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Formulation and Evaluation of Oral In Situ Gel of Metronidazole

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ABSTRACT

Efficient Helicobacter pylori elimination requires delivery of the antibiotic locally in the stomach. High dose of metronidazole (250 to 750 mg) is difficult to incorporate in floating tablets but can simply be given in liquid dosage form. By keeping the above observation in mind, we made an attempt to build up a new raft forming oral in situ gelling system of metronidazole with improved residence time using sodium alginate as gelling polymer to eliminate H. pylori. Methods: oral in situ gelling formulations were prepared using sodium alginate, xanthan gum, calcium carbonate, and sodium bicarbonate. Prepared formulations were evaluated for density, viscosity, floating lag time, floating duration, swelling index and in vitro drug release. Results. All formulations (F1–F12) showed floating within 180 s and had floating duration of more than 24 h. every formulations showed excellent pourability. It was observed that concentration of sodium alginate and xanthan gum had major influence on floating lag time, cumulative percentage drug release and other evaluation parameters. The batch F11 was optimized since it have good pourability with extended release of 10 hrs. Conclusion: oral in situ gelling system of metronidazole can be formulated by use of sodium alginate as a gelling polymer and xanthan gum as release retardant to control the drug release for more than 10 hrs.

Keywords: H. pylori , oral in situ, metronidazole, xanthan gum raft forming system.

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INTRODUCTION

Oral route is the most commonly adopted and most convenient route for the drug delivery. Oral route of administration has received more attention in pharmaceutical field because of the more flexibility in the designing of dosage form than drug delivery designs for other routes.[1]

Controlled Drug Delivery**:** The controlled drug delivery system is proposed to control on drug released in the body. In other words; this system attempts to regulate drug concentration within tissue or cell.[2]

Raft forming system which involves the creation of cohesive gel in contact with gastric fluids in which each part of liquid swells and forms a continuous layer called raft. Raft floats on the gastric fluids as of low bulk density produced by carbon dioxide (CO_2) formation. It contains a gel forming agent and alkaline bicarbonates or carbonates responsible for formation of CO_2 to make the system less dense and float on gastric fluids. This type of system contains a gel forming agent e.g. alginic acid, sodium bicarbonate and acid neutralizer, which create a foaming sodium alginate gel when it comes in contact with gastric fluids.[6,7]

In situ gel drug delivery system promotes simplicity and ease of administration, release of accurate dose as well as prolongation of residence time of drug in contact with mucosa.[9]. The creation of gels depends on factors like pH change, presence of ions, temperature modulation, and ultra violet irradiation, from which the drug released in a sustained and controlled manner [10].

Metronidazole a BCS class l drug is an antibiotic, amebicide, and antiprotozoale.[11] It is the drug of choice for mild-to-moderate Clostridium difficile infection. [12] Metronidazole is indicated for the management of Helicobacter pylori eradication therapy, as part of a multi-drug treatment in peptic ulcer disease .It is typically taken two or three times a day.[13]

The unionized metronidazole having ability to get reduced inter-cellulary, this reduced form covalently binds to DNA and destroy its helical structure which prohibit bacterial nucleic acid synthesis and resulting in bacterial cell death. [14]

The current research work deals with formulation and evaluation of sodium alginate and xanthan gum based floating oral *in situ* gel of metronidazole in which calcium carbonate was used as a source of Ca2+ ions and as a cross-linking agent.[15] The gel was evaluated for parameters like gel strength, density, floating lag time, floating duration, , pH, *in vitro* drug release, viscosity, drug content, and *in vitro* gelling capacity etc .

MATERIALS AND METHOD

Drug Metronidazole was purchased from Yarrow chem . (New Delhi, India). Sodium alginate,

sodium bicarbonate was purchased from Thomas and beker(St. Louis, USA). Xanthan gum was a gift from Hi media . sodium saccharin was purchased from chemy lab .Distilled water used in the formulations was of HPLC grade (Merck) and all other chemicals used in formulation were of analytical grade.

Method

Preparation oral *in situ* gelling solution

A in situ gel containing 250mg/10ml Metronidazole was prepared using different polymer concentration as shown in formulation table. Drug was dissolved in 60 ml of water in a beaker(A) which contain sodium bi carbonate, methyl paraben, propyl paraben at 70ºC on magnetic stirrer. Xanthan gum was socked for 2 hrs in remaining quantity of water in a beaker (B). Solution A was added to solution B at 70ºC.Calcium carbonate and sodium saccharine was added with flavoring agent at 40ºC.

