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Engineered Mucoadhesive Buccal Films of Repaglinide – Optimization and Quality Evaluation

Shraddha Anand Halarnkar*[1], Ramchandra Vishnu Keny[1]

1.Pharmaceutics Department, Goa College of Pharmacy, 18th June Road, Panaji-403001, Goa - India.

ABSTRACT

Buccal mucosa is a potential site for the delivery of drugs to the systemic circulation in a sustained manner; avoiding the first-pass metabolism. The goal of present investigation was to design and evaluate mucoadhesive buccal films of Repaglinide in order to avoid first-pass effect and release the drug for extended period of time. The Buccal films were formulated using polymers, Carbopol-971P and HPMC K4M, by solvent casting method; using PEG 400 as plasticizer. In this study, an attempt has been made to prepare Repaglinide inclusion complex; to enhance its solubility and was incorporated into the mucoadhesive buccal films in order to improve the bioavailability. A comparative study has been done between; uncomplexed Repaglinide loaded buccal films and inclusion complex loaded buccal films. The films prepared were evaluated for various physicochemical parameters and *in vitro* drug release. It was observed that inclusion complex loaded buccoadhesive films showed higher cumulative percentage of drug release at the end of 8 hours. The release data obtained was analyzed using various mathematical models. The Repaglinide loaded films followed zero order kinetics with principle mechanism of drug release being fickian diffusion. Whereas the inclusion complex loaded films followed zero order kinetics and exhibited non-fickian diffusion mechanism. HCC1 formulation having acceptable physicochemical parameters and with highest cumulative percentage of drug release (95.18%) at the end of 8 hours was chosen as optimum formulation and its short term stability studies revealed adequate stability of the formulation.

Keywords: Buccal patches, Repaglinide, Carbopol-971P, HPMC K4M, inclusion complex.

*Corresponding Author Email: Shraddha_halarnakar@rediffmail.com

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INTRODUCTION

In conventional oral routes of drug administration the rate and extent of absorption differs due to the various factors which may be drug itself, its formulation, presence of food, drug interactions, first-pass metabolism, and gastrointestinal pH. Apart from this, conventional routes of drug administration have many other demerits. Buccal delivery of drugs provides an attractive alternative to the oral route of drug administration. The oral transmucosal drug delivery by-passes liver and avoids pre-systemic elimination in the gastro intestinal tract and liver. Also drug absorption can be terminated in condition of drug toxicity by removing the buccal dosage form from buccal cavity. These factors make the oral mucosa a very attractive site for systemic drug delivery[1]. Buccal mucosa makes a more appropriate choice of site if prolonged drug delivery is desired because the buccal site is less permeable than sublingual site[2].

The buccal route is particularly suitable for those drugs, which are meant for chronic diseases, wherein the sustained or prolonged therapeutic effect is desired. One such chronic disease is diabetes mellitus, a major and growing health problem worldwide. A defective or deficient insulin secretary response, which translates in to impaired carbohydrate (glucose) use, is a characteristic feature of diabetes mellitus[3].

It has been reported that, controlled/prolonged release formulations containing a short-acting type oral blood glucose regulator expected to enable control of both PBG (Post-Prandial Blood Glucose) and FBG (Fasting Blood Glucose) for moderate and severe diabetes patients[4] Repaglinide is one such short-acting drug with half-life of about 1 hour requiring frequent administration. The total daily dose is also low (Max.16mg). It is also subjected to an extensive and highly variable hepatic metabolism following oral administration, with a reported bioavailability of 56%. Although Repaglinide is completely absorbed from gastrointestinal tract, it is degraded in intestine and poorly absorbed from upper intestinal tract[5]. Hence it is a suitable drug candidate for administration via buccal route.

Repaglinide is categorized under the BCS Class II i.e. it has poor solubility but high permeability. This poor solubility may cause poor dissolution and unpredicted bioavailability. To penetrate the buccal mucosa to a significant degree, a drug should exhibit biphasic solubility pattern i.e. the drug should be soluble in aqueous salivary fluid and should penetrate sufficiently through lipid membrane.

Repaglinide is practically insoluble in water. Hence in this study, an attempt has been made to prepare Repaglinide inclusion complex; to enhance its solubility and was incorporated into the

mucoadhesive buccal films in order to improve the bioavailability. A comparative study has been done between; the pure uncomplexed Repaglinide loaded buccal films and inclusion complex loaded buccal films.

MATERIALS AND METHODS

Materials

Repaglinide was obtained as gift sample from Biocon Ltd – Bangalore, Hydroxypropyl methylcellulose and Ethylcellulose from Colorcon Asia Pvt. Ltd. – Goa, Carbopol 971P from Lubrizol – Belgium, Polyvinyl pyrollidone K-30 from Glenmark Generics – Goa, Hydroxypropyl-β-cyclodextrin from Gangwal Chemicals – Mumbai and Polyethylene glycol 400 (Chemport - Mumbai). All the other reagents and solvents used were of analytical grade.

Methods

Drug-Excipient compatibility study

FT-IR Spectral analysis:

The IR spectra of pure drug, Repaglinide and its physical mixture with polymers were obtained using FT-IR spectrophotometer (Shimadzu/IR Prestige).

Differential scanning calorimetric (DSC) analysis:

Calorimetric characterization; of drug and its physical mixture with polymers were carried out using DSC (Mettler Toledo – DSC832e) instrument; and the data was processed using software, STAR SW 9.20 Version. DSC thermograms were recorded at heating rate of 10ºC /min in the range of 25 ºC – 150 ºC.

Chromatographic analysis by TLC:

TLC was used to study Drug-Liquid excipient (PEG 400) compatibility. Silica gel 60 F 254 TLC plate (normal phase) was used as stationary phase and a mixture of Chloroform: Methanol (9:1 V/V) was used as mobile phase. TLC plate was prewashed with methanol; dried and activated at 110 ºC for 30 min. Chromatographic chamber was saturated with mobile phase for 10 min. Then the activated TLC plate was spotted with the standard solution and test solution. The plate was then developed with mobile phase, air dried and spots were visualized under UV chamber (DESAGA) at 254nm. Finally Rf value was calculated[6, 7]

Part I

Formulation of buccoadhesive films of Repaglinide

The matrix type buccal films containing Repaglinide were prepared employing the solvent evaporation method. The dose per 2.25 cm^2 film was 3mg. The composition of film formulations is given in table 1.

Procedure:

Accurately weighed quantity of HPMC K4M was dissolved in solvent system of Methanol: Dichloromethane (1:1) mixture. Carbopol-971 (CP-971) was separately dissolved in methanol and added to HPMC solution. PEG 400 in concentration of 50% w/w of total polymer weight was added as plasticizer and mixed using magnetic stirrer. Accurately weighed quantity of Repaglinide was added to polymeric solution; and thoroughly mixed using magnetic stirrer. Then the polymeric drug solution was set aside for 4 hours to exclude entrapped air bubbles. The solution was then poured into the Petri plate and covered with inverted funnel to allow controlled evaporation of solvent at room temperature for 24 hrs. Films were further dried in hot air oven at 45ºC for 2 hrs to remove any residual solvent. Dried films were carefully removed, checked for any imperfections or air bubbles and cut into films of 2.25 cm^2. The films were wrapped in butter paper, further packed in aluminium foil and stored in desiccator; away from light.

Part II

Preparation of Inclusion Complexes of Repaglinide

Phase solubility studies: An excess amount of Repaglinide was added to 25 mL of aqueous solutions containing various concentrations of HP-β-CD (0 to 0.04 M) in 50 mL conical flask. The suspensions were vigorously shaken at room temperature in a rotary shaker for 3 days. After equilibrium was attained, the samples were filtered through a 0.45 µ Millipore membrane filter and suitably diluted with distilled water. Repaglinide concentration was determined using UV spectrophotometer, at 283nm. Phase solubility diagram was obtained by plotting a graph of molar concentration of Repaglinide solubilized against the molar concentration of HP-β-CD. The stability constant, *K*s, was calculated from the phase solubility diagrams using the following equation [8, 9,10]

$$Ks = \frac{\text{Slope}}{S_0\,(1\text{-Slope})}$$

Where, S_0 is the solubility of Repaglinide in the absence of HP-β-CD (intercept).

