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Research Paper

Formulation And Evaluation of a Combined Colon Targeted Novel Approach of Tinidazole for The Treatment of Amoebiasis.

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Abstract
This research aimed at creating a medication delivery method that targets the intestines to guarantee the precise release of Tinidazole where it is intended to act in the colon. Precise delivery is essential to improve therapeutic and reduce medication release in the upper gastrointestinal area. Tablets were developed with Tinidazole combined with a sugar component (mannitol) and pH-sensitive polymers, Eudragit E100 and Eudragit S100, utilizing the CODESTM method. The CODESTM approach was developed to overcome drawbacks linked to traditional pH- and microflora-dependent delivery systems. The coated tablets were used to obtain site-targeted release. Evaluations were conducted for both pre-formulation and post-formulation. Factors like weight fluctuation, film thickness, uniformity of content, variation in capsule content, and in vitro drug release were thoroughly evaluated to assess the performance of the formulation.of all the formulations created, formulation F8 showed the most positive outcomes. It included 9% Eudragit S100, 5% HPMC, and 6% Eudragit E100. This formulation demonstrated negligible drug release in gastric and intestinal pH environments, yet accomplished over 90% drug release under simulated colonic conditions. The CODESTM strategy effectively combined pH-sensitive and microbial-activated processes, demonstrating its appropriateness for colon-targeted distribution..The research determines that a colon-targeted drug delivery system for Tinidazole utilizing polysaccharides and pH-sensitive polymers represents a promising approach for localized colonic treatment. The CODESTM method successfully enables drug release in the colon while reducing early release in the stomach and small intestine, establishing it as a trustworthy technique for colon-targeted drug delivery.

Keywords: *CODESTM; Amoebiasis; Polysaccharide; Eudragit E and S100*

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I. INTRODUCTION

Systems designed for targeted drug delivery to the colon have garnered significant interest because of their promise in managing localized conditions like IBD. These systems are tailored to administer medications straight to the colon, thus improving treatment efficacy while minimizing overall side effects.

The colon creates a beneficial setting for drug administration because its enzymatic activity is relatively lower than that of the upper gastrointestinal tract. This feature is especially beneficial for medications that are prone to deterioration in the stomach or small intestine. Furthermore, extended duration in the colon increases drug uptake and boosts bioavailability.

Traditional oral drug delivery systems frequently struggle to attain site-specific targeting, leading to early drug release and diminished therapeutic effectiveness. To address these limitations, sophisticated methods like pH-sensitive, time-sensitive, and microflora-triggered systems have been created. Among these, the CODESTM technology combines pH-sensitive and microbial-triggered mechanisms, providing enhanced targeting efficiency.

Tinidazole is a potent antiprotozoal drug commonly utilized in treating amoebiasis. Nonetheless, traditional formulations might fail to deliver sufficient drug levels at the infection site within the colon. Consequently, creating a colon-targeted delivery method for tinidazole is crucial to improve its therapeutic effectiveness.

In this research, a new drug delivery system aimed at the colon was created utilizing a blend of Eudragit polymers and a hydrophilic protective layer. The formulation was assessed for its physicochemical characteristics, drug release properties, and appropriateness for focused colonic delivery. Targeted medicine delivery to the colon is advantageous for treating conditions related to the colon.

a) Medications that are harmed by stomach acid or broken down by pancreatic enzymes are less impacted in the colon.
b) Focused administration guarantees that the medication works directly at the impacted location, lowering the necessary dosage and lessening overall side effects.
c) It aids in minimizing extensive first-pass metabolism of steroids.

Advantages of delivery aimed at the colon.

a) Decreased dosing frequency,
b) Reducing the price of costly medications.
c) It also possesses an extended retention period and reacts to substances that improve the uptake of inadequately absorbed drugs.
d) This enhances adherence among patients.

