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FORMULATION, DEVELOPMENT AND CHARATERIZATION OF METRONIDAZOLE GASTRORETENTIVE IN-SITU FLOATING GEL

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Abstract
The aim of present work was formulation, development and characterization of metronidazole gastroretentive in-situ floating gel. The preliminary batches were formulated using sodium alginate, calcium carbonate, sodium citrate and distilled water. After preliminary preparation final formulations were prepared with different concentration of gelling agent (F1, F2, F3, and F4). The results shown that formula (F2) containing sodium alginate (1.5% w/v) and Xanthan gum (0.150% w/v) was the best formula regarding gelation time (5 seconds), floating duration (24 hours), pH (8.3), with drug release (99.80%) after 8 hrs, with the First Order release kinetic. It was concluded that the formulation of Metronidazole was gastroretentive floating *in-situ* gel, controls the release leading to improvement in drug absorption and bioavailability.

Key Words: Metronidazole, Sodium alginate, In-situ gel.

Introduction

Metronidazole is an antimicrobial agent that has been used in clinical medicine for 145 years. It was originally indicated for the management of infection caused by Trichomonas vaginalis and was then shown to be effective against other protozoal infections, such as amebiasis and giardiasis. To our knowledge, the first report on the effect of metronidazole for the management of anaerobic infections was published in 1962 by Shinn [1]. Gastro-retentive dosage forms (GRDFs) are designed to be retained in the stomach for a prolonged time and release their active ingredients and thereby enable sustained and prolonged input of the drug to the upper part of the gastrointestinal (GI) tract [2]. The gastro-retentive drug delivery systems can be retained in the stomach and contribute in improving the oral sustained delivery of drugs that have an

absorption window in a particular region of the gastrointestinal tract. [3]. These systems help in continuously releasing the drug before it reaches the absorption window, thus ensuring optimal bioavailability. The *in situ* gelling system is a type of mucoadhesive drug delivery system principally capable of releasing drug molecule in a sustained manner affording relatively constant plasma profile. These formulations are liquid at room temperature but undergo gelation when in contact with body fluids or change in pH. These have a characteristic property of temperature dependent, pH dependent and cation induced gelation. *In situ* gelling formulations are widely applicable for ocular, nasal, vaginal and oral therapy. It has several advantages as a dosage form for oral administration like maximum intimate contact of the drug at the absorption site, influenced rate of absorption, ease of preparation, homogeneity of drug distribution compared to other conventional suspensions [4-7].

MATERIALS AND METHOD

Metronidazole was a obtained from Wockhardt research center Aurangabad. Sodium Alginate, Xanthan gum, Calcium Carbonate, Methyl Paraben, Propyl Paraben and D-mannitol were purchased from Loba Chemie Pvt. Ltd. Mumbai.

Formulations of preliminary Batches:

The preliminary batches were formulated using Metronidazole, Sodium alginate, Sodium citrate and Calcium carbonate, Methyl Paraben, Propyl Paraben, D-mannitol, deionized water.

Table 01: Formulation of Preliminary Batches.

Ingredients	Formulation			
	PF1	PF2	PF3	PF4
Metronidazole	2.5	2.5	2.5	2.5
Sodium alginate	0.5	0.750	1.00	1.25
Sodium citrate	0.250	0.250	0.250	0.250
Calcium carbonate	0.500	0.500	0.500	0.500
D-mannitol	1.0	1.0	1.0	1.0
Methyl Paraben	0.09	0.09	0.09	0.09
Propyl Paraben	0.01	0.01	0.01	0.01
Distilled water	Up to 100ml	Up to 100ml	Up to 100ml	Up to 100ml

All quantities are in %w/v.

All formulations showing gelling time less than 1 minute and total floating time was 8 hours.

