DEVELOPMENT AND EVALUTION OF GASTRO RETENTIVE IN SITU ORAL FLOATING GEL OF ANTIPROTOZOVAL DRUG METRONIDAZOLE

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I. INTRODUCTION

In the field of pharmaceutical technology great efforts are being prefabrication of existing drug molecule capable of solving problems, stability, toxicity, etc. Oral dosage forms pose low bioavailability problems due to their rapid gastric transition from stomach, especially in case of drugs which are less soluble at alkaline pH of intestine. similarly some drugs having rapid gastric emptying time so frequent dose administration in such cases is increased to avoid this problem floating drug delivery system is developed. In situ gel forming drug delivery is a type of muco adhesive drug delivery system. These hydro gels are liquid at room temperature but undergo gelation when in contact with body fluids or change in p^H. Approaches of in situ gel drug delivery; there are three broadly defined mechanisms used for triggering the in situ gel formation of biomaterials : physiological stimuli (e.g., temperature and pH), physical changes in biomaterials (e.g., solvent exchange and swelling), and chemical reactions (e.g., enzymatic, chemical and photo initiated polymer. Metronidazole is used to treat amebiasis, vaginitis, trichomonas infection, giardisis, anaerobic bacteria and treponemal infections . it has also been proposed as a radiation sensitizer for hypoxic cells. Metronidazole is cytotoxic to facultative anaerobic bacteria such as Helicobacter pylori and gardnerella vaginalis, but the mechanism of this action is not well understood[1]. However, its activity against obligate anaerobes occurs through a four step process. Reduced intermediate particle interacts with intracellular targets-Cytotoxic intermediate particles interact with host cell DNA, resulting in DNA strand breakage fatal de stabilization of the DNA helix.

Infection with Helicobacter pylori (H. pylori) is a cofactor in the development of important gastrointestinal diseases including gastritis, peptic and duodenal ulcers, gastric adenocarcinoma and colorectal neoplasm (Inoue et al., 2014; Khalifa et al., 2010). About half a million new cases/year of gastric cancer, have been linked to H. pylori infection, and it has been predicted to be one of the top ten leading causes of death worldwide by 2020. There are various obstacles in the eradication of H. pylori infections, including low antibiotic levels and poor accessibility of the drug at the site of infection (Adebisi et al., 2015). It is believed that absorption of an antibiotic through the mucus layer, is more effective for H. pylori eradication than absorption through the

basolateral membrane (Prasanthi et al., 2011). Accordingly, preparing gastroretentive dosage forms is crucial for complete eradication of H. pylori. Some approaches have been proposed to increase the gastric residence time of anti H. pylori drugs. They include floating systems, e.g., tablets (Emara et al., 2014), minitablets (El-Zahaby et al., 2014a), liquid raft (Rajinikanth and Mishra, 2008; Rajinikanth et al., 2007; Dettmar and Lloyd-Jones, 1994), beads (Adebisi and Conway, 2013), microspheres (Tejaswi et al., 2011), mucoadhesion systems (Adebisi et al., 2015; Arora et al., 2012), and size increasing systems (El-Zahaby et al., 2014b). Among those systems, the floating raft system (FRS) is an advanced revolution in oral controlled drug delivery. It is a formulation of effervescent floating liquid with in situ gelling properties, which has been assessed for sustaining drug delivery and targeting. Moreover, the gels formed in situ remained intact for more than 48 h to facilitate sustained release of drugs (Ibrahim, 2009). The mechanism of the FRS involves the formation of a viscous cohesive gel in contact with gastric fluids, wherein each portion of the liquid swells forming a continuous layer called raft (Vinod et al., 2010). This layer floats on the gastric fluid because it has bulk density less than the gastric fluid, as low density is created by the formation of CO_2. So the system remains buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time (Pandey et al., 2012). The goal for designing this system is to reduce the frequency of dosing or to increase effectiveness of the drug by localization at the site of action, decreasing the dose required or providing uniform drug delivery. Metronidazole is an active adjunct in treatment of H. pylori (Emara et al., 2014). It offers the advantage of having pH independent activity (Prasanthi et al., 2011), unlike the anti-H. pylori antibiotic, clarithromycin (Rajinikanth and Mishra, 2008). The problems of bacterial resistance and side effects associated with metronidazole could be recovered by formulating gastroretentive floating system, since they provide adequate prolongation of drug release near the ecological niche of the bacterium (Rajinikanth et al., 2007). The aim of the present study was to prepare optimized floating system containing the anti-H. pylori drug metronidazole using the ion-sensitive in situ gel forming polymers sodium alginate and HPMC. The use of$CACO_3$ gas production and buoyancy, to optimize both the gelation capacity and release rate of the proposed metronidazole floating system was investigated.

