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RESEARCH ARTICLE

An Experimental Investigation based on a Novel Gastro-Retentive Raft Liquid Dosage Form in Tandem with Controlled-Release Strategies for Oral Delivery of Metronidazole

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SUMMARY

Peptic ulcers are lesions that form on the mucosal lining of the stomach and duodenum. The most common inducer of peptic ulcers is Helicobacter pylori (H. pylori) as the primary pathogen. The present study aimed to design and develop a novel gastro retentive raft liquid dosage formulation to prolong the gastric retention time of the medicament for the treatment of H. pylori infection. Metronidazole-loaded raft formulations were prepared using ion-sensitive in situ gel-forming polymers. The formulation was floated within 1 minute on the liquid surface of the in vitro model and maintained floatation for more than 24 hours. Among those formulations, formulation F-5 exhibited results within acceptable limits compared with the other batches of formulations. The in vitro drug release was 81.83 ± 0.54 % after 8 hours in 0.1 M HCl. The drug, metronidazole, was released from the dosage form slowly in the stomach. The formulation followed first-order release kinetics, and metronidazole was released from the formulation as a combination of diffusion and erosion mechanisms. Thus, the proposed metronidazole-loaded raft formulation would be a promising novel site-specific drug delivery system and has a potential utility in the therapy of peptic ulcers induced by H. pylori.

Key Words: *Floating drug delivery system, Metronidazole, Peptic ulcer, Stomach-specific delivery, H. pylori.*

Metronidazolün Oral Uygulaması için Kontrollü Salım Stratejileriyle Birlikte Yeni Bir Gastro-Retentif Raft Sıvı Dozaj Formuna Dayalı Deneysel Bir Araştırma

ÖZ

Peptik ülserler, mide ve duodenumun mukoza yüzeyinde oluşan lezyonlardır. Peptik ülserlerin en yaygın nedenlerinden biri, birincil patojen olarak Helicobacter pylori'dir (H. pylori). Bu çalışma, ilacın mide içinde kalış süresini uzatarak H. pylori enfeksiyonunu tedavi etmek amacıyla yeni bir gastro-retentif raft sıvı dozaj formülasyonu tasarlamayı ve geliştirmeyi amaçlamıştır. Metronidazol yüklü raft formülasyonları, iyon duyarlı in situ jel oluşturan polimerler kullanılarak hazırlanmıştır. Formülasyon, in vitro modelde sıvı yüzeyinde 1 dakika içinde yüzmeye başlamış ve 24 saatten fazla süreyle yüzme durumunu korumuştur. Bu formülasyonlar arasında, F-5 formülasyonu diğer formülasyon gruplarıyla karşılaştırıldığında kabul edilebilir sınırlar içinde sonuçlar göstermiştir. In vitro ilaç salım, 0.1 M HCl'de 8 saat sonra 81.83 ± 0.54 % olarak gerçekleşmiştir. Metronidazol, dozaj formundan midede yavaşça salınmıştır. Formülasyon birinci dereceden salım kinetiğini izlemiş ve metronidazol, formülasyondan difüzyon ve erozyon mekanizmalarının bir kombinasyonu olarak salınmıştır. Bu nedenle, önerilen metronidazol yüklü raft formülasyonu, bölgeye özgü yeni bir ilaç taşıma sistemi olarak umut vadetmektedir ve H. pylori'nin neden olduğu peptik ülserlerin tedavisinde potansiyel bir kullanıma sahiptir.

Anahtar Kelimeler: *Yüzen ilaç taşıma sistemi, Metronidazol, Peptik ülser, Mideye özgü taşıma, H. pylori.*

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INTRODUCTION

Peptic ulcers are lesions that form on the mucosal lining of the stomach and duodenum. The most common inducer of peptic ulcers is *Helicobacter* pylori (*H. pylori*) as the primary pathogen. Other non-pathogenic causes of peptic ulcers include the prolonged use of medicaments such as steroids, non-steroidal anti-inflammatory drugs (NSAIDs), anticoagulants, alcohol, spicy food, etc. Low antibiotic levels and limited medication accessibility at the site of infection are two of the many challenges in eliminating *H. pylori* infections (Adebisi et al., 2015). According to researchers, antibiotics absorbed via the mucus layer are more successful than those that are absorbed through the basolateral membrane in eliminating *H. pylori* (Nori et al., 2011). Hence, the nitroimidazole group of drugs like metronidazole is preferred for the treatment of anaerobic or facultative anaerobic microbes like *H. pylori*. *H. pylori* exhibits redox potential in the electron transport segments, which reduces the metronidazole nitro group to nitro radicals and leads to the production of toxic metabolites, which can disrupt *H. pylori* DNA replication (Hernández Ceruelos et al., 2019).

The application of nitro-imidazole derivatives, such as metronidazole, tinidazole, etc., is an active adjuvant antibiotic in the management of peptic ulcers caused by *H. pylori* (Emara et al., 2014). However, metronidazole has a plasma $t_{1/2}$ of 8 hours. Generally, 400 mg three times daily is prescribed in the treatment of *H. pylori*. The frequent administration of metronidazole aggravates the adverse effects such as anorexia, nausea, vomiting, peripheral neuropathy, and can develop bacterial resistance. The effects on the central nervous system are mainly dose-dependency related, which can negatively impact patient compliance (Tripathi, 2013). Therefore, it was thought to be worthy of formulating in site-specific dosage forms, which act locally at the site of disease occurrence. It would reduce the frequency of the therapeutic dose of the medicament, minimize the potential adverse effects as well as raise the efficacy of the treatment and patient compliance. Accordingly, preparing gastro retentive dosage forms is crucial for the complete eradication of *H. pylori*. Additionally, metronidazole offers the advantage of having pH-independent activity, unlike the other anti-*H. pylori* antibiotics such as clarithromycin. The researchers have made several attempts to eradicate microorganisms from the stomach completely (Koga, 2022; Nori et al., 2011; Rajinikanth & Mishra, 2008).

