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Design and characterization of bacteria friendly natural matrix tablet of zidovudine hydrochloride in colon cancer

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ABSTRACT

In present study, we have tried to develop the colon targeted matrix tablets of Zidovudine(AZT) hydrochloride for colon cancer using microbially triggered approach. Natural polysaccharide chitosan was used as a matrix forming agents for microbially triggered approach. Optimized ratio of Ethyl Cellulose, PVP-K30 and Starch different binders in varying concentration range were explored in different formulation batches. F8 batchwas found to be optimized in terms of providing higher drug release than other formulations among F1-F12 . It was seen that on increasing the concentration of binder the amount of drug release was decreased. 12% starch concentration provided the best release pattern and give maximum release upto 98.23 % till fourteen hrs.

Key words: Chitosan, Microbially, ethyl cellulose,colon cancer

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INTRODUCTION

Oral drug delivery system represents one of the frontier areas of controlled drug delivery system; such dosage forms are having a major advantage is patient compliance .Colonic drug delivery system belongs to oral controlled drug delivery system group that are capable of delivering in the colon by passing the gastric transit. Colonic delivery refers to targeted delivery of drugs into the lower gastrointestinal tract (GIT), primarily in the large intestine region i.e. colon. Targeted drug delivery to the colon ensures direct treatment at the disease site, lower dosing and fewer systemic side effects.[1,2] Recently, colon specific drug delivery system is intended for the local treatment of variety of bowel diseases like ulcerative colitis, Inflammatory bowel disease(IBD). Colon targeting can potentially be used for colon cancer or the systemic administration of drugs that are adversely affected by upper GIT [3]. Colon has a near neutral pH, longer transit time, less proteolytic enzyme activity. The properties of drug, type of delivery system, and interaction of drug with healthy or diseased gut are some of the important factors to be considered for successful colonic drug delivery [4] Moreover, Telomerase is a highly specialized reverse transcriptase enzyme and is a ribonucleoprotein composed of catalytic subunit hTERT, an RNA component hTR and group of associated proteins. The human holoenzyme telomerase is a ribonucleoprotein composed by a catalytic subunit, hTERT, an RNA component, hTR, and a group of associated proteins. Telomerase is normally expressed in embryonic cells and sometimes repressed in adult wood even upto around 85 % of solid tumers. The identification of the hTERT as a functional catalytic RT and prompted studies of inhibiting telomerase with the HIV RT inhibitor Azidothymidine(AZT) makes us to consider this drug Zidovudine (AZT) too as a potential target for anticancer therapy. This observation makes it a potential target for developing drugs that could be developed for therapeutic purposes.Since then, several studies have considered AZT for telomerase inhibition and have led to potential clinical

strategies for anticancer therapy.By considering the above facts, Zidovudine colonic drug delivery was designed and characterized for controlled release in order to improve the patient compliance in such a way that it reduces dosing frequency, reduces side effects and increases the bioavailability of the drug.[5]

The release rate will be controlled depending upon the type and concentration of the polymer that swells, leads to diffusion and erosion of the drug.[6-8] The investigation was concerned with design and characterization of Zidovudine (AZT) matrix based system for controlled release in order to improve efficacy and better patient compliance. Zidovudine is a dideoxynucleoside compound in which 3- hydroxy group on the sugar moiety can be replaced by group and this modification prevents the formation of phosphodiester linkages which are needed for the completion of nucleic acid chain. However, the main limitation to therapeutic effectiveness of Zidovudine is its dose-dependent hematological toxicity, low therapeutic index, short biological half-life of 0.8-1.5 hrs, and poor bioavailability 65%.[7-11].

MATERIALS AND METHODS

Zidovudine hydrochloride was obtained as a gift sample from Ranbaxy Laboratories, Gurgaon. PVP K30 were provided by Hi Media Labs Pvt Ltd. Lactose monohydrate was obtained from SD Fine ChemPvt Ltd. Enteric coating polymer Eudragit L100 and Eudragit S 100 were obtained from Degussa Pvt. Ltd., Mumbai. Other chemicals were of analytical grade and obtained from LobaChemie and Hi Media Labs Pvt Ltd.