The initial screening and selection of polymers were done based on their swelling and gelling properties and release behavior. From the initial observation two polymers were selected for further optimization of formulation batches. Polymers are Sodium alginate and Xanthan gum. The different concentration of each polymer was also further explored for their role in drug release along with their swelling capacity. (Table 2)

Characterization of oral *in situ* gel

Fourier transmission infrared (FT-IR) spectroscopy: [16]

The identity of drug was established by comparing IR spectrum of drug with reported spectrum of Metronidazole as shown in Fig & Table gives the functional groups. Using the KBR pellet, the IR spectrum of drug sample was recorded at a resolution of 4 cm^{-1} and the principle peaks were measured using FTIR Spectrophotometer.

Appearance [17,18] **:**

All formulations were evaluated for clearness by visual observation against a black and white surroundings.

PH [19]**:**

The PH of formulations was measured using a calibrated digital PH meter at RT. The measurement is carried out in triplicate and average values are taken.

Floating lag time[18]**:**

Floating lag time was carried out using 0.1 N HCL (pH1.2). The medium temperature was kept at 37ºC. 10 ml formulation was introduced into the dissolution vessel containing medium with no much disturbance. The time the formulation took to come out on the medium surface (Floating lag

time) and the time the formulation continuously floated on surface of the medium (Floating duration) were noted.

Sol to gel time[20]

In vitro gelation time was estimated by means of USP (Type II) dissolution apparatus containing 500 mL of 0.1N HCl (pH 1.2) at 37±0.5ºC it was observed that within part of seconds the solution transformed into gel later it floated in medium. As the formulation was comes in contact with 0.1N HCl, (pH 1.2) it changed from sol to gel and time was calculated.

Rheological behavior[21,22] **:**

The viscosities of the different formulations were determined by use of Brook field viscometer (Model RVDV-II+P). The samples (100 ml) were sheared at a rate of 100 rpm using spindle no 64 at room temperature. Viscosity estimation for each sample was performed in triplicate, and average was choosen.

Density [19,20]**:**

For stomach specific system density is an important consideration and it should be less than the stomach fluid density (< 1.004). The Densities of all formulations were calculated by forming gel of 10ml formulation was placed in measuring cylinder and weight of formed gel was noted with the help of calibrated balance. Finally, the densities of different formulations were calculated in triplicate.

Swelling index [20, 23]**:**

A gel of 100mg was formed and weighed precisely (W1). It was reserved in a beaker and 50ml of 0.1 N HCl was added. The beaker was set aside for 24 hrs. The weight of swollen matrix gel (W2) was found and swelling index was calculated by following formulae: Swelling Index = (W2-W1/W1)×100 Where, W1 = initial weight of gel (100mg), W2 = weight of bloated matrix after 24 hrs

Drug content [24,25]**:**

Ten mL of the formulation was added to 900 mL of simulated gastric fluid (0.1N–1HCl, pH 1.2) and stirred for 1 h on a magnetic stirrer. The solution was filtered, appropriately diluted with simulated gastric fluid and the drug concentration was calculated by use of a UV-visible spectrophotometer at 227nm against a suitable blank solution.

Measurement of in vitro drug release [26,27]

The release of metronidazole from the in-situ gel preparations was determined using USP dissolution test apparatus (USP XXIV) with a paddle stirrer at 50 rpm. This speed was slow enough to avoid the breaking of gelled formulation and will be maintaining with the mild agitation

conditions believed to exist in vivo. The dissolution medium used will 900 ml of 0.1 N HCl (pH 1.2), and temperature were maintained at 37 C. 10 ml formulation was added to the medium.. At each time interval, a precisely measured sample of the dissolution medium will removes and replace with prewarmed (37 C) fresh dissolution medium. Absorbance of metronidazole withdrawn samples was measured using UV Visible Spectrophotometer.

In vitro drug release kinetic studies [28,29]:

Kinetic model had described drug dissolution from In Situ Gel formulation where the dissolved quantity of drug is a function of experiment time. In order to study the exact mechanism of drug release from the formulation, drug release data was analyzed by Zero order, first order, korsmeyer peppas and Higuchi square root. The motive for selecting the most suitable model was chosen on the basis of goodness of fit test. The obtain data were processed for regression analysis by MS EXCEL statistical function.

Short -term stability studies [28,30]:

To assess stability of drug and formulation, stability studies were performed as per ICH guidelines. The optimized formulation was tested for a period of 3 months at different temperature and humidity conditions for their drug content and other parameters.

