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Physicochemical Properties and Glucose-Lowering Effect of An Insulin - *Aloe vera* Buccal Delivery System

(Sifat Fizikokimia dan Kesan Penurunan Glukosa daripada Sistem Penghantaran Bukal Insulin - *Aloe vera*)

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ABSTRACT

Subcutaneous insulin injection is one of the therapies in the treatment of diabetes mellitus. However, problems such as pain at the injection site and lipodystrophy present a challenge that will influence patient compliance. This study aims to develop and characterise buccal film formulations containing insulin, utilising the glucose-lowering and pharmaceutical properties of Aloe vera*. Characterisation tests such as morphology, rheological measurement, pH value, mechanical properties, and permeation test were performed on the optimal formulation. Assessment of the glucose-lowering efficacy was performed using alloxan-induced diabetic rabbits. Composition of the final film formulation included 3% w/w sodium carboxymethyl cellulose, 40% v/v glycerol, 70% v/v* Aloe vera*, 0.5% w/v mannitol, 0.125% w/v aspartame, 0.125% v/v Tween 80 and 15.3 mg insulin. The drug content has been determined to be 56.77 ± 8.55%. The formulation shows a low variation in weight and thickness measurements and acceptable physicochemical properties, in addition to sustained drug release for 6 h. The final film formulation that combined insulin and* Aloe vera *was effective enough to reduce blood glucose levels compared to the negative control (p > 0.05), despite fluctuation in the blood glucose levels throughout the study, and did not show any enhancement in reduction compared to the insulin-only film. In conclusion, the developed buccal film formulation has the potential to be used as a vessel for the delivery of insulin. Nevertheless, further studies are required to ensure the stability of the insulin* via *buccal route and stabilisation of insulin within the formulation.*

Keywords: Aloe vera*; buccal drug delivery; diabetes; insulin*

ABSTRAK

Suntikan subkutaneus insulin merupakan salah satu terapi untuk rawatan diabetes melitus. Namun begitu, masalah berkaitan penyuntikan seperti sakit pada tempat suntikan dan lipodistrofi menjadi satu cabaran yang akan mempengaruhi pematuhan pesakit. Tujuan kajian ini adalah untuk membangun dan mencirikan formulasi filem bukal yang mengandungi insulin, memanfaatkan kesan penurunan aras glukos dan ciri farmaseutik Aloe vera*. Pencirian seperti morfologi, pengukuran reologi, nilai pH, sifat mekanik dan ujian penembusan dijalankan pada formulasi filem optimum. Penilaian kadar penurunan glukosa darah dijalankan dengan menggunakan model arnab yang diaruh aloksan sebagai model untuk diabetes. Komposisi filem bukal terpilih terdiri daripada 3% b/b SCMC, 40% i/i gliserol, 70% i/i gel* Aloe vera*, 0.5% b/i manitol, 0.125% b/i aspartam, 0.125% i/i polisorbat 80 dan 15.3 mg insulin. Peratus kandungan insulin adalah 56.77 ± 8.55%. Formulasi ini menunjukkan variasi berat dan ketebalan filem yang rendah dan sifat fizikokimia yang memuaskan, di samping menunjukkan pelepasan dadah secara tertahan untuk tempoh enam jam. Filem terpilih yang menggabungkan insulin dan* Aloe vera *didapati berkesan dalam mengurangkan aras glukosa darah arnab berbanding kumpulan kawalan negatif (p >0.05), sungguhpun berlaku turun naik aras glukosa sepanjang tempoh kajian dan tidak menunjukkan penurunan dipertingkat berbanding filem insulin. Kesimpulannya, filem bukal yang dibangunkan mempunyai potensi untuk digunakan sebagai jasad penghantar insulin. Namun yang demikian, kajian lanjut diperlukan untuk menentukan kestabilan insulin yang dihantar melalui tapakjalan ini dan penstabilan insulin di dalam formulasi yang dibangunkan.*

Kata kunci: Aloe vera*; diabetes; insulin; penghantaran bukal dadah*

Introduction

Diabetes is a chronic endocrinological or metabolic disorder characterised by a higher level of blood glucose due to decreased or ceased insulin production (Abo-Youssef & Messiha 2013). According to the International Diabetes Foundation, diabetes currently affects 463 million people worldwide, and by 2045, this number will increase to 700 million (International Diabetes Foundation 2019). Insulin is an important pharmacological intervention to control blood glucose level in diabetics by facilitating the uptake of glucose (Ismail & Csóka 2017). It is still mainly administered through subcutaneous injection because of its instability, degradability, and low permeability, hence, absorption; in the gastrointestinal system (Alibolandi et al. 2016; Fonte et al. 2015). However, multiple daily insulin injections may cause infection in addition to psychological stress, leading to poor patient compliance (Fonte et al. 2015). These drawbacks have motivated researchers to develop safe and effective non-invasive strategies for insulin delivery (Ahmad et al. 2016, 2014). Numerous studies have attempted to deliver insulin across the buccal membrane while still maintaining its safety over repetitive use. Among them, Xu et al. (2002) successfully delivered insulin transbucally *via* a spray formulation with a bioavailability of 29.2% achieved using a combination of soybean lecithin and propanediol as permeation enhancers. Other attempts included conjugating insulin to cell-penetrating peptides to overcome the buccal barrier (Xu et al. 2020).

