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FORMULATION AND EVALUATION OF MUCOADHESIVE TABLETS OF METRONIDAZOLE USING ACID MODIFIED MUCIN

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ABSTRACT

The objective of this study was to determine the effect of acid treatment of mucin on the mucoadhesive properties and tablet parameters of metronidazole tablets. Mucin was extracted from the African giant snails (*Archachatina maginata*) by differential precipitation using acetone, air-dried and pulverized. Different portion of the mucin powder was treated with mineral acids (hydrochloric and nitric acids) and organic acid (acetic acid) at high and low concentrations and at varied times of 1 and 12 h. Ten batches of metronidazole tablets (M1-M10) were prepared with the modified mucin by direct compression. Their granules and tablets were evaluated for flow properties and tablets parameters respectively. Tablet mucoadhesion was determined using the mass flow rate method. All the batches of granules gave good flow characteristics with their angles of repose < 30°. The formulated tablets passed the weight variation test with hardness values within the range of 3.0 to 5.0 kp and friability of 0.7 to 2.9 % while the content of active drug met official compendial requirements. Tablets of HCl treated mucin gave mucoadhesion values of 2.80 g/sec irrespective of HCl concentration and time. This value was higher than those of the unmodified mucin tablets which gave 0.75 g/sec. Tablets of mucin treated by other acids gave mucoadhesion values of 0.39 g/sec, 0.46 g/sec and 0.19 g/sec for batches M7, M8 and M9 respectively. Tablets of mucin treatment using HCl gave improved mucoadhesive properties compared to the unmodified mucin. Thus, this knowledge can be improved upon to enhance the formulation of mucoadhesive dosage forms.

KEYWORDS: acid treatment, mucin, mucoadhesion, tablets, metronidazole.

INTRODUCTION

Polymers and their modified forms continue to find increased applications in drug delivery formulations. Most of these polymers are either obtained by synthesis or from natural sources. Examples of some modified products are sodium carboxymethyl cellulose from the incorporation of a sodium carboxymethyl group onto the cellulose backbone resulting in a profound change in its properties and hence diverse usage.[1] Other examples include microcrystalline cellulose obtained from the acid treatment of α-cellulose[2] and chitosan by the removal of the acetyl groups (deacetylation) of chitin with strong alkali treatment resulting in a polymer possessing bioadhesive properties.[3-5]

In recent times, products from animal sources such as mucin have been identified as useful materials in drug formulations.[6] Mucin possesses mucoadhesive property but this property can be improved upon or modified by treatment with different agents such that the medicament incorporated will achieve controlled release. The choice of modifying mucin is to potentially enhance its properties with the benefit of increasing the drug releasing profile in the gastrointestinal tract and hence improve administration and absorption. Various forms of modification have been attempted on mucin, of which PEGylation of mucin is the most prominent.[7-9] PEGylation is the modification of a protein, peptide or non-peptide molecule by the linking of one or more polyethylene glycol (PEG) chains. The PEG-drug conjugates have several advantages including a prolonged residence in the body, a decreased degradation by metabolic enzymes and a reduction or elimination of protein immunogenicity.[10]

Other modifications of mucin reported include thermal,[11] pH,[12] chemical,[13] physical,[14] etc. These modifications have been reported to affect mucin properties such as aggregation and gelation, stability, rheology, adhesive, crystallinity, viscosity, etc.[15] Mucin also is a matrix former,[16] this property will also be of benefit as incorporated drugs can also be released in a controlled or sustained manner.

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol) is an oral synthetic nitroimidazole antibiotic medication used for the treatment of infections caused by anaerobic bacteria and protozoa.[17] The increase frequency of administration of metronidazole in conventional dosage forms tend to reduce patient compliance and also lead to increase side effect of the drugs. The objective of this study was to determine the effect of acid modification of mucin on its bioadhesive properties and on some tableting parameters of metronidazole matrix tablets formulated with the modified mucin.

MATERIALS AND METHODS

Materials

The materials used were procured from local suppliers without further purification. All the reagents used were of analytical grade. Metronidazole (Huang Gang Yinhe Aarti Pharmaceutical Co. Ltd, China), sodium hydroxide (Titan Biotech Ltd, India), sodium chloride, hydrochloric acid, monobasic potassium phosphate and acetone (Merck, Germany), hydrochloric acid (May and Baker Ltd, England), methanol, lactose, magnesium stearate and talc were products of BDH Chemicals, England. Terrestrial Snails (*Archachatina marginata*, family - Arinidae) were purchased from a local market in Benin City, Nigeria.

