesiveness while ethyl cellulose being hydrophobic, as backing material. Ethyl cellulose has recently been reported as excellent backing material, given its low water permeability and moderate flexibility.

Inclusion complexes of Itraconazole were prepared in 1:1M ratios with β-Cyclodextrin using kneading method. The prepared complex was free flowing and off white in colour.

The Itraconazole complexes with β-CD presented better dissolution performance over pure drug in an *in vitro* test. According to these results, inclusion complexes prepared using with β- Cyclodextrin, at 1:1M ratio showed about 100 % drug release in 80min.

Evaluation for Buccal tablets of Itraconazole

The prepared buccal tablets were evaluated for thickness, hardness, friability, uniformity of weight, uniformity of drug content, surface pH determination, *in vitro* bioadhesive strength measurement, Mucoadhesion time measurement, swelling index, *in vitro* dissolution, *in vitro* drug permeation study and stability study.

Physicochemical Properties: It could be observed that all the prepared tablets fulfill the IP requirements for Physicochemical Properties. The flow pattern for trial formulations powder beds was found to be passable while increasing the amount of lubricants in final formulations gives excellent flow pattern for power beds of final batches. The hardness of prepared buccal tablets was found to be in the range of 4.14 to 4.71 kg/cm^2. Thickness was in the range of 2.8 to 3.3mm. The friability of all tablets was less than 1% i.e. in the range of 0.26 to 0.68 %. The percentage deviation from mean weights of all the batches of tablets was found to be within the prescribed limits as per IP. The low values in standard deviation indicates uniform drug content in all the batches prepared as observed from data table given in table 3-4.

Mucoadhesion time

Mucoadhesion time of tablets increases with increase in polymer content. Mucoadhesion test was performed using sheep buccal tissue. The time for tablet to detach from buccal tissue was recorded as mucoadhesion time. Formulations containing polymer HPMC alone like T_1, T_2 exhibited less mucoadhesion time (1 to 3 hrs) but the formulations containing carbopol alone and along with HPMC like T_3,T_4, T_5,T_6,T_7 and all the final formulations i.e. F_1 to F_6 exhibited mucoadhesion time more than 12 hours.

Bioadhesive Strength Measurement

Bioadhesion strength measurement of tablets indicated that the bioadhesive strength was proportional to carbopol content. The mean bioadhesive strength values after 3 min of contact time was 0.2934 N for formulation F_1. The values of bioadhesive strength [Table-10., Fig-33-34.] were decreased in the following order:

$T_7>T_6>T_4>F_6>F_3>T_5>F_5>T_3>F_2>F_4>F_1>T_2>T_1$.

Therefore, increasing carbopol concentration increases the bioadhesion. This increase in the bioadhesion could be due to the formation of secondary mucoadhesive bonds with mucin because of rapid swelling and interpenetration of the polymer chains in the interfacial region, while other polymers undergo only superficial bioadhesion. The peak detachment force was considered to be dependent on the formation of hydrogen bonds between the functional groups of the bioadhesive and the mucus. HPMC alone had poor adhesive properties, but when used in combination with Carbopol, its overall adhesion was increased. Very strong bioadhesion could damage the epithelial lining of buccal mucosa.

Swelling Index

In-vitro water uptake studies are of great significance as variation in water content causes a significant variation in mechanical properties of formulations. The capacity of the formulation to take up water is an important intrinsic parameter of the polymeric system in consideration to the release of the drug on the mucosal surface. Water absorbing capacity of system (SI after 6 hours.) decreased in the following order $T_7>T_6>T_5> F_6>F_5>F_4>T_4>F_3> T_2 > F_2 >F_1>T_3> T_1$; with decreasing concentration of Carbopol.

The Surface pH

The surface pH of all formulations was found to be within ±1 units of neutral pH hence these formulations should not cause any irritation in buccal cavity.

