Formulation and characterization of Itraconazole Mucoadhesive Microspheres

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Abstract: The Sustained Drug Delivery has been the mainstay of Pharmaceutical Research during the past few decades. Barrier related problem has been addressed by multiple approaches including alternative routes of administration. Harnessing the drug molecule for safer effective and improved therapeutics is the challenge which the Pharmaceutical Scientists are meeting successfully in fastly advancing and one of the most demanding 'Health Care Sector'.The novel drug delivery system needs to enhance the safety and efficacy of drug molecule by formulating a convenient dosage form for administration and to achieve better patient compliance. Mucoadhesive microspheres of itraconazole was able to enhancing the bioavailability of the drug at oral buccal site for the treatment of oral candidiasis. The mucoadhesive microspheres containing itraconazole formulation IMM5 is the best formulations with naturally occurring polysaccharide polymeric blend ain the composition of Drug : HPMC : Chitosan (1:1:1) that release more than 98.13 % of the drug in gastric environment in controlled and sustained manner upto 12 h.

Introduction: Drug delivery systems (DDS) that can precisely control the release rates or target drugs to a specific body site have had an enormous impact on the healthcare system. The last two decades in the pharmaceutical industry have witnessed an avant-garde interaction among the fields of polymer and material science, resulting in the development of novel drug delivery systems. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etc. which modulates the release and absorption

characteristics of the drug. Microspheres constitute an important part of these particulate DDS by virtue of their small size and efficient carrier characteristics [1]. However, the success of these novel DDS is limited due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the DDS with the absorbing membranes. It can be achieved by coupling bioadhesion characteristics to microspheres and developing novel delivery systems referred to as "bioadhesive microspheres" [2]**.** Bioadhesion in simple terms can be described as the attachment of a synthetic or biological macromolecule to a biological tissue. An adhesive bond may form with the epithelial cell layer, the continuous mucus layer or a combination of the two. The term "mucoadhesion" is used specifically when the bond involves mucous coating and an adhesive polymeric device, while "cytoadhesion" is the cell-specific bioadhesion. The mechanism of bioadhesion has been reviewed extensively [3]. Adhesion between mucin and mucoadhesive polymers is usually analyzed based on the molecular attractive and repulsive forces. Bioadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1–1000μm in diameter and consisting either entirely of a bioadhesive polymer or having an outer coating of it, respectively Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of bioadhesive properties to microspheres has additional advantages, e.g. efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of drugs to the absorption site achieved by anchoring plant lectins, bacterial adhesins and antibodies, etc. on the surface of the microspheres [4]. Bioadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye, nasal cavity, urinary and gastrointestinal tract, thus offering the possibilities of localised as well as systemic controlled release of drugs. Application of bioadhesive microspheres to the mucosal tissues of ocular cavity, gastric and colonic epithelium is used for administration of drugs for localised action [5]**.** Candidiasis can be a frequent and significant source of oral discomfort, pain, loss of taste, and aversion to food. Candida albicans carriage and a history of candidiasis are other significant risk factors for oral candidiasis. The infection is caused by candida albicans, a dimorphic fungal organism that typically is present in the oral cavity in a non-pathogenic state in

about one-half of healthy individuals [6]**.** Candidiasis is a common oral and perioral opportunistic infection that usually results from overgrowth of endogenous Candida fungal microorganisms. Excellent oral hygiene, including brushing and flossing of the teeth twice daily and maintenance of adequate intraoral moisture, is critical in the prevention of candidiasis recurrence in the susceptible patient [6-7]**.** Itraconazole is a white or almost white powder, practically insoluble in water, very slightly soluble in alcohol, freely soluble in dichloromethane, sparingly soluble in tetrahydrofuran. The proposed experimental work will be design and evaluate an mucoadhesive microspheres of itraconazole at oral buccal cavity as site of absorption of srug, which could be absorbed from the oral cavity. The present work hypothesized that drug was supply through the buccal route, which induce a quick onset of effect and enhanced bioavailability. The buccal mucosa offers several advantages for controlled drug delivery for extended periods of time. The mucosa is well supplied with both vascular and lymphatic drainage.