II. MATERIALS & METHODS

MATERIALS: Metronidazole (Lincholin pharmaceuticals ltd, India) HPMC (Shin Etsu chemicals . corporation ltd) Sodium alginate (S.D Fine chemicals.ltd) Calcium carbonate (S.D Fine chemicals.ltd)

METHODS:

Composition of Metronidazole floating in situ gel :

s.no		F 1	F 2	F 3	F 4	F 5	F 6

1	Metronidazole	100mg	100 mg	100 mg	100 mg	100 mg	100 mg
2	HPMC	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v
3	Sodium alginate	0.5% w/v	1% w/v	1.5% w/v	2 % w/v	2.5 % w/v	3 % w/v
4	Calcium carbonate	2 % w/v	2% w/v	2% w/v	2% w/v	2% w/v	2% w/v
5	Purified water	100ml	100 ml	100ml	100 ml	100ml	100ml

Preparation of in situ gel:

The active ingredient (Metronidazole) was passed from sieve # 60 while other in active ingredients were passed from sieve # 40. Dissolve HPMC in distilled water. Then calcium carbonate and metronidazole (100mg) was added to it while stirring so that there was proper and homogenous dispersion of the active ingredient in the solution. In other beaker water was heated to NMT 60^0C on hot plate, dissolve sodium alginate in it and cool it to 40^0C .This solution was added to HPMC solution or vice-versa. This solution was mix well, and after cooling to 40^0C. Volume was adjusted to 100% with distilled water. Finally, the mixture was mixed well to get the final preparation. Different formulation batches from F1 to F6 were prepared by taking different proportions of sodium alginate and calcium carbonate according the selected design (see above table). The active ingredient and HPMC concentrations were common (100mg & 0.5%) for the formulations.

III. EVALUTIONS

i.Determination of drug content

Accurately, 10 ml of in situ gel from different batches (equivalent to 20 mg of metronidazole) were measured and transferred to volumetric flask. To this 50-70 ml 0.1 N HCL was added and sonicated for 30min. Volume was adjusted to 100mL. Complete dispersion of contents were ensured, visually and filtered using Whatmann Filter paper. From this solution, 10ml of sample was withdrawn and diluted to 100 ml with 0.1N HCl. Contents of Metronidazole was determined spectrophotometrically at 277 nm using double beam UV visible spectrophotometer.

ii.PH measurement

The P^H was measured in each of the solution of sodium alginate based in situ solutions, using a calibrated digital p^H meter at 27^0C.

iii.In vitro gelling capacity

The *in -vitro* gelling capacity of prepared formulations was measured by placing 5 ml of gelation solution (0.1N HCl, p^H 1.2) in a 15 ml of borosilicate glass test tube and maintained at 37±1^{0}C temperature. One ml of colored formulation solution was added with the help of pipette. The formulation was transferred in such a way that places the pipette at surface of fluid in test tube and formulation was slowly released from the pipette. As the solution comes in contact with gelation solution, it was known immediately converted into stiff gel like structure. The gelling capacity of solution was evaluated on the basis of stiffness of formed gel.

