Original Research Article

Design and evaluation of colon targeted modified pulsincap delivery of 5-fluorouracil according to circadian rhythm

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Abstract

Introduction: A modified pulsincap dosage form of 5-fluorouracil was developed to target drug to colorectal carcinoma according to daily oscillations of rate-limiting metabolizing enzyme dihydropyrimidine dehydrogenase. **Materials and Methods:** The capsule body was made water insoluble by exposing the body to formaldehyde vapor. A mixture of granules containing drug, superdisintegrant, and osmogen was filled in the capsule body. A hydrogel plug was fitted to the mouth of the treated body, and the untreated cap was fitted to the body which was coated with Eudragit S100. Developed formulations were evaluated for *in vitro* drug release in 1.2 pH (2 h), 6.8 pH (3 h), and 7.4 pH (up to 12 h) buffer solutions. A 2^3 full factorial design was used for optimization in which the type of hydrogel plug (X1), the type of osmogen (X_2), and the type of superdisintegrant (X_3) were selected as independent variables while, cap opening time, percentage drug released in 5(Q_5), 6(Q_6), and 12(Q_{12}) h were taken as dependent variables. **Results:** Dissolution data were fitted to various models to ascertain the kinetic of drug release. Regression analysis and analysis of variance were performed for dependent variables. The results of the *F*-statistics were used to select the most appropriate model. **Conclusion:** Formulation F_1 containing sodium starch glycolate, potassium chloride, and hydroxypropyl methylcellulose K4M plug was considered optimum since it showed more similarity to the theoretical predicted dissolution profile (f_2 = 77.33). The studies indicate that the formulation was effective in providing *in vitro* colon targeted release and controlled release after predetermined lag time.

Key words: Colorectal carcinoma, dihydropyrimidine dehydrogenase, full factorial design, modified pulsincapt

INTRODUCTION

The drug of choice in the treatment of carcinoma of stomach, colon, rectum, breast, ovary, and urinary bladder is 5-fluorouracil. For several decades, 5-fluorouracil (5-FU) stood alone as the only chemotherapeutic agent with clinical activity against colorectal cancer. A convention method of administration is the continuous intravenous (IV) infusion. Numerous active 5-fluorouracil schedules are in clinical use, but inconsistent and unpredictable oral bioavailability has historically accepted in intravenous administration. On intravenous administration, 5-fluorouracil produces severe systemic toxic effects including gastrointestinal, hematological, neural, cardiac, and dermatological origin.[1,2] The drug, despite low lipid solubility, enters the cerebrospinal fluid and the brain. Most of this systemic side effects are due to the cytotoxic effect of 5-fluorouracil after reaching unwanted sites. For conventional IV infusion, relatively short-acting drug requires continuous therapy so the epidural catheter has to be left in body. This impairs patient mobility and may cause complications such as infections and internal bleeding.

Plasma half-life of 5-FU is 10–20 min because of rapid enzymatic degradation caused by dihydropyrimidine dehydrogenase (DPD) due to daily oscillations in circadian clock genes.[3] Krugluger[4] *et al.* found a peak in DPD activity in circulating mononuclear cells of both healthy and cancer patients between 10:00 p.m. and 1:00 a.m. During 5-FU chronotherapy regimens, 5-FU activity during early morning time seems to result in least damage to bone marrow and gut, greatest antitumor effect, and best survival. Some research has shown that 5-fluorouracil is best tolerated in the mid-portion of a sleep cycle. So against colon cancer, the drug would be administered between 1:00 a.m. and 10:00 a.m., when the healthy cells are at rest and the cancer cells are most active.[4]

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Access this article online

Quick Response Code:

Website: www.jpionline.org

DOI: 10.4103/2230-973X.85969

pH-dependent, time-dependent, or enzymatically controlled delivery systems are three major approaches of colon targeting. However, a disadvantage of the pH-dependent system is that a substantial amount of drug may be released in small intestine because the pH-difference between the small intestine and the large intestine not being very pronounced. Limitation of time-dependent release systems is that they are not able to sense any variation in the upper gastrointestinal tract (GIT) transit time due to the presence of food and disease conditions. Limitation of enzymatically controlled delivery system is a variation in the colonic flora. Alteration in composition of the GIT flora occurs under certain diseased states such as acute diarrhea, cholera, tropical spruce, inflammatory bowel disease (IBD), etc. The activity of the microbial enzymes is even more susceptible to diet, drug intake (particularly antibiotics and certain laxatives), and environmental factors.[5] Pulsincap delivery combines advantages of pH-dependent and time-dependent colon targeting and overcomes the disadvantages of conventional method of colonic delivery (pH, time, and microflora-assisted delivery).Modified pulsincap™ dosage form consists of a formaldehyde-treated water-insoluble body and a water-soluble cap. The amino groups in the gelatin molecular chain could react with an aldehyde group of formaldehyde by Schiff's base condensation reaction to produce a water-insoluble body. A mixture of drug, osmogen, and swelling agent is incorporated within the body, plugged with hydrogel plug and the cap is enteric coated to prevent variable gastric emptying. Water enters into the permeable body and the mixture of osmogen and superdisintegrant pushes the plug outside. After reaching the colon, enteric polymer dissolves and the plug is ejected from the body with the release of drug in colon.[6,7]