2.1Analysis of drug and preparation of calibration curve:
Standard plots of Zidovudine Hydrochloride were made by using series of standard solutions obtained by diluting the stock solution (100µg/ml) to calculate the amount of drug present in dissolution samples and to determine content uniformity. The calibration curve Zidovudine Hydrochloride was drawn at different pH 1.2, 6.8 and 7.4 buffer solutions taking a number of dilutions. The values of regression coefficient came out to be 0.999, 0.990 and 0.993 at pH 1.2, 6.8 and 7.4 respectively. Beer's law was found to be obeyed by the results obtained.

2.2Method of preparation of core tablets
All the batches of formulations were prepared using different binders in varying concentrations. All the ingredients required for the study were accurately weighed on a digital electronic balance and mixed properly to ensure uniform distribution of ingredients. Wet dough mass was prepared using sufficient quantity of isopropyl alcohol which was then passed through sieve no. 16 to form granules. The prepared granules were kept undisturbed for 3-4 hrs for air drying followed by drying in the oven at 45-50 ºC for 15-30 minutes. Dried granules were again passed through sieve no. 18 to get uniform size of granules and then magnesium stearate and talc were added. [6/11] Tablets weighing 300mg was prepared using minirotary tablet press (Fluid pack machinery) using 8 mm punch. Two types of punches were used, one flat punch and the other with the line of intersection for the preparation of two different shapes of tablets with same hardness of 4-5 kg/cm2. Two different shaped tablets were prepared for easy distinction between tablets of different batches during and after coating. To ensure the drug release only in large intestine, the prepared tablets were enteric coated by spray coating method using Eudragit L100 and S100 as enteric coating polymer.

Table 1: Experimental design for microbially triggered colon targeted Zidovudine Hydrochloride formulation

CONTENT	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Drug	300	300	300	300	300	300	300	300	300	300	300	300
Chitosan	21.6	7.2	14.4	21.6	14.4	21.6	14.4	21.6	21.6	21.6	21.6	21.6
PEG4000	12	6	12	18	12	18	12	18	12	12	12	12
EC	7.2	14.4	21.6	0	0	0	0	0	0	0	0	0
Starch	0	0	0	14.4	21.6	0	0	14.4	0	14.4	14.4	14.4
PVK 30	0	0	0	0	0	0	14.4	21.6	0	14.4	0	0
SSG	0	0	0	0	0	0	0	0	0	1.2	2.4	3.6
Mag St	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Talc	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Lactose	45.8	25.4	5	25.4	5	25.4	5	18.2	18.2	17	15.8	14.6

2.3Coating of tablets:
Before the enteric coating, seal coating is done to provide the moisture resistance property to the tablets. The seal coating is done with cellulose acetate solution.Enteric coating: Seal coated tablets were further coated with the

enteric polymer combination i.e. Eudragit L100 and Eudragit S100 to keep them intact in the acidic and small intestinal pH, and to release drug only at pH 6.8. Iso propyl alcohol and methylene chloride were used as solvent for the coating solution.

Table 2: Formula for the preparation of enteric coating solution

Ingredients	Quantity
Eudragit L100	60 gm
Eudragit S100	60 gm
IPA	0.84 Lt
Methylene Chloride	1.32 Lt
Color Sunset Yellow	6 gm

3.PREFORMULATION STUDIES

3.1Pre-compression studies of powder

The powder mixture of the formulations and prepared granules were evaluated to determine their flow properties by calculating different parameters like bulk density, tapped density, angle of repose, Carr's Index.

3.2Post compression evaluation oftablets

3.2.1Uniformity of weight: Twenty tablets were weighed individually and their average weight was also calculated. From the average weights of tablets, standard deviation and individual deviations were calculated.

3.2.2Hardness of Tablets: Six tablets from each formulation batch were selected randomly and their crushing strength (kg/cm2) was determined using Monsanto hardness tester.

3.2.3Friability: Six previously weighed tablets from each formulation were placed in Roche Friabilator for carrying out friability. Apparatus was run on 25 rpm for 4 minutes. Afterwards, tablets were taken out, dusted and weighed again. Friability of tablets was calculated from the formula:

$$\text{Percentage Friability} = \frac{\text{Initial weight-Final weight}}{\text{Initial weight}} \times 100$$

3.2.4Content Uniformity Test

Ten tablets from each formulation were tested for content uniformity test. Each tablet was individually triturated and dissolved in 100 ml of water. Drug content of all the formulations were calculated by potentiometrical analysis of tablets. It was concluded that the formulation batches passes the test for drug content.

