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Original Article

PRELIMINARY INVESTIGATION OF THE MUCOADHESIVE PROPERTIES OF THERMALLY MODIFIED MUCIN ON METRONIDAZOLE TABLETS

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ABSTRACT

Objective: To determine the effect of thermal treatment of mucin on the mucoadhesive and tablet parameters of metronidazole tablets.

Methods: Mucin was extracted from the giant African snails (*Archachatina maginata*) by differential precipitation using acetone, air-dried and pulverized. Modification of the mucin powder was carried out using a regulated water bath at 60 and 100 °C and at varied times of 1 and 12 h and a micro-wave oven at varied wattage (100-600 W) and time. Ten batches of metronidazole tablets (A1-A10) were prepared with the modified mucin by direct compression. Their granules were evaluated for flow properties and the tablets for weight uniformity, crushing strength, friability, drug content and *in vitro* studies. Tablet mucoadhesion was determined using the mass flow rate method.

Results: Granules of all the batches exhibited good flow characteristics with their angles of repose<30 °. Tablets formulated passed the weight variation test with hardness values above 4.0 kp and friability of 1.10-1.85 % while the content of active drug met official compendial requirements. Tablets of treated mucin at 60 °C for 1 and 12 h gave mucoadhesion values of 1.80 g/sec This value was higher than those of the unmodified mucin tablets which gave 0.70 g/sec. Tablets of micro-wave treated mucin gave mucoadhesion values of 1.0-1.30 g/sec, which were also higher than that of the unmodified mucin

Conclusion: The study shows that modification of mucin at 60 °C for 1 and 12 h may be considered as the most promising among the batches tested as their tablets had improved mucoadhesive properties compared to the unmodified mucin.

Keywords: Thermal treatment, Micro-wave, Mucin, Mucoadhesion, Tablets, Metronidazole.

INTRODUCTION

Mucins are the major macromolecular constituent of the mucous secretions that coat the oral cavity and the respiratory, gastrointestinal and urinogenital tract of animals and, in some amphibians, the skin. They are responsible for the viscoelastic properties of the secretions, providing protection for the exposed delicate epithelia surfaces from microbial and physical injuries. Secretory mucins are typically of very high molecular mass and have hundreds of O-linked saccharide constituting between 50-80 % of the molecule by weight [1-4].

Mucins are ubiquitous in many human tissues. They are also negatively charged. This makes mucin a good candidate for drug delivery as they can be conjugated to positively charged drug molecules and targeted to the respective tissue. Its biomaterial properties can readily be modified by the use of other cationic polymers such as chitosan. Apart from the modification of the biomaterials properties, cationic polymers help to stabilize mucin as it can readily degrade to its motifs [5].

It is highly biocompatible, non-toxic and easily biodegradable. Mucin is often used for modelling of mucoadhesive and bioadhesive systems [6]. Thus, the interpretation of various polymers at the mucin polymer interface at a temperature higher than the glass transition temperature is often used to explain the mechanism of mucoadhesion [7]. The molecular bridges which result between mucin-polymer interpretation account for the adhesive strength. Apart from these bridges, the electronic properties of mucin help in mucoadhesion. Mucin therefore has high potential as a pharmaceutical excipient if adequately harnessed [8].

The mucoadhesive property of mucin 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 thermally 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.

The objective of the study was to determine the effect of thermal modification on the bioadhesive properties of mucin and to evaluate 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 certified analytical grade. Metronidazole (Huang Gang Yinhe Aarti Pharmaceutical Co. Ltd, China), acetone, methanol, lactose, magnesium stearate and talc were products of BDH Chemicals, England. Terrestrial Snails (*Archachatina marginata*, family-Arionidae) were purchased from a local market in Benin City, Nigeria.

