



## FORMULATION OF METRONIDAZOLE MUCOADHESIVE TABLETS USING AZADIRACHTA INDICA GUM

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Article Received on 02/01/2021

Article Revised on 22/01/2021

Article Accepted on 12/02/2021

### ABSTRACT

The aim of this study was to formulate mucoadhesive tablets of metronidazole using HPMC and neem gum (NG). Neem gum was extracted from the stem bark of *Azadirachta indica* tree. Metronidazole granules were prepared by wet granulation technique with the extracted neem gum at varying concentrations of 25-50% w/w. The granules formulated were evaluated for micromeritic properties. The granules were then compressed to tablets at a compression pressure of 30 N/m<sup>2</sup>. The tablets were evaluated for hardness, friability, bioadhesive strength, *in vitro* dissolution studies and release kinetics. Drug-excipients compatibility studies were also carried out using Fourier Transform Infrared (FTIR) spectroscopy.

All formulated granules were free flowing with angle of repose between 25.1 - 30.6° and Carr's index of ≤ 11.2%. The granules were compressible with hardness of 6.4 - 8.2 kpa and friability of ≤ 0.81%, while the bioadhesive force was between 0.62 - 1.95 N. Increase in concentration of the binder resulted in a corresponding increase in tablet hardness and bioadhesive strength and decrease in percentage friability. All batches fitted into the Higuchi model release kinetics. FTIR studies showed that the excipients and the active pharmaceutical ingredient (API) were compatible. NG exhibited good mucoadhesive property and a synergistic mucoadhesive effect was observed with the addition of hydroxypropylmethylcellulose (HPMC).

**KEYWORDS:** Neem gum, mucoadhesion, metronidazole, mucosa.

### INTRODUCTION

Mucoadhesive drug delivery systems are dosage systems which utilize the property of bioadhesion of certain polymers which become adhesive on hydration and hence can be used for targeting a drug to a particular region of the body for extended period of time.<sup>[1]</sup> Bioadhesion is an interfacial phenomenon in which two materials, at least one of which is biological, are held together by means of interfacial forces. The attachment could be between an artificial material and biological substrate, such as adhesion between a polymer and a biological membrane. Mucoadhesion occurs when the polymer is attached to the mucin layer of a mucosal tissue.<sup>[2,3]</sup>

*Azadirachta indica* (neem) gum is naturally extracted from neem tree by incision or natural injury. It is a clear, bright and amber-coloured material, non-bitter in taste and soluble in cold water. It is majorly used as a bulking agent and for preparation of special purpose food for

diabetics owing to its antidiabetic effect.<sup>[4]</sup> The binding and mechanical properties of the gum have been reported by many researchers.<sup>[5]</sup> This study was carried out to evaluate the mucoadhesive property of neem gum when used as a binder in the formulation of metronidazole tablets.

The aim of this study was to formulate a mucoadhesive tablet of metronidazole which can retain the drug for prolonged duration by mucoadhesive nature of the dosage form and improve the bioavailability by virtue of slower release rate that avoid saturation of carrier-mediated transport of conventional dosage forms.

Metronidazole is an antibacterial, antiprotozoal, anti-amoebic and anti-trichomonal agent used in the treatment of trichomoniasis, amoebiasis and anaerobic infections. It has a half-life of 6 h and is frequently used in combination with other drugs for the eradication of *Helicobacter pylori* in *H. pylori*-induced ulcer. It is taken

400 mg three times daily for a duration of five (5) days, which is cumbersome to the patient.<sup>[6]</sup>

## MATERIALS AND METHODS

### Materials

Metronidazole (Aarti Pharmaceutical Co. Ltd, China), was used in the study as drug model, NG was extracted from the stem bark of *Azadirachta indica* tree, HPMC used was obtained from BDH Chemicals Ltd, England. All other chemicals were of analytical grades.