Screening of polymers [18,31]

Table 1 : Swelling Index and Gelling Properties of Polymers n=3

Sr no	Polymer	Gelling property	Swelling index(%)
1	Sodium alginate	+++	98.4±0.2
2	HPMC K4M	++	84.68±0.15
3	HPMCK100	++	89.01±0.19
4	Guar gum	++	91.03±0.21
5	Gellan gum	+	82.78±0.1
6	Pectin	+++	97.59±0.18
7	Methyl cellulose	+	87.2±0.09
8	Xanthan gum	+++	99.25±0.21

Fair = +; Good = ++; Excellent = +++

Table 2: Release of drug at different concentrations of polymers (n=3)

Sr no	Polymer	Concentration with 2.5% sodium alginate	In vitro drug release in 1 hr. (%)
1	HPMC K4M	1%	77.56±0.12
2	HPMCK100	1%	72.15±0.09
3	Guar gum	1.5%	67.84±0.14
4	Gellan gum	0.75%	92.30±0.18
5	Pectin	3%	69.26±0.08
6	Methyl cellulose	0.8%	86.45±0.17
7	Xanthan gum	1.5%	41.4±0.10

Table 3: Formulation table for Metronidazole floating in situ gel formulation in %w/v

Sr no	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
1	Drug	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
2	Sodium alginate	2.5	2	1.5	1	2.5	2	1.5	1	2.5	2	1.5	1
3	Xanthan gum	0.5	0.5	0.5	0.5	0.75	0.75	0.75	0.75	1	1	1	1
4	$CaCO_3$	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
5	$NaHCO_3$	1	1	1	1	1	1	1	1	1	1	1	1
6	MP:PP	9:1	9:1	9:1	9:1	9:1	9:1	9:1	9:1	9:1	9:1	9:1	9:1
7	Sodium saccharin	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
8	Distilled water	Qs.	Qs.	Qs.	Qs.	Qs.	Qs.	Qs.	Qs.	Qs.	Qs.	Qs.	Qs.

Figure 1: Different Formulation Batches (F1-F12)

RESULTS AND DISCUSSION

Calibration curve[32]

The calibration curve of metronidazole in 1.2 pH HCl was plotted (Fig: 5) and the regression coefficient was found to be 0.997

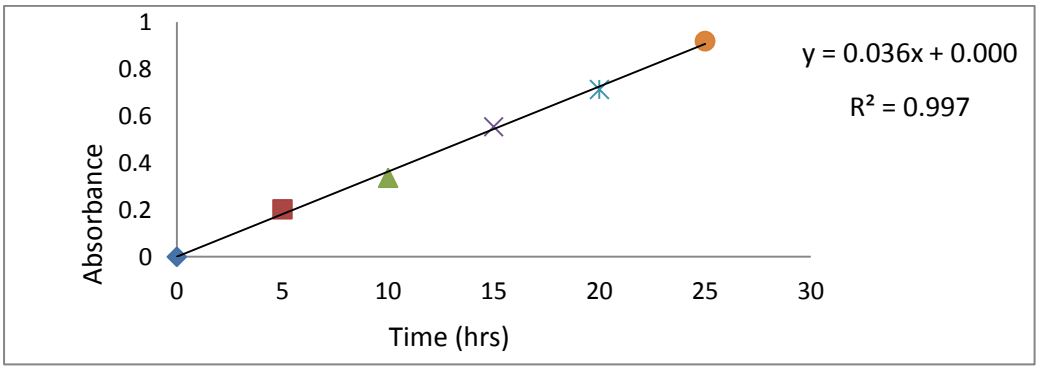

Figure 2: Calibration curve of Metronidazole

Fourier Transformed Infrared (FTIR) Spectroscopy and Differential Scanning Calorimetry (DSC) studies[33]

The pre formulation studies were conducted for drug and polymer interaction, these were evaluated with the help of FTIR and DSC. Based on these results drug and polymer interaction were not occurring.

The major functional groups in metronidazole are 3436.53 N-H Stretching, 3208.97 O-H Alcohols, 2970.8 CH_2 Stretching, 1722.12 C=O Aldehyde, 1688.37 C=N Imines, 1378.85 N=O (R-NO_2), 858.168 C-H (Aromatics), 765.601 C-H (Alkenes).

