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Research Article

FORMULATION AND EVALUATION OF BUCCO-ADHESIVE TABLETS OF INSULIN USING LOCUST BEAN GUM

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ABSTRACT

The present study is to develop a buccoadhesive drug delivery system of Insulin using natural polymer locust bean gum in order to overcome the poor permeability and ineffectiveness when administered orally. Insulin is a polypeptide anti-diabetic agent usually administered through injections. The use of these injections throughout life of a patient presents various problems, such as physical and psychic pain, hypertrophy or atrophy of the subcutaneous fat at an injection site. Also when taken orally results in short biological half life and ineffectivness due to enzymatic degradation and poor permeability. As insulin is a peptide drug, its delivery via the buccal oral cavity has several therapeutic advantages. Locust bean gum is a mucoadhesive polymer. It is useful as a stabilizer, adsorbent and demulscent therapeutically. Polyethylene glycol dimethyl ether 500 is used as permeation enhancer.Mucoadhesive buccal tablets of insulin are prepared by direct compression method. Buccal tablets are evaluated by certain parameters like dissolution, in vitro bioadhesion study drug permeation study and in vivo study. Results revealed that the tablet containing 5mg locust bean gum and 4 mg PEG, DME500 as an ideal formulation for mucoadhesive buccal delivery of insulin.

Keywords: PEG, DME500, insulin, locust bean gum, buccal adhesive.

INTRODUCTION

Diabetes mellitus is the most common endocrine disease. The frequency of diabetes mellitus in India is 1-2%. Insulin a polypeptide based pharmaceuticals chosen as an experimental drug, since it is the first line of choice for the treatment of type 1 diabetes. For chronic therapy involving peptide drugs, the oral mucosa of the buccal cavity appears to be attractive as it is easily accessible, dosage forms can be easily administered and even removed from the site of application.since patients are well adapted to the oral administration of drugs in general, and patient acceptance and compliance is expected to be good. Buccal mucosa is relatively permeable with a rich blood supply. An ideal buccal dosage form has these properties. It must maintain its position in the mouth for required time period; release drug in a controlled fashion, and provide the drug release in a unidirectional way towards the mucosa. This can be achieved by different approaches including castor oil hydrogenated as a coating material on all but one face .Locust bean gum as a mucoadhesive polymer and polyethylene glycol dimethyl ether 500 as a permeation enhancer in the formulation of buccoadhesive tablets

The present study was under taken to (a) prepare a new mucoadhesive buccal dosage form of insulin (b) evaluate the gum from ceratonia siliqua (locust bean gum) as a mucoadhesive component in buccal tablet (c) quantiate the plasma concentration of insulin

in presence and absence of a new permeation enhancer, polyethylene glycol dimethyl ether-500. In addition an attempt was made to overcome the one of the major limitations of buccal drug administraton i.e. in the event tat drug dissolved in saliva has potentially swallowed.

MATERIALS AND EQUIPMENTS

Materials

Insulin(Bovine Insulin),(24.5 I.U./mg)- Knoll Pharmaceuticals Ltd., Locust Bean Gum(Fluka Pharmaceuticals Ltd.), Polyethylene Glycol Dimethyl ether-500(Merck Pharmaceuticals Ltd.).

Equipments

Rotary tablet machine (Cadmach 16 station), Digital balance (Dhona 200D), UV spectroscopy (AUV-2092).

EXPERIMENTAL METHODS

Tablet Preparation

Core tablets were prepared by direct compression and coated with castor oil hydrogenated on all, but one face using a compression technique. Release of insulin was unidirectional occurring from only the uncoated tablet face.

Direct Compression

Core Tablets

The directly compressed tablets were prepared by initially mixing insulin and gum for 10 minutes. Subsequently calcium sulphate dehydrate, polyethyleneglycoldimethylether500 were incorporated and mixed for an additional 10 minutes. Finally magnesium stearate added and the mixing is continued for an additional 5 minutes.

Coating Granulation

Initially castor oil hydrogenated and talc were mixed for 4 minutes. Subsequently Calcium Sulphate Dihydrate is incorporated and the solid was mixed for additional 4 minutes. Finally Magnesium Stearate and colorant (tartrazine) were added and the mixing continued for an additional 2 minutes and pass through sieve no. 22. Resulting granules were dried at room temperature overnight.