Material And Methods

Preparation of mucoadhesive Microspheres: Itraconazole Mucoadhesive microspheres were prepared by Emulsification method. Hydroxy Propyl Methyl Cellulose (K4M) (Table 1) was dissolved Dichloromethane and Dehydrated alcohol (90%) (60:40 ratio). Add 3.0 gm of Itraconazole and Chitosan powder (Table 1) into polymeric solution mixture under stirring and mixture was blended for 24 hr. Then the suspension was slowly dispersed in 250 ml of light liquid paraffin containing 2% Span 80 at a stirring rate of 500 rpm. After 30 minutes of emulsification, solvents were evaporated gradually with the help of water-circulating vacuum pump until the microspheres were formed. The system temperature was kept at 20°C all through the process. The microspheres were washed with petroleum ether and vacuum dried at room temperature [8].

Table 1: Various combinations of IMM1 – IMM5 based mucoadhesive microspheres.

S. No.	Code	Ingredients	Drug : Polymer	Qty (mg)	Organic Solvent System	Stabilizing agent (PVA) (% w/v)

1	IMM1	Drug : HPMC	01:01	150:150	Dichloromethane: ethanol	1
2	IMM2	Drug : HPMC	01:02	100:200	Dichloromethane: ethanol	1
3	IMM3	Drug : Chitosan	01:01	150:150	Dichloromethane: ethanol	1
4	IMM4	Drug : Chitosan	01:02	100:200	Dichloromethane: ethanol	1
5	IMM5	Drug : HPMC:Chitosan	01:01:01	100:100:100	Dichloromethane: ethanol	1

Characterization of mucodhesive microspheres:

Shape and Surface Morphology: The surface characteristics were examined by means of scanning electron microscopy. The morphology, surface appearance, and inner structure of MS were examined more in depth by Scanning Electron Microscope analysis of samples.

Determination of Particle Size and Size Distribution: Particle size was determined by optical microscopy method using calibrated ocular eyepiece. Effects of process variables i.e., drug concentration, polymer concentration, stirring rate and stirring time on particle size and size distribution was studied.

Drug Encapsulation Efficiency: Entrapment efficiency was determined after removal of surface anchored drug. The surface anchored drug was removed by dispensing accurately weight amount of microspheres in 10 ml of pH 6.8 phosphate buffer for 10 min. with occasional shaking. The suspension was centrifuged at 3000 rpm for 5 min. and the supernatant was kept aside. The sedimented microspheres were retreated in the same manner and supernatant of this centrifuge was mixed with first supernatant and drug concentration was determined UV spectrophotometrically at 268 nm.

Degree of Swelling: Accurately weight microspheres were incubated with pH 6.8 phosphate buffer and allowed to swell upto a constant weight. The degree of weight gain was calculated by using equation DS= [Wmf – Wmi] / Wmi

where, DS is degree of swelling, Wmi is initial weight of microspheres and Wmf is final weight of microspheres.

In-vitro mucoadhesion studies: In-vitro mucoadhesion studies were carried out using Falling film technique. Freshly excised pieces of intestinal mucosa of rat was mounted onto tilted glass slide with an angle of 45° and washed with phosphate buffer saline for 30 min. at 30 ml/min. The 100 mg of dried microspheres were dispersed on the mucosal tissue and left for 20 min. for interaction. During this period the whole system was placed in a humidity chamber which was adjusted to 90% RH. At the end of period the system was washed for 5 min. at 22 ml/min. At the end of this process the detached particle were collected and weighted. The percent mucoadhesion was calculated using equation [9].

$$\% \text{ Mucoadhesion} = \frac{\text{Wt of sample- Wt of detached particles} * 100}{\text{Wt of Sample}}$$

In-vitro Drug Release: Drug release study was carried out using modified USP dissolution test apparatus using pH 6.8 phosphate buffer as a media. Cross linked microspheres bearing drug were suspended in dissolution media at 37±0.1°C. Samples were withdrawn periodically and compensated with same amount of fresh dissolution media. The samples were analyzed for drug content by measuring absorbance using UV spectrophotometer at 268 nm [10].

