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Application of *Brachystegia eurycoma* Gum-Egg Albumen Mixture in the Formulation of Modified Release Metronidazole Tablets

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ABSTRACT

The study was aimed at investigating the effects of *Brachystegia* gum (BG)-egg albumin (EA) mixture as binding agent on the qualities of metronidazole tablets. BG and EA were mixed at different ratios to generate fifteen binder mixtures (F1-F15), from which F1-F5, F6-F10 and F11-F15 were used at 2%, 4% and 6% w/w respectively to formulate metronidazole granules by wet granulation. The granules after micromeritic characterization were subsequently compressed into tablets. Quality control tests conducted on the tablets include: hardness, friability, weight uniformity and dissolution studies. Results reveal that granules flow rates ranged from 3.02 g/s (F4) to 4.73 g/s (F1); angle of repose ranged from 29.06° (F12) to 35.93° (F3); Carr's index values were between 7.49% (F9) and 25.43% (F12), while Hausner's ratio ranged from 1.08 (F9) to 1.34 (F12). Tablet hardness values were between 3.65±0.47 (F2) and 5.95±0.60 (F11). Tablets friability values were of magnitude ranging from 0.84 (F11) t0 21.73 (F1). The result of weight variation test revealed that only four batches (F4, F6, F9 and F15) met compendia specifications. Among the metronidazole tablet formulations, those containing binder at 2% w/w released the highest amount of drug, giving up to 90% drug release in 6 h. The least amount of metronidazole was released by tablets formulated with binders at 6% w/w from which only about 30% of drug was released in 6 h. The BG-EA mixture was found useful in imparting slow release profile on metronidazole tablets, which is desirable for some tablet formulations.

Keywords: *Brachystegia* gum, Egg albumin, slow release, metronidazole tablets.

INTRODUCTION

Pharmaceutical dosage forms may be developed in which the rate or site of release of active ingredient has in some way been modified compared with conventional release formulation. [1] Modified release dosage forms are formulations where the rate and/or site of release of the active ingredient are different from that of the immediate release dosage form administered by the same route. This deliberate modification is achieved by special formulation design and/or manufacturing methods. [2] Various types of modified release formulations have been developed to improve the patient compliance and also clinical efficacy of the drug. [3]

Modified release dosage forms are usually a single dose. This is advantageous because it is cost-effective, improves patient compliance, improve efficacy by reducing any variation in drug blood levels, and can ultimately use less of the active ingredient. [4] Types of modified release dosage forms have been reviewed. [2, 5-6]

Brachystegia eurycoma is an economic tree crop that grows in the tropical rainforest of West Africa. It belongs to the caesalpiniaceae family, the spermatophyte phylum and of the order of fabaceae. The crop is classified as legume with its pod containing seeds that are dicotyledonous. [7] *Brachystegia eurycoma* seed contains 56% carbohydrate, 15% crude fat, 9% protein, 4.5% ash and 2.9% crude fibre. [8] In some cultures, the seed flour is used as soup thickening and stabilizing agent and as emulsifying agent in food systems. [8-9] The seed gum from *Brachystegia eurycoma* compared favourably with commercial gums used in the food industry [10] and can be used as a binding agent in tablet formulation. [11] Egg albumin is available as dry powder and it is widely used in the field of foods and pharmaceutical formulation. As a natural polymer, it has recently attracted considerable attention in pharmaceutical field due to its safety. Egg albumin has been evaluated as drug carrier and its effect on drugs dissolution was studied for different oral formulations such as solid dispersion and microcapsules. [12]

Metronidazole occurs as white-to-pale-yellow crystals or crystalline powder. It is stable in air but darkens on exposure

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to light. It has a melting point between 159°C and 163°C. It is sparingly soluble in water, alcohol and chloroform and slightly soluble in ether. Its solubility at 20°C (g/100 mL) is 1.0 in water, 0.5 in ethanol and < 0.05 in ether and chloroform. It is soluble in dilute acids. [13] Metronidazole has long been the drug of choice for the treatment of trichomoniasis and, more recently in combination with iodoquinol, for the treatment of symptomatic amoebiasis, except in the brain. It is also the drug of choice for the treatment of vaginitis caused by *Gardnerella vaginalis*. It is the alternative drug treatment for giardiasis and guinea worm infection. The drug is currently of interest for the treatment and prophylaxis from infections caused by anaerobic bacteria. Metronidazole has also been reported to be of value in Crohn's disease. [13]

