EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 1
289-295
Year: 2022

FORMULATION AND EVALUATION OF FLOATING *IN-SITU* GEL CONTAINING METRONIDAZOLE

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Article Received on 26/10/2021 **Article Revised on 16/11/2021** **Article Accepted on 05/12/2021**

ABSTRACT
The aim of the present study was to formulate and evaluate a gastro-retentive oral *in-situ* gelling system of metronidazole. Sodium alginate based metronidazole floating *in-situ* gelling systems were prepared by dissolving sodium alginate in distilled water, to which varying concentrations of viscosity enhancing polymer (Ethyl cellulose), drug, and gas-forming agent(s) as calcium carbonate/and sodium bicarbonate were added and dissolved by stirring. Prepared formulae were evaluated for viscosity, floating behavior, drug content and *in-vitro* drug release behavior. Formulation variables such as the type and concentration of viscosity enhancing polymer, the concentration of gas-forming agents affected the formulation viscosity, floating behavior and *in-vitro* drug release. The prepared *in-situ* gelling formulations of metronidazole could float in the gastric conditions and release the drug in controlled manner. The prepared formulations appear to be promising drug delivery system for localized delivery of metronidazole for better treatment of peptic ulcer disease caused by H. pylori.

KEYWORDS: Peptic ulcer, Gastro-retention, *In-situ* gel, Sodium alginate, Metronidazole.

INTRODUCTION
Metronidazole, a nitro imidazole, exerts antibacterial effects in an anaerobic environment against most obligate anaerobes. Once metronidazole enters the organism by passive diffusion and gets activated in the cytoplasm of susceptible anaerobic bacteria, reduced of metronidazole takes place; this process includes intra-cellular electron transport protein such as ferredoxin, transfer of an electron to the nitro group of the metronidazole, and formation of a short-lived nitroso free radical. Because of this alteration of the metronidazole molecule, a concentration gradient is created and maintained which promotes the drug's intracellular transport. The reduced form of metronidazole and free radicals can interact with DNA leading to inhibition of DNA synthesis and DNA degradation leading to death of bacteria.[1]

Helicobacter Pylori (H. Pylori) is one of the most common pathogenic bacterial infections. It is associated with the development of serious gastroduodenal disease, including peptic ulcers, gastric lymphoma, and acute chronic gastritis. H. Pylori reside mainly in the gastric mucosa or at the interface between the mucous layer and the epithelial cells of the antral region of the stomach. Antibiotics required for eradication of H. Pylori are high in dose and in more frequencies. This is because of the low concentration of the antibiotic reaching the bacteria under the mucosa, instability of the drug in the low pH of gastric fluid, and short residence time of the antibiotic in the stomach, leading to incomplete eradication of H. Pylori.[2]

Floating Drug Delivery System (FDDS) is one of the novel systems of drug delivery. Various dosage forms are formulated in the form gastro retentive floating systems such as microspheres, micro beads, tablets, capsules, films etc. In-situ gelling system is a new trend in FDDS. In-situ gelling system have its application in different routes of administration like oral, nasal, ophthalmic, per-oral, rectal, vaginal and also parenteral route. Gastro retentive FDDS have bulk density lower than gastric fluid and hence remain buoyant in stomach without affecting the gastric emptying rate for a long period of time. While the system is floating on the stomach, the drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from the stomach besides a minimal gastric content needed to allow the proper achievement of the buoyancy retention principle, a minimal level of floating force (F) is also required to keep the dosage form reliably buoyant on the surface of the meal. To measure the floating force kinetics, a novel apparatus for determination of resultant weight has been in the literature. The apparatus operates by measuring continuously the force equivalent to F (as a function of

time) that is required to main submerged object. The object floats better if F is on the higher positive side. This apparatus helps in optimizing FDDS with respect to stability and durability of floating forces produced in order to prevent the drawbacks of unforeseeable intragastric buoyancy capability variations.[3]

MATERIAL AND METHOD

Materials

Metronidazole(Strides Arcolabs Ltd, Bangalore), Sodium Alginate, Ethyl Cellulose, Calcium Carbonate, Sodium Bicarbonate (SD Fine Chemical Ltd, Mumbai), all other chemicals used were of laboratory reagent grade (LR Grade).

