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Research Article

INFLUENCE OF HYDROXYPROPYL-β-CYCLODEXTRIN ON REPAGLINIDE RELEASE FROM SUSTAINED RELEASE BIOADHESIVE BUCCAL TABLETS

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ABSTRACT

Objective: The purpose of this investigation was to study the influence of cyclodextrin complexation on development of sustained release bio-adhesive repaglinide tablets for buccal delivery. **Methods:** Based on preliminary phase solubility studies, solid complexes prepared by freeze-drying method in 1:1 molar ratio were selected and characterized by Fourier transform infrared spectroscopy to corroborate the fact of complex formation. The sustained release repaglinide tablets were produced by direct compression and this drug or complexed –loaded hydrophilic matrices using HPMC, Sodium CMC and Carbopol as muco-adhesive polymers were assessed for *in vitro* bioadhesion strength, *in vitro* release modulation, surface pH, % moisture absorption and *ex vivo* permeation through porcine buccal membrane. **Results:** When the drug was incorporated as repaglinide-Hydroxypropyl-β-Cyclodextrin **(**HP-β-CD) freeze-dried product, total amount of drug permeated from the tablet through epithelium in about 12 hrs, displayed a constant release regimen after a transient period. The effect of HP-β-CD incorporation on the release mechanism was rationalized on the basis of the interplay of different physical phenomena: erosion and swelling of the tablet, drug dissolution, and complex formation. Formulation F10 showed % moisture absorption of 23.46 for 4hrs, surface pH 6.9±0.015, Peak detachment force 3.65±0.18 N, Work of adhesion 1.12±0.10 mJ, and *in vitro* drug release 98.31% in 6h. The feasibility of buccal administration of repaglinide was assessed by permeation experiments on excised mucosa of pig. The *ex vivo* permeation studies demonstrated that the matrix tablets containing repaglinide–HP-β-CD (F10) solid complex exhibited significantly higher drug permeation (92.18 % for 12hrs) compared to all of the other formulations tested, which could be attributed to both, the presence of the polymers, and the drug-cyclodextrin complexation. The flux was found to be increased by 1.12-1.37 folds with a permeability coefficient of 0.017-0.018. **Conclusion:** The results demonstrate that the formulations with inclusion complexes afford high utility as a trans-mucosal drug delivery system for improved drug release and permeability.

Keywords: Repaglinide, Freeze drying, HP-β-CD complexation, Bioadhesion, Ex-vivo permeation, Solubility

INTRODUCTION

Buccal mucosa is a potential site for the delivery of drugs to the systemic circulation. A drug administered through the buccal mucosa enters directly into the systemic circulation, thereby minimizing the first-pass hepatic metabolism and adverse gastro-intestinal effect [1]. However, permeability of drugs through oral mucosa is too low to allow plasma concentration to reach therapeutic levels. Buccal permeation can be improved by using various classes of transmucosal penetration enhancers such as bile salts, surfactants, fatty acids and derivatives, chelating agents and cyclodextrins[2].

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 flux due to a low chemical potential gradient, which is the driving force for transport. In this regard, cyclodextrins have emerged as an effective tool to increase drug release rate of sparingly soluble drugs once incorporated in sustained-release matrix-type systems made of different polymers [3]. These molecules are cyclic oligosaccharides with a hydrophilic outer surface and a hydrophobic central cavity. The hydrophilic exterior of the cyclodextrin molecules makes them water-soluble while the hydrophobic cavity provides a microenvironment for appropriate sized non-polar molecules [4]. CD can promote changes in erosion rate and hydrophilicity of the matrix [5], induce osmotic effects (observed in the case of polyionic CD) and pore formation [6], as well as modify drug effective mobility in the hydrated polymer [7].

The biopharmaceutical classification system (BCS) is used to group pharmaceutical actives depending upon the solubility and lipophilicity (permeability) characteristics of the drug. BCS class II compounds are poorly soluble but highly permeable, and they exhibit bioavailability that is limited by dissolution rate. The dissolution rate of BCS class II drug substances may be accelerated by improvement of the wetting characteristics of the bulk powder. Repaglinide (BCS class II compound), a carbamoylmethyl benzoic acid derivative, is a new anti diabetic agent from the class of glinides, used for the treatment of patients with type 2 diabetes mellitus by stimulating insulin secretion from pancreatic beta-cells. The substance is characterized by very low water solubility and a high lipophilicity (logP = 3.97) [8]. Repaglinide, S (+)2-ethoxy-4(2((3-methyl-1-(2-(1-piperidinyl) phenyl)- butyl) amino)-2-oxoethyl) benzoic acid, is chemically unrelated to the oral sulfonylurea insulin secretagogues (Figure 1). It is differentiated from other hypoglycemic agents such as sulphonyl ureas by its rapid and short duration of action. Following oral administration of repaglinide at clinically relevant doses, peak serum levels are reached within 30-60 min. Repaglinide is almost completely (98%) bound to plasma proteins, has a low tissue distribution and undergoes a very rapid elimination from the body, with a plasma half-life of less than 1hr. The mean absolute bioavailability is 56%. Repaglinide is completely metabolized by oxidative biotransformation and direct conjugation with glucouronic acid after either an IV or oral dose. Metabolites do not contribute to the glucose-lowering effect of repaglinide. Maximum daily dose of Repaglinide is 16mg and needs to be administered 4 times a day to maintain plasma drug levels. Patient compliance is compromised due to frequent administration of the drug. Hence it was selected as a model for buccal drug delivery and as being a poorly soluble drug repaglinide was complexed with β-cyclodextrins.

