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Effect of mucin extraction method on some properties of metronidazole mucoadhesive loaded patches

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Abstract

To evaluate the effects of mucin extraction method and plasticizer concentration on the bioadhesive strength and metronidazole release profile from mucin-based mucoadhesive patches. Mucin was extracted from the giant African snail *Archachatina marginata* by differential precipitation with acetone and alum. Various batches of metronidazole loaded mucoadhesive film patches were prepared with the precipitated mucin and varying volumes (0.2, 0.5, and 1 ml) of polyethylene glycol (PEG) as plasticizer. Properties evaluated include: thermal properties (DSC), weight uniformity, patch thickness, folding endurance, moisture content and uptake, bioadhesion, drug content and *in vitro* and *ex vivo* diffusion studies. DSC analysis showed no interaction between metronidazole and mucin irrespective of the means of extraction. Extraction of mucin with acetone and alum gave a percentage yield of 0.1 and 0.08 %w/w, respectively. Patch weight range from 0.17 - 0.28 g, moisture content (17 %) and moisture uptake was highest with patches prepared with acetone-precipitated mucin (up to 117 %) and decreased as PEG concentration was increased. All the patches showed bioadhesion values between 0.90 - 1.97 g/sec. Drug diffusion across rat fundus was highest at 74 % after 12 h from patches prepared from alum precipitated mucin containing least amounts of PEG. The potential for alternative extraction method of mucin (with alum) can be improved as suitable substitute for the expensive acetone extraction method.

Keywords: Bioadhesion; Drug diffusion; Mucin; Acetone-precipitate; Alum-precipitate

INTRODUCTION

Oral drug administration is the predominant route of drug delivery. However numerous drugs remain poorly absorbed when administered by this route which are due to low mucosal permeability of the drug, permeability restricted to a region of the gastrointestinal tract, low or very low solubility of the compound which results in low dissolution rate in the mucosal fluid and lack of stability in the GIT environment resulting in a degradation of the compound prior to its absorption (Moes, 2003). Different approaches have been proposed to prolong the residence time of delivery systems in the stomach of which bio or mucoadhesive delivery systems is among these several strategies to enhance the efficacy of orally administered drugs. Bioadhesion is said to increase the interaction between drugs and mucosa (Lavelle, 2001). The use of different polymers ranging from natural to synthetic polymers such as chitosan, alginate, collagen, albumin, metacrylic acid derivatives, etc in

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the formulation of mucoadhesive delivery systems have been reported (Goswami and Sharma, 2012; Amal *et al*., 2002; Quintanar-Guerrero *et al*., 2001).

Snail mucin is a natural mucoadhesive polymer owing to its biocompatibility, non-antigenic, nontoxic as well as biodegradability in the living system. Mucin is a constituent of the mucosa membrane in the body serving as a protective and physical barrier. It is also responsible in producing certain protective enzymes and use in pharmaceutical industries as drug delivery agent (Adikwu, 2006). It has been reported to possess wound healing properties (Adikwu and Alozie, 2007) and of much significance in cosmetology as an age renewing agent (Asante, 2013).

Several publications have documented approach in the precipitation of mucin from the slime of snail with acetone (Adikwu and Aneke, 2005; Adikwu, 2005; Momoh, *et al*., 2013). Interestingly, in our locality, the African giant snail is eaten as a cheap meat delicacy. In the preparation of the snail for consumption, the slime is usually washed off the snail with alum (potassium aluminium sulphate) or with a citrus fruit (lime). Alum is also used locally in the purification of water. Owning to the synthetic organic nature, high cost and inaccessibility of acetone, its use as a precipitating agent makes the mucin extraction process very expensive, unless the acetone is recovered after precipitation. The use of alum as a viable alternative in this process because its availability, acceptability as well as affordability is yet to be harnessed. The objective of this study was to investigate the effects of extraction method and plasticizer concentration on the bioadhesive strength of mucin and drug release profile of metronidazole mucoadhesive patches.

