



## Effect of Formulation on Buoyancy of Metronidazole Capsule

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### ABSTRACT

The present study was to develop a buoyant Hydrodynamically Balanced System (BHBS) for metronidazole (MN) using different formulations to show their effect on the floating time and in turn improving the sustained release pattern as a single unit floating capsule. The formulated blends were prepared using physical mixing of finely pulverized ingredients including the drug. The compatibility of different ingredients with the drug was studied using FTIR, DSC and XRPD techniques. The prepared BHBS capsules were also evaluated for drug content, weight uniformity and in vitro drug release. The drug release from different formulations relied on formulating ingredient, their concentrations and type of buoyant mechanism. Histopathological study. All formulations of MN sustained the drug release compared to capsule contains only pure MN.F7 depicted the best buoyant behavior. The histopathological study denoted a lack of metronidazole associated gastric mucosal cells injury in albino rats. The release data was best fit to first, diffusion model except F3 depicted first order kinetics.

**Keywords:** Effervescent system, Histopathological study, HPMC, Metronidazole.

### INTRODUCTION

Metronidazole is a limited spectrum antibiotic that actively deters the growth of protozoa, anaerobic gram-positive, and anaerobic gram-negative bacteria. The first use of this antibiotic was to suppress protozoans such as *Entamoeba histolytica*, *Giardia lamblia* and *Trichomonas vaginalis*. Further studies show that Metronidazole has been used to repress the growth of gram-negative anaerobes belonging to the *Bacteroides* and *Fusobacterium*, and gram-positive anaerobes such as *peptostreptococcus* and *Clostridia*. The advantages of this antibiotic is that it affects a high percentage of gram-negative bacteria and has greater tissue penetration. Furthermore, the gene *hefA* codes for the TolC efflux pump in *Helicobacter pylori* which is resistant to Metronidazole.<sup>1,2</sup>

Buoyant hydrodynamically balanced Systems remain buoyant in the stomach for a prolonged period of time and release the drug slowly at a desired rate<sup>3-5</sup> BHBS.

There are a number of approaches that can be used to prolong gastric retention time, such as floating drug delivery systems, also known as hydro dynamically balanced systems, swelling and expanding systems, polymeric bio adhesive systems, modified-shape systems, high-density systems, and other delayed gastric emptying devices.<sup>6-8</sup>

In the present study, an attempt was made to develop a gastro retentive floating capsules of metronidazole as single-unit floating capsules using two mechanisms. The first using low density polymers; the second is

formulating effervescent floating drug delivery systems generate gas (CO<sub>2</sub>). The effect of formulation and formulation technique on T<sub>90%</sub> was studied. We had tried to prolong the drug release and increase gastric residence time, to increase the anti protozoal therapeutic effect of the drug compared to multiple conventional oral dosage forms. The histopathological study was done to declare the effect of metronidazole on gastric mucosa in albino rats.

### MATERIALS AND METHODS

#### Materials

Metronidazole (MN), Hydroxypropylmethylcellulose (HPMC 4M), Chitosan, Sodium bicarbonate, Stearic acid, Polyethylene glycol (PEG6000), Buffer solutions, Sodium pentobarbital, Formaldehyde, Saline, Hematoxylin, Eosin were purchased from Sigma Chemical, Co., St. Louis. Albino rats were kindly supplied from King Abdulaziz University, KSA. All water used was distilled and de-ionized. All other chemicals were of reagent grade and used as received.

#### Methods

##### Selection of Polymers

Accurately weighed 250 mg of different low density hydrophilic polymers were added in appropriate size of empty hard gelatin capsule shell (size# 00). These polymers were tested for their floating time in dilute HCl (pH 1.2).



**Floating capacity**

Floating characteristics of the prepared formulations were determined by using USP 2 paddle apparatus at a paddle speed of 50 rpm in 900 ml of a 0.1 N HCl solution (pH=1.2) at 37±0.5°C for 9 h. The time during which the dosage form remains buoyant (floating duration) were measured.

**Formulation of Buoyant Capsules**

MN was weighed(200mg) and physically blended with different ingredients as illustrated in table 1 using mortar and pestle until the whole blend has the same color (homogenous mixing) then, filled into hard gelatin capsule (size #00) manually.

