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Original Research Article

Snail Mucin-Based Formulation of Ibuprofen for Transdermal Delivery

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Abstract

Purpose: To evaluate the bioadhesion properties and drug release profile of mucin-based ibuprofen (IBF) transdermal patches.
Methods: Mucin was extracted from the giant African snail, Archachatina maginata, by differential precipitation with acetone and alum. Various batches of IBF loaded transdermal film patches were prepared with the precipitated mucin and varying volumes (0.2, 0.5, and 1 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, in-vitro and ex-vivo (skin permeation) release profiles.
Results: Extraction of mucin with acetone and alum gave a mucin yield of 0.1 and 0.08% w/w, respectively. DSC analysis showed no interaction between the drug and excipients. There was an increase in the weight, thickness, folding endurance, drug content, moisture content and uptake with increasing volumes of PEG incorporated in the formulated patches. Moisture content and uptake values of patches made from mucin precipitated with alum decreased from 17 and 80 % with increasing PEG volumes to 7.7 and 50 %, respectively. All the patches showed bioadhesion values between 0.87 g/sec and 1.46 g/sec and in-vitro drug release gave 78 % after 2 h while ex-vivo diffusion across treated rat skin reached 74 % after 12 h.
Conclusion: Snail mucin showed promise as a transdermal drug delivery base in the formulation of IBF patches because of its bioadhesion property and drug release profile.

Keywords: Acetone-precipitate, alum-precipitate, bioadhesion, ibuprofen, in-vitro release, mucin.

Indexing: Index Copernicus, African Index Medicus

Introduction

A transdermal patch is a medicated adhesive formulation that is placed on the skin to deliver a specific dose of the medication through the skin into the bloodstream [1]. An advantage of transdermal bioadhesive drug delivery system over other routes such as oral, topical, intravenous, intramuscular, etc., is that the patch provides controlled release of medication into the patient systemic circulation. This is usually through either a porous membrane covering a reservoir of medication or through body heat melting thin layers of medication embedded in an adhesive [1]. Unfortunately, only medications whose molecules are small enough to penetrate the skin can be delivered by transdermal drug delivery systems.

Ibubrofen (IBF) is a common orally used medication because of its anti-inflammatory and analgesic properties. In order to minimize the gastrointestinal side effects usually associated with its oral administration, different dosage forms such as topical IBF gel, have been formulated. However, very few formulations can by-pass oral route of administration and still achieve systemic delivery. Transdermal delivery of IBF has been formulated in eutectic composition with a biocompatible emollient (shea butter) or a suitable common solvent (ethyl acetate) to produce O/W micro-emulsification in smart patches [2]. This was found to be cost-effective with a scalable technique to enhance permeation rates of IBF transdermal patches, but there was difficulty in dissolution of IBF to produce an effective oil phase. IBF transdermal patches using chitosan and hydroxyl propyl methyl cellulose (HPMC) [3] showed an optimum sustained release characteristics but the polymers used are quiet expensive. IBF loaded fiber mat patches inhibits IBF re-crystallization and has slow release rate of active ingredients [4]. However, mucin has often been used for drug modelling of

bioadhesive systems [5] as it is negatively charged making it a good candidate for drug delivery as it can be conjugated to positively charged drug molecules and targeted to various tissues. It is highly biocompatible, non-toxic and easily biodegradable. It is ubiquitous in many human and animal tissues and found in the intestine, eye, ovaries and salivary glands etc. and Its is]. The interaction of various polymers at the mucin-polymer interface is often used to explain the mechanism of mucoadhesion. The molecular bridges which result between mucin-polymer interaction account for the adhesive strength. Apart from these bridges, the electronic properties of mucin help in mucoadhesion thus making it to have a high potential as a pharmaceutical excipient [5].

The objective of this study was to assess the bioadhesive properties of transdermal patches formulated with snail mucin and to evaluate the *in-vitro* drug release profile of these patches loaded with the drug ibuprofen.