***In vitro* drug release study**

This study was performed using USP TDT 06 (paddle) dissolution test apparatus at 50 rpm using 900 ml of pH 6.8 Phosphate buffer + 0.5%

w/v SLS solution maintained at 37 ± 0.5^{0}C as dissolution medium. The results were shown in tables- 11 to 12. From the data, it is evident that as the proportion of polymers in the formulation increases, cumulative percent drug released was found to be reduced. Among the seven trial batches, formulation T_1 to T_4 have released 87 to 100% drug in 10 hours, whereas T_5 to T_7 formulations have released 70 to 75% drug in 10 hours. A higher diffusive flux develops as a consequence of the higher Solubilization rate operated by β-CD, which increases the amount of mobile species. Both these effects result in an enhanced release rate of drug.

It indicates that controlled release of drug can be obtained with increased in amount of polymers (HPMC K4M and carbopol 934P).

In seven trail formulations, T5 formulation has shown promising dissolution parameters ($t_{50\%}$=6.3 hours, $t_{70\%}$=8.9 hours) and good mucoadhesion time (> 12 hours).

From the data, it is evident that formulation F_1 has shown highly satisfactory values for dissolution parameters ($t_{50\%}$=3.9 hours; $t_{70\%}$ = 5.4 hours and swelling index =22.74 hours) and has released approximately 99.41% drug in 10 hours. Hence, formulation F_1 may be considered as the buccal tablet containing Itraconazole inclusion complex with β-CD for improved bioavailability.

Drug Release Kinetics

In-vitro drug release data of all the buccal tablet formulations was subjected to goodness of fit test by linear regression analysis according to zero order equations, Higuchi's and Korsmeyer-Peppas models to ascertain the mechanism of drug release.

From the data, it can be seen that except formulation T_1, T_2 and T_3 all the trial formulations containing combination of polymers HPMC and carbopol have displayed zero order release kinetics ('r' values in the range of 0.996 to 0.911). From Higuchi's and Peppas data, it is evident that the drug is released by non-Fickian diffusion mechanism except formulation containing HPMC and carbopol alone.

The values of 'r' for Higuchi's equation of factorial formulations range from 0.971 to 0.992. This data reveals that drug release follows non-Fickian diffusion mechanism. This is because as the proportion of polymers in the matrix increased there was an increase in the amount of water uptake and proportionally greater swelling leading to a thicker gel layer. Zero-order release from swellable hydrophilic matrices occurs as a result of constant diffusional path lengths. When the thickness of the gelled layer and thus the diffusional path lengths remain constant, zero-order release can be expected, as seen for formulations.

***In-vitro* Drug permeation study of formulation F_1**

Based on the results of all formulations, the F_1 formulation was selected for *in vitro* drug permeation studies. The oral mucosa of sheep resembles that of humans more closely in terms of structure and composition and therefore sheep buccal mucosa was selected for drug permeation studies. The results of drug permeation from buccal tablets through the sheep buccal mucosa reveal that Itraconazole was released from the formulation and permeated through the sheep buccal membrane and could possibly permeate through the human buccal membrane. The drug permeation was slow and steady and 68.47 ± 2.11% of Itraconazole could permeate through the buccal membrane in 10 hours with average flux of 139.28 µg/cm^{-2}/min.

The results, reported show that Itraconazole permeation through mucosa was quite good and increased in the presence of β-Cyclodextrin. This effect, in principle, can be attributed to both an increase of driving force for permeation due to the increase of Itraconazole apparent solubility in the presence of β- CD as well as to enhancing effect of β- CD. Thus β-Cyclodextrin has been suggested to act as penetration enhancers. They enhance the permeation of the drug by carrying the drug through the aqueous barrier towards the surface of the membrane, where the drug passes from the complex into the membrane. Addition of β- CD to the matrix increased the flux by increasing the solubility of Itraconazole, thus improving the diffusible form of the drug species at the tablet membrane interface. Though the complex did not penetrate the membrane, the drug in the complex was in rapid dynamic equilibrium with the "free" drug, thus continuously supplying the drug molecules to the membrane surface in a diffusible form.