Pharmaceutical scientists have designed various novel drug delivery systems to enhance the gastric residence time of anti-*H. pylori* medicaments. Thus, raft liquid, floating, swellable, mucoadhesive, high-density formulations, etc., are being developed to achieve gastro retention (Nori et al., 2011). Among the various novel gastro retentive drug delivery systems (GRDDS), raft liquid delivery dosage form is a progressed revolution in liquid oral controlled drug delivery. Raft dosage forms are liquid at room temperature but go through gelation once the pH changes, wherein each part of the liquid swells organizing a continuous layer called a raft (Vinod et al., 2010). Each layer of gel floats on the gastric fluid owing to its bulk density which is less than gastric fluids. It remains buoyant inside the stomach without being affected by the gastric emptying rate for a long duration of time, which has been assessed for sustaining as well as targeting the dosage form for drug delivery (Abou Youssef et al., 2015; Prajapati et al., 2013). The raft remains intact inside the stomach contents for more than 48 hours, raising gastric residence time and sustaining drug delivery in the gastrointestinal tract (GIT) (Ibrahim, 2009).

The goal of designing this raft formulation is to slowly release the medicament from the dosage form at a desired rate in the stomach. This raft formulation significantly prolongs the gastric residence time of a medicament, improves oral bioavailability, and simultaneously minimizes dosing intervals. Additionally, raft liquid formulation is simple to formulate as well

as cost-effective. Moreover, it is absorbed from the proximal part of GIT, so this raft formulation allows for prolonged drug release in the vicinity of the bacterium (Rajinikanth et al., 2007).

Keeping this concept in mind, the present study aimed to improve the gastric residence time of metronidazole via formulating a raft liquid formulation to eradicate *H. pylori* and control the release of the medicament into the local site of action. This dosage formulation could offer a revolutionary solution for the treatment of *H. pylori*.

MATERIAL AND METHODS

Chemicals

Metronidazole was obtained as a gift sample from Ozone Pharmaceutical Pvt. Ltd. Guwahati, India. Eudragit® NE 30D was procured from Yarrow Chem Product, Mumbai, India. Calcium carbonate ($CaCO_3$), sodium alginate, sodium citrate, and sodium bicarbonate ($NaHCO_3$) were procured from Himedia Ltd. Mumbai, India. Barium sulphate ($BaSO_4$) and Hydrochloric acid (HCl) were procured from Merck Specialities Pvt. Limited, Mumbai, India

Preparation of Metronidazole loaded gastro retentive raft forming formulation

Coating of Metronidazole

Metronidazole was initially dispersed in Eudragit® NE 30D and then dried in the hot air oven at 70 °C till completely dry. The achieved dried mixture was then kept in tightly closed containers until further use.

Preparation of the raft-forming formulation

According to Table 1 composition, the eight raft formulations were prepared (Abouelatta et al., 2018). Rafts were prepared by dissolving sodium alginate at the concentration of 1-3 % w/v in deionized water containing 0.17 % w/v of sodium citrate and 1 % w/v of $NaHCO_3$. Then $CaCO_3$ solution of (0.5-3 % w/v) different concentrations was with continuous stirring on a magnetic stirrer (Model AI-021, Alfa instruments, New Delhi, India). Then, previously coated metronidazole was suspended in the resulting solution with continuous stirring until it was thoroughly dispersed, and the final volume was made to 100 mL with deionized water. The obtained raft-forming formulations were stored in amber-colored containers and protected from light for further experimental study.

Drug excipients compatibility study of the model drug with excipients

It was carried on by Fourier transform Infrared spectroscopy (FT-IR) and Differential Scanning Calorimetry (DSC).

Differential Scanning Calorimetry

DSC was performed to measure the heat exchange during thermal transitions, which gave information on the structural properties of the ingredients. Metronidazole, metronidazole with Eudragit® NE 30D, and physical mixtures of metronidazole with all excipients were performed separately by using a Perkin Elmer JADE DSC (USA) instrument. The samples were kept in an aluminum pan and scanned at a speed of 10 °C $minute^{-1}$ at the temperature range of 20 °C to 300 °C under the inert nitrogen gas atmosphere. The thermograms were observed for any phase changes during the analysis process if there were any changes in thermograms compared to individual components used in formulation, indicating their interaction with each other.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR was performed to study the compatibility of ingredients. In this technique, the nature of the interacting force can be evaluated during the gelation process. The FT-IR spectra of metronidazole, metronidazole with Eudragit® NE 30D, and physical mixtures of metronidazole with all excipients were analyzed in Bruker Alpha FT-IR spectrophotometer, Germany, to distinguish if there is any interaction between drug and polymers.

Evaluation of post-formulation parameters and gastro retentive study of developed formulation

Rheological property of the raft formulations

The rheological property is the characteristic function of raft formulations. It was carried out on a rotating viscometer (Anton Paar Rheometer MCR 102SN81260812) at 25 ± 2 °C. Viscosity was measured at speeds ranging from 0.3 to 60 rpm.