Preparation of *In-Situ* Gelling Solution

The solutions of sodium alginate in different concentrations were Pprepared in deionized water, in which sodium citrate and calcium chloride were previously dissolved. The solutions were heated to 60˚C with constant stirring on a magnetic stirrer. It was then allowed to cool to 40˚C and later calcium carbonate in different concentrations was dispersed in the previous solution with continuous stirring. Metronidazole was then added to the resulting solution with continuous stirring. The solutions were then stored in amber colored bottles until use. The concentrations of calcium chloride and sodium citrate were kept constant in all the formulations.

Formulation of Final Batches:

Table 2: Formulation of Final Batches.

Name of Ingredients	Formulation Code			
	F1	**F2**	**F3**	**F4**
Metronidazole	2.5	2.5	2.5	2.5
Sodium Alginate	1.5	1.5	1.5	1.5
Xanthan gum	----	0.150	0.300	0.450
Sodium citrate	0.250	0.250	0.250	0.250
Calcium carbonate	0.500	0.500	0.500	0.500
Methyl Paraben	0.09	0.09	0.09	0.09
Propyl Paraben	0.01	0.01	0.01	0.01
Distilled water	q.s to 100ml	q.s to 100ml	q.s to 100ml	q.s to 100ml

All quantities are in %w

Evaluation of *In-Situ* Gelling System:

Measurement of Viscosity

The viscosities of the prepared solutions were determined by Brook field viscometer. The samples (100ml were sheared at a rate of 50 rpm/min using spindle number 3 at room temperature.

***In Vitro* Gelation Study**

The in vitro gelling capacity of prepared formulations was measured by placing 5ml of the gelation solution (SGF in a 15 ml borosilicate glass test tube and maintained at 37±1˚C temperature. 1ml of formulation solution was added with the help of pipette. The formulation was transformed in such a way that places the pipette at the surface of the fluid in test tube and formulation was slowly released from the pipette. As the solution comes into contact with the gelation solution, it was immediately converted into stiff gel like structure. The gelling capacity of the solution was evaluated on the basis of stiffness of formed gel and time period for which formed gel remains as such.

***In Vitro* Floating Study**

The floating ability of In-Situ gelling solution was determined in 500ml SGF (pH 1.2 in a beaker. Accurately measured 10ml of solution was added to SGF with mild agitation. Time required for adding solution (floating lag time and total floating time were measured.

Determination of Drug Content

Accurately measured 10ml of In-Situ gel was transferred to 100ml of volumetric flask. To this 70 ml of simulated gastric fluid was added and shake for 30 min, followed by sonication for 15min. Complete dispersion of contents were ensured visually and volume was made up to 100ml with simulated gastric fluid & filtered using whatman filter paper. From this solution, 10ml of sample was withdraw and diluted to 100ml with SGF. Contents of Metronidazole were determined spectrophotometrically at 305 nm using double beam UV-Visible Spectrophotomete (Lab India- UV3200).

***In Vitro* Drug Release study**

The in situ gel formulations were subjected to *In-vitro* dissolution studies using USP Type II (paddle dissolution apparatus at 37±0.5°C and 50 rpm speed. To mimic the Gastrointestinal conditions, as per the official recommendation of USFDA, 900 ml of 0.1 N HCL was used as dissolution medium. Aliquot equal to 5mL was withdrawn at specific time intervals (1hr, 2hrs, 3hrs, 4hrs, 5hrs, 6hrs, 7hrs, 8hrs, 9hrs 10hrs and 12hrs and replaced with fresh buffer. The aliquots were diluted and drug release was determined spectrophotometrically at a wavelength of 294 nm respectively by comparing with the standard calibration curve.

RESULT AND DISCUSSION

Evaluation of Preliminary Formulations

Table 3: Evaluation of Preliminary Formulation

Formulation code	Gelling time (seconds)	Floating lag time (seconds)	Total Floating time (hours)
PF1	16	20	>24
PF2	14	22	>24
PF3	11	15	>24
PF4	9	18	>24

From the above results it was concluded that as the concentration of calcium carbonate was increased formulation showed less gelling time, floating lag time and total floating time.