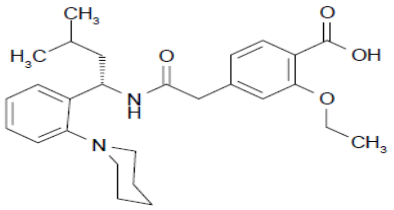

Figure 1: Chemical structure of repaglinide

The purpose of this study is to evaluate the possibility of increasing the solubility of repaglinide through complexation with cyclodextrin. Lyophilization was used as suitable method for obtaining inclusion complexes between repaglinide and cyclodextrin. In an attempt to develop a buccal tablet for the delivery of a poorly water-soluble repaglinide, we designed a matrix system based on a blend of HPMC K4M, HPMC K15M, Sodium CMC and Carbopol 947P as bioadhesive sustained release platform and hydroxypropyl-β-cyclodextrin (HP-β-CD) as modulator of drug release. In the first part of the study, we rationalized the effect of HP-β-CD on the release features of the system whereas in the second part we assessed the physicochemical parameters of repaglinide tablets as buccal delivery system.

MATERIALS AND METHODS

Materials

Repaglinide was gift sample from Dr.Reddy's laboratories, Hyderabad. Mannitol SD 200 was gift sample from Signet chemical corporation, Mumbai, India. Hydroxypropyl methyl cellulose (HPMC K4M, HPMC K15M), Sodium carboxy methyl cellulose and Carbopol 947P were gift samples from Dr.Reddy's laboratories, Hyderabad, India. All chemicals and reagents used were of analytical or pharmacopoeial grade.

Calibration curve of Repaglinide

Calibration curve for the estimation of the drug was constructed employing distilled water as medium (pH 7). The stock solution of the repaglinide (100µg/mL) prepared in methanol, was subsequently diluted with distilled water to obtain a series of dilutions containing 1, 2, 4, 6, 8, 10, 20 and 30 µg/mL of the drug. The absorbances of the above dilutions were measured using UV-Visible spectrophotometer at λmax of 226nm. These Calibration curves were used for estimation of repaglinide in the present study.

Phase solubility studies

Phase solubility studies of pure drug repaglinide with different concentrations of Hydroxy propyl β-cyclodextrin (0.1-5 millimoles) were performed by the method described by Higuchi and Connors [9]. Briefly, excess amount of the drug was added to 10mL of double distilled water containing various concentrations of Hydroxy propyl β-cyclodextrin (0.1-5 mM) taken in a stoppered glass vials. The suspensions were vigorously shaken at 25 ± 1°C for 3 days on a rotary shaker. After equilibrium was attained, the samples were filtered through a 0.45 µm Millipore membrane filter and suitably diluted. These samples were assayed for the drug content by UV-Visible spectrophotometer against blank in same concentration of HP-β-CD in water so as to cancel any absorbance that may be exhibited by the cyclodextrin molecules. The solubility experiments are conducted in triplicate.

The apparent 1:1 stability constant, *K*s, was calculated from the phase solubility diagrams using the equation:

$$Ks = \text{slope}/S_0\,(1 - \text{slope})$$

where S_0 is drug solubility in the absence of HP-β-CD (intercept).

Preparation of Freeze–dried solid inclusion complexes

Solid inclusion complexes were prepared by freeze-drying method. Systems were prepared in a stoichiometry 1:1 (drug:cyclodextrin) according to previous phase solubility studies [10]. 1mM cyclodextrin derivative was initially dissolved in distilled water and a 50% ethanolic solution containing repaglinide was then added stepwise to the aqueous solution of cyclodextrin derivative. It was stirred at 600 rpm for 6 hrs until a stable suspension was formed on magnetic stirrer to obtain complexation equilibrium. All the clear solutions were frozen at 2-8°C and the frozen solutions were lyophilized in a freeze-dryer (LYODEL) to obtain a solid complex for 72 hrs. These inclusion complexes were subjected to FT-IR studies to corroborate the extent of complex formation. The drug content of freeze dried product was assayed to calculate the equivalent amount of repaglinide to be taken.

