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Development and Characterization of Floating In-Situ Gelling System for Controlled Delivery of Metronidazole

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ABSTRACT
The drugs having a narrow absorption window in the gastrointestinal tract (GIT) when administered by oral route are often limited by poor bioavailability due to incomplete drug release and short residence time at the site of absorption. Novel drug delivery systems in the form of gastroretentive systems such as floating systems, mucoadhesive, high-density, expandable have been developed as they provide controlled delivery of drugs with prolonged gastric residence time. Liquid orals are more prone to low bioavailability because they are eliminated quickly from the stomach since they are subjected to faster transit from the stomach/ duodenum. The problems of immediate release and short gastrointestinal residence of liquids are eliminated by formulating as oral in situ gels as they provide the best means to overcome these problems The in situ gel dosage form is a liquid before administration and after it comes in contact with gastric contents due to one or more mechanisms gets converted to gel which floats on gastric contents. This achieves increased residence as well as sustained release.

Keywords :floating drug delivery, gastric retention, in-situ gel

I. INTRODUCTION

The development of floating in-situ gel systems has received considerable attention, mainly due to the advantages shown by these systems such as the ease of administration along with the ability of providing controlled action compared to conventional drug delivery systems; these factors led to reduced frequency of administration and therefore improved patient compliance and comfort. 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.

Oral route remains the preferred route for the administration of therapeutic agents because low cost of therapy and ease of administration leads to higher level of patient compliance. The high level of patient compliance has been observed in taking oral dosage forms that is due to the ease of administration and handling of these dosage forms.

Floating drug delivery systems are aimed to retain the drug in the stomach and are useful for drugs that are poorly soluble or unstable in intestinal fluids. The underlying principle is very simple i.e. to make the dosage form less dense than the gastric fluids so that it can float on them. However, this system is not ideal because its performance is highly dependent on the presence of food and fluid in the stomach.1

Metronidazole has been used as an antibiotic for several decades, with added anti-parasitic properties that set it apart from many other antibacterial drugs, allowing it to treat a wide variety of infections. It is available in capsule form, tablet form, topical form, and suppository preparations for the treatment of various infections. It is also used off-label in the treatment of Crohn's disease and rosacea, as a prophylactic agent after surgery, and in the treatment of Helicobacter pylori infection.

MECHANISM OF ACTION

The exact mechanism of action of metronidazole has not been fully established, however, it is possible that an intermediate in the reduction of metronidazole which is only made by anaerobic bacteria and protozoa, binds deoxyribonucleic acid and electron-transport proteins of organisms, blocking nucleic acid synthesis. Metronidazole is less than 20% bound to plasma proteins. The elimination half-life of metronidazole is 7.3 ± 1.0 after a single 500mg IV dose in healthy subjects. When administered orally in the tablet form, metronidazole is absorbed entirely absorbed, showing a good bioavailability.[2]

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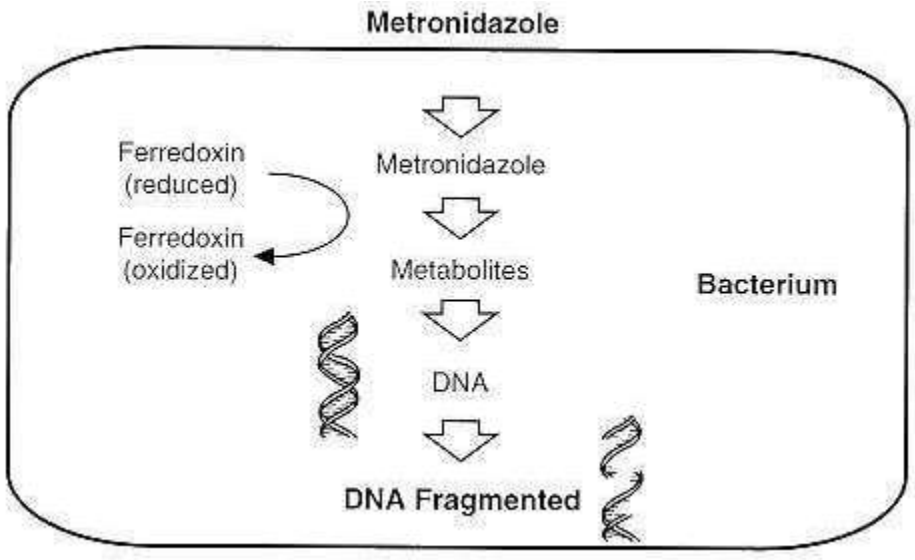

