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FORMULATION AND EVALUATION OF GASTRORETENTIVE DRUG DELIVERY OF METRONIDAZOLE IN-SITU GELLING SYSTEM

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ABSTRACT

Background: Controlled release refers to the use of delivery device with the objective of releasing the drug into patient body at a predetermined rate, or at specific times or with special release profiles. **Aim:** To formulate and evaluate *in-situ* gel system for various parameters related to drug release and floating behavior. **Results and discussion:** FT- IR studies showed that there is no interaction between the drug and excipients. The In-situ gels shows zero floating lag time and remained buoyant for more than 12 h achieving the gastric retention properties. All evaluation parameters were within the pharmacopoeial limits. The *in-vitro* drug release was sustained up to 12 h. **Conclusion:** The studies concluded that HPMC- Sodium Alginate with the calcium carbonate of 2% in- situ gels showed a good drug release and can be used as a gastroretentive drug delivery systems in view of their swelling characteristics in acidic pH and floating in stomach respectively. It might contribute better patient compliance while reduce frequency of dosing and by acceptable sustained-release dosage forms Metronidazole in the stomach to promote a fast and effective eradication of *H.pylori* to cure peptic ulcer. From the viscosity analysis, *In-vitro* buoyancy studies and release

kinetics studies it can be concluded that the formulation FA3 has better potential of sustainingdrug release with good gastric retention capability.

KEYWORDS: Gastroretentive drug delivery, Formulation, In-situ gels, Metronidazole, HPMC, Na-CMC, Methylcellulose, Sodium alginate.

INTRODUCTION

Conventional drug delivery system achieves and maintains the drug concentration within treatment only when taken several time a day. This often results in damagingside effects and leads to poor control of drug therapy and there is also significant fluctuation in drug level. Recently several technical advancement have been made, which have resulted in the development of new techniques for drug delivery. These techniques are capable of controlling the rate of drug delivery to achieve and maintain the concentration of administered drug within therapeutically effective range. Controlled drug delivery systems have been introduced to overcome the drawbacks of fluctuating drug levels associated with conventional dosage forms.[1-4] The concept of sustained or prolonged release of biologically active agents has been well appreciated and rationalized for decades. Controlled release refers to the use of delivery device with the objective of releasing the drug into patient body at a predetermined rate, or at specific times or with special release profiles.

Gastro retentive drug delivery

The importance of the controlled drug delivery system that releases the bioactive component over an extended period of time has long been recognized in pharmaceutical field. Of the major routes of drug delivery, oral route remains the most convenient and commonly employed means of introducing drugs to the systemic circulation. Recent advances in controlled release technology have made it possible to release drugs at a constant rate for days to years. Application of such controlled release technology to oral drug delivery however has been limited because the actual time for effective drug delivery is restricted by the gastrointestinal transit time which typically ranges from 6 to 8 hours depending upon various factors.[5-9] In pharmaceutical field, controlled/sustained release systems have been widely used in oral medication from as early as 1950s. A number of oral controlled drug delivery systems have been developed for this purpose and gastro retentive drug delivery system (GRDDS) is one more step forward in this regards. A major constraint in oral controlled release drug delivery is that not all drug candidates are absorbed uniformly throughout the gastro intestinal tract. Some drugs are absorbed in a particular portion of GI

tract only or are absorbed to a different extent in various segments of GI tract. Such drugs are said to have an absorption window.

Thus only the drugs which are released in the preceding region and in close vicinity to the absorption window are available for absorption. After crossing absorption window, the released drug goes to waste with negligible or no absorption. Thus the time available for drug absorption drastically decreases. Also most of the drugs are sparingly soluble or insoluble in gastric fluids. In these type of drugs dissolution is directly related to time available for solubilization and thus in such cases gastric retention time (GRT) or transit time becomes significant factor for drug absorption.[10] Also if the dosage forms pass through the drug absorption site before the complete release of loaded dose it will not perform satisfactorily. Thus attention must be given to prolonging the GRT to get complete drug release in GI tract. Hence a beneficial delivery system would be one which possesses the ability to control and prolong the gastric emptying time and can deliver drugs in higher concentrations to the absorption site (i.e. upper part of the small intestine). The gastro retentive drug delivery systems are designed on this rationale and prove really efficacious to deliver the drug candidates with above mentioned characters. The prolonged and sustained administration of the drug from GRDF to the stomach may be advantageous for local therapy in the stomach and small intestine by floating on surface of gastric fluid and provides an increased gastric residence time, resulting in prolonged drug delivery in gastro intestinal tract and increases local concentration of the drug effectively for complete eradication of H. pylori with better patients compliance.

Basic anatomy & physiology of gastrointestinal tract

The anatomy and physiology of GIT should be understood, while developing GRDDS. Factors affecting GI motility like pH, nature and volume of gastric secretion and gastric mucus plays important role while developing GRDDS. The stomach is J shaped enlargement of the GIT directly inferior to the diaphragm in the epigastric umbilical and left hypochondriac regions of the abdomen.[11-14] It is situated in the left upper part of the abdominal cavity immediately under the diaphragm. The stomach is a temporary storage area that mixes food with water and gastric juices to produce chyme, breaks food down physically and chemically, and controls release of the chyme into the small intestine via regulation of the pyloric sphincter. Specialized cells located throughout the gastric mucosa produce various substances. Goblet cell secretes mucus that protects the gastric mucosa. Parietal and chief

cells occur in the oxyntic and pyloric glands of gastric pits located in the fundus and the body of the stomach.[15-19] Parietal cells produce hydrochloric acid, which denatures proteins and kills bacteria, as well as intrinsic factor necessary for the absorption of vitamin B12. Chief cells secrete pepsinogen, an enzyme precursor that is converted to the proteolytic enzyme, pepsin, in gastric juice. Pyloric glands contain the gastrin producing G cells and mucous cells.