EXPERIMENTAL

Materials. Terrestrial Snails (*Archachatina marginata*, family Arinidae) were purchased from a local market in Benin City, Nigeria. Metronidazole was purchased from Huang Gang Yinhe Aarti Pharmaceutical Co. Ltd, China, acetone was a product of BDH Chemicals, England, polythylene glycol (PEG, Tween 80) was 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 Adikwu, *et al.*, 2005. 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. A total weight of 10 g of the snail bodies was subjected to washing by squeezing off the slime from the fleshy bodies repeatedly into a pool of 250 ml of water and decanted. This procedure was repeated 2 more times to give a total decanted pool of 1 litre. Mucin was precipitated out of the pooled washings using 2 L of acetone. The precipitate was filtered and lyophilized to give brownish flakes. The dried flakes were blended in an electric blender to give mucin powders. The powder was stored in an airtight container until use. The entire extraction process was repeated using a 2 % alum solution as precipitating liquid.

Preparation of drug loaded mucoadhesive film patches. Films of equal thickness and diameter were prepared by making a 10 %w/v aqueous dispersion of acetone precipitated mucin in a beaker. To the aqueous dispersion was added a 2 g quantity of metronidazole and 0.2 ml of polyethylene glycol (Tween 80) (Batch A). The same procedures were repeated with 0.5 and 1 ml of polyethylene glycol to give batches B and C, respectively. 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 an airtight container until required for use. This was

repeated with alum precipitated mucin to give batches D, E and F.

Evaluation of the patches

Differential scanning calorimetry (DSC). DSC thermographs were obtained using a Netzsch DSC 204F1 t-sensor/E apparatus (Netzsch, Germany). The crushed samples (1 to 2 mg) were placed in sealed aluminum pans with pierced lid. The equipment was set at a heating rate of 10 ^{o}C from room temperature to 350 ^{o}C under nitrogen gas at a flow rate of 70 ml/min.

Physical properties. The prepared mucoadhesive films of the various batches were microscopically and macroscopically examined for homogeneity and cracking tendency. Weight and dimensions were determined by individually weighing ten patches of 1 x 1 cm^2 from each batch by using a digital balance and the average weight of the 10 patches were calculated. The thickness of the patch from the various batches 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 the upper surface of the patch. The patches were observed until a development of a red color on the upper surface (Patel and Patel, 2007). 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 using the method of Attama *et al.*, 2000, with some modification. Freshly excised strip of rat stomach (fundus) was glued to the glass slide placed at an angle of 30° 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 purified water was added and shaken intermittently for 5 min until complete disintegration. One milliliter of the sample was withdrawn and diluted with 4 ml of 0.1 M HCl. The solution was filtered and the metronidazole content was then determined by measuring the absorbance at 276 nm against a similarly prepared blank of 0.1 M HCl.

In vitro and e*x vivo* release studies. The mucoadhesive patches from the different batches were evaluated using the USP paddle method over disc dissolution apparatus prescribed for mucoadhesive drug delivery 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 0.1 M HCl.

Aliquots of 5 ml of sample were withdrawn at 20, 40, 60, and 120 min, replacing with equal volume of the dissolution medium each time. The samples were then filtered and analyzed spectrophotometrically at 276 nm against a blank. The dissolution study was repeated with the patches placed and tied within a freshly excised section of rat fundus and agitated in the dissolution medium with aliquots collected for analysis at various time intervals up to 12 h.

Release kinetics. Data of *in vitro* release was fitted into different equations to determine the release kinetics of metronidazole from the mucoadhesive 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 metronidazole (Fig. 1a) and mucin (Fig. 1b) were characterized by sharp endothermic peak at 164 ºC and 60.5 ºC (melting points) respectively. A mixture of PEG, metronidazole, mucin (acetone-precipitated) (Fig. 1c) and PEG, metronidazole, 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. The sharp endothermic peak exhibited by metronidazole was still prominent in the mixture. The slight reduction observed was due to the masking or shielding effect of mucin. The DSC spectra of the different mucins were similar showing that formulation process did not affect the thermal property of the mucin produced.

Properties of metronidazole mucoadhesive patches. Table 1 shows the results of different properties of metronidazole mucoadhesive 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. The moisture values of the various patches also presented in Table 1 indicates 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 washing was also observed with increasing volumes of PEG for all the batches of the mucoadhesive patches.