Fig1:mechanism action of drug metronidazole

II. MATERIALS AND METHOD

Metronidazole is a gift sample from banglore, sodium alginate from rolex chemical industries Mumbai, micro crystalline cellulose lobapvt.ltd Mumbai, gum tragacanth from lobachemiepvt.ltd Mumbai, sodium bicarbonate and calcium carbonate fromqualigens fine chemicals Mumbai, all the chemicals were used for in-situ gel preparation.

METHOD: Metronidazole in-situ gel will be prepared by

- Hydrogel method
- Ionic cross linking method
- Ph triggered system

HYDROGEL METHOD:

Floating**insitu**gel can be prepared by hydro gel method by taking around 75%of water, a measured quantity of sodium alginate (SA)required to make a 2%w/v solution was dissolved in distilled water at 60℃ using heating magnetic stirrer. After cooling at below 40℃,appropriate amounts of polymer(EC), methyl paraben and propyl paraben(ratio of 9:1),the drug metronidazole along with gas generating agent (calcium carbonate with or without sodium bicarbonate)were dissolved uniformly into sodium alginate solution with continuous stirring. The stirring was continued after complete addition until uniform dispersion was obtained and dispersion was allowed to cool at room temperature. finally, the volume was adjusted to 100%with distilled water and mixture was mixed well to get final preparation which was stored in amber colour bottles until further use.

PREFORMULATION STUDIES:

Identification test:

Identification of metronidazole was done by UV, DSC, and FTIR and confirmed as per monographs.

solubilityanalysis:

solubility analysis of metronidazole was carried out in various solvents.as a results 10mg of metronidazole was dissolved in water, alcohol, acetic acid, and dichloromethane, and very slightly soluble in ether.

Melting point determination:

The capillary method is used for the determination of melting point of metronidazole. A little amount of compound was placed in thin walled capillary tube of about 10-15cm long and 1mm inside diameter and closed at one end. The capillary tube containing sample and thermometer is then suspended in oil bath containing liquid paraffin.so, they can be heated slowly and evenly. The temperature range over which the sample is observed to melt is taken as the melting point.

Drug –excipients interactions studies by fouriertransform infrared (FTIR):

The FT-IR spectrum of metronidazole has been recorded with a fourier transform IR with in spectrophotometer with in range of 4000-400$cm^{-1}$by utilizing the method of KBrpellet. This method took small amount of drug (approx..1mg) in a

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mortar, and KBr was added to it in the ratio1:10, followed by trituration with a pestle. the mixture was put in a dye cavity and pressed with KBr press under pressure of 4-5 tons, leading to thin film formation. This film was placed in the sample compartment, and FT-IR was performed in the mentioned range to obtained spectrum of the drug and the physical mixture of the excipients.

Thermal analysis by dsc:

DSC employed in a thermal analytical process to get the thermo program metronidazole by using aluminium pans. In this technique, an accurate weight of the sample was taken in the aluminium pans sealed tightly.to determine the thermal behaviour of the drug, the aluminium pans containing sample were heated at temperature range of 40-300℃, with a scanning rate of 20℃/min. nitrogen gas was purged continuously at a 40ml/min flow rate to provide an inert atmosphere.

Quantitative estimation of metronidazole by UV–spectrophotometric method

Determination of λ max

Metronidazole 20µg/ml concentration was prepared in 0.1N HCL. The solution was scanned from 400to200nm by uv spectrophotometer and spectrum was absorbed for absorption maxima.

Standard calibration curve of metronidazole

Calibration curve was constructed by using 0.1NHCL 100 mg accurately weighed metronidazole was dissolved in 0.1NHCL and the volume is made up to 100ml with 0.1NHCL and filtered using whatman filter paper and solution was used as standard solution.