Classification of gastro retentive drug delivery system

- High-density systems.
- Floating systems
- Hydrodynamically balanced systems: HBS
- Gas-generating systems
- Raft-forming systems
- Low-density systems
- Expandable systems.
- Superporous hydro gel.
- Mucoadhesive or Bioadhesive systems.
- Magnetic systems.
- Module assemblage technology.
- Pharmacokinetic and pharmacodynamic aspects of gastro retentive dosageforms

Pharmacokinetic aspects

- Absorption window validation that the drug is within the category of Narrow Absorption Window agents
- Enhanced bioavailability
- Enhanced first pass biotransformation
- Improved bioavailability due to reduced P-glycoprotein (P-gp) activity in the duodenum
- Reduced frequency of dosing
- Targeted therapy for local ailments in the upper GI tract
- Pharmacodynamic aspects:
- Reduced fluctuations of drug concentration
- Improved selectivity in receptor activation
- Reduced counter-activity of the body

- Extended time over critical concentration
- Minimized adverse activity at the colon

Applications of GRDDS

Sustained drug delivery

HBS systems can remain in the stomach for long periods and hence can release the drug over a prolonged period of time. The problem of short gastric residence time encountered with an oral CR formulation hence can be overcome with these systems.[20-22] These systems have a bulk density of G1 as a result of which they can float on the gastric contents. These systems are relatively large in size and passing from the pyloric opening is prohibited.

Site-specific drug delivery

These systems are particularly advantageous for drugs that are specifically absorbed from stomach or the proximal part of the small intestine, e.g., riboflavin andfurosemide.

Absorption enhancement

Drugs that have poor bioavailability because of site specific absorption from the upper part of the gastrointestinal tract are potential candidates to be formulated as floating drug delivery systems, thereby maximizing their absorption.

Aim

The study aimed to analyze the formulation and evaluation of gastroretentivedrug delivery of Metronidazole *In-Situ* gelling system.

Objectives

- To design and select a suitable formulation in sol form, as a vehicle forretaining drug in gastric region.
- To achieve gastric retention by conversion of the sol into floating gel this would float on to the gastric contents; So as to retain the drug in gastric region.
- To evaluate developed *in-situ* gel system for various parameters related to drug release and floating behavior.

Plan of work

- The following steps can be adopted to achieve the desired formulation
- Literature survey.

- Procurement of drug and polymers.
- Characterization of drug and polymers
- Preparation of standard calibration curve for Metronidazole by UV spectrophotometer.
- Compatibility study of drug & excipients by using suitable analytical technique.
- Preliminary trials using active pharmaceutical ingredients, polymeric agents and other adjuvants required for the formulation of the dosage forms with the desired characteristics.
- Optimization of the selected process variable based on the result of the preliminary study.
- In-vitro evaluation of Metronidazole in-situ gel formulation.

Physical evaluation of formulation

- Drug content and uniformity.
- P^H
- Rheology and Viscosity
- In-vitro floating ability
- In-vitro drug release
- Water uptake study
- Gel strength
- Spreadability
- Extrudability
- Stability study

METHODOLOGY

Table 1: List of materials used.

S. No	Materials	Uses
1	Metronidazole	Active ingredient.
2	Sodium alginate	Gelling agent.
3	Methyl cellulose	Dry binder Hydrophilic white powder
4	Hydroxy propylmethyl cellulose	Semisynthetic, Viscoelastic polymer.
5	Sodium carboxy methyl cellulose	Suspension thickening andStabilizing cellulose gum.
6	Calcium carbonate	Gas forming agent and ca^{2+} ion source.Antacid.
7	Sodium bicarbonate	Acidosis.
8	Methyl paraben	Preservative.
9	Propyl paraben	Preservative. Antifungal agent.

Table 2: List of equipments required.

Sr. No.	Equipment	Specification
1.	UV Spectrophotometer.	Shimadzu UV-1800, Japan
2.	FTIR	Shimadzu IR-Affinity-1, Japan.
3.	Dissolution apparatus	Electrolab TDT-08L, Bombay
4.	Brookfield viscometer	Model No.CAT200+
5.	Digital magnetic Stirrer	Remi Motors, Mumbai
6.	Digital pH Meter	Equiptronics EQ-610
7.	Digital Weighing Balance	Shimadzu AX200
8.	Stability Chamber	Thermolab India

Preformulation Studies

Metronidazole

1) Identification test: Identification test for Metronidazole was carried out by using IR spectroscopy & UV absorbance spectra.

a) For IR studies KBr powder was dried at 60 ^{o}C for one hour. The dried KBr powder was uniformly mixed with drug and IR spectra was taken for this mixture.

b) For UV identification of Metronidazole, the solution of concentration of 10µg/mlwas prepared in 0.1 N HCl. The solution was scanned from 200 to 400 nm and a spectrum was observed for absorption maxima.

Solubility: Slightly soluble in water, alcohol, acetic acid and in dichloromethane; very slightly soluble in ether.