(+) Gels after few minutes, Dispersed rapidly

(++) Gelation immediate remains for 12 hours

(+++) Gelation immediate remains for more than 12 hours

iv.In- vitro floating ability

The in vitro floating study was carried out using 900ml of 0.1 N Hcl, (p^H 1.2).The medium temperature was kept at 37^{0}C . Ten milli liter formulation was introduced into the dissolution vessel containing medium without much disturbances . 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 noted.

v.Measurement of water uptake by the gel

The water uptakes by the gel of the selected formulations of sodium alginate were determined by a simple method. In this study the in situ gel formed in 40ml of 0.1N Hcl was used. From each formulation the gel portion from the 0.1N Hcl was separated and the excess Hcl solution was blotted out with a tissue paper. The initial weight of the gel taken was weighed and to this gel 10 ml of distilled water was added and after every 30 minutes of the interval was decanted and the weight of the gel was recorded and the difference in the weight was calculated and reported.

Vi.In-vitro drug release study

The release rate of metronidazole from in situ gel was determined using USP XXIV dissolution testing apparatus I (basket covered with muslin cloth) at 50 rpm. This speed was slow enough to avoid the breaking of gelled formulation and was maintaining the mild agitation conditions believed to exist in vivo. The dissolution medium used was 900ml of 0.1 N HCl, and temperature was maintained at 37^{0}C. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus at 1,2,3,4,5,6,7 & 8 hrs of dissolution. The samples were filtered through Whatmann filter paper and contents of was determined spectrophotometrically at 277nm using double beam UV- visible spectrophotometer.

IV.RESULTS:

Table-1: Properties of in site gelling formulations

Formulation code	Drug content (%)	P^H	Graded gel response	Floating lag time (sec)	Duration of floating (hrs)
F 1	94.11±0.26	7.53	++	20	24
F 2	98.23±0.74	7.49	++	25	24
F 3	92.12±0.65	7.52	++	30	24
F 4	98.89±0.35	7.59	+++	25	24
F 5	95.15±0.63	7.54	+++	18	24
F 6	99.03±0.46	7.56	++	25	24

Table-2: Percent cumulative drug release of different batches.

Time (hrs)	F 1	F 2	F 3	F 4	F 5	F 6
0	0	0	0	0	0	0
1	21.02	20.05	18.02	19.67	19.92	14.45
2	28.96	26.74	21.23	29.94	22.25	19.56
3	32.24	31.12	37.29	32.2	29.85	23.46
4	41.45	38.56	43.5	38.34	32.12	28.82
5	49.87	46.52	48.5	41.54	39.18	32.82
6	58.89	51.54	52.95	44.56	41.56	38.76
7	61.24	59.87	55.67	47.89	44.58	42.02
8	68.05	62.08	59.05	52.09	50.03	45.20