### Methods

#### Extraction of NG

Crude neem gum was collected from the incised bark of *Azadirachta indica* tree at premises of the University of Benin. The collected neem gum was hydrated in sufficient amount of distilled water for three (3) days with intermittent stirring and extraneous materials were removed by filtering using a separation funnel. The gum from the filtered slurry was precipitated with 99% ethanol. Afterwards, the precipitated gum was filtered, washed several times with acetone, and dried in a hot air oven at 30°C for 96 h before milling and sieving with a mesh no. 60 (250 µm) and then stored in an air-tight container.<sup>[5]</sup>

#### Preparation of metronidazole granules

Mucoadhesive granules of metronidazole were prepared using the wet granulation technique. Three (3) batches were prepared using NG and HPMC (100, 150 and 200 mg). In each formulation, lactose, metronidazole and HPMC were mixed in the dry state in a mortar using the geometric mixing method. Then the binder mixture of the gum was used to wet mass the powder in the mortar. The damp mass formed was forced through a sieve mesh of 710 µm and dried at 60 °C for 30 min in a hot air oven. The granules were further passed through a sieve of mesh size 850 µm and evaluated for micromeritic properties.

#### Evaluation of prepared granules

The granules obtained were evaluated for micromeritic properties as follows:

**Angle of Repose:** The flow property of the granules was evaluated using the fixed funnel method to determine the static angle of repose. A funnel was fixed with its tip at a given height, above a flat horizontal surface on which a paper was placed. Granules (30 g) were carefully poured through a funnel, with the tip of the funnel covered with the index finger. The finger was removed and the granules were allowed to fall on the paper under the influence of gravity to form a pile. The circumference of the pile of the granules was drawn and the radius (r) of the pile was measured in centimeters (cm). The angle of repose of the granule was calculated, using Equation 1.

$$\text{Angle of repose } (\theta) = \tan^{-1}\left(\frac{h}{r}\right) \quad - - - \text{Equation 1}$$

Where  $\theta$  is the angle of repose, h is the height of powder heap in cm, r is the radius of base of the heap in cm.

**Bulk Density:** Five (5) gram of the granules was weighed into a clean, dry 10 ml measuring cylinder and the volume occupied by the granules was recorded as the bulk volume. The bulk density was determined using Equation 2.

$$\text{Bulk density} = \frac{\text{Weight of granules}}{\text{Bulk volume}} \quad - - - \text{Equation 2}$$

**Tapped Density:** Five (5) gram of granules was weighed into a clean, dry 10 ml measuring cylinder. The cylinder was then tapped 100 times from a constant height and the tapped volume was recorded. The tapped density was determined using Equation 3.

$$\text{Tapped density} = \frac{\text{Weight of granules}}{\text{Tapped volume}} \quad - - - \text{Equation 3}$$

**Carr's index and Hausner ratio:** The Carr's index (CI) was determined as described by Carr.<sup>[7]</sup> It was calculated by dividing the difference between the value of tapped density and bulk density by the value of tapped density. The result was expressed as percentage using Equation 4. Hausner ratio (HR) was determined by calculating the ratio of tapped density to bulk density using Equation 5

$$\text{Carr's index } (\%) = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \quad - - - \text{Equation 4}$$

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad - - - \text{Equation 5}$$

#### Formulation of metronidazole tablets

The prepared metronidazole granules were then compressed into tablets using a single punch tableting machine (Manesty machines) at a compression pressure of 30 N/m<sup>2</sup> after the addition of 1% w/w talc. The formula table is shown in Table 1.

**Table 1: Composition formula of metronidazole mucoadhesive tablets.**

S/N	Ingredients	Quantity
1	Metronidazole	400 mg
2	<i>Azadirachta indica</i> gum	25-50 w/w
3	HPMC	100 mg
4	Lactose	Qs
5	Talc	1%

#### Evaluation of the metronidazole tablets

**Weight uniformity:** In order to carry out this test, ten (10) tablets were randomly selected from each batch of tablets and weighed individually. The average weights as well as percentage deviation were computed. The tablets meet the USP test if not more than 2 tablets are outside

the percentage limits and if no tablet differs by more than 2 times the percentage limit.

**Hardness test:** The tablet hardness was determined by diametrical compression using the Campbell Electronics Hardness tester machine (HT30/50, India). The pressure required to fracture a tablet placed in the anvil of the hardness tester was determined. Three (3) tablets were used for the determination. The mean values and standard deviation were recorded.