Melting Point: The melting point of the drug was determined by using capillary method.

U V method of analysis

Construction of standard calibration curve

- Solvent employed : 0.1 N HCl
- 100 mg of Metronidazole was dissolved in 100 ml of 0.1 N HCl to give 1000 µg/ml stock solution.
- 10ml of above solution was then diluted 100 ml with 0.1N HCl to get 100 µg/ml stock solution.
- Further dilutions ranging from 5-30 µg/ml were prepared and analysed by UV Spectrophotometer.
- Spectrum was obtained by scanning the solution [15 µg/ml] over Range from
- 200-400nm.

- Absorption Maxima (λmax) = 277 nm was derived.
- The graph of absorbance v\s concentration in µg/ml was plotted. The r2 value of this graph was calculated to check the linearity of the absorbance against concentration.

Solubility study of the drug

Solubility is an important parameter for Preformulation studies because:

- It affects the dissolution of drug.
- Bioavailability of drug is directly affected by dissolution and absorption of drug by oral administration.
- Particle size, shape, surface area may affects the dissolution characteristics of drug henceit should be determined during Preformulation.

Metronidazole

Soluble in water (10 mg/ml at 20° C), ethanol (5 mg/ml), methanol, chloroform (<0.5 mg/ml), and DMSO (34 mg/ml at 25° C). The solubility of metronidazole in different pH medium is tabulated below

Table 3: Solubility study of the drug.

S.No	Media	Solubility mg/ml at roomtemperature
1.	Water	4.525
2.	0.1NHCL	2.105
3.	Phosphate buffer pH 4.5	0.0193
4.	Phosphate buffer pH 6.8	0.0256
5.	Phosphate buffer pH 7.4	0.0294

Methods for formulation of In-situ gel

There are various mechanism for the in gel formulation: physiologically changes (temperature and pH), chemically stimulates (ionic cross linking), physical change in biomaterial (diffusion of solvent and swelling).

Analytical method for evaluation of in-situ gels

- Determination of Drug Content
- pH Measurement
- In-Vitro Gelling Capacity
- In Floating Lag Time:
- In-Vitro Drug Release:
- Measurement of Water Uptake

- Fourier transform infra red spectroscopy (FT-IR)

Preparation of in-situ gel of Metronidazole

In-situ gel of Metronidazole was prepared using methylcellulose, Hydroxy propyl methylcellulose, Sodium Carboxy methyl cellulose, Sodium bicarbonate, Calcium Carbonate in the compositions given in Table 4.

Table 4: Formulation chart showing composition of each In-situ Gel.

Ingredients	F-1 in%w/v	F-2 in%w/v	F-3 in%w/v	F-4 in%w/v	F-5 in%w/v	F-6 in%w/v
Metronidazole	2.5	2.5	2.5	2.5	2.5	2.5
Sodium Alginate	2.0	2.0	2.0	2.0	2.0	2.0
Methylcellulose	0.6	0.8	-	-	-	-
Hydroxy propylmethylcellulose	-	-	0.6	0.8	-	-
Sodium Carboxymethyl cellulose	-	-	-	-	0.6	0.8
Calcium Carbonate	2	0.5	2	0.5	2	0.5
Sodium bicarbonate	0	1.5	0	1.5	0	1.5

Procedure

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. 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.[23] 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.

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.[23-24]

pH Measurement

In-situ solution formulation pH measure by using calibrated digital pH meter at room temperature.

In-Vitro Gelling Capacity

Evalution for gelling capacity can be measure by visualization method. In that method coloured solution of different formulations were prepared. in situ gelling formation was measured into 5ml of gelation solution (0.1 N HCL) in 15ml borosilicate glass tube at 37□±1 □c. In situ formulation was added in such a way that tip of pipette touch to gelation solution and solution release slowly during that time stiffness of gel and time duration to remain as such as a gel colour was added for the visualization purpose.

In situgelling capacity was categorized in three class based on gelation time and time period atthey remain as such. (+) gel after few minutes, dispersed rapidly (++) gelation immediate, remain for 12hr. (+++) gelation immediate, remain for more than 12hr.

In Floating Lag Time

In this parameter 10ml of in situ formulation was added into the 900ml dissolution vessel containing 0.1N HCL at 37□c.the time the formulation took to emerge on mediumsurface (floating lag time) and the time formulation constantly floated on surface of dissolution medium (duration of floating).

Figure 1: Photographs taken during the *in-vitro* floating study of Formulae F1& F2 in 100 mL 0.1 N HCl (pH 1.2).

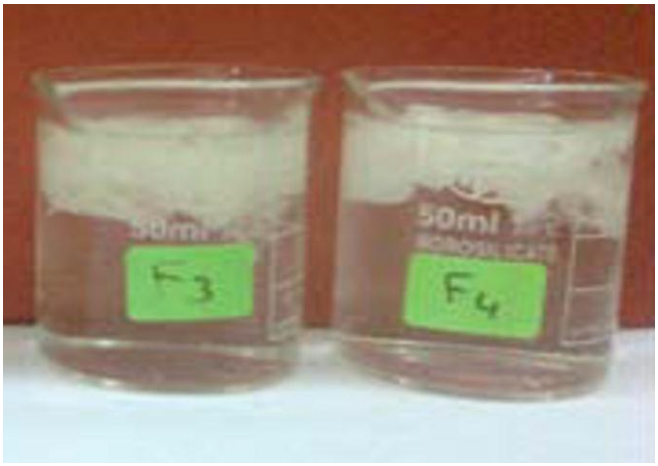


Figure 2: Photographs taken during the *in-vitro* floating study of Formulae F3 & F4 in 100 mL 0.1 N HCl (pH 1.2).