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

Preparation Of Floating Oral In-Situ Gel Containing Metronidazole

Floating in-situ gel formulations of metronidazole were prepared by using composition given in the table. In around75%water, a measured quantity of sodium alginate required to make a 2% (W/V) solution was dissolved in distilled water at 60℃ using heating magnetic stirrer. After cooling to below 40℃, appropriate polymer ,methyl paraben and propyl paraben (ratio 9:1), the drug, metronidazole (MTZ), along with gas generating agent (calcium carbonate with or with out sodium bicarbonate) were dissolved uniformly in to the sodium alginate solution with continuous stirring. The stirring was continued after complete addition of until uniform dispersion was obtained and 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 colour bottles until further use.[3]

COMPOSITION METRONIDAZOLE FLOATING ORAL IN-SITU GEL FORMULATIONS

Formulation codein %w/v	Drug mtz	Sodium alginate	mcc	Tragacanth	Calcium carbonate	Sodium bicarbonate
f-1	2.5	2.0	0.5	-	2.0	-
f-2	2.5	2.0	1.0	-	1.5	1.5
f-3	2.5	2.0	1.5	-	1.0	-
f-4	2.5	2.0	2.0	-	0.5	1.5
f-5	2.5	2.0	-	0.5	2.0	-
f-6	2.5	2.0	-	1.0	1.5	1.5
f-7	2.5	2.0	-	1.5	1.0	-
f-8	2.5	2.0	-	2.0	0.5	1.5

Table 1:composition metronidazole floating oral insitu gel formulation

EVALUATION

Determination of drug content

Accurately, 10ml of formulation (containing equivalent of 250 mg metronidazole) from different batches was measured and transfer in to 100ml volumetric flask. To this 50-70ml of 0.1NHCLwasadded and sonicated for 30min. volume was adjusted to 100ml.complete dispersion

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of contents was ensured visually and dispersion was filtered using whatman filter paper. From this solution, 10ml of sample was withdrawn and diluted to 100ml with 0.1NHCL.contents of metronidazole was measured and at maximum absorbance at 278nm using UV-visible spectrophotometer.[4]

PH MEASUREMENT

The ph of prepared formulation was measured using calibrated digital pH meter.[5]

INVITRO GELATION STUDY

To evaluate the formulations of their in-vitro gelling capacity, accurately measured 10ml of formulation was added to 100mloh 0.1Nhydrochloric acid (HCL, pH1.2) at 37℃ in a beaker with mild agitation that avoids breaking of formed gel. The in-vitro gelling capacity was graded in three categories on the basis of stiffness of formed gel, gelation time and time period for which the formed gel remains as such.

(+) gels after few minutes, dispersed rapidly.
(++) gelation immediate remains for few hours
(+++) gelation immediate remains for an extended period.[6]

MEASUREMENT OF VISCOSITY OF IN-SITUGELLING SYSTEM:

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

IN-VITRO FLOATING STUDY:

The in-vitro floating study was carried out by introducing 10ml formulation to beaker containing 100ml of 0.1N HCL, (pH1.2) at 37℃ without much disturbance. The time formulation took to emerge on the medium surface (floating lag time) and the formulation constantly floated on the surface of the dissolution medium (duration of floating) was recorded.[8]

IN-VITRO DUG RELEASE STUDY:

The dissolution studies were performed in triplicate using a the dissolution studies were performed in triplicate using a type II (paddle method) dissolution apparatus. The dissolution medium used was 900ml of 0.1N HCL (pH 1.2), maintained at 37℃. The stirring rate was adjusted to 50 rpm. The speed was believed to stimulate the in-vivo existing mild agitation and was slow to enough to avoid the breaking of gelled formulation. At predetermined time intervals, 10ml of samples were with drawn and replaced by fresh dissolution medium, filter through whatman filter paper, diluted and assayed at maximum absorbance at 278nm using UV- visible spectrophotometer.[9]

III. RESULTS

Solubility: Solubility of metronidazole in different pH can be listed below.

Sl.no	media	Solubility mg/mlat room temperature
1	Water	4.25
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

MELTING POINT:

method	Observed
Thiel's tube	159℃

QUANTITATIVE ESTIMATION OF METRONIDAZOLE BY UV-SPECTROPHOTOMETER

Determination of ג max of metronidazole

The ג max ofmetronidazole in 0.1N hcl buffer ph 1.2 was found to be 278nm and uv spectrum as shown in the figure.