**Friability Test:** The friability of five tablets was determined using Roche Friabilator (Erweka Germany). It is expressed in percentage (%). Five tablets were initially weighed and placed in the friabilator. The tablets were allowed to fall 100 times and then reweighed after removal of fines. The percentage of weight loss was calculated. The % friability of the tablets was calculated using Equation 6.

$$\text{Friability (\% loss)} = \left( \frac{W_1 - W_2}{W_1} \right) \times 100 \quad \text{--- Equation 6}$$

Where,  $W_1$  = weight of five randomly selected tablets before friabilation,  $W_2$  = weight of only the intact tablets after friabilation

**In vitro dissolution studies:** *In vitro* dissolution tests were performed in triplicate using dissolution tester (ST7, G.B. Caleva Ltd, England), USP dissolution apparatus 1 rotating at 50 rpm in 900 ml of 0.1 N HCl at  $37 \pm 0.5^\circ\text{C}$ . Aliquot of dissolution medium equal to 5 ml was withdrawn at specified time intervals and same volume of fresh dissolution medium was replaced. The withdrawn media were properly diluted and the concentration and percentage of metronidazole released at each time interval was determined using UV Spectrophotometer at a wave length of 276 nm by using the regression equation from the standard calibration curve.

**Ex-vivo Bioadhesion test:** This test was carried out by a modified method of Attama *et al.*, (2000). A 50 ml burette was clamped on to a retort stand and a stage clamped at an angle of  $30^\circ$  below the burette. A freshly excised cow ileum of about 4 cm was tapped to the stage; one tablet was weighed and placed on the exposed mucus surface. Normal saline was allowed to flow at a rate of 100 ml/min. The weight of fluid that detached the tablet was recorded and used to compute the mucoadhesive force. The mucoadhesive force was calculated thus;

$$N = \frac{w}{100} \times g \quad \text{--- 7}$$

Where N= Mucoadhesive force (Newton), w= weight of fluid that detached the tablet, g= acceleration due to gravity.

**In vitro release kinetics:** The data obtained from the dissolution studies of the mucoadhesive metronidazole tablets were subjected to various drug release models to determine the release kinetics. The models include: zero order, first order and Higuchi square root of time relationship.<sup>[8]</sup> The mechanism of drug release from the formulation was determined using Korsmeyer and Peppas models.<sup>[9,10]</sup> The linear regression coefficient ( $r^2$ ) for each rate order was computed. The dissolution profile was considered to have followed a specific release order if the  $r^2$  value was  $>0.95$ .

#### Drug-Excipient compatibility (FTIR Analysis)

Fourier Transform Infrared Analysis (FTIR) measurements of pure drug, physical mixture of the active ingredient and other excipients and optimized tablet formulation were done using an FTIR spectrometer (Shimadzu, Model-RT-IR8300). The potassium bromide (KBr) pellet method was used. The pellets were prepared on KBr-Press under hydraulic pressure of  $150 \text{ kg/cm}^2$ . The powder was compressed using a sigma KBr press into a tablet shape. The tablet was placed in the sample compartment and the IR scan was read. The samples were scanned at a range of  $4000\text{-}500 \text{ cm}^{-1}$  at the ambient temperature.

#### Statistical Analysis

All experiments were performed in triplicates for validity of statistical analysis and expressed as mean  $\pm$  standard deviation (SD). The hardness, friability, and mucoadhesive strength data were analyzed using GraphPad instat at a level of significance of  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Micromeritic properties of the granules

The results of the micromeritic properties of the formulated mucoadhesive tablets using NG are shown in Table 2. It was observed that all the granules produced with NG had angle of repose ranging from  $25.1 - 30.6^\circ$  while Carr's index values ranged from  $09.2 - 11.2 \%$ . The Hausner ratio was between  $1.10 - 1.13$ . The results of the micromeritic properties revealed that all the granules exhibited good flow which is essential in ensuring weight and content uniformities during tableting and capsule filing.