Figure 3: Photographs taken during the *in-vitro* floating study of Formulae F5 & F6 in 100 mL 0.1 N HCl (pH 1.2).

In Vitro Drug Release

The drug release was measured using USP dissolution apparatus I (basket covered with muslin cloth) at 50rpm. The speed of apparatus was maintain as slow as possible to avoid breaking of gelation formation and maintainmild agitation conditions to believe to exist in vivo condition.

900 ml of dissolution medium (0.1N HCL) at 37□ ±1 □c temperature. To that 5ml dissolution medium was pipette out at 1,2,4,6,8,10 and 12hour interval. And measured absorbance at particular wavelength of drug using uv-spectrophotometer.

Measurement of Water Uptake

The water uptake of selected formulation was determined by simple method. In this study 40ml of in situ gel formed in 40ml 0.1N HCL from all the formulation formed gel was separated and excess 0.1 NHCL was removed by tissue paper.

Before transfer gel formulation to water initial weight was taken and then added to 10ml water after every 30 min water was decant and weight the gel formulation. The data was calculated and reported.

Fourier transform infra red spectroscopy (FT-IR)

The FTIR spectra of the prepared formulations were recorded over the range of 400 - 4000 cm-1 by KBr pellet method using FT-IR spectrophotometer. The compatibility between the drug and the polymers were compared by FT-IR spectra.

RESULTS

Preformulation studies were carried out to investigate the physiochemical propertiesof the formulation.

Preformulation Studies

Qualitative estimation for Metronidazole by UV spectrophotometric method

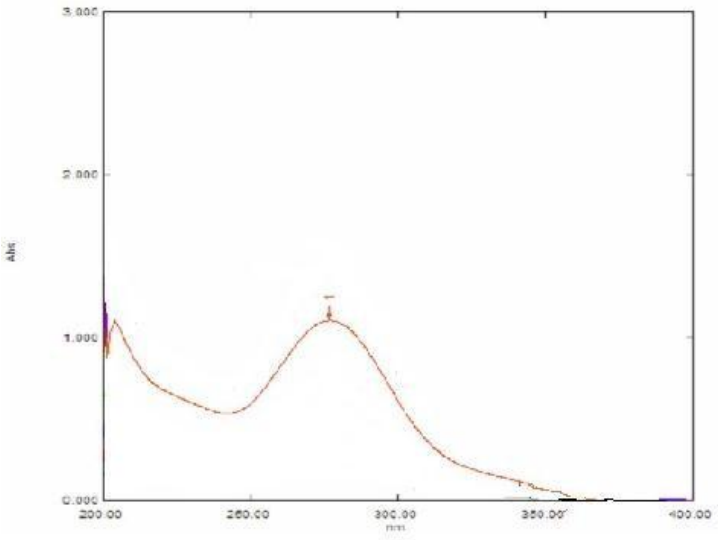


Figure 4: UV Spectrum of Metronidazole at 200-400 nm.

Medium: 0.1 N HCl (1.2 pH)

Standard stock: 1000 µg/ml

Working stock: 100 µg/ml

λ max : 200-400 nm

Table 5: Calibration curve of Metronidazole.

Sr.no.	Concentration(µg/ml)	Absorbance at 200-400nm
1	5	0.184
2	10	0.354
3	15	0.535
4	20	0.731
5	25	0.942
6	30	1.139

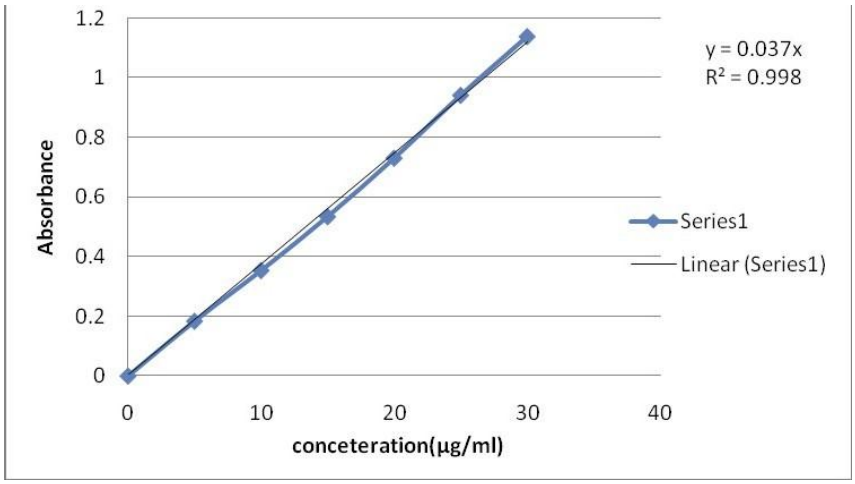


Figure 5: Standard calibration curve of Metronidazole.

From the standard curve, it was observed that the drug obeys Beer's law in concentration range of 5-30 µg/ml in 0.1N HCl. Drug shown good linearity with regression of coefficient (r^2 = 0.998) and equation for this line obtained was found to be (y=0.037x) which is used for the calculation of amount of drug in dissolution study and content uniformity.

