Original Article:

The Effect of Acid Modification of Porcine Mucin on Its Drug Release and Skin Permeation Properties in Insulin Transdermal Films

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ABSTRACT

Background: The transdermal delivery of insulin involving the use of polymers has been extensively reported. More recently, the use of mucoadhesive or bioadhesive polymers as an insulin base in its formulation is gaining attention possibly due to the penetration enhancing properties of the polymers.

Objectives: This study aimed at determining the effect of acid-modified porcine mucin powder on the release and permeation of insulin in transdermal films.

Methods: Various batches of insulin films were prepared by solvent casting method using polysorbate 80 as an emulsifying agent and acid-treated and untreated mucin powders as a base. The films were evaluated for their physical properties, folding endurance, moisture content and uptake, drug content, bioadhesion, *in vitro* release, *ex vivo* permeation, and *in vivo* glucose-lowering activity.

Results: The prepared insulin films had a weight range of 0.21-0.27 g, folding endurance of 101-103, moisture content and uptake of 13.73%-18.57% and 11.70%-22.30%, respectively, and drug content of 96%-101%. The bioadhesion of the films prepared with acid-treated mucin was within the range of 0.088-0.186 Nm^{-1} as against 0.055 Nm^{-1} of the films prepared with untreated mucin. The *in vitro* release profiles showed a release of 95% insulin from films prepared with untreated mucin within 2 h while the films made with acid-treated mucin gave a release of about 60%-73% over the same period, indicating a slower release. Animals that received acid-treated mucin-base insulin films showed delayed but sustained blood-glucose-lowering up to 70% and for films prepared with untreated mucin 55% within 12 h.

Conclusion: Insulin transdermal films prepared with acid-modified mucin powder gave superior bioadhesive strength values. They also showed improved drug permeation enhancing ability and achieving up to 70% blood glucose lowering in diabetic rats.

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Introduction

The transdermal delivery of insulin involving the use of polymers has been extensively reported [1-3]. More recently, the use of mucoadhesive or bioadhesive polymers as an insulin base in its formulation is gaining attention possibly due to the penetration enhancing properties of the polymers [4-6].

Diabetes mellitus is a chronic metabolic disorder that results from a failure of the body to produce the hormone insulin and or an inability of the body to respond adequately to circulating insulin. It has become the third most common disease that heavily threatens human health in the world, following cardiovascular diseases and cancers [7]. According to the International Diabetes Federation, approximately 463 million persons worldwide suffer from various forms of diabetes and the number is estimated to rise to 700 million by 2045 [8]. Insulin is not only vital in the treatment of insulin-dependent diabetes mellitus but is also widely used in the treatment of non-insulin-dependent diabetes mellitus. Insulin is mostly administered subcutaneously and therefore, much attention has been paid to the development of non-invasive routes of its administration.

Mucin is ubiquitous in many human and animal tissues and widely used in the formulation of specialized drug delivery systems, especially the mucoadhesive or bioadhesive types [9]. It is highly biocompatible, non-toxic, and biodegradable. It is a good candidate for drug delivery as it can be conjugated to positively charged drug molecules because of its negative charge. It is also used for bioadhesive drug delivery modeling systems. The use of the mucoadhesive properties of mucin has been extensively studied and its high potential as a pharmaceutical excipient needs to be explored further [10]. Previous studies have reported changes to the properties of mucin such as its aggregation and gelation, stability, rheology, adhesiveness, crystallinity, and viscosity when subjected to some modifications [11]. Some of these modifications have been harnessed in the formulation of bioadhesive tablets using acid and alkaline modified mucin resulting in increased tablets' bioadhesion and drug retardation [12, 13]. These improved properties may be beneficial to the transdermal formulation of drugs intended for release in a sustained manner. Hence, this study aimed at investigating the effects of acid modification of porcine mucin on insulin release and skin permeation from transdermal films formulated with the modified mucin powders.

Materials and Methods

Materials

The following materials were purchased from local suppliers and used without further purification: regular human insulin 100 IU/mL (Novo Nordisk, USA), porcine mucin and alloxan monohydrate (Sigma-Aldrich Chemical Company, Germany), polysorbate 80 (Tween® 80) (Carbowax Industrial Company, USA), hydroxypropyl methylcellulose (HPMC, E5 LV) (Shin Etsu Chemical Co. Ltd, Japan), sodium hydroxide, monobasic potassium phosphate and acetone (Merck, Germany), acetic acid (JHD Chemicals, China), concentrated hydrochloric acid and methanol (BDH Chemicals, England), pyridine (May and Baker Ltd, England) and Accu-Chek® Active test strips and glucometer (Roche, USA). All other reagents were of analytical grade.

