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FORMULATION DEVELOPMENT AND INVITRO EVALUATION OF INDINAVIR BUCCAL TABLETS

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ABSTRACT

The aim of the study was to explore the drug delivery system of indinavir for active anti retro viral therapy. A satisfactory attempt was made to develop buccal drug delivery system of Indinavir and evaluate it. From the reproducibility results obtained by the executed experiments it can be concluded that: The backing layer of ethyl cellulose was optimum for the unidirectional release of the drug. Influence of the formulation variables on hardness, drug uniformity, mucoadhesive strength, drug release is evident. Formulation F7 has successfully sustained the release of Indinavir in buccal cavity, with great mucoadhesive strength. The formulation F7 showed good pre compression and post compression parameters and follows zero order and higuchis kinetics.

Key words: Indinavir, buccal drug delivery system

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INTRODUCTION

Buccal cavity is part of mouth, anterior to cheeks and posterior to lips. The buccal glands are placed between the mucous membrane and buccinator muscle. The mucosal thickness of hard and soft palates, floor of the mouth, ventral tongue and gingivae measures at about 100–200 µm. While, the thickness of the buccal mucosa is measures to be 500–800 µm and is rough textured, hence suitable for retentive delivery systems. The average estimated turnover time for the buccal mucosa is 5-6 days. The epithelium is similar to stratified squamous epithelia found in rest of the body and is about 40–50 cell layers thick. Buccal mucosa lining layer is nonkeratinized stratified squamous epithelium and thick ness aprox 500-600 micro liter and surface area of 50.2 cm^2. The oral mucosa in general is somewhat leaky epithelia intermediate between that of the epidermis and intestinal mucosa. It is estimated that the permeability of the buccal mucosa is 4–4000 times greater than that of the skin[8]. Because of the diverse structures and functions of the oral mucosae there are considerable differences in permeability between different regions of the oral cavity. In general, the ranking is based on the relative thickness and degree of keratinization of the tissues. The sublingual mucosa being relatively thin and non-keratinized, the buccal thicker and non-keratinized, and the palatal intermediate in thickness but keratinized. The permeability of the oral mucosa decrease in the order [9] is like sublingual >buccal> palatal. The cells of the oral epithelia are surrounded by an intercellular ground substance, known as mucus which covers the

entire oral cavity. Mucus is bound to the apical cell surface and acts as a protective layer to the cells below. Mucus is a visco-elastic hydrogel, and primarily consists of 1–5 % of water insoluble glycoproteins, 95–99 % water, and several other components in small quantities, such as proteins, enzymes, electrolytes, and nucleic acids. This composition can vary based on the origin of the mucus secretion in the body. At physiological pH, the mucus network carries a negative charge due to the sialic acid and sulfate residues. Mucus can form a cohesive gel by binding epithelial surface as gel type layer. Thus mucus plays a role in mucoadhesion. The salivary pH ranges from 5.5 to 7 depending on the flow rate. At high flow rates, the sodium and bicarbonate concentrations increase leading to an increase in the pH. The daily salivary volume is between 0.5 to 2 liters. The salivary fluid plays a major role to hydrate oral mucosal dosage forms. This water rich environment of the oral cavity is the main reason behind the selection of hydrophilic polymeric matrices as vehicles for oral transmucosal drug delivery systems. These extrudes into the intercellular region of both keratinized and non keratinizedbuccal mucosa are barrier for penetration. The components of the membrane coating granules in both keratinized and non-keratinized epithelia are different. Membrane coating granules of keratinized layer consists of lipid stacks. The membrane coating granule lipids of keratinized epithelia include sphingomyelin, glucosylceramides, ceramides and other non polar lipids, however for non-keratinized epithelia, the major membrane coating granule lipid components are cholesterol esters, cholesterol, and glycosphingolipids. These extrudes into the intercellular region of both keratinized and non keratinizedbuccal mucosa are barrier for penetration. The components of the membrane coating granules in both keratinized and non-keratinized epithelia are different. Membrane coating granules of keratinized layer consists of lipid stacks. The membrane coating granule lipids of keratinized epithelia include sphingomyelin, glucosylceramides, ceramides and other non polar lipids, however for non-keratinized epithelia, the major membrane coating granule lipid components are cholesterol esters, cholesterol, and glycosphingolipids. The superficial layer of buccla mucosa represent the main barrier to the entry of substances from outside; the basement membrane also plays a role in limiting the passage of materials across the junction between epithelium and connective tissue. The charge on the constituents of the basal lamina may limit the rate of penetration of lipophilic compounds that can traverse the superficial epithelial barrier relatively easily. Barrier function of basal lamina is dependent upon the molecular weight of the permeant molecule and its reactivity with the barrier as well as the structural and functional factors of the barrier (1-5).

Indinavir is an antiretroviral drug for the treatment of HIV infection. Indinavir is a protease inhibitor with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Protease inhibitors block the part of HIV called protease. HIV-1 protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1. Indinavir binds to the protease active site and inhibits the activity of the enzyme. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Protease inhibitors are almost always used in combination with at least two other anti-HIV drugs.