Extraction of snail mucin

Mucin was extracted from the giant African land snails *Archachatina marginata* using the method described by Adikwu, *et al.* [9]. 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

The modification of mucin was carried out by weighing 10 g of mucin powder into 9 porcelain dishes (A1-A9). Porcelain dish A1 was placed in a thermo-regulated water bath operated at 60 °C for 1

h with the powders stirred at intervals for even distribution of heat. Dish A2 was subjected to the same treatment for 12 h. The procedure was repeated for dishes A3 and A4 at 100 °C.

Dishes A5-A7 were subjected to micro waving at 600 W, 300 W, 100 W respectively for 10 min while A8 and A9 were micro-waved at 100 W for 30 and 60 min respectively. The treated recovered mucin powders were kept in airtight containers prior to use. A schematic diagram of the modification process is shown in fig. 1.

Evaluation of the modified mucin powder

Solubility profile

The solubility of a 1 % w/v dispersion of the modified mucin powders was determined in distilled water, methanol and acetone at ambient temperature (28±2 °C). The dispersions were allowed to stand for 24 h before measurements were taken.

pH determination

A 1 %w/v dispersion of the modified mucin powder 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

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 0.5 °C per min until the sample melts and the melting temperature was recorded. Triplicate determinations were carried out per batch.

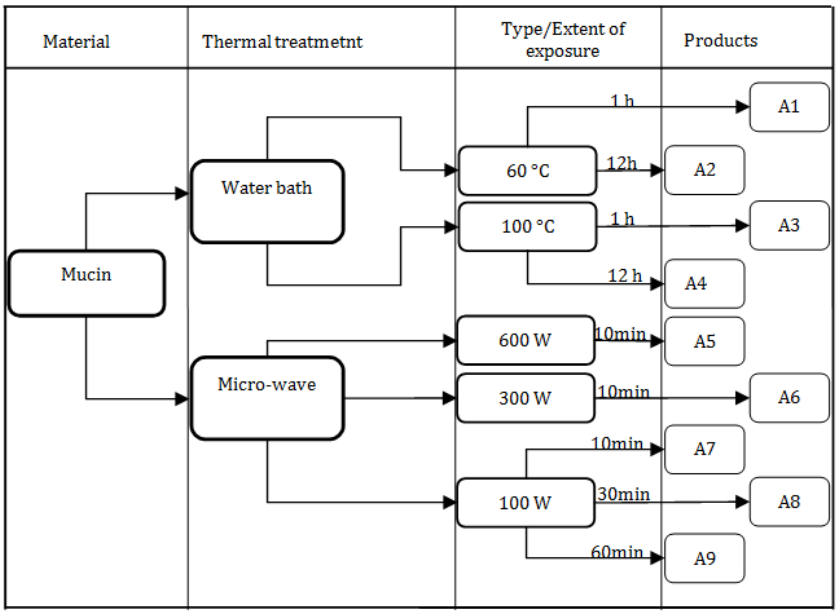


Fig. 1: Schematic diagram showing different stages of the thermal treatment

Preparation of mucoadhesive matrix granules and tablets

Various batches of the modified mucin granules and tablets were prepared by dry granulation using the formula in table 1. Ten (10) batches of metronidazole granules (A1-A9) and a control batch (A10) 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. 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 before compressing into tablets using a single punch tableting machine (Manesty Machines, UK) at a pressure of 40 arbitrary units (AU). One hundred tablets were prepared per batch. 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

Evaluation of granules and tablets

Bulk density

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.

Tapped density

The measuring cylinder containing the 30 g of granules was 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 the 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.

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

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 and dissolution rate [10].

Weight uniformity and friability

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 the percentage loss in weight.

Tablets hardness

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 simulated gastric fluid (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 (T70, PG Instruments Ltd, USA) of the filtrate determined at 275 nm using SGF as blank. The amount of the 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 the same temperature (37 ± 1 °C) of the dissolution medium. The samples were filtered and diluted with more volume of SGF. This was carried out for 60 min. The absorbances of the resulting solutions were measured at λmax of 275 nm. The concentration and the percentage of drug released at each time interval was determined using the equation from the standard calibration plot obtained 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.* [11], with some modification. Freshly excised albino rabbit ileum of about 10 cm was pinned on the 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 were 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 analysis was carried out using GraphPad InStat software version 3.10. Inferential statistical difference of the batches parameters was obtained using the student's t-test at the 5 % level of significance.