Preparation of inclusion complexes

Complexes were prepared in 1:1 molar ratio based on the results of phase solubility studies.

A. Kneading Method: Repaglinide and HP-β-CD in 1:1 molar ratio were transferred to a mortar pestle. The mixture was reduced in size by continuous stirring with pestle. Water-methanol mixture (3:1) ratio was added to the above physical mixture and continuously stirred until the thick slurry mass was formed. The thick slurry was kneaded for 45 min and dried in a hot air oven for 2 hrs at 50 °C. The dried mass were collected and passed

through 120 mesh, and packed it in a closed container [9, 11, 12].

B. Solvent evaporation method: Repaglinide and HP-β-CD in 1:1 molar ratio were dissolved in a common volatile solvent like methanol with continuous stirring. The solution was stirred until thick mass was obtained. Then the solvent was allowed to evaporate completely in hot air oven at 45 °C for 2h. The dried mass were passed through 120 mesh and stored in desiccator at room temperature until further use [9, 12 ,13].

C. Co-evaporation method: Repaglinide and HP-β-CD were weighed according to their corresponding 1:1 molar ratio. Repaglinide was dissolved in methanol and HP-β-CD in distilled water. Subsequently, alcoholic solution of the drug was slowly added to aqueous solution of HP-β-CD; followed by stirring at 300 rpm using magnetic stirrer at 37°C for 8 hrs. The resulting mixture was evaporated at a temp of 45 °C until dry. The dried mass was pulverized & sieved through 120 mesh [14]

Evaluation and characterization of prepared Inclusion Complexes of Repaglinide

The prepared inclusion complexes were evaluated for percentage yield, percent drug content and saturation solubility [9, 11]. And they were characterized by:

FT-IR spectroscopy

The IR spectra of Repaglinide, HP-β-CD and prepared complexes were obtained using FT-IR spectrophotometer (Shimadzu/IR Prestige). Absence of peaks; especially of hydrophobic groups due to inclusion into cyclodextrin cavity or appearance of new peaks or shift in peak position due to interaction; indicates complex formation.

Powder X-ray diffraction (P-XRD) studies

Powder X-ray diffraction technique has been extensively utilized to study the interaction between drug and HP-β-CD [11] and evaluates the residual crystallinity of the drug [15]. The diffraction studies were carried out in a powder X-ray diffractometer (Bruker Advance D-8). PXRD pattern of Repaglinide, its physical mixture with HP-β-CD and solid complexes were recorded between 2 θ = 5 to 45° at a voltage of 40 kV and a current of 40 mA.

Differential scanning calorimetric (DSC) studies

It was stated that when a guest molecule is incorporated in CD cavity; its melting, boiling and sublimation points are usually shifted to different points or disappear; additionally a change in heat of fusion may also occur [3, 16, 17]. DSC measurements; of Repaglinide and solid complexes were carried out using DSC (Mettler Toledo – DSC832e) instrument.

Dissolution study

Dissolution study of Repaglinide/HP-β-CD complexes was done in pH 6.8 buffer, using USP

type II dissolution apparatus (Lab India DS8000). Temperature was maintained at 37 ± 2 °C. Complexes equivalent to 5mg of Repaglinide and also 5mg of pure Repaglinide were taken and placed in muslin cloth bags. These bags were tied to paddles and dissolution was studied at 50 rpm for 60 min [11].

Scanning Electron Microscopy (SEM)

SEM was used to characterize the drug, HP-β-CD and inclusion complexes. Gold coated samples were scanned at 20 kV under scanning electron microscope (JEOL/JSM-5800LV).

Part III

Formulation of Inclusion Complex loaded Mucoadhesive Buccal Films

Complex prepared by Co-evaporation technique was optimized for the film formulation based on solubility, dissolution profile, X-Ray diffractogram and DSC thermograms.

Preparation of inclusion complex loaded buccal films

The same procedure; as for HC formulations; was followed in formulating these buccal films. The composition of film formulations is given in table 2.

Table 1: Composition of HC Mucoadhesive Buccal Films of Repaglinide

Formulation code	Repaglinide (mg)	HPMC K4M (mg)	CP-971 (mg)	PEG 400 (%w/w)
HC1	59	400	50	50
HC2	59	375	50	50
HC3	59	350	50	50
HC4	59	400	25	50
HC5	59	375	25	50
HC6	59	350	25	50
HC7	59	400	100	50
HC8	59	375	100	50
HC9	59	350	100	50
HC10	59	267	133	50
HC11	59	200	200	50
HC12	59	133	267	50
HC13	59	350	150	50
HC14	59	300	100	50

Table 2: Composition of Repaglinide-HP-β-CD Complex loaded Buccal Films

Formulation code	Repaglinide* (mg)	HPMC K4M (mg)	CP-971 (mg)	PEG 400 (%w/w)
HCC1	59	350	25	50
HCC2	59	350	50	50
HCC3	59	350	100	50
HCC4	59	375	25	50
HCC5	59	375	50	50
HCC6	59	375	100	50

*Inclusion complex equivalent to 59mg of Repaglinide

Evaluation of prepared Mucoadhesive buccal films

Physical appearance

All the film formulations were visually inspected for clarity, flexibility or any imperfections such as cracks or bubbles.

Weight uniformity

For the evaluation of weight uniformity, five films of 2.25cm^2 dimension from each formulation were taken and weighed individually on an electronic weighing balance. The results were analyzed for mean and standard deviation [18].

Thickness uniformity

Assessment of thickness was done on three films from each formulation by using micrometer screw gauge. And results were analyzed for mean and standard deviation [11].

Surface pH determination

For determination of surface pH, film of 2.25 cm^2 dimension from each formulation was kept in contact with 1ml of distilled water for 1 hr in a Petri plate. The pH was noted by bringing the electrode of pH meter in contact with the surface of swollen film, allowing it to equilibrate for 1min. The average of three determinations was calculated and analyzed for deviation [18, 19].

Content uniformity

Three films of 2.25 cm^2 dimension from each formulation were taken in separate 10ml volumetric flask and 2ml methanol was added to each volumetric flask. The flasks were shaken for 1 hr, in order to extract the drug. Then the volume was made up to the 10ml mark with phosphate buffer pH 6.8 and shaken until the entire film goes into solution. The solution was filtered and 1ml of filtrate was diluted to 10ml with phosphate buffer pH 6.8 and analyzed using UV spectrophotometer at 281nm [18]

Folding Endurance

Folding endurance of the films were determined by repeatedly folding a small strip of film at the same place; till it broke or folded up to 300 times manually, which was considered satisfactory to reveal good patch properties. This test was done on randomly selected three films from each formulation [18].