Methods

Quantitative Estimation of Metronidazole by UV-Spectrometric Method

Determination of Absorption Maxima

Metronidazole 20 µg/ml concentration was prepared in 0.1 N Hcl. The solution was scanned from 400nm to 200nm by UV spectrophotometer and a spectrum was observed for absorption maxima.

Standard calibration curve of Metronidazole

Calibration curve was constructed in 0.1N Hcl. 100 mg of accurately weighed Metronidazole was dissolved in 0.1N Hcl and the volume is made up to 100 ml with distilled water and filtered using Watmann's filter paper and the solution was used as standard stock solution.

- 1 ml of the above solution was diluted to 100 ml using 0.1 N Hcl and used as working stock solution.
- From the above solution 4, 8, 12, 16 and 20µg/ml UV spectrophotometer at 277nm.
- Calibration curve was plotted between concentration and absorbance and r^2 value of this graph was calculated to check the linearity of the absorbance against concentration.

Preparation of Floating Oral *In-Situ* Gel Containing Metronidazole

Floating *in-situ* gel formulations of metronidazole were prepared using compositions given in Table 1. In around 75% water, a measured quantity of sodium alginate (SA) 2 % (w/v) solution was dissolved in distilled water at 60°C using a heating magnetic stirrer. After cooling to below 40°C, appropriate amounts of polymer, methyl paraben and propyl paraben (ratio of 9:1), Metronidazole(MTZ), along with gas generating agent (calcium carbonate with or without sodium bicarbonate) were dissolved/ dispersed uniformly into the sodium alginate solution with continuous stirring. The stirring was continued after complete addition until a uniform dispersion was obtained and the dispersion was allowed to cool at room temperature. Finally, the volume was adjusted to 100% with distilled water and the mixture was mixed well to get the final preparation.[4]

Table 1: Composition of Metronidazole Floating Oral *In- Situ* Gel Formulations.

Formulation code	Drug (mg)	EC (%w/v)	$CaCO_3$ (%w/v)	$NaHCO_3$ (%w/v)	Sodium Alginate (%w/v)
F1	250	0.5	0.5	0.0	0.2
F2	250	1.0	1.0	0.5	0.2
F3	250	1.5	1.5	1.0	0.2
F4	250	2.0	2.0	1.5	0.2

EVALUATION OF PREPARED FORMULATIONS

Determination of drug content

Accurately, 10 mL of formulation (containing the equivalent of 250 mg metronidazole) from different batches was measured and transferred to 100 mL volumetric flask. To this 50-70 mL of 0.1 N HCl was added and sonicated for 30 min. Volume was adjusted to 100 mL. Complete dispersion of contents was ensured visually and the dispersion was filtered using Whatman Filter Paper. From this solution, 10 mL of sample was withdrawn and diluted to 100 mL with 0.1 N HCl. Contents of metronidazole was measured at maximum absorbance at 278 nm using UV-Visible Spectrophotometer at (Shimadzu UV-1800, Japan)[4]

pH Measurement

The pH of the prepared formulations was measured using a calibrated digital pH meter.[5]

In-vitro gelation study

To evaluate the formulations for their *in-vitro* gelling capacity, accurately measured 10 mL of formulation was added to 100 mL of 0.1N hydrochloric acid (HCl, pH 1.2) at 37° C in a beaker with mild agitation that avoids breaking of formed gel. The *In-vitro* gelling capacity was graded in three categories on the basis of stiffness of formed gel, gelation time and time period for which the formed gel remains as such.

(*) Gels after few minutes, dispersed rapidly.

(**) Gelation immediate remains for few hours.

(***) Gelation immediate remains for an extended period.[6]

Measurement of viscosity of *in-situ* gelling system

Viscosity of the dispersion was determined using a Brookfield digital viscometer. The samples (200 mL) were sheared at a rate of 100 rpm/min using spindle number 2 at room temperature. Viscosity measurement for each sample was done in triplicate, with each measurement taking approximately 30 seconds.[7]

In-vitro floating study

The *in-vitro* floating study was carried out by introducing 10 mL of formulation into a beaker

containing 100 ml of 0.1N HCl, (pH 1.2) at 37^0 C without much disturbance. The time the formulation took to emerge on the medium surface (floating lag time) and the time the formulation constantly floated on surface of the dissolution medium (duration of floating) were recorded.[8]

