

FORMULATION AND EVALUATION OF FLOATING ORAL IN-SITU GEL OF METRONIDAZOLE

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ABSTRACT

Objective: The objective of the present study was to formulate and evaluate a gastro-retentive *in situ* gelling system of metronidazole.

Methods: 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 (methylcellulose, hydroxypropyl methylcellulose, or sodium carboxymethylcellulose), 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.

Results: 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.

Conclusion: 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: Gastro-retention, *In situ* gel, Sodium alginate, Metronidazole.

INTRODUCTION

Over the past few years, the development of floating *in situ* gel systems has received considerable attention, mainly because of the advantages shown by these systems such as the ease of administration along with the ability of providing controlled and prolonged action compared to conventional drug delivery systems; these factors led to reduced frequency of administration and therefore improved patient compliance and comfort [1].

The *in situ* gel dosage form is a liquid before administration but converts into a gel that floats on gastric contents as it comes in contact with it. [2] Such gel conversions is due to one or more mechanisms such as physiological stimuli (e. g., temperature and pH), physical changes in biomaterials (e. g., diffusion of solvent and swelling), and chemical reactions (e. g., enzymatic, ionic and photo-initiated polymerization)[3].

Floating *in situ* gel drug delivery systems have been used to deliver many drugs which are used either for their systemic or for their local effects in the stomach [4].

Helicobacter pylori (*H. pylori*), one of the causative agents for bacterial infections in humans is responsible for several gastrointestinal diseases such as gastritis, gastric ulcer and gastric cancer.[5] *H. pylori* infection can be effectively cured by treatment with various combinations of antibiotics. More specifically, metronidazole, an effective antiprotozoal and antibacterial drug, 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 [6, 7].

In an attempt to prolong gastric residence time of metronidazole and therefore to improve its local effects in the stomach and achieve better eradication of *H. pylori*, certain floating systems were developed, including floating metronidazole beads [8-10]. Pellets [11]. And tablets [12, 13].

The objective of the present study was to develop metronidazole floating *in-situ* gel that remains in the stomach, resulting in an increased gastric residence time and thus increases local concentration of the drug for complete eradication of *H. pylori*.

MATERIALS AND METHODS

Materials

Metronidazole (supplied by Samarra Drug Industry, Iraq), sodium alginate (SA) and hydroxypropyl methylcellulose (HPMC) (Himedia laboratories, India), methylcellulose (MC) (BDH laboratories, UK), sodium carboxymethylcellulose (NaCMC) (BDH laboratories, UK), sodium bicarbonate and calcium carbonate (Riedel-deltaen, Germany). All other reagents were of analytical grade.

Methods

Preparation of *In-situ* Gel

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) required to make a 2 % (w/v) solution was dissolved in distilled water at 60°C using a heating magnetic stirrer (Velp Scientific a HSC, Italy). After cooling to below 40°C, appropriate amounts of polymer (MC, HPMC or NaCMC), methyl paraben and propyl paraben (ratio of 9:1), the drug, 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 which was stored in amber color bottles until further use [14].

Evaluation

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 (Biotech eng. UV-9200, UK) [15].

pH Measurement

The pH of the prepared formulations was measured using a calibrated digital pH meter (Schott Gerate, Germany)[16].

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 [17].

Measurement of viscosity of *in-situ* gelling system

Viscosity of the dispersion was determined using a Brookfield digital viscometer (NDJ-5S 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 [18].

Table 1: Composition of metronidazole floating in situ gel formulations*

Formulation code	MC (%w/v)	HPMC (%w/v)	NaCMC (%w/v)	CaCO ₃ (%w/v)	NaHCO ₃ (%w/v)
F1	0.6			2	0
F2	0.6			1.5	0.5
F3	0.6			1	1
F4	0.8			1.5	0.5
F5		0.6		2	0
F6		0.6		1.5	0.5
F7		0.6		1	1
F8		0.8		1.5	0.5
F9			0.6	2	0
F10			0.6	1.5	0.5
F11			0.6	1	1
F12			0.8	1.5	0.5

*All formulations were composed of metronidazole (2.5 % w/v), sodium alginate (2% w/v), methyl paraben (0.09 % w/v) and propyl paraben (0.01% w/v).

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°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 [19].

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 °C. 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 278 nm using UV-Visible Spectrophotometer (Biotecheng. UV-9200, UK) [20, 21].

RESULTS AND DISCUSSION

In this study, twelve formulations of sodium alginate based floating oral *in-situ* gelling system of metronidazole were prepared using sodium alginate as release-retarding gel-forming polymer.

Different types of viscosity enhancing polymers (MC, HPMC, and NaCMC) were 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 an

additional gas generating agent to enhance floating behavior of the *in situ* gelling systems of metronidazole.