The aim of the present investigation was to develop an oral rate-controlled targeted delivery of 5-fluorouracil which not only reduces systemic side effects, but also provides an effective and safe therapy for colon cancer with reduced dose and reduced duration of therapy according to circadian rhythm of the body. The present investigation is aimed to formulate a modified pulsincap with time of administration between 9:30 p.m. and 10:00 p.m. with a lag time of about 5 h considering gastric and intestinal emptying time as well as peak enzyme activity time (10 p.m.–1 a.m.). The developed formulation is expected to release the drug after 3:00 a.m. when dosage form is expected to reach in colon, while DPD activity is less. After the lag period, dosage form is expected to sustain the release of the drug for at least 6–7 h to eliminate the problem of short biological half life.

MATERIALS AND METHODS

Materials

5-fluorouracil was purchased from Sigma Life Science, USA. Hydroxypropyl methylcellulose K4M (HPMC K4M), crosscarmellose sodium, and Eudrgit® S100 were obtained from Yarrow Chem. Products (Mumbai, India). Potassium chloride, sodium chloride, sodium alginate, and sodium starch glycolate were obtained from Finar Chemical Limited (Ahmedabad, India). All other materials and chemicals used were of either pharmaceutical or analytical grade.

Methods

Preparation of formaldehyde-exposed hard gelatin capsule bodies

The hard gelatin capsules of 00 size were taken. The bodies of hard gelatin capsules were placed on a wire mesh. Formaldehyde (15%) was taken into a desiccator and potassium permanganate was added to it until vapor was produced. The reaction was carried out for 12 h after which bodies were removed and dried at 50 °C to ensure completion of the reaction between gelatin and formaldehyde vapor.[6] Reaction time was optimized by taking samples of capsules at 20-, 30-, 40-, 50-, and 60-min interval. The collected samples were assayed for the residual formaldehyde content. The effect of 0.1 N hydrochloric acid was performed on both the treated and untreated bodies.

Estimation of residual formaldehyde content in treated gelatin capsule bodies

The residual formaldehyde content in treated bodies was determined as per the method described by William.[8,9] Vapor hardened capsule body samples collected at 20-, 30-, 40-, 50-, and 60-min interval were cut into small pieces. Pieces of capsule samples were added separately to a mixture of 1 ml of 10% chromotropic acid solution and 10 ml of concentrated sulfuric acid in different test tubes. All test tubes were placed in a beaker filled with water for boiling. After cooling to room temperature, contents of test tubes were quantitatively transferred to a 100 ml volumetric flask and diluted up to the mark with distilled water. A blank was prepared in the similar way using 1 ml distilled water in place of pieces of body. Absorbance of sample was measured by colorimetry at 569 nm.

Preparation of sustained release granules containing 5-fluorouracil

The granules containing 5-fluorouracil were prepared to sustain the release of drug after predetermined lag time. Composition of granules per capsule is shown in Table 1. All the ingredients were passed through 100 # sieve and thoroughly mixed. The mixture was granulated through 20 # sieve using ethyl cellulose (20 cps) solution in ethanol as a binder. The prepared granules were dried at room temperature. The fines were separated using 60 # sieve to obtain 20–60 # sieve granules.

Characterization of sustained release granules

Granules were tested for flow property and content uniformity. The flow property was determined by a fixed height funnel method. For content uniformity, 100 mg granules containing 5-fluorouracil were crushed in a mortar, taken in a 100 ml volumetric flask and pH 7.4 phosphate buffer was added up to the mark. The flask was then placed on a magnetic stirrer at 100 rpm for 12 h. The flask solution was filtered through a 0.45 μm membrane filter, suitably diluted, and assayed at 266 nm using a Shimadzu UV 1800 double-beam spectrophotometer (Shimadzu, Kyoto, Japan).

