DEVELOPMENT AND EVALUATION OF FILMS BASED ON MICROEMULSIFIED INSULIN FOR BUCCAL DELIVERY USING MAISINE® OIL, ACACIA AND POLYVINYL ALCOHOL

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ABSTRACT

Insulin is a peptide hormone produced by beta cells in the pancreas which regulates the metabolism of carbohydrates, fats and, it is employed in the treatment of some forms of diabetes mellitus. It can be given as Intramuscular (I.M) injection due to its poor oral bioavailability. The aim of the present study was to develop a buccal film for the non-invasive delivery of insulin across the buccal cavity to the systemic circulation. The buccal films were formulated by dispersing microemulsions of insulin in bioadhesive, film-forming polymer dispersions and drying. The microemulsions contained varying concentrations of Maisine® oil and Phospholipon®90G, while the film-forming dispersions contained varying levels of polyvinyl alcohol and acacia. The prepared buccal films were evaluated for its thickness uniformity, folding endurance, % elongation, swelling index, thermal behaviour and *in-vivo* hypoglycaemic effects. The average thickness of the films was 0.4 mm, the folding endurance was found to be greater than 500 fold for each of the buccal films and the percentage elongation varied between 140 to 320%. The swelling index was found to be between 0.5 to 4.5%. The differential scanning calorimetry (DSC) results showed no drug-excipient interaction. In the *in vivo* studies, the optimized film showed a slow-acting profile in comparison to the glucose reduction capacity of I.M insulin administration.

KEYWORDS: Bioadhesive; hypoglycaemic; diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a metabolic disease in which there is high blood sugar level over a prolonged period. The metabolic disorder is usually caused by a defect in insulin secretion, insulin action or both.[1] Consequently insulin deficiency leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism. As the disease gradually progresses, a lot of tissue or vascular damage occur leading ultimately to severe diabetic complications such as, neuropathy,[2] nephropathy,[3] retinopathy,[4] cardiovascular abnormalities [5] and ulceration.[6] Insulin is a peptide hormone produced by beta cells in the pancreas which regulates the metabolism of carbohydrates, fats and, it is employed in the treatment of some forms of diabetes mellitus.

Although the short duration of action of rapid-acting regular insulin allows for better control over post-prandial glucose levels, it does not satisfy, alone, the basal insulin requirement in diabetic patients with low endogenous insulin reserve (type 1).[7]

Furthermore, insulin formulations usually show wide variations in absorption between different individuals (inter-subject variability), and in different injection sites. Different moments in the same subject can also result to different insulin effects. These differences in effects of insulin preparations occur in a manner which is directly proportional to the duration of their action.[8] This phenomenon is small, but still significant with Regular insulin, it is greater with NPH, Lente and greatest with Ultralente. Another problem encountered with insulin is their time-action profile. Their absorption gives rise to an insulin peak and a lowering effect on blood glucose, the major cause of nocturnal hypoglycemia which affects many insulin-treated patients.[9] The peak is followed by a significant drop in plasma insulin concentrations after an interval of about 7-8 h, resulting in morning hyperglycemia. An attempt was made to correct this distortion by administering long-acting insulin at bedtime, together with Regular forms. Although this strategy has improved basal hyperglycemia in many patients, enabling a reduction in dose, and consequently a reduced risk of nocturnal hypoglycemia, better results are still desired.

Usually insulin is administered using invasive routes including Intramuscular (I.M) and sub-cutaneous (S.C) administration. It is typically required that patients with diabetes should inject themselves with pre-calculated insulin doses, in some cases, several times daily. This procedure is usually difficult and painful which often lead to non-compliance or lack of adherence in patients. Self-administration of injectable insulin may also lead to accidental injury.

Based on these serious challenges and problems, there is urgent need to develop non-invasive delivery of insulin. A possible route is delivery through buccal cavity. Therefore, the aim of this present study is to develop and evaluate films based on microemulsified insulin for buccal delivery using maisine® oil, acacia and polyvinyl alcohol.

MATERIALS AND METHODS

Materials
Human Insulin (isophane insulin injection Torrent pharmaceuticals limited India), phospholipon® 90G (Phospholipid GmbH, Cologne, Germany), polyvinyl alcohol (Merck, Germany), Acacia (BDH, England), maisine®35–1(glyceryl monolinoleate) (Gattefosse, SAS France).

