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Original Article

Enteric Coated Sustained Release Mucoadhesive Tablet of Metronidazole for Targeted Colon Delivery: A Formulation and Evaluation Study

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ABSTRACT

Background: Colon-specific Drug Delivery Systems (CDDS) are useful for treating many localized conditions such as ulcerative colitis, Crohn's disease, irritable bowel syndrome, chronic pancreatitis and colonic cancer. In addition, the colon has the potential to serve as a potential site for the systemic absorption of several drugs used to treat diseases outside of the colon. Drugs, such as proteins and peptides, which are susceptible to degradation under highly acidic gastric conditions, can be absorbed systemically through the colonic mucosa. In order to achieve effective therapeutic outcomes, it is imperative that the designed delivery system specifically targets the drugs in the colon. **Objectives:** The objective of the current study is to formulate metronidazole sustained-release tablets that can be targeted to the colon because Metronidazole (MNZ) has good solubility at pH 1.2; hence, coating of the drug with the suitable pH dependent is done to prevent its release in the gastric region. **Materials and Methods:** Metronidazole sustained release tablets were manufactured by using the wet granulation method with hydroxypropyl methylcellulose k100 (HPMC K100), HPMC E-15, Locust bean gum, Sodium alginate and chitosan at different ratios and further coated with suitable polymers. The powdered blend was evaluated for adequate flow properties using Carr's compressibility index, tapped density, bulk density, angle of repose and Hausner's ratio before compression. The compressed tablets were then further evaluated for weight variation, hardness, friability, drug content, wash-off test and *in vitro* dissolution firstly in 0.1 NHCl followed by pH 7.4 phosphate buffer. **Results:** The friability conducted for every composition is within the appropriate range. The wash-off test F6 composition includes (sodium alginate and HPMC E-15) which shows the best adhesive strength among all the formulations. In the *in vitro* drug release study, the F6 formulation indicated better controlled delivery i.e., 95.34% in 16 hr. **Conclusion:** It was found that all of the formulations exhibited a good rate of drug release. Formulation F6 containing sodium alginate and HPMC E-15 exhibited better-controlled release than all other formulations i.e., 95.34% in 16 hr as a result drug release increases with an increase in polymers, exhibiting good mucoadhesive properties. The cumulative drug release percentage for all the formulations was observed to be sustained.

Keywords: Controlled delivery, Enteric-coated, *In vitro*, Metronidazole, Mucoadhesive.

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INTRODUCTION

Tablets are a prominent solid dosage form for treating many illnesses, generally taken orally. Drug bioavailability in oral administration varies on *in vitro* dissolution and gastrointestinal permeability.[1] In recent years, mucoadhesion drug delivery systems have gained prominence due to their propensity to extend residence time at the administration site and increase absorption by intricately contacting the underlying mucosa. The drug's bioavailability is enhanced by its vast surface area and rapid blood flow.[2] Bioadhesion occurs when two biological substances, one of which is bioadhesive, are bound together by interfacial forces over time. Mucoadhesion is the term for the phenomenon where the biological substrate is the mucosa covering surface tissues. Bio/mucoadhesive systems adhere to intestinal epithelial cell membranes or mucins, potentially expanding the Gastrointestinal retention drug delivery strategies that can be maintained in the gastric or intestine by enhancing drug-bio membrane interaction.[3]

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Sustained release drug delivery systems release medication constantly over an extensive duration of time to achieve a prolonged therapeutic consequence.[4] The purpose of developing oral prolonged or targeted delivery methods is to decrease the frequency of dose, improve drug efficacy by localizing at the site of action, reduce dosage and enhance patient adherence. Extended releasing dosage types allow improved control over the plasma drug levels, reduce dosing frequency, reduce side effects, boost efficacy and ensure consistent dosing.[5]