II. METHODOLOGY

The following preformulation studies was carried out;-

a) Identification
b) Organoleptic determination
c) Melting Point determination
d) Solubility determination of tinidazole
e) Analytical Method for the determination of tinidazole
f) λ max determination of Tinidazole.
g) Preparation of calibration curve of Tinidazole.

Compatibility studies

FTIR Study

An FTIR spectrophotometer was used to acquire the IR spectra. The drug and excipients were mixed in a 1:1 ratio to form the samples, which were then stored in sealed containers for a month. The samples' FTIR spectra were examined. produced using the potassium bromide pellet technique.

DSC

To determine whether the substances were compatible, a DSC research was conducted using the DSC-60 equipment. The medication and excipients were combined in a 1:1 ratio to create the samples.

PRECOMPRESSION PARAMETERS

The following precompression parameters were carried out;-

a) Angle of repose (θ)
b) Bulk density (Db)
c) Tapped density
d) Compressibility index (Carr's index)

e) Hausner's ratio

FORMULATION OF CORE TABLET

The required amount of bulk powder was measured and granulated through the wet granulation method using 2% w/w HPMC in distilled water as the binding solution. All the components were measured and mixed thoroughly then completely blended by passing it through a mesh number 60. A single station tablet press was then utilized to press the consistently blended materials into tablets using 6 mm round, flat, and plain punches. Weight fluctuation, hardness, friability, thickness, and disintegration in different media were a few of the quality control tests performed on core tablets.

Table No:1 Core tablet Formulation (CTF 1-9)

Ingredients	CTF1(mg)	CTF2(mg)	CTF3(mg)	CTF4(mg)	CTF5(mg)	CTF6(mg)	CTF7(mg)	CTF8(mg)	CTF9(mg)
Tinidazole	150	150	150	150	150	150	150	150	150
HPMC K100 M	5	5	5	5	5	5	5	5	5
Mannitol	7.5	15	22.5	30	37.5	45	52.5	60	67.5
Lactose	80	72.5	65	57.5	50	42.5	35	27.5	20
Talc	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
Magnesium stearate	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75

EVALUATION OF CORE TABLET

Density

Vernier calipers were used to measure the thickness of six tablets. Each tablet's thickness was measured to determine how far it differed from the standard value by ± 5%.

Hardness

The tablet's hardness was measured using the Monsanto Hardness Tester. From each batch, six tablets were selected and assessed; the average value and standard deviation were noted.

Friability

A Roche Friabilator was used to assess the tablets' friability. Ten tablets were weighed together before being placed in the chamber. The tablets were subjected to abrasion and shock during the 100 rotations of the friabilator because the plastic container holding the pills drops them six inches with each round.

The tablets are subsequently dusted and weighed again. The % friability (F) was determined using the formula below:

$$\mathbf{F = (W1\text{-}W2 / W1) \times 100}$$

Where,

W1 is the initial weight of the sample of twenty tablets before the test.

W2 is the weight of the tablet after the test.

Weight Variation

Twenty tablets were chosen at random, and the average weight was calculated. The weight of each tablet was then measured and compared to the average weight. After calculating the percentage deviation, the weight variation was examined. This process was used to calculate and document the weight fluctuation range for each batch of formulations. The following formula was used to compute weight variation.

$$\%\boldsymbol{Weight\ variation} = \boldsymbol{Individual\ wt.of\ tablet} - \boldsymbol{Average\ wt.of\ tablet} \times \mathbf{100}$$

Average weight variation range

Table no:2 Average weight variation

Average weight of Tablet	Percentage Deviation
80 mg or less	10
More than 80 mg but less than 250 mg	7.5
250 mg or more	5

Content Uniformity Test

Ten tablets were coarsely ground, and precisely calibrated amounts of powder equal to 100 mg of tinidazole were added to a 100 ml volumetric flask that held around 50 ml of methanol. To ensure the drug's total solubility, this combination was left for six hours while being periodically sonicated. The amount made with pH 6.8 phosphate buffer was completely mixed. A 0.22µm membrane filter was used to filter the solutions.

