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Transdermal Delivery of Bovine Serum Albumin using Snail Mucin

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The study aimed at evaluating the bioadhesion properties and penetration enhancing effect of mucin-based bovine serum albumin (BSA) transdermal patches. Mucin was extracted from the giant African snail *Archachatina maginata* by differential precipitation with acetone and alum. Various batches of BSA loaded transdermal film patches were prepared with the precipitated mucin and varying volumes (0, 0.2 and 0.5 mL) of polyethylene glycol (PEG) as plasticizer. Prepared patches were evaluated for weight uniformity, patch thickness, folding endurance, moisture content and uptake, bioadhesion, drug content and *in vitro* and *ex vivo* diffusion studies. Differential scanning calorimetry analysis showed no interaction between BSA and mucin. Mean weight range from 0.11±0.02 to 0.13±0.05 g, moisture content (32 %) and moisture uptake was highest with patches prepared with acetone-precipitated mucin (up to 129 %) and decreased as PEG concentration increased. All the patches showed bioadhesion values between 1.70 - 1.98 g/sec. Drug diffusion across treated rat skin was 47 % after 12 h from patches prepared from acetone-precipitated mucin. Thus, snail mucin showed promise as a transdermal drug delivery base in the formulation of BSA patches because of its bioadhesion property and penetration enhancing effect.

Keywords: Bioadhesion, drug diffusion, proteins, mucin, transdermal delivery

INTRODUCTION

Technological advances in drug discovery have resulted in increasing number of molecules including proteins and peptides as drug candidates. However, how to deliver drugs with satisfactory therapeutic effect, minimal side effects and increased patient compliance is a question posed before researchers, especially for those drugs with poor solubility, large molecular weight or instability [1]. A number of novel engineered drug delivery systems have been developed to address these problems [1-4]. Oral route is the most popular route of drug administration. Other routes such as pulmonary, nasal, ocular, buccal, rectal, vaginal and transdermal are commonly perceived as locally targeting, non-invasive and capable of minimizing systemic toxicity and thus they have attracted significant attention [1]. With newly developed technologies, they have also been considered for systemic drug delivery especially for biological drugs. For example, various routes of insulin administration such as oral and transnasal have been investigated and some products are in clinical trial while a few have already entered into the market [1-3].

There are certain barriers to overcome for drug to be delivered through these routes. For example, many drugs for oral delivery encounter extensive gut and hepatic metabolisms [3]. Skin is the barrier for transdermal drug delivery while the mucosal (intestinal, pulmonary, nasal, ocular, buccal, rectal and vaginal) are more permeable barriers compared to the skin [4]. However, poor mucosa absorption exists for drugs with large molecular weights or unsuitable hydrophilic/hydrophobic characteristics [5].

Bioadhesive materials have been widely used in transmucosal and transdermal delivery systems. To improve mucosal permeability, permeability enhancers such as bile salts and sucrose are added to mucoadhesive films as well [6]. The objective of this study was to investigate the modulatory effects of mucin and surfactant on transdermal protein delivery using mucin-based transdermal patches.

EXPERIMENTAL

Materials

Terrestrial snails (*Archachatina marginata*, Arinidae Family) were purchased from a local

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market in Benin City, Nigeria. Alum (sulphuric acid, aluminium potassium salt dodecahydrate) and acetone were purchased from BDH chemicals, England, while polyethylene glycol (PEG, tween 80) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, Germany. All other chemicals used were of reagent grade and were used without further purification.

Extraction of snail mucin

Mucin was extracted from the African giant land snails *Archachatina marginata* using the method described by Adiku *et al*. [7]. The snail shells were cracked and their fleshy bodies removed from the shells with the aid of a metal rod. Excretory materials accompanying the bodies were removed. The slime was then washed and squeezed off the fleshy bodies in repeated washings to give a total pool of 1 L. Mucin was precipitated out of the pooled washings using 2 L of acetone. The precipitate was filtered, lyophilized and blended in an electric blender to give mucin powders. The powder was stored in an airtight container until use. The same process was repeated using a 2 % alum solution as precipitating liquid.

