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Formulation And Evaluation Of Mucoadhesive Buccal Tablets (Repaglinide) For Management Of Diabetes

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Abstract

Repaglinide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus. The main objective of the study was to formulate and evaluate bioadhesive buccal tablets to avoid the first pass metabolism in liver. Bioadhesive buccal tablets were prepared by direct compression method using bioadhesive polymers like HPMC K4 M, HPMC K15 M, Chitosan, HPMC K100 M, Sodium CMC, Carbopol 974 P, Sodium Alginate, Gum karaya and Carbopol 941NF in different ratios. The physicochemical compatibility of drug and polymers was studied by FT-IR spectroscopy. Prepared tablets were evaluated for permeation study through porcine buccal mucosa, in vitro drug release, bioadhesion strength, swelling index, moisture absorbance, surface pH.

1. INTRODUCTION

Buccal Delivery involves the administration of drug through buccal mucosal membrane (the lining in the oral cavity).[1]The drug directly reaches to the systemic circulation through the internal jugular vein and bypasses the drugs from the hepatic first pass metabolism, which leads to high bioavailability.[2] A suitable buccal drug delivery system should be flexible and should posses good bioadhesive properties, so that it can be retained in the oral cavity for the desired duration. Bioadhesive formulations have been developed to enhance the bioavailability[8,9] of drugs that undergo substantial first pass hepatic effect and to control the drug release to a constant rate.[10] In addition ,it should release the drug in a controlled and predictable manner to elicit the required therapeutic response.[11-13] Various buccal mucosal dosage forms are suggested for oral delivery which includes: buccal tablets, buccal Patches and buccal gels.[14,15]

Advantages [6,9]

- Significant reduction in dose related side effects.
- It provides direct entry of drug into systemic circulation.
- Drug degradation in harsh gastrointestinal environment can be circumvented by administering the drug via buccal route.
- Drug absorption can be terminated in case of emergency.
- It offers passive system, which does not require activation.
- Rapid cellular recovery following local stress or damage.
- Ability to withstand environmental extremes like change in pH, temperature etc. Sustained drug delivery.
- The potential for delivery of peptide molecules unsuitable for the oral route.

General criteria for candidate's drug: [9,10]

One of the drug properties required for the practical buccal formulation will be high pharmacological activity or a low dose requirement. The Limit size of the dosage form should not exceed 12 cm^2 for buccal application or 3cm^2 for sublingual or gingival application. The following properties will make the drug suitable candidate for buccal delivery:

- In general, any drug with a daily requirement of 25mg or less would make a good candidate
- Relatively short biological half-life:- Drugs with biological half-life 2-8 hr will in general be good candidates for sustained release dosage forms
- The maximal duration of buccal delivery is approximately 4–8 hr
- Drug must undergo first pass effect or it should have local effect in oral cavity.
- Drugs susceptible to degradation:-Drug degradation either by stomach/intestinal enzymes or by first pass hepatic metabolism will be assured protection in buccal dosage form.
- Drug must undergo first pass effect or it should have local effect in oral cavity.

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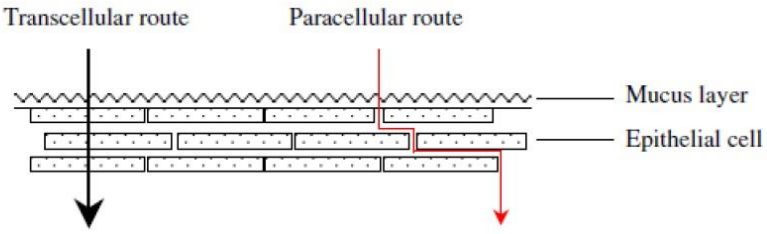
Absorption pathways

Studies with microscopically visible tracers such as small proteins and dextrans suggest that the major pathway across stratified epithelium of large molecules is via the intercellular spaces and that there is a barrier to penetration as a result of modifications to the intercellular substance in the superficial layers. However, rate of penetration varies depending on the physicochemical properties of the molecule and the type of tissue being traversed. This has led to the suggestion that materials uses one or more of the following routes simultaneously to cross the barrier region in the process of absorption, but one route is predominant over the other depending on the physicochemical properties of the diffusant.(11)

- Passive diffusion
  - Transcellular or intracellular route (crossing the cell membrane and entering the cell).
  - Paracellular or intercellular route (passing between the cells).
- Carrier mediated transport.
- Endocytosis.



Penetration routes in buccal delivery(26)

FACTORS IMPORTANT FOR BIOADHESION

I . Polymer related factors

1.Molecular weight:(9)

The inter penetration of polymer molecules is favorable for low molecular weight polymers where as entanglements are favored for high molecular weight polymers. The optimum molecular weight for the maximum bioadhesion depends on the type of polymers. Their nature dictates the degree of swelling in water in turn determines interpenetration of polymer molecules within the mucus. The bioadhesive force increases with the molecular weight of the bioadhesive polymer up to 100,000 and beyond this level there is not much effect.

2.Concentration of active polymer:(9,10)

There is an optimum concentration of polymer corresponding to the best bioadhesion. In highly concentrated systems the adhesive strength drops significantly. In fact, in concentrated solutions the coiled molecules become solvent poor and the chains available for interpenetration are not numerous.

3.Flexibility of polymer chain:(10)

As water-soluble polymers become crossed linked, the mobility of the individual polymer chain decreases. As the cross linking density increases, the effective length of the chain which can penetrate into the mucus layer decreases even further and mucoadhesive strength is reduced

4.Spatial conformation:(11)

Despite a high molecular weight of 19,500,000 for dextrans, they have similar adhesive strength to that of PEG with a molecular weight of 200,000.

5.Swelling:(10,11)

This characteristic is related to the polymer itself, and also to its environment. Interpenetration of chains is easier as polymer chains are disentangled and free of interactions. Swelling depends both on polymer concentration and on presence of water. When swelling is too great, a decrease in bioadhesion occurs. Such a phenomenon must not occur too early in order to lead to a sufficient action of the bioadhesive system. Its allows easy detachment of the bioadhesive system after the discharge of the active ingredient.

II. Environment related factors:[12]

1. pH:

It was found to have a significant effect on the mucoadhesion as observed in studies of polyacrylic polymers cross linked with –COOH groups. pH influences the charge on the surface of both mucus and polymers. Mucus will have a different charge density depending on pH because of differences in dissociation of functional groups on the carbohydrate moiety and amino acids of the polypeptide back bone.

2. Applied strength:

To place a solid bioadhesive system, poly (Acrylic acid /Divinyl benzene) or Carbopol 934, the adhesion strength increases with the applied strength. The pressure initially applied to the mucoadhesive tissue contact site can affect the depth of interpenetration.

