FORMULATION DEVELOPMENT OF EVALUATION OF ORAL MUCOSALCASEIN SALT FILM FOR THE ANTI DIADETIC OF INSULIN

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ABSTRACT:

The primary purpose of buccal mucoadhesive films is to bypass the gastrointestinal tract and liver, providing direct systemic drug absorption through the rich blood supply in the oral mucosa. By avoiding the first-pass metabolism in the liver, drugs can achieve higher bioavailability and faster onset of action, leading to more efficient treatment. Sodium caseinate has been successfully utilized as a suitable material for creating mucoadhesive films for buccal drug delivery systems. These mucoadhesive buccal patches, containing insulin as the drug, were developed and met satisfactory criteria in terms of pre formulation studies surface pH, bioadhesive strength, drug release, and permeation.

In particular, formulation F4 demonstrated excellent drug release performance, with 98.99% of the loaded insulin being released in vitro over an 11-hour period. Additionally, the permeation study showed that formulation F4 allowed 99.31% of the loaded insulin to permeate across the rabbit buccal mucosa over a more extended period of 54 hours.

To enhance drug release efficiency, the incorporation of 10% (w/v) sodium deoxycholate reduced the required release time from 54 hours to 26 hours, with 99.34% of the drug being released.

The in vitro permeation data analysis indicated that the insulin permeated through the mucosa following a first-order release pattern. This novel protein delivery system reduces the risk of interaction and incompatibility issues, making it a promising approach for buccal drug delivery applications.

KEY WORDS: Buccal mucoadhesive films, insulin, sodium caseinate, invitro permeation.

INTRODUCTION

Buccal mucoadhesive films are innovative drug delivery systems that adhere to the oral mucosa (the inner lining of the cheek) and release medication over an extended period. These thin, flexible films are designed to improve the therapeutic effectiveness of drugs and enhance patient compliance compared to traditional dosage forms like tablets or capsules. They offer several advantages, making them a popular choice for certain medications.

The primary purpose of buccal mucoadhesive films is to bypass the gastrointestinal tract and liver, providing direct systemic drug absorption through the rich blood supply in the oral mucosa. By avoiding the first-pass metabolism in the liver, drugs can achieve higher bioavailability and faster onset of action, leading to more efficient treatment.

The key components of buccal mucoadhesive films is Mucoadhesive Polymers a crucial ingredients responsible for the adhesive properties of the film, allowing it to cling to the wet mucosal surface of the mouth. Commonly used mucoadhesive polymers include hydroxypropyl methylcellulose (HPMC), sodium carboxymethylcellulose (Na CMC), polyvinylpyrrolidone (PVP), and chitosan.

Buccal mucoadhesive films offer a promising approach to drug delivery with potential benefits in terms of enhanced bioavailability, patient compliance, and reduced side effects. However, like any other drug delivery system, their development and formulation require careful consideration of the drug's physicochemical properties and the selection of appropriate mucoadhesive polymers and excipients

MATERIAL USED

Insulin is the pure drug procured from India Cadila Pharmaceutical Ltd., Dholka, India as gift sample. Hydroxy propyl methylcellulose (HPMC E15C; K4M) got as gift sample from Alembic Pharmaceuticals, Vadodara, India. Sodium caseinate procured from Clarion casein private limited. Mehsana, Gujarat India. Di-calcium Phosphate (DCP), Polyethylene glycol, Polyvinyl Pyrrolidone K30, Hydroxy Propyl cellulose, Hydroxy Ethyl Cellulose, Polyvinyl alcohol, cellulose acetate Hydrochloric acid, Sodium chloride (NaCl). Sodium Phosphate, Sodium deoxycholate, Sodium glycocholate Myrrh B- cyclodextrin from S.D. Fine Chemicals, Mumbai, India.

METHODOLOGY

Preparation of Insulin Patch

Among the various substrates for film formation including mercury, Teflon, glass and aluminium foil, mercury was found to give best result[95].The prepared solution of polymers and sodium caseinate containing drug have kept for constantstirring with glycerol (10% w/w of polymer) as a plasticizer. Then the mixture was poured in to a glass bangle of 18.08 sq. cm areas which was previously placed over mercury substrate in a petridish. The cut funnel was inverted over the Petri dish for the controlled evaporation at 30-35º C. After 12 hrs the dried patches were collected and stored in the desiccators. Different permeation enhancers with uniform concentration (10%) were added on the basis of previously conducted studies in the formulations and studied the effect on drug release characteristics.