Although the oral mucosa has many properties that make it an attractive site for drug delivery, it presents several challenges. Systems designed for local delivery to mucosal diseases need different pharmacokinetic behaviours for systemic applications. It has been shown, for example, that the oral mucosa can be 4 to 4000 times more permeable compared to the skin (Dixit & Puthli 2009). The buccal route is easily accessible for self-medication (Abruzzo et al. 2012; Zulfakar et al. 2016). In particular, mucoadhesive buccal films can ensure accurate drug dosing compared to liquid formulations and gels, which can be easily washed away by saliva and can be more comfortable (Abruzzo et al. 2012; Zulfakar et al. 2016).

Sodium carboxylmethyl cellulose (SCMC) is the sodium salt of carboxymethyl cellulose ether and is non-toxic, biodegradable, and soluble in both hot and cold water. Upon dissolution, the electrolytic process separates the carboxylmethyl cellulose (CMC) molecule into a cation of sodium and a polymer anion to produce CMC polyelectrolyte. Ions in solution interact through electrostatic forces (Behra et al. 2019). SCMC has been used as a suspending agent, thickener, protective colloid, and moisturizer (Lopez et al. 2015). This polymer relies on strong hydrogen bonds between carboxylate groups and tissues for mucosal adhesion (Wasilewska & Winnicka 2019).

This study aims to administer insulin through the buccal cavity *via* mucoadhesive films prepared from a combination of SCMC and gels obtained from *Aloe vera* leaves. *Aloe vera* is a perennial succulent xerophyte (Choi et al. 2003) and comprises many constituents such as amino acids, anthraquinones, enzymes, minerals, vitamins, lignins, monosaccharide, polysaccharides, salicylic acid, saponins and phytosterols (Choudhary et al. 2014). Many authors have supported the antidiabetic and antioxidant potential of *Aloe vera* in experimentally-induced diabetes (Ajabnoor 1990; Can et al. 2004). The glucose-lowering effects of *Aloe vera* were suggested to be mediated, at least partially, through its potent antioxidant effect (Boudreau & Beland 2006; Zulfakar et al. 2018). Therefore, in the present study, *Aloe vera* has been used along with insulin as a model drug to analyse its effectiveness as an anti-diabetic remedy.

Materials and Methods

Materials

Human insulin, alloxan monohydrate, trifluoroacetic acid HPLC grade, phosphate buffer saline, formalin solution, and sodium carboxymethyl cellulose were obtained commercially from Sigma Aldrich, Germany. Aloe vera (*Aloe barbadensis)* leaves, at an estimated age of one year, were purchased from PPA Bio Sdn. Bhd, Malaysia. Glycerine was purchased from Bendosen Laboratory Chemicals, Malaysia. Tween 80 was obtained from R&M Chemicals, Malaysia and aspartame from Supelco, Pennsylvania, USA. Acetonitrile was purchased from Merck Germany. Hydrochloric acid methanol was purchased from Friedmann Schmidt Pty Ltd., USA. All the materials used were of analytical grade.

Methods

Preparation of *Aloe vera* gel

Aloe vera leaves measuring 50-62 cm were first washed with tap water and then wiped dry with a lint-free cloth. Subsequently, the leaves were cut transversely and the outer cuticle removed with the aid of a knife. The leaves

were then homogenised using an Alba heavy-duty blender (model no. EBL-A1812G(SS)) (Malaysia). The bubbles that formed were removed, and the extract was subjected to centrifugation at 5,000 rpm at 5 °C for 30 min. The supernatant was then carefully isolated and filtered through Whatman® filter paper no. 1 using a Buchner funnel. The filtrate was then collected and frozen until further use.

DEVELOPMENT OF BUCCAL FILMS

The solvent casting method was employed to formulate buccal films; A 3% w/w solution of sodium carboxymethyl cellulose in de-ionised water was prepared. The polymer was stirred in water until a homogenous mixture was formed (labelled as SCB). Glycerine was used as a plasticiser at two different concentrations of 20 and 40% (of total polymer weight) to plasticise the films. 0.125% v/v Tween 80 was added in drop-wise manner to enhance buccal permeation while mannitol was added at 0.5% w/v as a cooling agent and aspartame at 0.125% w/v as a sweetening agent. Insulin, as an active ingredient, was incorporated at 15.3 mg into the optimal film formulation (SCF-I only). The ingredients for each formulation used in this are listed in Table 1. The mixtures were then left to stand to remove entrapped bubbles. 40 mL of the mixture was cast into a petri dish of 8.5 cm diameter and oven-dried at 40 °C for 24 h. The resulting films were then carefully peeled off. The film was then cut out into 2 × 2 cm squares using a sharp blade and stored in a desiccator until further use.