Dissolution Testing of Core Tablet of Tinidazole

Testing for dissolution of the main tablet of Tinidazole was conducted utilizing a pH 6.8 phosphate buffer as the dissolution medium. A dissolution study was conducted for approximately 4 hours at 37.5 °C and 50 rpm utilizing USP type II equipment. Five milliliters of sample were taken from the dissolution medium, and its absorbance was recorded at 318 nm using a UV spectrophotometer.

Preparation of CODESTM tablet

Core tablets were coated with three layers of polymer using a dip coating technique: the initial layer consisted of Eudragit E100 (3%, 6%, 9%) in isopropyl alcohol, the second layer was HPMC K100M (5%) in distilled water, and the final layer was Eudragit S100 (3%, 6%, 9%) in isopropyl alcohol. At the conclusion of every coating stage, tablets were dried in a hot air oven. The weight gain percentage of tablets post-coating was considered to reflect the thickness of the coating.

Investigation of *In Vitro* Disintegration

Disintegration is the process by which a tablet fragments into smaller pieces. I.P. standards-compliant disintegration test equipment was used to measure a tablet's in vitro disintegration time. Put one pill inside each of the six tubes in the basket. Put a disc in each tube and run the device with an immersion solution of pH 6.8 (simulated intestinal fluid) maintained at 37±2°C. The assembly needs to be raised and lowered at a pace of thirty cycles per minute in pH 6.8 while being kept at 37±2°C.

Evaluation of CODESTM Tablet

Following evaluation studies were done;-

a) Thickness
b) Hardness
c) Friability
d) Weight Gained after coating
e) Content Uniformity Test
f) *In vitro* drug release study

Type II paddle device (50 rpm, 37 ± 0.50 °C) was used for in vitro drug release investigations. Enteric coated tablets were subjected to an in vitro release investigation for two hours in a pH 1.2 (900 ml) acid buffer solution that mimicked stomach fluid (SGF). The dissolution media was then replaced with pH 7.4 phosphate buffer solution (900 ml), simulated intestinal fluid (SIF), and monitored for three hours. Next, pH 6.8 buffer solution (900 ml), simulated colonic fluid (SCF), was substituted, and the release was monitored for nineteen hours. Drug release was measured at different time intervals at 318 nm using the UV double beam spectrophotometer. For the in vitro drug release, a microbial culture of Lactobacillus sporogenes [108 ml] was added to all medium.

III.RESULTS

PREFORMULATION STUDY

Identification Of Drug

The sample spectrum was compared with the reference spectrum. There were no significant changes in the functional groups. The frequency of observed functional groups =CH, C-H, C=C, C=N, CH2, C-C, N=O, C-O, S=O, C-N are within the standard limits. The finger print region has not changed significantly. So the drug was identified as Tinidazole.

Organoleptic Evaluation

- Color : White or pale yellow
- Odour : Odorless or almost odorless
- Appearance: Crystalline powder

Determination Of Melting Point

The standard melting point of Tinidazole is in the range of 127-128°C. The observed value was 128°C which is within the range as per official monograph. So the drug was identified as Tinidazole.

Determination of Solubility

Solubility of Tinidazole

Table no:3 Solubility of tinidazole

Sl.No	Solvent	Solubility
1	Water	Insoluble

2	Methanol	Sparingly soluble
3	0.1N HCl	Soluble
4	Phosphate buffer 6.8	Soluble
	Phosphate buffer 7.4	Soluble

The drug's solubility was assessed by dissolving it in various solvents, including water, methanol, 0.1 N HCl, phosphate buffer saline 7.4, and 6.8. Table No. 13 presents the findings of the solubility analysis. It was sparingly soluble in methanol, very little soluble in water, and soluble in 0.1 N HCl, phosphate buffer saline 7.4, and 6.8.