3.3Scanning Electron Microscopy [13,14]

Surface morphology of the coated matrix tablets of Zidovudine Hydrochloride were examined using Scanning Electron Microscope (S-3400N, Hitachi Japan) with image analysis system. Prior to analysis, samples were gold sputter coated with to render them electrically conductive. Samples were analyzed before dissolution and after dissolution.

3.4Disintegration Test

Disintegration test was performed on each formulation for checking intactness of enteric coat. Disintegration apparatus (Electro lab Ltd., ED-2L) was used and I.P. method was followed. Three tablets of each formulation were tested for disintegration. Tablets were firstly tested in water for 5 minutes then in 0.1N HCL for 2 hours (simulating gastric transit time) to see the intactness to the coat. Afterwards, tablets were tested in the phosphate buffer pH 7.4 (simulating intestinal pH) till the coating dissolved. Temperature in each case was kept constant at 37^0C. The results of disintegration test and drug content are shown in table 4 below.

3.5In Vitro Drug release Study [15,16]

In Vitro Drug Release studies in pH 1.2 (Acidic Buffer) and pH 7.4 Phosphate Buffer Saline:

The ability of the formulated tablets to retard the drug release in the physiological environment of the stomach and small intestine was assessed by conducting drug release studies in simulated stomach and simulated intestinal pH

respectively. In vitro dissolution studies or drug release studies were performed for the Zidovudine Hydrochloride based matrix tablets using USP dissolution apparatus I (Basket Type, Electrolab tablet dissolution apparatus), with 50 rpm and 100 rpm at temperature 37 ± 0.50^{0}C in dissolution medium of 900 ml. In order to simulate the pH change along the gastrointestinal tract, dissolution media of pH 1.2 and 7.4 were used sequentially following the sequential pH change method. The tablets were studied in pH 1.2 acidic buffer (900 ml) for 2 hrs, as the average gastric emptying time is nearly 2 hrs. Then the tablets were tested in pH 7.4 buffer (900 ml) for 3 hrs, as the average small intestinal transit time is nearly 3 hrs. Samples each of 10 ml were taken. Afterwards, solution was filtered through 0.22 µm membrane filters and drug content was determined by UV method.

In Vitro Drug Release Study in Phosphate Buffer saline pH 6.8:
After performing the in vitro drug release studies in the simulated dissolution medium of pH 1.2 and 7.4, same formulations were tested in the dissolution medium having phosphate saline buffer of pH 6.8 for simulation of the colonic medium. This was done by replacing the 7.4 pH phosphate buffer with the 6.8 pH buffer solution. All the conditions for the in vitro drug release study were same as used in simulated gastric medium and simulated intestinal medium and drug content was also determined by the same method.

3.6Drug Release Studies in Presence of Rat Ceacal Content for chitosan based formulations [17,18]
Chitosan was used as a polymer in the formulations which is susceptible to microbes present in the colon. So dissolution rate studies were also performed using rat caecal content because of similarity with human colonic microflora to the rat's microbial environment of colon. The experimental protocol was under strict compliance of the CPCSEA guidelines. Wistar rats were used which were maintained on normal diet to simulate enzymes which specifically hydrolyze chitosan. For enzyme induction, chitosan aqueous dispersion (1 ml of 2% w/v dispersion) was administered to the rats daily for 6 - 7 days. Thirty minutes before the commencement of study, four rats were sacrificed, their abdomen were opened, caecal were isolated, ligated at both ends, cut loose and transferred immediately into phosphate saline buffer pH 6.8 bubbled with CO2 gas. Afterwards, caeacal bags were opened and their content were weighed and transferred to phosphate saline buffer to obtain 4% w/v rat caecal content. This caecal content was further used for the study. Due to the anaerobic nature of the bacterial content, all the operations were performed under the environment of CO2 gas.