MATERIALS AND METHODS

Materials

Metronidazole powder (Milpharm and Almus Pharmaceuticals, UK), lactose, egg albumin, acetone, methanol (BDH Chemicals, UK), *Brachystegia eurycoma* seed (was purchased from a local market in Elele, Rivers State, Nigeria), maize starch (M&B Lagos, Nigeria), chloroform (Fisher Scientific, UK), all other reagents are of analytical grade.

Methods

Extraction of *Brachystegia eurycoma* seed gum

Brachystegia eurycoma seeds were sun-dried and then powdered. The extraction was carried out according to a known method with some modification. [5] Two hundred and fifty grams (250 g) of the powdered seeds of *Brachystegia eurycoma* was dispersed in 3000 ml of distilled water for 24 h. The mixture was centrifuged and the sediment was discarded while the supernatant was kept. Acetone was added to the supernatant in the ratio of 1:1 to precipitate the gum. This was then centrifuged at 3000 g for 30 min, the supernatant was discarded and the sediment kept as gum. The gum was further washed with Acetone and then dried at 40^{o}C in an oven for about 48 h. The dry flakes were pulverized using a blender and stored in an air tight container.

Table 1: Mixing ratios of binders

Mixture Code	*Brachystegia* gum	Egg albumin
F1	1	1
F2	1	3
F3	3	1
F4	2	0
F5	0	2
F6	2	2
F7	1	3
F8	3	1
F9	4	0
F10	0	4
F11	3	3
F12	2	4
F13	4	2
F14	6	0
F15	0	6

Table 2: Formula for Metronidazole tablet

Ingredient	Quantity
Metronidazole	333 mg per tablet
Binder	2.0, 4.0, 6.0% w/w
Maize starch	5.0% w/w
Lactose	qs 500 mg
Magnesium stearate*	1% w/w

*Quantity is with respect to final granule weight

Preparation of BG-EA mixtures

The mixtures which were to be used as binders in wet granulation were prepared in accordance to the ratio shown in Table 1.

Formulation of metronidazole granules

The binders were combined in the ratios shown in Table 1 and with sufficient amount of distilled water the mucilage of each mixture was formed. Mixtures F1 to F5 were used at 2% w/w concentration to formulate the granules, while mixtures F6 to F10 were used at 4% w/w and mixtures F11 to F15 were used at 6% w/w concentrations. The granules were formulated with the formula shown in Table 2. In each batch, 100 tablets were formulated by mixing the required amounts of metronidazole, maize starch and lactose in a planetary mixer for 15 min. The powder mix was then quantitatively transferred into a mortar, moistened with the binder mucilage and kneaded with pestle to form a wet mass. The wet mass was sieved through an aperture of 1000µm, dried at 60°C in a hot air oven for 60 min. The granules were screened through sieve of aperture size 600 µm and further dried at 60°C for 60 min and then packed in air tight containers.

Study of granules flow properties

Flow rate

A stainless steel funnel of orifice diameter 1.0 cm was fixed using a clamp at a height of 10 cm over a sheet of graph paper on a flat horizontal surface, the orifice of the funnel was blocked with a stainless steel ruler, and then 15.0 g of each composite sample was loaded into the funnel. The ruler blocking the orifice was then removed at zero time and the time taken for the powder to completely flow out of the funnel was noted using a stop clock. The flow rate was evaluated using equation 1. Triplicate determinations were made and the mean reported.

$$\text{Flow rate} = \frac{\text{Mass of composite powder (g)}}{\text{Time of flow (s)}} \quad \ldots(1)$$

Determination of bulk and tapped densities

The bulk density of each powder sample was determined by pouring 20 g (M) of the powder into a 50 ml glass measuring cylinder and the bulk volume (V_o) determined. The bulk density (D_b) was then calculated from the relationship:

$$D_b = \frac{M}{V_0} \quad \ldots \quad (2)$$

Triplicate determinations were made and the mean values reported. [14]