Table 1. Formulation of Insulin Patch

Ingredients (%)	F1	F2	F3	F4	F5
Insulin (IU/ patch)	50	50	50	50	50
Sodium caseinate	3%	3%	3%	3%	3%
HPMC K4M		0.5 %	1%		
HPC				1%	
HEC					1%
Glycerol	10%	10 %	10 %	10%	10%
Water	5 ml	5ml	5ml	5ml	5ml

Table 2. Formulation of Insulin Patch with Permeation Enhancers

Ingredients (%)	P1	P2	P3	P4	P5
Insulin (IU/ patch)	50	50	50	50	50
Sodium caseinate	3%	3%	3%	3%	3%
HPC	1%	1%	1%	1%	1%
Sodium deoxycholate	10%				
Sodium glycocholate		10%			
Sodium laurate			10%		
Myrrh				10%	
B- cyclodextrin					10%

Glycerol (w/w of polymer)	10%	10%	10%	10%	10%
Water	5 ml	5ml	5 ml	5 ml	5 ml

EVALUATION PARAMETERS

Infrared (IR) Spectroscopic Analysis

Fourier–transform infrared (FTIR) spectra of moisture free powdered samples were obtained using a spectrophotometer (FTIR-8300, Shimadzu Co., Japan) by potassium bromide (KBr) pellet method (app. 5 mg sample in 200 mg KBr). The scanning range was 400–4000 cm^{-1} and the resolution were 1 cm^{-1}.

Differential Scanning Calorimetry (DSC) Analysis

DSC scans of the powdered samples were recorded using DSC- Shimadzu 60 withTDA trend line software. All samples were weighed (8-10 mg) and heated at a scanning rate of 10°C/min under dry nitrogen flow (100 ml/min) between 50 and 300° C. Aluminium pans and lids were used for all samples. Pure water and indium were used to calibrate the DSC temperature scale and enthalpic response.

Physical appearance

The films were observed visually for their physical appearance such as colour andtransparency.

Surface texture

The surface textures of the films were evaluated by pressing the film with finger.

Weight variation

Four films of each formulation were taken weighed by using single pan balance and average weight films were calculated and standard deviations were computed.

Thickness and size

our Films of each formulation were taken and the thickness of the film was measured using screw gauge at different places. The average film thickness and standard deviation were computed.

Drug content

Drug content uniformity was determined by tacking film area of 1.5 cm^2 from each formulation and it was placed in 50 ml of volumetric flask contained 50 ml of phosphate buffer of pH 6.6. It was kept aside for 6 hours and volume was made up to 100 ml with the buffer of pH 6.6. The content of insulin was calculated. Averages of three determinations were calculated.

Surface pH

Buccal patches were left on the surface of an agar plate, prepared by dissolving 2% (m/v) agar in warmed phosphate buffer of pH 6.6 under stirring and then pouring the solution into a Petri dish till gelling at room temperature. The surface pH was measured by means of a pH meter by bringing the glass electrode on the surface of the swollen patch. The mean of two readings was recorded.

Folding endurance test

The folding endurance of patches was determined using a modified USP tablet-disintegrating tester. The tester had fixed and movable jaws that mimicked a jaw's movement, with a rate of 28 strokes per minute. The distance between the jaws was 6 cm at the farthest point and 0.5 cm at the closest point. The 8 cm patch was clamped between the jaws, causing it to bend across the middle at the closest position and stretch at the farthest position. Each stroke of the movable jaw completed one bending and stretching cycle. The folding endurance was expressed as the number of strokes needed to either break or develop visible cracks on the patch. The test duration was 1 hour, totaling 1680 strokes.

Mucoretention time

The *in vitro* mucoretention time was determined by using a locally modified apparatus. A semi-circular sheet of 10cm length was kept at 30^{o} angle. The Rabbit intestinal mucosa was adhered to the center portion of the disc and the film was placed over it. The flow of phosphate buffer was maintained at 0.5 ml/min by using peristaltic pump. The time necessary for complete erosion or detachment of the patch from the mucosal surface was recorded (mean of triplicate determinations were recorded).

In vitro diffusion study

In-vitro diffusion studies were carried out in Franz diffusion cell with an internal diameter of 15 mm and a diffusion area of 1.8 cm^{2} through sigma dialysis membrane.The sigma dialysis membrane was hydrated by addition of distilled water and fixed to the one end which acts as a donor compartment. The assembly was filled with 50 ml of phosphate buffer of pH 6.6. The teflon coated magnetic bead was placed in the beaker and rotated at 50 rpm using magnetic stirrer and the temperature was maintained at $37\pm1\ ^{0}$ C. Samples of 1 ml were withdrawn at regular intervals and replace the volume with same buffer and maintained sink condition through the studies. Averages of duplicate readings were taken.