Infrared spectroscopy

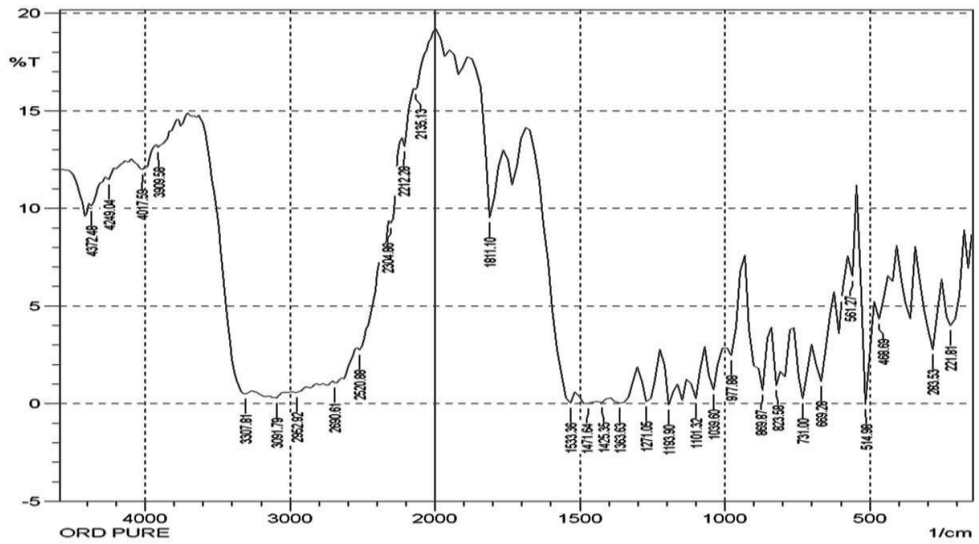


Figure 6: FTIR spectra of Metronidazole.

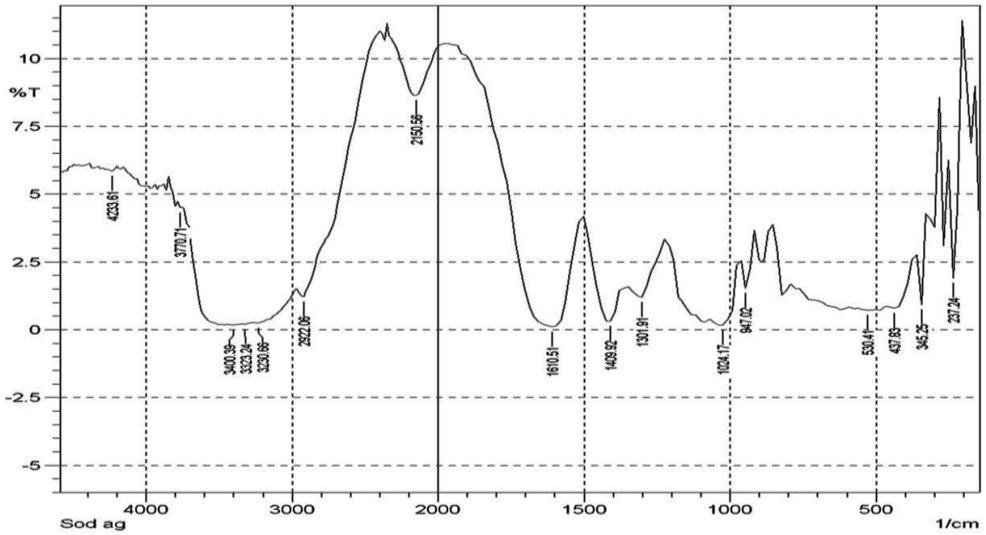


Figure 7: FTIR spectra of sodium alginate.

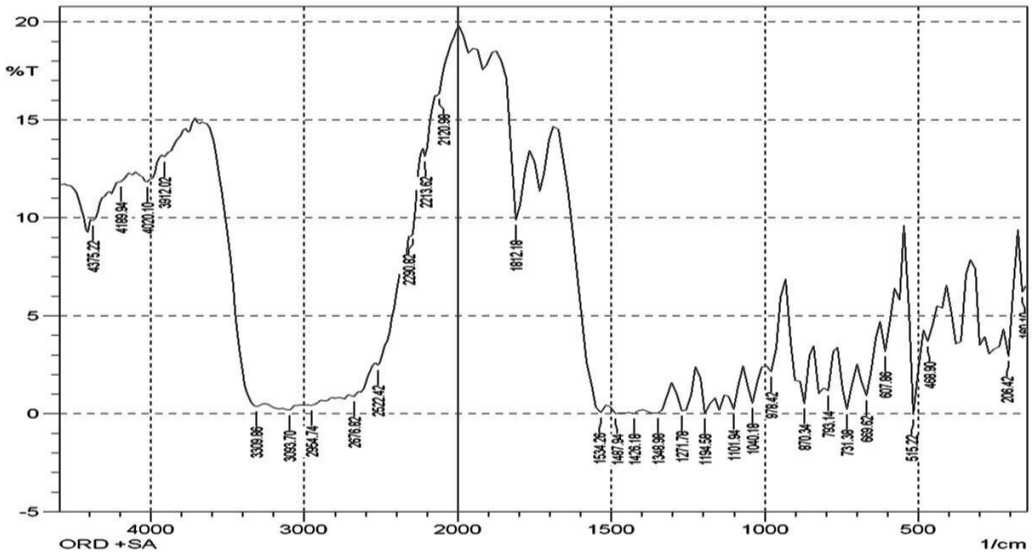


Figure 8: FTIR spectra of Metronidazole + sodium alginate.