***In-vitro* drug release study**

The dissolution studies were performed in triplicate using a type II (paddle method) dissolution apparatus. The dissolution medium used was 900 ml of 0.1 N HCl (pH 1.2), maintained at 37 ºC. The stirring rate was adjusted to 50 rpm. This speed was believed to simulate the in vivo existing mild agitation and was slow enough to avoid the breaking of gelled formulation. At predetermined time intervals, 10 mL samples were withdrawn and replaced by fresh dissolution medium, filtered through Whatman filter paper, diluted, and assayed at maximum absorbance at 277 nm using UV-Visible Spectrophotometer.[9]

RESULTS AND DISCUSSION

Determination of λ max of Metronidazole

The λ max of the Metronidazole in 0.1N HCl was found to be 277nm.

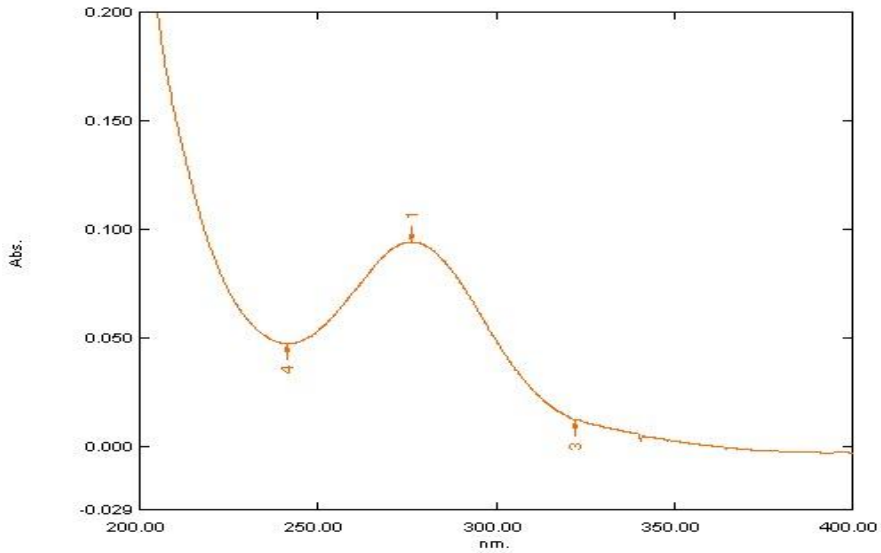

Fig 1: Absorption Maxima of Metronidazole in 0.1N HCl.

Standard calibration curve of Metronidazole

The absorbance of Metronidazole standard solutions containing 0-20 µg/ml of drug in 0.1N HCl was obtained. Calibration curve with regression value of 0.999 and slope 0.0263 was found. The curve was found to be linear in the range of 0-20 µg/ml at λ max 277nm.