Tensile Strength and % Elongation

The instrument used to measure the tensile strength was designed in our laboratory. A film strip of 3x1 cm was cut and its one end was fixed between clamps of a static end. The other end of film strip was attached to clamps of a movable block. A long thread was tied to the movable block, passed over the pulley; and the other end of thread was tied to a pan to hold the weights. A

small pointer was attached to the block, which travels over a scale on the base plate. The tensile strength was determined by gradually adding weights to pan in order to increase pulling force till the film was broken [20]. The weight required to break the film was noted as break force and the tensile strength was calculated by following formula:

$$\text{Tensile strength} = \frac{\text{Force (Kg)}}{\text{Initial cross-sectional area (cm}^2\text{)}}$$

The distance traveled by pointer before break of film was noted simultaneously and % elongation was calculated using following formula:

$$\% \text{ Elongation} = \frac{\text{(Final length – Initial length) x100}}{\text{Initial length}}$$

Swelling index

The preweighed film of 2.25 cm^2 dimension was placed on the surface of the agar plate (2% w/v) agar and allowed to swell in an incubator (Quality inst. & equipt.) maintained at 37 °C. Swelling index was determined at 2, 5 and 8 hrs. Swelling index was calculated as a function of weight [8, 21, 22] using the formula: SI = (Final weight – Initial weight) / Initial weight

Moisture Content Determination

The buccal film from each formulation was weighed accurately and kept in desiccator containing sodium hydroxide at room temperature. The films were weighed periodically until no further decrease in weight was recorded. The % moisture content was determined by calculating moisture loss using the formula [23]:

$$\% \text{ Moisture content} = \frac{\text{(Initial weight – Final weight)} \times 100}{\text{Final weight}}$$

Moisture Absorption Study

Films of 2.25 cm^2 dimension from each formulation were subjected to desiccation over sodium hydroxide at room temperature until no further decrease in weight was observed. This weight was recorded as initial weight. Dried films were exposed to 74.9% and 98% RH using sodium chloride and potassium dichromate saturated solution at room temperature. After 24 hrs the films were taken out and weighed. The percentage moisture absorption was calculated using the formula [24]:

$$\% \text{ Moisture absorption} = \text{(Final weight – Initial weight)} \times 100 / \text{Initial weight}$$

***Ex vivo* Mucoadhesive Strength**

The apparatus was locally assembled and was a modification of the physical balance. Fresh goat buccal mucosa was used as model mucosal membrane. The membrane was washed with distilled

water and then with simulated salivary fluid pH 6.8. The instrument broadly composed of modified physical balance in which the left pan was replaced by a small plastic pan and right pan was replaced with a plastic glass; which were vertically suspended. Below this plastic pan, a glass beaker was placed over a movable platform. A glass bottle filled with simulated salivary fluid; to which mucosal tissue was fixed; was placed in the glass beaker. The beaker was filled with SSF pH 6.8, in such a way that it just touches the surface of tissue. A buccal film of 2.25 cm^2 dimension was glued to lower surface of the plastic pan. The exposed film surface was moistened with 50µl of SSF pH 6.8 for 30 seconds; for initial hydration and swelling. The movable platform was raised upward until the film on glass contacts with mucosa. The preload of 50gm was placed in left plastic pan and whole assembly kept undisturbed for 3min (preload time) to establish the adhesion between film and mucosal tissue. After 3min, preload was removed and water was added drop wise to plastic glass until detachment of the film from mucosal surface took place. Weight of water collected in plastic glass was measured at the time of detachment. After each measurement the tissue was gently and thoroughly washed with SSF pH 6.8 and left for 5 minutes before taking reading. For each formulation fresh tissue was used. The mass in (gm) required to detach the patch from the mucosal surface gave the measure of Mucoadhesive strength. The experiment was performed in triplicate and analyzed for mean and standard deviation. The following parameters were calculated from Bioadhesive strength [18, 25, 26]

Force of adhesion (N) = Bioadhesive strength (gm) x 9.81/1000

Bond strength (N m^{-2}) = Force of adhesion/ Film surface area

***Ex vivo* Mucoadhesion Time**

The *ex vivo* residence time was evaluated using a locally modified USP disintegration test apparatus. Disintegration medium was composed of 800 ml simulated salivary fluid pH 6.8 maintained at 37ºC. A segment of goat mucosal membrane was glued to the surface of a glass slab, vertically attached to the apparatus. The Mucoadhesive film of 1cm^2 size (n=2) was hydrated with few drops of simulated salivary fluid pH 6.8 and brought into contact with the mucosal membrane. The glass slab was vertically fixed to the disintegration apparatus and allowed to move up and down so that the patch was completely immersed in the buffer solution at the lowest point and was out at the highest point. The time necessary for complete erosion or detachment of the film from the mucosal surface was recorded [8, 20, 27].

***In vitro* drug release study**

In vitro drug release studies were carried out using a modified Franz diffusion cell. The assembly consisted of an open ended glass tube; which acted as a donor compartment. A beaker containing

100ml of phosphate buffer pH 6.8 (dissolution media) was used as receptor compartment. A cellophane membrane was used as a barrier membrane between the donor and receptor compartment. The cellophane membrane was soaked for 24 hrs in Phosphate buffer pH 6.8 and was attached to one end of the open glass cylinder. The prepared drug loaded buccal film was placed on the cellophane membrane. And dipped in dissolution media present in receptor compartment such that membrane just touches the surface of dissolution media. The dissolution media was maintained at a temperature of 37±2ºC and hydrodynamics was maintained by stirring with a magnetic bead at 100 rpm. At every 1 hour interval, 5 mL of sample was withdrawn and replaced with 5 mL of fresh medium to maintain the sink condition. The samples were analyzed in UV spectrophotometer at 281 nm [27, 28, 29, 30].

Statistical analysis

The release data obtained was analyzed using mathematical models (zero order, first order, Higuchi and Peppas models) to evaluate the kinetics and mechanism of drug release. The model that best fits the release data is selected based on correlation coefficient (R) value in various models. The model that gives highest 'R' value is considered as the best fit of release data.

Stability studies

The optimized Mucoadhesive Buccal Films were wrapped in butter paper and further packed in aluminium packaging. The sealed films were subjected to room temperature and; elevated temperature and humidity conditions of 40 ± 2 ºC / 75 % RH for one month. At the end of 30 days, samples were withdrawn and evaluated for appearance, *ex vivo* mucoadhesive strength, drug content and *in vitro* drug release.

RESULTS AND DISCUSSION

Compatibility study

IR Spectral analysis: The IR spectrum of pure Repaglinide is shown in figure 1. The major peaks of the pure drug were retained in all the spectra with no substantial changes and have shown presence of all the major functional groups of the pure drug. This indicates that there is no interaction between the drug and polymers.

DSC analysis: The DSC thermograms of pure drug, Repaglinide is shown in figure 2. The DSC thermogram of the drug showed an endothermic peak at 136.05 ºC corresponding to its melting point and an onset temperature of 133.99 ºC. DSC thermograms of physical mixture did not show any significant difference in onset and melting point temperature. Thus it was proved that there was no positive evidence of interaction between the drug and utilized polymers.

TLC: TLC analysis was carried out to determine the Drug-Liquid excipient compatibility. The developed TLC plate was visualized under UV chamber at 254 nm. The Rf value of pure drug and its mixture with PEG 400 is found to be same i.e. 0.77. Thus it denoted absence of any possible interaction.