RESULTS AND DISCUSSION

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, acetone and distilled water. There was partial solubility of batch A1 in methanol and acetone and the unmodified mucin A10 in distilled water. There were reductions in the melting points of the modified batches with reference to the unmodified mucin while all the modified sample powders were alkaline in pH. The implications of these results could be that the modification carried out did not affect much of the physical properties of mucin as there could have been a distortion of mucin tertiary or quaternary structure from the modification without any effect on its primary structure.

Table 2: Some physicochemical parameters of the modified mucin powders

Batch	Solubility			Melting point	pH
	Methanol	Acetone	Water		
A1	+	+	-	85	7.68
A2	-	-	-	86	7.56
A3	-	-	-	86.5	7.60
A4	-	-	-	80	7.57
A5	-	-	-	81.5	7.40
A6	-	-	-	83	7.45
A7	-	-	-	84.5	8.45
A8	-	-	-	86	8.20
A9	-	-	-	87	7.90
A10	-	-	+	89.5	8.30

(-) Not soluble, (+) sparingly soluble

Flow properties of granules

The flow parameters of the formulated granules are shown in table 3. The results show comparable values among the batches of granules with no significant differences among them (p<0.05). The Carr's indices, Hausner's ratios and angles of repose suggest free flowing granules.

Tablet characteristics

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. The British Pharmacopoeia [10] specifies that not more than two of the individual weight should deviate from the average weight of tablets by more than±5 % and none should deviate by more than±10 %.

The mucoadhesive tablets of the different batches met this specification. All the batches of tablets had hardness values above 4 kp. Though this is an unofficial test, however a minimum hardness of 4 kp is desirable. This may be due to the fact that the concentration of the mucin was sufficient in binding the drug. Friability values of 0.8-1 % are frequently quoted as the upper level of acceptance for pharmaceutical products. Though, in direct compression formulation, values of up to 2 % or above have been reported. The results listed in table 4 show that all the tablet batches partially conformed to specifications. The friability test can also be adopted for the measurement of granule strength [12]. It is unlikely that in the normal life of a tablet it will be subjected to a compressive load large enough to fracture it. However, it may well be subjected to tumbling motion, e. g., during coating, packaging or transportation, which whilst not severe enough to break the tablet, may abrade small particles from its surface [13].

Table 3: Flow properties of formulated metronidazole bioadhesive granules

Batch	Bulk density (g/cm³)	Tapped density (g/cm³)	Carr's index (%)	Hausner ratio	Angle of repose (°)
A1	0.6442±0.02	0.7100±0.03	9.3	1.1	28.30
A2	0.6433±0.04	0.7095±0.09	9.3	1.1	31.21
A3	0.6621±0.02	0.7122±0.06	7.0	1.1	28.32
A4	0.6413±0.06	0.7099±0.04	9.7	1.1	30.02
A5	0.6513±0.10	0.7202±0.05	9.6	1.1	30.02
A6	0.6611±0.08	0.7213±0.08	8.3	1.1	29.01
A7	0.6512±0.09	0.7124±0.10	9.0	1.1	27.11
A8	0.6611±0.07	0.7193±0.11	8.1	1.1	29.14
A9	0.6121±0.06	0.7163±0.04	9.7	1.2	29.11
A10	0.6357±0.04	0.7026±0.02	9.52	1.2	29.17

Values are mean±standard deviation (n=3).