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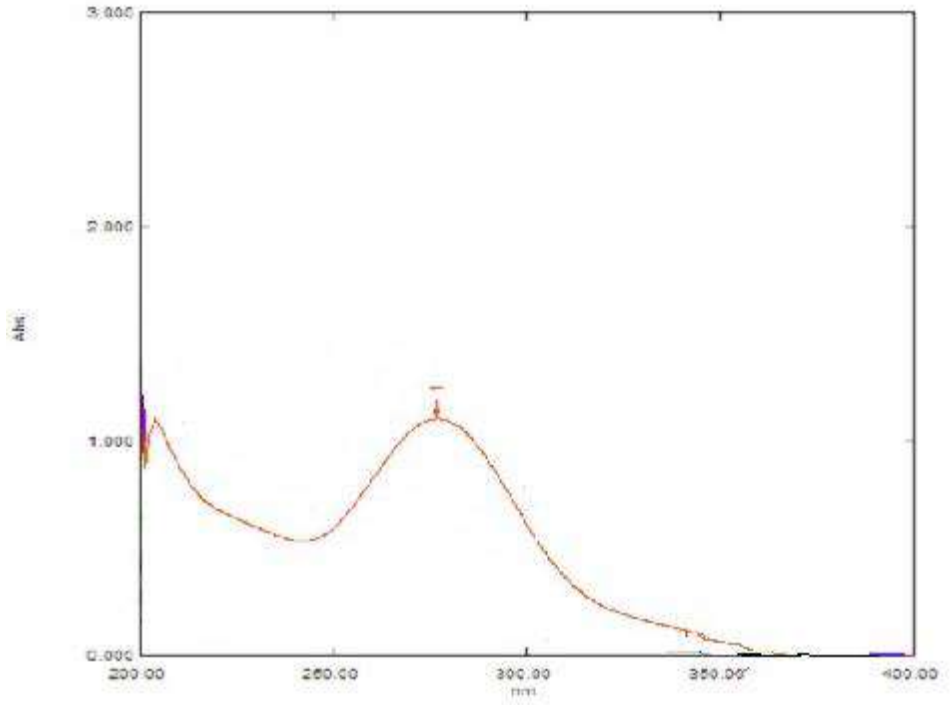

Fig:2λ max of metronidazole

STANDARD CALIBRATION CURVE OF METRONIDAZOLE

fromthe standard curve it obeys beer's law in concentration range of 0-20µg/ml in 0.1N HCL. Drug shows good linearity with regression co-efficient (r^2=0.9988)and the equation for this line obtained was found to be (y=0.0469x)which is used for the calculation of amount of drug in dissolution study and content uniformity.

CONC IN µgm/ml	ABSORBANCE WAVELENGTH AT 277nm
0µg/ml	0.0
4µg/ml	0.194
8µg/ml	0.350
12µg/ml	0.549
16µg/ml	0.753
20µg/ml	0.937

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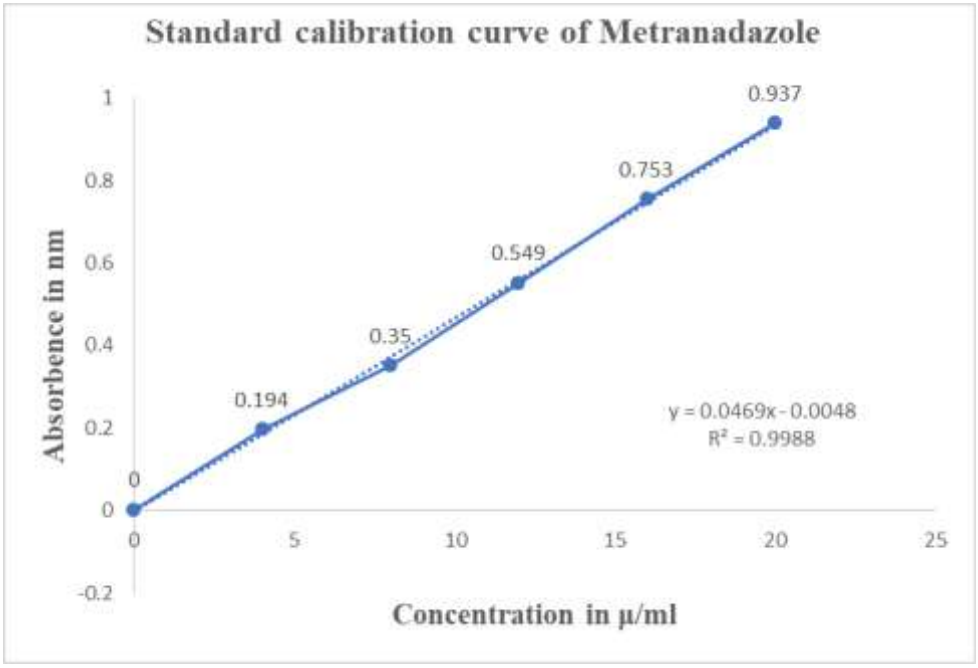