**Table 1:** Composition of BHBS Metronidazole capsule formulations (mg/capsule)

Ingredient	FD	F1	F2	F3	F4	F5	F6	F7	F8
Metronidazole	200	200	200	200	200	200	200	200	200
HPMC	-	120	160	100	90	110	130	75	85
Chitosan	-	70	30	70	80	60	40	100	85
Sodium bicarbonate	-	-	-	20	20	20	20	20	20
Stearic acid	-	5	5	5	5	5	5	5	5
PEG6000		5	5	5	5	5	5	-	5

**Table 2:** Kinetic analysis of the release data of different MN formulations

Formula	Model	R <sup>2</sup>	Slope	Y-Intercept	T <sub>90%</sub> (hrs)	Mechanism of Release
FD	Zero	0.750	19.68	73.76	0.79	Diffusion
	First	0.750	-1.29	1.72		
	Diffusion	0.817	39.91	54.49		
F1	Zero	0.898	19.43	62.50	1.35	Diffusion
	First	0.852	-0.50	1.69		
	Diffusion	0.938	42.43	40.77		
F2	Zero	0.940	28.30	59.60	1.00	Diffusion
	First	0.920	-2.15	2.70		
	Diffusion	0.973	55.92	33.33		
F3	Zero	0.875	20.38	52.99	1.63	First
	First	0.979	-0.54	1.88		
	Diffusion	0.936	48.58	26.07		
F4	Zero	0.920	16.94	45.80	2.52	Diffusion
	First	0.987	-0.406	1.96		
	Diffusion	0.972	45.52	17.80		
F5	Zero	0.950	17.01	37.74	2.95	Diffusion
	First	0.960	-0.37	2.04		
	Diffusion	0.982	47.66	7.41		
F6	Zero	0.986	11.49	33.94	4.89	Diffusion
	First	0.936	-0.21	2.01		
	Diffusion	0.992	37.54	6.61		
F7	Zero	0.973	8.48	30.90	6.98	Diffusion
	First	0.915	-0.179	2.11		
	Diffusion	0.984	33.17	2.30		
F8	Zero	0.950	9.944	36.37	5.30	Diffusion
	First	0.964	-0.200	2.013		
	Diffusion	0.989	35.31	8.59		

**Evaluation of BHBS capsules formulations****Weight Uniformity**

10 capsules were weighed individually and the average weight was determined. Test was performed according to the official method.

**Drug content**

The HBS capsules content was dissolved in ethanol and make up volume up to 100 ml with HCl pH 1.2 solution (SGF) and filter. The absorbance was measured at 289 nm after suitable dilution<sup>9</sup> by UV-Visible spectrophotometer (Shimadzu UV-1601, Japan).



**Infrared analysis (FTIR)**

The x-ray diffraction patterns of MN and F7 were performed in infrared spectrophotometer (Genesis II, Mattson, England). Radiation was provided by a copper target (Cu anode 2000W:1.5418 high intensity x-ray tube operated at 40 KV and 35MA). The monochromator was a curved single crystal (one PW1752/00). Divergence slit and receiving slit were 1 and 0.1 respectively. The scanning speed of goniometry (PW/050/81) USED WAS 0.02.20/5 and the instrument were combined with a Philips PM8210 printing recorder with both analogue.

**X-ray Powder Diffraction Analysis (XRPD)**

X-ray diffraction experiments were performed in a Scintag x-ray diffractometer (USA) using Cu K  $\alpha$  radiation with a nickel filter, a voltage of 45 kV, and a current of 40 mA. Diffraction patterns for MN and F7 were obtained.

**Differential scanning calorimetry (DSC)**

Samples were placed in Al pan and heated at rate of 25°C/min with indium in the reference pan, in an atmosphere of nitrogen up to a temperature of 400°C. The DSC study was performed for MN and F7.

**In vitro drug release studies**

The dissolution of different formulations compared to the plain drug, were determined using dissolution tester (VK 7000 Dissolution Testing Station, Vankel Industries, Inc., NJ) following the USP paddle method. All tests were conducted in 900 mL of 0.1N HCl (SGF) maintained at 37  $\pm$  0.5°C with a paddle rotation speed at 50 rpm. The amount of MN used was equivalent to 200 mg. After specified time intervals, samples of dissolution medium were withdrawn, filtered, and assayed for drug content spectrophotometrically at 289 nm after appropriate dilution with 0.1N HCl. The experiment was carried out in triplicate.