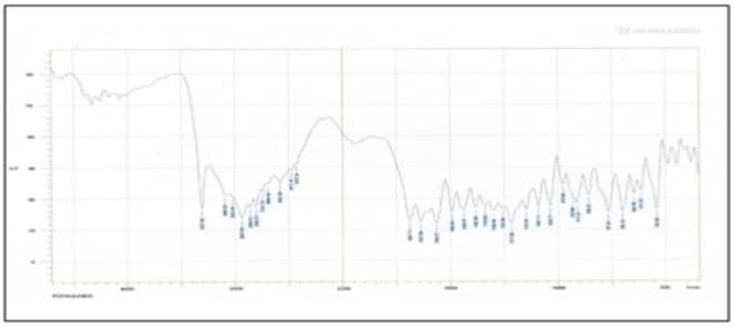

Figure 1: FT-IR Spectrum of pure Repaglinide

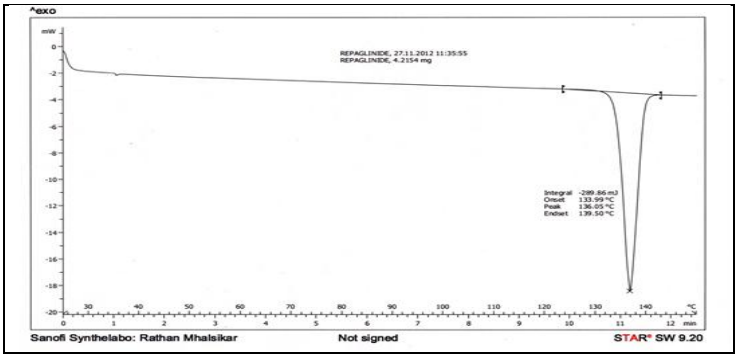

Figure 2: DSC Thermogram of pure Repaglinide

Characterization of prepared Inclusion Complexes of Repaglinide

Phase Solubility Study

The phase solubility diagram is shown in figure 3. The curve obtained can be classified as AL type according to Higuchi and Connors, because of linear increase in the solubility with R^2 value close to unity. The stability constant, Ks was found to be 566.69 M^{-1} which is within the range (200- 5000M^{-1}). This indicates that complex is quite stable. Hence 1:1 stoichiometry was assumed, considering the AL type of curve, with slope less than unity.

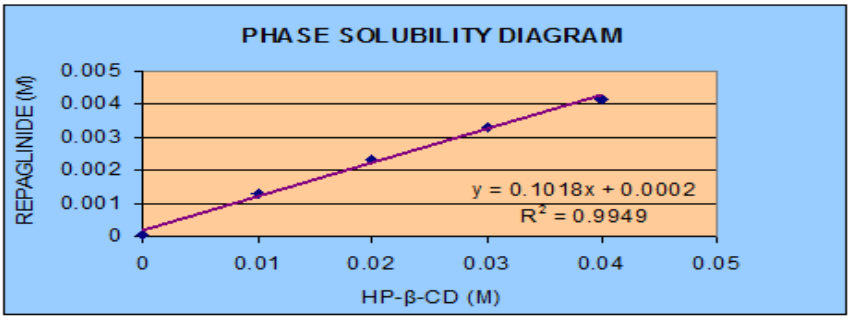

Figure 3: Phase Solubility diagram

Percentage yield, % Drug content of complex and Saturation Solubility Studies

The results are tabulated in table 3.

Table 3: Physical Evaluation parameters of complexes

Drug: HP-β-CD Complex	% Practical Yield	% Drug Content	Saturation Solubility(mg/ml)	%Increase in solubility
Pure drug	---	---	0.034	---
PM	85.00	99.62	0.049	145.00
KN	82.90	97.27	0.059	173.53
COE	90.23	98.90	0.082	241.18
SE	89.16	98.40	0.073	214.71

FT-IR spectroscopic studies

The IR spectrum of Repaglinide (Figure 1) reveals the presence of a peak at 3307.92 cm^{-1}, assigned to N-H stretching vibration and one at 1687.71 cm^{-1} corresponding to the carbonyl group. In the IR spectrum (Figure 4) of complex prepared by co-evaporation method (COE); the peak at 3307.92 cm^{-1} was not identified any more and the carbonyl band was shifted towards a lower wavenumber; suggesting the possibility of formation of hydrogen bonds between the hydroxyl groups of the host cavities and the Repaglinide carbonyl group. Thus the results indicate the occurrence of host-guest interactions.

X-Ray Diffraction (XRD) studies

The diffraction pattern of pure Repaglinide showed numerous distinctive sharp peaks indicating its crystalline nature. The overlay of X-Ray diffractograms of Repaglinide, HP-β-CD and solid complexes are presented in figure 5. The diffraction pattern of HP-β-CD showed a diffused pattern, indicating its amorphous nature. The XRD pattern of physical mixture of Repaglinide and HP-β-CD (PM) were found to be superimposition of diffractogram of single components, but the peak intensities were found to be much lower; possibly due to partial weak complexation. The inclusion complex prepared by kneading method (KN) presented a diffraction pattern quite similar to physical mixture with further reduction in intensity of crystalline peaks; indicating complex formation. The X-Ray diffractogram of complex prepared by solvent evaporation technique (SE) exhibited few of the diffraction peaks of crystalline Repaglinide; of extremely low intensity, indicating conversion of crystalline form of drug to partial amorphous state. Inclusion complex prepared by co-evaporation technique (COE) presented a very few of diffraction peaks which are very diffused; indicating properly formed inclusion complex. Thus COE technique was found to be more successful in complex formation.

DSC studies

The thermal analysis of Repaglinide revealed a single, sharp endothermic peak at 136.05°C

corresponding to its melting point; indicating a crystal polymorphic form (Figure 2). An evidence for the formation of inclusion complexes between Repaglinide and CDs was the change in the shape of the melting peak of Repaglinide and its shift towards lower temperatures. The COE complex (Figure 6), showed endothermic peak of less intensity that has been shifted towards the lower temperature; thus indicating formation of new mass.

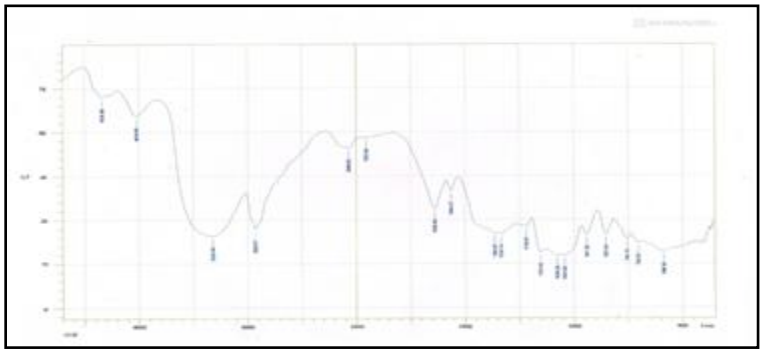

Figure 4: FT-IR spectrum of Repaglinide inclusion complex prepared by co-evaporation method

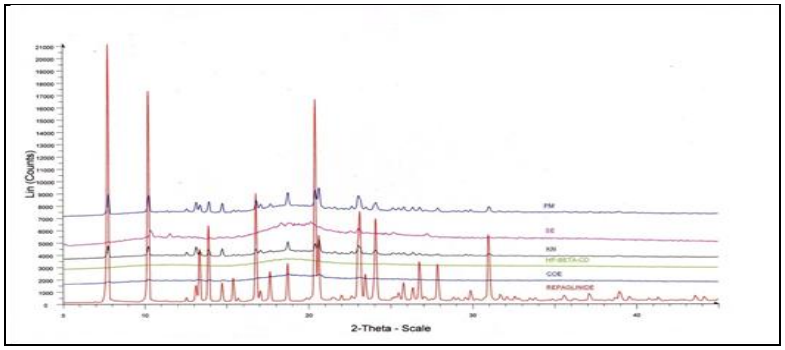

Figure 5: Overlay of X-Ray Diffractograms

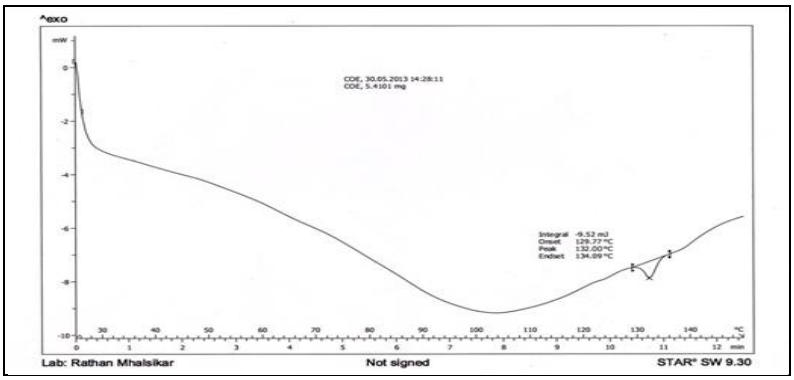

Figure 6: X-Ray diffractogram of inclusion complex prepared by co-evaporation method

Dissolution study

The inclusion complexes of Repaglinide showed pronounced enhancement in its dissolution, as shown in figure 7. Cumulative percentage of drug dissolved after 60 min was 24.81% for pure Repaglinide whereas for KN, COE and SE complexes it was 80.79%, 90.58% and 84.65%

respectively. This revealed that there was marked increase in dissolution rate of Repaglinide from complexes. It was observed that cumulative percentage of drug released after 60 min was maximum for complex prepared by co-evaporation technique (COE).