Twelve albino rats were obtained from pharmacology laboratory, University of Nigeria, Nsukka. The animals were housed in stainless metabolic cages and provided with standard diet and water *ad libitum*. The animals used in this study were cared for and all treatment procedures were performed in accordance with guidelines in animal ethics in Nigeria, which complied with Helsinki declaration.

Preparation of insulin microemulsion
Microemulsions of insulin were prepared using varying mixtures of Maisine® oil, Phospholipon®90G and insulin solution as presented in Table 1. Phospholipon®90G was employed as surface active agent and permeation enhancer.

The corresponding amount of Phospholipon®90G (P90G) for each batch was weighed out and heated to melt in a beaker on a water bath at 80 °C. The molten P90G was cooled to 60 °C and mixed with the required volume of Maisine® oil at that temperature for 3 min in the water bath. The lipid mixture was allowed to cool to ambient temperature and 1ml of insulin solution was added and mixed gently to form each batch.

Table 1: Formula for the preparation of four batches of insulin microemulsions.

Batch	A	B	C	D
Insulin(ml)	1	2	3	4
Maisine ® (ml)	2	2	1	1
Phospholipon90G (mg)	500	400	300	200

Preparation of film forming polymer dispersion and bioadhesive mucilage
Appropriate quantity polyvinyl alcohol (PVA) was dispersed in water and mixed gently to form a 16.7 %w/w polyvinyl alcohol solution. The PVA was weighed into a beaker and distilled water was added with gentle mixing until the PVA solution was formed.

Acacia powder was dispersed in warm distilled water and mixed gently to form a uniform dispersion of 16.7 %w/w acacia mucilage.

Preparation of bioadhesive film-forming polymer dispersion
Film-forming polymer dispersion and bioadhesive mucilage were mixed at a volume ratio of 9:1, and the solution was mixed gently and allowed to stand.

Formulation of insulin films for buccal delivery
Insulin films for buccal delivery were formulated by mixing insulin microemulsions, PVA dispersion and bioadhesive mucilage (bioadhesive film-forming dispersion) in a beaker and thereafter transferred into a petri dish (9 cm diameter and 1.2 cm height) with brief air-drying (30 min) and storage at 4 °C (not frozen). The different films were prepared using Table 2. For each batch of the insulin-loaded microemulsions (A, B, C and D), 1 ml of the microemulsion was mixed with 2, 4 and 8 ml of the bioadhesive film-forming dispersion.

Table 2: Formula for the formulation of insulin-loaded buccal films.

Film	Microemulsion batch	Volume of Insulin-loaded microemulsions (ml)	Bioadhesive film-forming dispersion (ml)
A1	A	1	2
A2	A	1	4
A3	A	1	8
B1	B	1	2
B2	B	1	4
B3	B	1	8
C1	C	1	2
C2	C	1	4
C3	C	1	8
D1	D	1	2
D2	D	1	4
D3	D	1	8

Characterization of insulin films
Differential Scanning Calorimetry (DSC)
A differential scanning calorimeter (NETZSCH DSC 204 F1, Germany) was used for thermal analysis of the films and excipients. The study was performed to detect any incompatibilities between film constituents and to investigate thermally–induced phase transition of the materials. The DSC was performed at a temperature range of 30-400°C and heating rate of 10 K/min on an aluminium pan with a pierced lid.

Physical Appearance and Properties
All the formulated films were inspected for clarity, colour, and smoothness. Also, other organoleptic properties such as odour were also observed.

Thickness uniformity and folding endurance of films
The thickness of each film was measured at different points of the films using a caliper and the average and variations were calculated accordingly.

Folding endurance was determined manually by folding a 1cm^2 strip of each film repeatedly at the same place on the film until a breaking point is reached. The number of times that the film could be folded at the same place without breaking or cracking is taken as the value for the folding endurance.[10]

Percentage elongation break test
The film samples (1 x 1cm) were placed on a metre rule and stretched until it breaks. The lengths were taken before and after stretching to its breaking point. The films were increasingly stretched and the percentage elongation break was determined by recording the length just before the break point and using the Equation 1:

$$Elongation \% = \frac{L1 - L2}{L2} \times 100 \dots\dots\dots\dots\dots .1$$

where L_1 and L_2 are the final and initial lengths of each strip.