Materials and Methods

Materials

Terrestrial snails *(Archachatina marginata*, family: Arinidae) were purchased from a local market in Benin City, Nigeria. Acetone was a product of BDH chemicals (Poole, England). Polythylene glycol (PEG, Tween 80) was purchased from Sigma-Aldrich (Germany). Ibuprofen powder was a gift from Fidson Drugs Limited (Lagos, Nigeria). 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*, [6]. Briefly, 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 then removed. A 200 mL quantity of water was introduced into a container containing 10 g of the snail's bodies and the slime was squeezed off the fleshy bodies repeatedly into the water. The water-slime mixture was decanted into another container and the process was repeated up to 4 times to give a total pool of 1 L of the water-slime mixture. Mucin was precipitated out of the pooled water-slime mixture using 2.5 L of acetone. The precipitate was filtered, air-dried for 4 day to give brown flakes. The flakes were blended in an electric blender to give mucin powders which was stored in an airtight container until use. This 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. To the aqueous dispersion was added a 2 g quantity of ibuprofen and 0.2 mL of polyethylene glycol (Tween 80) (Batch A). The same procedures were repeated with 0.5 mL and 1 mL of polyethylene glycol to give batches B and C respectively. The various dispersions were poured into petri dishes of 15 cm internal diameter and allowed to set into films by air drying. The casted films were sectioned into 1 cm^2 patches and thereafter stored in a desiccator until required for use. This was done with mucin precipitated with acetone (Batches A, B and C) and with alum (Batches D, E and F).

Evaluation of the patches

Transdermal films: The prepared transdermal films of the various batches were microscopically and macroscopically examined for some physical parameters including homogeneity and cracking tendency.

Differential scanning calorimetry (DSC): DSC thermographs 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 lid. The equipment was set at a heating rate of 10 ^oC from room temperature to 350 ^oC under nitrogen gas at a flow rate of 70 mL/min.

Dimensions: Ten patches of 1 x 1 cm^2 from each batch were weighed individually by using a digital balance and the average weight of the 10 patches were calculated and recorded. The thickness of the patch from 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 broke. The results were expressed as numbers of repeated folds.

Moisture content and uptake: Each patch was weighed and placed in a desiccator containing activated silica gel as desiccant. The patches were then withdrawn every 24 h and weighed until no further loss in weight was observed. 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 and small amount of amaranth powder was placed on upper surface of the patch. The patches were observed until a development of a red color on the

upper surface [7]. 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]. Freshly excised rat skin was glued to the glass slide placed at an angle of 30 ^{o}C 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. A burette was then 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 detaches 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 5 % NaOH 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 IBF content was then determined by measuring the absorbance at 266 nm against a similarly prepared blank of 5 % NaOH in phosphate buffer (pH 6.8).

***In-vitro* release studies**

The transdermal patches from the different batches were evaluated using the USP paddle method over disc dissolution apparatus prescribed for transdermal drug dosage systems. The dissolution test apparatus was maintained at 37± 0.5 ^{o}C 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 500 mL of 0.4 % sodium hydroxide in phosphate buffer (pH 6.8) as dissolution medium. Aliquots of 5 mL of sample were withdrawn at 20, 40, 60, and 120 min, filtered and analyzed spectrophotometrically at 266 nm against a blank. Equal volumes of fresh dissolution medium were used to replace those withdrawn for analysis.

***Ex-vivo* skin permeation studies**

This study was carried out using the principles of a Franz diffusion cell. A highly vascularised dorsal section of full thickness of an albino rat was used. The section was soaked in 5% NaOH for 30 min to remove the hair from the skin. It was defatted by soaking in acetone for 1 h. After complete defatting, it was soaked in pH 6.8 phosphate buffer overnight to make it a semi permeable membrane. 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 ^{o}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 with each withdrawal. The samples were then analyzed using a spectrophotometer at 266 nm against a blank.

Release kinetics

Data of *in-vitro* release was fitted into different equations to determine the release kinetics of IBF 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

DSC spectra

The DSC spectrum of pure IBF (Fig. 1a) and mucin (Fig. 1b) were characterized by sharp endothermic peak at 81.3 ºC and 82.9 ºC (melting point) respectively. A physical mixture of PEG, IBF, mucin (acetone-precipitated) (Fig. 1c) and PEG, IBF, mucin (alum-precipitated) (Fig. 1d); both exhibited endothermic peaks, although their peaks were appreciably broadened. The broad trough was as a result of mucin which is a complex glycoprotein material of natural origin. Part of it may also be as a result of loss of water.