The tapped density of each powder was determined using Stampf Volumeter (model STAV 2003, JEF Germany). Twenty grams (M) of each powder sample was poured through 45° angle into a 50 ml glass measuring cylinder and the heap of the powder levelled off horizontally with a spatula and the bulk volume V_o was read. The cylinder was then subjected to 500 taps mechanically and the volume, V_{500} of the powder column determined. Tapping was continued to 750 times and the new volume, V_{750} determined. The difference between V_{750} and V_{500} was calculated and if found to be < 2%, V_{750} was used to calculate tapped density (D_t) using the relationship:

$$D_t = \frac{M}{V_{750}} \quad \ldots \quad (3)$$

Triplicate determinations were made and the mean values reported. [14]

Evaluation of the flow characteristics of the powders

Hausner's Ratio (HR) and Carr's Index (CI) were evaluated using equations 7 and 8 respectively.

$$HR = \frac{D_t}{D_b} \quad \ldots (4)$$

$$CI = \frac{D_t - D_b}{D_t} \times \frac{100}{1} \quad \ldots (5)$$

Where D_t = tapped density; D_b = bulk density.

The static angle of repose, θ, was measured according to the BP (2005) fixed funnel and free standing cone method. [15] A glass funnel was clamped with its tip of internal diameter, 1 cm at a given height (h = 2 cm) above a graph paper placed on a flat surface. Twenty gram of powder sample was carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. The diameter (d) of the base of the cone was measured. This procedure was repeated three times for each powder/granules and the means were used to calculate the angle of repose for each powder using equation 6:

$$\tan\theta = \frac{2h}{d} \quad \ldots (6)$$

Where θ = angle of repose; h = height of powder heap; d = diameter of heap base.

Tablet formulation

The tablet machine (JC – RT - 24H, Jenn Chiang Machinery Co., LTD, Feng – Yuan, Taiwan) was set volumetrically using relevant granules to compress 500 mg granules and run to compress with a force of 15 KN. The granules were then lubricated with magnesium stearate at 0.5 % w/w and compressed. The resulting tablets were stored in air tight containers over silica gel for 72 h before relevant quality control tests were conducted on them.

Quality control tests conducted on the tablets

Tablet weight uniformity

Twenty tablets selected at random from each batch were individually weighed using the electronic balance (Mettler Toledo B154, Switzerland) to an accuracy of within ±1 mg. The mean weight and percentage deviation from the mean for each tablet were calculated.

Tablet hardness

The hardness values of ten tablets selected at random from each batch were determined at room temperature (32°C) by diametral compression using Eweka hardness tester (Karl Kolb, Erweka Germany). Results were taken from tablets that split cleanly into two halves without any sign of lamination. The hardness values were then converted to crushing strength by multiplying them by 10 m/s^2 which is acceleration due to gravity.

Friability of tablets

Ten tablets were de-dusted, weighed and placed in the friabilator (Copley/Erweka GmbH Type: TAR 20 Heusenstamm Germany). The apparatus was operated at 25 revolutions per minute (25 rpm) for 4 min. Thereafter the tablets were de-dusted and reweighed. The friability of the tablets was evaluated with equation 7:

$$\text{Friability} = \frac{W_i - W_f}{W_i} \times \frac{100}{1} \quad \ldots (7)$$

Where W_i = initial weight of the tablets before test; W_f = final weight of the tablets after test.

Tablets dissolution tests

Tablets dissolution tests were carried out according to USP XXIII basket method with an eight chambered dissolution test machine (Erweka Germany Type: DT 80) operated at 50 rpm for 360 min in 900 ml of 0.1 N HCl maintained at 37 ± 0.5°C . Five milliliter (5 ml) of dissolution fluid was withdrawn and replaced with 5 ml of fresh medium at the following intervals: 10, 20, 30, 40, 50, 60, 120, 180, 240, 300 and 360 min. Each withdrawn sample was filtered and its absorbance determined with the UV-Visible spectrophotometer at 277 nm using 0.1 N HCl as blank. Duplicate determinations were conducted and the mean values used to evaluate the percentage drug released using the Beer's plot equation for metronidazole: y = 0.0361x – 0.0108, r^2 = 0.9942, and applying the appropriate dilution factor.