Enteric coating systems prevent solid dose forms from disintegrating in the GI, which causes the enteric polymer to dissolve and the dose form to disintegrate in the small intestine. An enteric coating is a layer that is imposed on oral drugs to control where they are absorbed within the digestive system. Although the term "enteric" refers to the small intestine, enteric coatings restrict drugs from releasing before they reach the small intestine.[6] The pH variations between stomach pH 1-3 and intestinal pH 6-8 are used by the tablet's enteric coating. Metronidazole is referred to as the Nitroimidazole class of antibiotics. It is an appropriate drug for treating Amoebiasis, Giardiasis, Trichomoniasis and Anaerobic diseases.[7] This drug is intended to be administered to the colon to combat the trophozoites of Giardia lamblia and *E. histolytica*, which are found in the large intestine and caecum lumen and attach to colonic mucus and epithelial layers. Therefore, different Synthetic hydrophilic polymers are utilised to regulate drug administration and target it to the colon.[8] The limitation of this drug is its intake three times regularly for 5-7 days which may give poor patient adherence therefore in this research an attempt was made to reduce the dosage period by preparing mucoadhesive tablets using hydrophilic polymers such as HPMC K100, HPMC E-15, Sodium alginate, Locust bean gum and chitosan.

MATERIALS AND METHODS

Materials

Metronidazole was purchased from Yarrow Chem. Pvt. Ltd., Mumbai, Locust Bean Gum (LBG) was purchased from Pioneer Chemical CO. Delhi, HPMC k100, HPMC E-15, Sodium Alginate and Chitosan was acquired from Central Drug House Ltd., New Delhi.

Methods

Pre-Formulation Studies

Organoleptic characteristics

The organoleptic characteristics like color, taste, odor and physical form were examined using suitable approaches.[9,10]

Melting point

The melting point of Metronidazole was evaluated by adding a minimal amount into the capillary, Connecting it to the steam of a thermometer set in a warming bath, gently warming the water and measuring the temperatures at which melting begins and completes.[11]

Solubility analysis

The drug's solubility was determined in different solvent systems. A sufficient amount of drug was added to 5 mL of each solvent in a volumetric flask and shaken. The samples were then kept at room temperature for 24 hr. After 24 hr the samples were filtered, diluted and examined in a UV spectrophotometer for the absence or presence of drug particles.[12]

Preparation of standard curve in different solvents

25 mg drug was accurately weighed and dissolved in 25 mL of solvent. From the stock dilutions having a concentration of 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10 µg/mL were prepared. These dilutions were then observed in UV Spectrophotometer and absorbance was measured at 277 nm.

Drug-Polymer Interaction Study

Fourier transform infrared spectrum approach was used to detect any potential chemical interaction among the drugs and excipients using the diamond ATR (Model no 630) technique using Albunt FTIR spectrophotometer in the wavelength region of 100 to 4000 cm^{-1}. The procedure consists of dispersing the ATR spectrophotometer. Force is applied to the sample, pushing it into the diamond surface.[13,14]

XRD (X-ray Diffraction)

The X-ray diffraction technology is used to study physical properties like the composition of phases, crystalline arrangement, orientation of granules and solid samples. Samples were recorded over the 0.1-100 nm.[9]

Formulation of metronidazole sustained release mucoadhesive tablet

Metronidazole tablets were formulated by using the wet granulation method with different ratios of polymers as mentioned in Table 1. Drug and polymers are accurately weighted and pass through the sieve number 40. All the ingredients were combined and blended properly for 20-30 min for uniform distribution of drug. In a beaker, Locust Bean Gum (LBG) paste was prepared with water, 10% of the paste was then added to the ingredient mixture, forming a moist mass. The mixture was granulated by using the required amount of distilled water for granulation. The granules obtained were sized through sieve number 24 and dried in hot air oven for an hour at 60°C. The granules were blended with magnesium stearate, talc and aerosol for 10 min. The granules were then compressed by Cadmach multi-station automatic compression machine.[12] The diagrammatic representation is shown in Figure 1.