FTIR spectroscopic studies for drug-excipient compatibility

A Fourier Transform- Infra Red spectrophotometer (Spectrum BX series, 51658, Perkin Elmer BX, UK) equipped with spectrum v2.19 software by KBr pellet method was used to characterize the drug-excipient (binary mixture of drug: excipient 1:1 ratio) compatibility and extent of complexation. The instrument was operated under dry air purge. A base line correction was made using dried potassium bromide and then the spectra of the dried mixture of drug, formulation mixture and potassium bromide were recorded on FTIR. The spectrum for each sample was recorded at scanning speed of 2 mm/sec over the 4000-400 cm^{-1} spectral region with a resolution of 4 cm^{-1}[10].

Sustained release bioadhesive buccal tablets preparation

The tablets were prepared by direct compression of the repaglinide (4mg) or repaglinide-cyclodextrin complex (5.25mg binary systems containing 4mg of repaglinide) with mucoadhesive polymers like HPMC, Sodium CMC and Carbopol 947P in various ratios. Mannitol SD 200, magnesium stearate and talc were used as filler and lubricants respectively. Each component previously screened through #100 mesh, were mixed and compressed on a 16 station rotary tablet compression machine (Riddhi, Ahmedabad, India) using 8 mm circular flat beveled punches. For comparison purpose tablets containing uncomplexed drug with similar composition were also prepared. The detailed compositions of repaglinide tablet formulations are given in Table 1. Each tablet (250 mg) contained 4 mg of repaglinide, free or in complex form.

Weight and thickness variation

The weights of repaglinide buccal tablets were measured using digital balance (Denver, Germany). The average values and standard deviation were calculated. The thickness was measured using a digital screw gauge (Mitutoyo, Japan). Ten individual tablets from each batch were used and the average values were taken.

Hardness Test

Tablets require a certain amount of strength or hardness and resistance to withstand mechanical shocks. The hardness of tablet was measured by Pfizer hardness tester (Cadmach, India) and results were expressed in Kg/cm^2.

Assay of the tablets

Ten tablets were taken and powdered; powder equivalent to one tablet was taken and was allowed to dissolve in the medium by sonication for 30min. The solution was centrifuged and the supernatant was filtered through 0.22µ membrane filter. The absorbance of filtrate was measured using a UV-Visible spectrophotometer (Elico, India) at 226 nm against blank by suitable dilutions and drug content was analyzed.

In vitro dissolution studies

Dissolution from the repaglinide-CD complex and plain repaglinide buccal tablets were evaluated in distilled water using USP XXIII dissolution apparatus type-II (8 stations, Tab machines, India) at 37 ± 0.5°C stirring at 50 rpm. Dissolution studies of buccal tablets were performed using 500 mL of dissolution medium, by sticking the impermeable layer side onto the glass slide with the help of cyanoacrylate adhesive. 5mL samples were withdrawn at predetermined time intervals and analyzed spectrophotometrically at 226 nm against placebo tablet as blank. Results are reported as % released repaglinide fraction (ratio of released repaglinide to the total amount of repaglinide added to the medium) ±S.D. of three

replicates. The concentration of repaglinide in the surrounding medium was always below the saturation concentration by maintaining sink conditions.

Data analysis

In order to understand the mechanism and kinetics of drug release, the results of the *in vitro* drug release study were fitted to various kinetics equations like zero order (Time Vs Cumulative % release), first order (Time Vs log Cumulative % drug remaining), higuchi rate (Square root of time Vs cumulative % release), korsemeyer-peppas and release exponent (n) (log time Vs log cumulative % release). "n" value analyzed from slope of linear regression equation of Peppas represents the drug transport mechanism. n<0.5 indicates Fickian diffusion, 0.5<n<1 indicates non-fickian, n=1 indicates Case II transport and n> 1 indicates Super case II transport [11].

Bioadhesion studies

The bioadhesive parameters were determined at room temperature using a microprocessor based advanced force gauze with a motorized test stand, fitted with 5 kg load cell (Ultra Test, Mecmesin, West Sussex, UK) and equipped with a computer integrated data acquisition system. To the upper and the bottom support of the tensile apparatus two 15mm diameter aluminium discs were secured. The tablet was fixed to the lower support while the porcine buccal mucosa was fixed to the upper support by using a cyanoacrylate adhesive. The tablet was wetted with 0.1 mL of 1% mucin by using a micropipette. Then, the upper support was moved down at 30 mm/min and stopped when the force between the tablet and the mucosa was 2 N. After 10 min of contact, the crosshead moved upward at a speed of 0.5 mm/min. The peak detachment force (*F*) and the work of adhesion (*W*, area under the force/distance curve) were recorded. The temperature during the experiment was 23˚C and relative humidity 46–50%. Results are the mean of three force–elongation experiments.