Extraction of snail mucin

Mucin was extracted from the African giant land snails *Archachatina marginata* using the method described by Adikwu, *et al*.[18] 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 100 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 chilled 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.

Modification of mucin powder

Modification of mucin was carried out by weighing 10 g of mucin powder into 3 beakers (M1-M3). The contents of beakers M1 and M2 were mixed with 25 ml of 0.1 M hydrochloric acid solution while beaker M3 was treated with 25 ml 1.0 M HCl. The beakers were allowed to stand at room temperature and the contents of M1 and M3 were neutralized after 1 h while that of M2 after 12 h with equimolar volumes of sodium hydroxide solution. The neutralized contents of the beakers were first air-dried for 24 h and further dried under silica gel into flakes in a desiccator. The dried flakes was pulverized into powder and stored in an airtight container until use. Similar modification procedures were carried out with nitric acid (M4, M5, M6) and acetic acid (M7, M8, M9). A schematic diagram of the modification process is shown in Figure 1.

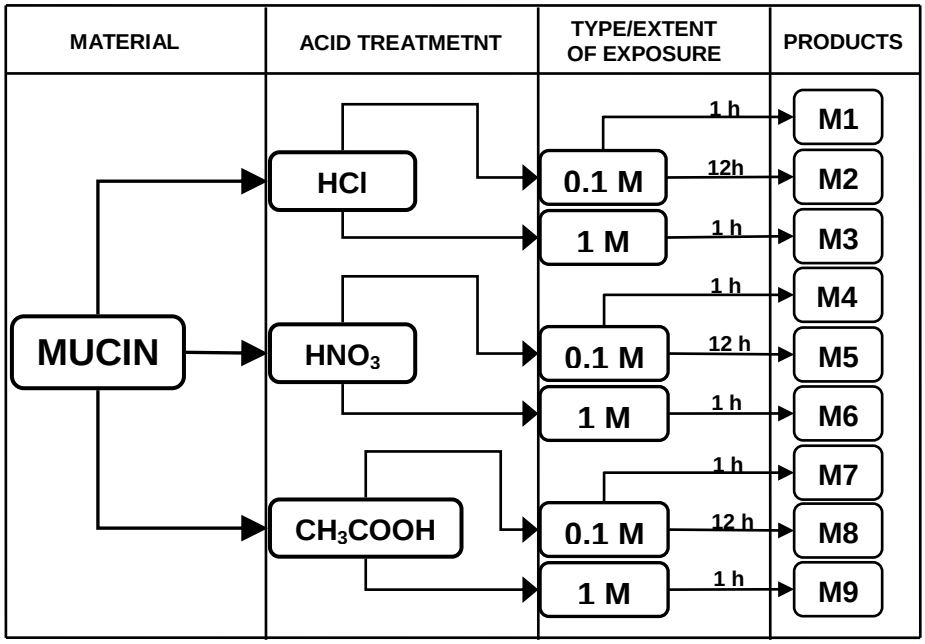

Figure 1: Schematic diagram of the acid treatment

Preparation of simulated gastric fluid (SGF) without pepsin

About 2.0 g of sodium chloride was dissolved in sufficient quantity of distilled water and 7.0 ml of concentrated hydrochloric acid was added. The resulting solution was adjusted to a pH of 1.2 and made up to 1 L with distilled water.[19]

Evaluation of the modified mucin powder

Solubility profile

The solubility of a 1 % dispersion of the modified mucin powders was determined in distilled water, methanol and chloroform. The dispersions were allowed to stand for 24 h and the extent of solubility noted.

pH determination

A 1 % dispersion of the modified mucin powders was prepared with distilled water and allowed to stand for 1 h with the container capped at room temperature. The pH of the resultant solution was determined in triplicates using a digital pH meter (HI 2215, Hanna Instruments, USA).

Melting point

The modified mucin powders were packed into a capillary tube sealed on one side and tapped on a hard surface for the powders to form a column at the bottom of the capillary tube. The tube was inserted into the heating block of a Gallenkamp melting point apparatus. The

temperature of the heating block was raised from room temperature at 1 °C per min until the sample melts. The melting temperature was recorded as the melting point. Triplicate determinations were carried out per batch.