3. Initial contact time:

The initial contact time between mucoadhesives and the mucus layers determines the extent of swelling and interpenetration of polymer chains. Along with internal pressure, the initial contact time can dramatically affect the performance of the system.

4. Selection of the model substrate surface:

The handling and treatment of biological substrates during the testing of mucoadhesives in an important factor, since physical and biological changes may occur in the mucus gels or tissues under the experimental conditions.

2. MATERIALS & METHODS

Materials

HPMC K4M, HPMC K15M, HPMC K100M, Chitosan, Sodium CMC, Carbopol 974P, sodium alginate, Gum karaya, Carbopol 941NF. All other chemicals used in the study were of analytical grade which are obtained from SURA Labs Pvt Ltd.

Method

Buccal Tablet Preparation

Buccal tablets were prepared by a direct compression method, before going to direct compression all the ingredients were screened through sieve no.100. HPMC K4M, HPMC K15M, HPMC K100M, Chitosan, Sodium CMC, Carbopol 974P, Sodium alginate, Gum karaya, Carbopol 941NF are the mucoadhesive and biodegradable polymers used in this preparation of buccal mucoadhesive drug delivery systems. Repaglinide was mixed manually with different ratios of HPMC K4M, HPMC K15M, HPMC K100M, Chitosan, Sodium CMC, Carbopol 974P, sodium alginate, Gum karaya, and Microcrystalline Cellulose as diluent for 10 min. In every formulation constant amount of PVPK30 was added as binding agent. The blend was mixed with aerosil and magnesium stearate for 3-5 min. Buccal tablets were compressed by a Single punch tablet machine. Tablet weight was kept constant 100mg, and the thickness of tablet was adjusted to 2.9 mm. Tablets were stored in air tight container away from the light for further studies. The Composition of 24 formulation of Buccal Tablets are mentioned in the following Table 1 & Table 2.

Table: 1

Ingredients (mg)	RF1	RF 2	RF3	RF4	RF5	RF6	RF7	RF8	RF9	RF10	RF11	RF12
Repaglinide	2	2	2	2	2	2	2	2	2	2	2	2
HPMC K4 M	12	16	20	-	-	-	-	-	-	-	-	-
HPMC K15 M	-	-	-	12	16	20	-	-	-	-	-	-
Chitosan	-	-	-	-	-	-	12	16	20	-	-	-
HPMC K100 M	-	-	-	-	-	-	-	-	-	12	16	20
Sodium CMC	-	-	-	-	-	-	-	-	-	-	-	-
Carbopol 974 P	-	-	-	-	-	-	-	-	-	-	-	-
Sodium Alginate	-	-	-	-	-	-	-	-	-	-	-	-
Gum karaya	-	-	-	-	-	-	-	-	-	-	-	-
Carbopol 941NF	6	6	6	6	6	6	6	6	6	6	6	6
PVP K30	5	5	5	5	5	5	5	5	5	5	5	5
MCC pH 102	71	67	63	71	67	63	71	67	63	71	67	63
Mg. Stearate	1	1	1	1	1	1	1	1	1	1	1	1
Aerosil	3	3	3	3	3	3	3	3	3	3	3	3
Total Weight (mg)	100	100	100	100	100	100	100	100	100	100	100	100

Table: 2

Ingredients (mg)	RF13	RF14	RF15	RF16	RF17	RF18	RF19	RF20	RF21	RF22	RF23	RF24
Repaglinide	2	2	2	2	2	2	2	2	2	2	2	2
HPMC K4 M	-	-	-	-	-	-	-	-	-	-	-	-
HPMC K15 M	-	-	-	-	-	-	-	-	-	-	-	-
HPMC K100 M	-	-	-	-	-	-	-	-	-	-	-	-
Chitosan	-	-	-	-	-	-	-	-	-	-	-	-
Sodium CMC	12	16	20	-	-	-	-	-	-	-	-	-
Carbopol 974 P	-	-	-	12	16	20	-	-	-	-	-	-
Sodium Alginate	-	-	-	-	-	-	12	16	20	-	-	-
Gum karaya	-	-	-	-	-	-	-	-	-	12	16	20
Carbopol 941NF	6	6	6	6	6	6	6	6	6	6	6	6
PVP K30	5	5	5	5	5	5	5	5	5	5	5	5
MCC pH 102	71	67	63	71	67	63	71	67	63	71	67	63
Mg. Stearate	1	1	1	1	1	1	1	1	1	1	1	1
Aerosil	3	3	3	3	3	3	3	3	3	3	3	3
Total Weight (mg)	100	100	100	100	100	100	100	100	100	100	100	100

3. Pre formulation Studies:

3.1. Physical Characterisation of Compressed blend:

After the Blend preparation there are many formulations and process variables involved in mixing and all these can affect the characterization of blends produced. Prior to compression, granules were evaluated for their characteristic parameter such as Tapped density, Bulk density, Carr's index, Angle of repose, Hausner's ratio. For Bulk density determination 30 gm of powder blend introduced into a dry 100 mL cylinder, without compacting and powder volume was recorded. Tapped density was obtained by using tapped density apparatus----, with a fixed drop of 14±2 mm at a nominal rate of 300 drops per minute. The cylinder was tapped 500 times initially followed by an additional tap of 750 times until difference between succeeding measurement is less than 2%. Compressibility index (carr's index), Hausner's ratio was calculated from the bulk and tapped density using the equations 1& 2

$$\textbf{Carr's index} = [(\rho_{tap}-\rho b)]/\rho_{tap}]\times 100 \quad (1)$$

$$\textbf{Hausner's Ratio} = \rho_{tap}/\ \rho b \quad (2)$$

Where, ρ_{tap} = Tapped density.
ρb = Bulk density.

3.2. Drug-excipient compatibility studies [14,15]

A Fourier Transform-Infra Red spectrophotometer was used to study the non-thermal analysis of drug-excipient (binary mixture of drug: excipient 1:1 ratio) compatibility. The spectrum of each sample was recorded over the 450-4000 cm^{-1}. Pure drug of Repaglinide, Repaglinide with physical mixture (excipients) compatibility studies were performed.

3.3. Analytical Quantification of Repaglinide in UV Spectroscopy

100 mg of Repaglinide was dissolved in small amount of phosphate buffers 6.8 and 7.4 seperately and make the volume up to 100 mL with phosphate buffer pH 6.8 and 7.4 in two Volumetric flasks. From this two primary stock (1mg/mL), 10 mL solution was transferred to another volumetric flask 1 made up to 100 mL with Phosphate buffer pH 6.8 and in another flask 2 with Phosphate buffer pH 7.4. From this secondary stock of two flasks 0.4 ,0.8, 1.2, 1.6, 2.0, 2.4 and 2.8 mL was taken separately and made up to 10 mL with phosphate buffer pH 6.8 and Phosphate buffer pH 7.4 to produce 4, 8, 12,16, 18, 20, 24, 28 µg/mL respectively. The absorbance was measured at 282 nm using a UV spectrophotometer.