Measurement of raft weight, volume, density, and buoyancy

The raft's weight, volume, and density were measured according to a modified method of Abbas et al. (2017), Bunlung et al. (2021), and Hampson et al. (2005). The maximum dose of the prepared raft formulation was transferred to 150 mL of 0.1 M HCl (pH1.2) in a glass beaker, which was previously maintained at the temperature of 37± 1 °C and waited for 30 minutes till the raft was formed. Before preparing the raft, each beaker was pre-weighed (W_1). The top of each raft was observed from the outer surface of the beaker, and the position of each raft reached on top was marked on the outside of the beaker. The total weight of the beaker containing the raft was noted after raft formation (W_2). The weight of each raft formation was calculated from the formula (Eq 1).

$$Raft\ wieght\ (g) = W_2\text{-}W_1 \quad \text{..... Eq 1}$$

Where, W_1 is the weight of the empty beaker, and W_2 is the weight of the beaker containing the raft.

The raft was transferred from the beaker to a measuring cylinder and the final volume of each raft was determined. We assumed that in the formula, the density of the subnatant liquid is the same as that of water. Finally, the density of each raft was calculated as per the following equation (Eq 2).

$$Raftdensity = \frac{Raftweight(g)}{RaftvOlume(ml)} \quad \text{..... Eq 2}$$

Each formulation was tested in triplicate. The density of each raft was expressed as g mL^{-1}

A buoyancy index was calculated as,

$$Raft\ bouncy(ml) = \frac{Raft\ Volume - Raft\ weight}{Raft\ weight} \quad \text{Eq 3}$$

Measurement of raft strength of the formulation

Raft strength is used to determine the firmness, consistency, cohesiveness, and gelling properties of the prepared formulation. The strength was with a modified balanced method. Here, rafts were developed in a 250 mL glass beaker under the same conditions as described above, but with an additional suspended L-shaped stainless-steel probe (diameter: 1.2 mm) placed in the center of the beaker, with the lower third immersed in acid. The beaker was positioned on the balance after 30 minutes of raft development. Then water was added dropwise until the raft surface was back drowned, and the force was recorded in grams. (Note: A double pan dispensing balance was modified to facilitate the measurement of raft strength, where one of the pans was replaced with the same volume of a beaker containing water) (Abbas et al., 2017; Hampson et al., 2005).

In vitro floating and gelling capacity of the raft formulation

The maximum dose of the prepared raft formulation was carefully and slowly transferred to 250 mL of a glass beaker, as above condition, but without suspending an L-shaped stainless steel probe, and waited for 30 minutes till the raft was formed. The gelation was noticed by visual observation. The time that the formulation takes to emerge on the medium, i.e., floating log time, and the duration of time during which the formulation continued to levitate on the surface of the medium, i.e., floating duration, were recorded. The floating behavior of each formulation was recorded in three categories based on the time and period for which the formed gel remained floating in a medium, such as the formation of the raft after a few minutes, dispersed rapidly, formation of immediate raft remains for 12 hours and formation of immediate raft remains for over 12 hours.

In vitro determination of drug release

Raft forming formulation is administered through the oral route; thus, the release rate of metronidazole from raft formulation was carried out using a USP type II rotating paddle dissolution apparatus (Abou Youssef et al., 2015) (Electro lab, Mumbai) in 900 mL 0.1M HCl at 37±1 °C and rotating speed of 50 rpm. 10 mL of the raft formulation was carefully injected using a disposable syringe into the dissolution vessel without much disturbance and in the paddle device, the raft was capable of floating freely on the surface of the liquid, due to the minimum share stress of the surface. 5 mL aliquots were withdrawn and replaced by fresh samples at predetermined fixed time intervals. The quantity of metronidazole in the withdrawn samples was analyzed by a UV Spectrophotometer (UV- 1800, Shimadzu Spectrophotometer, Japan) at approximately 276 nm. Tests were carried out repeatedly for each formulation three times, and data were reported as mean value ± S.D. The cumulative percentage release of the drug against time was plotted to depict the metronidazole release profile (Kerdsakundee et al., 2015).

In vitro drug release kinetics

To facilitate the easier comparison of each formulation and analyze the impact of various factors on drug release profiles, we calculated the cumulative percentage of drugs released at predetermined time intervals. Drug release data were analyzed according to various *in vitro* drug release kinetics models such as zero-order, first-order, Korsmeyer- Peppas, and Higuchi models (Abbas et al., 2017; Das et al., 2010).

In situ gel-forming activity in rabbit stomach using Roentgenography

The Roentgenography method is widely used to locate dosage forms in the GIT; therefore, it can be assumed and correlated with the duration of gastric evacuation along with the passage of dosage forms in the GIT. It involves radio-opaque markers such as $BaSO_4$ (Hooda, 2011; Prajapati et al., 2013).

Preparation of radio-opaque raft formulation

As mentioned previously, the optimized raft formulation was prepared and made radio-opaque by adding 500 mg of $BaSO_4$ in the same formulation instead of metronidazole. Then, the formulation was homogenously mixed to form a homogenous dispersion and finally stored in containers (El-Mahrouk et al., 2016).

Radiographic examination of optimized raft formulation in the rabbit model

The experiment was reviewed, approved (Approval No.-IAEC/DU/164), and conformed with the guidelines of the IAEC (Institutional Animal Ethics Committee) for Animal experiments.