Table 3: Flow properties of granules

Binder code	Concentration (%w/w)	Flow rate (g/s)	Angle of repose (°)	Carr's index (%)	Hausner's ratio
F1	2.0	4.73	32.62	11.66	1.13
F2	2.0	4.59	31.87	11.40	1.15
F3	2.0	3.15	35.54	13.65	1.17
F4	2.0	3.02	35.93	18.22	1.22
F5	2.0	4.54	34.78	22.21	1.29
F6	4.0	4.69	32.97	19.85	1.25
F7	4.0	4.54	34.78	17.37	1.21
F8	4.0	3.40	31.87	23.08	1.28
F9	4.0	3.20	35.22	7.49	1.08
F10	4.0	4.35	32.94	8.99	1.10
F11	6.0	3.91	32.57	20.81	1.26
F12	6.0	3.39	29.06	25.43	1.34
F13	6.0	3.50	30.26	22.78	1.30
F14	6.0	3.42	34.83	22.27	1.29
F15	6.0	4.55	32.93	17.08	1.21

Table 4: Some mechanical properties of tablets

Binder Code	No of tablets with % error > 5%	Hardness (Kg)	Friability (%)
F1	10	5.10±0.30	21.73
F2	7	3.65±0.47	2.28
F3	6	4.65±0.34	8.50
F4	0	3.80±0.42	10.03
F5	10	4.15±0.58	12.81
F6	2	4.20±0.35	6.44
F7	9	4.65±0.78	8.29
F8	4	4.05±0.44	5.77
F9	2	4.75±0.63	4.11
F10	7	5.90±0.57	5.70
F11	6	5.95±0.60	0.84
F12	5	5.85±0.47	1.99
F13	3	5.05±1.23	1.29
F14	5	4.25±0.82	0.87
F15	1	4.55±0.28	3.02

RESULTS

The flow properties of the granules are shown in Table 3. Granules flow rates ranged from 3.02 g/s (F4) to 4.73 g/s (F1); angle of repose ranged from 29.06° (F12) to 35.93° (F3); Carr's index values were between 7.49% (F9) and 25.43% (F12), while Hausner's ratio ranged from 1.08 (F9) to 1.34 (F12). Some mechanical properties of the tablets are shown in Table 4, with tablet hardness values lying between 3.65±0.47 (F2) and 5.95±0.60 (F11). Tablets friability values were of magnitude ranging from 0.84 (F11) t0 21.73 (F1). The result of weight variation test revealed that only four batches (F4, F6, F9 and F15) met compendia specifications. The dissolution profile of the various tablet formulations are depicted in Figures 1-4. Among the metronidazole tablet formulations, those containing binder at 2% w/w released the

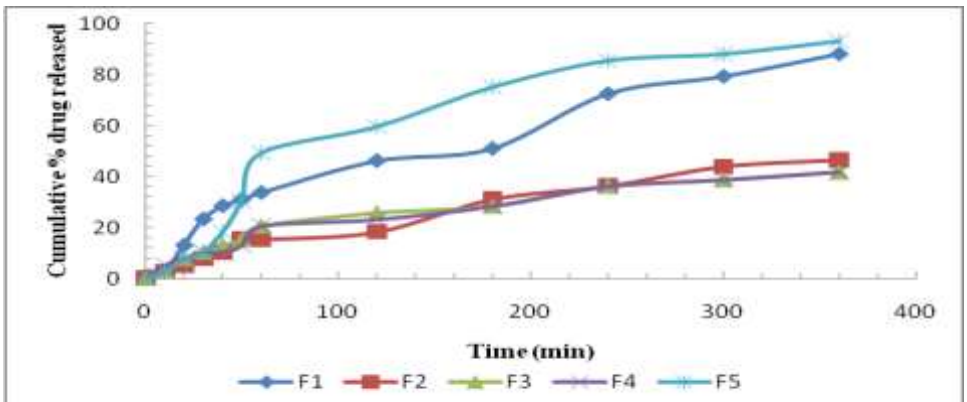

Fig. 1: Dissolution profile of metronidazole tablets formulated with binders at 2% w/w conc.