Preparation of Film coating solution

400 mg of polymer HPMC E-15 was dissolved in methanol to prepare a 0.4% coating solution for a particular formulation. The solution was then placed under stirrer for 15 min to obtain homogeneous dispersion. The prepared Metronidazole tablets were then poured into the coating solution and dried in hot air oven. 100 mL of methanol was added in a 500 mL sterile container, the required quantity of polymer Eudragit RL100 was added and left for overnight. After 24 hr, the mixture was filter out by using a muslin cloth and agitate for 10 min to achieve a homogeneous distribution of polymers. At the end excipients like PEG400 (plasticizer), Titanium dioxide (opacifier) and color were added to obtain a coating solution.

Coating Procedure

20 tablets were placed in a perforation pan, the solution was poured into a spray gun. The coating mixture was sprinkled froma specific distance with a constant speed. The temperature was kept constant and the tray was manually shaken. The procedure was continued until a consistent coat was obtained.

Evaluation parameters of Mucoadhesive sustained release Tablet

Pre compression studies

Before compression the powder blend was evaluated for pre compression studies using bulk density, tapped density, carr's compressibility index, hausner's ratio, angle of repose and flow properties.[15]

Bulk density

Bulk density of the powder was determined by transferring the sample through the funnel into a 100 mL graduated cylinder.[16] It can be measured by using formula:

$$\text{Bulk density} = \text{Weight of powder/ Bulk volume}$$

Tapped density

It can be calculated by transferring the sample through a funnel into a 100 mL graduated cylinder, which was then tapped from a height of 2.5 cm at an interval of 2-3 sec. After that, the samples volume was observed by using Electrolab's Tap Density Tester ETD-1020.[17] Tapped density was measured using formula:

$$\text{Tapped density} = \text{Weight of powder/ Tapped volume}$$

Carr's compressibility index

The flow rate and particle size distribution of a powder is related to compressibty. Powders having compressibility values less than 20% have good flow properties. A materials compressibility can be calculated by using carr's compressibility index.[18]

Compressibility index can be calculated by using formula:

$$\text{Carr"s compressibility index (\%)} = 100 \times (1 - \text{bulk density/tapped density})$$

Hausner's ratio

It is the ratio of tapped density to bulk volume and can be calculated using formula:

$$\text{Hausner"s ratio} = \text{tapped density/bulk density}$$

Angle of repose

Angle of repose can be performed by placing a 10 mm diameter funnel at a height of 2 cm. The sample was slowly moved downwards along the wall of the funnel until the top of the pile made contact with the stem of the funnel. The radius of the powder cone was measured and a circular shape was roughly formed around the bottom of the pile.[19]

Angle of repose was measured using formula:

$$\theta = \tan - 1(h/r)$$

Table 1: Formulation table of drug-loaded sustained release mucoadhesive tablets.

Formulation Code	Drug (mg)	HPMC K 100 (mg)	HPMC E-15 (mg)	Chitosan (mg)	Sodium Alginate (mg)	LBG (mg)	Talc (mg)	Mg Stearate (mg)
F1	400	80	70	-	80	65	2	3
F2	400	70	80	80	-	65	2	3
F3	400	-	70	70	80	65	2	3
F4	400	70	-	80	70	65	2	3
F5	400	115	-	115	-	65	2	3
F6	400	-	115	-	115	65	2	3
F7	400	80	-	150	-	65	2	3
F8	400	-	80	-	150	65	2	3