The bulk and tapped densities are a measure of the flow properties of the granules. Each of the batches showed relatively close bulk and tapped density values which indicates that the interparticulate interactions were less significant and granules were free flowing. Poor flowing materials frequently have greater interparticulate interactions and a greater difference between the bulk and tapped densities is often observed.<sup>[6]</sup>

**Table 2: Micromeritic properties of metronidazole granules.**

Formulation	Bulk Density (g/ml)	Tapped Density (g/ml)	Angle of repose (°)	Carr's Index (%)	Hausner Ratio
NG1	0.591 ±0.3	0.665 ±0.1	25.1 ±0.1	11.1 ±0.1	1.13 ±0.1
NG2	0.587 ±0.1	0.652 ±0.2	27.2 ±0.3	10.0 ±0.1	1.11 ±0.2
NG3	0.522 ±0.2	0.588 ±0.1	28.4 ±0.1	11.2 ±0.2	1.13 ±0.1
NG4	0.536 ±0.1	0.590 ±0.3	30.6 ±0.2	09.2 ±0.1	1.10 ±0.2

where NG1, NG2, NG3 are metronidazole tablets formulated with 100, 150 and 200 mg NG respectively and NG4 contains 100 mg each of NG and HPMC.

#### Tablet evaluation of metronidazole tablets

The results of the weight uniformity, hardness and friability tests are presented in Table 3. The weight uniformity tests results showed that all the batches complied with the weight uniformity test because for each batch, no individual tablet deviated from their respective mean values by more than ± 5% (Table 3). This will help to ensure that uniformity of dosage form is achieved because the active ingredient, metronidazole forms a greater part of the tablet unlike in cases of potent drugs administered in low doses where the excipients form a major component of the tablet.

The hardness values obtained from this study range from 5.0 to 8.0 kPa indicating tablets of good mechanical strength which plays a very important role in the transportation, packaging, shipping, disintegration and dissolution of the tablet. The tablets also showed an

increase in hardness with a corresponding increase in gum concentration. This is because an increase in binder concentration has been shown to increase particle-particle contact points resulting in the creation of more solid bonds. It also promotes a decrease in intra-granular and inter-granular voids with an increase in packing fraction causing breakage of granules and creating more contact points leading to an increase in the degree of bonding between particles, hence a higher hardness was obtained.<sup>[11,12]</sup>

The loss in total weight of the tablets due to friability was in the range of 0.57 to 0.78% in all the formulations and the values were less than 1%. Friability determines the mechanical strength of a tablet when exposed to mechanical shock and attrition. Tablets that lose less than 1% of weight are considered acceptable by pharmacopoeial standard. All the batches had good percentage friability values falling below the pharmacopoeia limit of 1%.<sup>[13]</sup> There was a decrease in percentage friability with an increase in gum concentration.<sup>[12]</sup>

**Table 3: Physicotechnical properties of metronidazole tablets.**

Formulation	Hardness (Kpa)	Friability (%)	Weight uniformity (g)
NG1	5.0 ± 0.1	0.78 ± 0.01	0.52 ± 0.01
NG2	6.0 ± 0.2	0.63 ± 0.01	0.57 ± 0.01
NG3	5.5 ± 0.5	0.68 ± 0.01	0.54 ± 0.01
NG4	8.0 ± 0.2	0.57 ± 0.01	0.53 ± 0.01

#### Mucoadhesive properties of metronidazole tablets

The results of the mucoadhesive properties of the tablets formulated using NG are presented in Table 4. NG exhibited good mucoadhesive property with mucoadhesive strength ranging from 0.62-4.95 N. Batch NG4 containing a combination of NG and HPMC 100 mg gave a synergistic mucoadhesive effect with a mucoadhesive strength of 4.95 N compared to the values obtained without the incorporation of HPMC. In this study, it was observed that an increase in the concentration of the gum resulted in an increase in the mucoadhesive strength of the tablet i.e. the gum concentration is directly proportional to the mucoadhesive strength of the tablet. This can be attributed to the increase in compaction and interparticulate forces of the gum with an increase in its concentration.<sup>[14]</sup>