Surface pH

The surface pH of the buccal tablets was determined in order to investigate the possibility of any side effects *in vivo*. As an acidic or alkaline pH may cause irritation to the buccal mucosa, care was taken to keep the surface pH as close to neutral as possible. The method adopted by Battenberg et al., [12] was used to determine the surface pH of the tablet. A combined glass electrode was used for this purpose. The tablet was allowed to swell by keeping it in contact with 5 mL of distilled water (pH 6.5 ± 0.05) for 2hrs at room temperature. The pH was measured by bringing the electrode in contact with the surface of the tablet and allowing it to equilibrate for 1 min.

Moisture absorption studies

Moisture absorption study for prepared batches was carried by using weight gain method on an agar plate. Initially, 1% agar solution was prepared on a hot plate by continuous stirring. This was then poured into a petri plate and allowed to solidify by cooling. Then buccal tablets were weighed individually (W1) and placed separately in Petri dish. At regular 1- hour time intervals until 4 hrs, the tablet was removed from the Petri dish and excess surface water was removed carefully using filter paper. The swollen tablet was then reweighed (W2) and the % moisture absorbed were calculated according to the equation:

$$\% \text{ Moisture uptake} = \frac{\text{Final weight (W2)} - \text{Initial weight (W1)}}{\text{Initial weight (W1)}} \times 100$$

Porcine Buccal Tissue Preparation

Porcine buccal mucosa was used as it resembles much better to the human buccal mucosa regarding permeability, barrier lipid composition, histology and ultra-structural organization [13]. Buccal mucosa from pigs weighing 70–100 kg was obtained freshly from a local slaughter house and it was used at least within 3 hrs after animal slaughtering. The mucosal membrane was excised by removing connective and adipose tissue and was equilibrated at 37 ± 0.1˚C for 30 min in distilled water.

***Ex vivo* permeation study**

The porcine buccal mucosa was carefully mounted in between the two compartments of Modified Franz Diffusion cell [14], with a diffusion area of 3.58 cm^2 and a compartment volume of 25 mL. The donor and the acceptor chambers were filled with distilled water at pH 7.0 ± 0.5 and allowed to equilibrate for 30 min. The donor chamber was emptied after the equilibration period and replaced with 3 mL of a solution containing 4mg of repaglinide alone, buccal tablets containing plain drug and buccal tablets containing complexed drug. The tablets were stuck to the mucosa in the donor side. Receiver medium was maintained at 37±0.1˚C under stirring of 400 rpm on a magnetic stirrer. From the receiver compartment, 2 mL aliquot was collected at predetermined time intervals and replaced by an equal volume of buffer solution. Analysis of samples was performed using a UV-Visible spectrophotometer at 226nm to determine the amount of drug permeated through the membrane. The repaglinide flux and permeability coefficient through the membrane was calculated using the equations:

$$J = dQ/A.dt$$

$$P = J/D$$

Where *J* is the steady-state flux, *A* is the diffusion area, P is the permeability coefficient and D is the dose of drug in µg.

RESULTS AND DISCUSSIONS

The complexation of repaglinide with HP-β-CD, the effect of HP-β-CD on the solubility and the type of phase solubility diagram were investigated by phase solubility studies. All the phase solubility diagrams can be classified as type AL according to Higuchi and Connors. Because the straight line had a slope less than unity in each case, the increase in solubility was due to the formation of a 1:1 molar complex in solution with drug. Thus the solubility of repaglinide was markedly enhanced by complexation with 1mM ratio of HP-β-CD and the results were indicated in Figure 2.

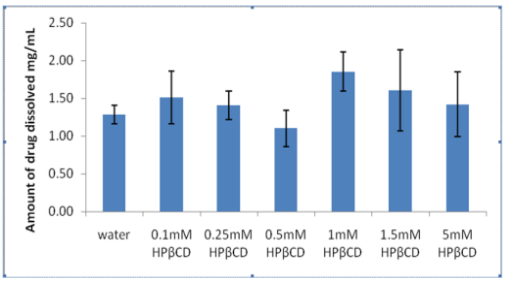

Figure 2: **Phase solubility studies of repaglinide in different milli molar ratios of HP-β-CD in water**

Freeze drying of solubilized repaglinide in cyclodextrin solution yielded a solid amorphous product. The assayed concentration of the drug in the solid product was found to be 98.9%.