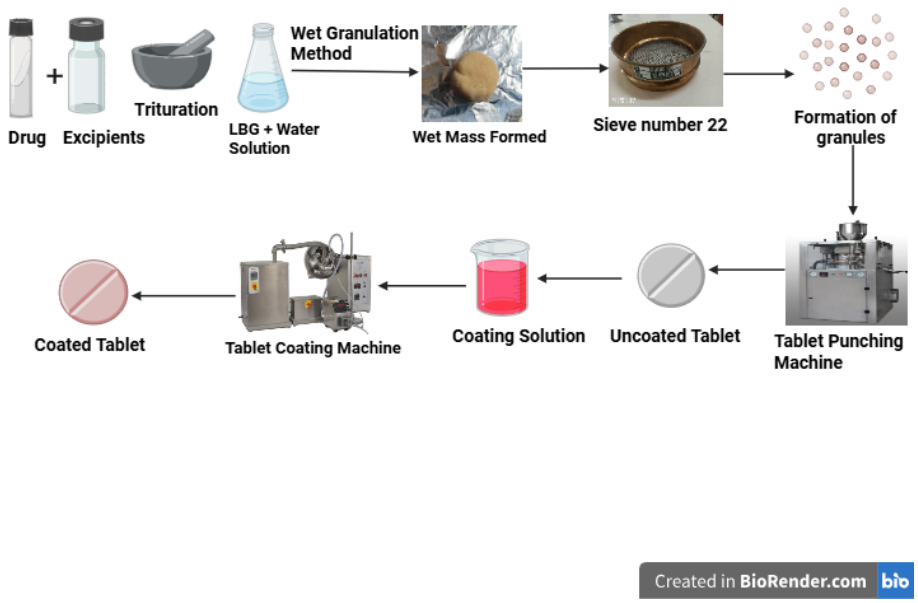

Figure 1: Preparation of metronidazole sustained release mucoadhesive tablet.

Post-compression studies

Weight Variation test (w)

A digital electronic balance was used to perform weight variation study, according to the method mentioned in Indian Pharamcopoeia (IP), twenty tablets from each formulation batch was taken and the total weight was assessed. The average weight was calculated. The weight variation for tablets more than 250 mg is ±5, individual weight of each tablet is also measured and weight variation was determined using formula.[20]

$$\% \text{ Weight variation} = (\text{weight of single tablet} - \text{Average weight of 20 tablets}) \times 100$$

Hardness (h)

Hardness is defined as the force which is required to break a tablet. Each batch's three tablets were examined for hardness using a Monsanto tester and average results were determined. The acceptable range for hardness is 5-7 kg/cm.[21]

Friability test (%F)

Roche's Friabilator was used for the friability tests. Twenty tablets from each batch were chosen and was examined. The test was performed for four minutes at a speed of 25 rpm (Electro lab, model EF1W, Mumbai). The tablets were then weighed again and the percentage of weight loss was determined. % Friability of tablets <1% is considered acceptable.

The % weight loss can be calculated using formula:

$$\% F = \frac{(W1 - W2)}{W1} \times 100$$

Thickness (t) and diameter

The diameter and thickness of the tablets were determined using Vernier caliper. From every batch, three tablets were randomly selected and tested.[22]

Drug content estimation

20 tablets were selected randomly and transferred into a mortar pestle. Phosphate buffer pH 7.4 was used to dissolve a quantity of powder equal to one drug dosage, which was then filtered using a Whatman filter paper. The solution was diluted and the absorbance was assessed at 319 nm using a UV spectrophotometer (Shimadzu, model UV-19001). Each observation was performed 3 times and average drug content was determined.[23]

Wash-Off Test

The tablets' mucoadhesive property were assessed using an *in vitro* adherence test technique called wash-off. Parts of the Intestinal membrane were put on a glass slide and attached with adequate support. Two tablets were tied to a slide and held by the arm of a USP tablet dissolving test equipment. The disintegrating testing equipment was operated at 37°C with a gradual and consistent upward and downward movement within the test solution (phosphate buffer pH 7.4). The separation of both tablets was noted down.[24]

In vitro drug dissolution Studies

USP paddle type II Dissolution apparatus (Electrolab) India Pvt. Ltd., Mumbai was used to carry out *in vitro* drug dissolution studies at a speed of 50 rpm in 900 mL of 0.1N HCl for the first 2 hr followed by phosphate buffer (pH 7.4) for the next 12 hr. The temperature was maintained at 37±0.5°C while the paddle was set

to rotate at 100 rpm. 5 mL of sample was taken at predetermined intervals, filtered through a membrane filter and examined through UV-visible spectroscopy at 319 nm. The absorbance readings were used to compute the cumulative percentage of drug release.[25]