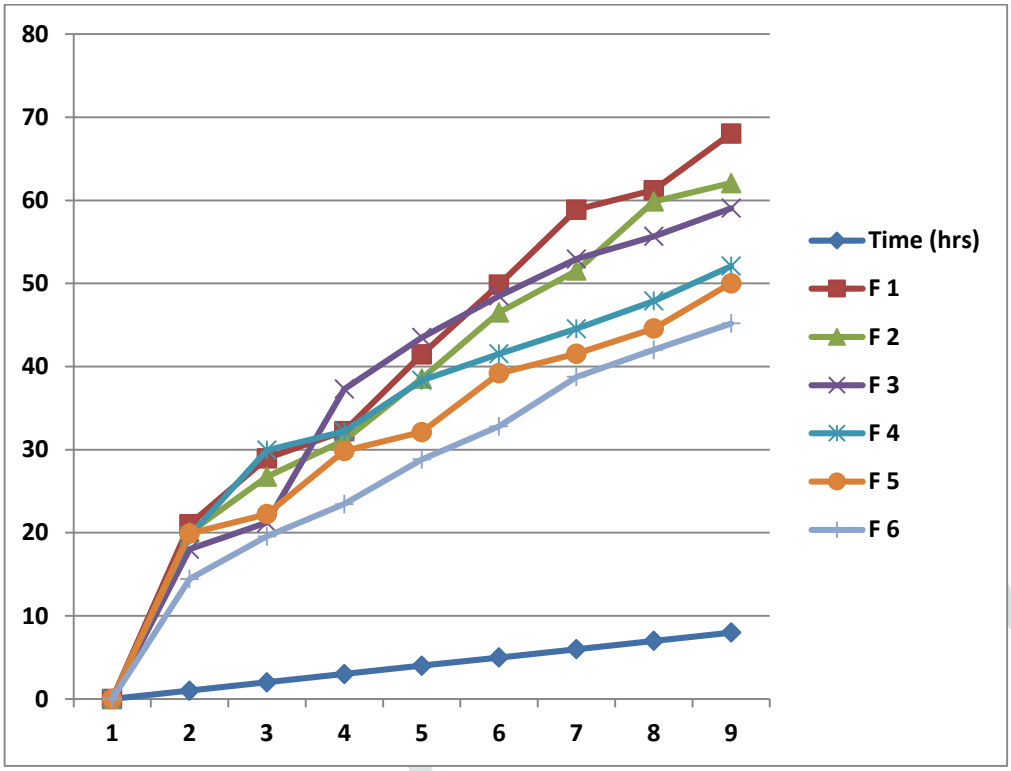


Figure-1

Table-3: Regression analysis data of formulations of Metronidazole

Formulation code	Regression coefficient	Zero order	First order	Higuchi equation	Peppas equation
F 1	r	0.961	0.966	0.961	0.972
F 2	r	0.960	0.985	0.960	0.987
F 3	r	0.923	0.938	0.923	0.952
F 4	r	0.877	0.978	0.877	0.959
F 5	r	0.877	0.987	0.915	0.987
F 6	r	0.957	0.966	0.957	0.970

Table-4: Percentage of water gain by different batches

Formulation code	Initial weight (gm)	Time (hrs)	% water gain
F1	50.34	0.5 1 1.5	5.435 5.306 5.345
F2	52.35	0.5 1 1.5	5.864 6.46 7.34
F3	48.94	0.5 1 1.5	5.89 6.678 5.675
F 4	55.48	0.5 1 1.5	5.897 7.865 5.674
F 5	52.64	0.5 1 1.5	8.724 9.235 11.65
F 6	53.24	0.5 1 1.5	5.876 5.244 5.887

V.DISCUSSION:

Determination of drug content:

This is one of an important requirement for any type of dosage form. Amount of the drug present in the formulation should not deviate beyond certain specified limits from the labeled amount. All formulations were found to having drug content in the range of 98-100% indicating homogenous distribution of drug throughout gel (Table-1) shows the drug content of the different batches.

P^H measurement

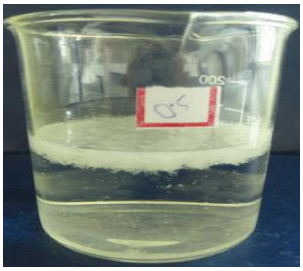
P^H was determined using calibrated digital p^H meter at 27^0C and it is listed in the (Table 1)

Gelling studies

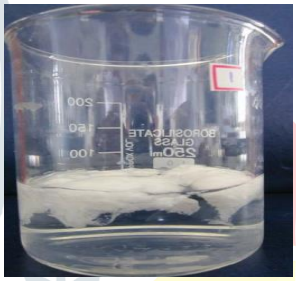
Gelling studies were carried out using 0.1N HCl, (p^H 1.2). In these studies the gelling capacity (extent and speed of gelation) for all formulation was determined. The in situ gel so formed should preserve it's integrity without dissolving or eroding so as to localize the drug at absorption site for extended duration. Gelation characteristic was assessed on an ordinal scale ranging between ---to +++ as shown table. After ingestion, the liquid polymeric solution should undergo a rapid sol-to-gel transition by means of ionic gelation (See Table 1)