Preparation of drug loaded transdermal film patches

Films of equal thickness and diameter were prepared by making a 10% w/v aqueous dispersion of mucin in a beaker. Bovine serum albumin (1 g) and 0.5 mL of PEG were added to the aqueous dispersion to give batch A. The same procedures were repeated with 0.2 mL of polyethylene glycol to give batch B and a control batch C with no PEG. The various dispersions were casted into petri dishes of 15 cm internal diameter. The cast films were air dried, sectioned into 1 cm^2 patches and thereafter stored in a desiccator until required for use. This was repeated with mucin precipitated with alum to give batches D, E and F.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) thermographs of BSA and the transdermal films were obtained using a Netzsch DSC 204F1 t-sensor/E apparatus (Netzsch, Germany). The samples (1 to 2 mg) were placed in sealed aluminum pans with pierced lids. The equipment was set at a heating rate of 10°C from room temperature to 350°C under nitrogen gas at a flow rate of 70 mL/min.

Evaluation of the transdermal films

The prepared transdermal films of the various batches were microscopically and macroscopically examined for some physical parameters such as homogeneity, cracking tendency, *et cetera*.

Dimensions

Ten patches of 1 x 1 cm^2 from each batch were weighed individual using a digital balance and the average weight of the 10 patches were calculated and recorded. The thickness of the various batches of patches was measured using a micrometer screw gauge at different spots on the surface of the patch and the average thickness was documented.

Folding endurance

This was determined by repeated folding and opening of the patches at the same point until it cracked or break. The results were expressed as numbers of repeated folds.

Moisture content and uptake

The patches from the various batches were weighed individually and placed in a desiccator containing activated silica gel as desiccant. The patches were then withdrawn every 24 h and weighed again to check for moisture loss. The process was continued until no further loss in weight was observed. The moisture content was calculated as a difference between initial and final weight with respect to the initial weight and expressed as a percentage. To determine the moisture uptake, a patch from each batch was weighed and placed on a soaked mass of cotton wool in a petri dish. The patches were observed until they were soaked up to the top surface. The patches were then reweighed and the moisture uptake for each of the patches was calculated as the difference between the final and initial weights with respect to the initial weight and expressed as a percentage.

Bioadhesion test

This test was carried out for each batch of patches by using a modified version of the method of Attama *et al*. [8]. The apparatus used consist of a burette clamped to a retort stand. A wooden support was used to position a glass slide at an angle of 30°. Freshly excised and treated rat skin was glued to the glass slide and the patch was placed on the exposed surface of the skin for a period of 15 min, to allow for polymer interaction and hydration. The burette was filled with water and then allowed to flow over the patch on the skin using lamina flow rate of 2 mL/sec until the patch detached from the excised rat skin. The mass flow rate of water (g/sec) was then used as a measure of bioadhesion. The test was carried out in triplicates and the average values recorded.

Drug content

A patch from each batch was cut into small pieces and placed in a 50 mL beaker and 10 mL of water was added and shaken intermittently for 15 min until complete dissolution. One milliliter of the sample was withdrawn and diluted with 4 mL of phosphate buffer (pH 6.8). The solution was filtered and the BSA content was then determined spectrophotometrically at λ_{max} of 279 nm (T70 PG Instrument, USA).

***In vitro* drug release studies**

The transdermal patches from the different batches were evaluated using the USP paddle over disc dissolution apparatus prescribed for transdermal drug delivery systems. The dissolution test apparatus was maintained at 37± 0.5 ºC and stirred at 50 rpm. Each of the patches was fixed on inverted glass petri-plate using cyanoacrylate adhesive allowing drug release only from the upper surface. This was placed at the bottom of the vessel containing 900 mL of 0.4 % sodium hydroxide in phosphate buffer (pH 6.8). Aliquots of 5 mL of sample were withdrawn at 20, 40, 60, and 120 min, replacing with equal volume of 0.4 % sodium hydroxide in phosphate buffer (pH 6.8). The samples were then analyzed spectrophotometrically at 279 nm.