The role of dissolution enhancement in increasing the rate of delivery is more relevant when the tablet is employed as transmucosal system since, differently from solution conditions; a very limited contribution to delivery derives from matrix erosion.

A successful design of a buccal delivery system should guarantee both an intimate contact with the mucosa for an adequate time interval and

proper release rates. Actually, before a drug passes through the mucosal barrier and reaches blood circulation, it should dissolve in the medium penetrating inside the buccal tablet. This step is generally critical for lipophilic drugs that, although being well absorbed, exhibit a slow dissolution rate in aqueous media. Buccoadhesive tablets containing Itraconazole inclusion complex with β-CD could therefore be of interest as a transmucosal delivery system due to their recognized bioadhesive properties and the possibility of improving release features of drugs poorly soluble in aqueous media. However, the incorporation of Itraconazole and β-CD a binary systems in the tablets for such an application should not impair the overall mucoadhesive properties of the system which is the inter diffusion of polymer chains and mucus components at interface. An overall evaluation of the mucoadhesive behavior of tablets shows good bioadhesive properties. Although containing considerable amounts of β- CD, and are suitable for transmucosal applications.

The feasibility of a buccal delivery for Itraconazole was preliminary assessed by measuring *in vitro* permeation of Itraconazole through sheep buccal mucosa.

Stability Studies

Stability study was performed on the promising formulation F_1 by storing the samples at 45±1°C for 3 months (90 days). The samples were tested for any changes in physical appearance and drug content at weekly intervals. *In vitro* drug release studies were performed at the end of 3 months storage. These results indicate that there were no significant changes in drug content and dissolution profile of the formulation F_1 during storage at 45^0C for 3 months.

Evaluation of Itraconazole buccoadhesive tablets
Table 3: Evaluation of Trial formulations

Formulation code	Mean Hardness Kg/cm^2	Thickness (mm)	Friability % w/w	Average weight (mg	Mean drug content %±SD	SI ± SD (after 6 hrs)	Mucoadhesion (time of detachment (hrs)	Tablet Surface pH
T_1	4.51	2.9	0.52	147.86	98.65 ± 0.76	20.08	1	7.12
T_2	4.41	2.9	0.64	148.37	95.36 ± 1.42	45.64	3	7.24
T_3	4.30	3.1	0.40	152.47	97.03 ± 0.55	21.17	>12	6.88
T_4	4.71	2.8	0.26	145.80	96.71 ± 3.07	51.17	> 12	6.17
T_5	4.62	2.9	0.27	144.64	97.85 ± 2.23	97.83	> 12	6.32
T_6	4.52	2.8	0.39	152.18	96.92 ± 1.77	112.24	> 12	6.35
T_7	4.56	2.9	0.53	146.54	97.69 ± 2.05	114.61	10	6.04

Evaluation of Itraconazole buccoadhesive tablets
Table 4: Evaluation of final formulations

Formulation code	Mean Hardness Kg/cm^2	Thickness (mm)	Friability % w/w	Average weight (mg	Mean drug content %±SD	SI ± SD (after 6 hrs)	Mucoadhesion (time of detachment hrs)	Tablet Surface pH
F_1	4.50	3.1	0.51	149.58	99.52 ± 1.60	22.74	>12	7.02
F_2	4.55	2.8	0.64	145.37	98.12 ± 1.08	33.36	>12	6.71
F_3	4.32	3.2	0.68	152.62	94.90 ± 0.81	48.35	>12	6.12
F_4	4.41	3	0.52	148.38	95.77 ± 0.08	56.52	> 12	6.11
F_5	4.29	3.1	0.61	149.89	101.14 ± 1.35	63.21	> 12	6.58
F_6	4.51	3.2	0.67	151.12	98.44 ± 0.68	75.56	> 12	6.21