ANALYTICAL METHOD FOR THE DETERMINATION OF TINIDAZOLE

Determination of λ max of tinidazole

The 10 µg/ ml sample was prepared and scanned between 200 to 400 nm. The drug showed maximum absorption at 278 nm in 1.2 HCl buffer, 320 nm in phosphate buffer 7.4 and 318 nm in 6.8buffer**.**

Preparation of Calibration Curve of Tinidazole in pH1.2 HCl buffer

Table no 4: Standard calibration curve data of Tinidazole in pH 1.2 HCl buffer

Sl.No	Concentration(µg/ml)	Absorbance(nm)
1	0	0
2	2	0.060
3	4	0.090
4	6	0.153
5	8	0.201
6	10	0.248
7	12	0.294
8	14	0.343
9	16	0.389
10	18	0.445
11	20	0.492

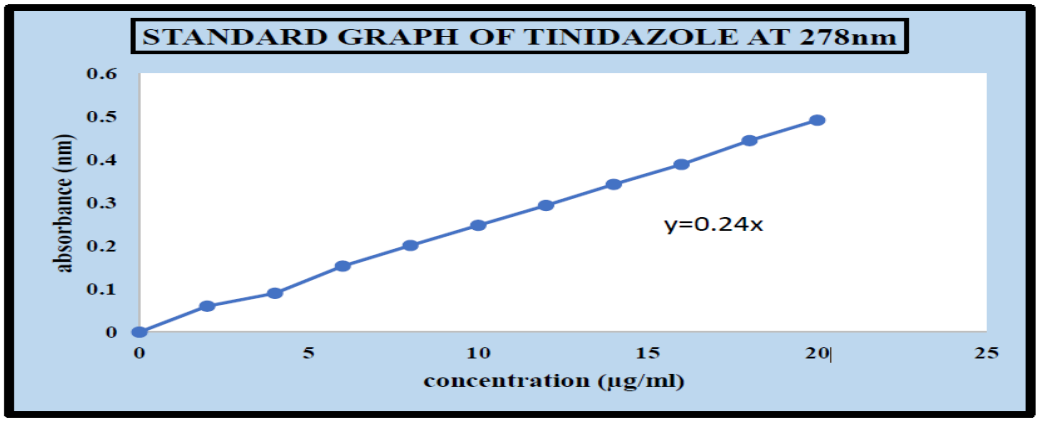


Fig no 1: Standard graph of Tinidazole at pH 1.2 HCl

FTIR

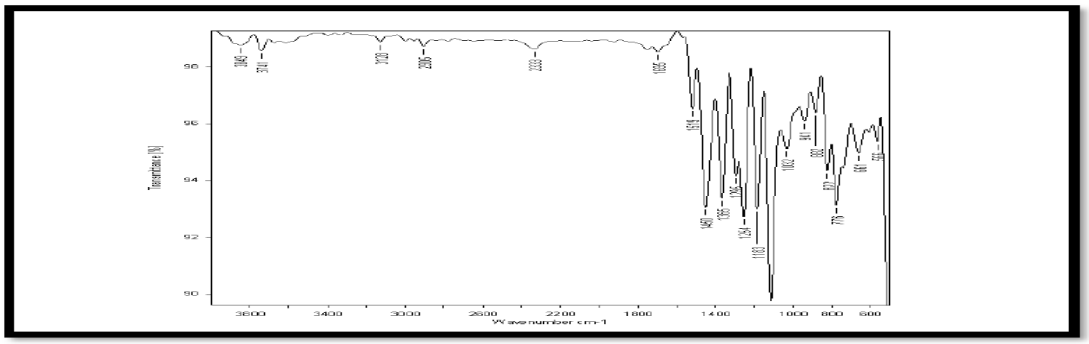


Fig no 2: FTIR Spectrum of Tinidazole (sample)

Comparison of FTIR Spectra of Tinidazole and excipients

As seen in Fig., FTIR analyses of the drug [Tinidazole] and the drug-excipient physical mixes revealed no appreciable variations in the frequency of Tinidazole's functional groups. The medication was therefore compatible with every excipient.