***Ex vivo* skin permeation studies**

This study was carried out using a highly vascularised dorsal section of full thickness skin of an adult albino rat. The section was soaked in 5 % NaOH for 30 min to remove the hair from the skin and thereafter defatted by soaking in acetone for 1 h. After complete defatting, it was soaked in pH 6.8 phosphate buffer overnight to equilibrate. The patches were pressed firmly to the semi permeable rat skin and tied to ensure adhesion throughout the experiment, forming the donor unit. The donor unit was introduced into a dissolution apparatus acting as the receptor compartment containing 500 mL phosphate buffer (pH 6.8) maintained at 37 ± 0.5 °C 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 equal volume of the receptor medium. Withdrawn samples were then analyzed spectrophotometrically at 279 nm.

Release kinetics

Data of *in vitro* release was fitted into different equations to determine the release kinetics of BSA from the transdermal patches. The kinetic equations used were zero order, first order, Higuchi and Korsemeyer-Peppas models to interpret the drug release mechanism from the patches.

RESULTS

Differential scanning calorimetry spectra

As shown in Figure 1, the DSC showed thermal degradation of BSA above 100 ºC (spectrum a) and high moisture content in mucin with a deep trough before 100 ºC (spectrum b). There were no observable modifications in thermal properties of the mixtures (spectra c and d).

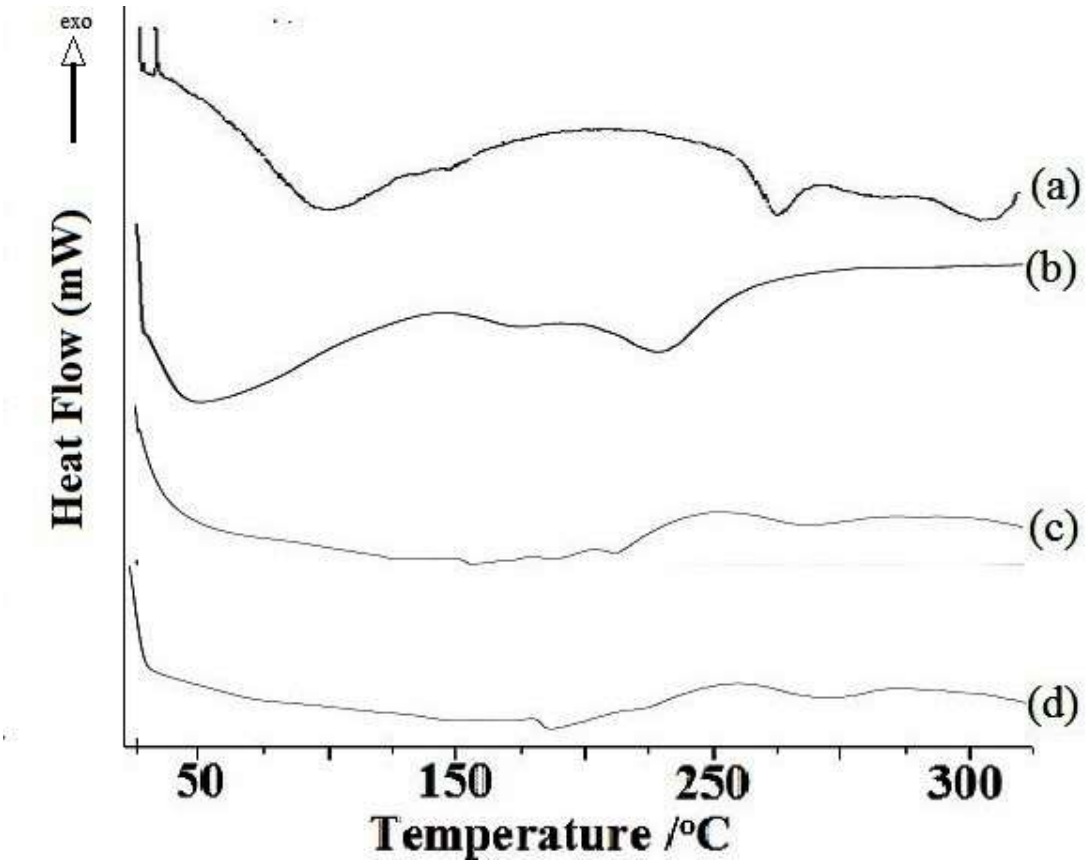

Figure 1: Differential scanning calorimetry thermograph of BSA (a), mucin (b) and a mixture of BSA and mucin precipitated by acetone (c) and alum (d).