Results and Discussion: The present study deals with preparation of microspheres by Emulsification and Solvent Evaporation Techniques which yielded microspheres with desired parameters and it has been investigated that buccal mucosa can also be a potential site for controlled delivery of macromolecular therapeutic agents, such as peptides, proteins and polysaccharides because of its accessibility and low enzymatic activity compared to the gastro-intestinal tract. The Drug Entrapment Efficiency of prepared mucoadhesive microspheres were studied for determination drug entrapment efficiency and the result was in the range of 81.71 % - 85.77 %. The Degree of Swelling of microspheres and swelling rate and percent mucoadhesion of mucoadhesive microspheres of itraconazole was in the range of 42.5 % - 53.9 %. *In-vitro* dissolution studies are a measure of quality control of product consistency and results can be evaluation of sustained release formulations. The Drug release for microspheres was evaluated in pH

6.8 phosphate buffer. The simulated gastric fluid was indicated that microspheres formulated using low drug to polymer ratio and high stirring speed yielded smaller microspheres which quickly release drug due to increase in surface area. Initial drug release may be from unencapsulated drug. The second phase of release process is slow and can be attributed to diffusion process of encapsulated drug from microspheres. The HPMC polymer based microspheres which were also evident from scanning electron microscopic observation showing non uniformity on surface. The mucoadhesive microspheres containing itraconazole formulation IMM5 is the best formulations with naturally occurring polysaccharide polymeric blend ain the composition of Drug : HPMC : Chitosan (1:1:1) that release more than 98.13 % of the drug in gastric environment in controlled and sustained manner upto 12 h. The in vitro drug release studies in simulated gastrointestinal fluids of pH 6.8 phosphate buffer and the observations are recorded in Table 3. Buccal mucoadhesive microspheres ITHPCH5 is the best formulations containing naturally occurring polysaccharide polymeric blend as Drug : HPMC : Chitosan (1:1:1) that release more than 98.13 % of the drug in gastric environment in controlled and sustained manner upto 12 h. Regression analysis was performed and the r^2 values suggested that the curves were fairly linear and slope values were computed from the graph. For all of the batches the value of release exponent “n” was > 0.89 indicating Super-case II transport mechanism (Figure 2). Regression analysis was performed and the r^2 values suggested that the curves were fairly linear and slope values were computed from the graph. For all of the batches the value of release exponent “n” was > 0.89 indicating Super-case II transport mechanism.

Table 2: Physical properties of mucoadhesive microsphere of itraconazole (IMM1 – IMM5)

S. No.	F. Code	dmean (µm)	Drug content	Encapsulation efficiency (%)	Swelling rate (%)	Percent Mucoadhesion
1	IMM1	361.45±0.540	428.1	85.62	42.5 ± 1.15	75.63 ± 0.018
2	IMM2	372.86±0.436	272.1	81.71	44.6 ± 1.18	77.64 ± 0.077
3	IMM3	371.15±0.495	431.2	86.24	47.7 ± 0.88	81.22 ± 0.123

4	IMM4	377.10±0.512	281.1	84.41	49.2 ± 1.38	84.64 ± 0.198
5	IMM5	382.12±0.436	427.8	85.56	53.9 ± 2.48	85.57 ± 0.208

Table 3: Dissolution data of buccal mucoadhesive microsphere of itraconazole (IMM1 – IMM5)

Time (h)	IMM1	IMM2	IMM3	ITMM4	IMM5
0	0	0	0	0	0
1	4.71	3.01	1.54	0.781	0.322
2	13.21	8.23	5.43	3.45	2.45
3	18.68	10.34	9.23	7.46	4.67
4	35.67	19.87	19.87	13.23	16.46
5	45.27	31.23	31.25	26.56	26.56
6	53.25	41.34	44.78	38.34	38.78
7	66.34	53.37	52.34	48.34	49.87
8	76.54	65.78	64.21	58.34	59.03
9	88.74	77.45	74.34	69.87	71.23
10	95.37	87.32	82.1	81.26	81.23
11	98.12	97.51	92.1	91.36	91.13
12	99.99	99.24	99.68	99.21	98.13