Preliminary screening

The preliminary screening was performed to optimize the

effect of Eudragit S100 concentration (P1–P4), osmogen concentration (P5–P8), superdisintegrant concentration (P7, P9, and P10) and different types of filler (P7 and P11) on cap opening time. Batches P1–P11 of preliminary screening are shown in Table 2. All batches were filled with sustained release granules, mixture of osmogen and superdisintegrant, and lastly with filler. The mouth of the body was fitted with the compressed hydrogel plug. The hydrogel plug was compressed using a double rotary tablet compression machine, Karnavati Engg. Pvt. Ltd., Ahmedabad, in 7-mm diameter punch with compression force 2 kg/cm^2. A direct compression method was used for preparing the hydrogel plug. Then, a cap was placed onto the body. The mouth of the body was fitted with a compressed hydrogel plug (7 mm diameter). Then a cap was placed onto the body. Only the cap was coated with different concentrations of Eudragit S100 solution in acetone containing Sudan IV as a coloring agent. Average percentage weight gain after Eudragit coating was 7.94%.

In vitro dissolution study

The *in vitro* dissolution study of 5-fluorouracil pulsincap was performed using an USP apparatus (model TDT-08T, Electrolab, Mumbai, India) fitted with a paddle (50 rpm) at 37 ± 0.5 °C. Dissolution media were 900 ml of 0.1 M HCl for 2 h (since average gastric emptying time is 2 h) and 900 ml of phosphate buffer pH 6.8 for 3 h (average small intestinal transit time). After 5 h, the dissolution medium was replaced with pH 7.4 phosphate buffer (900 ml) and tested for the drug release up to 12 h. At the predetermined time intervals, 10 ml samples were withdrawn, filtered through a 0.45 μm membrane filter, diluted, and assayed at 266 nm using a Shimadzu UV 1800 double-beam spectrophotometer (Shimadzu, Kyoto, Japan). Cumulative percentage drug release was calculated using an equation obtained from a calibration curves.[1,2,6,9] *In vitro* dissolution profile of batches F_1–F_8 is shown in Figure 1.

Optimization of variables using full factorial design

A 2^3 randomized full factorial design was used in this study. In this design three factors were evaluated, each at two levels, and experimental trials were performed for all eight possible combinations. The type of hydrogel plug (X_1), the type of osmogen (X_2), and the type of superdisintegrant (X_3) were selected as independent variables while, cap opening time, percentage drug released in 5 (Q_5), 6 (Q_6), and 12 (Q_{12}) h were taken as dependent variables. The formulation layout for the factorial design batches (F_1–F_8) is shown in Table 3.

Kinetic modeling of dissolution data

The dissolution profile of all batches were fitted to various models such as zero order, first order, Higuchi,[10] Hixon Crowell,[11] Korsmeyer and Peppas,[12] to ascertain the kinetic of drug release. The method described by Korsemeyer and Peppas was used to describe the mechanism of the drug release.

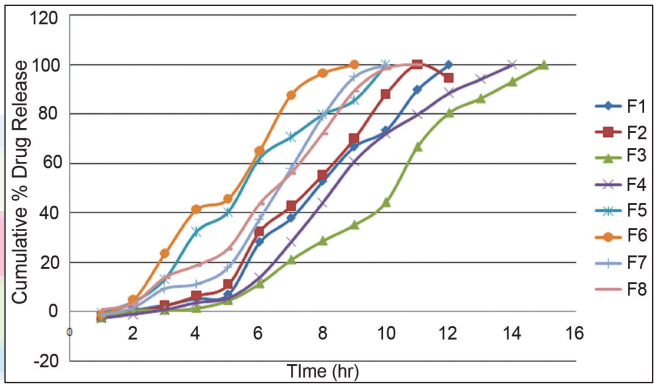

Figure 1: *In vitro* dissolution profile of prepared formulations (F_1–F_8).

Table 1: Composition and characterization of granules containing 5-fluorouracil per capsule

Composition of granules					
Ingredients	5-Fluorouracil	HPMC K4M	Ethyl cellulose	Lactose	Ethyl cellulose in ethanol
Quantity (mg)	50	20	10	15	5 (as binder)

Characterization of granules						
Parameter	Bulk density (g/ml)	Tapped density (g/ml)	Angle of response (θ)	% Carr's index	Hausner's ratio	% Assay
Observation	0.66	0.79	22.7	16.45	1.19	88

HPMC K4M: Hydroxypropyl methylcellulose K4M.