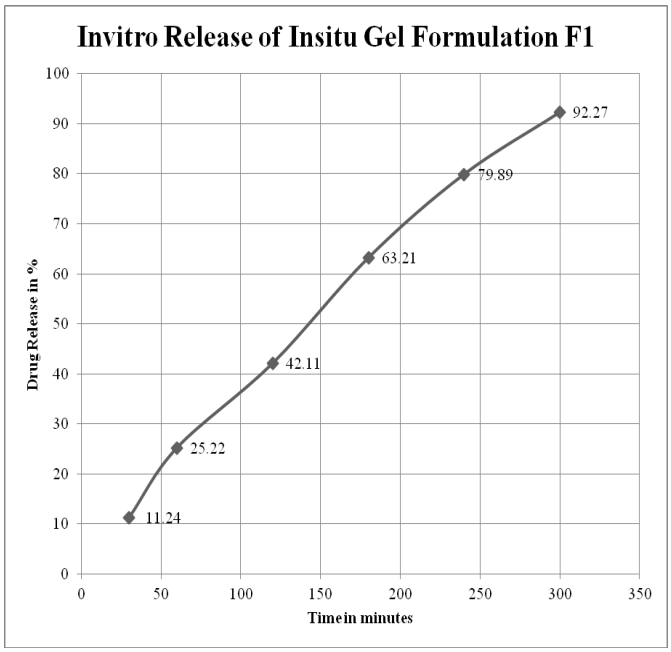
Table 6: Properties of in situ gelling formulations.

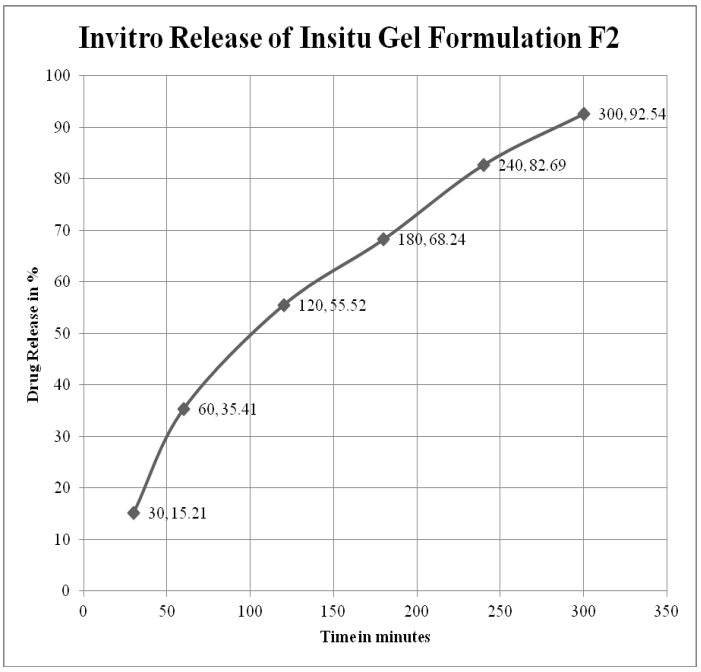
FormulationDetails	F-1	F-2	F-3	F-4	F-5	F-6
Drug Release	92.27	92.54	93.14	99.54	92.17	89.75
pH	7.3	7.8	7.5	7.58	7.43	7.92
Gel Response Grade	++	+++	++	+++	+++	+++
Floating Lag Time inmins	< 10	< 01	< 08	< 02	< 08	< 02
Floating duration inHrs	>12	>12	>12	>12	>12	>12
Viscosity in cps	521	485	568	521	568	591

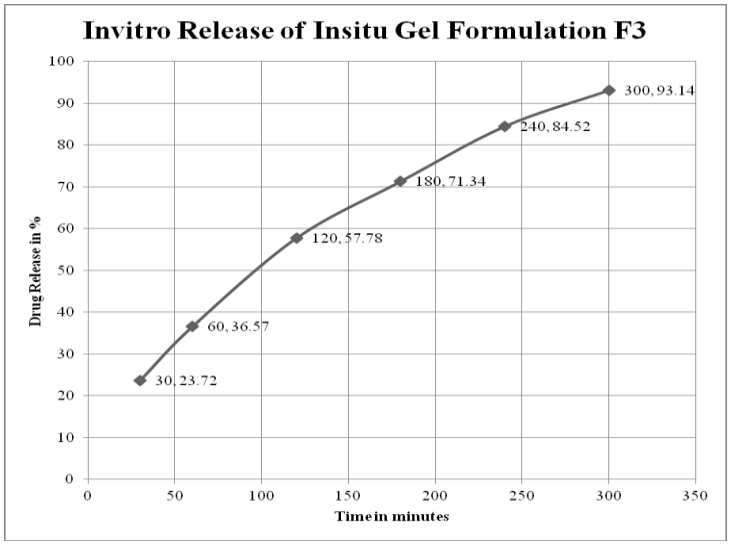
Regarding the effect of sodium bicarbonate on drug release, comparing the drug release profiles of formulations containing sodium bicarbonate (F1, F3, F5) to formulations without sodium bicarbonate, a proportional increase in drug release profile can be observed with increasing amounts of sodium bicarbonate. Such observations were not only observed for