Acid treatment of porcine mucin powder

The acid modification was carried out as earlier described [14]. Ten grams of mucin powder was weighed into each of 5 beakers (A1-A5). The contents of beaker A1 were left untreated while those of A2 and A3 were mixed with 25 mL of 0.1 and 1.0 M acetic acid solution respectively; beakers A4 and A5 were treated with 25 mL of 0.1 and 1.0 M hydrochloric acid solution, respectively. All beakers were allowed to be at room temperature for 24 h after which the contents of only beakers A2-A5 were neutralized with equimolar volumes of sodium hydroxide solutions and rinsed repeated with distilled water until neutral to litmus. The neutralized contents of the beakers were dried under vacuum at 30°C into flakes. The dried flakes were pulverized into powder and characterized before being used in the formulation of transdermal films. The acid-treated and untreated mucin powders were subjected to the following characterizations such as UV scans, pH, solubility profiles, melting points, particle size determination, differential scanning calorimetry, Fourier transform infrared spectroscopy, scanning electron microscopy, powder x-ray diffraction, and powder bioadhesion studies. Results from these characterizations have been reported earlier [14].

Formulation of transdermal films

In the preparation of the transdermal films, the amount of insulin in a 1 x 1 cm square transdermal film was first calculated using the area of the base of a Petri dish. The target amount of insulin per film was estimated to be 10.0 IU. Using the solvent casting method and the formula in Table 1, a dispersion of the calculated amounts

of mucin and hydroxypropyl methylcellulose (HPMC) powders was prepared in a beaker with 200 mL warm distilled and allowed to stand for an hour for swelling of the polymers.

A separate dispersion of the required volumes of insulin solution and polysorbate 80 in 300 mL of distilled water was added to the aqueous dispersion of mucin/HPMC mix and mixed vigorously using a hot plate magnetic mixer at 400 rpm until a uniform dispersion was achieved. The resulting dispersion was cast on a glass Petri dish and air-dried at room temperature for 48 h [15].

For each batch, an aqueous dispersion of mucin and HPMC powders was prepared in 200 mL warm distilled water in a beaker and left for an hour for swelling of the polymers to occur. The calculated amount of insulin was added to the aqueous polymeric dispersion after levigating with the required volume of polysorbate 80. The dispersion was cast on a glass Petri dish and air-dried at room temperature for 48 h. The film was carefully removed from the Petri dish, checked for any imperfections, and cut into the required size (1×1 cm^2) to deliver the equivalent dose of 10.0 IU per film. Blank films containing no insulin were also prepared for each batch to act as a control in their evaluations. The film samples were stored in between aluminum foils in airtight containers until further analysis.

Evaluation of transdermal films

Physical properties

The prepared transdermal films of the various batches were macroscopically examined for homogeneity and cracking tendency. The thickness of the transdermal films was determined using a Gallenkamp micrometer screw gauge at different points of the film and the average thickness was calculated. To determine their weight variation, 10 films of 1.0 cm^2 were individually weighed using a digital balance (Shimadzu, Japan) and the average weight was calculated [16].

Folding endurance

Folding endurance was determined by folding and opening the film at the same point repeatedly until it broke. The result was expressed as the number of folds endured before breakage [17].

Moisture content

The films (n=3) were weighed individually and kept in a desiccator containing anhydrous calcium chloride. The films were brought out intermittently and re-weighed until their weights were constant. The moisture content was calculated as the difference between the initial and final weights, concerning the final weight, expressed as a percentage [18].

Moisture uptake

The films (n=3) were weighed accurately and placed in a 100% relative humidity chamber. The films were brought out intermittently and re-weighed until there was no further weight gain. The moisture uptake was calculated as the difference between final and initial weights with respect to initial weight, expressed as a percentage [18].

Drug content

Preparation of standard calibration curve

Standard solutions of insulin were prepared by serially diluting a 1.0-mL stock solution of insulin equivalent to 100 IU/mL with distilled water to obtain 10 different concentrations. The absorbance of the serial dilutions was measured at a wavelength of 275 nm using a UV/Visible spectrophotometer (T70 PG Instrument Ltd, USA). The plot of the absorbance versus concentration was made.

A film from each batch was cut into small pieces and placed in a 50-mL beaker and 10 mL of distilled water was added and shaken intermittently for 5 min until complete dissolution of the pieces of films. One milliliter of the sample was withdrawn and diluted with 4 mL of phosphate buffer pH 6.8. The solution was filtered and the insulin content was then determined by measuring the absorbance at 275 nm against phosphate buffer pH 6.8 as blank.