Drug content

The percent drug content for all formulations was determined and are shown in Table 2. The drug content was found to be in the range of 92-98% for all the formulations indicating uniform distribution of drug.

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 7.2-7.95 as shown in Table 2.

In Vitro gelation study

Gelling studies were carried out using 0.1N HCl (pH 1.2) and the obtained data were represented in Table 2. 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 [22].

Formulations containing calcium carbonate alone produce stiffer floating *in situ* gels than those containing CaCO₃ and NaHCO₃. 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. Similar observations were noted by Hasan et al. [23] who concluded that as the percentage of NaHCO₃ increases, the gel integrity decreases.

Viscosity studies

The formulation should have an optimum viscosity that will allow ease of administration and swallowing as a liquid and produces satisfactory gel strength for use as a delivery vehicle. Results of viscosity for formulations F1 to F12 are shown in Table 2.

The formulations showed a viscosity order of NaCMC > MC > HPMC. In addition to the influence of the type of viscosity enhancing

polymer added, it was observed that increasing the concentration of the viscosity enhancing polymer in the formulation simultaneously increased the viscosity for all polymer types studied.

Increasing calcium carbonate content in the formulation increased the viscosity at all polymer types studied. Since the calcium carbonate is present in the formulations as insoluble dispersion, an increase in its concentration proportionally increased the number of particles dispersed, thus contributing to increased viscosity. [24]

Table 2: Properties of *in situ* gelling formulations

Formulation code	Drug content (%)	pH	Graded Gel response	Floating lag Time (min)	Duration of floating (hr)	Viscosity (cp)
F1	96.22	7.3	+++	10	> 12 hr	401.86
F2	98.00	7.26	+++	< 1	> 12 hr	360.55
F3	95.75	7.8	+++	< 1	> 12 hr	299.90
F4	94.78	7.23	+++	< 1	> 12 hr	730.56
F5	96.20	7.40	+++	5	> 12 hr	218.35
F6	92.00	7.58	+++	< 1	> 12 hr	214.7
F7	93.50	7.2	+++	< 1	> 12 hr	198.55
F8	94.20	7.32	+++	< 1	> 12 hr	320.3
F9	95.20	7.43	+++	6	> 12 hr	838.96
F10	96.00	7.95	+++	< 1	> 12 hr	742.20
F11	94.25	7.27	+++	< 1	> 12 hr	641.40
F12	95.00	7.93	+++	< 1	> 12 hr	1567.16

In vitro Floating study

The formulated floating *in situ* gelling system of metronidazole employed NaHCO₃ or CaCO₃ as a gas-generating agent. The *in vitro* floating test revealed the ability of all formulae to maintain buoyant for more than 12 h (Table 2 and Figure 1).

Regarding the floating lag time, it was observed that formulae containing NaHCO₃ had instantaneous floating behavior and had significantly shorter ($p < 0.05$) floating lag times than formulae containing CaCO₃ 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 (CO₂). The evolved CO₂ gas was entrapped in the gel causing floating. Incorporation of sodium bicarbonates improves floating behavior by providing an additional source for CO₂ gas generation [25, 26].

The observed behavior suggests that the gel formed by the combination of sodium alginate with the investigated polymers, enabled efficient entrapment of CO₂ gas producing a buoyant preparation with shorter floating lag time which can retain in the stomach for a longer time period and assist controlled release of the drug.

In vitro drug release study

The *in vitro* release study of metronidazole from all twelve formulae in 0.1 N HCl (pH 1.2) was conducted for a period of 6 hours and the results were shown in figure 2. The highest drug release of 89.81 % was observed with formula F7 (SA 2 %, HPMC 0.6 %, CaCO₃ 0.5 % and NaHCO₃ 1.5 %) and the lowest drug release of 55.40 % was observed with formula F9 (SA 2 %, NaCMC 0.6 %, CaCO₃ 2 %). The release of drug from these formulae was characterized by an initial phase of high release (burst effect) followed by a second phase of moderate release. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics [27].

The influence of using different types of viscosity enhancing polymers (MC, HPMC and NaCMC) with sodium alginate on *in vitro* drug release is shown in Figure 2 a, b and c, respectively. The pattern of drug release seen from formulae containing MC (F1 through F4, figure 2a), HPMC (F5 through F8, figure 2b) and NaCMC (F9 through F12, figure 2c) showed that the release of metronidazole was different when using different types of polymers and was in the following order: HPMC > MC > NaCMC. This suggests that the choice of the polymer base added is of obvious importance for achieving a desired drug release. The higher viscosity of NaCMC compared to MC

and HPMC promote the formation of highly viscous gels upon contact with aqueous fluids which will produce more retardation in drug release rate.