Bioadhesive Strength measurement

Table 5: Bioadhesive Strength measurement

Formulation Code	Bioadhesive Strength [Newton] [Mean, n=3]
T1	0.2634
T2	0.2841
T3	0.2984
T4	0.3013
T5	0.3325
T6	0.3510
T7	0.3819
F1	0.2802
F2	0.3029
F3	0.3108
F4	0.3120
F5	0.3249
F6	0.3781

Table 6: *In-vitro* Drug release data of Trial formulations T1, T2, T3, T4, T5, T6 and T7

Time (Hrs	T1 Cumulative* percent drug released ±SD	T2 Cumulative* percent drug released ±SD	T3 Cumulative* percent drug released ±SD	T4 Cumulative* percent drug released ±SD	T5 Cumulative* percent drug released ±SD	T6 Cumulative* percent drug released ±SD	T7 Cumulative* percent drug released ±SD
0.5	70.65±1.38	42.04±1.25	15.14±1.73	13.45±1.32	10.09±1.23	8.41±0.92	8.41±0.92
1	85.79±1.15	57.19±.1.83	21.86±1.44	16.85±0.38	15.14±0.26	13.45±1.22	13.45±1.22
2	95.88±0.64	68.97±1.32	30.28±0.54	21.86±1.12	18.50±1.28	20.18±1.24	20.18±1.24
3	97.57±0.17	77.38±0.80	40.37±1.52	30.28±1.09	26.91±1.29	30.28±0.82	30.28±0.82
4	-	84.11±1.64	52.14±1.43	37.09±1.67	35.32±0.16	37.00±1.24	37.00±1.24
5	-	89.15±0.74	74.01±1.32	62.24±1.77	42.05±1.26	38.69±1.94	42.05±1.09
6	-	94.20±1.42	79.06±0.43	68.97±1.81	47.10±1.55	45.42±1.24	50.46±1.89
7	-	97.57±0.65	85.79±1.46	80.70±1.21	53.82±1.46	53.83±1.43	58.87±1.77
8	-	99.25±0.81	89.15±1.35	89.15±0.64	63.92±1.15	60.56±0.32	65.60±1.03
9	-	-	90.84±0.83	92.52±0.41	70.65±1.37	67.28±1.54	74.01±0.87
10	-	-	-	94.93±0.95	71.88±1.23s	77.37±1.54	-

[*Average of three determinations]

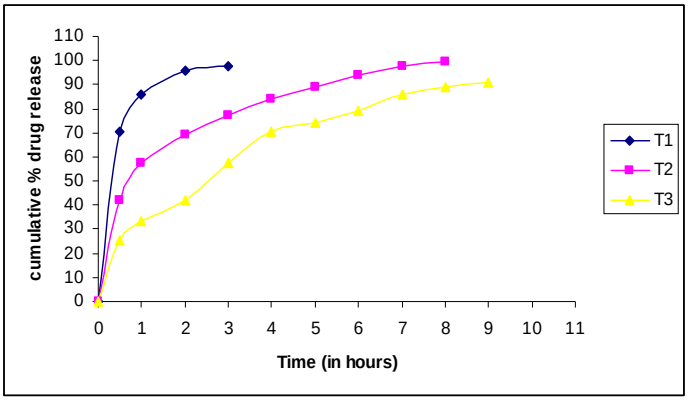

Fig. 3: Cumulative Percent Drug Released Vs Time Plots (Zero Order) of formulations T_1, T_2 and T_3