Film bioadhesion

Mass flow method

This test was carried out for each batch of the films by using a modified version of the Attama et al. method [19]. The apparatus used consisted of a separating funnel clamped to a retort stand. Below the separating funnel, a wooden support was used to position a glass slide at an angle of 30° with a treated rat skin glued to the glass slide. The skin was treated by soaking a freshly excised section of an albino rat skin in 5% NaOH solution for 30 min to remove the hair from the skin. A film was placed on the exposed surface of the skin for 15 min to allow for film-skin interaction and hydration. The separating funnel was filled with water, which was allowed to flow over the film on the skin at a constant lamina flow rate

until the film detached from the excised rat skin. Using Equation 1, the total of the force needed to detach the film was used as a measure of bioadhesion:

1. $F=mg$ $A=\pi r^2$

Where m=total mass of water used as calculated from the volume of water collected at the base of the setup (kg) and g=acceleration due to gravity (9.8 m/s^2)

Tensiometric method

The tensiometric determination was performed using a tension balance (Model-OS, White Electrical Instrument Co Ltd, UK) adapted to measure bioadhesive strength. A 1×1 cm^2 film was glued to a glass plate of the same size hanging from the lever arm of the tensiometer. An excised and treated rat skin was glued to the bottom of the watch glass and placed on the platform of the zeroed tensiometer. The platform was moved up until the rat skin made contact with the film. A 15-min contact time was allowed for the interaction between the rat skin and the film. The glass plate was raised using a dial on the tensiometer until the film just detached from the surface of the rat skin. The force required to raise the glass plate from the surface of the rat skin was read off from the balance scale in Nm^{-1}. The average of three determinations was recorded.

Dissolution studies

In vitro release studies

Insulin release from the transdermal system was evaluated using the USP Apparatus 5 (paddle over disc method) prescribed for Transdermal Drug Delivery Systems (TDDS). The dissolution test apparatus (Caleva ST7, UK) used was thermostated at 32±0.5°C and stirred at 50 rpm. The film was fixed on an inverted glass Petri dish using cyanoacrylate adhesive allowing the drug to release only from the upper surface and was placed at the bottom of the vessel containing 500 mL of phosphate buffer pH 6.8. Aliquots of 5 mL of sample were withdrawn at 20, 40, 60, and 120 min intervals while replacing with an equal volume of the dissolution medium each time. The samples were then filtered and analyzed spectrophotometrically at 275 nm against phosphate buffer pH 6.8 as blank.

Ex vivo (skin permeation) studies

This study was carried out using a modified dissolution apparatus in place of a Franz Diffusion Cell. A highly vascularised dorsal section of full-thickness skin of an adult albino rat (male, 250 g) was treated to impact semi-permeability property. The section was soaked in 5% NaOH solution for 30 min to remove the hair from the skin and thereafter defatted by soaking in acetone for 1 h. After defatting, it was soaked in pH 6.8 phosphate buffer overnight to equilibrate. A film was pressed firmly to the semi-permeable rat skin to form the donor unit. The donor unit was tied to ensure adhesion throughout the experiment and placed in the basket unit of a dissolution apparatus. The donor unit was lowered into the dissolution medium (acting as the receptor compartment) containing 500 mL of phosphate buffer pH 6.8 maintained at 32±0.5ºC and stirred at 50 rpm. Aliquots of 5 mL of sample were withdrawn from the receptor compartment at various time intervals up to 12 h, replacing with an equal volume of the receptor medium. Withdrawn samples were then analyzed spectrophotometrically at 275 nm. The procedure was repeated with the treated rat skin replaced with a synthetic membrane with a molecular weight cut-off of 3500 (Membra-Cel®). Triplicate determinations were carried out each time and the cumulative percentage of insulin released was calculated.

Animal handling

Healthy albino rats of either sex weighing 200-250 g were purchased and allowed to acclimatize for two weeks in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. Ethical approval for the study was obtained from the Ethics Committee on the Use of Animals for Experiments, Faculty of Pharmacy, University of Benin, Benin City, Nigeria (EC/FP/018/20). The animals were treated according to the principle established for the care and use of laboratory animals [20].

In vivo release studies

The rats were induced with diabetes using alloxan at a dose of 150 mg/kg body weight [21]. The drug was administered subcutaneously through their abdominal muscle and was left for 72 h. To prevent hypoglycaemic shock within the first 24 h, the animals were administered with 10% glucose solution orally. After 72 h, experimental diabetes was confirmed in the rats using the Accu-Check® Active glucometer (Roche, USA), and fasting blood glucose level greater than 200 mg/dL (11.1 mmol/L) was taken as being diabetic [22].