EVALUATION OF BUCCOADHESIVE TABLETS OF INSULIN

In-Vitro Bioadhesion Study

Isolated Oesophagus Preparation

Pigs weighing 90-100 kg used .Immediately after slaughter, the oesophagus were removed and transported to laboratory in tyrode solution, kept at -4^{0}C. During experiments the solution was aerated with pure oxygen and kept at 37^{0}C Segments 6-7cm long were cut from the oesophagus. The segments were mounted in organ bath with a volume of 60ml.The lower end of the oesophageal segment was tied off and the upper end was attached around a glass tube(diameter 15mm).The solution in the organ bath was changed at intervals of about 30 minutes**.**

Marvola Apparatus

In order to measure the adhesion of a dosage form to the oesophagus marvola developed two types of apparatus. These apparatuses use segments of pig oesophagus maintained at 37˚C in oxygenated tyrode solution.The solid dosage form (tablet or hard gelatin capsule) under study is attached to a prescription balance ,and inserted in the oesophagus.It is then progressively deattached by increasing the charge on the opposite tray of the balance .

Recording of Adherence

A hole (diameter of 1mm) was drilled in the products to be tested.The product was attached to a copper wire(diameter 0.25mm) and placed, using a plastic tube as an applicator,in the Oesophageal preparation for a fixed time.The force needed to deattach the product was measured using a modified prescription balance. This force was used as a parameter for adherence.The force (F) in newtons was calculated by the equation.

$$F = 0.00981 W/ 2$$

Where

W= amount of water in the beaker in grams. The water flow rate was 280gm min^{-1} (Corresponding to 1.37N min^{-1})

In-Vitro Drug Permeation Study

Modified Flow Through-Diffusion Cell

The apparatus was based on a modification of a flow-through diffusion cell. It consists of two compartments, the donor and receptor compartments. The receptor compartment, with an effective volume of 6 ml. The receptor cell also consisting a outer jacket with an

outlet and inlet arrangement to permit the flow of water maintained at 37^{0}C and in addition with arrangement for aeration to retain the viability of the tissue.The solution in the receptor compartment is stirred by a teflon coated magnetic bead driven by synchronous meter. Samples can be replaced through a sampling port connected with receptor compartment. Both these compartments are closed tightly by securing springs over the hooks, made on the sites of both the compartments, so that there was no lekage from the apparatus.

Permeation Study

Immediately before each experiment, a mucosal specimen was thawed at room temperature at about 10 minutes, rinsed twice in isotonic kcl. Then mucosal specimen was mounted between donar and receptor compartment with the mucosal surface facing the donar site. The formulated muco adhesive buccal tablets were stuck on the mucosal surface using 25µl of phosphate buffer pH 7.38 and a weight of 10 gms for 30 sec. The temperature of receptor compartment was maintained at 37±1˚C. The receptor solution (6ml of phosphate buffer pH 7.38) was stirred with a magnetic stirrer at a constant speed throughout the experiment. Aliquot of the receptor fluid (1.5ml) were withdrawn at predetermined time intervals, and replaced immediately with same volume of fresh fluid Samples were analysed by HPLC for the amount of drug permeated.

In-Vivo Study

Intramuscular Insulin Study

Albino rabbits weighing 2-2.5 kg are used for the present study. The rabbits were fasted for 18 hrs before the experiments. To determine the relative bioavailability of insulin of the mucoadhesive buccal tablets in comparision with that from intramuscular administration, the area under the curve of plasma insulin levels in albino rabbits for 300 minutes after injection with a dose of 2.5u / kg was obtained.

Determination of Fasting or Basal Concentration of Insulin in the Plasma of Albino Rabbits

In order to determine the fasting or basal concentration of insulin and also to exclude the effect of stress to the rabbit by the application of buccal tablet following experiments were conducted. Albino rabbits weighing 2-2.5kg were used for the present study. The rabbits were fasted for 18 hrs before the experiments and were allowed water. Animals were lightly anesthetized by an i.v. injection of phenobarbitone (6%) (50-60mg dose per kg). Following induction of anesthesia catheter was placed in the marginal year vein for blood sample collection. A 2ml blood sample was obtained at 30, 60, 120, 180, 240, 300 minutes every day for 3 days following the application of buccal tablets containing no insulin. All the blood samples were centrifuged at 3000 rpm for 10 minutes to separate the plasma and the retrieved plasma was stored at 20^{0}C until the time of analysis.The sampling time vs concentration values are shown in table (3).