Fig3:standard calibration curve of metronidazole

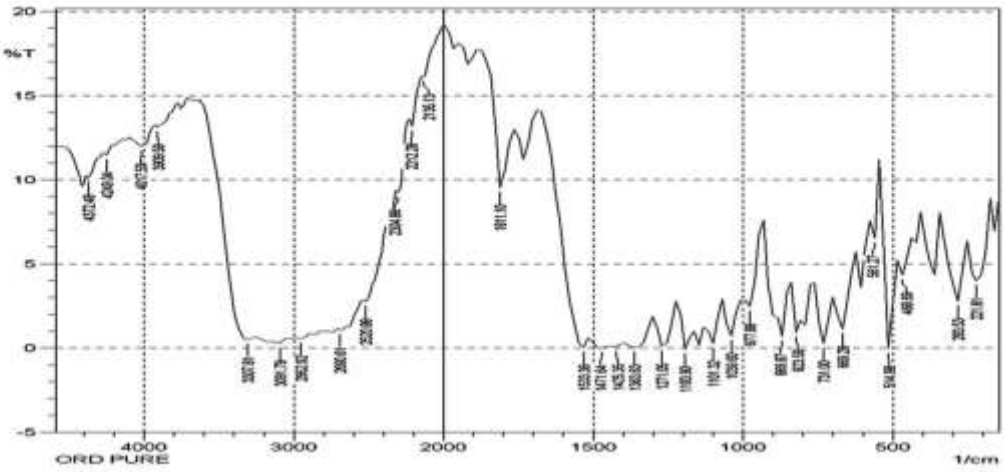

Fig4:FTIR spectra of Metronidazole

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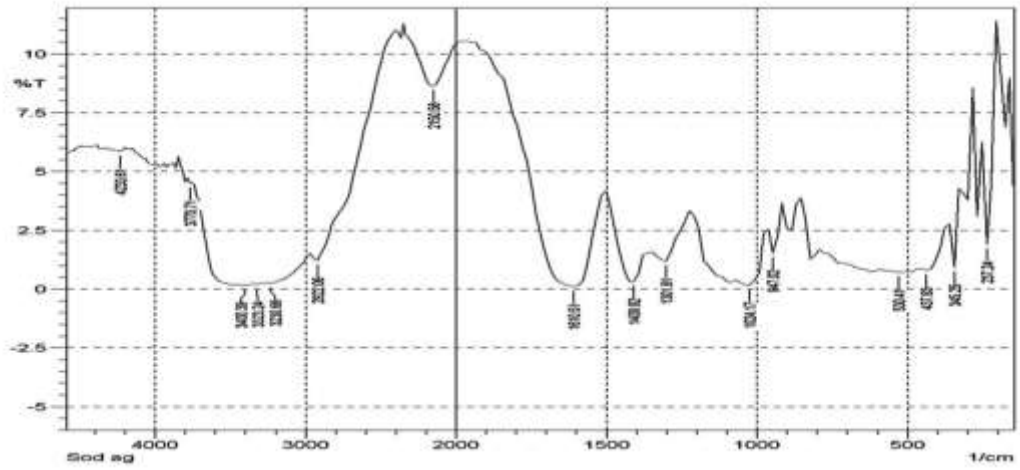