DSC Studies

DSC Curve of pure Tinidazole

Drug [Tinidazole] and drug-excipient physical combinations were the subjects of the DSC investigations. Figures Nos. 19 and 20 present the findings. The profile of tinidazole with a melting point of 128˚C was displayed in the recorded DSC thermograms. The drug had a melting point of 128ºC when mixed with excipients. The medicine and excipients are compatible when the melting point stays nearly unchanged.

PRECOMPRESSION PARAMETER OF CORE MATERIAL

Pre-formulation assessments were conducted on the prepared powder mixture to examine its physical and chemical attributes and confirm its appropriateness for tablet manufacturing via direct compression. Evaluated metrics included angle of repose, bulk density, tapped density, Carr's compressibility index, and Hausner's ratio, as presented in the accompanying table. The mixture displayed an angle of repose between 26° and 29°, reflecting excellent flow characteristics; Carr's index values of 7.8–11.1% and Hausner's ratio of 1.08–1.12 further demonstrate strong flowability and compressibility, making it ideal for tableting.

Table no 5: Values of angle of repose, bulk density, tapped density,Compressibility Index and Hausner's ratio

F Code	Angle of repose	Bulk density	Tapped density	Carr's Index	Hausner's ratio
F1	29.94 ±0.12	0.32± 0.02	0.36± 0.21	11.11± 0.32	1.12 ±0.11
F2	28.92± 0.16	0.34± 0.21	0.37 ±0.32	8.1 ±0.21	1.08± 0.25
F3	28.76± 0.2	0.32 ±0.24	0.36± 0.12	11.1 ±0.34	1.12± 0.14
F4	27.91± 0.24	0.35 ±0.31	0.38± 0.15	7.8± 0.1	1.08 ±0.24
F5	26.23 ±0.32	0.36± 0.21	0.4 ±0.23	10.1 ±0.26	1.11 ±0.14
F6	27.31 ±0.24	0.33 ±0.32	0.37 ±0.12	11.2± 0.32	1.13 ±0.12
F7	28.63± 0.54	0.32± 0.26	0.41± 0.23	10.3± 0.21	1.12 ±0.22
F8	27.76 ±0.52	0.34± 0.24	0.36± 0.22	10.2± 0.24	1.09 ±0.14
F9	28.41± 0.12	0.36 ±0.22	0.38 ±0.26	11.1± 0.33	1.11± 0.17

EVALUATION OF CORE TABLETS

Table No 6: . Thickness, Hardness, Friability of core tablet.

F Code	Thickness(nm)	Hardness(kg/cm2)	Friability
F1	2.73± 0.07	4.5 ±0.67	1.010± 0.00
F2	2,67 ±0.06	4.7 ±0.35	0.505 ±0.01
F3	2.64 ±0.05	4.6± 0.61	1.003± 0.04
F4	2.72± 0.06	4.7± 0.42	1.021 ±0.01
F5	2.68 ±0.05	4.8± 0.62	1.032 ±0.03
F6	2.71 ±0.02	4.6± 0.46	1.027± 0.01
F7	2.70 ±0.06	4.4± 0.52	1.031± 0.03
F8	2.72± 0.05	4.6 ±0.64	1.027± 0.01
F9	2.68± 0.03	4.5± 0.51	1.029 ±0.04

Table no 7: Results of Weight variation, Content uniformity and Disintegration time of core tablet

CTF Code	Weight variation(mg)	Drug content(%)	Disintegration time(sec)
CTF1	248 ±0.03	89.32 ±0.15	47 ±0.01
CTF2	247 ±0.02	92.67± 0.23	49± 0.05
CTF3	251± 0.05	95.51± 0.14	45± 0.07
CTF4	249± 0.03	93.46 ±0.21	44 ±0.06
CTF5	251 ±0.06	94.38 ±0.34	45± 0.03
CTF6	251± 0.03	96.43± 0.42	44 ±0.05
CTF7	249± 0.05	95.31± 0.16	46± 0.03
CTF8	251± 0.02	98.12 ±0.21	45± 0.04
CTF9	251 ±0.04	96.04± 0.31	46± 0.06

The hardness value of core tablets of all formulation were in the range between 4.5 to 4.8kg/cm2. The friability of all formulations was determined. The friability value of none of the formulation exceeds the limit. The thickness of all tablets was between 2.6-2.7 mm showing fairly uniform tableting. The *in vitro* disintegration time of different tablets are given in the table. All the formulation passes the content uniformity, average tablet weight is within the limit as per IP specifications.