Kinetics release data

The drug release kinetics was determined by fitting the *in vitro* release data to various mathematical models, including zero-order, first-order, Higuchi, Hixson-Crowell cube root and Korsmeyer-Peppas models using DD solver. The model with the highest R2 coefficient, indicating the best fit to the experimental data and was selected to describe the drug release mechanism of the system under investigation.[26]

RESULTS AND DISCUSSION

Pre-Formulation Studies

Metronidazole was analyzed for various pre-formulation specifications including organoleptic characteristics, solubility, melting point and drug excipient compatibility. The drug was found white crystalline powder with no taste and odour. Drug's melting was reported to be between 160-163°C. The observed solubility of metronidazole in distilled water was found to be 10 mg/mL in 0.1 NHCl was 3.9 mg/mL in and in Phosphate Buffer 7.4 is 5 mg/mL.

Preparation of standard curve in different solvents

The standard curve of the drug metronidazole was prepared in different solvents such as in 0.1 NHCl and in phosphate buffer pH 7.4. The r^2 value was found to be 0.9884 and 0.9902 respectively. The observed r^2 value suggested linearity in the equation.

Drug-Polymer Interaction Study

FTIR spectra were used to analyze drug compatibility with polymers. The peaks obtained in the spectra of drug and polymer mixtures correlate with each other as shown in Table 2. This indicates that the drug was compatible with the formulation components and shows no interaction between drugs and polymers.

XRD (X-ray Diffraction)

In X-ray Diffraction (XRD) analysis, the results indicate that the drug and polymers in their pure form is crystalline in nature. When the drug is converted into a formulations, the XRD graph in Figure 2 shows the transformation of formulations like F1, F6 and F3 to an amorphous form. This change is significant because amorphous forms generally have higher solubility and bioavailability as compared to their crystalline form.

Table 2: Comparison of the peak of functional group of pure drug with drug and excipient mixture.

	Wavelength (cm^{-1})			Interpretation
Sl. No.	Drug	Drug+ LBG	Drug+ HPMC K100	
1	3838	3838	3838	O-H Stretching
2	3201	3311	3281	N-H Stretching
3	2849	2749	2851	C-H Stretching
4	2523	2523	2522	C-H Stretching
5	1074	1073	1073	CH_2 twisting (vibration)

Table 3a: Pre-Compression Evaluation.

Formulation Code	Bulk density (gm/mL)	Tapped density (gm/mL)	Carr's Index (%)	Hausner's ratio*	Angle of repose (Θ)*	Flow Property
1	0.377±0.03	0.380±0.05	7.08±0.09	1.065±0.04	24.31±0.26	Good
2	0.322±0.04	0.332±0.02	6.66±0.15	1.063±0.05	26.20±0.14	Good
3	0.308±0.03	0.322±0.03	6.19±0.12	1.065±0.02	29.13±0.34	Good
4	0.314±0.03	0.330±0.06	6.48±0.14	1.073±0.06	22.13±0.26	Good
5	0.308±0.03	0.332±0.05	8.30±0.17	1.093±0.08	26.78±0.18	Good
6	0.388±0.04	0.424±0.05	10.38±0.20	1.127±0.07	25.79±0.24	Good
7	0.354±0.05	0.381±0.04	7.09±0.13	1.065±0.03	21.52±0.14	Good
8	0.282±0.05	0.318±0.04	8.68±0.07	1.086±0.03	26.95±0.15	Good

Evaluation of sustained release mucoadhesive tablet

Pre-Compression Studies

The flow characteristic is an important parameter for ensuring consistent dose distribution in tablets. The powder mixture of all formulations was examined to ensure good flow characteristics. The powder mixture was evaluated for various pre-compression studies like bulk density, tapped density, carr's compressibility index, Hausner's ratio, angle of repose and flow properties. The results showed good flow properties which are desirable for the compression of tablets as given in Table 3.