Properties of bovine serum albumin transdermal patches

Table 1 shows the properties of the BSA transdermal patches. There were variations in the dimensions and folding endurance of the patches from batch to batch. The acetone-precipitated mucin patches gave higher weight values than those of alum-precipitated mucin. The folding endurance of the various patches indicated increase in folding endurance with decreasing volumes of PEG in the acetone-precipitated mucin patches. The moisture values of the various patches also presented in Table 1 indicates that the batches prepared with alum-precipitated mucin had higher moisture content but less moisture uptake values than those prepared with acetone-precipitated mucin and the moisture uptake of the patches increased with higher volumes of polyethylene glycol in all the patches.

The bioadhesion results also show a higher resistance to washing with increasing volumes of PEG for all the batches of the transdermal patches. There was an increase in the drug content with increasing volumes of PEG in all the batches of the patches. The drug content of the patches was affected by the inclusion of increasing volume of surface active agent (PEG). Such agent (PEG) alters the distribution of drugs between the hydrophobic and hydrophilic domain of the snail mucin residue and may cause emulsification at the interface between these two phases [9].

***In vitro* and *ex vivo* release results**

The *in vitro* release studies of BSA patches fixed on a glass dish and through rat skin are shown in Figures 2 and 3. The *in vitro* result showed no significant difference ($p > 0.05$) in BSA diffusion from the patches prepared from alum-precipitated mucin while acetone-precipitated mucin showed variable release profile significantly influenced by the concentration of polyethylene glycol in the formulation.

Table 1: Properties of mucin-bovine serum albumin transdermal patches

Parameter	Batch					
	A	B	C	D	E	F
Weight (g±SD)	0.12 ± 0.004	0.13 ± 0.05	0.11 ± 0.005	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.02
Thickness (mm±SD)	1.24 ± 0.16	1.60 ± 0.20	1.49 ± 0.29	1.60 ± 0.43	1.01 ± 0.49	1.43 ± 0.06
Folding endurance (n)	1.67 ± 0.58	2.00 ± 1.00	2.33 ± 1.15	1.00 ± 0.00	1.00±0.00	1.00 ± 0.00
Moisture content (%)	25.19	23.11	16.00	18.65	23.54	32.69
Moisture uptake (%)	129.67	116.70	57.08	80.00	69.57	34.76
Drug content (%)	10.66	10.50	9.59	10.00	9.83	9.77
Bioadhesion (mass flow rate, g/sec)	2.10	1.98	1.20	1.79	1.77	0.87

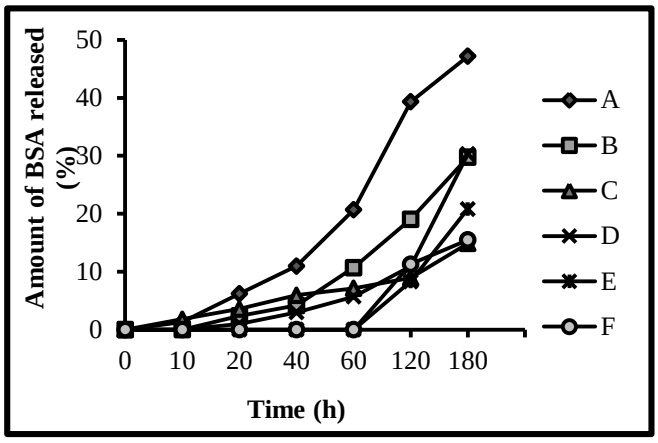

Figure 2: *In vitro* release of BSA from mucin based transdermal patches.

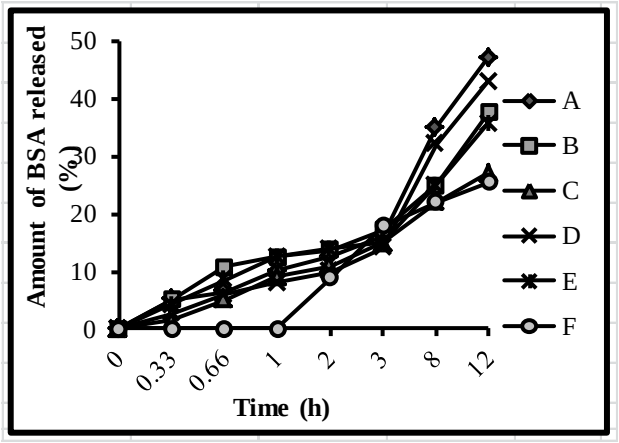

Figure 3: Diffusion of BSA from mucin based transdermal patches through rat skin.