PHYSICAL APPEARANCE

All the films produced were evaluated for their physical characteristics such as colour, opacity, and smoothness. Only films that were transparent or translucent, flexible, and smooth were used for further studies.

MEASUREMENT OF MECHANICAL PROPERTIES

The samples were analysed using a universal testing machine of the model 5567 (Instron Corp., USA), according to the American Society for Testing and Materials D 882-02 guidelines for films less than 1.0 mm in thickness. All the samples were conditioned for at least 40 h at 23 ± 2 °C and 50 ± 5% relative humidity before analysis. They were cut out around a standard template in a dumbbell shape, of which the gauge length was 30 and 5 mm across and fixed between grips. The rate of grip separation was set at 12.5 mm min^{-1}, and the films were stretched to the breaking point. Parameters such as tensile strength, elastic modulus, elongation at the breakpoint, percentage elongation and strain were calculated for the film formulations.

RHEOLOGICAL MEASUREMENT

The rheological properties were obtained using a Gemini 200 rheometer (Malvern Instruments Ltd., UK) with a cone-and-plate measuring system. The shear rate was ramped up from zero to 300 s^{-1} in one minute and then ramped down from 300 to zero s^{-1} in the same interval. A cone of 2°/55 mm was used, and the measurements were obtained at ambient conditions and repeated three times. The rheograms were then plotted and analysed to explain the flow behaviour of the formulations studied. The apparent viscosity was recorded at the highest shear rate (300 s^{-1}).

pH VALUE

Prior to pH measurement, the buccal films were allowed to swell for 2 h at room temperature in 2 mL of simulated salivary fluid (SSF) made up of 0.01 M phosphate-buffered saline (PBS) (NaCl 0.138 M, KCl 0.0027 M) per litre of deionised water adjusted with orthophosphoric acid to a pH of 6.75. The surface pH was then measured using a pH meter (Mettler Toledo International Corp., Switzerland).

FIELD EMISSION SCANNING ELECTRON MICROSCOPY (FESEM)

The surface and cross-section morphology of the films after the addition of each excipient were analysed by the FESEM model, Merlin (Carl Zeiss Inc., Germany). To prepare the samples, the films were first kept in a desiccator at a low relative humidity of approximately 7% using silica gel for 48 h and then fractured using liquid nitrogen. The sample was then mounted onto an aluminium stub and sputter-coated with iridium for 1 min. It was loaded into the instrument and scanned by the electrons emitted by the field emission source. The surface and cross-section images of the films were then used for structural evaluation.

ex vivo DETERMINATION OF INSULIN IN *A. vera* BUCCAL FILM PREPARATION OF PORCINE BUCCAL MUCOSA

Drug-release studies were carried out to determine the release of insulin from the optimised formulation (SCF-I). Porcine buccal mucosa obtained from a local butcher was washed with normal saline. Then, using a sharp scalpel blade, excess fat and connective tissues were carefully removed from the substrate. The buccal mucosa was

wrapped in an aluminium foil and stored at -20 °C for further analysis.

DRUG PERMEATION EXPERIMENT

The transbuccal permeability of the insulin released from the optimised buccal film (SCF-1) was evaluated using vertical Franz diffusion glass cells (PermeGear Inc., USA) with 5.0 mL receptor volume and a diffusional area of 1.0 cm^2 utilising porcine buccal mucosa. The receptor compartment was filled with phosphate-buffered saline (PBS) and continuously stirred with a magnetic bar at 130 rpm. The porcine buccal membrane was carefully sandwiched between the receptor and the donor compartments that were then clamped together. The temperature was equilibrated at 37 ± 0.5 °C using circulating water to closely mimic the human body temperature. The set-up was equilibrated for half an hour before the start of the experiment. The buccal film was cut into discs of 1.15 cm diameter and then applied to the surface of the buccal tissue. 0.5 mL of SSF was added to the donor compartment to simulate *in vivo* physiological conditions. The donor top was subsequently covered with a paraffin film, and the receptor arm was capped throughout the experiment to prevent evaporation. At predetermined time points for 6 h (0.5, 1, 1.5, 2, 3, 4, 5, and 6 h), the whole recipient content was sampled using a plastic syringe and then replaced with an equal volume of preheated PBS at 37 °C to maintain the sink conditions. The samples containing insulin were analysed using reverse-phase high performance liquid chromatography (RP-HPLC).

REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The chromatographic system consisted of RP-HPLC (Waters Corp., USA) with Waters XBridge™ C18 column (250 × 4.6 mm, 5 µm). The mobile phase for insulin analysis consisted of 0.1% v/v trifluoroacetic acid (TFA) in water: absolute acetonitrile (67: 33) was delivered at a flow rate of 1 mL/min with an injection volume of 20 µL. Wavelength was set at 214 nm and concentration of the insulin permeated was determined against a standard curve.

ANALYSIS OF PERMEATION DATA AND RELEASE KINETICS

The cumulative amount of the drug permeated through the tissue per unit area was calculated and plotted as a function of time. The flux (Q) of the drug was then calculated from the slope of the linear portion of the curve. The apparent permeability coefficient, K_p, was calculated as follows (1):

$$K_p = Q / C_D \quad (1)$$

where Q is the flux of the drug; and C_D is the drug concentration in the donor chamber.

The release kinetic model describing the dissolution profile of the buccal films was predicted using the DDSolver. Different kinetic models such as zero order (cumulative amount of drug permeated vs. time), first order (log cumulative percentage of drug remaining vs. time), Higuchi model (cumulative percentage drug permeated vs. square root of time) and Korsemeyer-Peppas model (log cumulative percentage drug permeated vs. log time) have been used to describe the mechanism of drug release kinetics. A correlation coefficient (R^2) closest to one was employed as the key to determining the model with the best linear fit.

in vivo DETERMINATION OF HYPOGLYCAEMIC PROPERTIES OF BUCCAL FILMS

DEVELOPMENT OF DIABETIC RABBIT MODEL

All the experimental protocols were approved in advance by the UKM Animal Ethical Committee at Universiti Kebangsaan Malaysia with the approval number FF/2018/MOHD HANIF/25-JULY/940-SEPT.-2018-NOV.-2018. The environmental conditions were maintained at 20 ± 2 °C and with a 12 h light-dark cycle. Both male and female New Zealand long-eared white rabbits weighing 3.0 ± 0.5 kg were obtained and placed in an animal handling room. Before the start of the experimental procedure, the rabbits were allowed to acclimatize for at least 7 days in individual metal cages. To induce diabetes, 5% of alloxan solution dissolved in normal saline was injected into the ear vein of the rabbits at a dosage of 100 $mgkg^{-1}$. The diabetic model was developed successfully after 48 h if the blood glucose of the rabbits exceeded 15 $mmolL^{-1}$. For the *in vivo* studies, an equivalent amount of insulin from SCF-I formulation was also loaded into SCG2 formulation to analyse the influence of the *Aloe vera* gel and to distinguish it from the insulin effect. The rabbits were randomly divided into 4 groups of 6 rabbits each, as shown in Table 2. The rabbits were fasted but allowed access to water *ad libitum* for 12 h before the experiment. The tested rabbits were each anaesthetised by an intramuscular injection of the KTX mixture. The mixture was prepared by dissolving 250 mg of Zoletil 50 into a fluid mixture with 250 mg of ketamine and 250 mg of xylazine. Before film application, 50 µL of distilled water was dropped into the rabbit's oral cavity; then, the required size of the film preparation was applied to the buccal cavity bilaterally in halves.

DETERMINATION OF BLOOD GLUCOSE

To determine the hypoglycaemic rate, the blood glucose was assayed with a Medisafe Fit C blood glucose meter (Terumo Corp., Japan). The film was administered once daily for 10 days. The bodyweight of the rabbits was measured at the beginning and then on the 1st, 3rd, 5th, 8th and 10th day of buccal treatment while fasting; the blood glucose levels were recorded at the beginning and then a day after the treatment on the 1st, 3rd, 5th, 8th and 10th day. At the end of the experiment, the animals were sacrificed, and histological studies were performed.

HISTOPATHOLOGY

Histological studies were carried out to assess the effect of the buccal films on the buccal mucosa. The buccal mucosa exposed to the films was excised into small pieces approximately 1 × 1 cm in size. The tissues were then fixed with 10% buffered formalin to later embed in paraffin wax. The embedded tissue samples were serially sliced into 5 µm of thickness sections with RM2235 microtome (Leica Microsystems Nussloch GmbH, Germany). The sections were then stained with haematoxylin and eosin to observe the histopathological features of the buccal tissues (Hussain et al. 2013).

STATISTICAL ANALYSIS

All the data were presented as mean ± standard deviation. The data were analysed with either paired t-test or independent t-test and ANOVA, followed by the Scheffe test for parametric data. Meanwhile, Kruskal-Wallis followed by Mann-Whitney U was used for non-parametric data. The results were considered significant at a p-value of < 0.05. Statistical analysis was performed using IBM SPSS Statistic Version 23.0 (IBM Corp., USA).