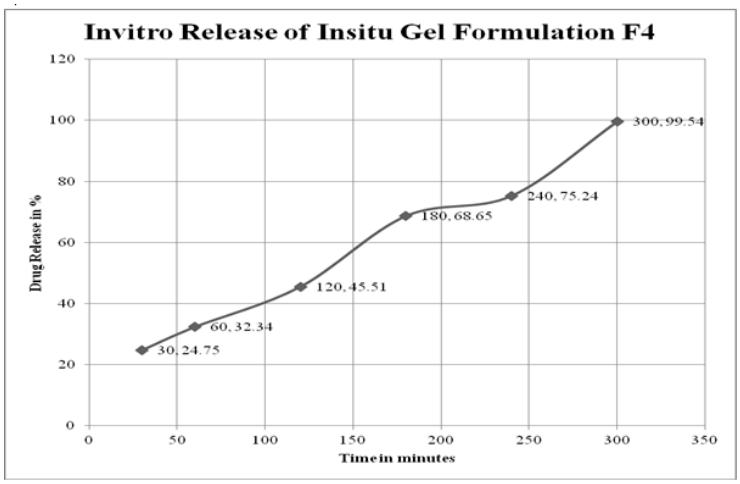
formula containing MC but were also apparent with formulae containing HPMC or NaCMC.

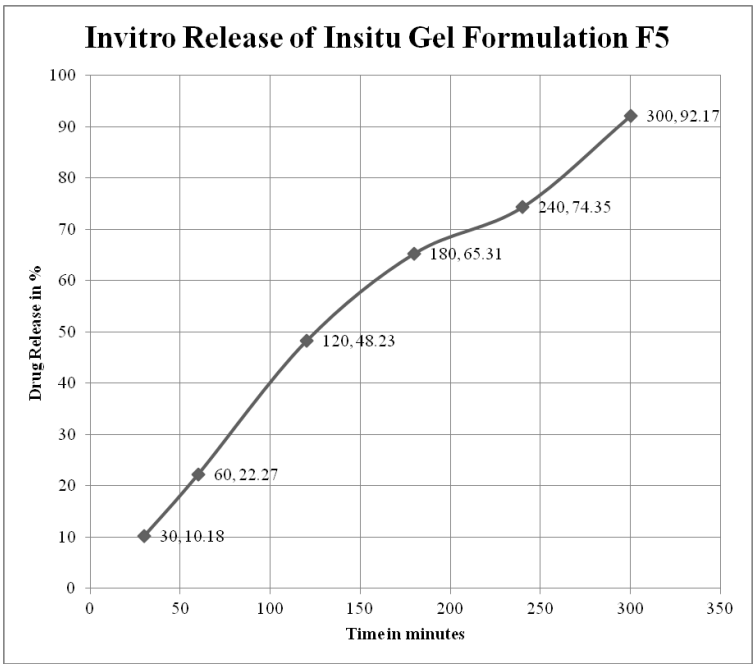
The reason for the increase in drug release when using higher amounts of sodium bicarbonate may be because of weaker gelation properties occurring with the presence of sodium ions in the formulation compared to stronger gelation effect produced in the presence of calcium ions.






Invitro Release of Insitu Gel Formulation F3
Drug Release in %
Time in minutes
30, 23.72
60, 36.57
120, 57.78
180, 71.34
240, 84.52
300, 93.14


Invitro Release of Insitu Gel Formulation F4
Drug Release in %
Time in minutes
30, 24.75
60, 32.34
120, 45.51
180, 68.65
240, 75.24
300, 99.54


Invitro Release of Insitu Gel Formulation F5
Drug Release in %
Time in minutes
30, 10.18
60, 22.27
120, 48.23
180, 65.31
240, 74.35
300, 92.17

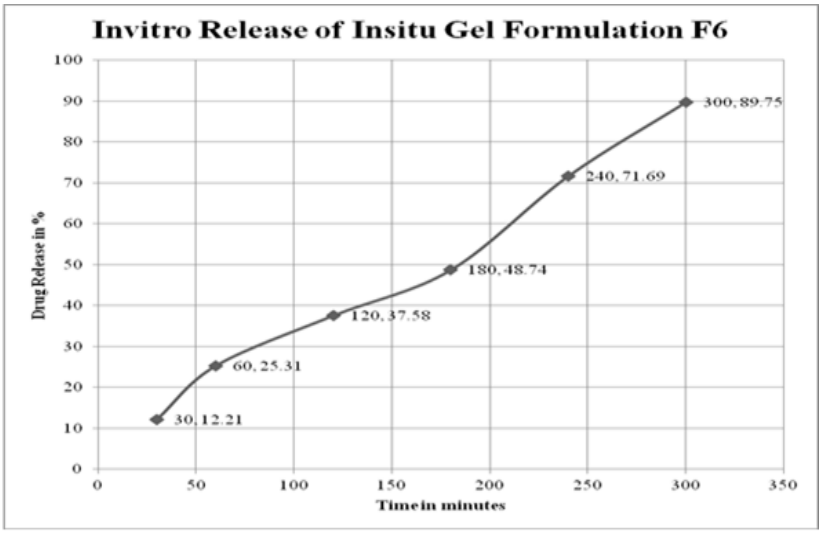


Fig. 9: Invitro release of Insitu gel formulations F1-F6.