In vivo gel-forming activity was carried on through the X-ray method in young and healthy male albino rabbits (6–8 weeks, 2.0 - 2.2 kg). They were kept for one week in an animal house in spacious, labeled cages maintained in standard laboratory conditions at a temperature of 26 °C ± 2 °C and relative humidity of 44–56 % with 12 hours of light and dark cycle prior to the experiment acclimatizing them and were given food as well as water *ad libitum*. None of the animals had symptoms or a history of GI disease. The rabbits were kept in fast condition for 24 hours with free access to water prior to the commencement of the experiment, which maintained the constant GI motility. In this study, the first X-ray image of the rabbit was taken to ensure the absence of radio-opaque compounds in the GIT. The formulation prepared for radiography was orally administered to rabbits with the help of a gastric lavage tube. The rabbits were not allowed to eat during the experiment, but water *ad libitum* was provided.

In this model, 5 mL of the optimized formulation, instead of the drug, was orally administered to each rabbit. Radiographs were taken from the ventral portion of the rabbit at predetermined time intervals af-

ter dosing. The X-ray generating unit (Allengers Mars 3.5/4.2 Mobile X-ray machine) was set at 50 kv, 100 mAm, and 1 second. Radiographs were taken to assess gel formation and demonstrate gastric retention for an extended period (El-Mahrouk et al., 2016).

Statistical analysis

All the tests were expressed in triplicates, and the results are shown in mean ± standard deviation. All statistical analyses were performed in GraphPad Prism 7.04. Statistical significance within the variables was determined by employing a one-way ANOVA. The correlation between the cumulative percent *in vitro* drug release profile, raft strength, and viscosity was analyzed using Pearson's correlation (two-tailed test). The significance level was studied at $p < 0.05$.

RESULTS AND DISCUSSION

Drug-excipients compatibility study

Differential scanning calorimetric study

The DSC thermogram of metronidazole (Supplementary Figure 1) showed a sharp endothermic peak at 170.58 °C. When metronidazole was coated with Eudragit® NE 30D (Supplementary Figure 1), a sharp endothermic peak was observed at 168.76 °C, where a slight change was observed compared to the uncoated metronidazole peak in its melting point range. In sodium alginate, a broad endothermic peak appeared at 109.20 °C (Supplementary Figure 1), assigned to the loosely bound water release from the molecule. In Ca_2CO_3 (Supplementary Figure 1), the thermogram did not exhibit any endothermic or exothermic peak within 50-300 °C. The $NaHCO_3$ (Supplementary Figure 1) showed an endothermic peak at 165.23 °C (starting from 142.98 °C and ending at 216.76 °C), which may be characterized by the evaporation of adsorbed water from $NaHCO_3$.

The sharp endothermic peak of metronidazole and the broad endothermic peak of sodium alginate were slightly shifted (Supplementary Figure 1) at 167.43 °C and 112.17 °C in the physical mixtures of different excipients in the ratio of 1:1. The slight shift in the melting endotherm of metronidazole could be due to drug mixing with the contaminant existing in the excipients. The endothermic peak of each excipient, as well as the physical mixture of excipients with metronidazole, clearly depicts the integrity. It remained intact in crystalline form, confirming the absence of interaction between the metronidazole and excipients used in the raft formulation.

Fourier Transform Infrared (FT-IR) spectroscopy

The FT-IR spectra of metronidazole showed the typical characteristics peak [Supplementary Figure 2(A)] of aromatic -C-H stretching, aromatic -C-N-, aromatic -N-O- of NO_2 stretching, and -O-H stretching band at 3093 cm^{-1}, 1261 cm^{-1}, 1531 cm^{-1}, 1361 cm^{-1}(i.e., 3° -N-O- group is attached to an aromatic ring) and 3679 cm^{-1} in their respective position. All the characteristic bands of metronidazole were also present in the FT-IR spectrum of Eudragit® NE 30D coated as well as physical mixture [Supplementary Figure 2 (B&C)].

Physical characteristics of raft forming formulation

The formulations were liquid, which could be easily swallowable, rapidly transforming into a gel raft in the stomach. The following were the physical attributes of the developed raft-forming systems-

Measurement of raft strength, weight, volume, and raft density

After the liquid formulation was poured in 0.1M HCl (pH 1.2), it sank to the bottom of the medium and formed a gel, as illustrated in Figure 1.

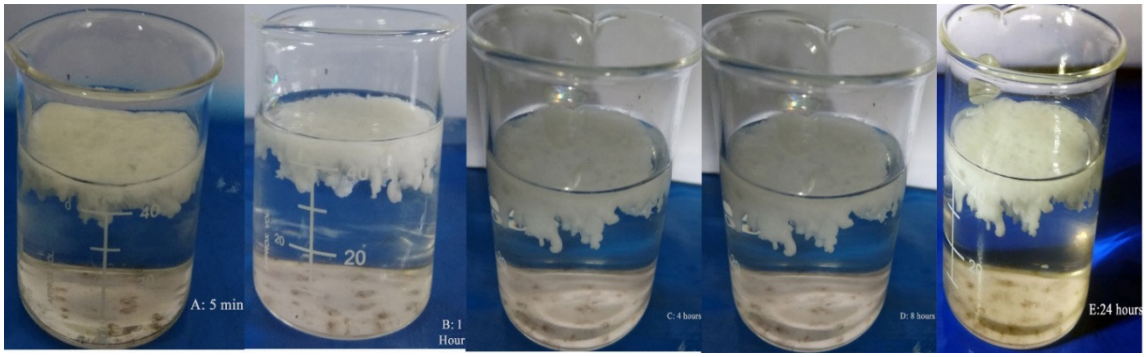

Figure 1. Floating behavior of the metronidazole-loaded raft liquid formulation.