Table 2: Batches for preliminary screening

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Drug granules* (mg)	112	112	112	112	112	112	112	112	112	112	112
KCl (osmogen) (mg)	100	100	100	100	60	80	100	120	100	100	100
Sodium starch glycolate (superdisintegrant) (mg)	q.s.	q.s.	q.s.	q.s.	100	100	100	100	50	150	100
Filler	–	–	–	LAC	LAC	LAC	LAC	LAC	LAC	LAC	MCC
Concentration of Eudragit S100 coating solution (%)	3	4	5	6	4	4	4	4	4	4	4
Response											
Average cap opening time (min)	270±10	330±10	450±10	480±10	390±10	360±10	330±10	240±10	450±15	310±10	570±20

*Contains quantity equivalent to 50 mg of 5-fluorouracil; LAC indicates lactose; MCC indicates, microcrystalline cellulose (PH 101 grade)

Comparison of dissolution profiles for selection of optimum batch

The similarity factor (f_2) given by SUPAC guidelines for a modified release dosage form was used as a basis to compare dissolution profiles. The dissolution profiles are considered to be similar when f_2 is between 50 and 100. The dissolution profiles of products were compared using f_2 which is calculated from the following formula:

$$f_2 = 50 \times \log\left\{\left[1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n} w_t(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad (1)$$

Where n is the dissolution time and R_t and T_t are the reference (here this is the theoretical dissolution profile of 5-fluorouracil) and test dissolution value at time t.[13]

Drug excipients interaction study

The Fourier transform infrared (FTIR) technique has been used to study the physical and chemical interactions between the drug and excipients used. FTIR spectrum of 5-fluorouracil, HPMC K4M, ethyl cellulose, lactose, and a physical mixture of 5-fluorouracil:HPMC K4M:ethyl cellulose:lactose was recorded using a KBr mixing method on a FTIR instrument available at the central instrument laboratory of the institute (FTIR-1700, Shimadzu, Kyoto, Japan).

In vivo study of prepared formulation

The prepared formulation was tested for an *in vivo* study to check the passage of the dosage form throughout the GIT. The purpose of the *in vivo* study was to find the location of the capsule during its passage through the GI tract. In this study, drug granules were replaced with barium sulfate. The dosage form was prepared in the similar manner as optimized formulation. An X-ray study was performed at the Sanjivani imaging centre, Ahmedabad. The volunteer with overnight fasting was taken for the study. The laxative was given to the volunteer before 12 h of the study to completely empty the GIT content. The X-ray study was performed at 2-h, 3-h, 5-h, and 8-h interval.

RESULTS AND DISCUSSION

Estimation of residual formaldehyde content in treated gelatin capsule bodies

The orally tolerated limit of formaldehyde is 0.1%.[14] The residual amount of formaldehyde was 0.0081% after 50-min heat exposure time for the completion of reaction. After 50 min, the residual formaldehyde level was near constant. Since 50 min reaction time was optimum, the residual amount of formaldehyde remained in the capsule was safe for oral intake. The effect of 0.1 N hydrochloric acid was performed on both treated and untreated capsule bodies. The treated bodies were remained unaffected by 0.1 N HCl for 24 h while untreated bodies collapsed within 15 min.

Characterization of sustained release granules

Granules containing 5-fluorouracil exhibited good flow property and the drug content of the granules was found to be 88% as evident from Table 1.

Preliminary screening

The evaluation results for cap opening time showed that concentration of Eudragit S100 coating solution, osmogen, superdisintegrant, and filler had significant effect on cap opening time [Table 2]. Results of batch P1–P4 showed that average cap opening time was delayed with increasing the Eudragit S100 concentration. For colon targeted release, 5:30 h (~330 min) is considered the ideal lag period. Eudragit 4% solution (Batch P2) was considered optimized coating solution with cap opening time of about 330 ±10 min. Osmogen alone had an unpredictable effect on cap opening time. Therefore, the mixture of osmogen and superdisintegrant was used in investigation. From the results of P5–P11, it was concluded that 100 mg KCl and 100 mg sodium starch glycolate (Batch P7) were optimum with ~5:30 h lag time. Average cap opening time was decreased with increasing the concentration of osmogen and superdisintegrant. The result of batch P11 showed that microcrystalline cellulose (PH 101 grade) delayed the lag time up to ~9 h.