**Effect of release modifiers**

Stearic acid and Peg6000 were used 5mg each in the formulation to study their effect on the in vitro drug release study (Table 1). The release modifiers were added to the powder blend, physically mixed in mortar and pestle for 15 min and filled into hard gelatin capsule (size #00) manually.

**Histopathological Study****Material and Method**

This experimental work was carried out over a 2 weeks period and comprised 20 normal healthy growing male albino rats of uniform strain and aged between 2-3 months and weighing 180 $\pm$ 20 gm. All animals were housed 5 in each cage under the same environmental conditions with food and water ad libitum.<sup>10</sup> The rats were left without handling for one week before the study to allow their climatic acclimatization. Thereafter, they were separated equally into 2 groups, A and B, each included 10 rats as follows:

Group A (control group): each rat gavaged with 1 ml of distilled water daily for 2 consecutive weeks. Group B (Metronidazole-treated group): each animal gavaged daily with designated sustained release metronidazole capsule for 2 consecutive weeks at a single dose of 200 mg/kg body weight. All animals were weighted and fasted for 4 hours before their gavage. Twenty-four hours after administration of the last dose, all rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally) before scarification. Laparotomy was performed and each dissected stomach was fixed in 10% formal saline for 2 weeks then processed for Hematoxylin-Eosin (Hx & E) staining<sup>11</sup>

**Kinetic analysis of release data**<sup>12,13</sup>

To analyze the mechanism of drug release from different formulations the in vitro dissolution data were fitted to different model dependent kinetics and model independent approaches like zero order, first order and diffusion model.

**RESULTS AND DISCUSSION****Floating capacity**

The polymers selected were of inherent low specific gravity to formulate an excellent BHBS to achieve the required prolonged release drug delivery system for the aqueous soluble MN. The floating capacity of different BHBS was not less than 8 hours for F6 –F8.

**Evaluation of Buoyant Formulations****Weight Uniformity**

The average weight of capsules within each formulation was found to be uniform. This indicated uniform filling of powder blend during capsule filling. Not more than two of the individual weights deviated from the average weight by more than 7.5% and none deviated by more than twice that percentage, which provided good weight uniformity.<sup>14,15</sup>

**Drug Content**

The values for drug content in all formulations were found to be uniform among different batches of the floating drug delivery system (FDDS) and ranged between 97.5 and 101.2% of the theoretical value as per USP.<sup>16</sup> The value ensures a good uniformity of the drug content in the capsules.

**Infrared Spectra Analysis**

Figure 1 illustrated the spectra of MN and F7. The spectra showed no shift in IR bands in functional group region and the fingerprint regions of both drug and F7 are not superimposed. This confirmed the absence of chemical interaction and a physical change in MN.

**DSC Studies**

DSC thermogram of pure MN in figure (2), showed sharp endothermic peak at 164.68°C and in F7 the endothermic peak appeared at 162.20°C. Slight shifting of endothermic



peak of the drug to the left side indicates a little reduction in melting point of drug. This indicates that both viscosity of matrix and degree of cross-linking of the polymers eventually control the rate of drug release.