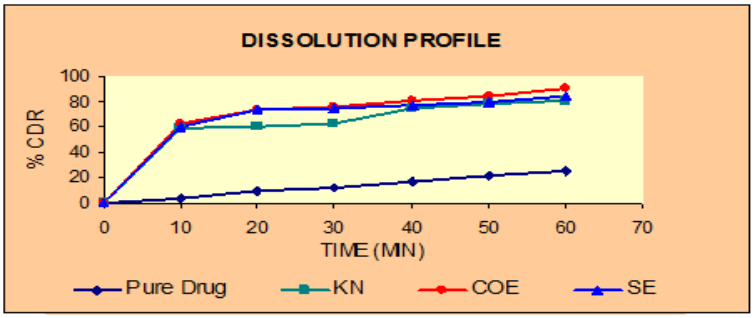

Figure 7: Dissolution profile of pure Drug and Inclusion complexes

Scanning Electron Microscopic (SEM) Study

Photomicrograph of Repaglinide, HP-β-CD and COE complex as shown in figure 8, revealed crystalline nature of the drug, wherein the crystals are flat tabular shaped; whereas is present as irregularly shaped mass. SEM Image of COE complex revealed formation of inclusion complex; since the drug crystals can no more is seen.

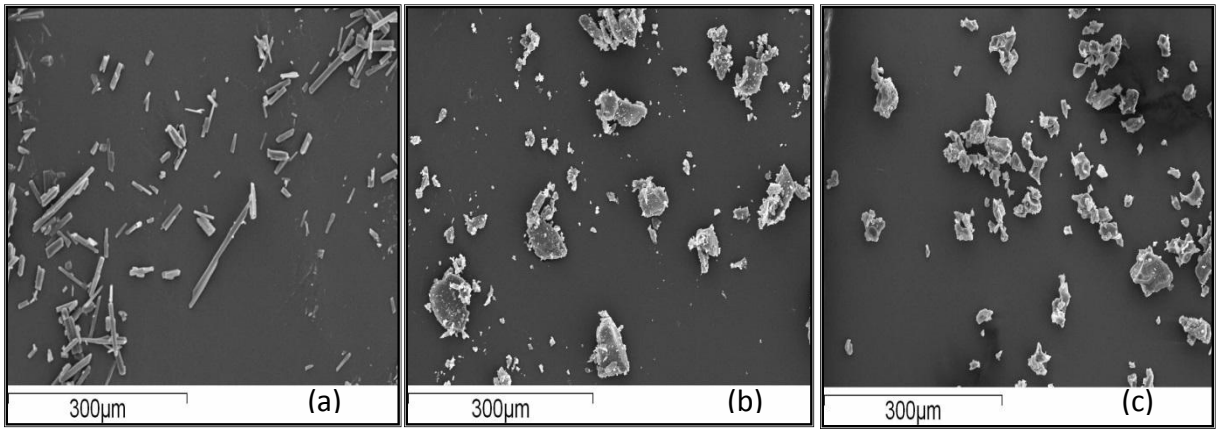

Figure 8: SEM image of (a) Repaglinide (b) HP-β-CD (c) COE

Evaluation of prepared Mucoadhesive buccal films

Physical appearance

All the film formulations were translucent, flexible and non-tacky; except HC11 and HC12, which were found to be tacky.

Weight uniformity

The average weight of five films from each batch of formulation is reported in table 4. Weight ranged between 31.06mg – 38.84mg. There was no much variation in weight of films from each batch, indicating uniformity in weight.

Table 4: Physicochemical Evaluation Data of Buccal Film Formulations

Formulation code	Weight (mg) Mean ± SD	Thickness (mm) Mean ± SD	pH	Folding endurance
HC1	34.48 ± 1.096	0.262 ± 0.008	6.74 ± 0.025	>300
HC2	32.58 ± 0.813	0.260 ± 0.005	6.68 ± 0.040	>300
HC3	31.26 ± 0.748	0.248 ± 0.007	6.58 ± 0.037	>300
HC4	33.72 ± 0.426	0.243 ± 0.010	7.03 ± 0.025	>300
HC5	31.06 ± 1.062	0.237 ± 0.003	6.81 ± 0.075	>300
HC6	29.70 ± 0.738	0.236 ± 0.003	6.75 ± 0.026	>300
HC7	37.94 ± 1.021	0.283 ± 0.010	6.44 ± 0.051	>300
HC8	36.72 ± 1.044	0.262 ± 0.007	6.34 ± 0.035	>300
HC9	35.42 ± 0.614	0.255 ± 0.005	6.27 ± 0.060	>300
HC10	32.08 ± 0.763	0.237 ± 0.004	6.32 ± 0.046	>300
HC11	32.14 ± 1.264	0.237 ± 0.003	6.21 ± 0.040	---
HC12	31.94 ± 1.426	0.236 ± 0.001	6.09 ± 0.115	---
HC13	38.84 ± 0.477	0.284 ± 0.009	6.24 ± 0.031	>300
HC14	31.88 ± 1.132	0.244 ± 0.003	6.35 ± 0.020	>300
HCC1	38.44 ± 0.301	0.242 ± 0.002	6.77 ± 0.047	>300
HCC2	41.02 ± 0.277	0.253 ± 0.002	6.63 ± 0.047	>300
HCC3	44.46 ± 0.163	0.258 ± 0.0001	6.32 ± 0.061	>300
HCC4	41.04 ± 0.158	0.241 ± 0.001	6.85 ± 0.050	>300
HCC5	42.84 ± 0.130	0.264 ± 0.001	6.72 ± 0.030	>300
HCC6	46.16 ± 0.116	0.265 ± 0.001	6.41 ± 0.040	>300

Thickness uniformity

The average thickness of 3 films from each batch of formulation is reported in table 4. Thickness ranged between 0.236mm – 0.284mm. There was no much variation in thickness of films from each batch, indicating uniformity in thickness.

Surface pH determination

The average pH value of 3 determinations from each formulation is reported in table 4. The surface pH varied between 6.09 – 7.03. The surface pH of all the films was found to be in acceptable pH range.

Content uniformity

The drug content ranged between 96.13% – 98.28 %. The results are tabulated in table 5.

Folding endurance

Films from all the formulations except HC11 and HC12; did not show any cracks even after folding for more than 300 times. Hence it was taken as the end point. Thus it was proved to possess good Folding endurance. Since HC11 and HC12 were tacky folding endurance could not be found. Results are reported in table 4.

Tensile strength and % Elongation

The results are tabulated in table 5. Tensile strength ranges between 27.85 – 101.18 Kg/cm^{-2} and

% Elongation from 4.44 – 44.44 %. It has been observed from the results that, tensile strength and % elongation decreases with decreasing total polymer concentration. It was also found that with increasing HPMC amount, tensile strength and % elongation increases while with increase in CP -971 amount, tensile strength and % elongation decreases. No much difference was found in tensile strength of complex loaded films when compared with its corresponding uncomplexed drug loaded films.