The buccal route was chosen because of its good accessibility, robustness of the epithelium, facile removal of the dosage form, relatively low enzymatic activity, and natural clearance mechanism for elimination of the drug from buccal area, satisfactory patient compliance and avoidance of first pass hepatic metabolism. The route provides intimate contact between dosage form and absorbing tissue thereby resulting in high drug concentration in a local area and hence high drug flux through the absorbing tissue and drugs showing poor and unpredictable absorption from the stomach and intestine can be administered via the oral mucosa. So an attempt has made to formulate Indinavir as the buccal tablet. As the drug is directed through buccal region, drug reaches into systemic circulation directly. The aim of the present investigation is to develop and characterize sustained release buccoadhesive tablets of Indinavir.

MATERIALS AND METHODS

Pre Formulation Studies

Pre formulation studies are used for investigation of chemical and physical properties of drug and excipients. Main objective of the study is to develop information to develop stable formulation.

Compatibility Studies

To investigate any possible interactions between the drug and excipients used, the FT-IR spectra of pure drug and its physical mixture with different polymers were carried out using thermo Electron Corporation (Nicolet IR 200 FTIR) spectrophotometer. The samples were prepared as KBr (potassium bromide) disks compressed under a pressure of 150 lbs. The wave number range is selected between 500 - 3500cm^{-1}.

Preparation of Phosphate buffer pH 6.8

Weigh accurately 100 mg of Indinavirwas dissolved in 100 ml of volumetric flask using dissolution medium (phosphate buffer) which gives concentration of 1000 µg/ml. Then 10ml of stock solution was taken and diluted to 100 ml which gives a concentration of 100 µg/ml, from this stock solution subsequent dilutions were made in phosphate buffer ph 6.8 in order to get 50µg/ml, 60µg/ml, 70µg/ml, 80 µg/ml, 90 µg/ml and 100 µg/ml. Absorbance of these solutions were measured at λ max 260nm using UV-Visible spectrophotometer and standard curve was plotted.

Formulation of Mucoadhesive Tablets of Indinavir (6-10)

In this work, direct compression method has been employed to prepare buccal tablet with HPMC and Guar gum as polymers because with the dry granulation and wet granulation the hardness of tablets has increased because of which rate of drug release got decreased. For one tablet accurately weighed 300mg was used in the formulation. All the ingredients were accurately weighed and passed through mesh#60. In order to mixall ingredients thoroughly Drug, polymers, mannitol, micro crystalline cellulose, Aspartame were blended geometrically in mortar and pestle for 10minutes then magnesium stearate were mixed for 1-2 min (Table-1).

Table-1 Composition of Indinavir buccal tablets

Ingredients(%)	F1	F2	F3	F4	F5	F6	F7
Indinavir(mg)	400	400	400	400	400	400	400
Hydroxyl propyll methyl cellulose	5	5	5	--	--	--	5
Guar gum	--	--	--	5	5	5	5
Acacia	5	5	5	5	5	5	5
Sodium alginate	5	10	15	5	10	15	5
Micro crystalline cellulose	qs	qs	qs	qs	qs	qs	qs
Sodium saccharine	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Magnesium stearate	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Total weight	600	600	600	600	600	600	600

Determination of the *Ex Vivo* Residence Time

The ex vivo residence time was found using a locally modified USP disintegration apparatus. The disintegration medium was composed of 800 ml pH 6.8 phosphate buffer maintained at 37°C. The sheep buccal tissue was tied with thread to the central stand. The buccal tablet was hydrated with 0.5ml of pH 6.8 phosphate buffer and then the hydrated surface was brought in contact with the mucosal membrane. The tissue was allowed to run in such way that the tablet completely immersed in the buffer solution at the lowest point and was out at the highest point. The time taken for complete erosion or dislodgment of the tablet from the mucosal surface was noted.

***In Vitro* drug release study**

In vitro drug release study of mucoadhesive tablets were performed using standard USP dissolution apparatus type II (lab india USP XXII). The bowls of the dissolution apparatus was filled with 500ml of phosphate buffer pH 6.8 and maintained at a temperature of 37±0.5^{0}C. For each time interval 5ml sample withdrawal and replacement of fresh media at predetermined time interval. The collected samples were filtered through the 0.45µm 59millipore filter. The samples were analyzed for drug content using double beam UV spectrophotometer at 260nm.

RESULTS AND DISCUSSION

Characterization of Blend

The blends for Bucoadhesive tablets were characterized with respect to angle of repose, bulk density, tapped density, Carr's index, and drug content. Angle of repose was less than 35° and Carr's index values were less than 15 for the blend of all the batches indicating excellent to good flowability and compressibility. Hausner's ratio was less than 1.0 for all the batches indicating excellent to good flow properties. The drug content was more than 98 % for all the blend of different formulations (Table-2).