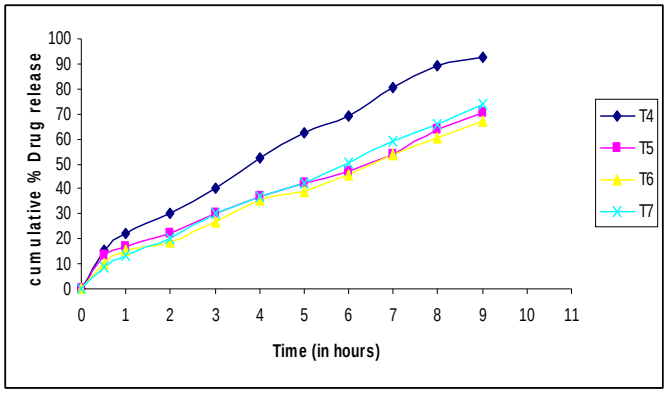

Fig. 4: Cumulative Percent Drug Released Vs Time Plots (Zero Order) of formulations T_4,T_5, T_6 and T_7

Table 7: *In-vitro* Drug release data of factorial formulations F_1, F_2, F_3, F_4, F_5 and F_6

Time (Hrs)	F1	F2	F3	F4	F5	F6
	Cumulative* percent drug released ±SD	Cumulative* percent drug released ±SD	Cumulative* percent drug released ±SD	Cumulative* percent drug released ±SD	Cumulative* percent drug released ±SD	Cumulative* percent drug released ±SD
0.5	11.13±1.24	09.31±0.14	08.71±1.22	10.19±1.22	10.52±1.22	12.71±1.09
1	18.09±1.35	15.81±1.23	14.28±0.56	16.38±0.44	18.31±0.73	17.24±0.52
2	29.31±1.26	22.51±0.15	21.90±1.12	18.20±0.56	30.02±1.32	21.55±1.63
3	38.15±1.10	32.30±1.24	27.39±0.76	29.63±1.86	37.41±0.98	31.08±1.08
4	50.54±1.25	52.00±1.22	39.17±1.34	38.41±0.43	40.61±1.98	37.28±1.13
5	63.70±0.12	64.14±2.01	46.28±0.43	45.39±1.56	49.52±0.34	41.16±1.66
6	81.22±1.42	69.28±0.11	52.62±1.65	58.33±0.46	61.73±1.23	60.41±0.56
7	95.62±1.33	73.49±0.24	62.19±1.32	61.23±1.54	65.82±0.44	66.70±1.39
8	98.01±1.31	79.98±0.32	69.15±0.11	66.18±1.74	70.12±0.63	72.90±0.31
9	99.99±1.23	92.13±1.91	78.39±1.32	72.31±1.33	76.59±1.66	74.68±0.30
10	-	95.16±0.78	85.21±1.61	76.48±1.32	79.00±1.01	77.20±1.24

[*Average of three determinations]

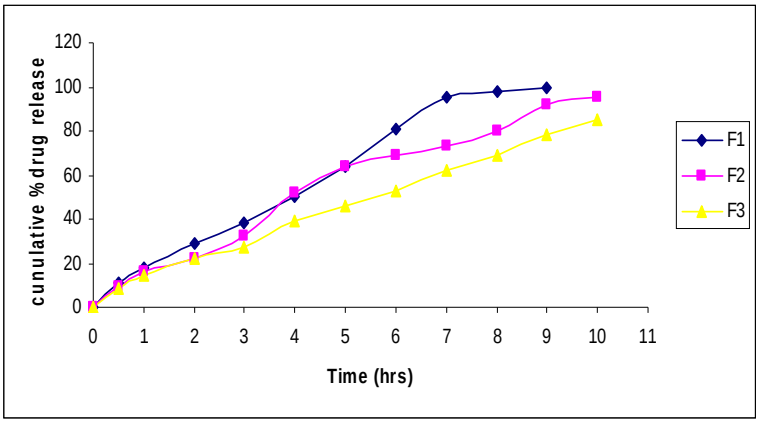

Fig. 5: Cumulative Percent Drug Released Vs Time (Zero Order) of formulations F_1, F_2 and F_3