Table 5: Physicochemical Evaluation Data of Buccal Film Formulations

Formulation code	% Drug content Mean ± SD	Tensile strength (Kg/cm^{-2}) Mean ± SD	% Elongation Mean ± SD
HC1	97.1 ± 01.44	79.39 ± 2.042	38.88 ± 4.16
HC2	98.02 ± 0.73	71.57 ± 1.770	29.08 ± 5.09
HC3	98.28 ± 1.13	62.87 ± 2.32	23.33 ± 3.34
HC4	97.53 ± 1.60	101.18 ± 2.61	44.44 ± 1.92
HC5	96.87 ± 1.26	87.86 ± 1.26	37.80 ± 5.09
HC6	97.46 ± 1.36	80.68 ± 0.79	32.22 ± 1.92
HC7	97.61 ± 0.63	62.30 ± 2.42	30.00 ± 3.33
HC8	97.37 ± 1.54	60.62 ± 5.51	25.55 ± 1.93
HC9	96.13 ± 1.38	49.99 ± 2.66	18.90 ± 3.85
HC10	96.73 ± 1.25	41.43 ± 1.28	8.88 ± 3.85
HC11	97.70 ± 2.09	37.97 ± 0.50	8.89 ± 1.93
HC12	98.10 ± 0.95	27.85 ± 2.17	4.44 ± 1.94
HC13	98.28 ± 0.67	38.97 ± 0.44	13.33 ± 3.34
HC14	97.56 ± 1.76	41.89 ± 1.76	6.66 ± 3.33
HCC1	97.23 ± 2.15	78.71 ± 0.64	34.44 ± 1.92
HCC2	97.30 ± 1.70	63.44 ± 0.59	24.44 ± 3.85
HCC3	96.40 ± 2.09	52.06 ± 2.09	18.89 ± 1.92
HCC4	96.56 ± 1.82	86.59 ± 0.45	38.88 ± 3.85
HCC5	97.27 ± 1.56	71.34 ± 1.16	27.78 ± 5.09
HCC6	97.47 ± 1.82	62.05 ± 1.95	21.11 ± 1.92

Swelling index

Swelling study of the prepared buccal films was carried out as a function of weight increase due to swelling; at 2hrs, 5hrs and 8hrs. The results are tabulated in table 6. It has been observed that swelling index increases with increasing amount of CP-971. This might be due to its rapid swelling and gel forming capacity upon contact with aqueous media, absorbing a great quantity. It is also seen that; with increase in HPMC content or increase in total polymer amount, swelling index decreases.

Table 6: Swelling Index data as a function of weight of Buccal Films

Formulation code	Weight Swelling Index		
	SI(2hrs)	SI(5hrs)	SI(8hrs)
HC1	0.97	2.24	3.88
HC2	1.01	2.32	4.03

HC3	1.12	2.83	4.36
HC4	0.88	2.09	3.26
HC5	1.07	2.61	4.08
HC6	1.03	2.77	4.16
HC7	1.15	2.88	4.54
HC8	1.19	3.06	4.75
HC9	1.27	3.19	4.96
HC10	1.30	3.28	5.28
HC11	1.35	3.40	5.37
HC12	1.44	3.61	5.75
HC13	1.29	3.20	5.17
HC14	1.33	3.19	5.25
HCC1	1.10	2.89	4.17
HCC2	1.18	3.91	4.33
HCC3	1.29	3.33	4.77
HCC4	1.12	2.18	4.09
HCC5	1.09	2.54	4.06
HCC6	1.26	3.21	4.64

Moisture Content Determination

The % Moisture Content ranged between 2.25 – 8.25 %. The results are tabulated in table 7. Moisture Content was found to be slightly more for HCC films in comparison to its corresponding uncomplexed drug loaded films.

Table 7: Moisture Content and Moisture Absorption data of Buccal Films

Formulation code	**% Moisture content**	**%Moisture Absorption after 24 hours**	
		@ RH 75%	**@ RH 98%**
HC1	3.18	1.91	10.69
HC2	2.45	1.83	9.39
HC3	2.43	1.22	7.78
HC4	3.08	1.65	8.87
HC5	2.25	1.61	6.37
HC6	2.82	1.21	6.64
HC7	4.64	2.79	11.85
HC8	3.23	2.65	9.86
HC9	3.51	2.56	9.75
HC10	4.84	2.64	10.36
HC11	7.16	3.00	14.84
HC12	8.25	3.30	14.65
HC13	3.50	2.43	9.26
HC14	3.22	2.58	10.75
HCC1	2.89	1.84	7.33
HCC2	2.74	1.99	7.75
HCC3	3.49	2.56	9.79
HCC4	3.02	2.01	7.52
HCC5	3.39	1.94	9.51
HCC6	4.88	2.79	10.51

Moisture Absorption Study

The Moisture Absorption study was carried out at two different humidity conditions i.e. at RH 75% and RH 98%. The % Moisture absorption values varied between 1.21 – 3.30 % (at RH 75%) and 6.37 – 14.65% (at RH 98%). The results are tabulated in table 7. Moisture uptake capacity was found to be increased with increase in total polymer content, increase in CP-971 content (due to its hygroscopic nature) and increase in HPMC content (due to its hydrophilic nature). A slight increase in moisture uptake capacity was seen for HCC formulations, when compared to its corresponding uncomplexed drug loaded films, possibly due to overall increase in hydrophilicity.

***Ex vivo* Mucoadhesive strength**

The results are tabulated in table 8. CP-971, being an excellent mucoadhesive polymer; have shown increase in strength with its increasing concentration. Also increase in HPMC content has shown an increase in bioadhesive strength. A slight increase in strength was observed in all the complex loaded formulations, in comparison to its corresponding uncomplexed drug loaded films.

Table 8: *Ex vivo* Mucoadhesive Strength and Mucoadhesion time data of Buccal Films

Formulation code	Bioadhesive strength (gm) Mean ± SD	Force of adhesion (N)	Bond strength (Nm^{-2})	*Ex vivo* Residence Time(min) Mean ± SD
HC1	53.73 ± 1.94	0.527	2342.22	482.5 ± 3.54
HC2	49.97 ± 1.25	0.490	2177.78	438.5 ± 2.12
HC3	49.43 ± 0.61	0.485	2155.56	432.5 ± 3.54
HC4	46.23 ± 1.00	0.453	2013.33	420 ± 7.07
HC5	43.10 ± 2.14	0.423	1880.00	433 ± 4.25
HC6	37.63 ± 0.47	0.369	1640.00	430 ± 7.07
HC7	70.16 ± 0.65	0.688	3057.78	487.5 ± 3.53
HC	65.77 ± 3.25	0.645	2866.67	479 ± 1.41
HC9	65.26 ± 2.08	0.640	2844.44	468 ± 10.61
HC10	72.27 ± 0.66	0.711	3160.00	472.5 ± 10.61
HC11	74.53 ± 3.08	0.731	3248.89	468.5 ± 12.02
HC12	79.93 ± 1.10	0.784	3484.44	468 ± 18.38
HC13	68.33 ± 2.21	0.670	2977.78	489 ± 5.66
HC14	67.50 ± 2.29	0.662	2942.22	475.5 ± 7.78
HCC1	39.43 ± 0.83	0.387	1720.00	415 ± 7.07
HCC2	50.30 ± 0.40	0.493	2191.11	423 ± 2.83
HCC3	66.37 ± 1.40	0.651	2893.33	457.5 ± 3.54
HCC4	43.80 ± 1.61	0.429	1906.67	417.5 ± 3.54
HCC5	50.73 ± 0.87	0.498	2213.33	429.5 ± 6.36
HCC6	67.27 ± 2.00	0.659	2928.89	475.5 ± 7.78

***Ex vivo* Mucoadhesion time**

Observations related to the *ex vivo* residence time for all the films, indicated adequate attachment to the mucosal surface for about 7 hours and more. The results are reported in table 8. Results have shown increase in Mucoadhesion time with increasing CP-971 amount.