Table 3: Formulation and evaluation of batches in the 2^3 full factorial design

Batch code	Variable levels in coded form			Cap opening time (min)	Q_5 (%)	Q_6 (%)	Q_{12} (%)	f_2
	X_1	X_2	X_3					
F_1	−1	−1	−1	340	6.96	28.3	100	77.33
F_2	1	−1	−1	310	10.86	32.46	100	64.55
F_3	−1	1	−1	390	4.42	11.32	80.49	36.40
F_4	1	1	−1	370	5.44	13.91	88.62	54.51
F_5	−1	−1	1	205	40.41	62.11	100	30.34
F_6	1	−1	1	180	45.53	64.92	100	24.61
F_7	−1	1	1	260	17.80	37.50	100	39.86
F_8	1	1	1	225	25.31	43.52	100	39.87
TP				330	5.00	30.00	100	
Coded values	**Actual values**							
	X_1		X_2		X_3			
	(Type of hydrogel plug		Type of osmogen		Type of superdisintegrant			
−1	HPMC K4M		Potassium chloride		Sodium starch glycolate			
1	Sodium alginate		Sodium chloride		Crosscarmellose sodium			

HPMC K4M: Hydroxypropyl methylcellulose K4M; TP: Theoretical profile.

Table 4: Summary of results of regression analysis

For cap opening time (COT)							
Response (COT)	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}
FM	285	−13.75	26.25	−67.5	−1.30E−14	−1.25	−1.25
RM	285	–	–		–	–	–
For Q_5							
Response (Q_5)	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}
FM	19.5907	2.1932	−6.349	12.671	−0.0615	−4.358	0.964
RM	19.5907	–	–	12.671	–	–	–
For Q_6							
Response (Q_6)	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}
FM	36.754	1.94	−10.1924	15.259	0.2042	−1.3092	0.2602
RM	36.754	–	−10.1924	15.259	–	–	–
For Q_{12}							
Response (Q_{12})	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}
FM	96.13	1.016	−3.861	3.861	1.0162	3.8612	3.8612
RM	–	–	–	–	–	–	–

FM: Full model, RM : Reduced model

Full factorial design

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 + b_{123}X_1X_2X_3 \quad (2)$$

where Y is the dependent variable, b_0 is the arithmetic mean response of the eight runs, and b_i is the estimated coefficient for the factor X_i. The main effects (X_1, X_2, and X_3) represent the average result of changing 1 factor at a time from its low to high values. The two-way interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The three-way interaction terms ($X_1X_2X_3$) show how the response changes when three factors are simultaneously changed. The dissolution profile for eight batches showed a variation (i.e., cap opening time ranging from 180 to 390 min and drug released after 6 h ranging from 11.34% to 64.92%). The data indicate that the release profile of the drug is strongly dependent on the selected independent variables. The fitted equations (full and reduced) relating the responses, cap opening time, Q_5, Q_6, Q_{12} to the transformed factor are shown in Table 4. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e., negative or positive). Table 5 shows the results of analysis of variance (ANOVA), which was performed to identify insignificant factors. Data were analyzed using Microsoft® Excel. .

R^2 value for cap opening time, Q_5, Q_6, and Q_{12} are 0.9988, 0.9980, 0.9989, and 0.9789, respectively, indicating good correlation between dependent and independent variables. The reduced models were developed for response variables by omitting the insignificant terms with $P > 0.05$. The terms with $P < 0.05$ were considered statistically significance and retained in the reduced model. The coefficients for full and reduced models for response variables are shown in Table 4.

Full and reduced model for cap opening time

The significance levels of the coefficients b_1, b_2, b_{12}, b_{23}, and b_{13} were found to be P = 0.114, 0.060, 1.0, 0.7048, and 0.7048, respectively, hence they were omitted from the full model to generate a reduced model. The results of statistical analysis are shown in Table 4. The coefficient b_3 was found to be significant at $P < 0.05$; hence they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficients b_1, b_2, b_{12}, b_{23}, and b_{13} contribute significant information to the prediction of cap opening time.[15] The results of model testing are shown in Table 5. The critical value of F for $\alpha = 0.05$ is equal to 230.16 (df = 5, 1). Since the calculated value (F = 28.2) is less than critical value (F =230.16), it may be concluded that the term b_1, b_2, b_{12}, b_{23}, and b_{13} do not contribute significantly to the prediction of cap opening time and can be omitted from the full model to generate the reduced model.

Full and reduced model for Q_5

The significance levels of the coefficients b_1, b_2, b_{12}, b_{23}, and b_{13} were found to be P = 0.18, 0.065, 0.94, 0.095, and 0.38, respectively, so they were omitted from the full model to generate a reduced model. The results of statistical analysis are shown in Table 4. The coefficient b_3 was found to be significant at $P < 0.05$; hence they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficients b_1, b_2, b_{12}, b_{23}, and b_{13} contribute significant information to the prediction of Q_5. The results of model testing are shown in Table 5. The critical value of F for $\alpha = 0.05$ is equal to 230.16 (df = 5, 1). Since the calculated value (F = 29.95) is less than the critical value (F = 230.16), it may be concluded that the term b_1, b_2, b_{12}, b_{23}, and b_{13} do not contribute significantly to the prediction of Q_5 and can be omitted from the full model to generate the reduced model.