The floating lag time and duration of floating are shown in Table 1. All the formulations were floated on the surface within 60 seconds. The formed *in situ* gel of all the raft formulations maintained floatation for more than 24 hours, encouraging further *in vivo* study. Figure 1 shows the floating behavior of the optimized metronidazole raft formulation at different time intervals in a rabbit stomach.

The raft strength and density of the formulation are presented in Table 2. The raft strength was found within the range of 2.48 ± 0.41 to 8.76 ± 0.13 g**.** The density was found within the range from 0.33 ± 0.03 to 0.93 ± 0.07 $g.cm^{-3}$.

Measurement of rheological properties of raft formulation

The flow behavior was studied according to Farrow's equation: Log D = N Log S- Log η, where, D implies shear rate (sec^{-1}), S implies shear stress (Pa), N is Farrow's constant, and η is the viscosity (Pa. s). The flow index (N) is characteristic for each formulation, as N = 1 indicates Newtonian behavior, while N is less than one (N < 1), indicating a thickening shear rate. If N is greater than one (N > 1) indicates a thinning shear rate (Table 3) (Abouelatta et al., 2018) (The viscosity shear rate profiles of the metronidazole raft forming optimized formulation are illustrated in Supplementary Figure 3).

In vitro drug release study

The metronidazole release profiles of raft formulation are shown in Figure 2 (A, B). The *in vitro* drug releases were 87.53 ± 2.87%, 84.37 ± 1.04%, 95.96 ± 0.01%, 90.27 ± 1.93 %, 81.83 ± 0.54%, 84.78 ± 0.98%, 88.57 ± 1.81% and 92.24 ± 0.94% from F-1, F-2, F-3, F-4, F-5, F-6, F-7 and F-8 formulation respectively after 8 hours in 0.1M HCl medium. The 8-hour release values are mentioned considering the half-life of Metronidazole.

Table 1. Physical characteristics of the batches of raft forming formulation

Formulation Code	Floating lag time (in Seconds)	Duration of floating (in Hours)	Raft strength (g)	Raft weight (g)	Raft volume (mL)	Density ($g.mL^{-1}$)	Buoyancy index
F-1	7	>24	4.05 ± 0.43	5.16 ± 0.11	6.96±0.14	0.74±0.026	0.34±0.10
F-2	7	>24	6.56 ± 0.16	2.32±0.17	5.81±0.31	0.394±0.055	1.50±0.22
F-3	5	>24	3.97 ± 0.56	1.89±0.17	5.69±0.13	0.33±0.032	1.86±0.12
F-4	6	>24	6.65 ± 0.31	3.18±0.24	5.20±0.43	0.61±0.035	0.67±0.06
F-5	16	>24	3.74 ± 0.23	6.04±0.27	6.44±0.22	0.93±0.070	0.16±0.07
F-6	56	>24	8.76 ± 0.13	6.63±0.15	12.24±0.08	0.54±0.028	0.84±0.13
F-7	45	>24	5.98 ± 0.22	5.53±0.12	9.73±0.12	0.56±0.030	0.61±0.26
F-8	6	>24	2.84 ± 0.41	2.02±0.19	5.29±0.24	0.38±0.041	1.47±0.35

Table represent mean ± SD, n=3

Table 2. Composition of raft formulations

Composition of Raft	Formulation Code and quantity in percentage (W/V)							
	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8
Sodium alginate	3	1	1	1	3	3	3	1
Sodium bicarbonate	0.5	0.5	1	1	1	1	0.5	0.5
Calcium carbonate	0.5	3	0.5	3	0.5	3	3	0.5
Sodium citrate	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Coated API	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Table 3. Rheological properties of the batches of raft formulation

Formulation Code	Viscosity of sols (Pa. s)	N (Furrow's Constant of sols)	Flow behavior	Zeta Potential (milli volts)
F-1	0.0175±0.001	1.001	Thixotropic	45.7
F-2	0.0148±0.015	1.021	Thixotropic	43.2
F-3	0.003±0.001	1.01	Thixotropic	38.2
F-4	0.003±0.001	1.01	Thixotropic	43.1
F-5	0.231±0.37	1.06	Thixotropic	41.0
F-6	0.021±0.001	1.025	Thixotropic	45.7
F-7	0.024±0.001	1.010	Thixotropic	46.4
F-8	0.003±0.006	1.0101	Thixotropic	43.0

Table 4 - Correlation coefficient (r^2) and diffusional exponent (n) of raft forming formulation

Formulation Code	Drug release kinetic correlation coefficients (r^2)				Release exponent (n)
	Zero order	First order	Higuchi	Korsmeyer–Peppas	
	r^2	r^2	r^2	r^2	
F-1	0.6665	0.9462	0.8903	0.141	0.663
F-2	0.7082	0.9727	0.8939	0.083	0.45
F-3	0.5616	0.7496	0.9603	0.707	0.674
F-4	0.6037	0.9157	0.9497	0.727	0.811
F-5	0.7354	0.9715	0.8752	0.763	0.781
F-6	0.7339	0.9755	0.8436	0.172	0.581
F-7	0.6532	0.9141	0.9188	0.808	0.45
F-8	0.5824	0.8989	0.9378	0.099	0.543