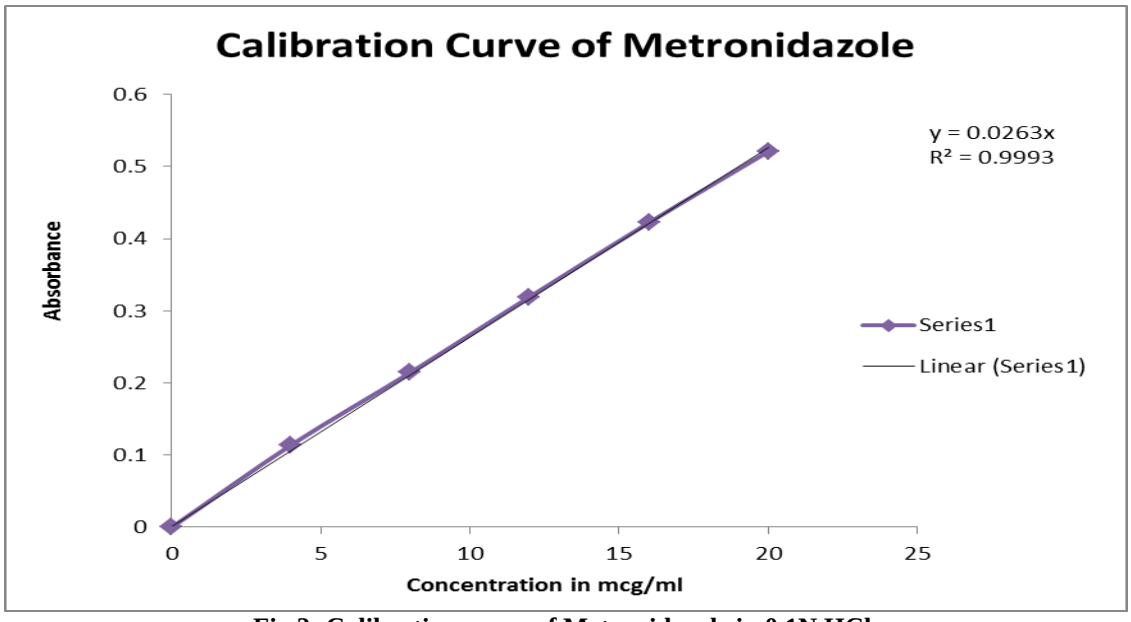

Fig 2: Calibration curve of Metronidazole in 0.1N HCl.

Comparison of Formulation FI to F4

In this study, four formulations of sodium alginate based floating oral *in-situ* gelling system of metronidazole were prepared using sodium alginate as release-retarding gel-forming polymer. Viscosity enhancing polymers (EC) was added to sodium alginate solution in an attempt to improve viscosity and to obtain slower drug release than those formulations containing sodium alginate alone. Calcium carbonate was used as a source of calcium ions and as a gas generating agent, it was used in different concentrations to determine its optimum concentration; in addition, sodium bicarbonate, also used in different concentrations, was included in some formula as a formed gel, gelation time and time period for which the formed gel remains as such.

Table 2: Comparison of *in-vitro* dissolution trials.

Time (Hrs)	% Cumulative Drug Release			
	F1	F2	F3	F4
0	0.0	0.0	0.0	0.0
1	34.62	32.69	31.25	26.92
2	37.16	34.74	33.29	30.87
3	40.67	38.73	36.79	33.40
4	42.89	40.93	39.95	36.04
5	47.67	43.90	44.34	38.93
6	50.85	48.87	48.31	42.77
7	56.41	54.01	52.39	46.62
8	64.69	60.31	58.52	51.81

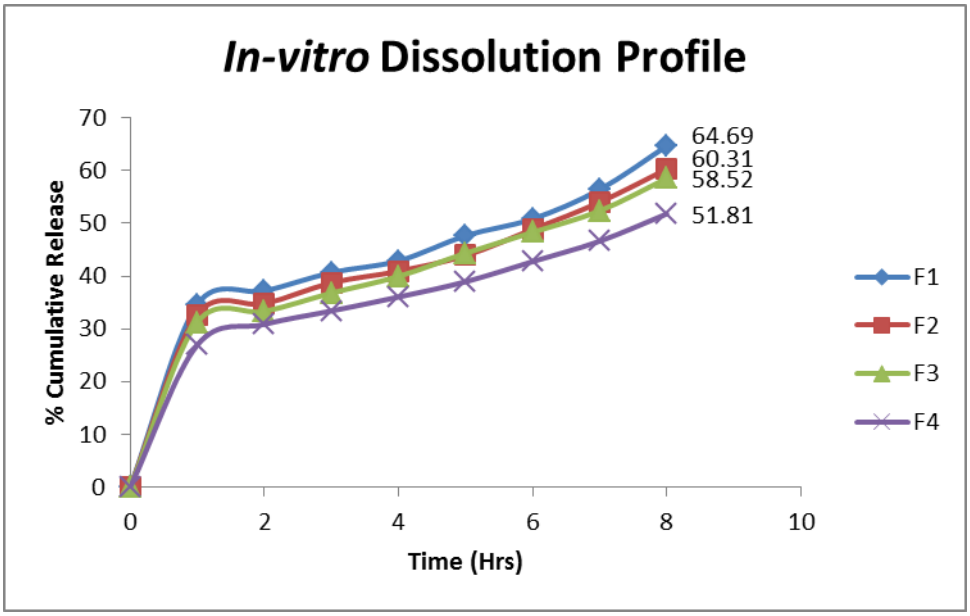

Fig. 3: Comparison of *in-vitro* dissolution profiles of F1 to F4.

***In-situ* gelling formation of metronidazole**

Table 3: Properties of *in-situ* gelling formulations.