Buccal insulin study

The potential of the mucoadhesive buccal tablets to deliver insulin to the systemic circulation was evaluated by conducting the following experiments. There were three groups of rabbits. Rabbits weighing 2-2.5 kg were used for present study. The rabbits were fasted for 18 hrs before the experiments and were allowed water ad libitum. Animals were lightly anesthetized by an i.v. injection of phenobarbitone (6%) (50-60mg dose/kg). Following induction of anesthesia a catheter was placed in the marginal year vein for blood sample collection. A 2ml blood sample was obtained at 0, 30, 60, 120, 180, 240, 300 min following the application of the buccal tablets. After the collection of each blood sample the cannula was flushed with 0.2ml of a 10% (v/v) heparin / normal saline solution to keep the cannula open. The light plane of anesthesia was maintained by i.v. injection of one- third of the initial dose of phenobarbitone as needed. All the blood samples were centrifuged at 3000 rpm for ten minutes to separate the plasma and the retrived plasma was stored at 20^0 c until the time of analysis.

RESULTS AND DISCUSSION

In-vitro Bioadhesion Study

The profile showing the mean values of the force of detachment of the buccal tablets following their applications to isolated oesophagi is shown in table (4) and graph (1). It can be noted that the mean values of the force of detachment increased with time and were greater for tablets containing 15mg of locust bean gum as compared to the tablets with 5 and 10 mg of locust bean gum and also

presence of other excipients for tablet did not appear to affect the bioadhesive strength.

In vitro Drug Permeation Study

The in-vitro drug permeation studies carried out indicates the influence of gum concentration and presence of enhancer on the permeation of drug. The cumulative release (μ U / cm^2) over 300 minutes and permeability co-efficient of drug from buccal tablets (I_1 to I_6) were determined and are summarized in table (5, 6) and graph (2). The cumulative release and permeability co-efficient of drug was found to be decrease with increase in gum concentration (I_1 , I_2 , I_3) and also the cumulative release and permeability co-efficient of drug was found to be increased in presence of permeation enhancer (I_2 , I_4, I_6,).

Buccal Insulin Study

The plasma insulin concentrations Vs time profiles following administration of buccal tablets (I_1 to I_6) are shown in graph (3). The relevant pharmacokinetic parameters are listed in table (8). The plasma insulin concentration Vs time profiles was found to be decreased with increase in gum concentration (I_1 ,I_3, I_5) and also plasma insulin concentration Vs time profile was found to be increased in presence of permeation enhancer.

Bioavailability of insulin following I.M. administration and buccal administration in healthy albino rabbits

The plasma insulin concentration Vs time profiles following intramuscular and buccal administration of insulin are shown in graph (3). The relavent pharmacokinetic parameters are listed in table (8) following intramuscular administration the area under the plasma concentration curve was 2136.43μ U hrs/ml as compared to 143.83, 230.1, 95.5, 172.7, 82.29, 141 μ U hrs/ml following buccal administration(I_1 to I_6). This compared to relative bioavailabity (Fr) of 0.4, 0.67, 0.28, 0.5, 0.24 and 0.4%

CONCLUSION

The delivery of drugs via the buccal mucosa of the oral cavity has several therapeutic advantages. The results from this study clearly shows that the tablet containing 5mg LBG and 4mg PEGDME-500, as an ideal formulation for mucoadhesive buccal delivery of insulin.Finally, although the amount of insulin absorbed was low in the present dosage form, we did confirm that insulin absorbed at the oral mucosa of the buccal cavity .Further investigations may improve the bioavailability of insulin in the present dosage form,as regards both the extent of absorption and duration of enhanced blood level.