RESULTS AND DISCUSSION

PHYSICAL APPEARANCE

The physical appearance of the film formulations was one of the criteria for the selection of an optimal polymeric and plasticiser concentration. It was found that 3% sodium carboxymethyl cellulose-prepared film was smooth, thin, flexible and translucent, which made it an ideal candidate for buccal drug delivery. It was observed that the thickness of polymeric films increased with the addition of glycerine as a plasticiser, *Aloe vera* gel and the remaining excipients ($p > 0.05$). The film containing the maximum concentration of glycerine and *Aloe vera* gel (SCF-B) were chosen to load the insulin due to their thickness and to facilitate the loading of a maximum amount of the drug. The insulin was only loaded in the final formulation (SCF-I) that was of maximum thickness to ensure maximum drug loading capacity and better mechanical properties. The buccal films retained the same physical appearance after loading the insulin. The prepared films were thin, flexible, smooth, and translucent.

MECHANICAL CHARACTERISATION

The mechanical characterisation was performed throughout the preparation phase of the final product to analyse the changes that took place because of the addition of different concentrations of the plasticiser and the *Aloe vera* gel. The tensile strength and durability of the film were found to decrease as the glycerine concentration increased. The same trend was observed in the elasticity modulus, which also decreases with increase in the concentration of the plasticiser. In addition, an increase in the concentration of the plasticiser increased elongation till the breaking point, percentage elongation and strain. A glycerine concentration of 40% w/w (SCG2) was selected for further study based on the higher strain values compared to the glycerine concentration of 20% (SCG1). The results also showed a lower modulus of elasticity for SCG2 and a longer percentage elongation at a higher breakpoint ($p < 0.01$) than SCG1. The buccal films showed a decrease in rigidity and tensile strength at higher *Aloe vera* concertation. The increase in the gel concentration (SCA3) caused a significant decrease in the elastic modulus compared to SCA2 ($p < 0.01$) and SCA1 ($p < 0.05$) formulations. Furthermore, the percent elongation at SCA3 and the breaking point were also significantly higher ($p < 0.01$) compared to the lower concentrations. The 70% w/w concentration of *Aloe vera* gel was found to be optimal for the final film formulation. The insulin was loaded in the blank formulation SCF-B and, after loading the drug, labelled as SCF-I. The mechanical characterisation of all the formulations along with the insulin-loaded formulation is given in Table 3.

RHEOLOGICAL MEASUREMENT

The viscosity and rheological behaviour of the final film mixture loaded with insulin was assessed. It was observed that the formulation exhibited shear-thinning, also known as pseudoplastic behaviour, and approached the Newtonian behaviour with higher shear rates. The apparent viscosity measured at a maximum shear rate of 300 s^{-1} for the formulation was 0.02 Pa.s.

pH

The pH of the film formulation was measured as 6.5 ± 0.2. Saliva has a normal pH range of 6.2-7.6 with 6.7 being the average pH (Baligar et al.2013). The results indicate that the insulin-loaded oral film formulation was suitable for buccal administration.

FESEM

The formulations were observed under FESEM to examine the surface morphology of the formulations. The changes to the surface morphology were observed throughout the preparation stage of the final formulation. The surface of the SCB film (containing only 3% polymer) was smooth and homogeneous without pores and cracks. After the addition of the plasticiser, although there were no pores or cracks, the surface of the film appeared to be coarser. After the *Aloe vera* gel was added, the *Aloe vera* fibres changed the surface morphology completely, and it appeared rough and non-uniform under electron microscopy. After the addition of all the excipients, needle-shaped crystals appeared that can be attributed to the addition of mannitol, a natural morphology of mannitol crystals (Koner et al. 2015). After the addition of insulin, the formulation became smoother, and the absence of cracks indicated the compatibility among all the ingredients. Cross-sectional images of the formulations were also taken to confirm the findings and understand the morphology throughout the formulation (Figure 1).

ex vivo PERMEATION STUDIES

Permeation studies were conducted to find out the mechanism by which the drug could be released and permeated across the buccal surface. Insulin content for the optimised formulation was determined at 56.77 ± 8.55%. During the permeation studies, the film was significantly swollen, making a pathway for the insulin to randomly diffuse out and release from the formulation. The formulation showed compliance with the Korsemeyer-Peppas model ($R^2 = 0.9821$). The diffusional coefficient 'n' value was 0.65, indicating that diffusion is indeed the dominant mechanism of drug permeation and is classified as non-Fickian diffusion. The cumulative permeation of insulin is shown in Figure 2. Cumulatively, 48.96 ± 10.71 μgcm^{-2} insulin was released, with a flux value of 3.550 ± 1.803 $\mu gcm^{-2}h^{-1}$. LOD and LOQ for the HPLC method are 0.171 and 0.518 μgmL^{-1}, respectively. Accuracy of this method, expressed as % recovery, ranged from 97-103%, with a relative error, RE = 0.3. Precision, expressed as %relative standard deviation, RSD = 1.69%.