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

Batch	Weight uniformity (g)	Hardness (kp)	Friability (%)	Drug content (%)
n	**20**	**10**	**10**	**20**
A1	0.51±0.04	4.86±0.42	1.34±0.003	99.5
A2	0.52±0.03	4.48±0.54	1.42±0.007	98.8
A3	0.53±0.02	4.55±0.30	1.43±0.008	101.4
A4	0.52±0.02	4.94±0.59	1.20±0.007	98.4
A5	0.52±0.03	4.92±0.59	1.26±0.003	100.2
A6	0.52±0.02	4.94±0.59	1.36±0.008	98.6
A7	0.52±0.03	4.00±0.97	1.32±0.007	100.2
A8	0.52±0.20	4.43±0.39	1.85±0.009	97.5
A9	0.54±0.20	4.28±0.49	1.25±0.007	98.0
A10	0.52±0.02	4.56±0.31	1.10±0.012	99.5

Values are mean±standard deviation

Bioadhesion

The results of bioadhesion of tablets (fig. 2) showed that the batches A1 and A2 tablets gave the highest bioadhesion; this was closely followed by A3. The A10 batch of tablets prepared with the unmodified mucin powders gave the least bioadhesive effect. There was a significant increase in the bioadhesion properties of the thermally modified mucin. The mucin modified using moist heat over a thermostated water bath produced tablets with greater bioadhesion properties than those prepared using micro-wave assisted modification. It is likely that the higher humidity of modification of batches A1-A3 led to irreversible softening and increased gelling tendency of the mucin powder. In the micro-wave assisted, dry heat was involved and the sudden exposure of the mucin powder to dry heat may have resulted in hardening of the powder particles hence achieving either a reduction or no significant effect on the bioadhesion property of the mucin powder despite the intensity or duration of heat applied.

There was also no significant difference between the times of modification as the A1 and A2 batches gave similar bioadhesion properties since the desired levels of hydration and softening of the mucin polymer were achieved within 1 h. There would therefore be no need to heat for up to 12 h.

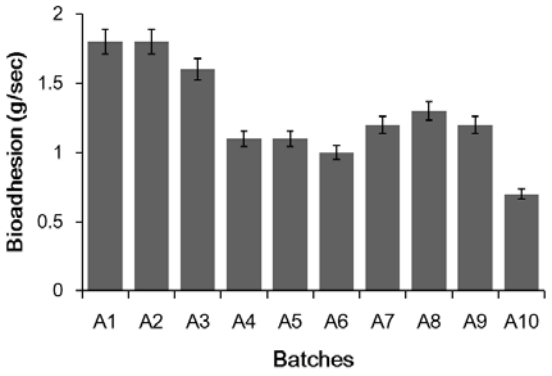
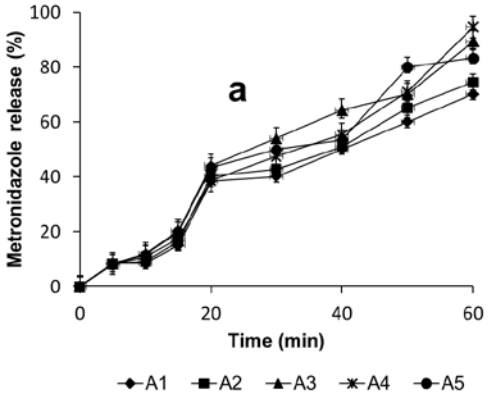


Fig. 2: Bioadhesion of the different batches of thermal modified mucin. Data are expressed as the mean±SD (n= 3)

***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 fig. 3a and b. There were variations in the drug release profile among the different batches of the tablets. There was some level retardation in the release of metronidazole from the matrix tablets in the first 10 min. However, these delays by the different batches did not significantly affect the outcome of the overall release profiles.