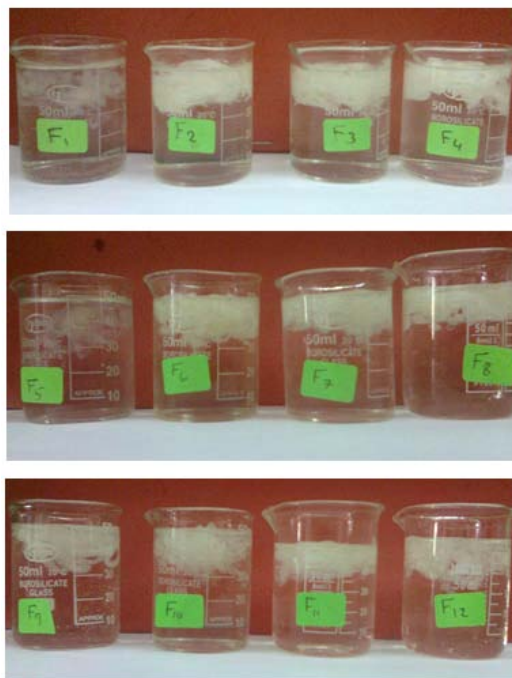


Fig. 1: Photographs taken during the *in vitro* floating study of Formulae (F1-F12) in 100 mL 0.1 N HCl (pH 1.2)

Besides the polymer type, the polymer concentration can control the drug release. In the MC series (figure 2 a), formula F4 containing 0.8 % of MC released about 72.97 % in 6 hours compared to 78.02 % release seen with formula F2 containing 0.6 % of MC. In a similar way, formula F8 and formula F12 (figure 2, b and c) containing 0.8 % HPMC and 0.8 % NaCMC, respectively released about 82.18 % and 67.20 % of metronidazole compared to 87.79 % and 70.23 % released by formula F6 and F10 containing 0.6 % of HPMC and 0.6 % NaCMC. It can be concluded that an increase in concentration of

viscosity enhancing polymer resulted in decreased 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. [28]

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.

As shown in figure 2, formulae F1, F5 and F9 containing CaCO_3 in a concentration of 2 % had slower drug release profiles than the rest of formulae containing lower percentages of 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. These results are in good agreement with the observations of Rohith et al. [29] gastro-retentive *in situ* gelling liquid formulation of ranitidine.

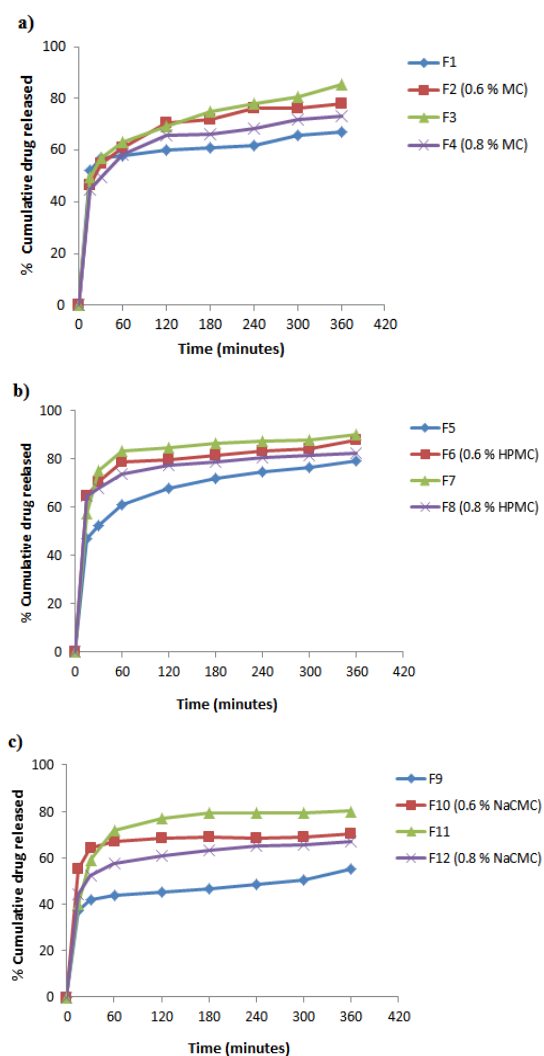


Fig. 2: Comparison of *in vitro* dissolution profiles of F1 to F12.

a) Formulations containing MC, b) Formulations containing HPMC, and, c) Formulations containing NaCMC.

Regarding the effect of sodium bicarbonate on drug release, comparing the drug release profiles of formulations containing sodium bicarbonate (F2 to F4, figure 2a) to formulations without

sodium bicarbonate (F1), a proportional increase in drug release profile can be observed with increasing amounts of sodium bicarbonate. Such observations were not only observed for formula containing MC but were also apparent with formulae containing HPMC or NaCMC.

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.

CONCLUSION

In the present study, various *in situ* gelling liquid oral formulations of metronidazole were prepared. The study has shown that by 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*.

CONFLICT OF INTERESTS

Declared None

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