Full and reduced model for Q_6

The significance levels of the coefficients b_1, b_{12}, b_{23}, and b_{13} were found to be P = 0.19, 0.79, 0.27, 0.74, respectively, so they were omitted from the full model to generate a reduced model. The results of statistical analysis are shown

Table 5: Calculation for testing the model in portions

For cap opening time						
	df	SS	MS	F	R^2	
Regression						
FM	6	43,500	7250	145	0.9988	F_{cal} = 28.2
RM	1	36,450	36,450	30.8	0.837	F_{tab} = 230.16
Error						
FM	1	50	50	–	–	df (5, 1)
RM	6	7100	1183.33	–	–	
For Q_5						
	df	SS	MS	F	R^2	
Regression						
FM	6	1804.99	300.83	86.59	0.998	F_{cal} = 29.95
RM	1	1284.59	1284.59	14.71	0.7103	F_{tab} = 230.16
Error						
FM	1	3.47	3.47	–	–	df (5, 1)
RM	6	523.88	87.31	–	–	
For Q_6						
	df	SS	MS	F	R^2	
Regression						
FM	6	2738.68	456.45	159.84	0.9989	F_{cal} = 3.929
RM	2	2693.8	1346.89	141.07	0.9825	F_{tab} = 224.58
Error						
FM	1	2.86	2.86	–	–	df (4,1)
RM	5	47.73	9.55	–	–	
For Q_{12}						
	df	SS	MS	F	R^2	
Regression						
FM	6	382.608	63.77	7.72	0.978862	
RM	–	–	–	–	–	
Error						
FM	1	8.26	8.26	–	–	
RM	–	–	–	–	–	

Df indicates degree of freedom; SS: Sum of squares; MS: Mean of squares; R2: Regression coefficient; FM: full model; RM: Reduced model.

Table 6: Kinetic treatment of dissolution data

	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
Zero order								
b	12.13	12.50	11.24	12.68	6.78	4.77	10.43	10.14
a	−44.99	−42.70	−60.09	−58.70	23.66	49.89	−12.43	−11.39
R^2	0.9972	0.9863	0.9977	0.9902	0.9620	0.7870	0.9072	0.9491
First order								
b	0.906	0.086	0.134	0.126	0.036	0.025	0.066	0.0616
a	0.956	1.037	0.330	0.543	1.598	1.740	1.293	1.329
R^2	0.9810	0.9750	0.9859	0.9376	0.9569	0.7677	0.8748	0.9303
Higuchi								
b	71.82	74.24	65.95	75.53	41.05	29.21	63.11	60.89
a	149.91	−151.49	−155.50	−169.71	−36.90	5.66	−106.70	−101.64
R^2	0.9963	0.9884	0.9675	0.9953	0.9705	0.8149	0.9265	0.9617
Hixon–Crowell								
b	0.266	−0.260	−0.332	−0.332	−0.121	−0.083	−0.206	−0.062
a	3.106	2.934	4.308	3.938	1.340	0.870	2.208	1.329
R^2	−0.9898	−0.9811	−0.9932	−0.9617	−0.9591	−0.7741	−0.8871	0.9303
Korsemeyer and Peppas								
a	1.962	−1.836	−3.008	−2.774	−0.775	−0.538	−1.420	−1.316
n	1.837	1.747	2.698	2.598	0.743	0.530	1.388	1.272
R^2	0.9942	0.9899	0.9926	0.9673	0.9763	0.8238	0.9184	0.9609

b: Slope, a: Intercept, R2: Correlation coefficient, n: Diffusion exponent.

in Table 4. The coefficients b_2 and b_3 were found to be significant at $P < 0.05$; hence they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficient b_1, b_{12}, b_{23}, and b_{13} contribute significance information to the prediction of Q_6. The results of model testing are shown in Table 5. The critical value of F for $\alpha = 0.05$ is equal to 224.58 (df = 4, 1). Since the calculated value ($F = 3.93$) is less than the critical value ($F = 224.58$), it may be concluded that the term b_1, b_{12}, b_{23}, and b_{13} do not contribute significantly to the prediction of Q_6 and can be omitted from the full model to generate the reduced model.