Evaluation of Formulation

Physical Appearance and pH:

All the prepared sodium alginate and Xanthan gum based *in situ* solution of Metronidazole evaluated for their clarity and the type of the solution. All the solutions are light brown in appearance and showed no visible particles or lumps in the preparation. After administration of the prepared solution in (0.1N HCL, pH1.2) also checked the time required for gel formation and type of gel formed. The pH was measured in each of the solution of sodium alginate based *in situ* solution of Metronidazole, using a calibrated digital pH meter. The measurement of pH of data were in triplicate and the Average values given in Table 04. All the formulations are within required pH range suitable for absorption.

Table 04: pH of prepared *In-situ* gel formulation

Formulation code	F1	F2	F3	F4
pH	8.1	8.3	8.7	9.1

All the formulation has an alkali pH. Maximum pH was observed in F4 as 9.1 and minimum pH was observed in F1 as 8.1. The pH is one of the important environmental parameter for floating *insitu* gel. These formulations are polymeric dispersion in aqueous system which undergoes spontaneous gelation in response to change in pH after application at the target site. The entire pH sensitive polymers acidic or basic group either accepts or release protons in response to change in environmental pH. Swelling of *in-situ* gel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups.

Viscosity

Table 05: Viscosity **of prepared *In situ* gel formulations**

Formulation code	F1	F2	F3	F4
Viscosity (Solution)	17±0.20	21±0.21	31±0.29	39±0.35
Viscosity (Gel)	240±0.15	330±0.51	410±0.32	650±0.18S

The viscosity of the formulations increased with an increase in sodium alginate and Xanthan gum concentration. This phenomenon is a consequence of increasing chain interaction with an increase in polymer concentration. Calcium carbonate, which is the source of cations, increased the viscosity of the formulation. It was observed that calcium carbonate concentration had a significant effect on viscosity. The calcium carbonate is present as insoluble dispersion in the formulation. Hence, an increase in the concentration, probably enhanced the number of particle dispersed, thus contributing to the increased viscosity.

In-vitro gelling capacity

Table 06: Gelling capacity of prepared *In situ* gel formulations

Formulation code	F1	F2	F3	F4
Gelling capacity	+++	+++	+++	+++

Gelling studies were carried out using simulated gastric fluid (pH 1.2). In this study the gelling capacity for all formulations were determined. The *in situ* gel so formed should preserve its integrity without dissolving or eroding so as to localize the drug at absorption site for long duration. It was represented in Table 06, that F1, F2, F3, F4 were shown rapid gel formation and integral gel structure even after more than 12 hours, which upon increasing the concentration of Sodium alginate and Xanthan gum in this study.

In vitro Gelation study

The formulations gelled within 4 seconds after contact with simulated gastric fluid (pH 1.2) and the gelling time was ranged from 4-9 seconds Table 07.

Table 07: In vitro gelation study of prepared *In situ* gel formulations

Formulation code	**F1**	**F2**	**F3**	**F4**
In vitro gelation	4	7	8	9

Drug content

The drug content of all (F1-F4) formulations is given in Table 08. It ranges in between 93.82%-98.79%. The values are acceptable as per United State Pharmacopoeia standards.

Table 08: Drug content study of prepared *In situ* gel formulations

Formulation code	**F1**	**F2**	**F3**	**F4**
Drug content	93.82%	98.79%	96.18%	97.01%

It ranges in between 93.82%- 98.79%. The values are acceptable as per United State Pharmacopoeia standards.

In vitro dissolution studies

The batch F2 showed highest percentage of drug release at the end of 8 hours. The Table 15 shows drug release of different batches.