***In vitro* and *ex vivo* release studies.** The *in vitro* and *ex vivo* release profiles of metronidazole 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 metronidazole was observed after 2 h for batch C patches. The *ex vivo* diffusion across rat fundus also showed variable results. There was however a significant difference ($p < 0.05$) among metronidazole diffusion from the different patches. As polyethylene glycol concentration increased, metronidazole diffusion decreased in patches prepared with acetone precipitated mucin but this result was opposite for patches prepared from alum-precipitated mucin. Nevertheless, the batch D patches reached 74 % drug release within 12 h.

Release kinetic data analysis showed high R^2 values for First order and Korsmeyer Peppas suggesting that the release of the drug follows a first order kinetics with a Non-Fickian diffusion mechanism.

DISCUSSION

The average weight of the patches from alum-precipitated mucin was higher than that of acetone-precipitated mucin patches while the reverse was the case with the thickness of the patches. The reason for these variations could be attributable to the quality of the mucin precipitated. This showed that possibly residual alum retained by the mucin imparted on the weight and texture. Acetone is usually not significantly retained because it is lost through evaporation.

Table 1: Properties of mucin/metronidazole mucoadhesive patches

Batch	Weight (g±SD)	Thickness (µm±SD)	Folding Endurance (n)	Moisture Content (%)	Moisture Reuptake (%)	Drug Content (%)	Bioadhesion (mass flow rate, g/sec)
A	0.25 ± 0.005	1.29 ± 0.10	2 ± 0.57	17.33	50	0.746 ± 1.02	0.90
B	0.17 ± 0.003	1.78 ± 0.19	3 ± 0.68	13.73	72	0.866 ± 0.48	0.97
C	0.21 ± 0.005	1.79 ± 0.23	3 ± 0.71	12.00	117	0.981 ± 0.79	1.39
D	0.28 ± 0.004	1.51 ± 0.31	1 ± 0.35	8.57	32.3	0.982 ± 0.67	1.28
E	0.22 ± 0.003	1.35 ± 0.08	3 ± 0.39	13.64	50	0.951 ± 0.87	1.17
F	0.22 ± 0.004	1.54 ± 0.25	3 ± 0.43	9.10	100	0.863 ± 0.54	1.11

Table 2: R^2 values for different release models

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas	
	R^2				n
A	0.7907	0.9703	0.9973	0.9969	0.6442
B	0.5873	0.9143	0.9718	0.9818	0.4545
C	0.4116	0.9620	0.9630	0.9852	0.3811
D	0.8845	0.9985	0.9906	0.9969	0.6442
E	0.6872	0.9568	0.9842	0.9842	0.5139
F	0.6702	0.9121	0.9635	09709	0.5365

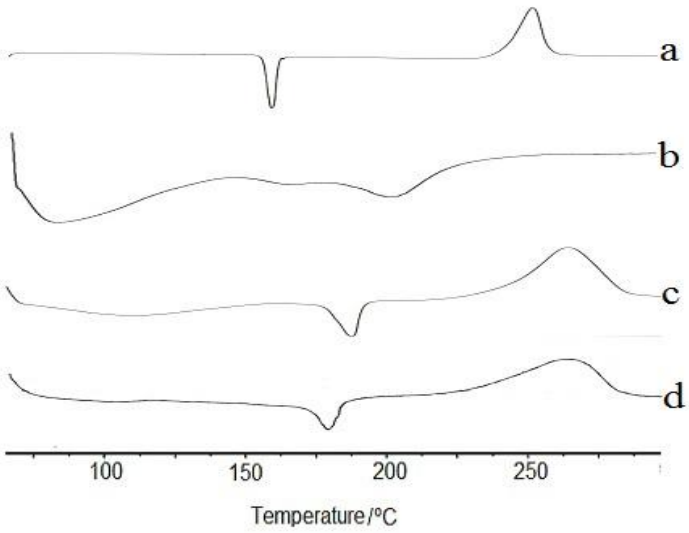

Figure 1: DSC thermograph of metronidazole (a), mucin (b) and a patch prepared with metronidazole and mucin precipitated by acetone (c) and alum (d)