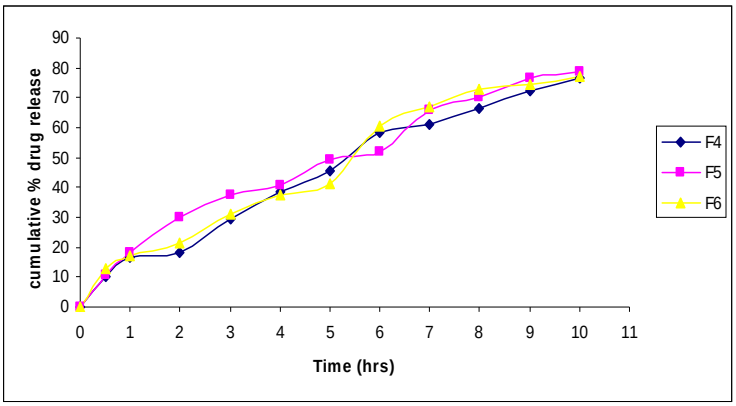

Fig. 6: Cumulative Percent Drug Released Vs Time (Zero Order) of formulations F4, F5 and F6

Table 8: *In-vitro* Drug permeation study of formulation F_1

TIME (in hrs)	Cumulative Percentage of Drug permeated across sheep buccal mucosa ±SD
0.5	10.06±1.65
1	13.42±2.08
2	19.91±1.98
3	25.92±1.76
4	30.92±1.48
5	34.91±1.08
6	48.12±1.91
7	55.32±2.21
8	61.87±1.82
9	66.55±1.67
10	68.47±2.11

Average Flux = 141.28 µg/cm^{-2}/min

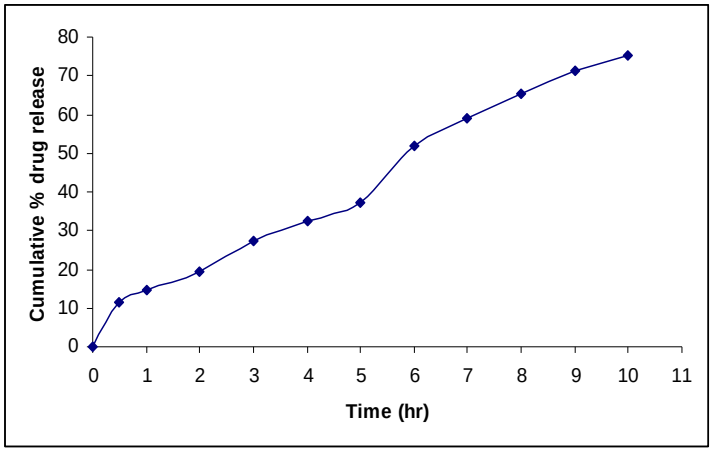

Fig. 7: Cumulative Percent Drug permeated across sheep buccal mucosa Vs Time Plots (Zero Order) of formulations F_1

Table 9: Dissolution and Swelling Index Parameter for the Trial Formulations

Formulation code	$t_{50\%}$ (hours)	$t_{70\%}$ (hours)	SI (after 6 hours)	Cumulative percent drug release in 10 hours
T_1	0.5	0.8	18.19	97.57
T_2	0.6	2.2	35.50	99.25
T_3	2.4	3.7	25.38	90.84
T_4	3.7	6.2	43.65	92.52
T_5	6.5	9.0	79.17	70.65
T_6	6.6	9.3	109.00	67.28
T_7	5.8	8.4	113.52	74.01

Table 10: Dissolution and Swelling Index Parameter

Formulation code	$t_{50\%}$ (hours)	$t_{70\%}$ (hours)	SI (after 6 hours)	Cumulative percent drug release in 10 hours
F_1	4.0	5.5	24.32	99.99
F_2	3.9	6.3	35.50	95.16
F_3	5.6	8.0	50.14	85.21
F_4	5.4	8.6	55.07	76.48
F_5	5.0	7.8	59.19	79.00
F_6	5.3	7.5	64.50	77.20
F_7	5.6	9.0	72.55	72.19
F_8	5.9	9.2	78.26	71.56
F_9	7.0	9.5	85.35	66.81