Formulation code	Drug Content (%)	pH	Graded gel response	Floating lag time(min)	Duration of floating(hr.)
F1	86.35	7.6	***	< 1	> 12
F2	82.74	7.4	***	< 1	> 12
F3	82.34	7.2	***	< 1	> 12
F4	81.44	7.3	***	< 1	> 12

Drug content

The percent drug content for all formulations was determined and is shown in Table 2. The drug content was found to be in the range of 81-86% for all the formulations indicating uniform distribution of drug. The formulation F1 shows the maximum drug content. (Fig 4)

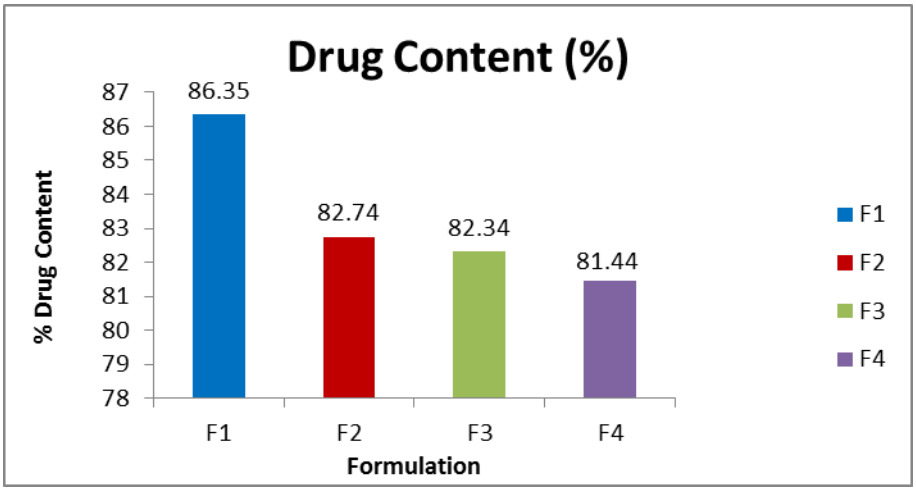

Fig 4: Percent Drug Content of Formulation F1 to F4.

PH Measurement

Measurement of pH is very important for oral preparations; otherwise, it leads to irritation to the throat. All the formulation has a pH around neutral or slightly alkali. The pH of formulations was found in the range of 7.2 - 7.6 as shown in Table 3.

***In-vitro* gelation study**

Gelling studies were carried out using 0.1N HCl (pH 1.2) and the obtained data were represented in Table 3. All formulations showed immediate gelation upon contact with acidic medium and the formed gel preserved their integrity. Gelation occurs when the insoluble calcium carbonate solubilizes when it comes in contact with acidic medium releasing carbon dioxide and calcium ions. The calcium ions interact with the anionic polymer (sodium alginate) in the formulation causing instantaneous gelation and provide a gel barrier that restricts drug release. Formulations containing calcium carbonate alone produce stiffer floating *in-situ* gels than those containing $CaCO_3$ and $NaHCO_3$. This is due to the internal ionotropic gelation effect of calcium on sodium alginate. In comparison, increasing the amounts of sodium bicarbonates in the formulations reduced gel integrity and produced gels with loose structural appearance.

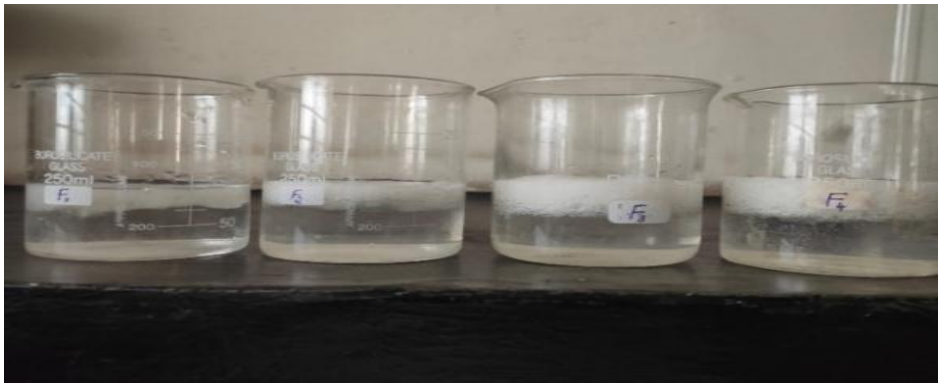

Fig 5: Photograph taken during the *in-vitro* gelation study.