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Table 1: Composition

S. No.	INGREDIENTS	FORMULATION
1	Insulin	4mg
2	Calcium sulphate dihydrate	136mg
3	Locust bean gum	5mg
4	Poly ethylene glycol dimethylether500	4mg
5	Magnesium Stearate	1mg

Table 2: Composition of Mucoadhesive Buccal Tablets – Composition of Core tablet

S. No.	Ingredients	Formulation(Weight in mg)					
		I_1	I_2	I_3	I_4	I_5	I_6
1	Insulin	4	4	4	4	4	4
2	Calcium Sulphate Dihydrate	140	136	135	131	130	126
3	Locust Bean Gum(LBG)	5	5	10	10	15	15
4	Polyethylene Glycol Dimethyl Ether 500 (PEGDME-500)	-	4	-	4	-	4
5	Magnesium Stearate	1	1	1	1	1	1
	Total weight	150	150	150	150	150	150

- I_1 – Tablet containing 5 mg LBG.
- I_2 – Tablet containing 5mg LBG and 4mg PEGDME-500
- I_3- Tablet containing 10mg LBG
- I_4 – Tablet containing 10mg LBG and 4 mg PEGDME-500
- I_5 – Tablet containing 15 mg LBG
- I_6 - Tablet containing 15mg LBG and 4 mg PEGDME-500

Table 3: Mean fasting or basal concentration of insulin in the plasma of albino rabbits

S. NO.	SAMPLING TIME (min)	CONCENTRATION [μ U/ML]
1	30	20.67 ± 1.25
2	60	20 ± 0.816
3	120	22 ± 0.816
4	180	21 ± 0.816
5	240	21.67 ± 1.70
6	300	22± 1.63

Each value represents the mean ± S.D of three determinations

Table 4: Invitro Bioadhesion Profiles of Formulated Mucoadhesive Buccal tablets (I_1 to I_6)

Time (Min)	Force of detachment (N)					
	I_1	I_2	I_3	I_4	I_5	I_6
5	0.833 ± 0.125	0.83 ± 0.125	1.37 ± 0.287	1.38 ±0.30	1.8 ±0.163	1.8 ± 0.163
10	1.1 ± 0.170	1 ± 0.386	1.5±0.450	1.5 ±0.450	2 ±0.163	2 ±0.163
20	1.53 ±0.330	1.53 ± 0.330	2.0 ±0.163	2.0 ± 0.163	2.53 ±0.368	2.5 ±0.580
30	2 ± 0.163	2.1 ± 0.294	2.53 ±0.368	2.53 ± 0.368	3 ±0.374	3 ±0.374
45	2.3 ± 0.294	2.3 ± 0.294	2.9 ±0.572	2.9 ±0.572	3.9 ±0.573	3.87 ±0.580
60	2.5 ± 0.408	2.5 ± 0.408	3.1 ±0.50	3.1 ±0.50	4.3 ±0.455	4.33 ±0.464

- Each value represents the mean ± S.D of three determination

Table 5: In-Vitro Permeation Profiles of formulated Muco adhesive Buccal Tablets (I_1 to I_6)

Sampling time(min)	Cumulative Amount Of Drug Release (μ U/cm²)					
	I_1	I_2	I_3	I_4	I_5	I_6
30	199.8 ± 2.87	313.8 ± 3.68	168 ± 4.08	288 ±2.94	139.8 ±3.68	256 ±4.11
60	279.8 ± 3.86	428.3 ± 2.95	241.8 ±2.87	390 ±3.74	206.97 ±5.00	350 ±5.19
120	299.3 ±2.055	441.5 ± 2.45	272 ±2.94	425.5 ±4.5	223 ±5.72	371.5 ±1.63
180	342.5 ± 3.27	492 ± 3.68	289.5 ±3.3	454.3 ±4.19	238.8 ±68	388.8 ±3.68
240	372 ± 2.45	520.5 ± 2.45	302.5 ±3.3	462 ±3.86	246.5 ±4.08	396.5 ±3.27
300	388.8 ± 3.68	537 ± 4.32	313 ±4.32	468.5 ±3.86	253.5 ±4.9	403.5 ±3.27

- Each value represents the mean ± S.D of three determination

Table 6: The Permeability Coefficient of Formulated Mucoadhesive Buccal Tablets (I_1 to I_6)

Formulation	P_x10^{-7}cm/s
I_1	0.91 ±0.643
I_2	1.13 ±0.550
I_3	0.45 ±0.547
I_4	0.68 ±0.479
I_5	0.23 ±0.755
I_6	0.45 ±1.07