Further evidence of the complex formation was obtained by FT-IR spectroscopy. The infrared (FT-IR) spectra were obtained using a perkinelmer FT-IR spectrometer at resolution 4 cm^{-1} from 4000 to 400 cm^{-1} (presented in Figure 3). The typical IR spectrum of pure repaglinide reveals the presence of a peak at 3309.22 cm^{-1}, assigned to N-H stretching vibration and one at 1686.50cm^{-1}, corresponding to the carbonyl group. Upon complexation, the repaglinide characteristic absorption peaks were not identified any more in the IR spectra or slightly shifted towards a lower wavenumber due to host-guest interactions, suggesting the formation of hydrogen bonds between the functional groups of repaglinide and the hydroxyl group of the host cavity. We consider the results obtained by IR Spectroscopy to serve as proof for the formation of inclusion complexes between HP-β-CD and repaglinide. Characteristic bands of repaglinide were well retained in the IR spectrum of drug-polymer mixture without any new bands, indicating that there was no change in the structure of drug. On the basis of above report, it was concluded that repaglinide is compatible with polymers used.

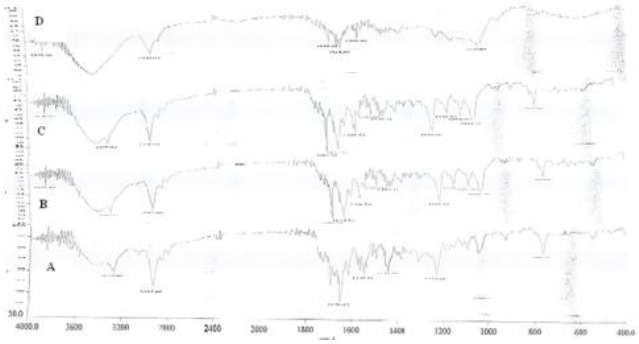

Figure 3: FTIR spectrum of (A) Repaglinide pure drug (B) Repaglinide with HPMC (C) Repaglinide with Sodium CMC (D) Repaglinide- HP-β-CD complexed freeze dried product

In the present study, buccoadhesive tablets of repaglinide free or in the complex form were prepared with the objective of improving patient compliance by reducing frequency of administration. A total number of fourteen formulations were prepared by four different bioadhesive polymers, HPMC K4M, HPMC K15M, Sodium CMC and Carbopol 947P containing the same amount of the drug alone or in the complexed form (Table 1).

Table 1: Composition of various formulations of repaglinide

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
Repaglinide	4	-	-	4	-	-	-	4	-	-	4	-	-	-
1mM FD Drug	-	5.25	5.25	-	5.25	5.25	5.25	-	5.25	5.25	-	5.25	5.25	5.25
Mannitol	216	206.75	214.75	220	222.75	218.75	210.75	208	198.75	206.75	220	226.75	218.75	210.75
HPMC K4M	24	32	24	-	-	-	-	-	-	-	-	-	-	-
HPMC K15 M	-	-	-	20	16	20	28	-	-	-	-	-	-	-
Sod CMC	-	-	-	-	-	-	-	32	40	32	-	-	-	-
Carbopol 947P	-	-	-	-	-	-	-	-	-	-	20	12	20	28
Magnesium stearate	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Talc	3	3	3	3	3	3	3	3	3	3	3	3	3	3

***Freeze Dried Drug equivalent to 4mg Drug; All Amounts Represented in mg**

The results of weight variation, thickness, hardness and drug content of formulations were given in Table 2. The weights and thicknesses of tablets prepared by direct compression technique were within the limits as per IP specifications. A good content uniformity was observed among all the formulations.

Table 2: Process parameters: Weight variation, Thickness, Hardness and drug content

Formulation code	Thickness (mm)	Weight Variation(mg)	Hardness (Kg/cm²)	%Drug content
F1	4.64±0.024	249±0.20	4.3±0.15	99.74
F2	4.64±0.110	247±0.24	4.9±0.04	101.17
F3	4.69±0.020	248.6±0.15	5.4±0.08	99.69
F4	4.51±0.024	258.1±0.50	6.1±0.05	98.04
F5	4.61±0.032	249.2±0.30	5.2±0.24	99.58
F6	4.35±0.030	250.0±0.35	5.0 ±0.05	100.39
F7	4.67±0.090	252.8±0.25	6.5±0.08	99.74
F8	4.40±0.015	251.8±0.55	6.4±0.12	100.17
F9	4.62±0.050	243.3±0.50	7.7±0.10	98.69
F10	4.50±0.035	251±0.60	3.8±0.08	98.04
F11	4.40±0.015	250.1±0.55	5.5±0.21	100.94
F12	4.46±0.020	256.4±0.75	4.5±0.13	98.75
F13	4.73±0.035	245.5±0.15	5.0±0.33	99.66
F14	4.64±0.010	251.4±0.20	5.5±0.13	99.62

***All the values are expressed as mean, n=10**

In vitro dissolution profiles of various formulations prepared are shown in Figure 4a & 4b. Differences in the drug release rate from the tablets can be attributed to the presence of different polymers (HPMC K4M, HPMC K15M, Sodium CMC and Carbopol 947 P) and cyclodextrin. Dissolution of pure drug based tablets was slower compared to the tablets containing the cyclodextrin complex. The drug complex dissolves easily in a hydrated polymeric environment, resulting in a higher diffusional driving force and faster drug release. Due to poor aqueous solubility, only a limited amount of drug can dissolve inside the hydrated polymeric matrices. Incorporation of HP-β-CD in the matrix improved the drug solubility and dissolution rate. The dissolved HP-β-CD in the gel matrix formed a complex with drug, and improved its solubility. The solubilization due to the in situ complex formation was the main reason for enhanced drug release from HP-β-CD containing polymeric matrices.