***In-vitro* floating study**

The formulated floating *in-situ* gelling system of metronidazole employed $NaHCO_3$ or $CaCO_3$, regarding the floating lag time, it was observed that formulation containing $NaHCO_3$ as a gas-generating agent, the *in-vitro* floating test revealed the ability of all formulations to maintain buoyant for more than 12 hr. (Table 3 and Figure 6).

Regarding the floating lag time, it was observed that formulation containing Sodium bicarbonate had instantaneous floating behavior and had significantly shorter ($p < 0.05$) floating lag times than formulation containing $CaCO_3$ alone as a gas-generating agent. The basic mechanism behind floating was because calcium carbonate solubilized and effervesced upon contact with acidic medium, releasing calcium ions and carbon dioxide (CO_2). The evolved CO_2 gas was entrapped in the gel causing floating. Incorporation of sodium bicarbonates improves floating behavior by providing an additional source for CO_2 gas generation.

The observed behavior suggests that the gel formed by the combination of sodium alginate with the investigated polymers, enabled efficient entrapment of CO_2 gas producing a buoyant preparation with shorter floating lag time which can retain in the stomach for a longer time period and assist controlled released of the drug.

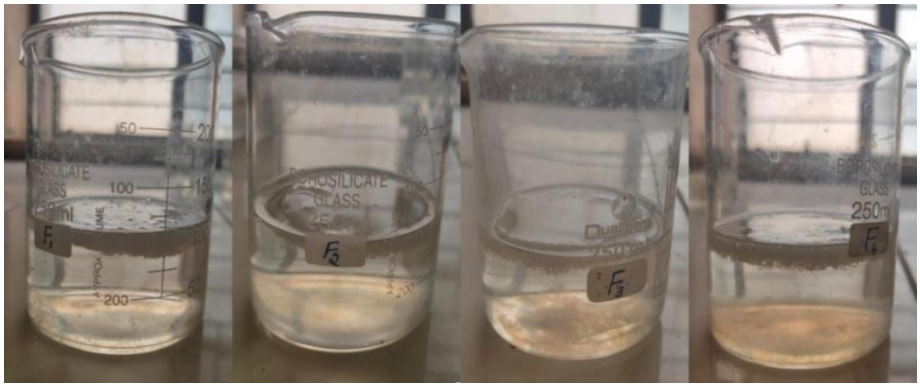

Fig 6: Photograph taken during *in-vitro* floating study for 12 hours.

***In-vitro* drug release study**

The *in-vitro* release study of metronidazole from all four formulations in 0.1N HCl was conducted for period of 8 hours and results were shown in figure 7. The highest drug release was observed with formulation F1 and lowest drug release was observed with formula F4.

The influence of using different concentration of viscosity enhancing polymer (EC) with Sodium alginate on *in-vitro* drug release is shown in figure. The higher viscosity of EC, it promote the formulation of highly viscous gels upon contact with aqueous fluids which will produce more retardation in drug release rate.

Besides the polymer type, the polymer concentration can control the drug release. In the formulation F4 containing 2.0% of EC release about 51.80% in 8 hours compared to 64.69% release seen with formulation F1 containing 0.5% of EC. It can be concluded that an increase in concentration of viscosity enhancing polymer resulted in decrease cumulative drug release, this is a reflection of increased gel strength seen when using higher polymeric concentrations due to more available polymeric chains for crosslinking with the calcium ion.

Calcium carbonate (0.5–2 %) was used as a gas generating agent and as a source of cations for gelation in the formulation. Using a concentration of 0.5 % $CaCO_3$ produced desired floating duration but higher concentrations were used in an attempt to have more retarded drug release.

In F4 concentration of 2 % had slower drug release profiles than the F1 containing lower percentage $CaCO_3$. This indicates that the drug release decreased as the concentration of calcium carbonate in the formulation was increased. Such behavior may be attributed to the fact that as the concentration of calcium ions increases, cross-linking also increases leading to formation of a stronger gel, which results in more restricted and slower drug release.

Regarding the effect of sodium bicarbonate on drug release, comparing the drug release profiles of formulations containing sodium bicarbonate (F1 to F4) to formulations without Sodium bicarbonate (F1), a proportional increase in drug release profile can be observed with increasing amounts of sodium bicarbonate.