The drug release studies were carried out using the same USP dissolution rate test apparatus with slight modifications. The experiments were carried out in a 250 ml beaker immersed in water maintained in the jars of the dissolution test apparatus. Initial studies were carried out in 300 ml of 0.1N HCL (pH 1.2) for 2 hours followed by phosphate saline buffer pH 7.4 for 3 hours. Afterwards, drug release studies were performed using 400 ml of pH 6.8 phosphate saline buffer having 4% w/v rat caecal content prepared by adding 16 gm of caecal content to the dissolution medium of pH 6.8. The experiment was performed for 7 hours in pH 6.8 completing an overall time period of 12 hrs. with continuous supply of CO2 to provide anaerobic environment.

At different time interval, samples were withdrawn without a prefilter and was replaced with the same dissolution medium freshly bubbled with CO2 gas to maintain the sink condition. Afterwards, each withdrawn samples was diluted with phosphate saline buffer pH 6.8. Then samples were centrifuged and supernatant was removed using bacteria proof filters (G5) and the filtrates were analyzed for drug concentration under UV spectrophotometer.

RESULTS AND DISCUSSION

The results observed through pre-compression studies for bulk density, tapped density, Hauser's ratio and angle of repose of powder formulation were not satisfactory therefore granules were prepared to improve flow properties. The bulk density values were found to be in the range of 0.565 – 0.722g/cm3, while the corresponding Tapped density values were in the range of 0.643 – 0.816g/cm3. Angle of repose was also found to be less than 25 indicating excellent flow properties. Hence it was concluded that wet granulation can be the desired method for tablet formulation.

Results for the uniformity of weight were found to be within the range (i.e. ± 7.5%) provided by Indian Pharmacopoeia. The hardness came out to be in range 4.25±0.54 kg/cm^2 to 5.11±0.26 kg/cm^{2}for uncoated tablets and 7.16±0.30 kg/cm^2 to 7.91±0.31 kg/cm^2 for coated tablets. The friability range for uncoated tablets was from 0.41% to 0.74% and for coated tablets it was from 0.13% to 0.35% which was within the limits provided by Indian Pharmacopoeia (i.e. ± 1%). The results are mentioned in the table 3 below. Disintegration test was performed on

three tablets of each formulation. From the results it was clearly seen that the coat remained intact for first two (02) hrs. in the acidic media preventing the drug release completely and gets dissolved in the phosphate buffer at a time period of around 192 to 267 minutes. Results of disintegration and drug content studies are shown in Table 4. The results of in vitro dissolution studies show that no drug was released from the formulation during first three (3) hrs of study indicating the effectiveness of the enteric coat in preventing drug release in upper GIT region. After 3 hours of dissolution a small amount of drug was found to be released in the dissolution medium containing phosphate buffer saline at pH 7.4.

Table 3: Results of Various In Vitro Post Compression Parameters

Sr. No	Batch code	Wt. Variation (gm)		Hardness (kg/cm2)		Friability(%)	
		Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
1	F1	388.40±4.42	423.05±5.20	4.57±0.50	7.60±0.36	0.58	0.22
2	F2	354.81±3.68	421.69±6.51	4.66±0.24	7.58±0.42	0.72	0.35
3	F3	354.81±7.32	438.28±4.66	5.03±0.55	7.91±0.31	0.49	0.18
4	F4	381.20±5.06	442.54±4.71	4.50±0.42	7.47±0.53	0.45	0.16
5	F5	354.8±4.51	439.95±3.03	4.84±0.31	7.33±0.25	0.53	0.24
6	F6	366.82±3.07	433.62±5.76	4.25±0.54	7.16±0.30	0.56	0.21
7	F7	347.62±6.41	438.31±7.22	5.11±0.26	7.89±0.48	0.41	0.13
8	F8	395.97±5.03	441.26±4.38	4.68±0.32	7.60±0.29	0.74	0.30
9	F9	353.60±6.54	437.51±5.19	4.42±0.28	7.41±0.56	0.67	0.29
10	F10	382.24±6.68	442.01±6.43	4.39±0.41	7.24±0.48	0.48	0.19
11	F11	368.33±6.17	446.28±3.80	4.70±0.33	7.82±0.34	0.70	0.32
12	F12	368.38±5.10	440.94±4.65	4.36±0.57	7.55±0.23	0.62	0.26