**Table 4: Mucoadhesive strength of metronidazole tablets.**

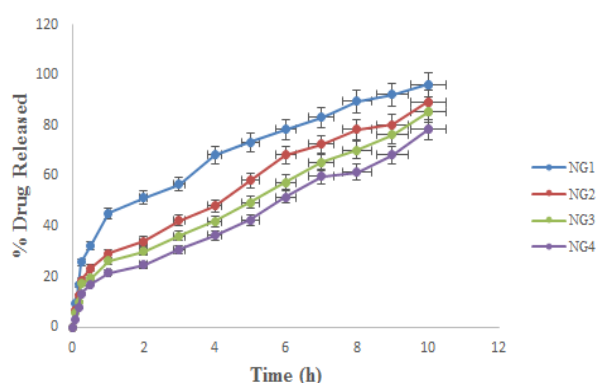
Formulation	Mucoadhesive strength (N)
NG1	0.62 ± 0.01
NG2	0.83 ± 0.02
NG3	1.12 ± 0.01
NG4	4.95 ± 0.01

#### Release profile of mucoadhesive tablets of metronidazole

The *in vitro* drug release profiles of the mucoadhesive tablets of metronidazole formulated using NG are shown in Figure 1. The drug release from batch NG1 showed a faster release of drug content compared to the other batches (NG2-NG3). It was also observed that there was a decrease in the rate of release of the drug content as the concentration of the gum increased. For example, batch NG1 released about 51.4% of its drug content within 2 h while batches NG2 – NG3 released about 31.4 and 30.1% respectively of their drug contents in 2 h. There



was a more sustained release of drugs from batch NG4 – containing HPMC as it released 24.7% of its drug content in 2 h. This shows that the release profile of the tablet was concentration dependent. The higher the concentration of the gum, the more sustained the release of drug content from the tablet.<sup>[12]</sup> It was also observed that the time to attain maximum release in the mucoadhesive tablets also reduced as the concentration of the gum increased. The dissolution parameters are presented in Table 5. For instance, maximum drug released ( $m_{\infty}$ ), time to achieve maximum release ( $t_{\infty}$ ) and dissolution rate ( $m_{\infty}/t_{\infty}$ ) for batch NG1 was 96.3%, 10 h and 9.63% h<sup>-1</sup> respectively while the corresponding values for batch NG4 was 78.5%, 10 h and 7.85% h<sup>-1</sup>. The higher the concentration of the gums, the slower the drug release from the formulations studied.



**Figure 1: Drug release profile of metronidazole mucoadhesive tablets formulated with neem gum.**

**Table 5: Dissolution parameters of metronidazole mucoadhesive tablets.**

Batches	$m_{\infty}$ (%)	$t_{\infty}$ (h)	$m_{\infty}/t_{\infty}$ (%h <sup>-1</sup> )
NG1	94.5	10	9.50
NG2	87.2	10	8.72
NG3	83.4	10	8.34
NG4	78.2	10	7.82

**Table 6: Regression coefficient values for different release models.**

Formulations	Zero Order		First Order		Higuchi model		Korsmeyer - Peppas	
	R <sup>2</sup>	K0	R <sup>2</sup>	K1	R <sup>2</sup>	Kit	R <sup>2</sup>	n
NG1	0.890	8.064	0.986	0.115	0.988	28.676	0.400	0.488
NG2	0.959	8.209	0.984	0.081	0.990	26.874	0.483	0.532
NG3	0.975	7.411	0.969	0.069	0.978	25.116	0.561	0.542
NG4	0.875	8.190	0.977	0.141	0.978	29.287	0.392	0.476

### FTIR Spectroscopy

The FTIR spectra of pure metronidazole powder and admixture of metronidazole and the polymers are shown in Figure 2. From the spectra, the major peaks of the pure metronidazole sample remained in the same range in the spectra of the formulations containing metronidazole, neem gum and HPMC. This shows that there was no alteration in the peaks of the pure metronidazole sample hence no alteration of the functional groups of the pure sample or interaction