The diabetic animals fasted overnight were divided into 11 groups of 4 animals each but had access to water before the experiment. The first 5 groups of rats received different batches of the insulin loaded films equivalent to 20 IU on their shaved skin and secured in place using plaster adhesives with the section of the adhesive cov-

ering the film lined with backing polyethylene material to prevent insulin absorption by the adhesive. The next 5 groups of rats, acting as negative control received different batches of the blank transdermal films, while the last group of rats, acting as the positive control, received insulin equivalent to 20 IU subcutaneously. The animals were anesthetized 15 min before drug administration by intravenous injection of thiopental (3 mg/kg body weight). Insulin absorption was monitored based on the effects on the blood glucose level. Blood samples were obtained by the tail snipping method at 1-h intervals for the first 2 h, then at 2-h intervals for the next 6 h, and finally at 12 and 24 h following film administration for all groups of animals. Blood glucose level was determined immediately after sampling using the glucometer and expressed as a percentage of the initial level, before drug administration. The percentage of glycaemic change in the experimental animals was calculated using Equation 2:

$$2.\ Glycaemic\ change\ (\%) = \frac{Initial\ Conc - Final\ Conc}{Initial\ Conc} \times 100$$

Data analysis

All experiments were conducted at least in triplicate and the results expressed as mean of triplicate determinations±SD. Statistical analysis was carried out using Microsoft Excel, version 2013. Differences between means were determined by 1-way Analysis of Variance (ANOVA) at a P level of <0.05 as significant.

Results

Physicochemical properties of the transdermal films

Some physicochemical properties of the formulated insulin transdermal films are shown in Table 2. The results showed some batch to batch variations in the dimensions (weight and thickness) and folding endurance of the films which were not significant but their moisture content (12.00%-17.33%) and moisture uptake (11.04%-22.30%) values showed some level of significant variations (P<0.05). The drug content values of the films ranging from 98% to 101% showed a 2.0% maximum deviation from the mean drug content and these variations in the drug content of the films were within acceptable limits.

Bioadhesive property of the transdermal films

The bioadhesive strengths of the films prepared with the different batches of the unmodified and modified mu-

Table 1. Formula for the preparation of insulin transdermal films

Treatment	Acid Strength (M)	Batch	Insulin (100 IU/mL) (mL)	Mucin (g)	HPMC (g)	Tween 80 (mL)
Untreated	0	A1	2.77	4.0	1.0	0.5
Acetic acid	0.1	A2	2.77	4.0	1.0	0.5
	1.0	A3	2.77	4.0	1.0	0.5
Hydrochloric acid	0.1	A4	2.77	4.0	1.0	0.5
	1.0	A5	2.77	4.0	1.0	0.5

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Table 2. Properties of the insulin transdermal films

Batch	Mean±SD					
	Weight (g)	Thickness (mm)	Folding endurance (n)	Moisture content (%)	Moisture uptake (%)	Drug content (%)
A1	0.25±0.005	1.30±0.10	102±0.57	17.33±0.88	11.04±0.34	98±1.02
A2	0.24±0.003	1.50±0.19	103±0.68	13.73±1.44	17.22±0.35	101±0.48
A3	0.21±0.005	1.45±0.23	103±0.71	12.00±0.73	15.70±0.26	98±0.79
A4	0.24±0.004	1.51±0.31	101±0.35	13.57±1.62	22.30±0.58	98±0.67
A5	0.22±0.006	1.35±0.08	103±0.39	13.64±0.81	15.10±1.01	98±0.87

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Table 3. Bioadhesion values of the formulated films

Batch	Mean±SD	
	Mass Flow	Tensiometer
	mN	Nm^{-1}
A1	0.0064 (0.0024)	0.075 (0.011)
A2	0.0074 (0.0030)	0.088 (0.005)
A3	0.0081 (0.0025)	0.096 (0.002)
A4	0.0151 (0.0022)	0.186 (0.006)
A5	0.0111 (0.0012)	0.155 (0.008)

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cin powders are presented in Table 3. The results show bioadhesion values ranging from 0.0064 to 0.0151 mN for the mass flow method and from 0.075 to 0.186 Nm^{-1} using the tensiometer. The A4 batch of films exhibited the highest bioadhesion with values of 0.0151 mN and 0.186 Nm^{-1}, closely followed by the A5 batch of films. The A1 batch of films containing unmodified mucin gave the least bioadhesion values.

In vitro release profiles

The *in vitro* release profiles of the various batches of the transdermal films are shown in Figure 1. The results showed that all prepared films had variable release profiles depending on the type of mucin powder used in the formulation. There was a decrease in drug dissolution from the transdermal films moving from batches A1 to A5. Up to 95% release of insulin was observed after 2 h from the batch A1 films giving the highest release within the time frame and a 60% release for the batch A5 films giving the lowest release within the same period.