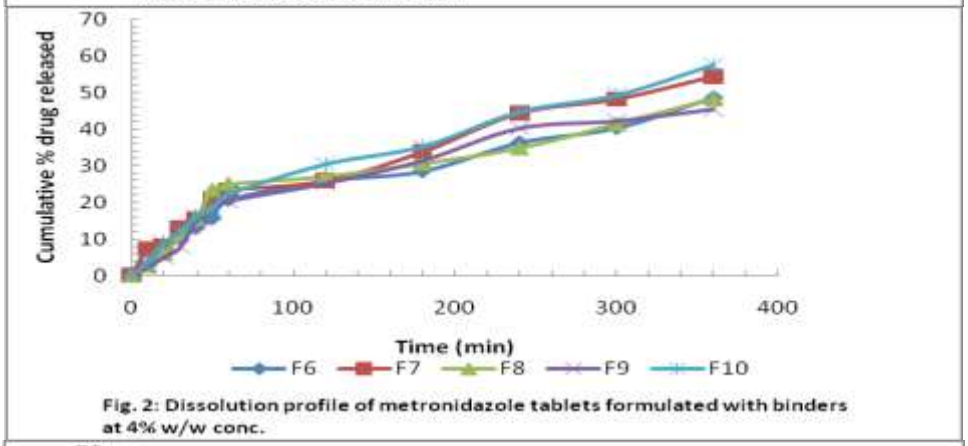

Fig. 2: Dissolution profile of metronidazole tablets formulated with binders at 4% w/w conc.

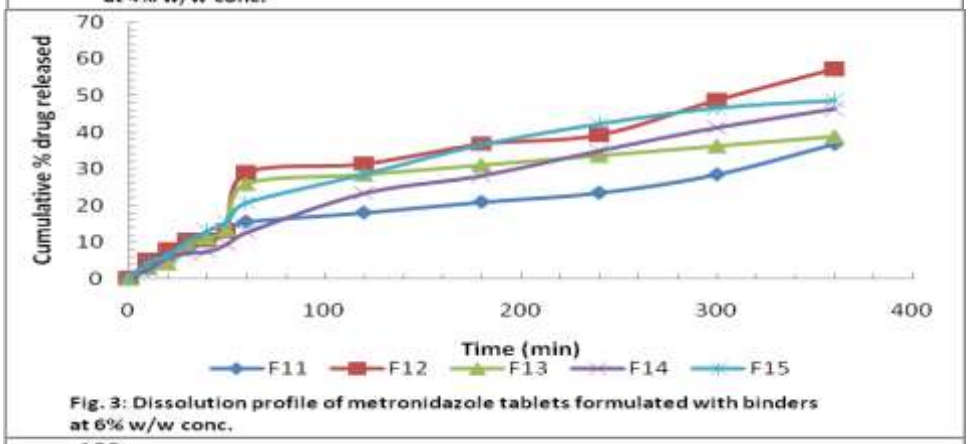

Fig. 3: Dissolution profile of metronidazole tablets formulated with binders at 6% w/w conc.