Drug release kinetics

Drug release kinetics study showed R^2 values ranging from 0.6 to 0.9 (Table 2). There were higher values for first order and Higuchi release models suggesting that the release of the drug follows a first order kinetics with a diffusion-controlled mechanism.

Table 2: R^2 values for different release models

Batch	R^2			
	Zero Order	First Order	Higuchi	Korsmeyer Peppas (n)
A	0.9720	0.9859	0.9300	0.9552 (1.2080)
B	0.9866	0.9899	0.8885	0.9868 (1.1877)
C	0.8866	0.9474	0.9530	0.9679 (0.6709)
D	0.8873	0.8915	0.7431	0.9420 (1.2097)
E	0.8070	0.8688	0.6606	0.6799 (1.0350)
F	0.8471	0.9047	0.7176	0.6800 (1.0159)

DISCUSSION

During formulation, mucin softens in water to form a mesh within which the drug is entrapped. Mucin based BSA patches had smooth surfaces and retained the basic colour of mucin (brown) while alum precipitated mucin produced slightly textured surfaces. There was no significant change in the mean weights of patches irrespective of mucin precipitation method or PEG concentration.

BSA was used in this formulation as a model protein. One of the trusts of the formulation is that moisture content and uptakes should be minimized during storage and optimised during drug release. The results of moisture content showed no significant difference ($p > 0.05$) in the different patches irrespective of the method of extraction of mucin employed or the amount of plasticizer used. The moisture uptake on the other hand revealed a higher level in patches prepared with mucin extracted with acetone. Evidently the acetone was completely lost during drying leaving purer mucin while the alum was removed only by rinsing (which may not be very efficient). The possibility of residual alum components in the mucin could have altered the surface texture and moisture uptake capacity. Polyethylene glycol also facilitates moisture uptake by forming a bridge between atmospheric moisture and polymer material. Hence increased levels of PEG in patch formulations may not necessarily be beneficial.

Patches prepared using acetone-precipitated mucin entrapped more BSA thereby having higher drug content per unit area. This was due to two major factors; acetone being organic facilitated the removal of more mucin from solution as has been reported in an earlier study [10]. This mucin contains less of extracting and interfering materials and hence created more void for the drug to fill. Furthermore, increasing PEG concentration resulted in increased amount of drug per patch. Although this increase was neither proportional to PEG concentration nor significantly different per batch, it is likely as a result of an increase in the affinity between PEG and BSA leading to molecular displacement of mucin within a unit area of the patch.

The BSA diffusion profile was determined using two different experiments. BSA being a protein must diffuse across barriers to get to the site of action. *In vitro* release revealed between 20-50 % drug release after 2 h irrespective of the amount of PEG and the type of mucin used. This suggests that up to 20 % of the drug will be sufficiently available for absorption across skin barrier within the first 2 h of administration.

Batches A and D (with the highest amounts of PEG) showed the highest diffusion across treated rat skin barriers with over 40 % BSA diffused after 12 h, when compared to other batches. This was due to increased solubilisation of the drug and mucin in the diffusion medium due to higher amounts of PEG in these batches. Hence in patch formulations, it would be needful to determine the optimal concentration of plasticizer that would yield stable and efficient diffusion profile of the drug. Mucin appeared to have also facilitated drug diffusion across membrane due to its membrane binding properties and as an absorption enhancer [11]. There seems to be a synergy between low concentrations of PEG and the mucin base to facilitate drug diffusion. This was revealed by the fact that the source of mucin (method of extraction) influenced significantly the amount of drug that diffuse with acetone precipitated mucin patches yielding the highest amount of BSA.

CONCLUSION

Snail mucin can therefore be harnessed in the formulation of transdermal delivery devices of protein based drugs. Furthermore, plasticizer such as PEG plays a significant role in facilitating drug stability and absorption.

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