in vivo GLUCOSE-LOWERING ACTIVITY OF BUCCAL FILM

No significant differences were observed in the glucose levels of all the treatment groups at the beginning of the study. After 30 min of the treatment, an increase in the blood glucose levels in all the groups was seen. This increase indicated the highest glucose levels compared to baseline levels in all the treatment groups throughout the whole duration of the study. The glucose levels were subsequently reduced almost to the baseline at t = 1 h, where it remained steady throughout the study duration. The hypoglycaemic effect was seen in Group I (insulin-only film), where the lowest blood glucose levels compared to all the other groups were recorded. At the end of the study, the blood glucose levels for this group showed a 27.89% reduction. Meanwhile, the combined insulin - *Aloe vera* group (IA), which was expected to show a more pronounced hypoglycaemic effect, exhibited fluctuating levels but was effective in reducing the blood glucose levels compared to the negative control group ($p > 0.05$). The weight of the rabbits also fluctuated throughout the study, which can be attributed to the induced diabetes and the observed fluctuation in blood glucose. The weight and glucose level changes throughout the study period are shown in Figure 3.

The histopathology of the epithelium (for all the groups demonstrated the integrity of epithelium, and no significant changes in the structural morphology of the tissues that could be attributed to diabetic effects, or as a result of the applied film, except for some slight desquamation in the vehicle control. This may be contributed by slight abrasion during the application of the film. Nevertheless, the histological profile assured that the film is safe to be used.

TABLE 1. Composition of buccal films

ID	Aloe Vera gel % v/v	Sodium carboxy-methyl cellulose % w/v	Glycerin % w/w (of total polymer weight)	Tween 80 % v/v	Aspartame % w/v	Mannitol % w/v	Insulin (mg)
SCB	-	3	-	-	-	-	-
SCG1	-	3	20	-	-	-	-
SCG2*	-	3	40	-	-	-	-
SCA1	50	3	40	-	-	-	-
SCA2	60	3	40	-	-	-	-
SCA3	70	3	40	-	-	-	-
SCF-B	70	3	40	0.125	0.125	0.5	-
SCF-I	50	3	40	0.125	0.125	0.5	15.3

*For *in vivo* studies, SCG2 was loaded with insulin as a representative of an insulin only (no *Aloe*) film to determine the influence of aloe on the formulation

TABLE 2. Rabbit diabetic model treatment group for the *in vivo* glucose lowering activity determination

Group	Treatment
Group D	Untreated; negative control
Group V	Blank film (SCF-B); vehicle control
Group I	Insulin-only film (SCG2)
Group IA	Insulin- *Aloe vera* film (SCF-1)

TABLE 3. Mechanical characterisation of buccal film formulations

Formulation	Tensile strength (MPa)	Modulus elasticity (MPa)	Elongation till break point (%mm^{-2})	Percentage elongation (%)	Strain	Tear resistance (N)
SCB	0.13 ± 0.03	2420.78 ± 651.30	0.06 ± 0.02	8.71 ± 3.47	$5.48 \times 10^{-5} \pm 1.11 \times 10^{-5}$	22.02 ± 6.46
SCG1	0.05 ± 0.01	802.58 ± 74.86	0.12 ± 0.02	18.07 ± 2.94	$5.66 \times 10^{-5} \pm 7.92 \times 10^{-6}$	6.85 ± 1.42
SCG2	0.01 ± 0.01	160.78 ± 81.32	0.22 ± 0.03	32.72 ± 3.96	$9.09 \times 10^{-5} \pm 1.56 \times 10^{-5}$	2.05 ± 0.81
SCA1	0.03 ± 0.01	132.84 ± 62.99	0.32 ± 0.03	47.98 ± 5.15	$2.60 \times 10^{-4} \pm 1.32 \times 10^{-5}$	5.09 ± 2.12
SCA2	0.02 ± 0.01	99.24 ± 27.81	0.31 ± 0.03	46.95 ± 4.50	$2.32 \times 10^{-4} \pm 7.28 \times 10^{-5}$	3.62 ± 1.81
SCA3	0.02 ± 0.01	24.76 ± 10.37	0.45 ± 0.03	67.29 ± 4.05	$6.97 \times 10^{-4} \pm 2.75 \times 10^{-4}$	2.55 ± 1.21
SCF-B	0.02 ± 0.00	11.15 ± 3.36	0.49 ± 0.06	73.83 ± 9.21	$2.08 \times 10^{-3} \pm 8.84 \times 10^{-4}$	3.17 ± 0.70
SCF-I	0.06 ± 0.03	325.90 ± 258.16	0.13 ± 0.05	19.56 ± 7.27	$1.97 \times 10^{-4} \pm 1.30 \times 10^{-4}$	9.65 ± 5.05