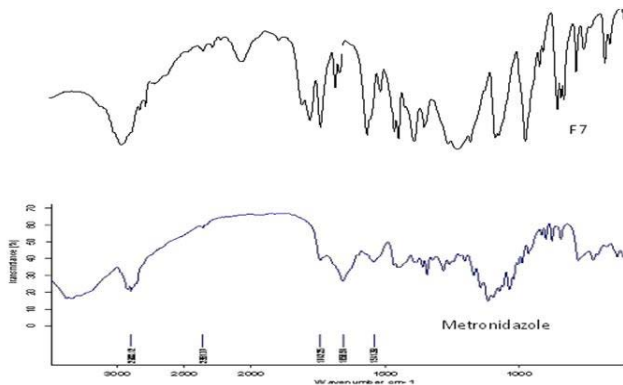


Figure 1: FTIR spectra of plain MN and F7

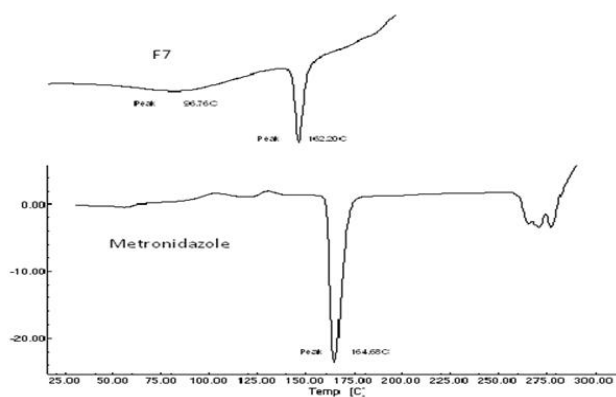


Figure 2: Thermograms of MN and F7

**XRPD Studies**

XRPD pattern of MN and formulation F7 showed in figure (3). It revealed that the intensity of the peaks for the pure drug was sharp, but when it was incorporated into the polymer matrix, the intensities of the peaks decreases due to decrease in crystalline properties of MN.

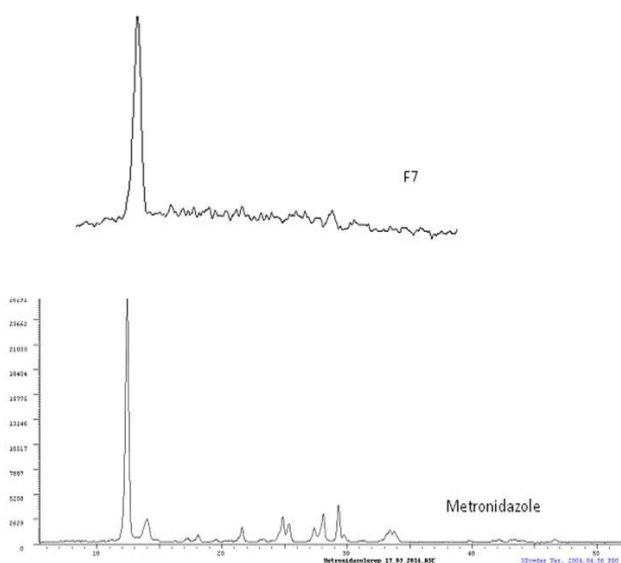


Figure 3: XRPD of RH and F7

**In-vitro Drug Release Study**

Figure 4 depicted the release pattern of MN from different formulations. F7 depicted the ideal drug release pattern during the release time; the released amount of MN after 9 hours was 100%. This is due to presence of a blend of HPMC, Chitosan and effervescent floating system which controlled the drug release throughout the polymeric matrix by virtue of a high degree of cross-linking and increased viscosity moreover the absorbed carbon dioxide evolved from the reaction of sodium bicarbonate with HCl. Presence of HPMC and Chitosan as drug carriers retard MN release to a great extent because Chitosan is a cationic polymer insoluble in acid medium which swells forming stiff matrix retarding MN release. Also presence of stearic acid increased the toughness of the matrix. FD, F1, F2, F3, F4, F5, F6 and F8 depicted their maximum drug release (100%) after 1, 2, 1.5, 2.5, 3.5, 4, 6 and 7.5 hours respectively. This is due to the lower polymer concentration and/or absence of effervescent system. Incorporation of PEG600 in formulations accelerated drug release compared to F7. This is due to its channeling effect.

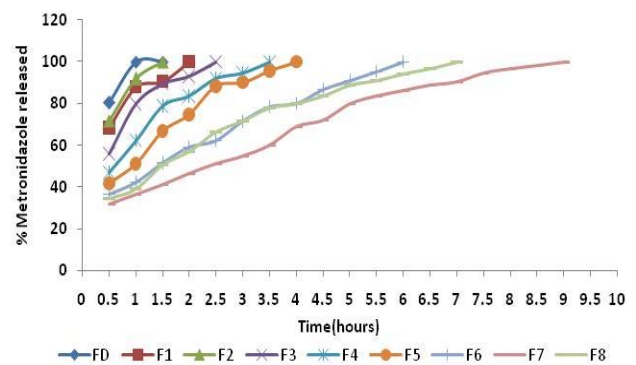


Figure 4: Release pattern of different formulations of metronidazole in buffer solution of pH 1.2 at 37±0.5°C