DISCUSSION

The principal prerequisites of raft formulations are optimum viscosity of the prepared liquid sol, gelling, and floating capacities. The formulation should have an optimal viscosity that will allow easy swallowing as a liquid dosage form. Thus, it undergoes a rapid sol-gel transition and floats owing to the ionic reaction. Furthermore, the *in situ* formed gel should preserve its integrity without dissolving or eroding. The viscous raft formulation is being floated in the stomach for a prolonged period to locally encourage a sustained release of medicament. Eudragit NE 30 D, a copolymer of methyl acrylate, enables the pH-dependent release of metronidazole through salt formation. Metronidazole coated with Eudragit NE 30 D forms a thin film in the raft formulation, is insoluble in the GI tract, has extremely low permeability, and exhibits pH-independent swelling (Ahmed et al., 2020; Thakral et al., 2012). The sodium alginate can form a floating gel mass or raft in the stomach, an essential ingredient of anti-reflux products such as Gaviscon Advance, Gastrocote, Gaviscon Liquid, Paptac, and Rennie Duoa, etc. intended to work by a barrier action. It has also imparted mucoadhesive properties in the formulation (Hampson et al., 2005). Also, $NaHCO_3$ and $CaCO_3$ have an antacid function, so the formulation has an acid-neutralizing capacity (Shakya et al., 2013).

Sol-to-gel transformation of sodium alginate occurs due to either monovalent or divalent cations in the acidic pH (Siew et al., 2005). Sodium alginate

is freely dissociated into alginate and Na^+ ion at an aqueous media and forms double helices at room temperature when the solution has a viscosity near that of water. The helices are weakly connected by the Van der Waals attraction force. When gel-promoting cations are present, some helices associate with cation-mediated aggregates, which cross-link the polymer. For instance, the divalent ions, calcium, are superior to monovalent cations in encouraging the gelation of the polysaccharide (Tang et al., 1997). This is the result of the internal ionotropic gelation effect of Ca^{2+} on the polymer used (Choi et al., 2002). In the present work, $CaCO_3$ was used as a source of Ca^{2+} and a gas-generating ingredient. While insoluble in water, $CaCO_3$ dissolves in acidic media (pH 1.2- 3.5). When the formulation is in contact with the acidic medium of the stomach, it produces CO_2, which may get entrapped and protected within the gel layer formed by cross-linking with the polymeric alginate chain in an acidic medium. The Ca^{2+} interaction with alginate can create a firm and strong gel network that can entrap the CO_2 more efficiently (Jiang et al., 2015). Simultaneously, Na^+ from sodium alginate is exchanged with divalent Ca^{2+}, which can lead to a low-viscosity solution to a gel by forming an egg-box structure. This interaction would be essential to create a strong, coherent raft in the post-prandial stomach weakly acidic medium. $NaHCO_3$ was used to avoid any internal ionotropic gelation effect of calcium on alginates during storage (Poncelet et al., 1999). Various literature has reported that the amount of $NaHCO_3$ and $CaCO_3$ are directly related to the floating lag time. If the amount of $NaHCO_3$ and $CaCO_3$ is increased, floating lag time decreases due to the production of a significant amount of CO_2. Moreover, increasing the amount of alginate resulted in increased weight, which then requires a prolonged period to achieve a density below that of the gastric fluids and float. In contrast, $NaHCO_3$ and $CaCO_3$ reduced the floating lag time owing to the increase in CO_2 produced by an increased amount of $NaHCO_3$, which resulted in a shorter time to reach a suitable density. At the same time, the drug loading did not affect the floating ability.

Thus, sodium citrate (0.17% w/v) was added to the formulation as a chelating agent; it complexes with the free Ca^{2+} ions. It prevents premature gelation, which may occur during storage, and only releases it in the stomach's acidic environment (Abou Youssef et al., 2015; Abouelatta et al., 2018). The formulation thus remains in the liquid phase until it reaches the stomach, where gelation is instantaneous. Optimum quantities of $CaCO_3$ that guaranteed fluidity during storage, then immediate gelation and floatation, were determined by preliminary tests.

The density of a raft formulation was less than the density of gastric content (~1.0597 $g.mL^{-1}$) (Table 2). This result confirmed that the raft was buoyant in the GI fluid and remained withheld in the stomach, facilitating the local release of medicament. The buoyancy of the raft can be anticipated to enhance the effectiveness of the product in resisting reflux since a more buoyant raft would be more likely to displace corrosive gastric content in the upper part of the stomach, and it would also be less likely to be emptied along with the meal. The buoyancy of the raft was generated by the entanglement of CO_2 in the gel network. Additionally, the gel elevated and retained buoyant on the surface of the medium. The floating raft was favorable as it behaved as a hindrance to avert gastric fluid reflux into the esophagus.

Different alginate concentrations disturbed the raft density. The raft density rises with the enhancement of the alginate content. However, all raft formulations could form a gel and float in an acidic medium. The amount of CO_2 did not affect the gelled raft density, although the weight of $CaCO_3$ raised the raft volume.

The raft formulations showed thixotropic due to the sodium alginate solution exhibiting a shear-thinning behavior or pseudoplastic flow of the viscosity resulting in an inverse proportion to the shear rate. The viscosity of the formulation was enhanced with the increment in the alginate concentration. On the other hand, the amount of metronidazole did not affect the viscosity of the formulation.