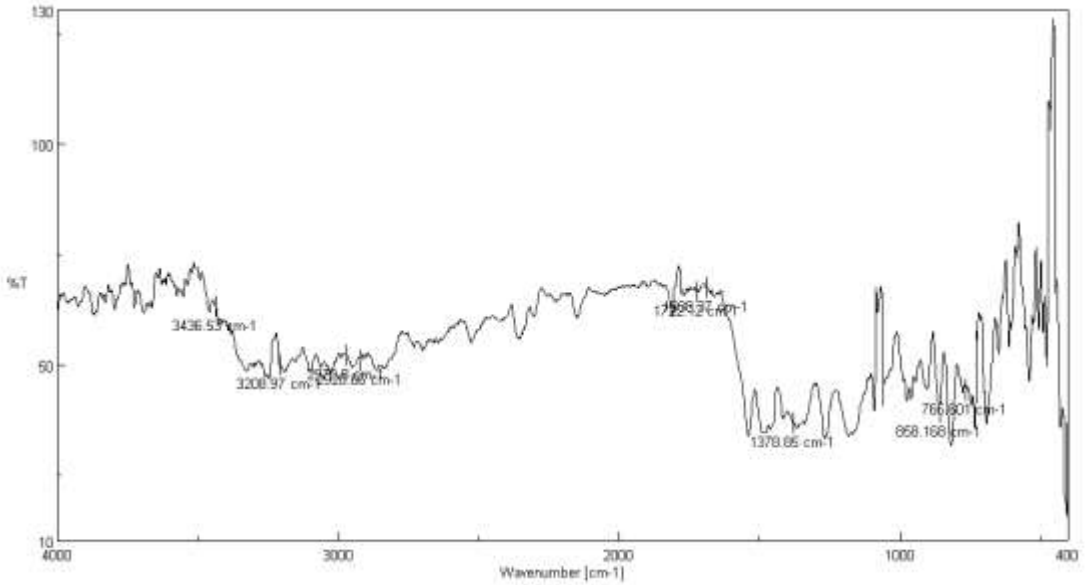

Figure 3: FTIR Spectrum of Metronidazole

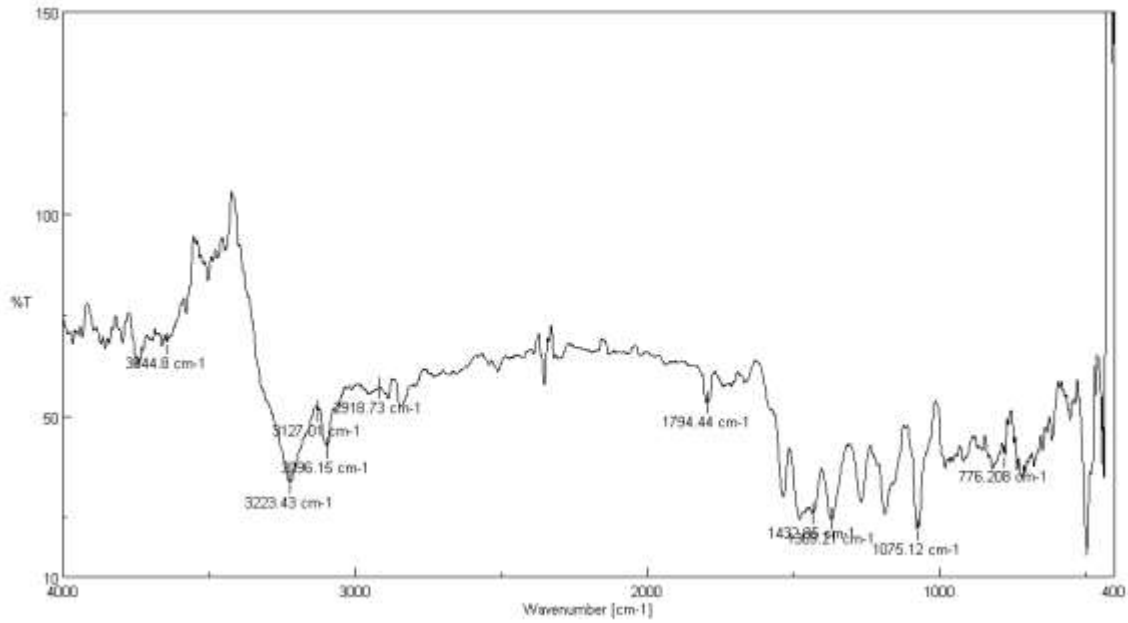

Figure 4: FTIR Spectrum of Metronidazole and sodium alginate

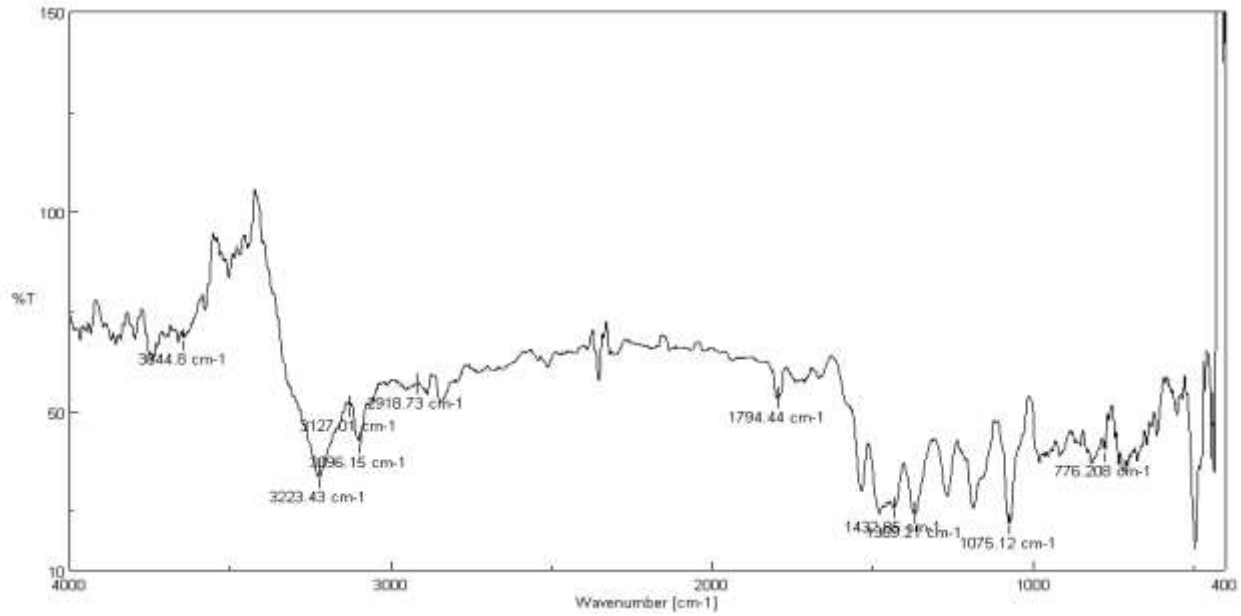

Figure 5: FTIR spectrum of Metronidazole and Xanthan Gum

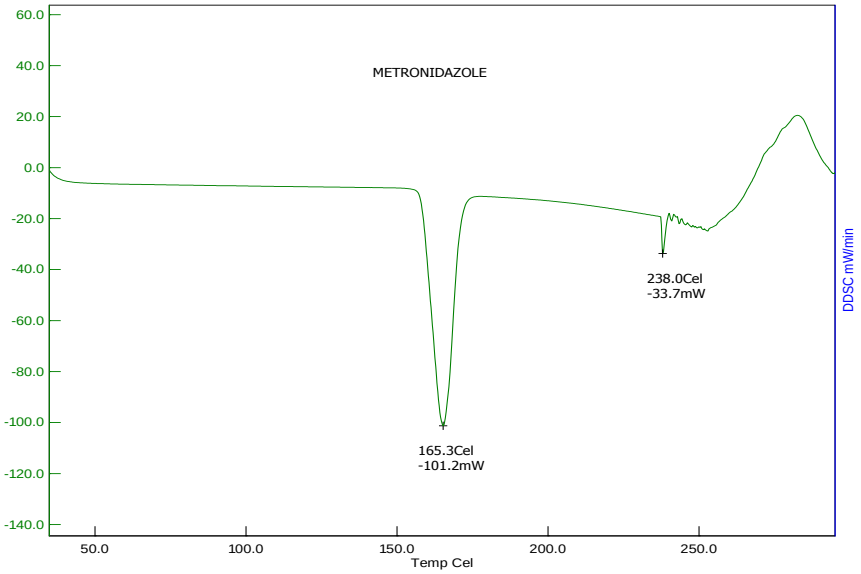

Figure 6: DSC of Metronidazole

Physical Appearance, PH and Drug Content

All formulations (F1-F12) were found to have creamy appearance and it was found that pH was in the acceptable range of 7-8.The percent drug content of all formulations was present in the range of 96.5-99.8%,which shows loss of drug during the formulation .