The reason for the increase in drug release when using higher amounts of sodium bicarbonate may be because of weaker gelation properties occurring with the presence of sodium ions in the formulation compared to stronger gelation effect produced in the presence of calcium ions.

Fig 7: Photograph taken during *in-vitro* drug release study.

SUMMARY

Metronidazole is an effective antiprotozoal and antibacterial drug, and is commonly used in conjunction with either amoxicillin or clarithromycin and an acid suppressor (usually a proton pump inhibitor) or an H2-receptor antagonist (E.g., ranitidine) to eradicate *H. Pylori* infection.

The present study was aimed to develop prolong gastric residence time of metronidazole and therefore to improve its local effects in the stomach and achieve better eradication of *H. Pylori*.

The various formulations containing ethyl cellulose (EC) polymer in different concentration is prepared. The prepared *in-situ* floating, gel gelation and floating study was determined by *in-vitro* gelation study and *in-vitro* floating study.

The result illustrated that the floating oral *in-situ* gel formed by F4 formulation containing $NaHCO_3$ (2%) remains longer period in stomach, resulting in an increase gastric residence time and thus increases local concentration of the drug for complete eradication of *H. Pylori*.

CONCLUSION

From the results it can be concluded that modifying parameters like the type and concentration of viscosity enhancing polymer, concentration of gas generating agent, the release can be modulated to the desired rate.

By observing various evaluation parameters for the studied formulations, it can be stated that incorporation of sodium bicarbonate in an appropriate amount was able to shorten the floating lag time and variation in concentration of calcium carbonate influences viscosity and drug release behavior from *in-situ* gel.

The prepared floating *in-situ* gel of metronidazole has the feasibility of sustaining the drug release while remaining in the stomach. It appears to be promising as a stomach specific delivery system of metronidazole for better treatment of peptic ulcer disease caused by *H. Pylori.*

REFERENCES

1. Jackson EA, Cardoni AA, Stranz MH, Bradley WE. New Drug Evaluations: Metronidazole (Flagyl IV®, Searle). Drug Intell. Clin. Pharm, 1981 Nov; 15(11): 838-846.
2. Emara LH, Abdou AR, El-Ashmawy AA, Badr RM, Mursi NM. In vitro evaluation of floating matrix tablets of amoxicillin and metronidazole for the eradication of helicobacter pylori. Int J Pharm Pharm Sci, 2012; 4(3): 671-681.
3. Sharma N, Agarwal D, Gupta MK, Khinchi M. A comprehensive review on floating drug delivery system.Int. J. Pharm, 2011; 2(2): 428-441.
4. Thomas LM. Formulation and evaluation of floating oral *in-situ* gel of metronidazole. Int J Pharm Pharm Sci, 2014; 6(10): 265-269.
5. Vipul V, Basu B. Formulation and characterization of novel floating in-situ gelling system for controlled delivery of ramipril. Int. J. Drug Deliv, 2013; 5(1): 43.
6. Jahangir MA, Saleem MA, Shahab MS, Muheem A, Saleem S. Formulation and Evaluation of Novel Floating *In-situ* Gelling System of Macrolide Antibiotic Using Different Gelling Polymers. J. Drug Deliv. Ther, 2015; 5(5): 48-55.
7. Patel DM, Patel DK, Patel CN. Formulation and evaluation of floating oral in situ gelling system of amoxicillin. Int Sch Res Not, 2011; doi:10.5402/2011/276250:1-8.
8. Maheswaran A, Padmavathy J, Nandhini V, Saravanan D, Angel P. Formulation and evaluation of floating oral in situ gel of diltiazem hydrochloride. Int J App Pharm, 2017; 9(1): 50-53.
9. Antony JE, Nair SS. Formulation and evaluation of stomach specific floating in situ gel of clarithromycin. Int. J. Pharm. Sci. Res, 2020; 11(3): 1479-1487.
10. Rathod HJ, Mehta DP. A review on pharmaceutical gel. Int. J. Pharm. Sci. Res, 2015; 1(1): 33-47.
11. Bashir R, Majeed A, Ali T, Farooq S, Khan NA. Floating oral in-situ gel: a review. J. Drug Deliv. Ther, 2019; 9(2): 442-448.