Table 4: Results of In Vitro evaluation parameters of all batches

S. No.	Batch Code	Disintegration Time (min.)*	Drug Content (%)**
1.	F1	260.76±3.45	96.43 ± 0.680
2.	F2	251.87±4.51	96.76 ± 0.850
3.	F3	266.35±5.73	97.65 ± 0.654
4.	F4	255.26±4.62	100.4 ± 0.173
5.	F5	228.50±3.20	97.6 ± 0.901
6.	F6	267.18±1.64	95.8 ± 0.541
7.	F7	250.69±2.34	97.83 ± 0.907
8.	F8	246.63±3.80	98.23 ± 0.251
9.	F9	257.54±4.77	99.13 ± 0.208
10.	F10	205.42±2.88	100.6 ± 0.556
11.	F11	201.51±3.90	95.8 ± 0.632
12.	F12	192.38±5.72	98.83 ± 0.568

4.1 Effect of different binders and concentration on drug release from Zidovudine Hydrochloride matrix tablets

In the formulations F1, F2 and F3, Ethyl cellulose was used in 6%, 12% and 18% concentration. The drug release was decreased on increasing the binder concentration. The % CDR at 12th hour for three formulations F1, F2 and F3 were found to be very low. Therefore it was decided to change the binder solution keeping all other factors same in the formulations. Batch F4 and F5 were prepared using Starch as binder in concentrations 12% and 18% respectively. The formulations F6 and F7 were prepared by using PVP-K30 as binder solution in concentrations 12% and 18%. The % CDR of batch F7 was found less than that of batch F6 during the study of 12 hours. It may be due to the presence of higher amount of binder which decreased the drug release behavior of formulations. The batches F8 and F9 were prepared using starch and PVP K30 as binder respectively while keeping Chitosan and PEG 4000 concentration same in order to compare the drug release pattern from the two formulations to select the best binder for our study. Ethyl Cellulose was discarded because it showed very less amount of % CDR and thus these two binders were compared to find out the best binder.

4.2 In Vitro Drug Release Study of Formulation F8 in Presence of Rat Ceacal Content.

From the dissolution study of the microbially triggered Zidovudine Hydrochloride(AZT) formulations, F8 batch was found to be optimized in terms of providing higher drug release than other formulations. So to check the vulnerability of this formulation to colonic enzymes, in vitro drug release study was also carried out at colonic pH 6.8 with 4% w/v rat ceacal content and with antimicrobial treated group as shown below in figure 1. In the presence of rat ceacal content, the formulation showed higher drug release proving the effect of colonic microflora on the

formulations but no increase in drug release was found in drug treated group.