In Vitro Gelation Study[24]

Gelling studies were conducted using 0.1N HCl (pH 1.2) and the obtained data were shown in Table 4. All formulations showed instantaneous gelation when it make contact with acidic medium and formed gel maintained its integrity. Gelation occurs by solubilization of insoluble calcium

carbonate in acidic medium releasing carbon dioxide and calcium ions. The calcium ions act together with the anionic polymer eg. Sodium alginate in the formulation which causes instantaneous gelation and provide a gel barrier that control drug release. % Swelling index of different formulations were shown in table 5

Table 4: Evaluation parameters of formulations (n=3)

Formulations	Appearance	PH	Floating lag time (Sec)	Floating buyoncy time (hrs)	Drug content(%)
F1	+++	7.6±0.057	110±0.81	>24	98±0.081
F2	+++	7.3±0.06	150±1.05	>24	99±0.082
F3	+++	7.4±0.08	180±1.82	>24	98.3±0.08
F4	+++	7.7±0.077	120±1.25	>24	99.2±0.084
F5	+++	7.2±0.09	60±1.2	>24	99.8±0.081
F6	+++	7.5±0.088	90±1.05	>24	97.1±0.080
F7	+++	7.6±0.091	108±1.8	>24	98.5±0.079
F8	+++	7.8±0.099	188±1.5	>24	99.6±0.083
F9	+++	7.5±0.079	88±2.20	>24	98.5±0.079
F10	+++	7.4±0.1	100±1.61	>24	96.5±0.081
F11	+++	7.8±0.18	120±0.81	>24	97.8±0.080
F12	+++	7.6±0.063	155±1.85	>24	98±0.081

+ Poor, ++ Acceptable, +++ Good

Viscosity Studies[34]

The formulation should have favorable viscosity that will permit ease of administration and swallowing as a liquid and produces acceptable gel strength. Results of viscosity for formulations F1 to F12 are shown in Table 5.

Density

The main condition of any floating system is that it should have density lesser than that of gastric contents (~1.004).The density of all floating in situ gel formulations were less than that of gastric contents.gm/cm3 This can obviously be a indicator to the floating ability of the formulations. The densities of F1-F12 formulations were found to be 0.792 to 0.998 gm/cm3 (Table 5).

Table 5: Evaluation parameters of formulations (n=3)

Formulations	Sol to gel (sec)	Viscosity cps	Density	Swelling index (%)
F1	5±0.61	360.9±0.05	0.871±0.09	92.85±0.1
F2	3±0.67	401.9±0.06	0.982±0.1	90.63±0.3
F3	3±0.7	537.9±0.07	0.792±0.3	95.0.3±04
F4	2±0.59	551.9±0.09	0.961±0.5	91.88±0.1
F5	2±0.66	557.55±0.1	0.884±0.2	96.02±0.3
F6	4±0.57	635.9±0.8	0.985±0.09	93.25±0.2
F7	3±0.70	641.9±0.1	0.978±0.08	91.05±0.1

F8	2±0.61	730.6±0.09	0.964±0.05	92.5±0.1
F9	5±0.59	643.4±0.12	0.992±0.06	98.03±0.5
F10	3±0.64	635.9±0.08	0.998±0.04	94.55±0.3
F11	2±0.70	737.8±0.05	0.891±0.09	96.86±0.2
F12	4±0.68	743.8±0.11	0.968±0.06	94.02±0.1

In-Vitro Drug Release[35]

The in-vitro drug release of the in situ floating gel was carried in 0.1N HCl from 0.5 to 10 hrs by USP type-II apparatus. The plot of percent Cumulative drug release v/s time (hrs) was plotted as shown in figure 8, 9, and 10. Initially, the drug was released at a more rapidly due to the burst effect. The drug release from the gel was seen by an initial phase of high release (burst release). However, drug was release was at a slower rate in the second phase due to swelling behavior of gelation polymer, which results in moderate release rate. The initial burst release was significantly reduced with an increase in polymer concentration.

Table 6: Dissolution Study of different formulation batches (n=3)

Hour	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	80.9±0.12	76.8±0.08	77.8±0.15	50±0.14	46.7±0.2	58±0.18	55.8±0.1	68.6±0.094	43.93±0.11	26.30±0.09	40.3±0.1	52.2±0.18
1	87.79±0.10	84.85±0.1	79.25±0.12	66.95±0.11	59.38±0.16	70.7±0.2	67.52±0.096	88.16±0.17	65.32±0.17	33.79±0.1	47.84±0.2	64.58±0.098
2	86.80±0.11	96.78±0.09	87.12±0.1	78.99±0.09	69.67±0.09	83.22±0.11	76.46±0.12	96.16±0.1	74.83±0.2	44.66±0.2	56.27±0.17	78.89±0.1
3	88.35±0.13	98.84±0.12	91.07±0.08	87.33±0.06	75.29±0.08	89.83±0.09	79.99±0.09	96.86±0.08	83.96±0.14	50.25±0.087	66.58±0.11	87.65±0.17
4	89.830.11	98.20±0.08	92.94±0.06	97.80±0.08	79.047±0.2	93.104±0.12	81.86±0.07	102.3±0.084	85.41±0.09	56.25±0.096	71.82±0.096	97.50±0.09
5	90.970.±10	99.89±0.1	95.08±0.1	98.95±0.1	80.69±0.14	95.30±0.09	88.23±0.1		90.51±0.087	63.36±0.10	77.60±0.1	99.92±0.088
6	91.36±0.2	101.78±0.06	98.42±0.09	101.2±0.09	90.8±0.1	96.11±0.05	91.17±0.15		91.85±0.069	69.13±0.11	80.92±0.17	101.05±0.078
7	94.42±0.15				90.44±0.081	98.83±0.1	94.23±0.18		92.83±0.074	69.39±0.2	82.38±0.1	
8	97.49±0.2				96.56±0.096	96.91±0.08	97.92±0.05		94.26±0.065	70.15±	85.67±0.098	
9					102.08±0.05				96.64±0.1	83.28±	87.04±0.087	
10									98.81±0.098	88.155±0.14	93.25±0.099	