Properties of IBF transdermal patches

Table 1 shows the results of different properties of IBF 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 increasing volumes of PEG in the patches. Moisture values of the various patches indicated that the batches prepared with acetone-precipitated mucin had more moisture content than those prepared with alum-precipitated mucin and the moisture content and uptake of the patches increased with higher volumes of polyethylene glycol in acetone-precipitated mucin patches, but decreased in the alum-precipitated mucin patches. The results of bioadhesion indicated that higher resistance to

Table 1: Properties and drug contents of mucin/ibuprofen transdermal patches

Parameters	Batch					
	A	**B**	**C**	**D**	**E**	**F**
Weight (g±SD)	0.12±0.04	0.22±0.05	0.28±0.05	0.10±0.30	0.18±0.06	0.09±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 (%)	16.00	11.11	18.19	17.65	11.54	7.69
Moisture Uptake (%)	116.70	126.67	123.08	80.00	69.57	50.00
Drug content (%)	9.59	10.50	10.66	9.77	9.83	10.00
Bioadhesion (mass flow rate, g/sec)	0.87	0.90	1.06	1.20	1.42	1.46

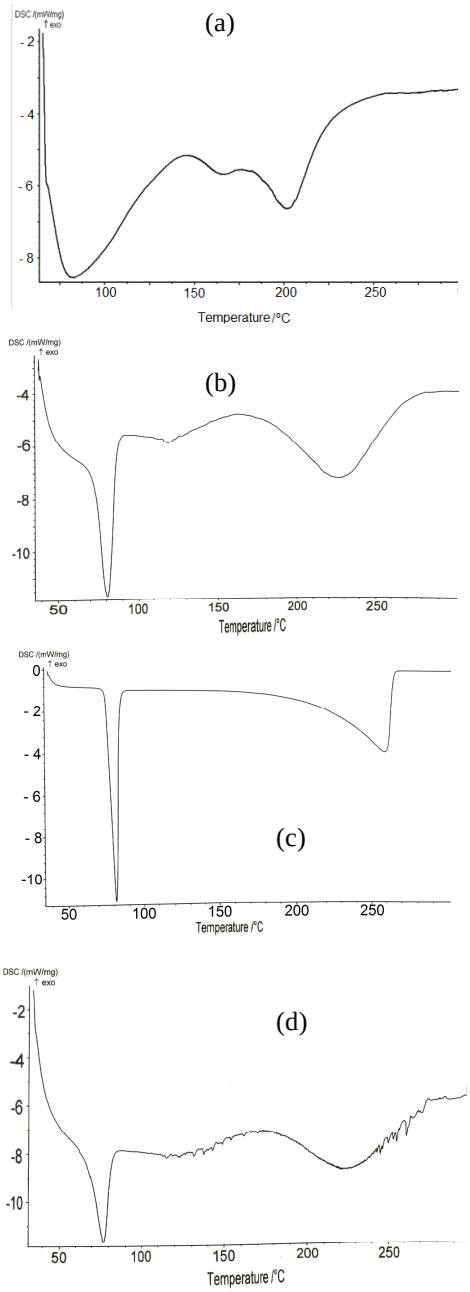

Figure 1: DSC thermograph of IBF (a), mucin (b) and mixture of IBF and mucin precipitated by acetone (c) and alum (d)

washing was also observed with increasing volumes of PEG for all the batches of the transdermal patches.

In-vitro and *ex-vivo* release results

The *in-vitro* and *ex-vivo* (skin permeation) release profiles of IBF patches are shown in Figures 2 and 3 respectively. The *in-vitro* results showed that all the patches prepared from both acetone and alum precipitated mucin had variable release profiles depending on the concentration of polyethylene glycol in the formulation. In general, there was increased drug dissolution as polyethylene glycol concentration increased. Up to 78 % release of IBF was observed after 2 h for batch E patches. The *ex-vivo* diffusion across rat skin also showed variable results. There was however a significant difference ($p < 0.05$) among IBF diffusion from the different patches. As polyethylene glycol concentration increased, IBF diffusion decreased in batches prepared with acetone precipitated mucin but this result was opposite for batches prepared from alum-precipitated mucin. Nevertheless, the batch F patches reached 74 % drug release within 12 h.

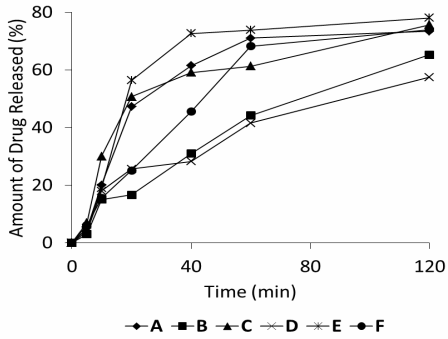

Figure 2: *In-vitro* diffusion release of IBF from mucin based transdermal patches on glass dish