Table 09: In vitro drug release of different batches

Sr.No.	**Time (hr.)**	**F1 (%)**	**F2 (%)**	**F3 (%)**	**F4 (%)**
1	0	0	0	0	0
2	1	10.09	12.90	10.20	11.60
3	2	25.31	21.69	18.92	15.25
4	3	29.08	29.85	26.25	28.15
5	4	40.82	38.55	39.83	36.46
6	5	56.21	60.30	57.63	58.35
7	6	64.53	81.99	74.51	75.31
8	7	71.31	92.81	89.98	89.58
9	8	95.98	99.80	96.89	96.85

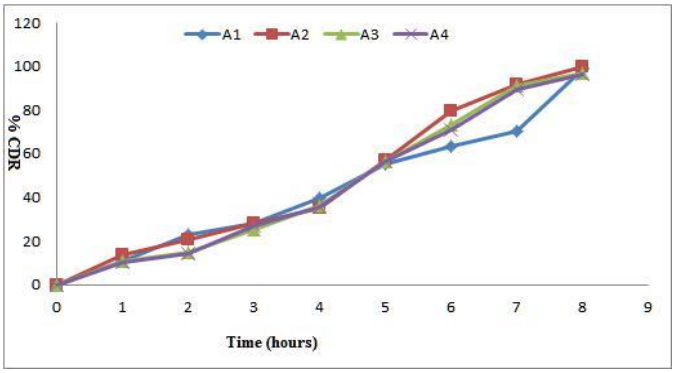

Figure 01: In vitro drug Dissolution profile of formulation batches

Drug Release Kinetic

The drug release data were evaluated by the curve fitting method using PCP-Disso software. In-vitro release data were fitted to various mathematical models such as zero order, first order, Higuchi and Korsemeyer-Peppas model in order to understand the mechanism of drug release and the release rate from dosage forms. Table 10 illustrates the correlation of dissolution data to different models of release kinetic. This result indicated that most formulations exhibit diffusion mechanism in drug release accompanied by acceptable regression value for zero order. Kinetic model which best fit zero order equation was most suitable for controlled release formulation.

Table 10: Regression coefficient of Models for Formulation Batches

Formulation Code	R^2				
	Zero Order	First order	Matrix	Hixson-Crowell	Krosmeyer Peppas
F1	0.9803	0.9489	0.9231	0.9258	0.9358
F2	0.9802	0.9495	0.9334	0.9318	0.9433
F3	0.9688	0.9421	0.9280	0.9410	0.9428
F4	0.9810	0.9449	0.9501	0.9419	0.9489

SUMMERY AND CONCLUSION

The formulation was liquid before administration and produce rapid gelation upon contact with gastric fluid. This study involved formulation of Metronidazole oral solution which undergoes gelation upon direct contact with gastric fluid and floated using primary polymer as sodium alginate in combination with secondary polymer as Xanthan gum. The calcium carbonate present in the formulation as insoluble dispersion was dissolved and released carbon dioxide on reaction with acid of the stomach (Artificial SGF) and the *in situ* released calcium ions result in formation of gel with floating characteristics. The results showed that formula (F2) containing sodium alginate (1.5% w/v) and Xanthan gum (0.150% w/v) was the best formula regarding gelation time (7 seconds), floating duration (24 hours), pH (8.3), with drug release (99.80%) after 8 hrs, with the First Order release kinetic. It was concluded that the formulation of Metronidazole was *in-situ* gastroretentive floating gel, controls the release leading to improvement in drug absorption and bioavailability. Amongst the various concentrations of polymers used in the study, *in situ* gel were formulated by using sodium alginate and Xanthan gum as a polymer and calcium carbonate was used as cross linking agent. The formulation containing sodium alginate (1.5%w/v) and Xanthan gum (0.150%w/v) showed satisfactory results with respect to floating lag time, in vitro floating time, gelling capacity and drug release. The formulation shown floating time and gelation capacity remains more than 12 hours. The in vitro release studies revealed that initially there was burst effect then followed by sustained release for 12 hours and percentage drug release was found to be 99.80% of optimized F2 formulation. Based on the optimization results it was concluded that the objective of formulating floating *in situ* gel based gastroretentive drug delivery of Metronidazole has been achieved.

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