3.4. Solubility Studies [1,11]

The solubility of Repaglinide in phosphate buffer solution pH 6.8 was determined by phase equilibrium method. An excess amount of drug was taken into 20 mL vials containing 10 mL of phosphate buffers (pH 6.8). Vials were closed with rubber caps and constantly agitated at room temperature for 24 hr using rotary shaker. After 24 hr, the solution was filtered through 0.2 µm Whitman's filter paper. The amount of drug solubilized was then estimated by measuring the absorbance at 282 nm using a UV spectrophotometer. The standard curves for Repaglinide were established in phosphate buffer (pH 6.8) and from the slope of the straight line the solubility of Repaglinide was calculated. The studies were repeated in triplicate (n = 3), and mean was calculated.

4. Evaluation of Buccal Tablets: [12,13]

4.1. Physicochemical characterization of tablets:

The prepared Repaglinide buccal tablets were studied for their physicochemical properties like weight variation, hardness, thickness, friability and drug content (assay).

4.2. Weight variation:

The weight variation test is done by taking 20 tablets randomly and weighed accurately. The composite weight divided by 20 provides an average weight of tablet. Not more than two of the individual weight deviates from the average weight by 10 %. The percent deviation was calculated using the following formula:
% Deviation = (Individual weight – Average weight / Average weight) X 100

4.3. Tablet Thickness:

The thickness and diameter of the tablets was determined using a Digital Vernier caliper. Ten tablets from each formulation were used and average values were calculated.

4.4. Tablet Hardness:

Six tablets were taken from each formulation and hardness was determined using Monsanto hardness tester and the average was calculated. It is expressed in Kg/cm2.

4.4. Friability:

A sample of preweighed tablets was placed in Roche friabilator which was then operated for 100 revolutions at a speed of 25 rpm for 4 minutes, dropping the tablets to a distance of 6 inches in each revolution. Percent friability (% F) was calculated as

$$\text{Friability (\%)} = \frac{\text{Initial weight of 10 tablets – final weight of 10 tablets}}{\text{Initial weight of 10 tablets}} \times 100$$

$F\ (\%) = [Wo\text{-}W/W_O] \times 100$
Where, W_0 is the initial weight of the tablets before the test and
W is the final weight of the tablets after test.

4.4. Assay:

Six tablets of each formulation were taken and amount of drug present in each tablet was determined. Powder equivalent to one tablet was taken and added in 100 mL of pH 6.8 phosphate buffer followed by stirring for 10 minutes. The solution was filtered through a 0.45µ membrane filter, diluted suitably and the absorbance of resultant solution was measured by using UV-Visible spectrophotometer at 282 nm using pH 6.8 phosphate buffer.

5. ***In vitro*** **release studies:**

The drug release rate from buccal tablets was studied using the USP type II dissolution test apparatus. Tablets were supposed to release the drug from one side only; therefore an impermeable backing membrane was placed on the other side of the tablet. The tablet was further fixed to a 2x2 cm glass slide with a solution of cyanoacrylate adhesive. Then it was placed in the dissolution apparatus. The dissolution medium was 500 mL of pH 6.8 phosphate buffer at 50 rpm at a temperature of 37 ± 0.5 °C. Samples of 5 mL were collected at different time intervals up to 8 hrs and analyzed after appropriate dilution by using UV Spectrophotometer at 282 nm.

6. Kinetic Analysis of Dissolution Data: [14,15]

To analyze the *in vitro* release data various kinetic models were used to describe the release kinetics.
1. Zero – order kinetic model – Cumulative % drug released versus time.

$$\mathbf{A_t = A_0 – K_0 t}$$

Where, A_t = Drug release at time‘t’.
A_0 = Initial drug concentration
K_0 = Zero – order rate constant (hr^{-1}).

2. First – order kinetic model – Log cumulative percent drug remaining versus time.

Log C = log C0 – Kt / 2.303

Where, C = Amount of drug remained at time‘t’.
C0 = Initial amount of drug.
K = First – order rate constant (hr-1).

3. Higuchi’s model – Cumulative percent drug released versus square root of time.
$Q = [D\varepsilon / \tau (2A - \varepsilon Cs) Cst]^{1/2}$
Where, Q = Amount of drug released at time‘t’.
D = Diffusion coefficient of the drug in the matrix.
A = Total amount of drug in unit volume of matrix.
Cs = the solubility of the drug in the matrix.
ε = Porosity of the matrix.
τ = Tortuosity.
t = Time (hrs) at which ‘q’ amount of drug is released.

4. Korsmeyer equation / Peppa’s model – Log cumulative % drug released versus log time.
$M_t / M_a = Kt^n$

Where, M_t / M_a = the fraction of drug released at time‘t’.
K = Constant incorporating the structural and geometrical characteristics of the drug / polymer system.
n = Diffusion exponent related to the mechanism of the release.

7. Swelling Studies:

Buccal tablets were weighed individually (designated as W_1) and placed separately in Petri dishes containing 15 mL of phosphate buffer (pH 6.8) solution. At regular intervals (0.5, 1, 2, 3, 4, 5 and 6 hr), the buccal tablets were removed from the Petri dishes and excess surface water was removed carefully using the filter paper. The swollen tablets were then reweighed (W_2**)**. This experiment was performed in triplicate. The swelling index (water uptake) calculated according to the following Eq**.**

$$\text{Swelling index} = \frac{(W_2 - W_1)}{} \times 100$$

***8. In vitro* bioadhesion strength:**

Bioadhesion strength of tablets were evaluated using a microprocessor based on advanced force gauge equipped with a motorized test stand (Ultra Test Tensile strength tester, Mecmesin, West Sussex, UK) according to method describe as it is fitted with 25 kg load cell, in this test porcine membrane was secured tightly to a circular stainless steel adaptor and the buccal tablet to be tested was adhered to another cylindrical stainless steel adaptor similar in diameter using a cyanoacrylate bioadhesive. Mucin 100 µl of 1 % w/v solution was spread over the surface of the buccal mucosa and the tablet immediately brought in contact with the mucosa. At the end of the contact time, upper support was withdrawn at 0.5mm/sec until the tablet was completely detached from the mucosa. The work of adhesion was determined from the area under the force distance curve.