Kinetic analysis of release data[36,37,38,39]

The dissolution drug release profile was plotted as cumulative % drug release v/s time curve as depicted in figure 8, 9, 10. The dissolution data so obtained was fitted to different kinetic models for example Zero Order, First order, Higuchi, Korsmeyer-Peppas models. Results were shown in table 7. The model that best fitted the release data was preferred based on the correlation coefficient value (^{r2}) obtained from various kinetic models. In-vitro drug release profile from optimized formulation could be best expressed by Korsmeyer-Peppas and Higuchi equation as plot showed maximum linearity with r^2 value 0.921-0.994.

Table 7: Kinetic release data of different model for formulation F11

Model	Slope	Coefficient of regression(R^2)
Zero order	5.081	0.921
First order	-0.086	0.969
Korsmeyer peppas	0.28	0.994
Higuchi	20.85	0.984

Stability studies [39,40,41]

Finally, optimized F 11 batch was subjected to stability studies according to the ICH guidelines and results of the various evaluation parameters were shown Table 8, 9 & 10. After stability studies, the formulations F 11 was more stable at accelerated condition.

Table 8: Stability studies at 25^{0}C and 60% RH

Parameters	Initial	1 month	2 month	3 month
Appearance	+++	+++	+++	+++
PH	7.8±0.18	7.9±0.09	7.9±0.9	7.8±0.9
Floating lag time	120±0.81	121±0.14	125±0.2	130±0.1
Floating duration	>24	>24	>24	>24
Viscosity	737.8±0.05	738.5±0.08	738±0.12	737±0.09
Density	0.891±0.09	0.887±0.18	0.878±0.13	0.880±0.10
Swelling index	96.86±0.2	96.5±0.1	96.3±0.03	95.9±0.02
Drug content	97.8±0.080	97.2±0.11	97.6±0.06	96.6±0.9
In vitro drug release	93.25±0.099	94.01±0.2	93.8±0.02	92.3±0.01

Table 9: Stability studies at 30^{0}C and 65%RH

Parameters	Initial	1 month	2 month	3 month
Appearance	+++	+++	+++	+++
PH	7.8±0.18	7.7±0.10	7.8±0.08	7.8±0.2
Floating lag time	120±0.81	119±0.15	122±0.1	125±0.02
Floating duration	>24	>24	>24	>24
Viscosity	737.8±0.05	736.5±0.1	736.9±0.1	735.9±0.09
Density	0.891±0.09	0.877±0.08	0.879±0.1	0.880±0.09
Swelling index	96.86±0.2	95.95±0.08	96.3±0.08	95.5±0.1
Drug content	97.8±0.080	96.2±0.01	96±0.09	96.6±0.1
In vitro drug release	93.25±0.099	92.01±0.06	94.2±0.08	93.2±0.09

Table 10: Stability studies at 40^{0}C and 75%RH

Parameters	Initial	1 month	2 month	3 month
Appearance	+++	+++	+++	+++
PH	7.8±0.18	7.6±0.09	7.7±0.08	7.8±0.01
Floating lag time	120±0.81	129±0.14	123±0.3	130±0.05
Floating duration	>24	>24	>24	>24
Viscosity	737.8±0.05	740.5±0.12	739.2±0.09	738.2±0.1
Density	0.891±0.09	0.899±0.2	0.889±0.12	0.890±0.10
Swelling index	96.86±0.2	95.85±0.09	96.2±0.09	95.06±0.19
Drug content	97.8±0.080	96.54±0.08	95.9±0.08	94.9±0.12
In vitro drug release	93.25±0.099	94.89±0.1	93.65±0.2	92.50±0.10