Swelling Index (SI)
The buccal films were dried until constant weight using desiccators containing anhydrous calcium chloride at room temperature for one day. The films were immersed in 100 ml of distilled water at 37 ^{0}C and weighed after removal of excess water by pressing it gently between two filter papers (W_2). The re-weighed films were retained in the desiccators and allowed to be dried to constant weight. (W_3). Swelling index (S.I) was calculated from the Equation 2.[11]

$$S.I = \frac{W_2 - W_3}{W_3} \dots\dots\dots\dots\dots\dots\dots .2$$

Where W_2 is the weight of immersed film and W_3 is the weight of redried film.

***In vivo* study**
Albino rats were used in the in vivo study. The animals were divided into three different groups (A, B, C). Group A (the test group) was treated with optimized test batch (D_2), Group B (negative control group) was treated with bioadhesive polyvinyl alcohol film, group C (positive control group) was treated with I.M insulin injection (0.25 ml) equivalent to 10 I.U. of insulin from vial. A 1x1 cm size of test insulin-loaded film contains an equivalent 10 I.U of insulin.

The animals were fasted over-night and the basal blood glucose levels were determined using glucose meter. A 1x1 cm size of test films was placed on the upper buccal cavity of the rats (Groups A and B). A 0.25 ml (10 I.U) quantity of insulin from vial was administered to the positive control groups (Group C) through the thigh of their hind limbs and the blood glucose level of the three groups were determined after 2, 5 and 8 h and were recorded.

RESULTS AND DISCUSSION

Organoleptic and Physical properties
The prepared mucoadhesive buccal films were white, smooth, uniform, and flexible with the drug droplets evenly dispersed.

Folding endurance, % elongation, thickness and weight of film
All the batches of buccal films showed folding endurance greater than 500 (Table 3) which indicated high flexibility and mechanical strength. The percent elongation was in the range of 140-320% indicating strong bond formation of the polymers. It was observed that with increase in bioadhesive film-forming mucilage concentration, the percent elongation (Table 3) and plasticity of the films gradually increased. Reports have suggested that differences in composition of hydrogels causes changes in percentage breaking elongation.[12] The average thickness of the films was 0.4 mm, and the weight of a 1×1 cm film was around 50 mg. These mechanical and elastic attributes of the buccal films are important for physical stability and integrity since the human jaw and buccal cavity is always in motion. Furthermore, these simple physical tests, together, simulate the different directions of motion that the buccal cavity can undergo. The folding endurance test mimics the folding and contractive motion while the elongation test simulates the stretching motion of the buccal area. The result of the tests then showed that the films can withstand these motions.

Swelling index
The swelling index did not follow any definite pattern (Table 3). This can be attributed to the negligible time spent in the aqueous medium (just dip and withdraw) since the buccal application of the films do not require its total immersion in a considerable volume of medium. This then implies that the swelling index is now dependent on the opposing effects of increasing bioadhesive film-forming polymers and decreasing P90G concentration (Table 1 and 2) on the small volume of water absorbed within the very short time it is immersed in aqueous medium. Usually the hydrogel achieves an equilibrium state when the force of absorbing water balances the opposing elastic force of the network.[13] Generally, very low levels of water was integrated into the film matrix, although batch D2 (high insulin, low P90G and 1:4 insulin-bioadhesive film-former ratio) had as much as 4.5% swelling.

Drug-excipients compatibility study (thermal analysis)

The DSC thermograms (Fig. 1) revealed no physical or chemical interactions of the components of the formulated film. No new peak was observed in the thermograms. All the peaks represented the components while amorphous materials showed diffused peaks. The materials basically displayed glass transitions and amorphous structures with usually broad, very short or diffused peaks. There was no sign of emergence of a large, sharp peak and this signifies chemically stability.

Table 3: Folding endurance, swelling index and % elongation of the mucoadhesive films.