The amount of drug permeated was determined by removing samples (5 ml aliquots), from the receptor compartment using a micro syringe at appropriate time intervals followed by their HPLC analysis. The volume withdrawn was replenished with an equal quantity of pre-

warmed receptor solution. The samples were analysed by HPLC(Shimadzu, Japan) at 214 nm according to the procedure mentioned in USP-24 using 25 cm x 4.5 mm, L1 packing (5µm) Rp-18 Column and phosphate buffer pH 2.3: acetonitrile (74:26) as the mobile phase.

Ex-vivo diffusion study

Ex-vivo drug release studies were carried out for the selected formulation by using goat cheek pouch membrane. In this method goat cheek pouch was attached to one end of donor compartment of the area of 1.5 cm^2 was selected and the above procedure was repeated.

Kinetic modeling and Mechanism of drug release

Data obtained form in vitro drug release studies were fitted to various kinetic equations. The kinetic models used are zero order, first order and Higuchi equation. The following plots were made for appropriate model.

- Q Vs t (zero order kinetic model)
- Log (Qo - Qt) Vs t (first order kinetic model)
- Qt Vs √t (Higuchi model)
- Korsmeyer-Peppas. $\frac{M_t}{M_\infty} = kt^n$

RESULTS AND DISCUSSION

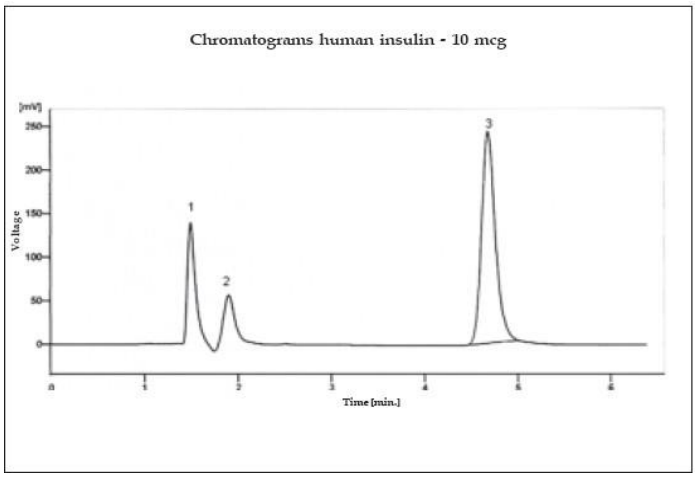

Figure 1. Chromatogram of human insulin

Table 3. Calibration curve of Insulin

Concentration(µg/ml)	Peak area
1	148945 (1304)
2	378341(1264)
4	820048(1382)
6	1219315(1483)
8	1554187(1475)
10	1921223(1748)
Correlation coefficient = 0.997 Absorbance = 19578 × concentration - 3883	
Values in parenthesis indicates standard deviation (n = 3)	