Preparation of granules and tablets

Various batches of the modified mucin granules were prepared using dry granulation method according to the formula in Table 1. Ten (10) batches of metronidazole granules (M1-M9 and a control batch (M10) containing the unmodified mucin) were prepared using lactose as diluent. The ingredients of each batch were weighed and passed through a 710 mm mesh screen (Endecotts, England) prior to mixing. The screened quantities of metronidazole, mucin powders and lactose were transferred into a mortar and mixed intimately with a pestle. Specific screened quantities of magnesium stearate and talc were added stepwise and mixed thoroughly. The powder blend was slugged using a heavy-duty tableting machine (Karl Kolh Technical Supplies, Germany) and the resulting tablets were broken up into granules with a mortar and pestle. The granules were evaluated for pre-compression parameters.

The granules were compressed into tablets using a single punch tableting machine (Manesty Machines, UK) at a pressure of 40 arbitrary units (AU). Tablets from the various batches were evaluated for post-compression parameters.

Table 1: Formula for the preparation of metronidazole mucoadhesive matrix granules/tablets

Ingredients	Quantity/Tablet (mg)	Quantity/Batch (g)
Metronidazole	200	20
Mucin	200	20
Lactose	100	10
Magnesium stearate	25	2.5
Talc	25	2.5

Granule properties

Bulk and tapped densities: A 30 g quantity of the granules was poured gently into a 100 ml graduated measuring cylinder. The volume of the granules was read and the bulk density calculated. The measuring cylinder was then tapped 100 times on a wooden platform. The volume was noted and used in calculating the tapped density. Carr's index and Hausner's ratio were computed from the bulk and tapped density values obtained.

Angle of repose: The hollow tube method was used. A short hollow tube of 3 cm in internal diameter sitting on a circular horizontal surface of same diameter was filled with granules. The tube was withdrawn vertically and excess granules allowed to fall off the edge of the circular horizontal surface. The height of the heap was measured. The angle of repose, θ, was calculated using Equation 1.

$\theta = tan^{-1} (h/r)$ - - - - - 1

Where h is the height of the heap of granules and r is the radius of the circular base

Tablet evaluations

The following tests were carried out on the compressed tablets using standard procedures: tablet weight uniformity, friability, hardness test, content of active and dissolution rate.[20]

The weight of each of 20 tablets was determined from each batch using an electronic balance (College B154, Mettler Toledo, Switzerland) and the mean weight and standard deviation were computed. Ten weighed tablets were then placed in the drum of a friabilator (Erweka GmbH, Germany) revolving at 25 rpm which exposed the tablets to rolling and repeated shock resulting from free fall within the apparatus. After 4 min, the tablets were brought out, dedusted and reweighed. The weight was then recorded and friability calculated as percentage loss in weight.

The hardness of each of ten tablets per batch was determined (Campbell Electronics, Model HT-30/50, India). The mean hardness and standard deviation were calculated.

Content of active drug

Twenty (20) tablets were randomly selected from each batch and crushed to fine powder. A quantity of the powdered tablets equivalent to 200 mg metronidazole was weighed and dissolved in about 50 ml of SGF in a 100 ml volumetric flask. The volume was made up with more SGF and allowed to hydrate for 5 h. Necessary dilutions were carried out to obtain a final concentration of 100 µg/ml, the solution was thereafter filtered through a Whatman No 1 filter paper and the absorbance of the filtrate determined at 275 nm (T70, PG Instruments Ltd, USA) using SGF as blank. The amount of drug was determined using the equation from the standard calibration plot obtained from pure metronidazole powder.

Dissolution studies: The dissolution profiles of the metronidazole tablets were determined using the BP basket method for the various batches of the tablets (Caleva ST7, UK). A dissolution medium of 900 ml of SGF maintained at 37 ± 1 °C with a basket revolution of 50 rpm was used. A 5 ml aliquot of the dissolution medium was withdrawn at various intervals and replaced with an equivalent volume maintained at same temperature (37 ± 1 °C) of the dissolution medium. The samples were filtered and diluted with more volume of SGF. This was carried out until dissolution was completed. The absorbances of the resulting solutions were measured at λmax of 275 nm. The percentage of drug released at predetermined time interval was computed using the values obtained from the standard calibration plot for the pure drug. A minimum of triplicate determinations was carried out for all batches and the results were reported as mean ± SD.

Bioadhesion test

This test was carried out for each batch of tablets using the method of Attama *et al*.,[21] with some modification. Freshly excised albino rabbit ileum of about 10 cm was pinned on a plastic support at an angle of 30° and a tablet was placed on the exposed hydrated mucus surface for a period of 5 min, to allow for tablet mucus interaction and hydration. A burette was then filled with simulated gastric fluid and then allowed to flow over the tablet using lamina flow rate of 2 ml/sec until the tablet detaches from the excised ileum. The mass flow rate of SGF (g/sec) was then used as a measure of bioadhesion. The test was carried out in triplicates and the average values recorded.