Table-2 Physical Properties of Pre-compression Blend

Formulatios	Angle of repose (°)	Bulk Density (g/mL)	Tapped Density (g/mL)	Carr's Index (%)	Hausner's ratio
F1	25.11	0.326	0.334	2.39521	1.02454
F2	24.6	0.334	0.348	4.022989	1.041916
F3	22.4	0.387	0.442	12.44344	1.142119
F4	26.3	0.331	0.338	2.071006	1.021148
F5	25.1	0.328	0.342	4.093567	1.042683
F6	29.3	0.452	0.516	12.4031	1.141593
F7	20.4	0.325	0.341	4.692082	1.049231

Swelling Index

The swelling behavior of a buccal adhesive system is an important properties uniform and prolonged release and effective mucoadhesion. The swelling index study indicated that the rate of swelling was directly proportional to carboxy methyl cellulose and Carbopol 934 content. Swelling index was calculated with respect to time. The swelling index gives an indication of the relative moisture absorption capacities of polymers and whether the formulations maintain their integrity after moisture absorption. The results of present formulation were tabulated in the table-3.

Table-3 Results of Percent swelling Index

Time (hrs)	F1	F2	F3	F4	F5	F6	F7
1	10.21	14.32	12.93	8.71	12.93	11.62	7.87
3	30.47	36.76	33.52	22.37	28.31	32.15	19.34
6	80.69	80.07	80.21	57.24	79.47	77.28	60.85
8	101.5	103.8	88.31	100.9	93.83	98.02	99.17

***In-vitro* drug release study**

The In-vitro drug release study has been done for various formulations (F1-F7). The different ratios of polymers were used. The results shown 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 F_1 F2 and F_4 have released 92%, 84, and 94% drug release in 8th hr respectively, F_3, F5 formulations have drug release of 96% and 92% drug release in 6th hr respectively where as F6 Showed a drug release of 94% of drug release in 7thhr respectively. Among all F7 was optimized based on sustained drug release and highest drug release at 97% at 8th hr (Table-4 and fig-1).

Table-4 *In-vitro* drug release study

Time(hrs)	F1	F2	F3	F4	F5	F6	F7
0	0	0	0	0	0	0	0
1	25	17	34	26	34	30	34
2	34	26	46	38	45	41	40
3	45	32	50	48	53	50	49
4	52	38	79	69	76	67	64
5	68	50	82	76	81	79	76
6	74	66	96	84	92	81	81
7	80	72	--	90	--	94	89
8	92	84	--	94	--	--	97

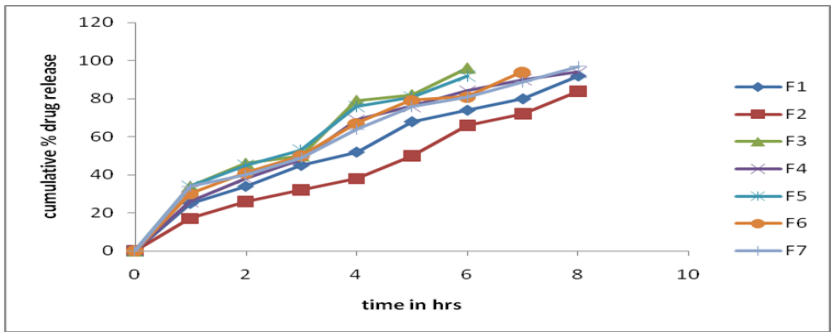

Fig-1 *In-Vitro* Drug Release for Formulations

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, Higuchi's and Korsmeyer-Peppas models to ascertain the mechanism of drug release. From the above data, it can be seen the formulation, F7 have displayed zero order release kinetics ('r^2 value of 0.9475). From Higuchi's and Peppas data, it is evident that the drug is released by non-Fickian diffusion mechanism. The values of 'r' for Higuchi's equation of factorial formulations have r^2 value of 0.984. 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.

Stability Studies

Results from stability studies indicate that the formulated Indinavir bucoadhesive tablet are stable for a period of 3 months under 2 different conditions at 25±2^{0}c, 65±5%RH and 40±2^{0}c and 75±5%RH. There were no remarkable changes were observed during the period of storage.

CONCLUSION

From the reproducibility results obtained by the executed experiments it can be concluded that the backing layer of ethyl cellulose was optimum for the unidirectional release of the drug. Influence of the formulation variables on hardness, drug uniformity, mucoadhesive strength, drug release is evident. Formulation F7 has successfully sustained the release of Indinavir in buccal cavity, with great mucoadhesive strength. The formulation F7 showed good pre compression and post compression parameters and follows zero order and higuchis kinetics. After the Stability studies the optimized formulation has showed the same amount of drug release. Based on the all experiment results it can be concluded that carboxy methyl cellulose and carbopol 934P containing buccal formulation would be the suitable candidate for mucoadhesive drug delivery of Indinavir

with sustained release properties for the treatment of ulcer. The ethyl cellulose which is used in formulation for applying unidirectional released showed desired results.

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