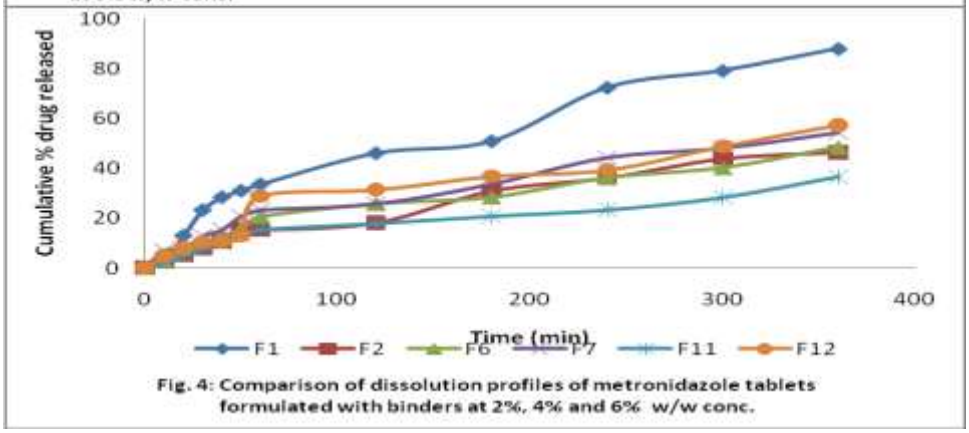

Fig. 4: Comparison of dissolution profiles of metronidazole tablets formulated with binders at 2%, 4% and 6% w/w conc.

highest amount of drug, with albumin alone giving up to 90% drug release in 6 h. (Fig. 1). The least amount of metronidazole was released by tablets formulated with binders at 6% w/w (Fig. 3). And among these ones, formulating with *Brachystegia eurycoma* gum alone resulted in the release of the smallest amount of drug (Figures 3 & 4).

DISCUSSION

The flowability of a powder is an important property influencing several drug manufacturing steps. Flowability is affected by the physical properties of the powder, such as particle size and shape, the loading experienced by particles (gravity, interaction with air flow and container etc.), the

current state of the powder (i.e. tap, free flowing etc.) and the processing environment (e.g. humidity). Particles larger than 250µm usually flow freely while particles below 100µm are generally cohesive and prone to flowability problems. [16] The flow indices of the granules (Table 3) indicate that they possess passable flow properties. [17] These types of flow properties may be improved by the addition of glidants to the granules prior to compaction or filling into capsule shells. Tablet hardness values were within official recommendations [18], except for tablets formulated with F2 or F4 binder whose hardness values were less than 4Kg (Table 4). Tablet hardness influences other mechanical properties of tablets like friability and disintegration. Table 4 however shows that in the present study, tablet hardness did not have any predictable influence on friability. This observation is not immediately explainable but may be tied to the interaction between the two binders whose admixture was employed in the formulation. Only two batches, F11 and F14 passed the test for friability. [19] Four batches, F4, F6, F9 and F15 passed the weight uniformity test (Table 4). [20] The inability of the others to meet the specification may be due to poor flow properties of their granules. Again, one of the main reasons for weight variation is inconsistent working height of lower punches, or alternatively may be traced to fluctuations in the compression pressure of the tableting machine since this has been shown to influence tablet weight variation. [21-22]

The results of dissolution studies reveal that all the batches exhibited slow release of the drug over 6 h (Figures 1-4). Although F5 released as much as 90% of drug (Fig. 1), it did not meet the requirement for classification as conventional tablet (should release ≥ 70% of drug in 60 min). [23] It is therefore obvious that both *Brachystegia* gum and egg albumin individually imparted slow release on the formulated tablets even at low concentration of 2% w/w. The slowest release was observed in tablets formulated with F11at 6% w/w. Tablets formulated with Brachystegia gum or egg albumin alone at 6% w/w concentration released more drug than F11. This suggests that there may be interaction between the two excipients to form a more occluding complex polymer mix even when mixed simply as was the case in this study. Comparison of dissolution of tablets formulated with *Brachystegia* gum-egg albumin reveals that F1 stands out (Fig. 4) as the best mixture composition for the formulation of slow release metronidazole, since it was able to release approximately 90% of drug within 6 h. Except where targeted release to the colon is the case, delayed release of metronidazole beyond 6 h may not be particularly advantageous in ailments that are normally treated with metronidazole. However, multi- dose unit system of metronidazole may be formulated using conventional metronidazole granules mixed with slow release granules and filled in capsules for extended release coverage and for better therapeutic outcome.

Brachystegia gum-egg albumin mixture when used as a binding agent successfully slowed down the release of metronidazole from tablets. The mixture is a better retardant of drug release than either excipient used alone. Employing the F1 (1:1, *Brachystegia* gum : egg albumin) at 2% w/w concentration was found to be good for the slow release of metronidazole from tablets in contrast to other mixture ratios which slowed the drug's release beyond acceptable limits for common metronidazole indications.

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