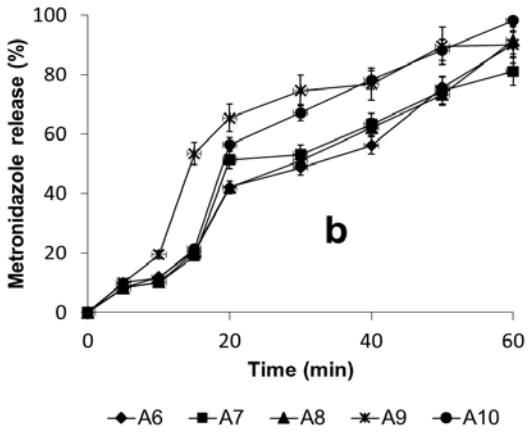


Fig. 3a-b: Dissolution profile of the different batches of the metronidazole tablets in SGF. Data are expressed as the mean±SD (n= 3)

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)			
A1	0.9510	0.9538	0.9699	0.9084 (11.240)
A2	0.9547	0.9570	0.9666	0.9090 (12.150)
A3	0.9589	0.9614	0.9221	0.9186 (14.366)
A4	0.9778	0.9779	0.8043	0.8908 (16.443)
A5	0.9556	0.9573	0.9386	0.9064 (14.211)
A6	0.9765	0.9641	0.9363	0.9359 (13.319)
A7	0.9157	0.8842	0.9635	0.9081 (10.976)
A8	0.9757	0.9501	0.9180	0.9292 (13.787)
A9	0.7646	0.7812	0.9518	0.9013 (03.934)
A10	0.9320	0.9329	0.8914	0.9064 (16.835)

CONCLUSION

The study shows that modification of mucin at 60 °C for 1 and 12 h may be considered as the most promising among the batches tested with the highest mucoadhesion values. Inclusion of modified mucin in the batches also showed some promise in controlling drug release from the matrix tablet. Therefore matrix tablet made from these modifications will ensure optimum therapeutic level with minimum adverse effect and may find usefulness in mucoadhesive formulations of metronidazole for the gastrointestinal tract and vagina mucosa.

CONFLICT OF INTERESTS

No conflict of interest associated with this work

REFERENCES

1. Bansil R, Turner BS. Mucin structure, aggregation, physiological functions and biomedical applications. Curr Opin Colloid Interface Sci 2006;11:164-70.
2. Perez-Vilar J, Mabolo R. Gel-forming mucins. Notions from *in vitro* studies. Histol Histopathol 2007;22:455-64.
3. Perez-Vilar J, Robert LH. The structure and assembly of secreted mucins. J Biol Chem 1999;274 Suppl 45:31751-4.
4. Roussel P, Delmotte P. The diversity of epithelial secreted mucins. Curr Org Chem 2004;8:413-37.
5. Adikwu MU. Evaluation of snail mucin motifs as rectal absorption enhancer for insulin in non-diabetic rat models. Biol Pharm Bull 2005;28 Suppl 9:1801-4.
6. Adikwu MU. Mucin and their potentials. Trop J Pharm Res 2006;5:581-2.
7. Samyuktha RB, Satyanarayana L, Brahma RA. Mucin a natural glycoprotein with effective coating potentials. Pharmanest 2014;5 Suppl 6:2469-71.
8. Adikwu MU. Multifarious potentials of tropical animal-derived biopolymer in drug delivery: Lesson from the African snail mucin. In: Biopolymers in Drug Delivery: Recent Advances and Challenges, Sharjah, UAE: Bentham Science Publishers; 2009. p. 27-38.
9. Adikwu MU, Aneke KO, Builders PF. Biophysical properties of mucin and its use as a mucoadhesive agent in drug delivery: current developments and future concepts. Nigerian J Pharm Res 2005;4:60-9.
10. British Pharmacopoeia Vol. I and II. London: The Pharmaceutical Press, Her Majesty's Stationer Office; 2003. p. 249-52.
11. Attama AA, Adikwu MU, Okoli ND. Studies on bioadhesive granules I: Granules formulated with *Prosopsis africana* (Prosopsis) gum. Chem Pharm Bull 2000;48:734-7.
12. Ofoefule SI. A Textbook of Pharmaceutical Technology and Industrial Pharmacy. Nigeria: Samakin Enterprises; 2002. p. 1-65.
13. Armstrong NA. Functionality related tests for excipients. Int J Pharm 1997;155:1-5.