Post Compression Studies

The sustained release tablets were prepared by using wet granulation method and then characterized for various post compression studies like, weight variation, thickness, diameter, % friability and drug content. All the formulations showed good satisfactory results, which were within the required limits as shown in Table 3. The % friability of all prepared tablets was within the limit which is less than 1%. The weight variation of all prepared formulations was within the limit of ±5%. The average thickness and hardness of tablet were in the range of 6.2-7.1 mm and 6.2-7.6 kg/cm^2, the drug content of all the formulations was found to be within the USP limit (85-115).

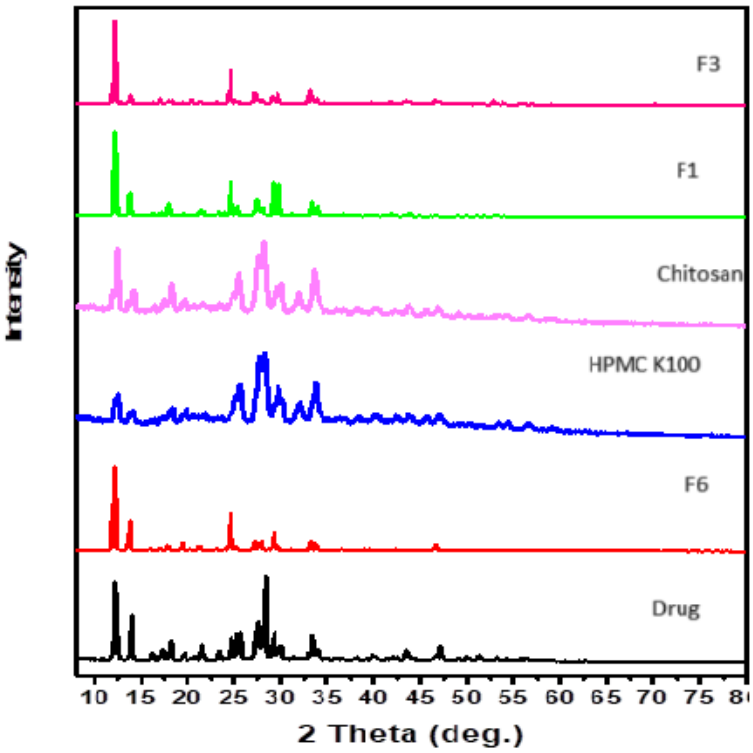

Figure 2: XRD of drug and formulations.

Table 3b: Post-compression studies.

Formulation Code	Weight Variation (mg)	Hardness (kg/cm^2)	Friability (%)	Thickness (mm)	Drug Content %	Wash off test
F1	Passed	6.9±0.12	0.54±0.015	6.8±0.02	97.55±0.21	410
F2	Passed	7.0±0.24	0.50±0.017	6.4±0.12	95.71±0.15	402
F3	Passed	6.6±0.08	0.42±0.014	6.9±0.009	99.85±0.34	365
F4	Passed	7.4±0.15	0.62±0.015	6.2±0.024	98.22±0.42	430
F5	Passed	6.2±0.25	0.72±0.016	6.8±0.031	96.65±0.16	360
F6	Passed	7.6±0.34	0.61±0.026	6.4±0.015	95.22±0.09	467
F7	Passed	6.4±0.17	0.52±0.026	6.7±0.019	98.85±0.48	355
F8	Passed	7.4±0.35	0.44±0.025	7.1±0.008	99.32±0.26	415