Full model for Q_{12}

The significance levels of the coefficients b_1, b_2, b_3, b_{12}, b_{23}, and b_{13} were found to be $P = 0.50$, 0.163, 0.163, 0.499, 0.163, and 0.499, respectively. Coefficients b_1, b_2, b_3, b_{12}, b_{23}, and b_{13} do not contribute significantly to the prediction of %drug release after 12. Therefore, generation of the reduced model from the full model is not possible. The results of statistical analysis are shown in Table 4.

Kinetic modeling of dissolution data

The kinetics of the dissolution data were well fitted to zero order, Higuchi model, and Korsemeyer–Peppas model for batches F_1–F_5 as evident from regression coefficients Table 6. Batches F_6 and F_7 followed the Korsemeyer–Peppas and Higuchi model, respectively, while batch F_8 followed both the models. In the case of the controlled or sustained release formulations, diffusion, swelling, and erosion are the three most important rate controlling mechanisms. Formulation containing swelling polymers show swelling as well as diffusion mechanism because the kinetic of swelling include relaxation of polymer chains and imbibitions of water, causing the polymer to swell and changing it from a glassy to rubbery state. The diffusion exponent n is the indicative of mechanism of drug release from the formulation. For a swellable cylindrical (tablet) drug delivery system, the n value of 0.45 is indicative of Fickian diffusion controlled drug release, n value between 0.5 and 0.85 signifies anomalous (non-Fickian) transport, n value of 0.85 indicates case II transport, and n value greater than 0.85 indicates super case II transport.[16,17] The value of diffusion exponent n for factorial formulations F_1, F_2, F_3, F_4, F_7, and F_8 are greater than 0.85 [Table 6] indicating super case II transport drug release from the formulations while the values for formulations F_5 and F_6 are between 0.5 and 0.85 [Table 6] indicating anomalous non-Fickian transport.

Comparison of dissolution profiles for selection of optimum batch

The values of similarity factor (f_2) for batches F_1–F_8 are shown

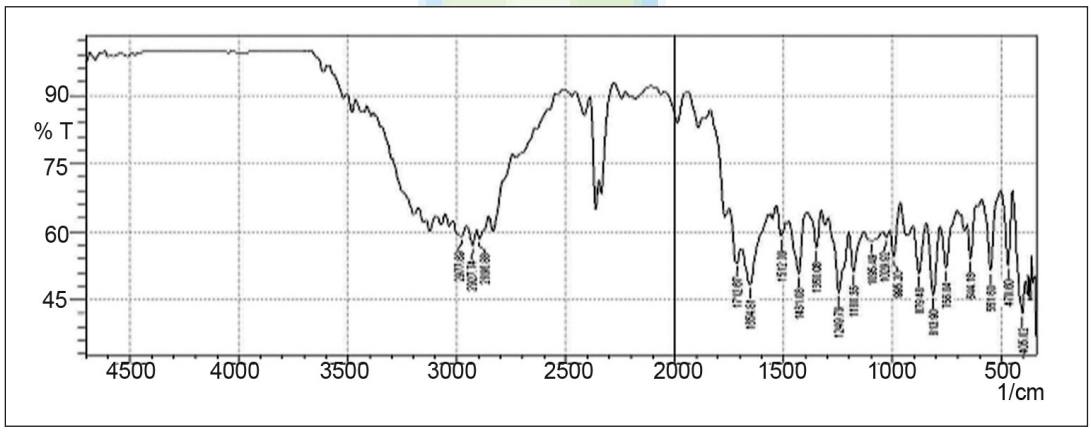

Figure 2: FTIR spectrum of 5-fluorouracil

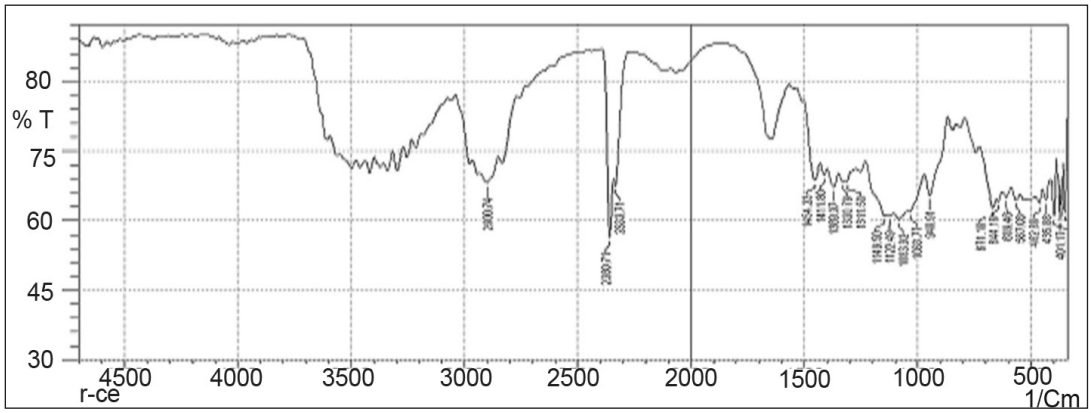

Figure 3: FTIR spectrum of HPMC K4M

in Table 3. The batch F_1 showed a maximum value of f_2 (77.33), hence was selected as the optimum batch.