***In vitro* release study of Repaglinide loaded buccal films**

The *in vitro* release profile of all the HC formulations (HC1 to HC14) is shown in figure 9 to 12. Cumulative percentage of drug release (%CDR) at the end of 8 hours was found to be highest for HC14 i.e. 83.24% and least for HC13 i.e. 42.05%. The two polymers i.e. HPMC K4M and Carbopol-971 were taken as 2 variables and their effect on drug release was studied.

Effect of HPMC on drug release: It was observed that with increase in HPMC proportion; the cumulative percentage of drug release at the end of 8 hours was reduced. HPMC can act as a hydrophillic matrix; controlling the diffusion of drug from the matrix.

Effect of Carbopol-971 on drug release: % CDR at the end of 8 hours was found increased, with increasing proportion of CP-971. This might be due to lower gel strength of Carbopol matrices compared to HPMC matrices[31] The CP-971 is an acrylic acid polymer and its combined polymeric matrices with HPMC impart lower microenvironment pH within gel layer[31]; which may improve the solubility of some weakly basic drugs. Four batches were prepared keeping the total polymer amount constant i.e. HC10, HC11, HC12 and HC14; and varying the CP-971 concentration as 33%, 50%, 66% and 25% respectively. From the release data of these 4 formulations it was observed that; Carbopol inclusion level of upto 25% as contained in HC14, showed its positive effect on drug release; with highest %CDR of 83.24%. As CP-971 concentration was increased to 33%, 50% and 66%; a decrease in % CDR of 67.9%, 50.59% and 54.87% respectively was observed. This might be due to excessive swelling of CP-971. An excess radial swelling of HC11 and HC12 was observed during the *in vitro* release study. So there might be a possibility of drug molecules being also diffusing in radial direction, availing less drug molecules to diffuse axially.

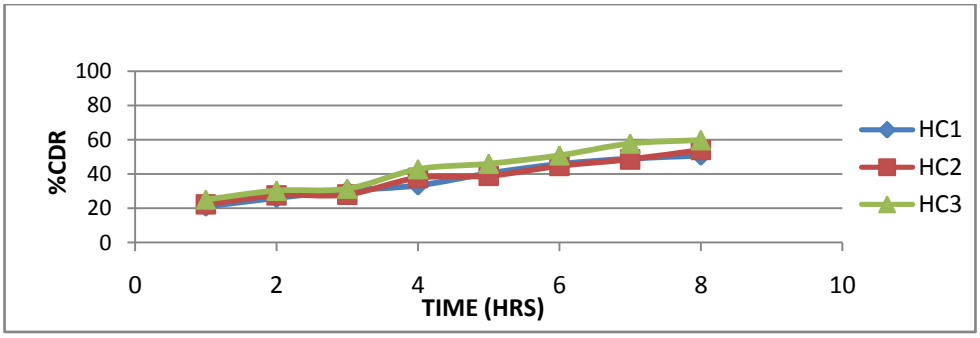

Figure 9: *In vitro* Drug Release profile of HC1, HC2 and HC3

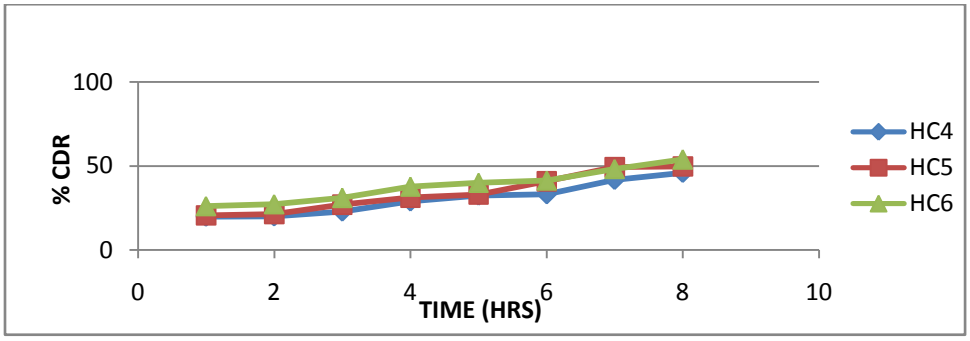

Figure 10: *In vitro* Drug Release profile of HC4, HC5 and HC6

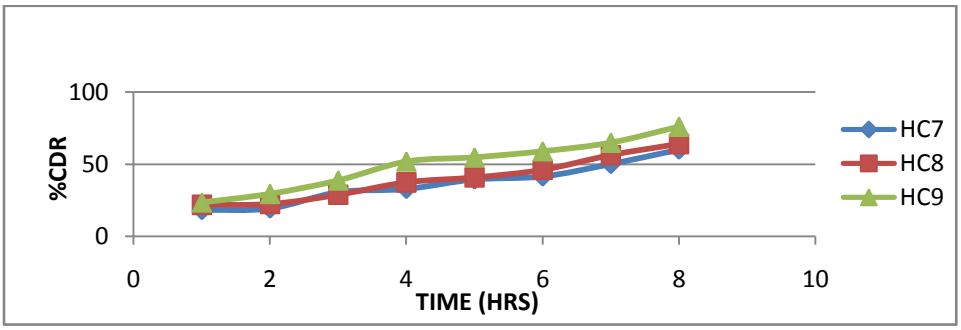

Figure 11: *In vitro* Drug Release profile of HC7, HC8 and HC9

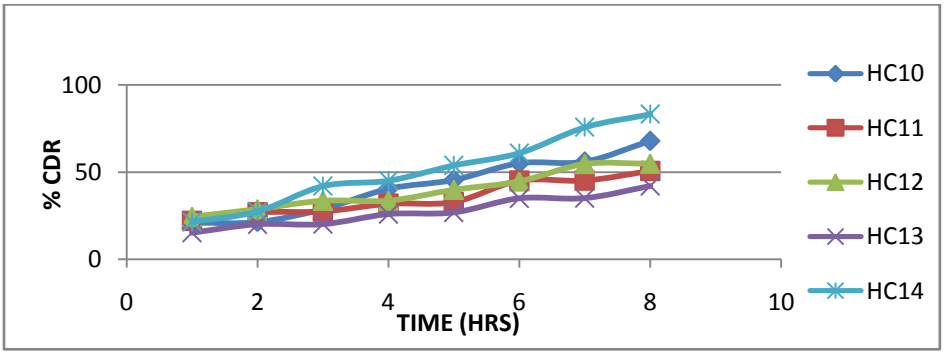

Figure 12: *In vitro* Drug Release profile of HC7, HC8 and HC9

Kinetic modeling of *in vitro* release data of HC Buccal Films

The *in vitro* release data of all HC film formulations was fitted to five mathematical models viz. Zero, First, Higuchi and Korsmeyer-Peppas and were subjected to linear regression analysis. The results obtained are tabulated in table 9. The R values for zero order plots were higher as compared to first order plots; which indicated that all HC film formulations followed zero order kinetics. The data was also best fitted to Higuchi model with correlation coefficient ranging between 0.9478 – 0.9938; indicating diffusion as drug release mechanism. Further data was treated to korsmeyer-Peppas model; where diffusion exponent "n" confirmed the release mechanism. The principle mechanism of drug release was fickian diffusion from all the HC formulations (n value varied from 0.352 to 0.47); except HC7, HC8, HC9, HC10 and HC14 (n value varied from 0.543 to 0.635); which exhibited non-fickian diffusion as release mechanism.