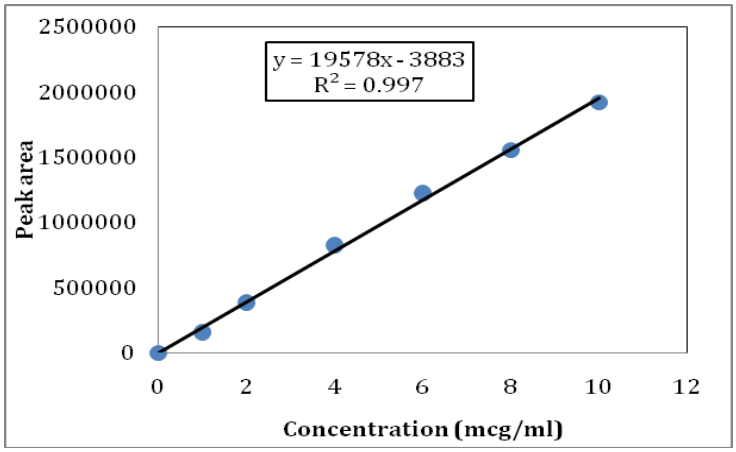

Figure 2. Calibration curve of Insulin

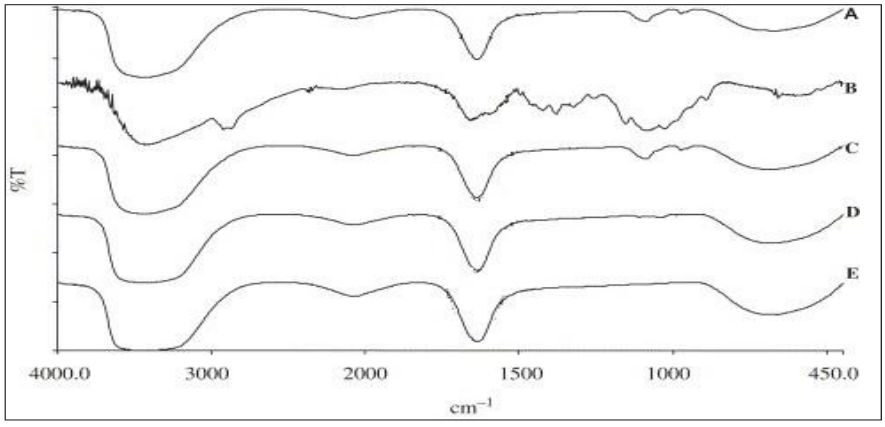

Figure 3. FTIR overlay of different formulations: (A) Insulin, (B) Insulin + HPMC, (C) Insulin + HPC, (D) Insulin + HPC + Sodium deoxycholate, (E) Insulin + HPC + Sodium deoxycholate solution

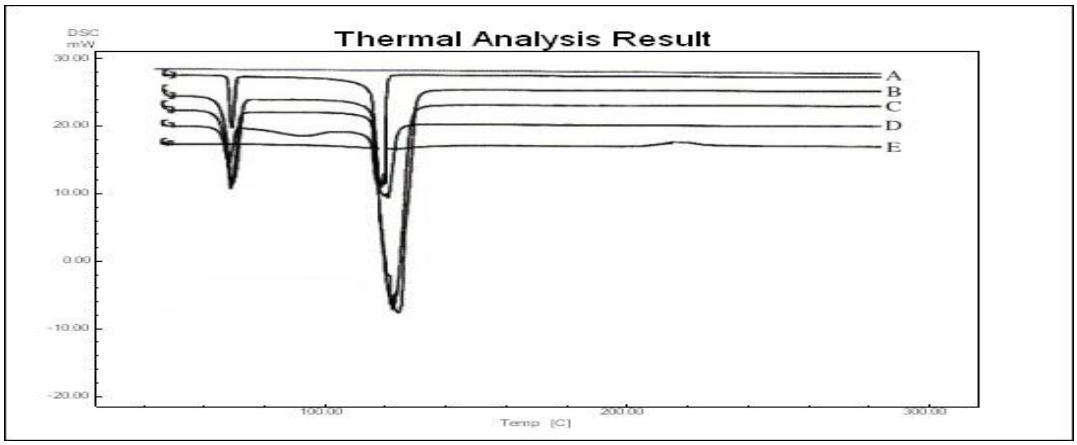

Figure 4. Overlay of DSC thermograms from 0 to 300 °C at a heating rate of10 °C/min: (A) Insulin, (B) Insulin + HPMC, (C) Insulin + HPC, (D) Insulin + HPC + Sodium deoxycholate, (E) Insulin + HPC + Sodium deoxycholate solution.

Table 4. Physico-chemical characteristics of mucoadhesive buccal films

Formulation Code	Appearance	Surface texture	**Average weight (mg± SD)	**Thickness (mm)	*Folding endurance	*Surface pH	*Drug content
F4	-	Smooth	202.11± 0.13	104.09±0.35	> 300	6.8± 0.4	99.67±0.3
P1	-	Smooth	211.08± 0.43	122.9± 0.32	> 450	6.6± 0.5	98.24±0.2
P2	+	Smooth	213.12± 0.34	126.12±0.59	> 450	6.5± 0.3	99.88±0.4
P3	-	Smooth	206.25± 0.76	134.9± 0.50	> 450	6.9±0.5	98.87±0.2
P4	+	Smooth	208.71± 0.98	138.21±0.48	> 450	6.7±0.5	99.07±0.3
P5	-	Very Smooth	207.53± 0.12	133.6 ± 0.12	> 450	6.8±0.3	98.87±0.2