***IN VITRO* DRUG RELEASE STUDY OF CORE TABLET**

In-vitro drug release studies of the prepared tablets were carried out in three stages with microbial culture. The first stage used 900 ml simulated gastric fluid (pH 1.2) for 2 hours, followed by 900 ml simulated intestinal fluid (pH 7.4) for 3 hours, and finally 900 ml simulated colonic fluid (pH 6.8) for 19 hours, giving a total study period of 24 hours.

Drug release in pH 1.2 was almost negligible, except CTF-1 and CTF-2 which showed about 1.5% release in the first 2 hours. In intestinal fluid, formulations CTF-1, CTF-4 and CTF-7 (3%, 6% and 9% Eudragit S100 with 3% Eudragit E100) showed 10.07%, 3.17% and 1.83% release respectively after 5 hours. Similar decreasing release was observed for CTF-2, CTF-5, CTF-8 and CTF-3, CTF-6, CTF-9 with increasing concentrations of Eudragit E100 and S100. Overall, drug release decreased as polymer concentration increased.

Effect Of Eudragit S100 On The Drug Release:

Figure 24 illustrates the dissolution behavior of formulations CTF1, CTF4, and CTF7, which incorporate 3%, 6%, and 9% coating levels of Eudragit S100 combined with 3% Eudragit E100. As the Eudragit S100 coating thickness increased, the duration required for full coating dissolution extended, resulting in lower cumulative drug release. Figures 25 and 26 depict the dissolution curves for formulations coated with 6% and 9% Eudragit E100, revealing a comparable trend: higher polymer concentrations led to progressively reduced drug release percentages.