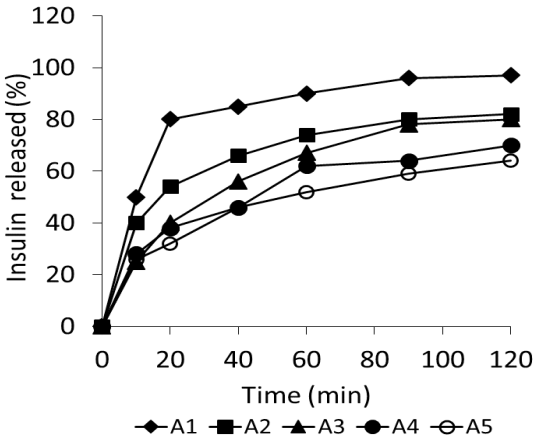

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Figure 1. *In vitro* release profiles of insulin from mucin based transdermal films

Ex vivo release profiles

The *ex vivo* skin permeation/diffusion profiles of insulin from the different batches of transdermal films across rat skin and synthetic membrane are shown in Figure 2. The results showed variable permeation profiles for both the rat skin and synthetic membrane, but there was, however, a more controlled or orderly rate of diffusion of insulin from the films across the membrane. Films prepared with mineral acid (HCl) treated mucin powders (A5) gave the highest percentage of insulin diffused across rat skin and synthetic membrane with 80% and 66%, respectively. Generally, the synthetic membrane showed a lower percentage of diffused insulin than the rat skin. There were significant differences ($P<0.05$) in the insulin diffusion from the different films in both the rat skin and membrane setup.

In vivo release profiles

Figure 3 shows the percentage blood glucose concentration vs time curves of animals dosed with the various batches of the insulin transdermal films, while Table 4 presents the pharmacodynamic parameters calculated from the various curves. The results show a slight increase in the percentage of blood glucose concentration within the first hour in all films except for the subcutaneously administered insulin. Though all animals received the same amount of insulin in the different formulation batches, a variable blood-glucose-lowering rate was observed between 2 and 6 h. Animals that received the A4 and A5 films showed the highest blood-glucose-lowering effect within 24 h with AAC (area above the plasma glucose levels vs time curve) of 1165 and 1274 %h, GLmin

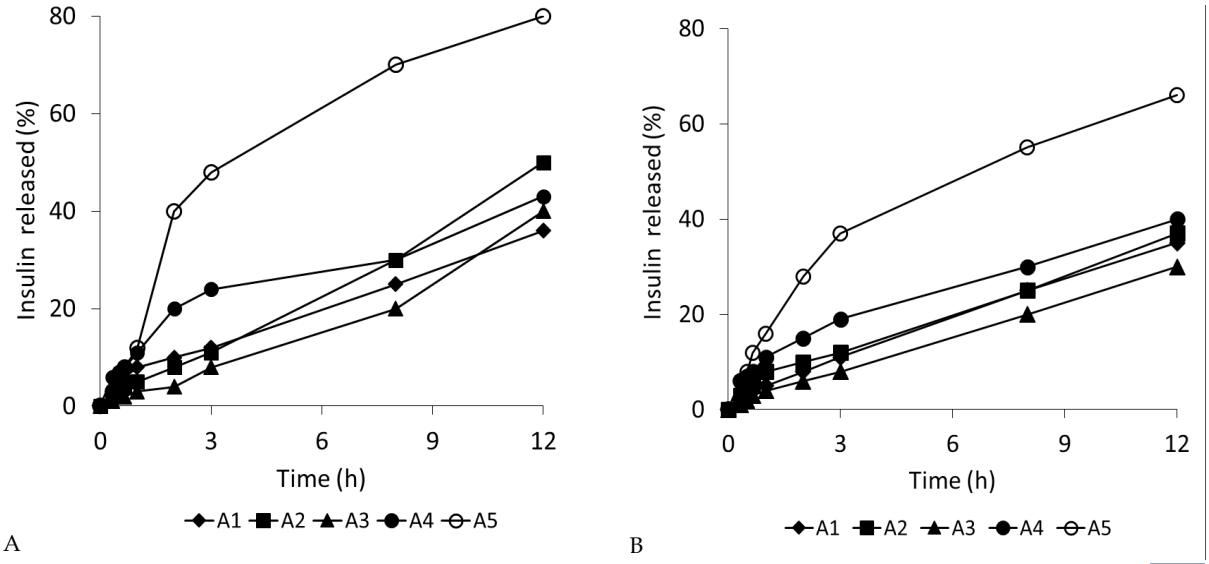

Figure 2. *Ex vivo* permeation profiles of insulin across rat skin (A) and synthetic membrane (B) from mucin based transdermal films

(minimum glucose level attained) of 30% and 35%, and a GLowmax (maximum glucose lowering attained) of 65% and 70%, respectively. Animals that received the A1 and A2 films showed the least blood-glucose-lowering effect within 24 h with AAC of 950 and 994 %h, GLmin of 45% and 48%, and a GLowmax of 55% and 52 %, respectively.