### Release kinetics and mechanism of drug release from the mucoadhesive tablets

The results of the various release kinetics for mucoadhesive metronidazole tablets are presented in Table 6. The results obtained from the dissolution studies were fitted into zero order, first order, Higuchi, Korsmeyer and Peppas release models in order to determine the release kinetics of the different formulations. The *in vitro* release profiles of the mucoadhesive metronidazole tablets simulated the Higuchi release model as the plot showed the highest coefficient regression ( $r^2$ ) values of 0.978-0.990 compared to the zero and first order release models which had  $r^2$  values within the ranges of 0.875-0.975 and 0.969-0.986 respectively. This shows that the drug released from the tablets were mainly by Higuchi's model which states that the amount of drug released is dependent on the square root of time.<sup>[12]</sup> This is in line with studies conducted by Higuchi, 1963 who in analyzing the mechanism of drug release from matrices, postulated two mechanisms which are dissolution and diffusion controlled mechanisms. The natural gums in the mucoadhesive tablets studied controlled the dissolution and gelation rate of the drug in the matrix being hydrophilic and the diffusion of the drug through the gel.

The data obtained were fitted into Korsmeyer and Peppas equation in order to determine the mechanism of release. The formulation showed poor linearity with  $r^2$  values ranging 0.392-0.561. Since the  $r^2$  values were consistent with Higuchi's model, it was expected that the mechanism of drug release from matrix tablet was diffusion controlled. The release exponent ( $n$ ) for the metronidazole tablets ranged from 0.46-0.54. All the formulations had their release exponent ( $n$ ) > 0.45; hence their release mechanism was by Non-Fickian diffusion. This indicates that diffusion was the dominant mechanism of drug release.

between the pure sample and the other excipients used in the formulation.

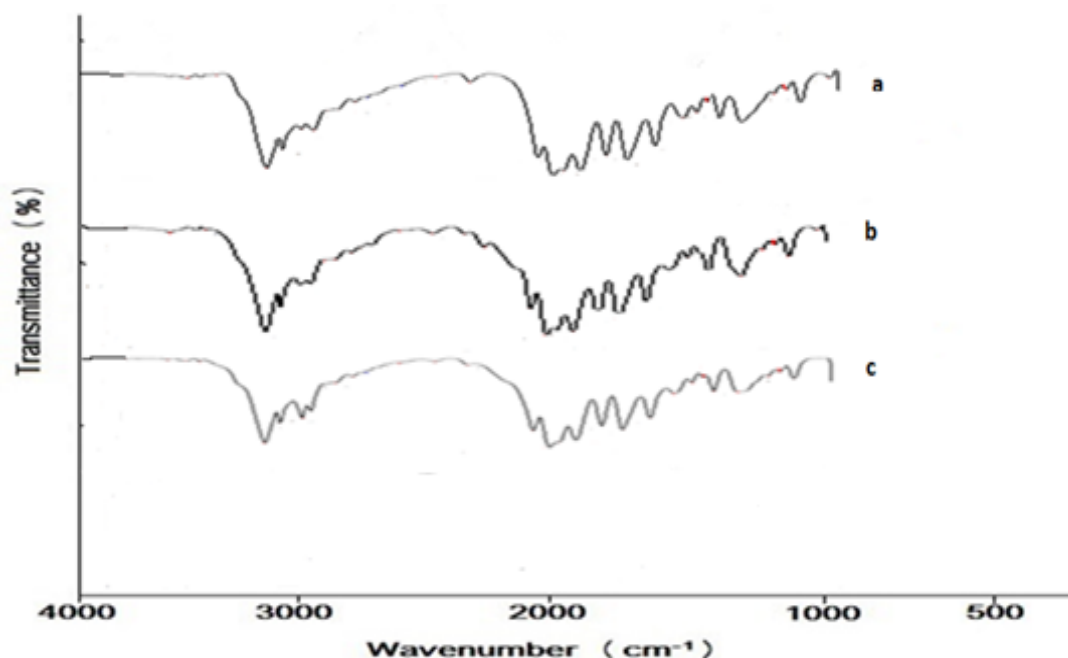


Figure 2: FTIR spectra of (a) pure metronidazole sample (b) metronidazole and neem gum (d) metronidazole, neem gum and HPMC.

## CONCLUSION

Mucoadhesive tablets of metronidazole were successfully formulated using neem gum. A combination of the neem gum and HPMC exhibited synergistic mucoadhesive properties retarding the release of metronidazole from the dosage form which will be beneficial in the formulation of sustained drug delivery systems.

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