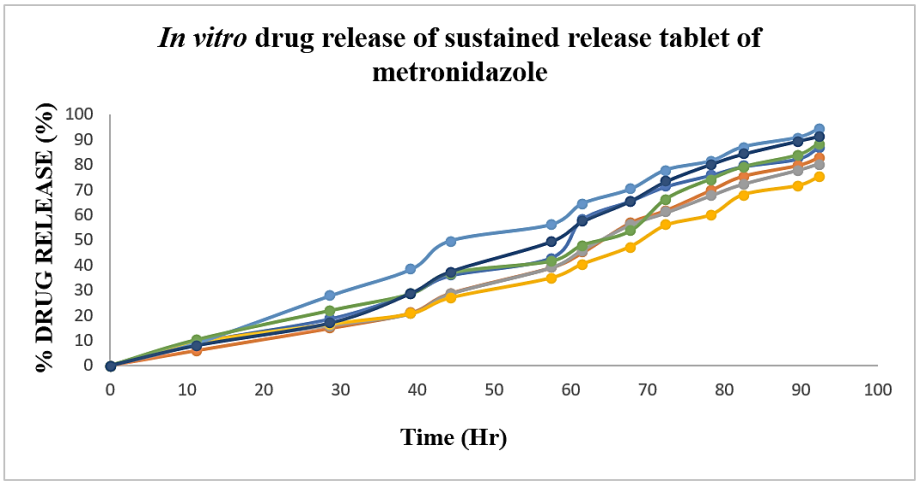

Figure 3: Dissolution Profile for the Formulation (F1-F8).

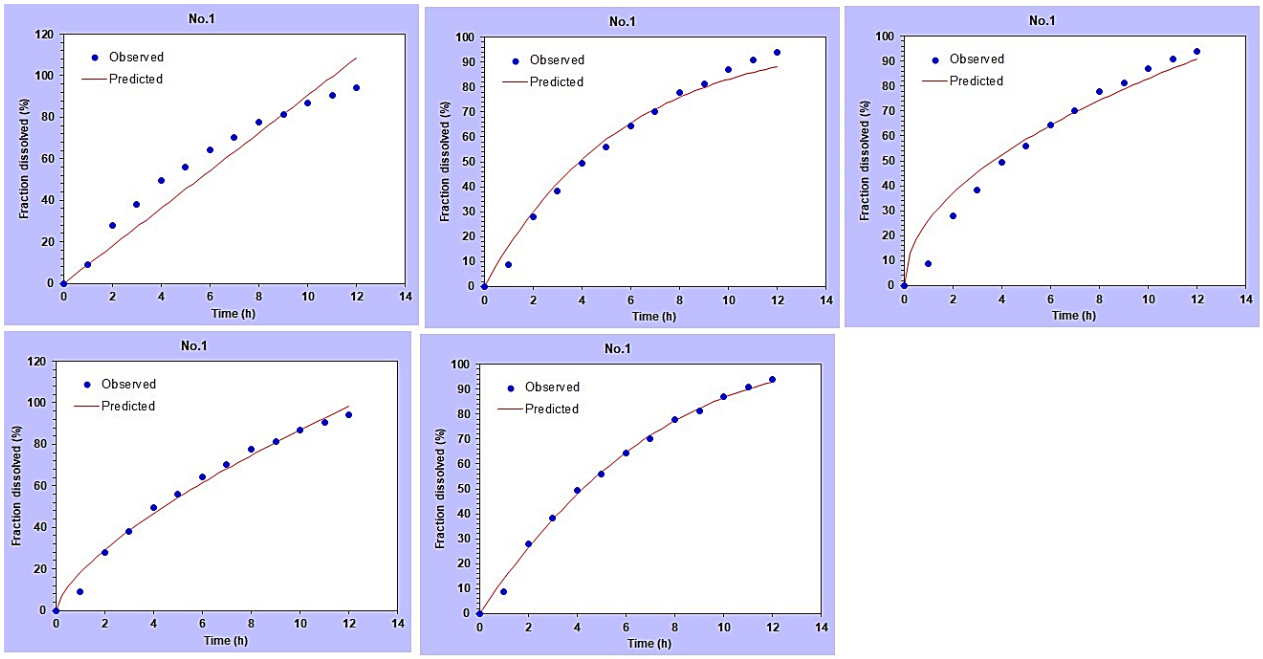

Figure 4: Kinetic Modeling study F6 A) Zero order kinetics B) First order kinetics C) Higuchi kinetics D) Korsmeyer Peppas kinetics E) Hixon Crowell Kinetic.