The control group of animals dosed with blank transdermal films containing no insulin showed no reduction in the percentage glucose level within the 24 h period of the experiment. There was an initial increase in blood glucose levels at the start of the experiment from 100% to 107% in the first to the fourth hour and remained more or less at that level till the end of the experiment in 24 h giving an AAC of -45 %h, GLmin of 107% and a GLowmax of -7%.

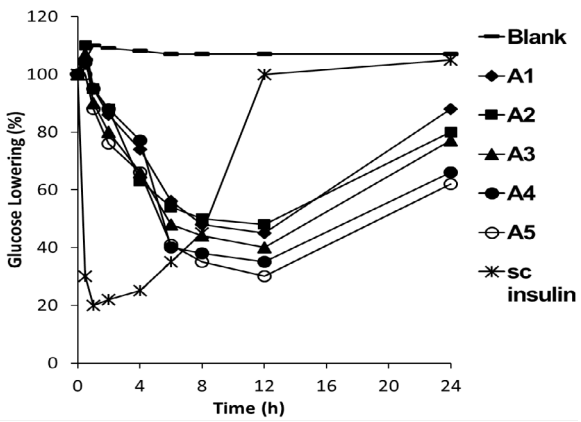

Figure 3. Effect of insulin from mucin-based transdermal films on blood glucose level

Discussion

The formulation and evaluation of insulin transdermal films from acid-modified mucin powder were carried out in this study. The non-significant batch to batch variations in some of the physicochemical properties of the formulated films could be the result of proper selection of the amounts of the formulation excipients. The moisture content values of the various films showed some significant variations ($P<0.05$). The values showed films prepared from the acid-treated mucin (A2-A5) having less moisture than those containing the untreated mucin (A1). Also, the films from the modified mucin powders showed a higher percentage of moisture uptake. This property could be attributed to the modification process affecting more the hydrophilic region of the mucin molecule and resulting in drier films.

Despite the variation in moisture content and uptake, the films exhibited acceptable drug content which could be as a result of the inclusion of polysorbate 80 in the formulations and consequently and effectively emulsified the drug in the films. A previous report showed that polysorbate 80 favors the distribution of drugs between the hydrophobic and hydrophilic domains of mucin residue and may cause emulsification at the interface between these two phases [23].

Furthermore, the bioadhesion results of the films revealed that bioadhesion was generally higher with films prepared with mineral acid (HCl) modified mucin than those prepared with an organic acid (acetic) modified

Table 4. Pharmacodynamic parameters from the insulin transdermal films

Batch	AAC (%h)	Tmin (h)	GLmin (%)	GLowmax (%)
A1	950	12	45	55
A2	994	12	48	52
A3	1119	12	40	60
A4	1165	12	35	65
A5	1274	12	30	70
BLANK	-45	4	107	-7
SC INSULIN	977	1	20	80

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AAC: Area above the plasma glucose levels vs time curve; Tmin: The time taken to achieve the minimum glucose concentration; GLmin: The minimum glucose level attained; GLowmax: Tthe maximum lowering attained at time Tmin.

mucin. Bioadhesion occurs because of the attractive interaction of two surfaces (with at least one of the surfaces being a bioadhesive material), then followed by a succession of events dependent on the nature of the bioadhesive material. In this study, the first stage of events would involve intimate contact between the film and the rat skin, resulted from the good wetting of the film or its swelling. The second stage after contact would be the penetration of the film material into the crevices of the rat tissue surface to initiate weak chemical bonds in the final stage. Since attractive interactions arise from van der Waals forces, electrostatic attractions, and hydrogen bonding [24], the modification of mucin achieved with the mineral acid (HCl) must have enhanced the ability of mucin molecules to form stronger hydrogen bonds in its interaction with the rat skin.

The *ex vivo* diffusion profiles of the films did not correspond with that of their *in vitro* with the A5 batch of films that exhibited the lowest *in vitro* insulin release in 2 h giving the highest insulin diffusion *ex vivo* in 12 h. This observation could be attributed to a superior interaction between the modified mucin used in the formulation of the A5 batch of films and the rat skin or the membrane resulting in increased bioadhesion and facilitating penetration of insulin or diffusion through them. Mucin has been known as a penetration enhancer by its affinity to biological surfaces leading to skin-mucin interaction and the formation of molecular bridges and adhesion to the epithelium [25].

Results from the *in vivo* release studies showed that the films caused an initial increase in the blood glucose levels of all the experimental animals in the first hour of testing. This would be the lag time required for drug diffusion and penetration across the intact rat skin into the blood circulation. Although the blood-glucose-lowering effect of the films in the rats was in varying degrees, the analysis showed that the differences in the effect of the A4 and A5 batches of films were significant ($P<0.05$) when compared with the result from the A1 films.