The peak detachment force was maximum force to detach the tablet from the mucosa.

$$\text{Force of adhesion} = \frac{\text{Bio adhesion strength}}{1000} \times 9.8$$

$$\text{Bond strength} = \frac{\text{Force of adhesion}}{\text{Surface area}}$$

9. Surface pH:

Weighed tablets were placed in boiling tubes and allowed to swell in contact with pH 6.8 phosphate buffer (12 mL). Thereafter, surface pH measurements at predetermined intervals of 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h were recorded with the aid of a digital pH meter. These measurements were conducted by bringing a pH electrode near the surface of the tablets and allowing it to equilibrate for 1 min prior to recording the readings. Experiments were performed in triplicate (n=3).

10. Moisture absorption:

Agar (5% m/V) was dissolved in hot water. It was transferred into Petri dishes and allowed to solidify. Six buccal tablets

from each formulation were placed in a vacuum oven overnight prior to the study to remove moisture, if any, and laminated on one side with a water impermeable backing membrane. They were then placed on the surface of the agar and incubated at 37 °C for one hour. Then the tablets were removed and weighed and the percentage of moisture absorption was calculated by using following formula:

$$\% \text{ Moisture Absorption} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

RESULTS & DISCUSSION

Preformulation study

FTIR Compatibility Studies:

FTIR spectra of pure drug and formulation with other ingredients were recorded. The spectrum of each sample was recorded over the 450-4000 cm^{-1}. There was no appearance or disappearance of any characteristics peak in the FTIR spectrum of drug and the polymers used. This shows that there is no chemical interaction between the drug and the polymers used. The FTIR Spectra of pure Repaglinide drug and polymer was compared with the FTIR spectrum of drug and optimised in the Figures 1,2.

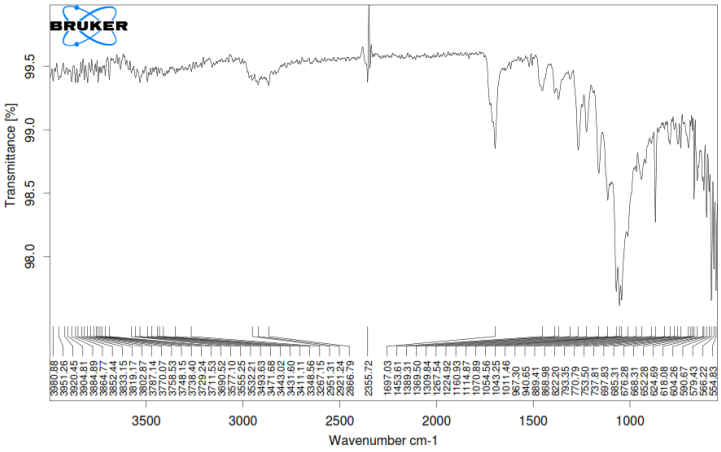


Fig 1: FTIR studies of pure drug Repaglinide

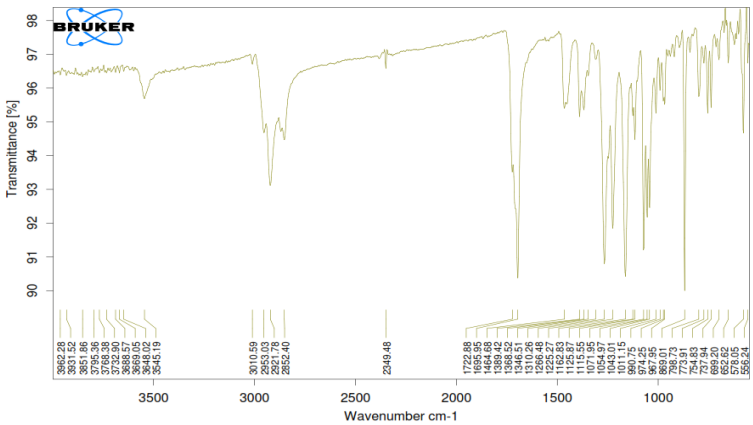


Fig 2: FTIR compatibility studies of optimized formulation

Solubility studies:

The solubility of the Repaglinide in phosphate buffer pH 6.8 is 88 µg/mL and it was selected as the suitable media for the release studies because the pH of the phosphate buffer pH 6.8 is nearer to that of buccal mucosa pH. The results revealed that the solubility of the Repaglinide was increased from pH 6.8 to 7.4. The studies were shown in the Table3.

Table 3: Solubility studies of Repaglinide

Medium	Amount present (µg/mL)
Distilled water	22.59
Phosphate buffer pH 6.8	88
Phosphate buffer pH 7.4	94

Standard graph in phosphate buffer pH 6.8 (λ_{max} 282 nm):

The standard graph of Repaglinide showed good linearity with R^2 of 0.999, which indicates that it obeys "Beer- Lamberts" law in the range 0-28 µg/mL. Its linearity is shown in Table 4 and Figure 3.

Table 4: Standard graph of Repaglinide in phosphate buffer pH 6.8

Concentration (µg/mL)	Absorbance
0	0
4	0.102
8	0.198
12	0.275
16	0.365
20	0.444
24	0.532
28	0.622

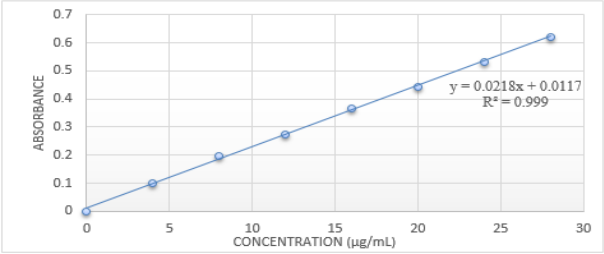


Figure 3

Characterization of Precompression Blend:

The precompression blend for Buccal tablets were characterized with respect to Angle of repose was less than 33.65°, Carr's index values were less than 19.39 and Hausner's ratio was less than 1.24 for all formulations indicating good to fair flow ability, compressibility and flow property shown in Table 5.

Table 5: Physical Properties of Precompression Blend

Formulation Code	Angle of repose (θ)	Bulk density (g/mL)	Tapped density(g/mL)	Carr's index (%)	Hausner's ratio
RF1	25.49	0.214	0.251	14.74	1.17
RF2	26.24	0.308	0.364	15.38	1.18
RF3	29.05	0.276	0.322	14.28	1.16
RF4	26.97	0.341	0.388	12.11	1.13
RF5	29.25	0.324	0.376	13.82	1.16
RF6	32.27	0.320	0.397	19.39	1.24
RF7	33.65	0.521	0.629	17.17	1.20
RF8	33.21	0.518	0.627	17.38	1.21
RF9	26.56	0.422	0.506	16.60	1.19
RF10	28.75	0.481	0.572	15.90	1.18
RF11	27.33	0.475	0.566	16.07	1.19
RF12	25.38	0.524	0.599	12.52	1.14
RF13	26.43	0.412	0.483	14.69	1.17
RF14	24.77	0.488	0.537	9.12	1.10
RF15	26.42	0.439	0.521	15.73	1.18
RF16	28.19	0.559	0.649	13.94	1.16
RF17	29.58	0.331	0.393	15.77	1.18
RF18	28.73	0.362	0.428	15.42	1.18
RF19	30.45	0.386	0.473	18.39	1.22
RF20	26.43	0.375	0.442	15.15	1.17
RF21	19.29	0.434	0.497	12.67	1.14
RF22	21.25	0.520	0.582	10.65	1.11
RF23	26.27	0.487	0.561	13.19	1.15
RF24	25.49	0.494	0.566	12.72	1.14

Each value represents the mean value (n =3).