Table 5. Mucoretention studies for insulin buccal films

Formulation code	Mucoretentiontime (Minutes)
F4	550 ± 5

P1	545 ± 6
P2	521 ± 4
P3	530 ± 5
P4	505 ± 2
P5	498 ± 3

Table 6. Invitro drug release profiles of insulin loaded film

Time (hrs.)	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	21.26	16.87	13.21	15.82	17.41
2	37.98	27.23	21.02	18.91	25.67
3	54.09	42.22	35.62	33.55	38.00
4	66.12	54.81	48.41	42.43	49.79
5	85.27	66.29	59.15	54.16	61.12
6	98.47	82.35	71.88	61.80	69.65
7	98.12	98.11	89.28	72.47	78.62
8		98.05	98.73	83.29	89.54
9			98.64	91.13	97.61
10				98.73	97.22
11				98.99	
12					

Table 7. Ex vivo drug release profiles of insulin loaded film

Time (hrs.)	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	6.21	5.40	4.80	4.50	6.04
2	12.84	10.23	9.84	8.72	11.36
3	19.28	14.33	14.13	12.37	16.24
4	24.79	18.05	17.21	16.13	19.63
5	29.67	22.42	20.78	19.84	22.75
6	34.78	26.78	24.15	22.03	25.50
7	38.97	31.36	28.51	24.53	28.80
8	43.41	35.02	31.48	27.06	31.13
9	47.38	38.41	35.01	30.58	34.90

10	51.72	41.78	38.47	32.71	36.00
11	54.58	45.87	42.35	34.58	38.75
12	56.49	49.37	44.84	36.32	40.88
18	66.09	59.87	53.24	44.20	51.02
24	75.54	68.87	62.09	52.24	60.87
30	84.12	77.99	71.30	61.56	69.55
36	92.23	87.97	82.1	72.29	79.65
42	99.10	95.44	92.33	81.05	88.75
48	99.05	98.14	99.74	90.67	98.08
54			99.67	99.31	98.34
60				99.03	

Table 8. Ex-vivo diffusion study of insulin film with permeation enhancers

Time (hrs.)	P1	P2	P3	P4	P5
0	0	0	0	0	0
2	10.34	9.34	7.34	6.34	4.34
4	18.23	16.23	13.23	9.23	7.23
6	24.1	21.1	18.1	15.1	11.1
8	30.28	28.28	25.28	20.28	16.28
10	36.61	33.61	31.61	26.61	21.61
12	46.61	41.61	37.61	30.61	27.61
14	60.11	49.11	45.11	38.11	33.11
16	69.23	57.23	51.23	46.23	39.23
18	77.29	65.29	59.29	53.29	45.29
20	85.21	73.21	66.21	59.21	52.21
22	93.07	81.07	72.07	67.07	58.07
24	97.61	88.61	79.61	71.61	65.61
26	99.34	97.10	88.46	82.28	74.27
28	99.25	98.26	99.08	91.32	83.11
30		98.34	99.10	98.25	92.66
32				98.22	99.76
34				99.32	99.67

Kinetic modeling and Mechanism of drug release

Table 9. Results of model fitting for prepared batches

Batches	Zero order		First Order		Higuchi		Korsmeyer's peppas.	
	K	r^2	k	r^2	K	r^2	N	r^2
P1	10.36	0.955	0.037	0.989	0.740	0.986	0.648	0.987
P2	8.76	0.814	0.031	0.996	0.843	0.992	0.549	0.971
P3	8.00	0.785	0.030	0.970	0.891	0.995	0.560	0.976
P4	7.95	0.814	0.028	0.990	0.895	0.983	0.657	0.993
P5	7.79	0.677	0.027	0.976	0.914	0.996	0.591	0.974

CONCLUSION

Sodium caseinate were made a suitable candidate to prepare mucoadhesive film for buccal drug delivery systems. Mucoadhesive buccal patches of protein salt containing insulin were developed to a satisfactory level in terms of surface pH, bioadhesive strength, drug release and permeation. In the formulation F4, 98.99% of the loaded drug was released *in vitro* from the drug delivery system; over a period of 11 h. Formulation F4 has shown permeation of 99.31% of the loaded insulin across the rabbit buccal mucosa over a period of 54 h. With the use of 10% (w/v) sodium deoxycholate, the time required to show the 99.34% drug release was decreased from 54 hrs to 26 hrs. Analysis of the *in vitro* permeation data revealed that insulin permeated across the mucosa following a first order release pattern. The delivery of protein by the protein developed a novel system which reduces the chance of interaction and incompatibility problem.

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