The raft formulations exhibited raft strengths within the same range as commercial antacid products (between 1.1 to 16.5 g) (Hampson et al., 2005). Moreover, it was noticed that raft was present in the rabbit's stomach more than 7 hours after taking the X-ray through oral feeding (Figure 3). It indicates that the developed formulation could resist GI peristalsis. Thus, a formulation with the highest strength (F6, F7) resists breakup for the longest time, whereas a formulation with low raft strength (<5 g) has little or no resistance to raft breakup in the resilience test.

The capability to retain the raft within the gastric cavity constituted the exclusive attribute, in addition to the necessity for it to withstand peristaltic contractions. The force (g) exerted for extracting the probe transpires through the strength of the gel raft. Consequently, the robustness of the raft is contingent upon the concentrations of sodium alginate and $CaCO_3$. This investigation has elucidated that an escalation in the concentration of sodium alginate and $CaCO_3$ results in enhanced raft integrity, attributable to an increase in its density as well as augmented cross-linking interactions among adjacent alginate chains.

***In vitro* drug release study**

The impacts of all components in the raft-forming formulations on metronidazole release are represented in Figure 2 (A, B). Coating metronidazole with Eudragit® NE 30D played a key role in the release from the raft formulation. The *in vitro* drug release pattern could be partitioned into two steps. The initial step involved a burst release, likely due to incomplete gelation and rapid drug release upon contact with 0.1M HCl (pH 1.2). Radiological observation revealed that the burst release effect was not due to raft damage (Figure 3). After the raft was formed entirely, the residual metronidazole was limited and gradually released from the gel network, representing the second phase; the drug was released moderately in this phase.

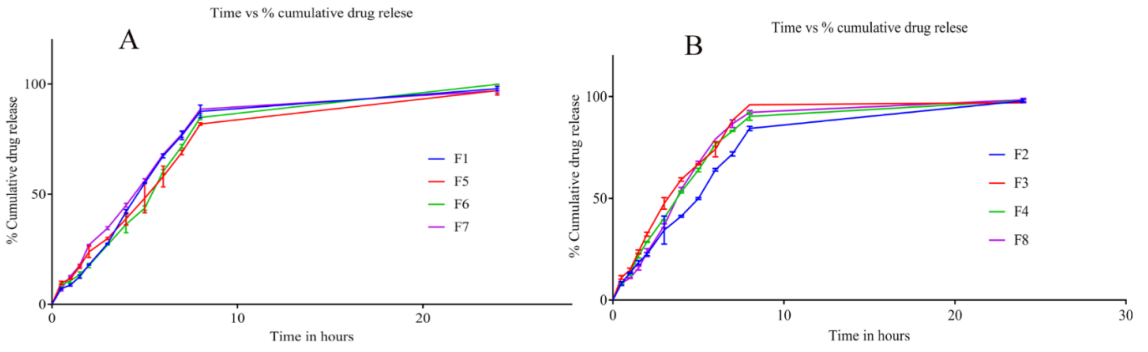

Figure 2. (A) Effect of Sodium alginate concentration on the *in vitro* metronidazole release of the raft forming systems incorporating Eudragit NE30D coated metronidazole; (B) Effect of $CaCO_3$ concentration on the *in vitro* metronidazole release of the raft forming systems incorporating Eudragit NE 30D coated metronidazole. Bars represent mean ± SD (n = 3)

The amount of metronidazole released at 30 and 60 minutes did not differ significantly at varying concentrations of alginate. This suggests that the drug was not effectively trapped within the gel network and was released into the media independently of the polymer matrix control.

Nevertheless, the rate of metronidazole release after a duration of 60 minutes exhibited a pronounced reduction with an elevation in alginate concentration. The increased concentration of alginate augmented the density of the polymer matrix and contributed to the formation of a more viscous gel raft. Conse-

quently, the drug was subjected to an extended path length and time for diffusion as a result of the gel network's properties. The quantity of $CaCO_3$ exerted a minor influence on the drug release characteristics of the raft formulations. Nonetheless, the presence of Ca^{2+} facilitated the formation of a more robust raft in conjunction with sodium alginate, while the CO_2 gas generated within the gel network resulted in a gel of enhanced permeability, which in turn improved the release rate. As a result, the elevated concentration of $CaCO_3$ yielded a greater quantity of Ca^{2+} and produced increased amounts of CO_2, which collectively exerted a negligible overall impact on the drug release profile.

***In vitro* drug release kinetics**

The drug loading in the formulation did not significantly affect the *in vitro* release profile of the raft formulation. The release profile was analyzed to identify the most appropriate mechanisms for elucidating the release characteristics by utilizing linear regression analysis alongside various kinetic models. The model exhibiting the highest coefficient of determination (i.e., r^2 approaching 1) was deemed the most suitable kinetic model. The Korsmeyer-Peppas model parameter 'n' represents the release exponent linked to the drug release mechanism. The raft formulation adhered to first-order release kinetics (Table 4). The release exponents (n) for the formulations ranged from 0.45 to 0.81, indicating that the metronidazole release from the formulation occurs via a combination of diffusion and erosion mechanisms (i.e., a non-Fickian diffusion mechanism where $0.45 < n < 0.89$) (Das et al., 2010; Dhar et al., 2020).