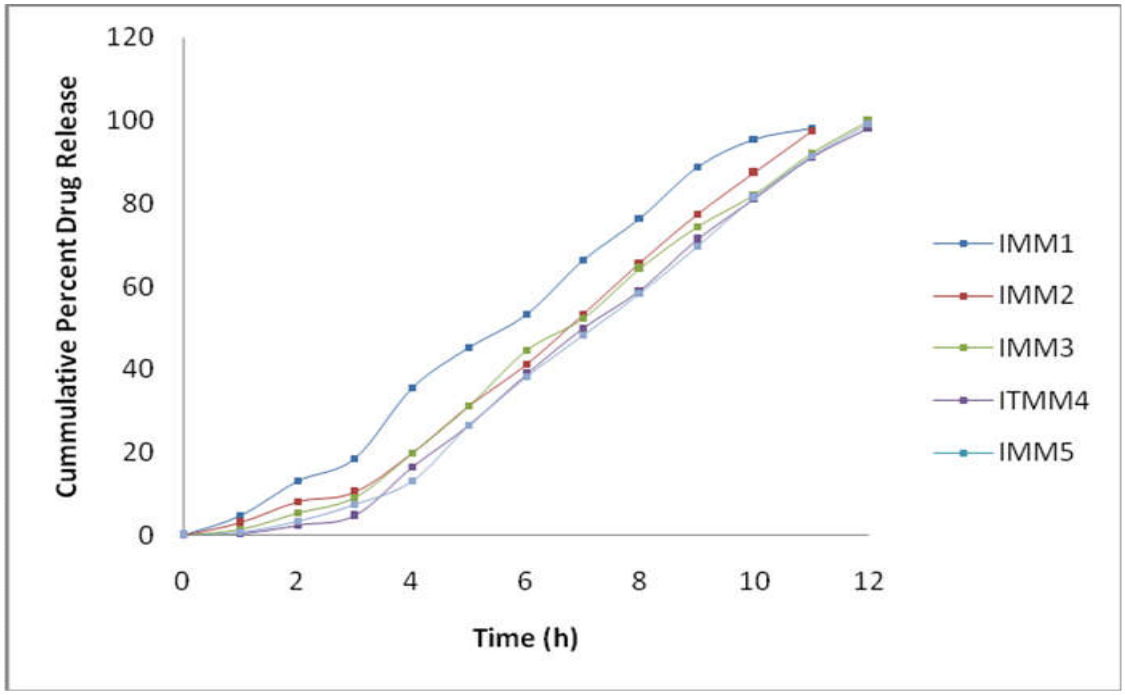

Figure 1: Zero-order kinetic dissolution data of mucoadhesive microsphere of itraconazole (IMM1 - IMM5)

Summary and Conclusion: Candidiasis is one of the most common, treatable oral mucosal infections seen in persons with human immunodeficiency virus (HIV) infection or acquired immune deficiency syndrome (AIDS). Candidiasis can be a frequent and significant source of oral discomfort, pain, loss of taste, and aversion to food. Candida albicans carriage and a history of oral candidiasis are other significant risk factors for oral candidiasis. The infection is caused by candida albicans, a dimorphic fungal organism that typically is present in the oral cavity in a non-pathogenic state in about one-half of healthy individuals. Buccal Delivery of drugs provides an attractive alternative to the oral route of drug administration, particularly in overcoming deficiencies associated with the latter mode of dosing. It is also possible to administer drugs to patients who cannot be dosed orally via this route and suitable buccal drug delivery system should be flexible and possess good bioadhesive properties, so that it can be retained in the oral cavity for the desired duration. In addition, it should release the drug in a controlled and predictable manner to elicit the required therapeutic response.

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