Fig5:FTIR spectra of sodium alginate

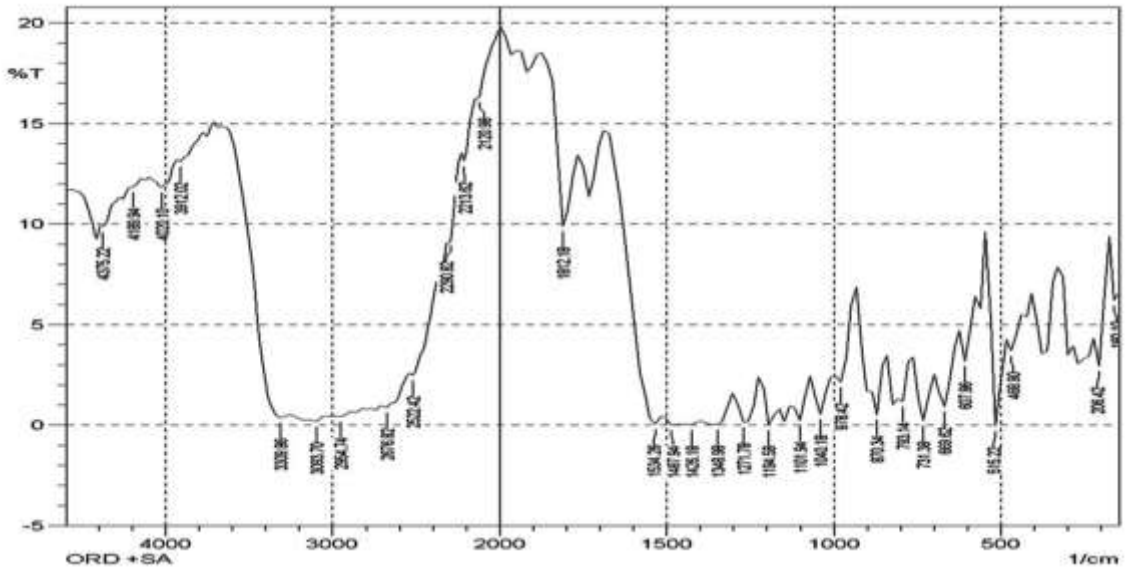

Fig6:FTIR spectra of Metronidazole + sodium alginate

Table 2:Properties of in situ gelling formulations

Formulation code	Drug content	Drug release	ph	Gel response grade	Duration of floating	Viscosity in cps
f-1	89.47	85.125	7.3	+++	>24	521
f-2	87.291	81.375	7.8	+++	>24	485
f-3	95.208	91.685	7.5	+++	>24	568
f-4	97.291	95.8125	7.58	+++	>24	521
f-5	88.208	86.625	7.43	++	>24	564
f-6	94.1666	93.1875	7.92	++	>24	591
f-7	80.1041	77.625	7.83	++	>24	596
f-8	84.7916	82.6875	7.94	++	>24	598

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Drug content

The percentage of drug content of all the formulation was determined and are shown in the table. The drug content was found to be80-97% for all theformulation indicating the uniform distribution of drug.