Physicochemical Characterization of Buccal Tablets:

Acceptable physicochemical properties were observed for the prepared buccal tablets all the formulated tablets passed the weight variation test within the limits as per USP, thickness varied from 2.00 to 2.98, hardness compression force

applied (5.0 – 6.2 kg/cm²), friability was below 1%, assay values in the range of 97.42 to 100.14 of all compressed tablets were within the limits as per USP shown in Table 6.

Table 6: Physico-chemical parameters of Repaglinide buccal tablets

Formulation Code	Weight variation (mg)	Thickness (mm)	Hardness (kg/cm$^{2)}$	Friability (%)	Assay (%)
RF1	100.12	2.61	5.1	0.62	98.61
RF2	99.86	2.14	5.9	0.54	99.74
RF3	100.25	2.81	6.0	0.38	98.32
RF4	98.68	2.36	5.6	0.49	99.64
RF5	100.21	2.80	6.2	0.51	99.56
RF6	99.86	2.91	5.4	0.37	97.42
RF7	99.57	2.05	5.3	0.61	98.61
RF8	100.08	2.34	6.1	0.59	99.82
RF9	100.31	2.62	5.9	0.34	99.24
RF10	99.87	2.19	5.7	0.52	98.46
RF11	97.59	2.00	6.1	0.49	99.29
RF12	99.38	2.78	5.4	0.63	99.52
RF13	100.25	2.81	5.3	0.71	98.68
RF14	100.47	2.54	5.5	0.65	99.87
RF15	99.34	2.98	5.6	0.48	98.46
RF16	100.09	2.33	5.8	0.59	99.81
RF17	99.37	2.50	6.1	0.39	99.37
RF18	98.65	2.18	5.7	0.51	100.14
RF19	97.34	2.51	5.3	0.48	99.52
RF20	100.19	2.64	5.0	0.28	98.64
RF21	100.02	2.08	6.2	0.34	100.01
RF22	99.89	2.11	5.4	0.29	99.64
RF23	99.72	2.34	5.8	0.41	98.37
RF24	100.05	2.52	5.9	0.62	99.81

Each value represents the mean value (*n* =3).

***In vitro* drug release studies:**

In vitro drug release studies revealed that the release of Repaglinide from different formulations varied according to the type and ratios of the matrix forming polymers.From the dissolution studies, it was observed that as the concentration of repaglinide increases drug release was prolonged indicating the drug release retarding ability of the HPMC K100M. Formulation RF11 containing drug to HPMC K100M in the ratio of 1:8 and extended the drug release up to 6 h and was considered as preliminary optimized formulation (Table7 to 14) & (Figure 4 to 9).

Table 7. Repaglinide formulations with

Time (hrs)	Cumulative percentage drug release		
	RF1	RF2	RF3
0	0	0	0
1	51.35	43.64	31.85
2	68.18	53.25	42.15
3	76.92	62.98	57.62
4	85.06	68.16	61.22
5	90.15	78.50	67.85
6	93.73	80.14	72.98

Table 8. Repaglinide formulations with HPMC K4M HPMCK15M

Time (hrs)	Cumulative percentage drug release		
	RF4	RF5	RF6
0	0	0	0
1	41.12	47.35	44.36
2	52.81	59.19	56.91
3	61.63	65.02	62.89
4	69.19	79.56	70.05
5	75.06	82.18	76.20
6	85.14	96.36	94.64

Table 9. Formulations with HPMC K100 M.

Time (hrs)	Cumulative percentage drug release		
	RF7	RF8	RF9
0	0	0	0
1	38.68	46.35	55.05
2	46.55	52.19	64.56
3	58.62	65.56	73.25
4	62.18	73.29	83.53
5	65.72	79.86	89.50
6	72.35	83.21	98.42

Table 10. Formulations with Chitosan.

Time (hrs)	Cumulative percentage drug release		
	RF10	RF11	RF12
0	0	0	0
1	33.83	52.15	31.97
2	48.16	61.83	45.56
3	56.93	74.19	53.82
4	60.18	84.43	65.19
5	66.08	96.19	77.34
6	71.11	99.43	82.97

Table 11. Formulations with Sodium CMC

Time (hrs)	Cumulative percentage drug release		
	RF13	RF14	RF15
0	0	0	0
1	53.18	41.81	31.92
2	63.34	52.96	44.75
3	74.19	60.47	50.29
4	80.62	67.36	56.09
5	86.36	72.97	67.79
6	96.17	81.83	73.34

Table12. Formulations with Carbopol974P

Time (hrs)	Cumulative percentage drug release		
	RF16	RF17	RF18
0	0	0	0
1	30.96	42.62	53.39
2	46.14	48.92	64.14
3	51.83	53.06	73.81
4	57.23	62.53	81.08
5	64.09	73.85	87.43
6	71.18	81.32	95.27

Table 13. Formulations with Sodium alginate

Time (hrs)	Cumulative percentage drug release		
	RF19	RF20	RF21
0	0	0	0
1	38.89	55.31	45.91
2	45.17	60.85	62.03
3	50.36	75.96	68.75
4	56.89	82.36	75.19
5	61.26	87.19	92.43
6	64.19	97.54	95.79

Table 14. Formulations with Gum Karaya

Time (hrs)	Cumulative percentage drug release		
	RF22	RF23	RF24
0	0	0	0
1	50.38	39.62	32.46
2	68.17	52.92	44.11
3	74.63	58.06	53.38
4	80.27	63.53	56.19
5	86.88	70.85	62.76
6	92.09	82.32	70.39