Each value represents the mean ± S.D of three determinations

Table 7: Mean plasma insulin concentration in healthy albino rabbits following intramuscular administration of insulin (2.5u/kg)

S.NO.	SAMPLING TIME (min)	CONCENTRATION μ U/ML
1	30	750 ± 1.63
2	60	700 ± 2.45
3	120	650 ± 2.45
4	180	600 ± 1.63
5	240	500 ± 2.45
6	300	400 ± 1.63

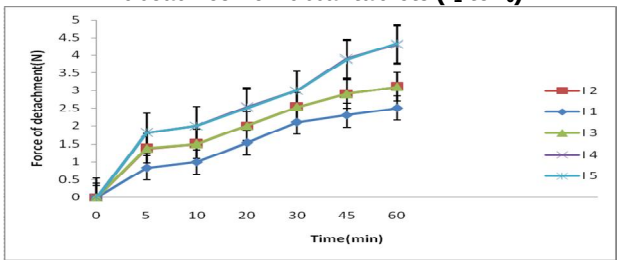
• Each value represents the mean ± S.D of three determinations

Table 8: Bioavailability of insulin following I.M. administration and buccal administration in healthy albino rabbits: Mean pharmacokinetic parameters of insulin following buccal (I_1 to I_6) Administration (100U/ rabbit)

Pharmacokinetic parameters					
Route	$C_{max(\mu U/ml)}$	$T_{max(hrs)}$	$K_{cl(hrs^{-1})}$	$T^{1/2}$(hrs)	AUC 0 – T* μ U hrs/ml
Buccal					
I_1	38 ± 3.56	3 ± 0.00	0.138 ± 1.98	5.0 ± 0.542	143.83 ± 0.83
I_2	58 ± 4.5	3 ± 0.00	0.115 ± 0.0047	6.0 ± 0.155	230.1 ± 0.39
I_3	32.2 ± 0.47	3 ± 0.00	0.134 ± 0.00	5.17 ± 0.00	95.5 ± 10.25
I_4	53.3 ± 2.05	3 ± 0.00	0.108 ± 0.0029	6.4 ± 1.27	172.7 ± 7.00
I_5	21.67 ± 2.83	3 ± 0.00	0.129 ± 0.0094	5.37 ± 0.660	82.29 ± 3.81
I_6	45.2 ± 2.50	3 ± 0.00	0.104 ± 0.0020	6.6 ± 0.136	141 ± 5.44

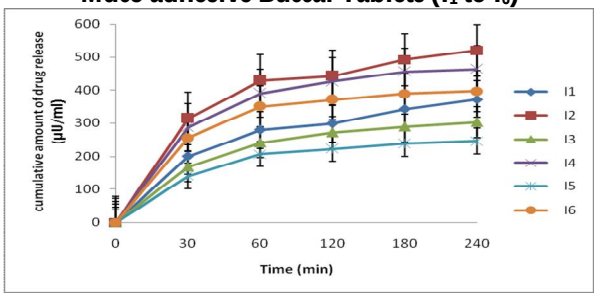
• Each value represents the mean ± S.D of three determinations

Graph 1: Invitro Bioadhesion Profiles of Formulated Mucoadhesive Buccal tablets (I_1 to I_6)



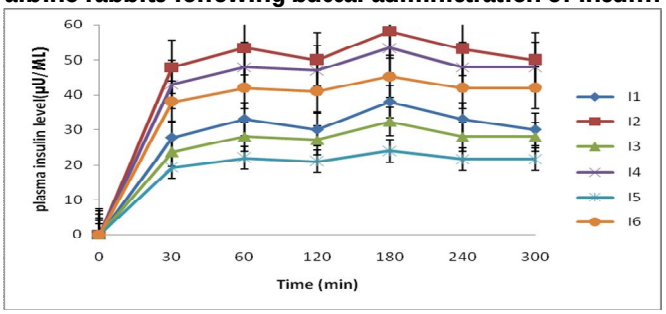
• Each value represents the mean ± S.D of three determinations

Graph 2: In-Vitro Permeation Profiles Of Formulated Muco adhesive Buccal Tablets (I_1 to I_6)



• Each value represents the mean ± S.D of three determination

Graph 3: Mean plasma insulin concentration in healthy albino rabbits following buccal administration of insulin



• Each value represents the mean ± S.D of three determinations

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