Release kinetics

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

Statistical analysis: Data obtained were analysed using GraphPad InStat software version 3.10. The statistical difference of the batches parameters were subjected to the student's t-test at the 5 % level of significance.

RESULTS

Physicochemical properties

Results from the physicochemical tests on the modified mucin powders are shown in Table 2. The solubility profiles at room temperature shows no solubility of all the batches in methanol with batches M1 and M3 showing solubility in chloroform. There were some level of solubility of some of the batches in chloroform and water. There were reductions in the melting points of the modified batches with reference to the unmodified mucin which were however not significant while all the modified sample powders were alkaline in pH.

Granule properties

The flow properties of metronidazole granules formulated with the modified mucin powders are shown in Table 3. The angle of repose for all the batches of granules fell within the range of 27.08º to 29.56º and compressibility index was found to be in the range 8.01 to 14.40 % while the Hausner ratio of the batches were in the range of 1.042 to 1.168. Monographs specify an angle of repose between 20º to 30º and Hausner ratio of < 1.25 as indicative of granules with good flow properties. This shows that the granules from all the batches exhibited good flowability.

Table 2: Some physicochemical parameters of the modified mucin powders

Batch	Solubility			Melting Point	pH
	Methanol	Chloroform	Water		
M1	-	++	+	85	7.68
M2	-	+	+	86	7.56
M3	-	++	+	87	7.60
M4	-	+	+	80	7.57
M5	-	-	+	82	7.40
M6	-	+	+	83	7.45
M7	-	-	-	85	8.45
M8	-	-	-	86	8.20
M9	-	-	-	87	7.90
M10	-	-	+	90	8.30
(-) Not soluble, (+) sparingly soluble, (++) soluble					

Table 3: Flow properties of formulated metronidazole bioadhesive granules

Batch	Bulk Density (g/cm^3)	Tapped Density (g/cm^3)	Carr's Index (%)	Hausner Ratio	Angle of Repose (°)
M1	0.6612 ± 0.08	0.7201 ± 0.09	8.01 ± 0.11	1.127 ± 0.12	29.56 ± 0.30
M2	0.6281 ± 0.05	0.7030 ± 0.02	10.65 ± 0.88	1.119 ± 0.18	28.29 ± 0.51
M3	0.6001 ± 0.02	0.7011 ± 0.08	14.40 ± 0.90	1.168 ± 0.08	29.23 ± 1.01
M4	0.6221 ± 0.10	0.7011 ± 0.05	11.26 ± 0.01	1.042 ± 0.16	27.98 ± 0.61
M5	0.7001 ± 0.05	0.8121 ± 0.05	13.79 ± 0.51	1.160 ± 0.04	28.35 ± 0.45
M6	0.6721 ± 0.03	0.7510 ± 0.03	10.50 ± 0.11	1.057 ± 0.22	28.10 ± 0.41
M7	0.6281 ± 0.09	0.7121 ± 0.10	11.79 ± 0.31	1.134 ± 0.10	27.08 ± 0.61
M8	0.6722 ± 0.04	0.7490 ± 0.04	10.25 ± 0.61	1.069 ± 0.05	29.18 ± 0.80
M9	0.6723 ± 0.06	0.7542 ± 0.07	10.85 ± 0.80	1.061 ± 0.14	28.32 ± 0.45
M10	0.6357 ± 0.02	0.7026 ± 0.10	9.52 ± 0.40	1.105 ± 0.04	29.17 ± 0.01

± Standard deviation

Tablet properties

The result of some of the physicochemical parameters of the bioadhesive tablets prepared for the various batches of the active drugs is shown in Table 4. All the formulated tablets passed the weight variation test as the variation was within the British Pharmacopoeia specification,[20] i.e., that not more than two of the individual weights should deviate from the average weight by more than ±5 % and none should deviate by more than ±10 %. The hardness of the entire tablets was within the range of 3.0 to 5.0 kp. The friability of the formulations ranged from 0.7 to 2.9 % while the content of active drug in all the samples were within the range required by the BP, that is, not less than 90.0 % and not more than 110.0 % of the labeled content.[20]

Table 4: Some physicochemical parameters of the different batches of tablets formulated