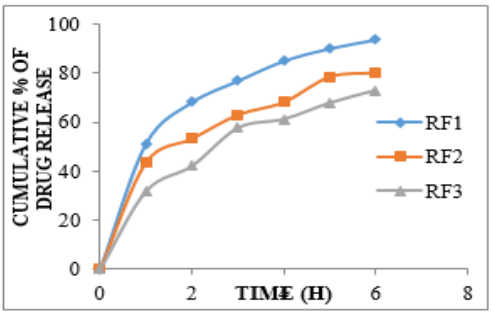


Figure 4

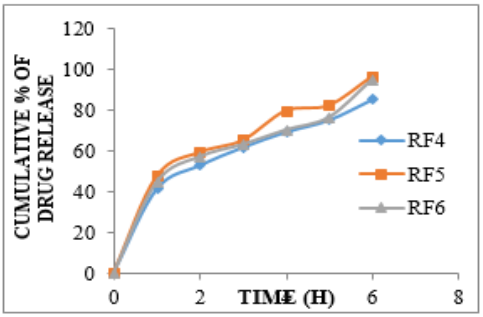


Figure 5

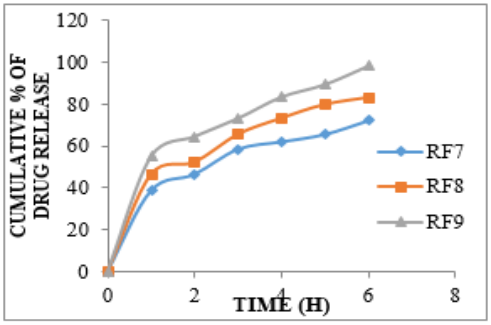


Figure 6

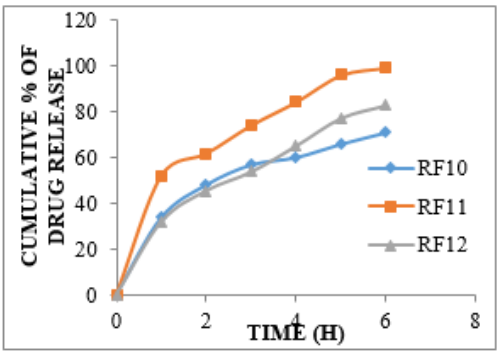


Figure 7

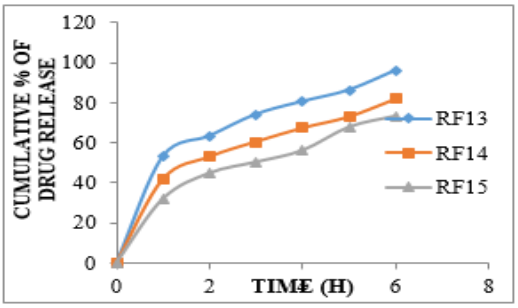


Figure 8

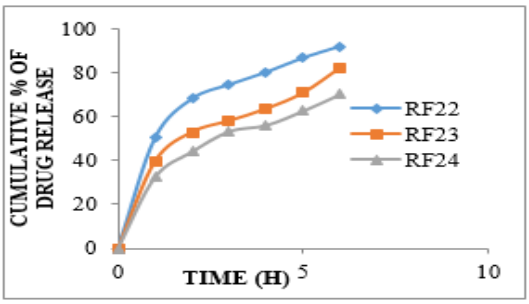


Figure 9

Surface pH Study:

The surface pH of the formulations was found to be 6.0 to 6.9 and the pH was near to the neutral. These results suggested that the polymeric blend identified was suitable for oral application and formulations were not irritant to the buccal mucosa. Surface pH values for all the formulations shown in Table-----.

Moisture absorption test:

Moisture absorption of the mucoadhesive buccal tablets is in the range of 42.1% to 86.9%, which shows that the tablets have suitable moisture absorption capacity. The highest moisture absorption of formulation was RF11.

***Ex vivo* bioadhesive strength measurement:**

The results revealed that the carbopol 974 P containing formulations showed better residence time than the other polymer formulations shown in Table.

***Ex vivo* bioadhesive strength measurement:**

From the results, finally It was concluded that, the more bioadhesive strength of formulation was RF11. Hence the formulation with optimized bioadhesive strength should be chosen i.e. formulation containing HPMC K100M shown in Table 14.

Table 14: *Ex vivo* residence time, Moisture absorption, Surface pH, Bioadhesive strength values of Repaglinide buccal tablets.
Each value represents the mean value (*n* =3)

Formulation code	*Ex vivo* residence time	Moisture absorbance	Surface pH	Bioadhesive strength	
				Peak detachment force (N)	Work of adhesion (mJ)
RF1	4Hrs 32 min	56.3	6.2	1.28	0.52
RF2	4Hrs 45 min	62.9	6.8	2.62	0.42
RF3	5Hrs 10 min	71.2	6.1	2.38	0.36
RF4	4Hrs 45 min	43.1	6.0	2.10	0.71
RF5	4Hrs 58 min	50.6	6.5	2.54	0.39
RF6	5Hrs 25 min	68.2	6.9	2.67	0.54
RF7	4Hrs 18 min	65.7	8.5	1.36	0.38
RF8	4Hrs 36 min	70.5	8.6	2.01	0.49
RF9	4Hrs 42 min	72.6	8.5	2.36	0.54
RF10	5Hrs 30 min	76.1	6.3	2.16	0.62
RF11	6Hrs 49 min	80.0	6.5	2.99	0.89
RF12	7Hrs 10 min	86.9	6.4	2.51	0.19
RF13	5Hrs 35 min	46.6	6.9	1.97	0.27
RF14	5Hrs 48 min	52.1	6.2	2.64	0.39
RF15	5Hrs 53 min	61.8	6.4	2.82	0.30
RF16	8Hrs 48 min	44.6	6.3	2.71	0.42
RF17	9 Hrs 23 min	42.1	6.2	2.61	0.40
RF18	9 Hrs 53 min	53.6	6.0	3.30	1.24
RF19	4Hrs 43 min	56.1	6.1	2.41	0.31
RF20	4Hrs 59 min	62.8	6.8	1.66	0.38
RF21	5Hrs 50 min	68.5	6.3	1.89	0.20
RF22	5Hrs 12 min	46.4	6.4	1.91	0.35
RF23	5Hrs 26 min	50.1	6.9	2.15	0.42
RF24	5Hrs 41 min	53.9	6.2	2.45	0.58

Swelling Studies of buccal tablets:

Therefore, formulations containing HPMC K 100M showed higher swelling index values (higher water uptake) of all the formulations were given in (Table 15 & 16). Swelling behavior of buccal tablets of all formulations as a function of time is shown in Figures 10 to 17.