Table 9: Release kinetics data of Buccal Films

Formulation code	Zero order		First order		Higuchi		Peppas	
	R	K	R	K	R	K	R	n
HC1	0.9929	4.489	0.9932	0.072	0.9881	17.42	0.9984	0.448
HC2	0.9903	4.502	0.9875	0.074	0.9773	17.325	0.9682	0.427
HC3	0.9892	5.25	0.989	0.094	0.9938	19.506	0.9691	0.443
HC4	0.9779	3.873	0.9694	0.057	0.9478	14.637	0.9277	0.42
HC5	0.9817	4.615	0.9728	0.072	0.9571	17.545	0.9451	0.463
HC6	0.986	3.962	0.9775	0.066	0.9646	15.115	0.9483	0.352
HC7	0.9843	5.797	0.9683	0.096	0.9654	22.17	0.9635	0.582
HC8	0.9873	6.197	0.9695	0.109	0.9607	23.513	0.9501	0.543
HC9	0.9882	7.249	0.9785	0.153	0.987	28.231	0.9875	0.571
HC10	0.9869	6.96	0.9763	0.127	0.9702	26.677	0.9555	0.611
HC11	0.9704	4.099	0.9627	0.065	0.9459	15.579	0.9369	0.393
HC12	0.98	4.547	0.9648	0.078	0.9586	17.343	0.9467	0.396
HC13	0.9808	3.671	0.9747	0.052	0.961	14.024	0.9604	0.47
HC14	0.993	8.802	0.9391	0.189	0.9767	31.631	0.9855	0.635
HCC1	0.9917	9.550	0.9043	0.336	0.9697	36.840	0.9813	0.613
HCC2	0.9821	10.042	0.9059	0.299	0.9477	37.779	0.8912	0.631
HCC3	0.9917	10.350	0.8920	0.265	0.9684	36.641	0.9875	0.736
HCC4	0.9893	10.461	0.9278	0.329	0.9622	39.674	0.9503	0.677
HCC5	0.9915	8.901	0.9574	0.227	0.9748	34.124	0.9752	0.622
HCC6	0.9934	8.813	0.9688	0.215	0.9795	33.885	0.9816	0.632

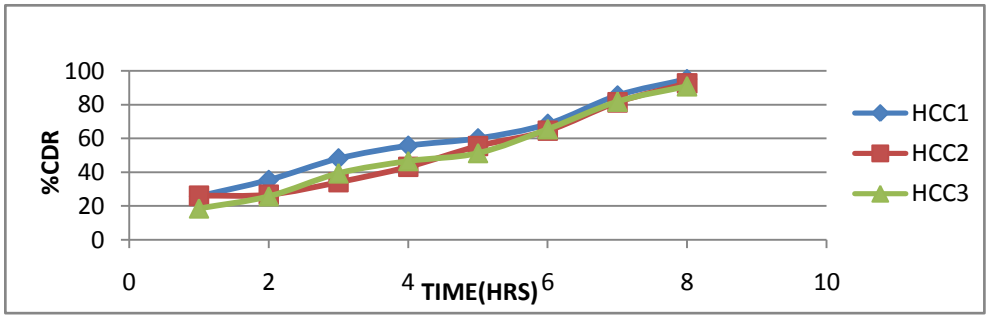

Figure 13: *In vitro* Drug Release profile of HCC1, HCC2 and HCC3

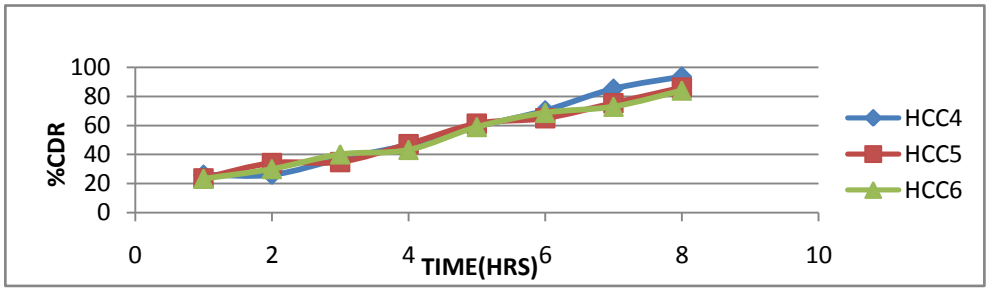

Figure 14: *In vitro* Drug Release profile of HCC4, HCC5 and HCC6

***In vitro* release study of Repaglinide inclusion complex loaded buccal films**

The *in vitro* release profile of all the HCC formulations (HCC1 to HCC6) is shown in figure 13 and 14. Cumulative percentage of drug release (% CDR) at the end of 8 hours was found to be

highest for HCC1 i.e. 95.18 %. It was observed that; with increase in HPMC proportion, % CDR at the end of 8 hours was decreased. Further, as the concentration of CP-971 was increased; a decrease in % CDR was observed. In inclusion complex loaded films; the CP-971 showed no effect on the drug release. This might be due to inclusion of hydrophobic portion of the drug within the cyclodextrin cavity, which is responsible for its higher solubility at lower pH. Overall it was observed that, cumulative percentage of drug release was increased with decrease in total polymer amount. Since the highest % CDR was given by HCC1 (95.18 %) among all the formulations and also showed acceptable physicochemical properties of the film; it was chosen as an optimum formulation.

Kinetic modeling of *in vitro* release data of HCC Buccal Films

The *in vitro* release data of all HCC film formulations was fitted to four mathematical models viz. Zero, First, Higuchi and Korsmeyer-Peppas. The results obtained are tabulated in table 9. The R values for zero order plots were higher as compared to first order plots; which indicated that all HCC film formulations followed zero order kinetics. The data was also best fitted to Higuchi model with correlation coefficient ranging between 0.9477 – 0.9795; indicating diffusion as drug release mechanism. Further data was treated to korsmeyer-Peppas model; where diffusion exponent "n" confirmed the release mechanism. The principle mechanism of drug release from all the HCC formulations was non-fickian diffusion, as confirmed from 'n' value that varied from 0.613 to 0.736.

Stability studies

Results of stability studies are reported in table 10.

Table 10: Stability data of an Optimized Film Formulation (HCC1)

Parameters	**Storage conditions**	
	Room temperature	**40 ± 2 ºC / 75 % RH**
Appearance	Translucent and flexible	Translucent and flexible
% Drug Content	97.30	96.97
Bioadhesive Strength (gm)	40.18	39.50
% Cumulative Drug Release	95.27	94.03

CONCLUSION

A comparative study was carried out between the pure Repaglinide loaded and inclusion complex loaded Buccal films. It was observed that the inclusion complex loaded Buccal films (HCC) showed higher cumulative percentage of drug release (%CDR) at the end of 8 h as compared to pure drug loaded Buccal films. Among the complex loaded Buccal films, HCC1 film formulation showed highest %CDR (95.18 %) at the end of 8 hrs. Also the physicochemical

parameters were found to be acceptable. All the formulations showed quite a good mucoadhesion for approximately 7 hrs and more. The surface pH of all the films was found to be in acceptable neutral pH range i.e. 6.09 – 7.03. From the study; the Buccal Drug Delivery System was found to be promising and successful in delivering the antihyperglycaemic drug, Repaglinide for prolonged duration of time with increased bioavailability. Thus sustaining the therapy with reduction in dose and dosing frequency. The Repaglinide Inclusion Complex loaded Buccal films was able to substantially increase the percentage of drug release from the films; thereby increasing the bioavailability. Kinetic treatment of in vitro release data revealed that all HC film formulations followed zero order release kinetics and Fickian diffusion as the release mechanism; except HC7, HC8, HC9, HC10 and HC14 which exhibited non-Fickian diffusion mechanism. The reason for exception could not be attributed to any of the formulation parameter. The HCC Buccal films followed zero order kinetics and non-Fickian diffusion as the principle mechanism of drug release. The stability study results of optimized HCC1 buccal film revealed that; the film formulation remained physicochemically stable. The *in vitro* release data was also in agreement with the pre-stability release data.

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