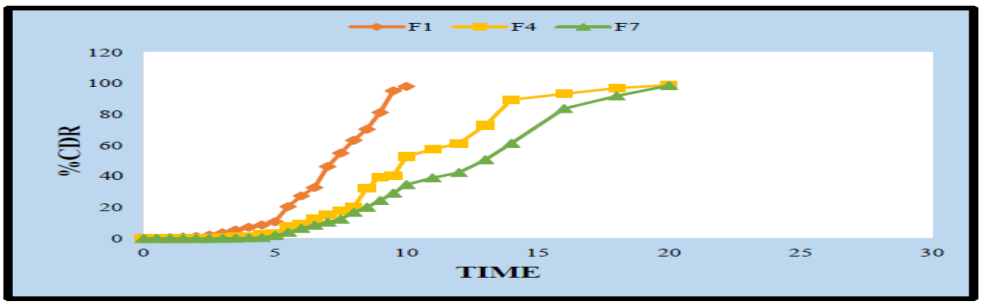


Fig no 3: Dissolution Profile of 3, 6, and 9% Eudragit S100 and 3% Eudragit E100

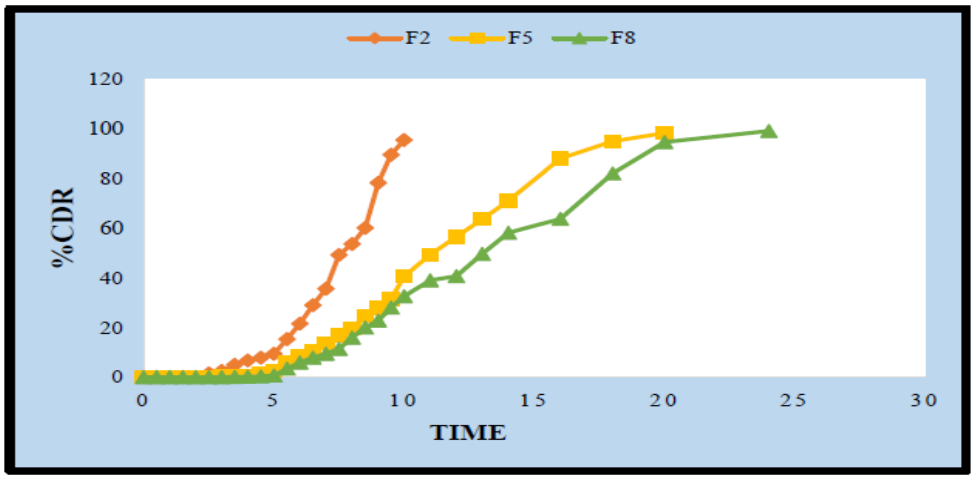


Fig no 4: Dissolution Profile of 3, 6, and 9% Eudragit S100 and 9% Eudragit E100

Effect of Eudragit E100 on drug release :

The polymer concentration clearly has a major influence on the tablets' drug release behavior. Among the nine formulations tested, CTF8—featuring 9% Eudragit S100 and 6% Eudragit E100 coatings—stood out as the optimal choice, since it achieved the highest release at colonic pH and nearly complete (100%) drug release within 24 hours, compared to CTF9, which only reached 83.98% release by that time.

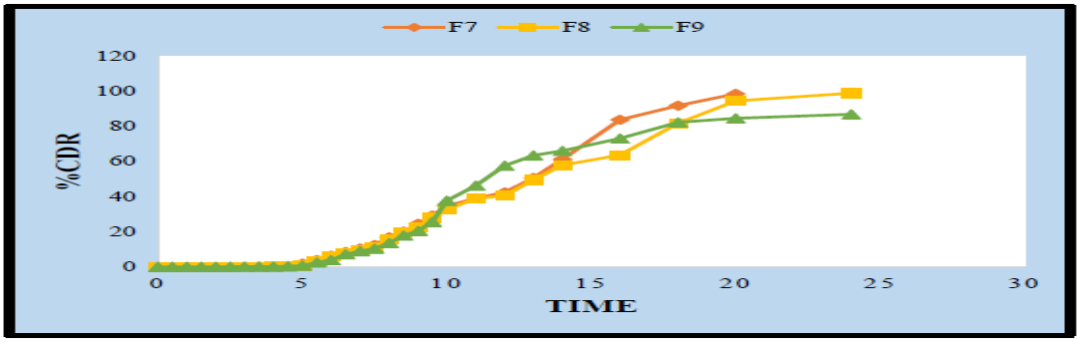


Fig no 5: Dissolution profile of CTF 7,CTF 8 and CT F9

IV.CONCLUSION

Amoebiasis, caused by Entamoeba histolytica, mainly impacts the colon, and successful treatment necessitates sufficient drug levels at the infection site. Standard Tinidazole tablets do not provide adequate medication to the colon and can lead to side effects because of inconsistent gastrointestinal transit time. A colon-targeted drug delivery system was created to address these limitations, utilizing a mix of pH-sensitive polymers and a polysaccharide-based method triggered by microbes (CODESTM concept).

Among the different formulations created, Formulation F8, which includes 9% Eudragit S100, 5% HPMC, and 6% Eudragit E100, showed the best results. In vitro drug release experiments verified that this formulation successfully reduced drug release in the stomach and small intestine, while ensuring optimal drug release in the colonic setting. The blend of pH responsiveness and microbial activation guaranteed accurate and targeted delivery to the colon.

Consequently, the colon-targeted Tinidazole delivery system effectively increased localized drug release, potentially boosting therapeutic effectiveness, decreasing dosing frequency, and reducing systemic side effects. This method stands as a hopeful and efficient tactic for addressing intestinal amoebiasis and various colonic infections.

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