Table 15: Swelling studies of buccal tablets

Time (hr)	% Swelling index											
	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	RF9	RF10	RF11	RF12
0	0	0	0	0	0	0	0	0	0	0	0	0
1	38.63	31.91	28.36	21.23	32.46	28.82	30.90	42.92	58.83	29.19	42.46	35.82
2	48.97	42.65	35.73	38.60	42.20	40.49	45.93	59.31	66.39	38.53	57.20	51.49
3	56.16	54.53	44.15	46.01	55.85	46.96	55.56	62.05	72.2	42.16	65.34	58.96
4	68.41	67.92	58.86	56.83	65.41	56.46	63.12	72.96	81.16	56.73	74.12	63.49
5	83.69	75.90	62.21	64.07	77.86	62.32	67.94	87.56	89.42	63.98	85.59	78.35
6	92.79	86.19	75.36	76.29	91.59	86.79	72.26	93.72	97.73	76.46	98.53	86.72

Each value represents the mean value (*n* =3)

Table 16: Swelling studies of buccal tablets

Time (hr)	% Swelling index											
	RF13	RF14	RF15	RF16	RF17	RF18	RF19	RF20	RF21	RF22	RF23	RF24
0	0	0	0	0	0	0	0	0	0	0	0	0
1	45.19	31.82	28.19	28.59	33.92	48.83	51.09	55.53	58.96	56.74	45.02	36.20
2	57.56	45.49	35.82	39.96	40.31	56.03	62.56	66.90	61.17	65.90	52.26	49.12
3	68.15	50.96	42.54	45.34	56.99	68.99	67.85	73.14	70.45	79.35	65.62	51.52
4	78.30	66.46	56.19	65.71	72.25	76.34	73.42	81.64	78.24	84.39	71.75	62.64
5	87.84	72.39	68.06	76.43	83.06	87.92	81.63	89.76	83.41	87.01	77.26	65.91
6	96.93	84.72	75.96	82.35	93.13	95.67	85.74	94.53	90.63	96.34	81.79	72.20

Each value represents the mean value (*n* =3)

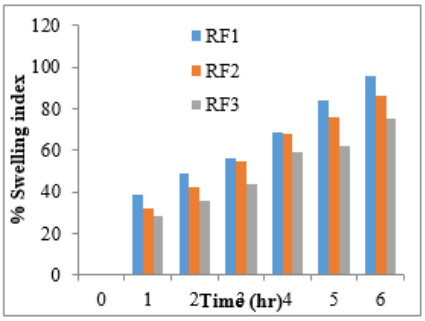


Figure 10

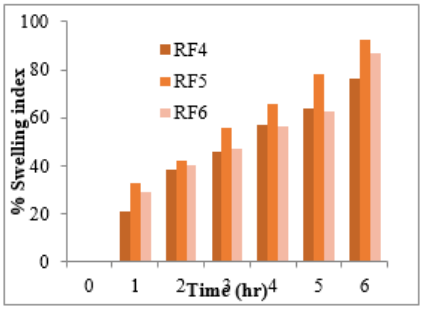


Figure 11

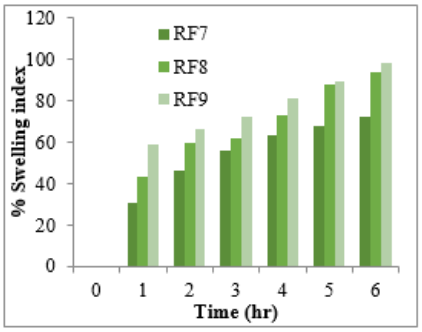


Figure 12

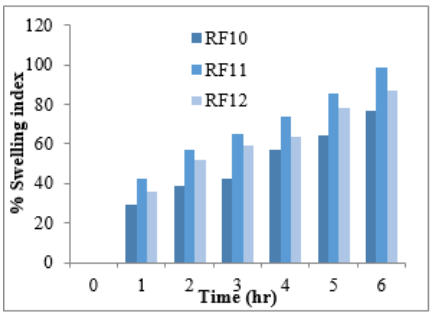


Figure 13

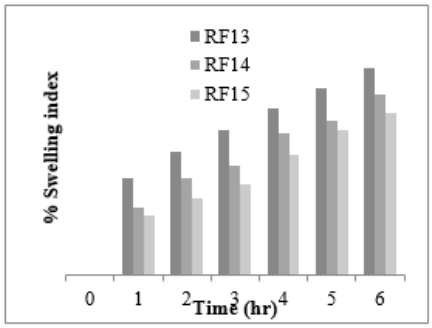


Figure14

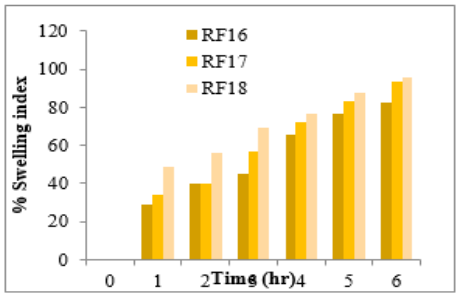


Figure 15

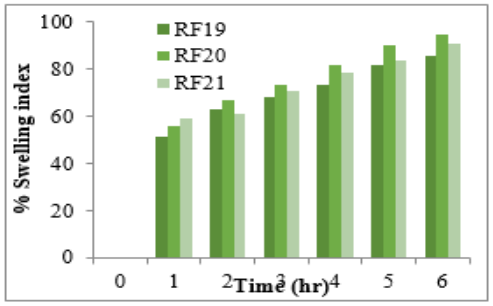


Figure 16

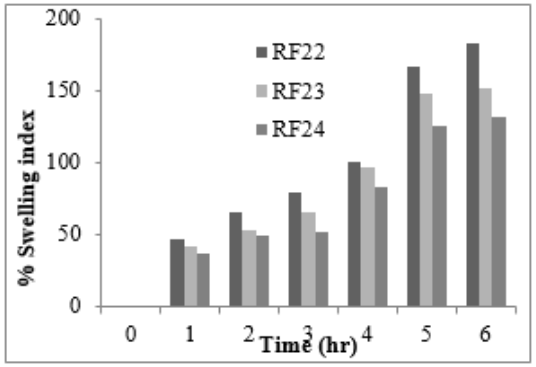


Figure 17

***Ex vivo* permeation studies of Repaglinide buccal tablet:**

The formulations containing HPMC K4M (RF1), HPMC K 100M (RF11) and Carbopol 974 P (RF18) showed highest flux at 6th hr i.e., 0.488 mg hr^{-1}cm^{-2}, 0.522 mg hr^{-1}cm^{-2} and 0.487 mg hr^{-1}cm^{-2} respectively shown in Table 17, 18.