Sample	Folding endurance	Swelling index (%)	% Elongation (%)
A1	>500	3.00	140
A2	>500	1.00	200
A3	>500	0.50	220
B1	>500	2.00	200
B2	>500	0.70	210
B3	>500	1.25	240
C1	>500	0.50	180
C2	>500	1.75	210
C3	>500	1.40	170
D1	>500	0.80	200
D2	>500	4.50	240
D3	>500	0.30	320
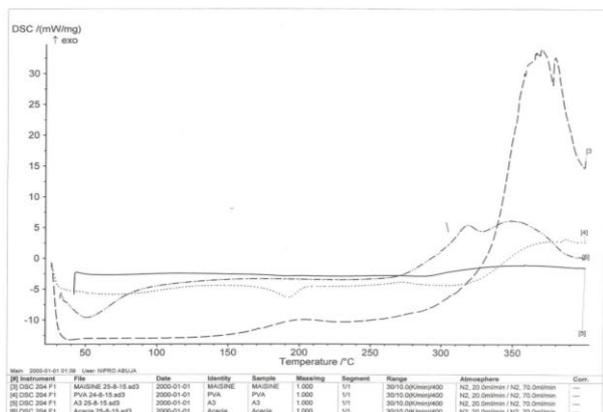
Fig. 1: DSC thermograms of representative film, Maisine®, Acacia, PVA).

Comparative evaluation of the hypoglycemic effect of insulin administration (I.M) and a selected buccal film

The hypoglycemic effect of intramuscular (I.M) insulin administration was compared with a selected batch (D2) using equivalent concentration of insulin contained in 1x1cm of cut buccal film (10 I.U. of insulin) which is equivalent to 0.25 ml of Human insulin solution (40 I.U. ml). A 1x1 cm size of PVA mucoadhesive film without insulin was used as negative control.

The prepared insulin buccal films showed slow-acting characteristics (Fig. 2) different from that of I.M insulin administration which had a faster onset of action and adequate bioavailability more than that of the buccal film which may be as a result of prolonged release or insufficient absorption. The insulin-loaded buccal films showed higher reduction in glucose levels of the rat blood after 2 h of administration compared to the negative control up to around 7.5 h. This revealed that the buccal films were capable of releasing insulin in a manner that successfully led to its absorption. During the initial 2 h of administration the test film and placebo film showed small but equal reduction in glucose level and this may actually signify a delayed release of insulin from the test film. This profile of the test buccal film may signify its possible application in the prevention of nocturnal hypoglycaemia and early morning hyperglycaemia that may be associated with use of rapid-acting insulin products alone as mentioned in the introduction. Between 2 to 7.5 h, the buccal film continuously reduced glucose level and released insulin relative to the placebo. During this period, insulin solution showed a steady decline in glucose reduction although values were still higher than those of the insulin film.

A possible application based on the relative glucose reduction profile of insulin solution (positive control) and the test film is the co-administration of both rapid-acting solution and buccal film at bed time. The co-administered product would be similar in action to a sustained release insulin product with a high loading dose and a maintenance dose.

Differences in blood flow around the region of insulin administration may also affect its onset of action.[7] Absorption is associated with capillary area and muscle contraction and vasodilation around the site of administration significantly affect the speed of glucose reduction action of the insulin. Reports have shown that individual variability in insulin absorption is very significant and this can cause differences in the levels of daily insulin absorption.[14]

The glucose reduction profile of the test insulin film shows that increase in the loaded dose may increase the glucose reduction and extend the duration of action.
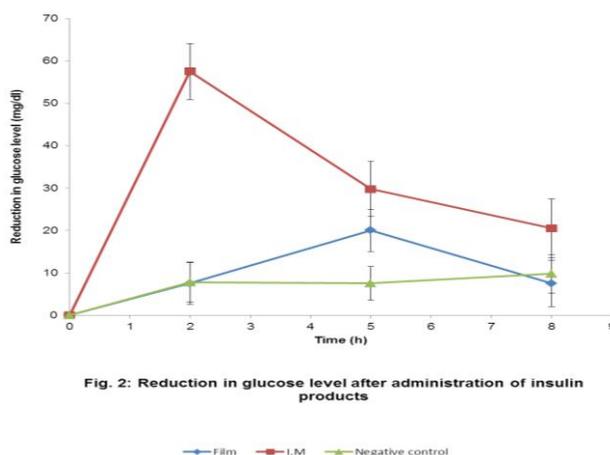
Fig. 2: Reduction in glucose level after administration of insulin products

CONCLUSION

The formulated insulin film for buccal delivery showed favourable physical, chemical and mechanical properties. The test films provided slow-acting insulin delivery in rats. In the *in vivo* studies, the optimized film showed a slow and delayed-action profile in comparison to the I.M administration of insulin. It was concluded that microemulsified insulin films based on PVA can be co-administered with insulin injections or serve as a promising alternative to insulin injections, especially in terms of self-administration, convenience, possible prolonged action and nocturnal use.

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