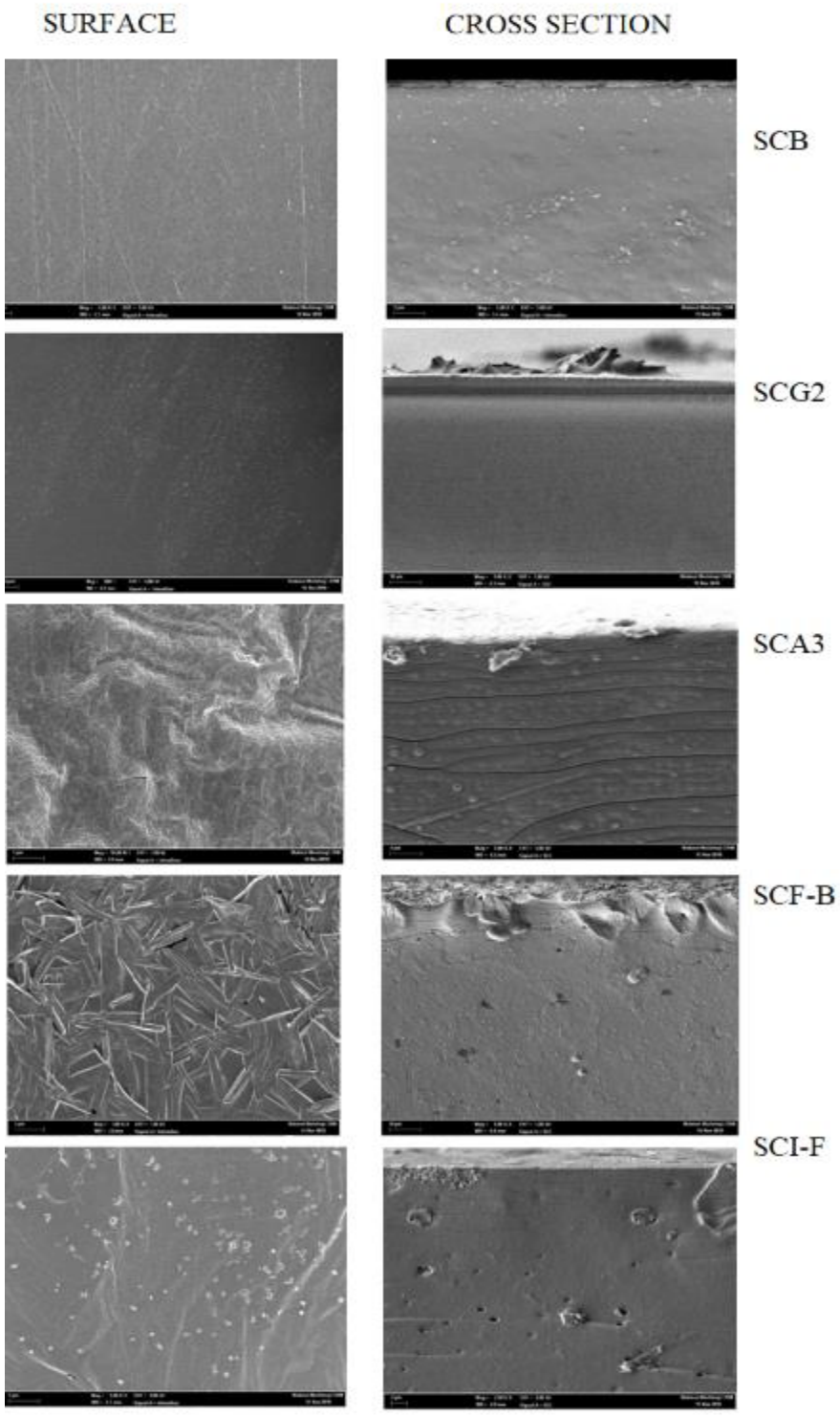

FIGURE 1. FESEM images for surface and cross-sectional morphology of buccal film formulations. Addition of insulin produced films with smooth surface, and the absence of cracks signified compatibility between the ingredients