Therefore, the effect of A4 and A5 batches of transdermal films on blood glucose-lowering may be attributed to the modification carried out on the mucin powder, which enhanced its potential as an absorption enhancer. Even though there was no correlation between the *in vitro* and *in vivo* release of the films, the *in vivo* insulin release seems to correspond with that of their *ex vivo* release. Apart from the earlier mentioned affinity of mucin to biological surfaces which the modification process could have increased bioadhesion and consequently more insulin release in these batch of films via mucin structural modification [14], the possibility of a synergy afforded by the modified mucin, between insulin and the polysorbate 80 used in the preparation of the films should be taken into account in explaining the likely mechanism of increased permeation.

An earlier study reported increased drug diffusion through rat skin from a mucin transdermal formulation of Bovine Serum Albumin (BSA). The study postulated that the increase resulted from a synergy between BSA and the polysorbate 80 used as a plasticizer in the formulation, where the plasticizer may have facilitated the solubilization of BSA and enhanced its transport across the rat skin [26]. Therefore, the increased bioadhesion of the A4 and A5 films prepared with the acid-modified mucin powders over the A1 batch of films that contained unmodified mucin could not be solely responsible for their increased permeation enhancing effects but in com-

bination with the synergy that may exist between insulin and polysorbate 80 in the transdermal formulations.

Transdermal formulations using acid-modified mucin showed superior bioadhesive properties in comparison to the formulations containing unmodified mucin. Their insulin films formulated with modified mucin achieved up to 70% blood glucose lowering in diabetic rats after 8 h and sustained to 12 h compared to the subcutaneous insulin which achieved 80% blood glucose lowering in 30 min and sustained it for 2 h. This result indicates the superior penetration enhancing and controlled release properties of acid-modified mucin powder.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the Faculty of Pharmacy, University of Benin (Code: EC/FP/018/20).

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Authors' contributions

Conceptualization and Study Design: Sylvester O. Eraga and Matthew I. Arhewoh; Investigation, data collection and writing of original draft: Sylvester O. Eraga; Supervision and writing (review & editing): Matthew I. Arhewoh and Magnus A. Iwuagwu.

Conflict of interest

The authors declared no conflict of interest.

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References

[1] Ling MH, Chen MC. Dissolving polymer microneedle films for rapid and efficient transdermal delivery of insulin to diabetic rats. Acta Biomater. 2013; 9:8952-61. [DOI:10.1016/j.actbio.2013.06.029] [PMID]

[2] Nguyen TM, Lee S, Lee SB. Conductive polymer nanotube film for fast and controlled ex vivo transdermal drug delivery. Nanomedicine. 2014; 9:2263-72. [DOI:10.2217/nnm.13.153] [PMID]

[3] Hadebe SI, Ngubane PS, Serumula MR, Musabayane CT. Transdermal delivery of insulin by amidated pectin hydrogel matrix film in streptozotocin-induced diabetic rats: Effects on some selected metabolic parameters. Plus One. 2014; 9:e101461. [DOI:10.1371/journal.pone.0101461] [PMID] [PMCID]

[4] Avadi MR, Sadeghi AM, Mohamadpour DN, Dinarvand R, Atyabi F, Rafiee-Tehrani M. Ex vivo evaluation of insulin nanoparticles using chitosan and arabic gum. ISRN Pharm. 2011; 2011:860109. [DOI:10.5402/2011/860109] [PMID] [PMCID]

[5] Sadhasivam L, Dey N, Francis AP, Devasena T. Transdermal patches of chitosan nanoparticles for insulin delivery. Int J Pharm Pharm. 2015; 7(5):84-88. https://www.researchgate.net/publication/281662233_Transdermal_patches_of_chitosan_nanoparticles_for_insulin_delivery

[6] Yu W, Jiang G, Zhang Y, Liu D, Xu B, Zhou J. Polymer microneedles fabricated from alginate and hyaluronate for transdermal delivery of insulin. Mater Sci Eng C Mater Biol Appl. 2017; 80:187-96. [DOI:10.1016/j.msec.2017.05.143] [PMID]

[7] World Health Organization (WHO). Global Report on Diabetes: Technical report. [Cited: 2016 April 21]. Geneva, Switzerland; 2016. https://www.who.int/publications/i/item/9789241565257

[8] International Diabetes Federation (IDF). Diabetes Atlas 2019. 9th edition. International Diabetes Federation, Brussels, Belgium. [cited: 2020 Mar 25]. Available from: https://www.diabetesatlas.org/en/

[9] Samyuktha RB, Satyanarayana L, Reddy AB. Mucin a natural glycoprotein with effective coating potentials. Pharmanest. 2014; 5(6):2469-71. https://www.pharmanest.net/abstract.php?article_id=341&title=MUCIN%20A%20NATURAL%20GLYCOPROTEIN%20WITH%20EFFECTIVE%20COATING%20POTENTIALS