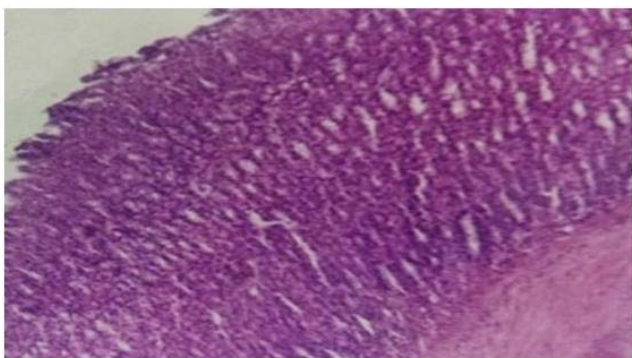
**Histopathological Study**

No deaths were recorded in any of the experimental groups during the studied period.

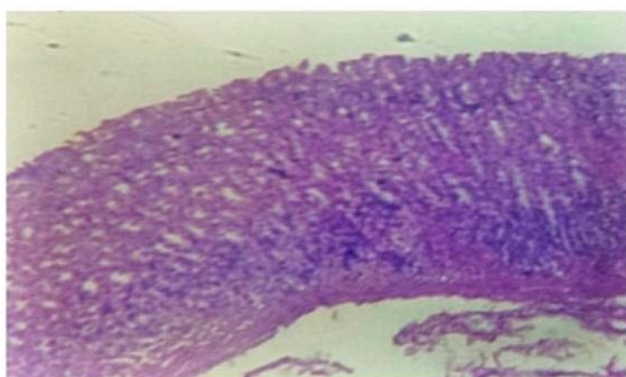
Light microscopic assessment of the stomachs sections prepared from control and metronidazole-treated rats (photomicrograph 1 and 2, respectively) revealed normal histological architecture of the gastric fundus tissue in both groups, when compared together, as demonstrated by ordinary appearance of mucosal, submucosal, and muscularis cellular layers as well as glands and connective tissue arrangements.

Irrespective of the clinically observed gastrointestinal disorders induced by mitroimidazole derivatives<sup>17</sup> including oral administration of mitronidazole<sup>18</sup> that may occur in acute overdose as well as with chronic therapeutic doses<sup>19</sup> the histological effects of metronidazole on gastric structure is scarce or unreported. The present experimental study denoted a lack of metronidazole associated gastric mucosal cells

injury. Metronidazole is not only safe on gastric cellular composition but also has a beneficial protective effect on gastrointestinal mucosal cells against non-steroidal anti-inflammatory drugs<sup>20-22</sup> and ethanol producing gastroenteropathy in humans and rodents.



**Figure 5:** A photomicrograph of a section in rat's stomach from control group showing the ordinary architecture of the tissue with relatively thick mucosa that consists of three layers namely the surface simple columnar epithelium, the lamina propria, and muscularis mucosa (Hx & E stain, original magnification x 100).



**Figure 6:** A photomicrograph of a section in rat's stomach from metronidazole-treated group showing the ordinary architecture of the gastric mucosa closely resemble that of the control group (Hx & E stain, original magnification x 100).

#### Kinetic Analysis of Release Data

The drug release from polymer matrix was eventually due to diffusion mechanism except F3 depicted a drug release according to first order kinetics.

#### CONCLUSION

It was concluded that, on the basis of in vitro drug release patterns and  $T_{90\%}$  that the formulations F7 showed an ideal drug release profile and suitable  $T_{90\%}$  (6.98 hrs) to control the drug release as compared to capsules containing only pure MN. The increased polymer concentration in the formulations decreased the rate of MN released. Evolved carbon dioxide as well as polymers increased the floating time. The incorporation of hydrophobic and hydrophilic release modifiers in the formulations greatly affected the release pattern of MN from the polymer matrix. Histopathological studies

revealed that MN has no side effect on gastric mucosa of albino rats over the extended time.

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