Table 11: Drug content Data of Stability Formulation (F_1)

Trial No.	1st Day (%)	15th Day (%)	30th day (%)	45th Day (%)	90th Day (%)
I	99.52	98.97	98.45	97.85	96.79
II	100.12	98.80	98.40	97.59	97.93
III	99.35	99.89	98.62	98.75	97.78
Mean ($\overline{X}$)	99.66	99.22	98.49	98.06	97.50
SD	0.404	0.586	0.115	0.60	0.619

Table 12: *In vitro* Release Data of the Stability Formulation (F_1)

Time (Hrs)	Cumulative* Percent of Drug Released ± SD at 45 ± 1^{0}C	
	1st Day	90th Day
01	11.13±1.24	10.55±1.25
02	18.09±1.35	16.75±0.97
03	29.31±1.26	27.99±0.08
04	38.15±1.10	37.49±0.42
05	50.54±1.25	49.88±0.76
06	63.70±0.12	61.56±0.58
07	81.22±1.42	80.39±0.90
08	95.62±1.33	93.92±0.84
09	98.01±1.31	97.65±1.58
10	99.99±1.21	97.98±1.36

*Average of three determinations

CONCLUSION

It has been shown that the incorporation of β-cyclodextrin in buccoadhesive tablets of Itraconazole with hydrophilic matrix intended for the delivery of poorly soluble drugs can be a suitable strategy to release features of the system while maintaining good bioadhesive properties. Cyclodextrin are responsible for an increase in the erosion rate of the tablet and an improved dissolution of the drug inside the polymeric matrix. This latter effect is the crucial factor, which determines the increase of release rate from the tablets in solution as well as an increase in the amount of Itraconazole permeated through sheep buccal mucosa. This systems turns to be of great potential as buccal delivery system in view of the possibility of tailoring release features while maintaining good bioadhesive properties.

The phase solubility analysis studies indicated formation of inclusion complex of Itraconazole with β-Cyclodextrin. Phase solubility analysis indicated A_L type of phase diagram suggesting formation of 1:1 M complex of Itraconazole and β-CD. The stability constant values for the Itraconazole and β- CD complexes were 6461.835 M^{-1}.

In this work, inclusion complexes of Itraconazole were prepared using β-cyclodextrin, these inclusion complexes were prepared by kneading (Physical mixing) method.

These complexes were characterized by spectroscopic studies such as UV spectroscopy, IR spectroscopy and X-RD, Differential scanning calorimetery (DSC). These indicated absence of significant drug: carrier interactions. The DSC thermo gram of Itraconazole: β– Cyclodextrin (1:1 M) complex, showed considerable reduction in the intensity and / or broadening of the Itraconazole endotherm at around 162°C suggesting the successful inclusion of Itraconazole in the β- Cyclodextrin cavity.

Mucoadhesive buccal tablets of Itraconazole with controlled release can be prepared by direct compression method using β-CD as a drug carrier and HPMC K4M, carbopol 934P as mucoadhesive polymers also if amount of polymer in the tabled increases, the drug release rate decreases, where as swelling index and mucoadhesion time increases.

Most of the designed formulations of Itraconazole buccal tablets displayed zero order release kinetics, and drug release follows non – Fickian diffusion mechanism.

From the results of experiments, formulation F_1 containing, HPMC K4M 10 mg and carbopol 934P 5 mg and it released 99.41 % drug in 10 hours.

Stability studies of formulation F1 indicate, that there are no significant changes in drug content and dissolution parameters values after 3 months and when stored at 45 ±10C.

The formulation F1 can be considered as promising Buccoadhesive tablets containing inclusion complex of Itraconazole with β-CD providing zero order drug release over period of 10 hours.

In-vitro Drug permeation study of formulation F_1 suggested that β-Cyclodextrin act as penetration enhancers by increase in fluxes in BDDS of Itraconazole.

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