Table 4a: *In vitro* Drug Release of Formulations F1-F8.

Time (hr.)	% Drug Release							
1	F1	F2	F3	F4	F5	F6	F7	F8
2	11.31	9.03	6.01	8.56	9.20	8.89	10.30	8.00
4	28.74	18.55	14.86	15.95	16.47	27.76	21.89	16.99
6	44.33	35.74	28.67	28.49	26.96	49.56	36.80	37.33
8	61.51	58.09	44.84	45.86	40.34	64.39	47.82	57.21
10	72.42	71.21	61.73	60.81	55.82	77.81	66.20	73.24
12	82.50	79.18	75.38	72.23	67.96	84.98	79.01	80.20
14	89.44	86.55	84.56	87.98	80.43	90.23	85.97	85.12
16	91.29	90.32	91.33	93.76	89.04	95.34	92.34	90.77

Table 4b: Kinetic release data.

Released model	R^2	MSC
Zero-order	0.9137	1.9589
First model	0.9860	3.7771
Higuchi	0.9551	2.6116
Korsmeyer-peppas	0.9874	3.7257
Hixon-Crowell	0.9969	5.2850

Wash-Off Test

The detachment time of the formulations was found to be in the range of 355 min to 467 min. It indicates that all the formulations possess good adhesion properties as shown in Table 3.

In vitro drug release

The *in vitro* drug release data for all formulations were mentioned in Table 4 and Figure 3. It was found that all of the formulations exhibited a good rate of drug release. According to the release data, F6 shows better-sustained release effect i.e., 95.34% in 16 hr, this is because of the higher quantity of mucoadhesive polymer in F6 HPMC E-15 and sodium alginate therefore, the formulation gave sustained drug release.

Kinetics Analysis of Drug Release

The kinetic analysis of the *in vitro* release of drug data of optimized formulation F6 was carried out using DD solver as shown in Figure 4. The correlation coefficient (R^2) and Sum of Square Residues (SSR) result for each model was calculated and mentioned in Table 4. The optimised formulation shows Hixon-Crowell model while this model has the highest R^2 equal of 0.9969 and has the lowest SSR value of 35.5962. Amongst all models examined, the Model Selecting Criteria (MSC) of 5.2850 was shown to be the highest, implying that the properties of drug release are well specified by the Hixon-Crowell model.

CONCLUSION

In this, study HPMC K100, HPMC E-15, Locust bean gum, Sodium alginate and chitosan were used to prepare metronidazole sustained-release tablets. It was found that all of the formulations exhibited a good rate of drug release. According to the release data, F6 shows a better-sustained release effect i.e., 95.34% in 16 hr, this is because of the higher quantity of mucoadhesive polymer, in formulation F6 HPMC E-15 and sodium alginate was used in the highest quantity therefore, the formulation gave sustained drug release. Hence, it is a great choice for prolonged release of drugs, will improve patient compliance, enhance drug bioavailability and have low side effects.

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ABBREVIATIONS

CDDS: Colon-specific Drug Delivery Systems; **MNZ:** Metronidazole; **HPMC K100:** Hydroxypropyl methylcellulose K100; **XRD:** X-ray Diffraction; **LBG:** Locust bean gum; **IP:** Indian Pharamcopoeia; **SSR:** Sum of Square Residues; **MSC:** Model Selecting Criteria.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Colon-specific drug delivery systems (cdds) are effective for addressing several localized illnesses, including ulcerative colitis, crohn's disease, irritable bowel syndrome, chronic pancreatitis, and colonic cancer. Metronidazole sustained-release tablets are formulated to achieve a desired pharmaceutical concentration in the blood or at the intended site for a certain period of time. The wet granulation approach was used to prepare different blends of matrix tablets of metronidazole. All the developed formulations were able to provide sustained drug release of 16 hr. Henace, an excellent choice for prologed release of drugs, will improve patient compliance with enhanced bioavailability and reduced side effects.

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