Batch	Weight Uniformity (g)	Hardness (kp)	Friability (%)	Content of Drug (%)	Bioadhesion (g/sec)
M1	0.548 ± 0.008	4.56 ± 0.353	0.8 ± 0.15	98.0 ± 0.21	2.80 ± 2.40
M2	0.552 ± 0.009	4.91 ± 0.743	0.9 ± 0.10	99.0 ± 0.15	0.19 ± 1.45
M3	0.545 ± 0.014	3.09 ± 0.256	0.9 ± 0.02	99.5 ± 0.11	2.85 ± 1.22
M4	0.553 ± 0.013	3.17 ± 0.106	1.3 ± 0.20	98.8 ± 0.22	0.39 ± 0.99
M5	0.551 ± 0.009	3.67 ± 0.309	1.9 ± 0.05	101.4 ± 0.14	0.46 ± 1.10
M6	0.555 ± 0.013	3.44 ± 0.341	1.4 ± 0.12	98.4 ± 0.12	0.19 ± 0.80
M7	0.541 ± 0.015	3.81 ± 0.331	2.3 ± 0.06	100.2 ± 0.18	0.62 ± 0.50
M8	0.542 ± 0.015	3.85 ± 0.242	2.9 ± 0.55	97.5 ± 0.20	0.64 ± 0.90
M9	0.554 ± 0.015	3.56 ± 0.366	1.9 ± 0.20	98.0 ± 0.20	0.26 ± 0.85
M10	0.551 ± 0.010	5.00 ± 0.800	0.7 ± 0.08	99.5 ± 0.10	0.70 ± 0.50

± Standard deviation

Bioadhesion

The results of bioadhesion of tablets (Table 4) showed that the batch M3 tablets gave the highest bioadhesion; this was closely followed by M1. The M2 and M6 batches of tablets prepared with mucin powders modified with 0.1 M hydrochloric acid for 12 h and 1 M nitric acid for 1 h respectively gave the least bioadhesive property.

***In vitro* drug release**

In-vitro drug release studies revealed differences in the release of metronidazole from the different tablet formulations in SGF as shown in Figures 2a and b. There were variations in the drug release profile amongst the different batches of the tablets. There were initial bursts within the first 5 min of between 10-20 % followed by a lag in the release of metronidazole from all the matrix tablets. Batches M4, M5 and M7 showed delays up to 20 min and those of M8 and M9 lasted up to 30 min while M1 and M2 delayed up to 40 min. Batch M3 showed a steady release of its drug content after an initial 10 min delay while batch M10 exhibited a steady release from the onset.

The correlation coefficient (r^2) values of the metronidazole bioadhesive tablets are shown in Table 5. The result indicates that the relationship between drug release and time is not linear in SGF, suggesting a slow release kinetic and a diffusion controlled release mechanism.

Table 5: Correlation coefficient (r^2) of the dissolution studies

Batch	Release Kinetic				
	Zero order	First order	Higuchi	Korsemeyer-Peppas	
	r^2				n
M1	0.9182	0.9206	0.8800	0.7865	15.537
M2	0.9205	0.9226	0.8308	0.8119	12.169
M3	0.7121	0.8611	0.9782	0.9642	2.4665
M4	0.6690	0.7573	0.9680	0.8517	6.9969
M5	0.8432	0.8667	0.9682	0.8835	13.806
M6	0.8645	0.8828	0.6428	0.8005	7.4919
M7	0.5571	0.7190	0.8478	0.8570	1.7084
M8	0.9232	0.9409	0.9496	0.9042	12.395
M9	0.9227	0.9231	0.9189	0.8416	20.983
M10	0.6854	0.9135	0.9253	0.9744	2.1241