[10] Adikwu MU, Okafor JO. Evaluation of mucin as a release enhancer for rectal delivery of glibenclamide. Curr Drug Deliv. 2006; 3:243-54. [DOI:10.2174/156720106777731064] [PMID]

[11] Bansil R, Turner BS. Mucin structure, aggregation, physiological functions and biomedical applications. Curr Opin Colloid Interface Sci. 2006; 11:164-70. [DOI:10.1016/j.cocis.2005.11.001]

[12] Eraga SO, Arhewoh MI, Obazeiki, RE, Iwuagwu MA. Formulation and evaluation of mucoadhesive tablets of metronidazole using acid modified mucin. Eur J Pharm Med Res. 2015a; 2:33-46. https://www.researchgate.net/publication/343305098_Formulation_and_evaluation_of_mucoadhesive_tablets_of_metronidazole_using_acid-modified_mucin

[13] Eraga SO, Arhewoh MI, Okogbenin CO, Iwuagwu MA. Investigation of the effect of alkali modification of mucin on some properties of metronidazole bioadhesive tablets. J Appl Sci Environ Manage. 2015b; 19:427-33. [DOI:10.4314/jasem.v19i3.12]

[14] Eraga SO, Arhewoh MI, Iwuagwu MA. Characterization of acid treated porcine mucin and its effects on mucin bioadhesion. Trop J Nat Prod. 2018; 2(2):74-9. [DOI:10.26538/tjnpr/v2i2.4]

[15] Sanjay JK, Mangesh RB, Santosh KM. Development and evaluation of carvedilol loaded transdermal drug delivery system: In vitro and in vivo characterisation study. Drug Dev Ind Pharm. 2012; 38(12):1530-7. [DOI:10.3109/03639045.2012.656271] [PMID]

[16] Dorwal D. Formulation and in vitro evaluation of transdermal films of methyl salicylate. Int J Pharm Pharm Res. 2012; 4(2):4208-17. https://www.researchgate.net/publication/281663560_Formulation_and_in-vitro_evaluation_of_transdermal_patches_of_methyl_salicylate

[17] Sashikant DB, Mrugendra BP. Formulation of transdermal film of carvedilol by using novel polymers. Pelagia research library. Der Pharma Chem. 2011; 2(2):185-9. https://www.imedpub.com/articles/formulation-of-transdermal-patch-of-carvedilol-by-using-novel-polymers.pdf

[18] Saxena M, Mutalik S, Reddy MS. Formulation and evaluation of transdermal films of metoclopramide hydrochloride. Indian Drugs. 2006; 43(3):740-5. https://www.researchgate.net/publication/280138500_Formulation_and_evaluation_of_transdermal_patches_of_metoclopramide_hydrochloride

[19] Attama AA, Adikwu MU, Okoli ND. Studies on bioadhesive granules I: Granules formulated with Prosopsis africana (Prosopsis) gum. Chem Pharm Bull. 2000; 48(5):734-7. [DOI:10.1248/cpb.48.734] [PMID]

[20] National Institute of Health: Office of Laboratory Animal Welfare. PHS Policy on Humane Care and Use of Laboratory Animals. Revised 2015. https://olaw.nih.gov/policies-laws/phs-policy.htm

[21] Katsumata K, Katsumata Y, Ozawa T, Katsumata K (Jr). Potentiating effect of combined usage of three sulfonylurea drugs on the occurrence of alloxan diabetes in rats. Horm Metab Res. 1993; 25(2):125-6. [DOI:10.1055/s-2007-1002058] [PMID]

[22] Rheney CC, Kirk JK. Performance of three blood glucose markers. Ann Pharmacother. 2000; 34(3):317-21. [DOI:10.1345/aph.19187] [PMID]

[23] Zhai J, Day L, Aguilar MI, Wooster TJ. Protein folding at emulsion oil/water interfaces. Curr Opin Colloid Interface Sci. 2013; 18(4):257-71. [DOI:10.1016/j.cocis.2013.03.002]

[24] Mortazavi SA, Carpenter BG, Smart JD. An investigation of the rheological behaviour of the mucoadhesive/mucosal interface. Int J Pharm. 1992; 83(1-3):221-5. [DOI:10.1016/0378-5173(82)90025-4]

[25] Adikwu MU. Evaluation of snail mucin motifs as rectal absorption enhancer for insulin in non-diabetic rat models. Biol Pharm Bull. 2005; 28(9):1801-04. [DOI:10.1248/bpb.28.1801] [PMID]

[26] Arhewoh MI, Eraga SO, Maroh O. Transdermal delivery of bovine serum albumin using snail mucin. East Central Afric J Pharmaceutical Sci. 2014; 17(1):18-24. https://www.researchgate.net/publication/343152001_