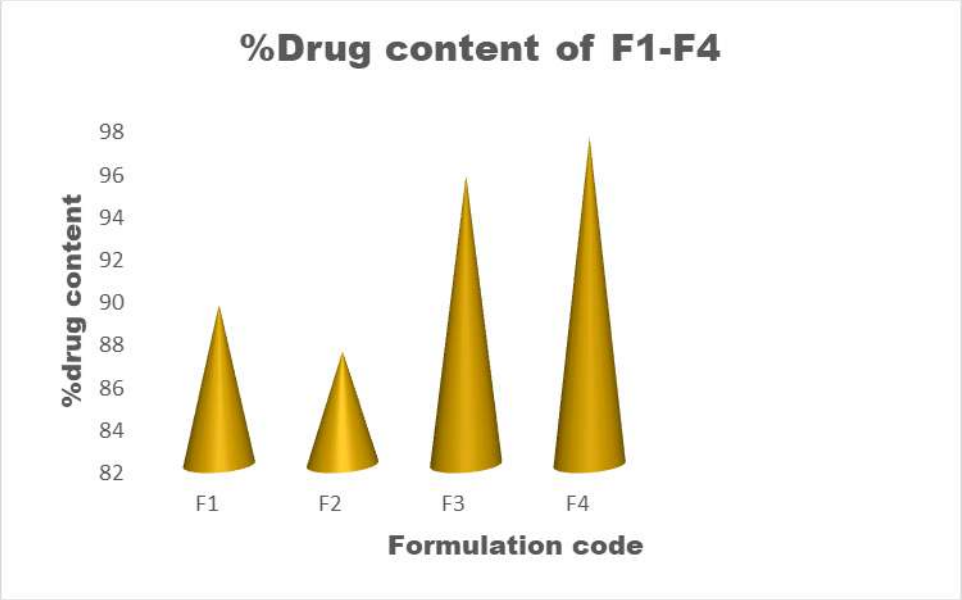

Fig7: percentage of drug content from f1-f4

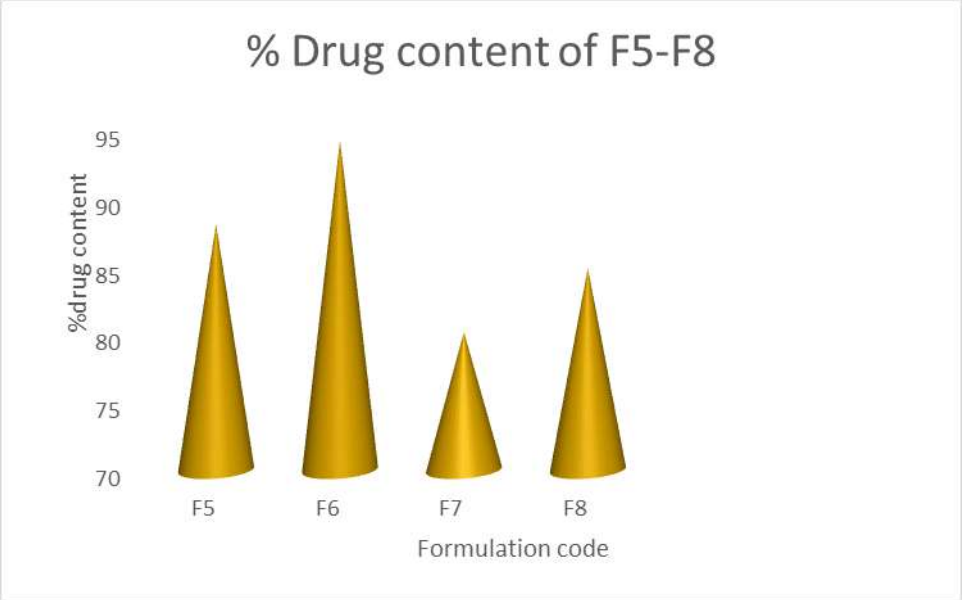

Fig8:percentage of drug content from f5-f8

Drug release

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.

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Percentage of drug release

Time in hours	Formulationf-1	Formulation f-2
1	27.185	17.625
2	31.5	25.875
3	40.125	36.375
4	51.9375	48.1875
5	57.375	55.5
6	70.875	63.375
7	87.375	72.375
8	85.125	81.375

Time in hours	Formulation f-3	Formulation f-4
1	28.785	27.1875
2	32.6025	31.5
3	41.185	40.125
4	53.5375	51.9375
5	59.5375	57.375
6	72	70.875
7	86.4375	87.375
8	91.6875	95.8125

Time in hours	Formulation f-5	Formulation f-6
1	24.5625	29.4375
2	33.375	35.25
3	41.8125	42.5625
4	49.5	51.5625
5	58.3125	58.875
6	68.25	72.1875
7	77.8125	83.625
8	86.625	93.1875

Time in hours	Formulation f-7	Formulation f-8
1	25.3125	27.375
2	34.5	32.25
3	38.8125	42.5625
4	49.6875	52.6875
5	55.875	58.6875
6	65.8125	72.1875
7	72.1875	78
8	77.625	82.6875