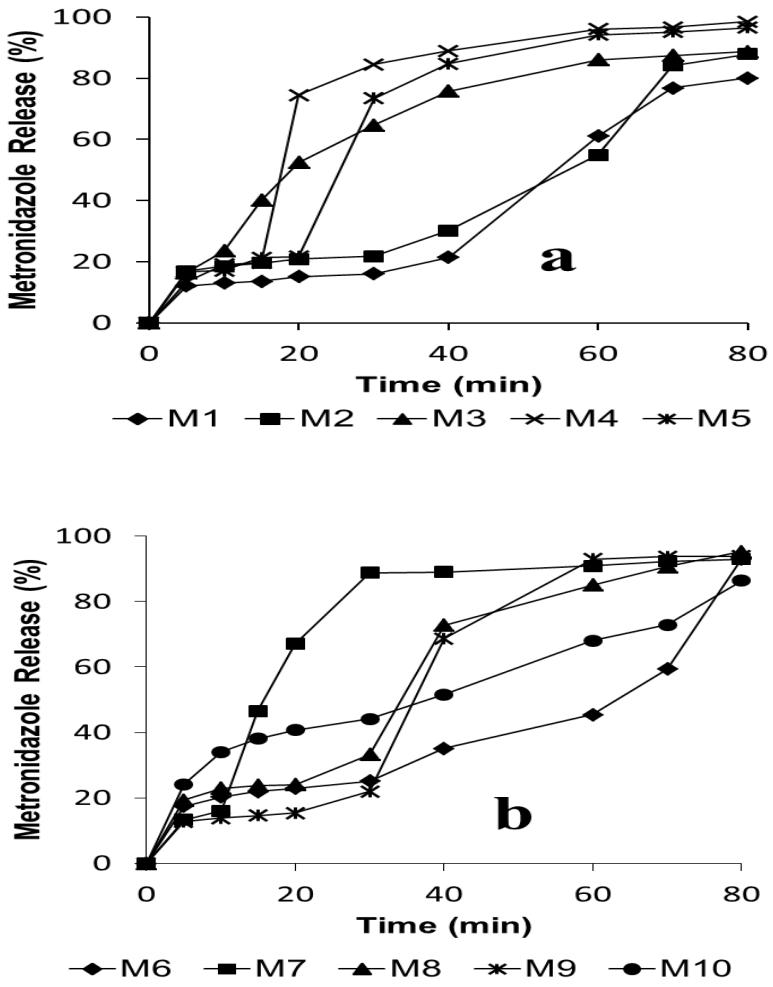

Figure 2a & b: Dissolution profile of the metronidazole tablets in SGF

DISCUSSION

The search for newer and better bioadhesive polymers is an ongoing process and one of such processes is by modification of existing polymers to achieve newer molecules with improved bioadhesive properties. In this attempt of modification, any other property acquired by the new substance is an added advantage. This process has led to the discovery of high functional excipients having improved bioadhesion, binding and direct compression ability.

In this study, an attempt was made to modify mucin by acid treatment and formulate mucoadhesive tablets with the modified products. Granules prepared with modified and unmodified mucin show good flow properties with all the batches having angle of repose below 30°. These findings are similar with earlier reports by Eraga *et al.*[9] The tableting properties were not significantly affected by the modification process with weight uniformity, hardness and drug content uniformity that met BP specifications. Batches M1 and M3 tablets show a significant improvement in their bioadhesion when compared with the unmodified

M10 batch of tablets. Their improved bioadhesion can be attributed to the effect of the specific acid type on mucin and in this case HCl.

The duration of modification process of 12 h and higher acid concentrations did not yield products with significantly different bioadhesion properties. Hence resultant changes were achieved within 1 h of exposure to acid. HCl specifically modified the mucin to increase bioadhesion due to higher equimolar reactivity when compare to nitric and acetic acids. It should however, be noted that acid treatment did not destroy the observable granules and tableting properties of mucin from this present investigation.

The *in vitro* pattern of release of drugs from the mucoadhesive tablets suggests that the modified products of the various batches of tablets seem to confer some degree of retardation on drug release, a quality that is ideal for sustained release formulations. But the mechanism and kinetics of drug release shows no obvious pattern. The R^2 (correlation coefficient) values of the bioadhesive tablets suggests a slow release kinetic and a diffusion controlled release mechanism. This is in line with Higuchi,[22] who in analyzing the mechanism of drug release from matrices, postulated two processes that usually prevail; dissolution controlled and diffusion controlled mechanisms.

In hydrophilic matrices, the tablets swelled when they came in contact with moisture. In this study, penetration of water into the tablet and diffusion of dissolved drug out or through the gelled layer are the two limiting processes of drug liberation from matrices,[23] accounting for the initial lag that was observed during dissolution. After sufficient penetration, there was a burst in M7, M8 and M9 probably due to subsequent swelling. The initial release of 5-10 % metronidazole was due to the drugs on the surface of tablets that were immediately eroded on contact with the release medium.

CONCLUSION

This study shows that acid treatment of mucin base using HCl affects its bioadhesive property. Tablets prepared from mucin modified with 0.1 M and 1 M HCl treatment for 1 h showed an improved mucoadhesive activity over and above the unmodified mucin Thus, this knowledge can be improved upon to enhance the formulation of mucoadhesive pharmaceutical dosage forms using HCl modified mucin as matrix former.

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