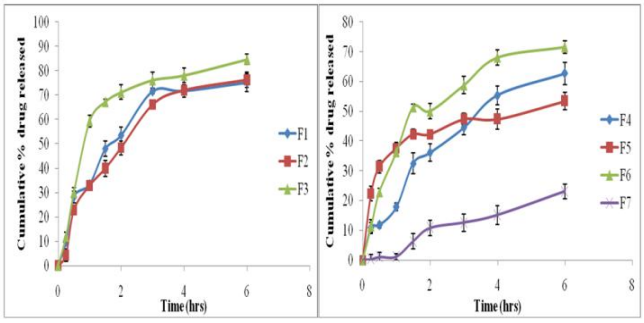

Figure 4a: Comparative *in vitro* drug release profiles (F1-F7) of repaglinide buccal tablets

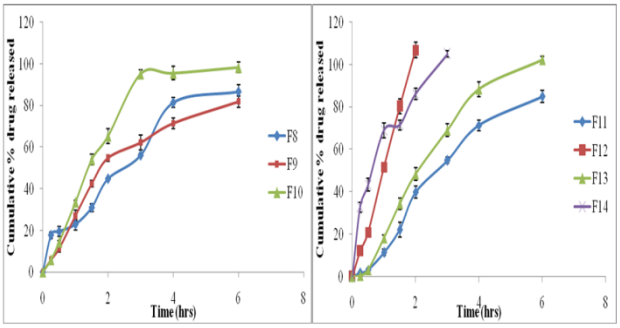

Figure 4b: **Comparative *in vitro* drug release profiles (F8-F14) of repaglinide buccal tablets**

From the *in vitro* dissolution results, formation of inclusion complexes by freeze-drying technique showed a dramatic improvement in drug dissolution efficiency (84.68%, 71.58%, 98.31% and 102.36% in 6hrs) against the pure drug (75.05%, 62.66%, 86.62% and 84.95% in 6hrs) with various hydrophilic polymers like HPMC K4M (1:6), HPMC K15 M (1:5), sodium CMC (1:8) and carbopol 947P (1:5) respectively. The differences observed in drug release as a function of the type of polymer and cyclodextrin complexation were related to the differences in water absorption and erosion behavior of the tablets in the release medium. On the basis of these results obtained in the preliminary screening studies, the batches containing drug: HPMC K4M in the ratio of 1:6, drug: HPMC K15M in the ratio of 1:5 and drug: sodium CMC in the ratio of 1:8 showed the better drug release profile. Hence, they were selected for further studies.

In the formulations optimized (F3, F6, F10 and F13), a Higuchi's square root of time equation showed a significantly better fit than other equations (Table 3). The dissolution data were analyzed for release mechanism using the equation proposed by Korsmeyer. In the optimized tablets containing only HPMC, the values of '*n*' were 0.18-0.35 indicating that the transport of drug from the tablets was governed by Fickian mechanism whereas for the optimized formulation containing sodium CMC, the value of '*n*' was 0.60. Finally the formulation containing carbopol 947P has '*n*' value of 0.96, which is indicative of anomalous or non-Fickian release, i.e. the drug release proceeded by diffusion as well as erosion of the polymer and hence excluded from the study.

Table 3: Release kinetics and correlation coefficients of repaglinide buccal tablets

Formulation Code	Zero order R^2	First order R^2	Higuchi R^2	Korsemeyer-Peppas R^2	n –value
F1	0.7856	0.8778	0.9428	0.8905	0.4532
F2	0.8464	0.9386	0.9602	0.9544	0.5094
F3	0.649	0.8294	**0.9709**	0.8669	**0.187**
F4	0.9169	0.9704	0.976	0.9294	0.6579
F5	0.6014	0.7049	0.8523	0.9576	0.1812
F6	0.7844	0.8958	**0.9503**	0.9167	**0.3586**
F7	0.9641	0.9708	0.8945	0.8048	1.4829
F8	0.9363	0.9526	0.9457	0.9674	0.7882
F9	0.878	0.9802	0.9606	0.9263	0.5872
F10	0.8247	0.925	**0.9395**	0.8747	**0.6078**
F11	0.9591	0.9855	0.9174	0.9383	1.1249
F12	0.9974	0.9374	0.8917	0.9994	1.0523
F13	0.9508	0.943	**0.9529**	0.9322	**0.9608**
F14	0.8806	0.9657	0.9905	0.9177	0.398