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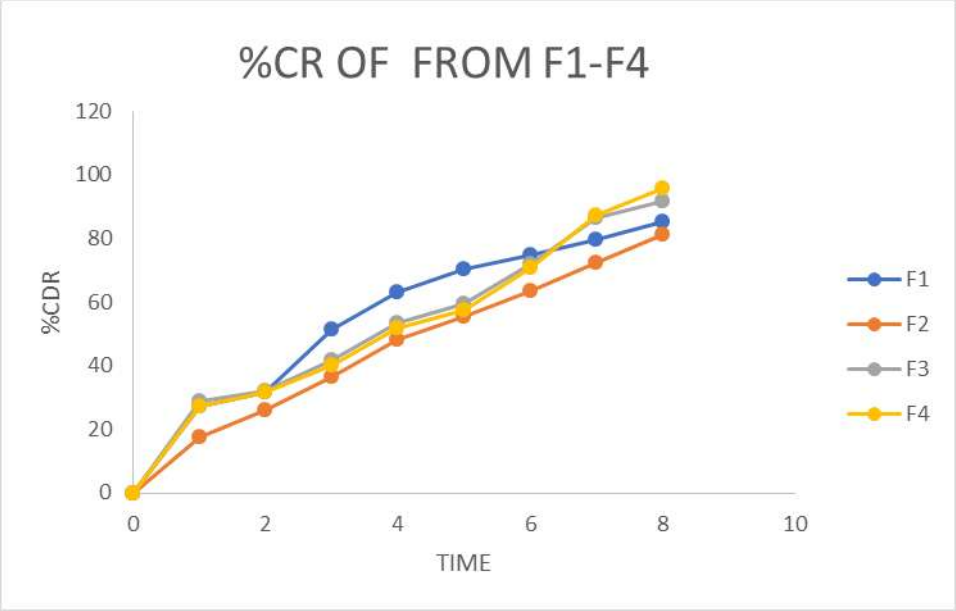

Fig 9:percentage of drug release from f1-f4

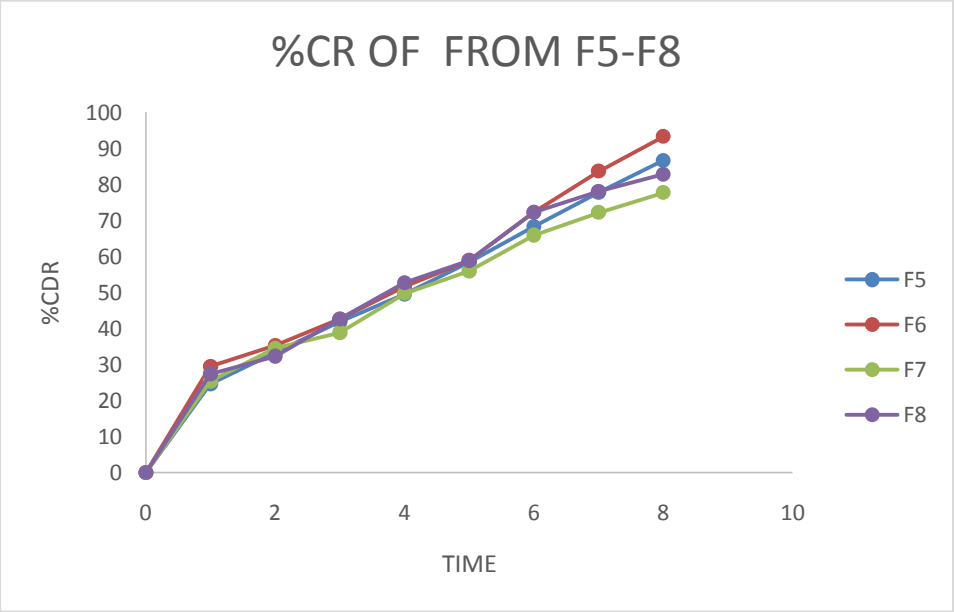

Fig9: percentage of drug release from f5-f8

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

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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. who concluded that as the percentage of NaHCO3 increases, the gel integrity decreases.

In-vitro Floating study: The formulated floating in-situ gelling system of metronidazole employed NaHCO3 orCaCO3as a gas-generating agent. The in vitro floating test revealed the ability of all formulae to maintain buoyant for more than 24h(Table 2 and Figure1).

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 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. 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.

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 F8 are shown in Table 2.

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.

IV. CONCLUSION

From the present study carried out on Metronidazole floating in-situ gel system, the following conclusion can be drawn.

- 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 Ca2+ 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.pyloriinfections.

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