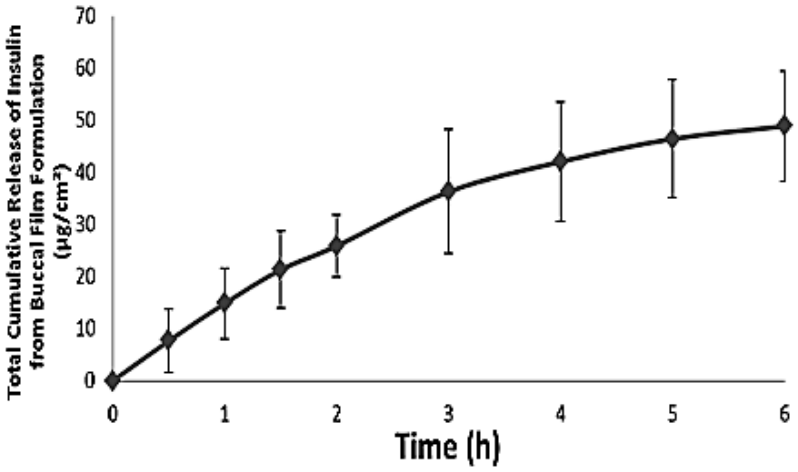

FIGURE 2. % cumulative release of insulin from optimised buccal film formulation. The optimised film exhibited Korsemeyer-Peppas release kinetics with non-Fickian diffusion as the dominant release mechanism

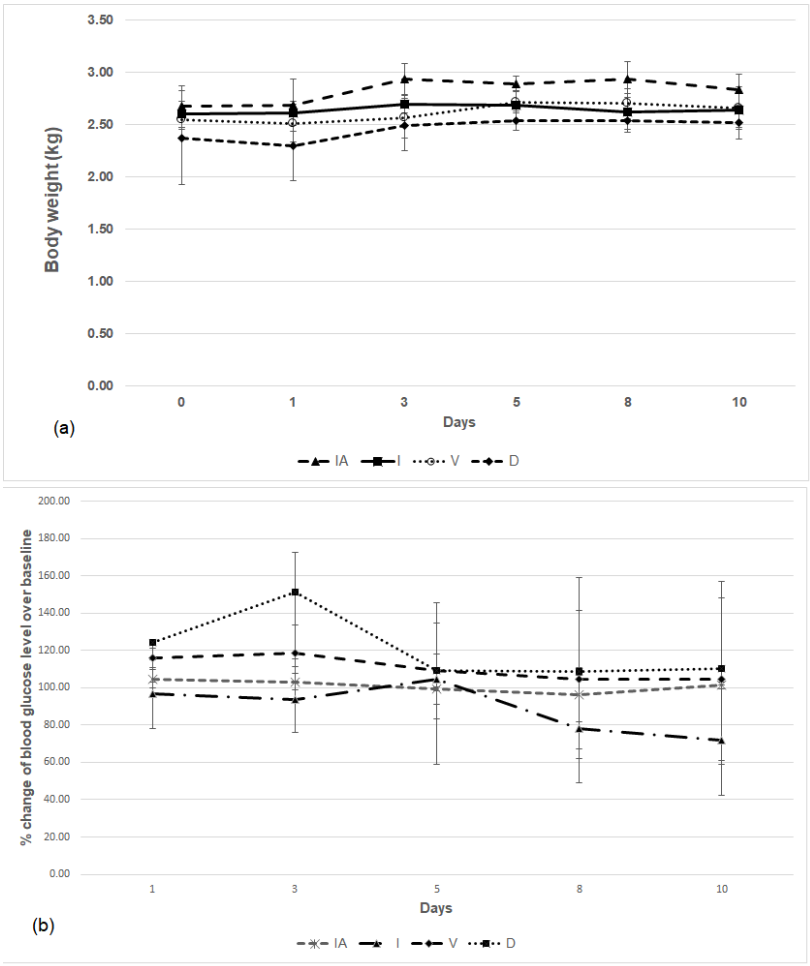

FIGURE 3. Changes in rabbit body weight (a) and blood glucose level compared to baseline (b) during the *in vivo* anti-diabetic studies throughout 10 days. The combined insulin-*Aloe vera* film (IA), despite fluctuating levels, successfully reduced blood glucose compared to the negative control (D)

Conclusion

This study had attempted to formulate a buccal drug delivery system containing insulin as an alternative to subcutaneous injection. Sodium carboxymethyl cellulose, a mucoadhesive polymer, along with *Aloe vera* gel was utilised to formulate an optimised film formulation (SCF-I) for buccal drug delivery. The physical and chemical characterisation of the film formulation was conducted to find out the mechanical strength, adhesiveness, pH suitability, and morphology using scanning electron microscopy. The cumulative drug release and its permeation mechanism across the mucosa were determined, and anti-diabetic studies were conducted on alloxan-induced diabetic rabbit models. The SCF-I formulation displayed significantly ($P > 0.05$) higher hypoglycaemic activity from the negative control, but *Aloe vera* did not appear to influence any anti-diabetic activity or increase any hypoglycaemic effect. The film was determined histologically to not damage the buccal cavity, hence safe to be used. Thus, it can be said that more studies are required to obtain the effective synergetic effects of the insulin - *Aloe vera* combination.

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