An overall evaluation of the mucoadhesive behaviour of optimized formulations on pig buccal mucosa was performed by determining the force of detachment (N) versus time. Swelling of the polymer contributed to the interpenetration of mucus and made bioadhesion possible [15]. Adhesion tests (detachment force and the work of adhesion) for the formulations optimized are shown in Table 4. The bioadhesive forces of buccal tablets were affected by the nature of the polymer. 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. The bioadhesive behavior of the tablets has been characterized by means of the work of adhesion, *W*, i.e. the area under the force–elongation curve. HPMC alone had poor adhesive properties. It was evident that the peak detachment force and the work of adhesion values increased in the presence of Sodium CMC and Carbopol 947P in the tablets due to increased physical entanglement, producing a broader force/distance curve, and thus increased the work of adhesion values. Considering the overall properties of complexed drug containing tablets, it can be concluded that the formulations containing HPMC K4M, HPMC K15M, Sodium CMC, Carbopol 947P display good bioadhesive properties, although containing considerable amounts of HP-β-CD, and are suitable for transmucosal applications.

Table 4: Bioadhesive strength parameters and surface pH values of optimized formulations

Formulation code	Peak detachment force(N)	Work of adhesion(mJ)	Surface pH
F3	2.30±0.06	0.540±0.13	6.8±0.010
F6	3.08±0.03	0.743±0.11	6.8±0.515
F10	3.65±0.18	1.12±0.10	6.9±0.015
F13	4.62±0.17	1.33±0.14	6.7±0.515

***All the values are expressed as mean, n=3**

The surface pH of the buccal tablets was determined in order to investigate the possibility of any side effects *in vivo*. As an acidic or alkaline pH may cause irritation to the buccal mucosa, it was determined to keep the surface pH as close to neutral as possible. Surface pH of the optimized formulations was found to be 6.7±0.515 to 6.9±0.015 as presented in Table 4. The pH was found to be near to the neutral. From the results it was found that, the formulation does not cause any irritation to the buccal mucosa.

Appropriate swelling property of a buccal device is essential for uniform and prolonged release of drug and proper mucoadhesion [16]. All the formulations showed an increase in weight due to water uptake. The % moisture absorption of the prepared buccoadhesive tablets showed moisture absorption rates in the order: F13 > F10 > F3 > F6 (Table 5). The high uptake of water in the case of the F13 may be due to the faster hydration rate of polymers. This could be attributed to the Carbopol property to retain water and form a thick swollen mass. HP-β-CD also promoted hydration of the polymeric system acting as a channeling or wicking agent. The tablet formulated with sodium CMC (F10) rapidly absorbed water and showed a higher degree of swelling compared to the tablet formulated with HPMC (F3, F6). Ionization of carboxylic groups of polymer with subsequent repulsion and relaxation of the polymer chains resulted in an increase of water uptake and hence of the % moisture absorption with time. The presence of phosphate ions retarded the HPMC hydration [17]. Formulations with carbopol and sodium CMC showed higher % moisture absorption values, this is

because the water swellable polymers can absorb more water and swell to higher extent than that of water insoluble polymers.

Table 5: Percentage of moisture absorption of optimized repaglinide buccal tablets

Time (hrs)	% Moisture absorbed			
	F3	F6	F10	F13
0.25	2.29±0.15	2.27±0.07	1.15±0.24	18.3±0.96
0.5	2.68±0.34	3.4±0.05	3.07±0.10	25.67±0.61
1	4.98±0.09	4.92±0.13	6.5±0.06	55.17±0.21
2	10.72±0.11	8.56±0.51	13.4±0.34	69.34±0.54
3	17.24±0.26	14.39±0.19	20±0.21	
4	21.83±0.42	16.66±0.28	23.46±0.18	

***All the values are expressed as mean, n=3**

Physicochemical, swelling and erosion, bioadhesion properties for these formulations were studied and the results revealed that the optimized formulations (F3, F6 & F10) demonstrated good bioadhesion with acceptable moisture absorption ratio. Hence, they were selected for the *ex vivo* permeation studies through porcine buccal mucosa.