Assortment of optimized formulation

The chosen optimized formulation was based on the significant characteristics ($p < 0.05$, general appearance, raft strength, viscosity, and *in vitro* drug release profile) of the raft formulation. Among the parameters mentioned above, the F-5 formulation was found to be a suitable formulation in all aspects ($p < 0.05$). The strength of the raft was found to be 3.74 ± 0.23g, which assured that the raft has adequate mechanical strength as well as constant drug release from the gel network. The viscosity of the formulation was 0.93 ± 0.070 Pa.s, which was less than GI fluid. The floating lag time was less than 60 seconds, ensuring that the formulation floated immediately after oral administration (Figure 1). The *in vitro* drug release was 81.83 ± 0.54 % after 8 hours in 0.1M HCl.

***In vivo* gel-forming activity in rabbit stomach using Roentgenography**

Figure 3 demonstrates the appearance of gel formation in rabbit stomach after oral administration of $BaSO_4$ disperses optimized formulation. The presence of $BaSO_4$ in the stomach confirms the GI retentive property of the drug-free optimized formulation. The amount of gel was reduced over time, as shown in Figure 3. The findings indicated a gradual degradation of the synthesized gel, thereby underscoring the enhanced gel integrity of the optimized formulation.

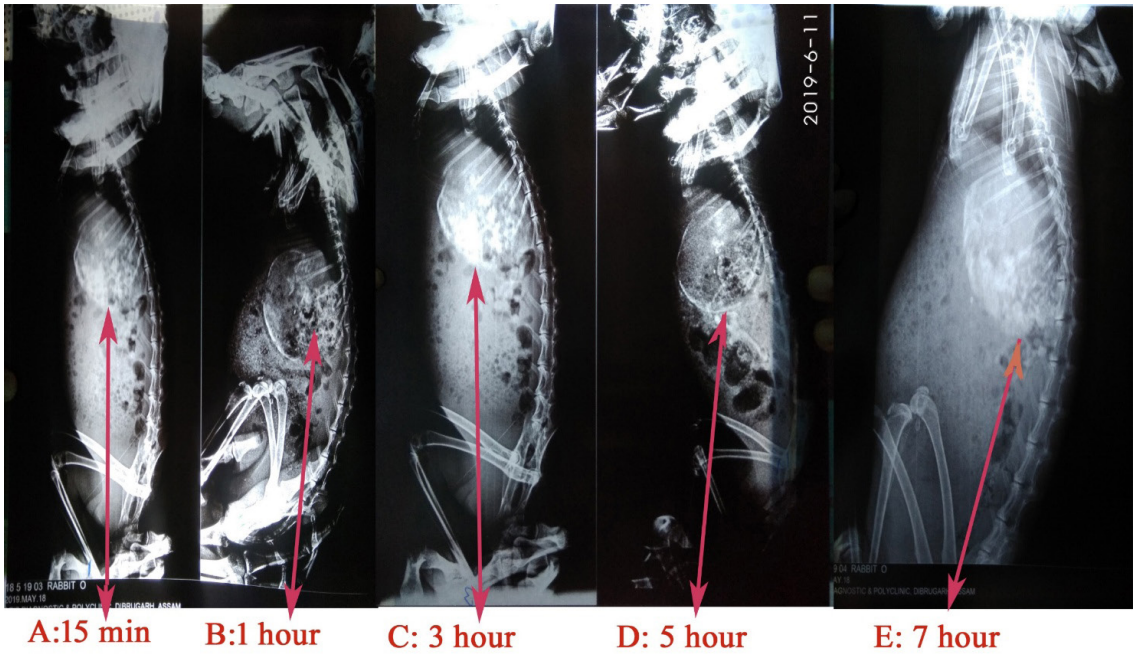

Figure 3. X-ray images of rabbit stomach at different time intervals after oral admiration raft liquid

CONCLUSION

This study successfully formulated a gastro retentive raft-forming formulation incorporating metronidazole. The optimized F-5 formulation contains 3% w/v of sodium alginate, 0.5% w/v of $CaCO_3$, and 1% w/v of $NaHCO_3$. The formulation was characterized by having an optimum viscosity that will permit smooth ingestion as a liquid dosage form, which undergoes a rapid sol-gel transition and floating due to ionic interaction. Excellent floating behavior and controlled release profile for more than 8 hours were observed.

The drug, metronidazole, was released from the dosage form slowly in the stomach, significantly extending its residence time, which may facilitate its direct antibacterial action and consequently enhance the healing of chronic gastric ulcers and stimulate mucosal regeneration in the affected area. Furthermore, the administration interval for metronidazole would be minimized, resulting in enhanced efficacy compared to conventional oral dosage formulations. Therefore, the proposed raft formulation would be a promising site-specifying dosage form for treating peptic ulcers caused by *H. pylori*. Also, it could be suitable for geriatrics who find difficulty swallowing other solid dosage forms of metronidazole, thus improving efficacy due to enhancing patient compliance. Moreover, the thick barrier of raft formulation on top of the GI fluid may prevent or minimize gastroesophageal reflux disease symptoms. This study has illustrated the potential utility of a novel raft liquid formulation for stomach-specific delivery.

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AUTHOR CONTRIBUTION STATEMENT

We declare that this study was done by the authors named in this article. HS & HKS were involved in the conceptualization; TJ& HS were contributed to the methodology, involved in the formal analysis, and investigation; HS & AKG contributed to writing original draft preparation and writing, review and editing; HKS was involved in the supervision, contributed to the project administration and funding acquisition. All the authors read and approved the final manuscript.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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