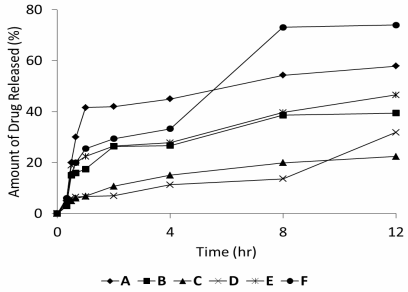

Figure 3: *Ex-vivo* diffusion of IBF from mucin based transdermal patches across treated rat skin

Release kinetic showed R^2 values ranged from 0.5 to 0.9 (Table 2). There were higher values for First order and Higuchi 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	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2			
A	0.5716	0.7186	0.6396	0.5921
B	0.7405	0.9665	0.8441	0.7425
C	0.8593	0.8494	0.9823	0.9593
D	0.9092	0.9283	0.9152	0.8854
E	0.7914	0.9192	0.9840	0.7686
F	0.8817	0.9572	0.9235	0.8682

Discussion

Very few formulations have been successfully produced as transdermal delivery devices for commercial purposes mainly because of their inability to overcome the skin diffusion barrier. In this study, the patches formulated showed an increase in the weight, thickness, folding endurance, drug content, moisture content and uptake with increasing volumes of PEG incorporated in the formulated patches with good moisture content, uptake and bioadhesion values. *In-vitro* and *ex-vivo* drug release were as high as 78 % and 74 % within 2 and 12 h respectively.

The effect of increasing volume of surface active agent (PEG) on the moisture content and uptake, drug content and bioadhesion of the patches prepared with acetone or alum precipitated mucin can be attributed to the surfactant (PEG) activity of altering 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]. This effect leads to an increase in the interfacial area across which partitioning can occur and, therefore increase the amount of drug incorporated. The high moisture uptake of the patches from acetone-precipitated mucin also indicates that this type of mucin with PEG facilitated hydration of the patches than the alum precipitated one. The increase in the folding endurance of the acetone precipitated mucin patches with increase in PEG volumes is due to the plasticizing property of PEG [10] and this is in line with Madhulatha and Naga [3] who worked with glycerol as plasticizer.

The increasing bioadhesive strength of the patches with increasing PEG volumes confirm the bioadhesive properties of snail mucin due to its electronic properties (negatively charged) and the molecular bridges, which result between mucin/polymer interpenetration [11]. These results were similar for all the patches irrespective of the method of precipitation of the mucin base which indicates that the properties of mucin are retained despite the two methods of extraction.

Although the release profiles of IBF from the patches on glass disk were similar, the increase in release with increasing volumes of PEG could be as a result of a reduced surface tension of the patches by the incorporated PEG, facilitating interpenetrations of the dissolution medium and resulting in increased solubility of IBF in the aqueous medium and diffusion of the drug out of the patch. Similarly, for the *ex-vivo* release of IBF using treated rat skin, where lesser amounts of the drug was released, the probable reason would be that the solubility of IBF increases more in favour of the aqueous phase and hence its partitioning to the organic layer of the rat skin is reduced, hence lesser amounts of the drug diffuse across the rat skin. But the reasons for the decrease release of the drug in acetone precipitated mucin patches and increase in alum precipitated mucin patches are not clear. It may probably be as a result of possible interaction between residual alum and the polyethylene glycol and the skin layer. It can therefore, be inferred that polyethylene glycol can facilitate solubilisation and drug diffusion up to a certain concentration beyond which it will become counter-productive. The diffusion of IBF across treated rat skin implied that Ibuprofen can diffuse through the skin and be absorbed into systemic circulation if formulated into mucin patches with low concentrations of PEG.

Snails are cultivated locally for their fleshy part as cuisine delicacies mainly in the Southern region of Nigeria. The slime of the snails are washed off and thrown away as waste products. The use of this waste product as excipients in pharmaceutical formulation will increase its demand and this will in turn boost the economic viability of snail cultivation.

Conclusion

This study showed that snail mucin can be used as a base for transdermal drug delivery for the formulation of ibuprofen patches. Significant bioadhesive properties and drug diffusion across treated rat skin suggest possibility of achieving systemic absorption through the skin. Snail mucin can therefore be harnessed in the formulation of bioadhesive ibuprofen transdermal patches as a specialized drug delivery system.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be

borne by us. MIA conceived, designed, supervised the study and contributed in the manuscript write-up, SOE co-supervised the laboratory work, collected and analysed the data, and also contributed in the manuscript write-up, PFB also co-supervised the laboratory work and participated in analysis of the data while UAU carried out the laboratory work.

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