Table 7: Drug release in %.

Time inmins	Drug release in %	
	Formulation F1	Formulation F2
30	11.24	15.21
60	25.22	35.41
120	42.11	55.52
180	63.21	68.24
240	79.89	82.69
300	92.27	92.54

Table 8: Drug release in %.

Time inmins	Drug release in %	
	Formulation F3	Formulation F4
30	23.72	24.75
60	36.57	32.34
120	57.78	45.51
180	71.34	68.65
240	84.52	75.24
300	93.14	99.54

Table 9: Drug release in %.

Time inmins	Drug release in %	
	Formulation F5	FormulationF6
30	10.18	12.21
60	22.27	25.31
120	48.23	37.58
180	65.31	48.74
240	74.35	71.69
300	92.17	89.75

DISCUSSION

The aim of present study was an attempt to "Formulate and evaluate the gastroretentive drug delivery of Metronidazole in-situ gelling system" for complete eradication of H.pylori, it compliance to the patients and improved therapeutic performance of the drug over conventional dosage forms.

In this study, six 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.[25-28] 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. The drug content was found to be in the range of 92-98% for all the formulations indicating uniform distribution of drug.

pH Measurement

The 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.

In-Vitro gelation study

Gelling studies were carried out using 0.1N HCl (pH 1.2) and the obtained data were represented in Table 4 and Table 6. All formulations showed immediategelation 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 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. whoconcluded that as the percentage of $NaHCO_3$ increases, the gel integrity decreases.

In-vitro Floating study

The formulated floating *in-situ* gelling system of metronidazole employed $NaHCO_3$ or$CaCO_3$as a gas-generating agent. The *in vitro* floating test revealed the ability of all formulae to maintain buoyant for more than 12 h. Regarding the floating lag time, it was observed that formulae containing $NaHCO_3$ had instantaneous floating behavior and had significantly shorter ($p < 0.05$) floating lag times than formulae 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.[29-30] 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. The results were shown in figure 1-3.

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

The *in-vitro* release study of metronidazole from all six formulae in 0.1 N HCl (pH 1.2) was conducted for a period of 5 hours and the results were shown in figure 2. The highest drug release of 99.54 % was observed with formula F4 (SA 2 %, HPMC 0.8 %, $CaCO_3$ 0.5 % and $NaHCO_3$ 1.5 %) and the lowest drug release of 89.75 %was observed with formula F6 (SA 2 %, NaCMC 0.8 %, $CaCO_3$ 0.5 %,$NaHCO_3$ 1.5%). 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. 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 & F2), HPMC (F3 & F4, figure 2b) and NaCMC (F5 & F6,) showed that the

releaseof 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 polymerbase 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.

Besides the polymer type, the polymer concentration can control the drug release. In the MC series, formula F2 containing 0.8 % of MC released about 92.54 % in 5 hours compared to 92.27 % release seen with formula F1 containing 0.6 % of MC.

Similarly formula F3 and formula F4 containing 0.6 % HPMC and 0.8 % HPMC, respectively released about 93.14 % and 99.54 % of metronidazole compared to 92.17,and 89.75 % released by formula F5 and F6 containing 0.6 % of NaCMC and 0.8 % 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. 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 attemptto have more retarded drug release.

As shown in figure, formulae F1, F3 and F5 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.[31]

The effect of sodium bicarbonate on drug release, comparing the drug release profiles of formulations containing sodium bicarbonate (F1, F3, F5) to formulations without sodium bicarbonate, 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 Na CMC. 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. Hence from the above results we can conclude that it is possible to formulate gastro retentive *in-situ* gels of Metronidazole for treatment of *H.pylori* infections and it release the drug in sustained manner. The results were shown in Table 7-9.

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 F6. 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. The results were shown in Table 6.

CONCLUSION

The study concluded that A lesser floating lag time and prolonged floating duration could be achieved by varying the concentration of polymer and calcium carbonate. Both polymer and calcium carbonate have contributing effect on the floating performance and the *in-vitro* drug release pattern. As the concentration of the calcium carbonate increases the lag time of the solution to form buoyant gel decreases. Ideally lag time should be such that the gel is formed and it floats onto the gastric contents, so that it is not swept away with peristaltic movements. The *in-vitro* drug release profiles obtained for all the different formulations with different combinations shows efficient controlled released profile of the drug. Solution with minimum polymer concentration formed slimy or fragmented gels which resulted into burst release and poor sustained release effect. As the concentration of the polymer increases the drug release decreases significantly. This shows that polymer concentration along with Ca^{2+} ion concentration is a considerable factor in sustained released formulation. From the, pH, *in-vitro* buoyancy studies, viscosity analysis, water uptake, gel strength, drug release and release kinetics studies it can be concluded that the combination FA3 has better potential of sustaining drug release with good gastric retention capability. From the prepared *in-situ* gel selected the best formulation w.r.t.

organoleptic additives and stabilizers can serve as better alternative of administering drugs for sustained release with added advantage of liquid oral formulations. As per ICH guidelines the stability study of formulations were carried out results shows no significant changes in floating lag time, drug content and%drug release. Hence from the above results we can conclude that it is possible to formulate in situ gels of Metronidazole using sodium alginate for treatment of eradication of*H.pylori* infections.

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