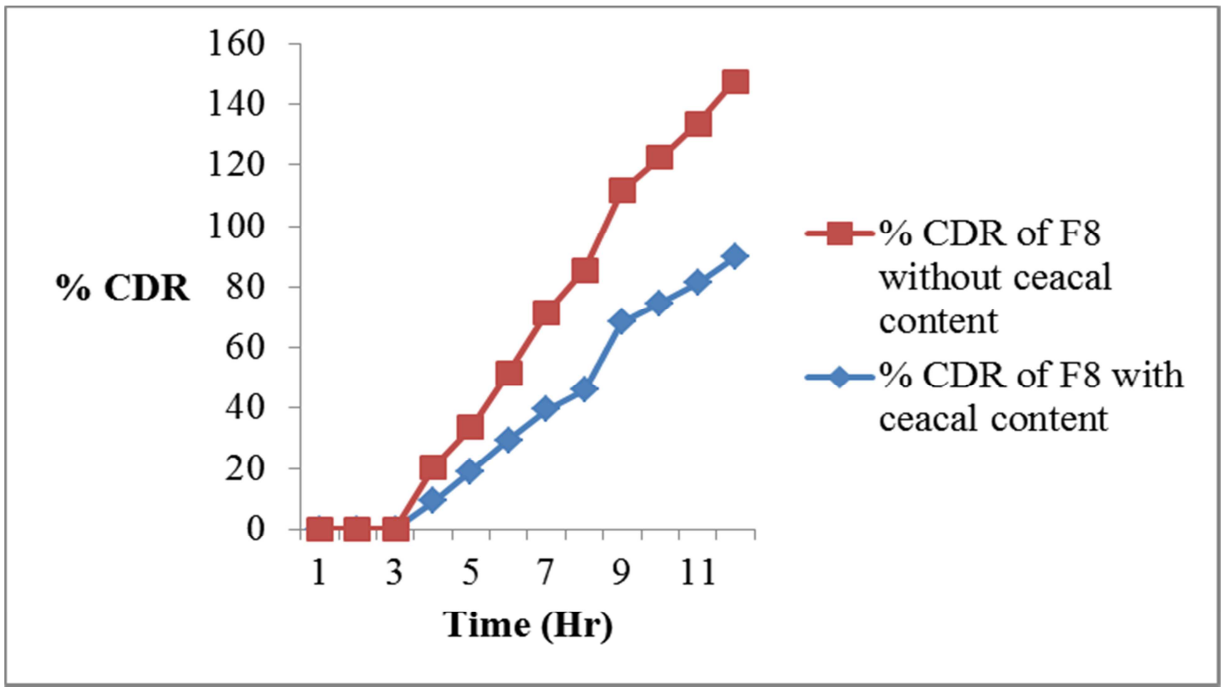

Fig1: In Vitro Drug Release Study of Formulation F8 in Presence of Rat Ceacal Content

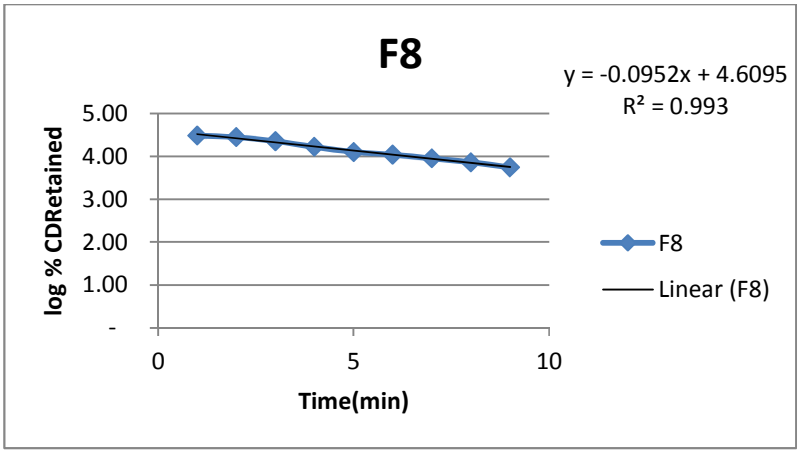

Fig 2: % CDR of formulation F8 with and without the presence of 4% ceacal contents

4.3 In vitro release kinetics predication by mathematical modeling:

To establish the order and mechanism of drug release, dissolution data of the optimized formulation F8 was fitted to four different models named as zero order model, first order model, Higuchi model and koresmeyerpeppas model. First order release model was found to be most appropriate according to data fitted values with r^2 value 0.993 and anomalous behavior came out to be the release mechanism of drug from the dosage form.

Table 5: Values of r^2 obtained from different kinetic models applied to Microbially triggered formulation Formulation F8

Kinetics	Zero Order Kinetics	Ist Order Kinetics	Higuchi Kinetics	KoresmeyerPeppas
r^2 value	0.978	0.993	0.983	0.912

CONCLUSION

From the above study it was concluded thatEthyl cellulose as binder was not considered appropriate as it caused very low drug release from the formulation i.e. nearly 40%. The 6% Chitosan concentration was not considered good for later studies as it was considered that such low concentration may not be adequate for showing the microbial action.PEG 4000 when incorporated in the formulation causes enhanced drug release by its hydrophilic property, but when used in further higher concentration delayed disintegration time of tablets was seen. Thus a middle value of PEG was considered suitable for our study. Starch was used in two concentrations 12% and 18% and decreased drug release was found with increasing concentration of starch. PVP-K30 was also a good binder but the results showed less efficacy of PVP-K30 binding action than that of starch when used in same concentrations. Thus formulation F8 was considered best as the % CDR was found maximum at Thus formulation F8 was considered best as the % CDR was found maximum at 12 hour of study. The binder starch in 12% concentration was proven best among Ethyl Cellulose and PVP K30. Chitosan 18% provided the desired results and PEG 4000 in 10% concentration was good. Rat caecal content results depicts that optimized formulation can extended the drug release upto 14 hrs.

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