In vitro Floating ability

Time taken by formulation to emerge on the medium surface (floating lag time) and time for which formulation continuously floated (duration of floating) are shown in the released CO_2 was entrapped in gel network producing buoyant formulation and then calcium ion reacted with sodium alginate produced a cross linked 3-D gel network and swelled structure that might further diffusion of CO_2 and drug molecule and resulted in extended period of floating and drug release respectively.



F1(0.5%)



F2(1%)

F3(1.5%)

F4(2%)

F5(2.5%)

F6(3%)

In vitro drug release

The effect of polymer concentration on *in vitro* drug release from in situ gels is shown in **(figure 1**) A significant decrease in the rate and extent of drug release was observed with the increase in polymer concentration and is attributed to increase in the density of the polymer matrix and also an increase in the

diffusion path length which the drug molecules have to traverse from various release patterns of formulations can be judged. Role of sodium alginate was primarily in formations of sol-gel phenomenon, but it also did affected release from formulations to some extent. From all above mentioned information, **F-5 was considered to the optimized formulation** (Table-2)

Drug release kinetics

The *in vitro* drug release data was subjected to goodness of fit test by linear regression analysis according to zero, first order, Higuchi, korosemeyer-peppas models to ascertain the mechanism of drug release. The results of linear regression analysis of data including regression coefficient are summarized in (Table-3). When the regression coefficient (r) value of zero and first or plots were compared, it was observed that the (r) value of zero order plots were found to be in the of 0.877-0.961 where as (r) values of first order plots were found to be in the range of 0.966-0.987 indicating drug release from all the formulations were found to follow first order kinetics. The good fit of the Higuchi model to the dissolution profile of all the formulations suggested that diffusion is the predominant mechanism limiting drug release since the (r) values of Higuchi were nearer to unity. The *in vitro* dissolution data as log cumulative percent drug release vs log time were fitted to Korsemeyer-peppas equation, values of the exponent (n) was found to be in the range of 0.952-0.987 indicating that the drug release is by non Fickian diffusion mechanism.Among various **formulations F5 was regarded as ideal form exhibited flotation lag time of 18 sec with floatation time for 24 hrs.**

Water uptake study

Release of the drug from the polymer matrix depends on the amount of water associated with the system. The release of the drug may involve the penetration of water into the matrix and simultaneously release of the drug via diffusion or dissolution. The water associated with the formulation at any point in the time can be determined by thermo gravimetric analyzer but in this present study a simple test was done for the selected batches of sodium alginate based in situ gel of metronidazole. Water uptakes by the different batches were listed in (Table-4) From the table it is clear that water uptake by F 5 batch at 1.5 hours is 11.65% and it is having maximum water uptake capacity.

VI. CONCLUSION

In this study gastric specific in situ gel of Metronidazole, which is used in the treatment of Gastro intestinal diseases, prepared by using HPMC and sodium alginate as a release retardant, it was found that increase in concentration of polymer decreases the drug release .Calcium carbonate acts as cross linking agent. **Optimized formulations F4 (2% sodium alginate), F5 (2.5% sodium alginate), F6(3% sodium alginate) were** liquid

before ingestion and underwent rapid gelation when it reaches the stomach, the formulations having good in situ gelling capacity, optimized formulations were showed sustained drug release over 8hrs period.

Hence from above results we can conclude that it is possible to formulate in situ gel of Metronidazole for gastric specific drug delivery by using HPMC & Sodium alginate.

CONFLICTS OF INTEREST:

There is no financial conflict of interest. This project has not been submitted to any funding agency. This research project is a part of the my graduation (B-pharmacy) dissertation work in the area of Pharmaceutical Sciences.

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