Ex vivo permeability studies are a useful tool to assess the potential of a localized anatomical site as a route for drug delivery. However, *ex vivo* conditions should simulate the *in vivo* situation as closest possible. The permeation test was aimed at investigating the drug passage through the semipermeable membrane to which the tablets adhered. *Ex vivo* permeation study of Repaglinide drug solution containing 4mg of repaglinide alone, buccal tablets containing plain drug and buccal tablets containing complexed drug through the porcine buccal mucosa was performed using Modified Franz diffusion cell and membrane assembly, at 37°C ± 0.5°C and 400 rpm by using magnetic stirrer. The samples were collected at predetermined time intervals (0.5, 1.0, 1.5, 2.0, 3.0, 4, 6, 8, 10 and 12hrs) and the analysis was carried out to determine the drug permeated.

In the investigation of integrity of buccal mucosa, Phenol red was used as marker compound and is not expected to permeate through the porcine membrane. Absence of phenol red in the receiver compartment indicated the intactness of the buccal membrane. This data suggests that the tissue could be isolated successfully and integrity of buccal mucosa was maintained during the permeation studies. The principle here is that phenol red being a very large molecule, cannot permeate the buccal mucosa and enter the acceptor chamber unless the mucosa is damaged.

Table 6: Ex vivo parameters of repaglinide buccal tablets through porcine buccal mucosa

Parameters	Drug solution	Cumulative amount of percentage drug permeated					
		With HPMC K4M		With HPMC K15M		With Sodium CMC	
		F1	F3	F4	F6	F8	F10
Flux (μg.hr^{-1} cm^{-2})	90	64.12	72.05	51.54	70.92	57.11	75.02
Permeability coefficient (P)	0.022	0.016	0.018	0.012	0.017	0.014	0.018

***All the values are expressed as mean, n=3**

The profiles of drug permeation from the buccal tablets are shown in Table 6 & Figure 5. Flux values were determined from the steady state region of the diffusion profiles obtained using the linear regression analysis. The differences in the plot of drug permeated and flux values of repaglinide from the tablets could be attributed to the polymers used, and the presence of HP-β-CD. The cumulative % of drug permeated from formulation F3 containing complexed repaglinide with HPMC K4M over 12 hrs through epithelium was 90.13% and was greater than the % of drug permeated from formulation F1 containing plain drug alone. Formulation F6 containing complexed repaglinide with HPMC K15M showed an increase in cumulative % drug permeated from 55.50% to 85.28% when compared to the formulation F4 containing plain drug. At the end of 12hrs, the cumulative % drug permeated was 81.13% and 92.18% from plain tablets (F8) and tablets containing complexed repaglinide with Sodium CMC (F10) respectively. This effect, in principle, can be attributed to an increase of driving force for permeation due to the increase of drug apparent solubility in the presence of HP-β-CD as well as to an enhancing effect of HP-β-CD. Cyclodextrins have 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 HP-β-CD to the matrix increased the flux by increasing the solubility of drug, thus improving the diffusible form of the drug species at the tablet membrane interface. The flux was found to be increased by 1.12-1.37 folds with a permeability coefficient of 0.017-0.018. Higher penetration rate of medium into the complex containing tablets may also contribute to the improved release rate.

The drug was released gradually from the formulations over a period of 12hrs. The formulation comprising repaglinide-HP-β-CD complex and sodium CMC (RT10) demonstrated a complete and sustained release associated with an enhanced buccal permeation characteristics.

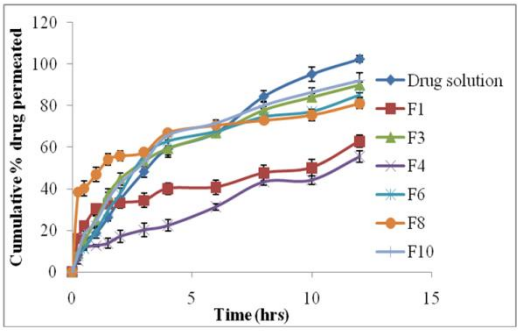

Figure 5: Comparative *ex vivo* drug release profiles of repaglinide buccal tablets through porcine buccal mucosa

CONCLUSIONS

In conclusion, the influence of HP-β-CD on the drug release from buccal tablets was investigated in order to overcome the formulation problems connected with the design of sustained release systems containing poorly soluble drugs. Results of characterization of freeze dried system revealed that the formation of inclusion complex between repaglinide and HP-β-CD has improved its solubility and dissolution rate. Buccal tablets formulated with swellable hydrophilic polymer Sodium CMC containing complexed repaglinide showed better drug dissolution and permeability across buccal mucosa as compared to those containing plain drug.

Repaglinide was released from the polymer matrices in a constant mode over the passage of time, thus providing a prolonged effect. Thus it can be concluded that the incorporation of cyclodextrins in a sodium CMC-based hydrophilic matrix intended for the delivery of poorly soluble drug repaglinide can be a suitable strategy to optimize the release features of the system while maintaining good bioadhesive properties. Along with avoidance of first pass metabolism, incorporation of complex into buccal tablet may lead to increased bioavailability of repaglinide.

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