Table 17: % Drug permeation of Repaglinide *Ex vivo* permeated buccal tablets

Time (hr)	Cumulative amount of Repaglinide through buccal mucosa (mg)											
	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	RF9	RF10	RF11	RF12
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0.55	0.41	0.36	0.42	0.54	0.43	0.21	0.35	0.53	0.25	0.59	0.43
2	0.68	0.56	0.43	0.56	0.67	0.57	0.38	0.46	0.64	0.43	0.68	0.55
3	1.13	0.98	0.81	0.97	0.84	0.64	0.46	0.53	0.75	0.61	0.76	0.68
4	1.61	1.25	1.12	1.14	1.10	0.83	0.81	0.93	1.24	0.86	1.21	0.93
5	1.72	1.32	1.24	1.36	1.19	0.91	0.99	1.24	1.36	0.93	1.55	1.19
6	1.86	1.65	1.36	1.42	1.26	1.12	1.03	1.39	1.53	1.10	1.99	1.53
*Flux	**0.488**	**0.433**	**0.356**	**0.372**	**0.330**	**0.293**	**0.270**	**0.364**	**0.401**	**0.288**	**0.522**	**0.401**

Each value represents the mean value (*n* =3) **indicates units for flux: mg hr^{-1}cm^{-2}**

Table 18: % Drug permeation of Repaglinide *Ex vivo* permeated buccal tablets

Time (hr)	Cumulative amount of Repaglinide through buccal mucosa (mg)											
	RF13	RF14	RF15	RF16	RF17	RF18	RF19	RF20	RF21	RF22	RF23	RF24
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0.39	0.32	0.23	0.19	0.29	0.39	0.18	0.39	0.35	0.46	0.35	0.26
2	0.56	0.49	0.34	0.26	0.38	0.54	0.22	0.42	0.40	0.58	0.46	0.31
3	0.89	0.73	0.52	0.37	0.52	0.86	0.33	0.58	0.51	0.69	0.57	0.43
4	0.99	0.86	0.61	0.89	0.93	1.57	0.46	0.72	0.64	0.86	0.65	0.51
5	1.65	1.24	1.12	0.92	1.21	1.73	0.62	1.26	1.12	1.35	1.12	0.89
6	1.78	1.30	1.27	0.99	1.82	1.86	0.76	1.54	1.39	1.65	1.36	0.98
*Flux	**0.466**	**0.340**	**0.332**	**0.259**	**0.477**	**0.487**	**0.199**	**0.403**	**0.364**	**0.432**	**0.356**	**0.256**

Each value represents the mean value (*n* =3)

RELEASE KINETICS:

The optimized formulation such as HPMC K100 M (RF11) follows Zero order and Higuchi order of release kinetics governed by Fickian diffusion mechanism shown in Table 19,20 and in Figure 18-22.

Table 19: Release kinetics and correlation coefficients

Formulation code	Mathematical models (Release kinetics)					
	Zero order	First order	Higuchi	Hixson-Crowell Kinetics	Korsemeyer – peppas	
	R^2	R^2	R^2	R^2	R^2	n
RF11	0.904	0.799	0.992	0.926	0.983	0.449

Table20: RF11 Release kinetics

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
42.46	1	1.000	1.628	0.000	1.760	42.460	0.0236	-0.372	57.54	4.642	3.861	0.781
57.20	2	1.414	1.757	0.301	1.631	28.600	0.0175	-0.243	42.8	4.642	3.498	1.144
65.34	3	1.732	1.815	0.477	1.540	21.780	0.0153	-0.185	34.66	4.642	3.260	1.381
74.12	4	2.000	1.870	0.602	1.413	18.530	0.0135	-0.130	25.88	4.642	2.958	1.684
85.59	5	2.236	1.932	0.699	1.159	17.118	0.0117	-0.068	14.41	4.642	2.433	2.208
98.53	6	2.449	1.994	0.778	0.167	16.422	0.0101	-0.006	1.47	4.642	1.137	3.505

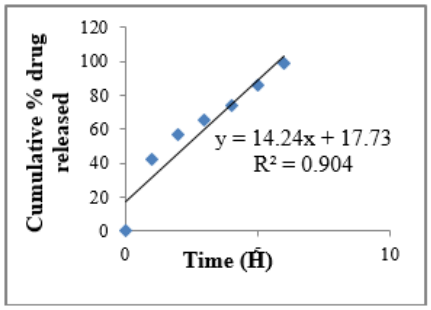


Figure 18: Zero order release kinetics

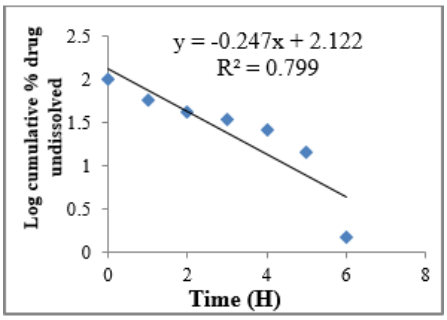


Figure 19: First order release kinetics

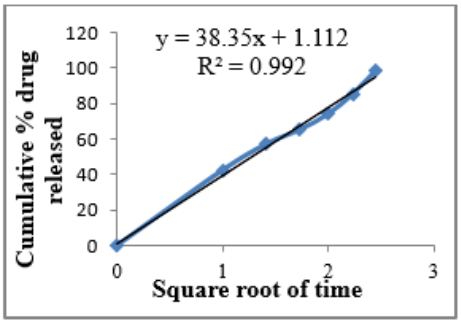


Figure 20: Higuchi order release kinetics

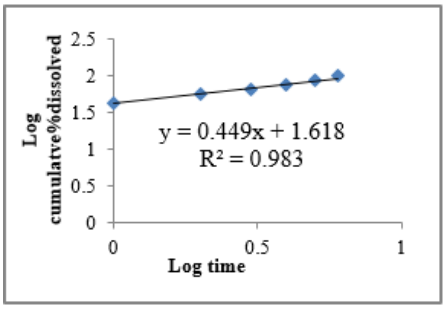


Figure 21: Peppas release kinetics

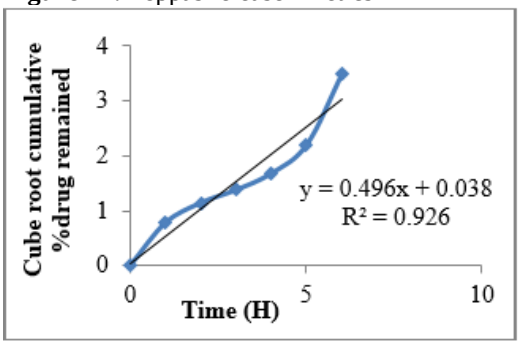


Figure 22: Hixson-Crowell Kinetics

CONCLUSION:

Among the prepared formulations, the formulation containing HPMC K 100M (RF11) was found to be best formulation which showed the higher flux 0.522 mg $hr^{-1}cm^{-2}$ than the pure drug solution (0.288 mg $hr^{-1}cm^{-2}$), and bioadhesive strength of 2.99 N (peak detachment force) and 0.89 mJ (work of adhesion). From the results of this study, it may be concluded that the combination of HPMC K 100M and Carbopol 974P polymers are suitable for developing bio adhesive buccal tablets of Repaglinide.

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