Figure 7: Optimized Batch F 11

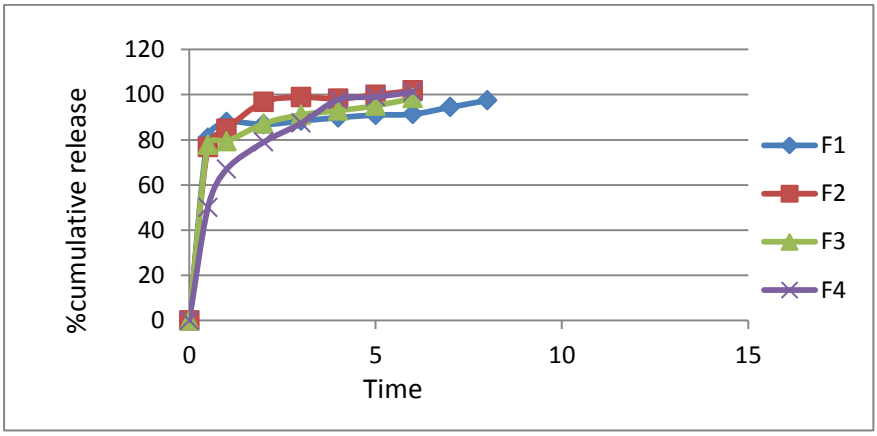

Figure 8: In vitro drug release of formulation F1-F4

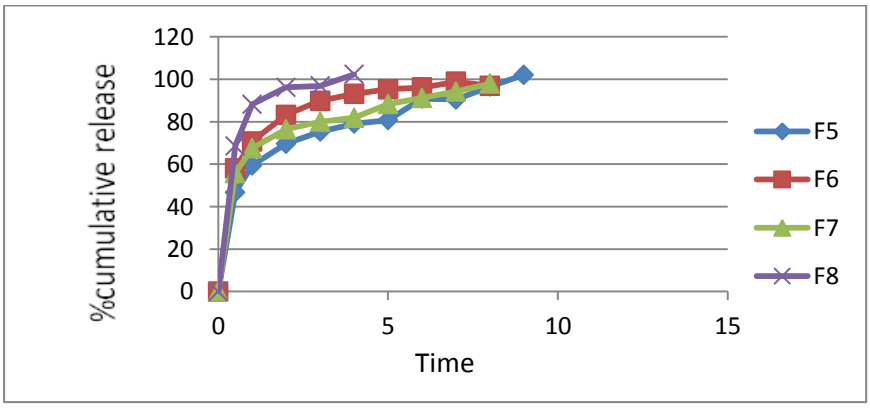

Figure 9: In vitro drug release of formulation F5-F8

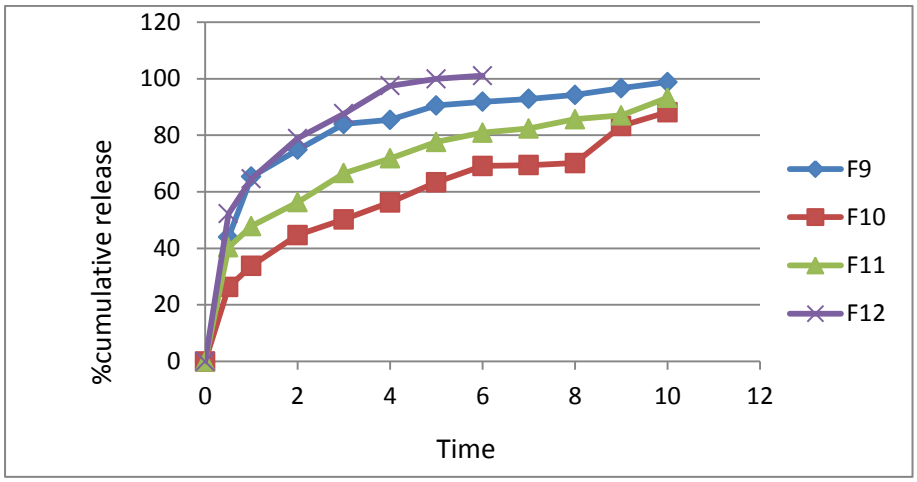

Figure 10: In vitro drug release of formulation F9-F12

CONCLUSION

Controlled release floating in situ gel formulation of Metronidazole was prepared and evaluated Using different evaluation parameters and it was found that batch F11 was optimized and shows Drug release over a period of 10 hrs.Retention time of drug in stomach was increases hence site Specific delivery of Metronidazole was achieved Hence this formulation can helpful for better eradication of H pylori infection. Kinetics studies are done and optimized batch shows release mechanism by korsmeyer peppas model. Stability studies for optimized batch are done for 3 months; optimized formulation is stable over various conditions.

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