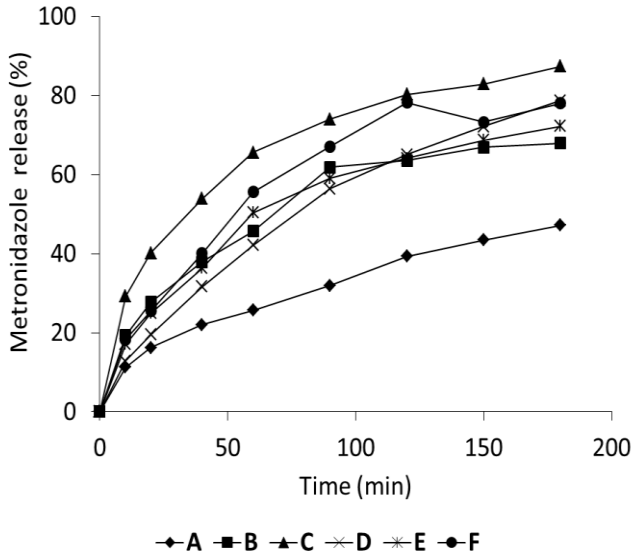

Figure 2: *In vitro* release of metronidazole from mucin based mucoadhesive patches

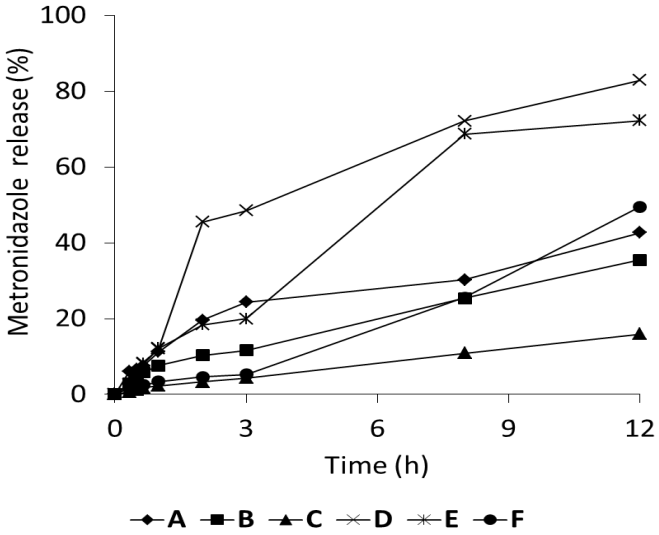

Figure 3: *Ex vivo* diffusion of metronidazole from mucin based mucoadhesive patches across rat fundus

The variations in the percent moisture content and uptake of the various acetone/alum precipitated mucin-metronidazole patches could be the result of the astringent property of alum. Also the increase in moisture content and uptake with increase in the volume of PEG in both acetone/alum precipitated mucin patches is indicative of the humectant property of PEG. As PEG concentrations increase, the amount of moisture absorbed also increase, thus higher volume of PEG affect the resultant property of the snail mucin hence minimal volumes are essential in enhancing the effect of snail mucin.

The higher bioadhesiveness observed with patches from acetone precipitated mucin was due to its loosely packed molecular constituent thus allowing penetration/ permeation of water molecules through its pores, hence more of hydration, interaction and interpenetration which are the basis for bioadhesion (Roy and Prabhakar, 2010). In addition, the mucoadhesive materials are

activated by the presence of moisture. Moisture plasticizes the system, allowing the mucoadhesive molecules to break free and to link up by weak van der Waals and hydrogen bonds (Smart, 2005). It is likely that the astringent property of alum resulted in the production of patches of closely packed constituents, decreasing pores for hydration and interpenetration as well as interaction.

The *in vitro* release studies of metronidazole from the different mucoadhesive patches on glass dish and through rat fundus showed a significant release of drug which increased with time, with an increase in PEG. The release of the drug implies that the drug can be release in a steady control pattern deposited wholly and absorbed at a control rate through the stomach wall. In addition, patches prepared with alum precipitated mucin had a higher release of the drug than those of acetone precipitated mucin patches through the rat fundus wall.

Conclusion

An alternative cheap mode for the precipitation of mucin from snail has been reported. Alum-precipitated mucin showed comparable properties to acetone-precipitated mucin. There is however, issue with purity as residual alum may influence the property of extracted mucin.

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