Drug excipients interaction study

Drug-excipients interactions play a vital role in the release of drug from formulation. The pure 5-fluorouracil and its mixture with HPMC K4M, ethyl cellulose, and lactose was mixed separately with IR grade KBr and were scanned over a range of 400–4500 cm^{-1} using a FTIR instrument (FTIR-1700, Shimadzu, Kyoto, Japan). The drug exhibits peaks due to the cyclic ketonic group, the secondary amino group, the C=C stretching, and C–F group. It was observed that there were no changes in these main peaks in the IR spectra of a mixture of drug and polymers [Figures 2–5]. The FTIR study revealed no physical or chemical interactions of 5-fluorouracil with HPMC K4M, ethyl cellulose, and lactose as evident from Figure 6.

In vivo study of prepared formulation

The digital X-ray photographs of GIT are shown in

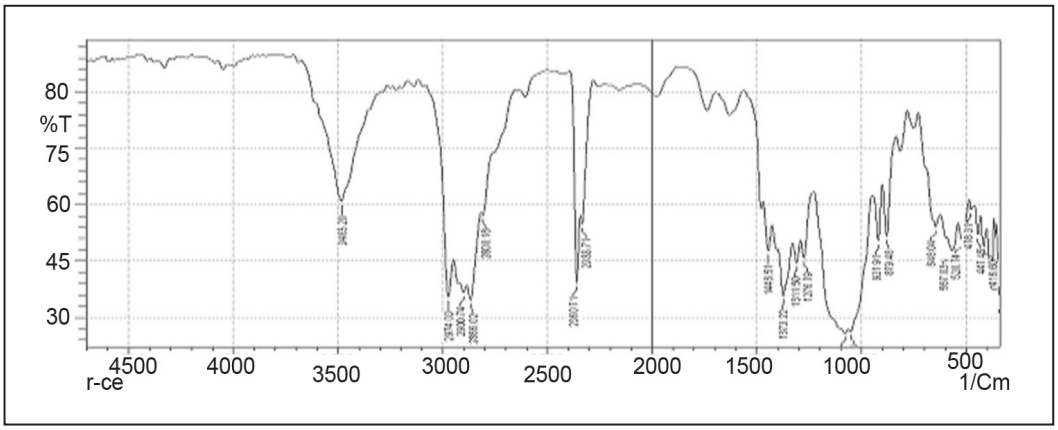

Figure 4: FTIR spectrum of ethyl cellulose

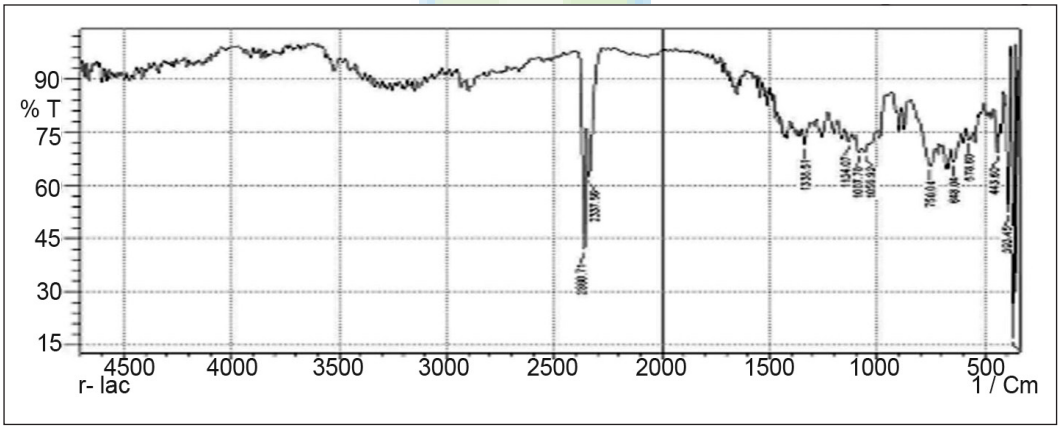

Figure 5: FTIR spectrum of lactose

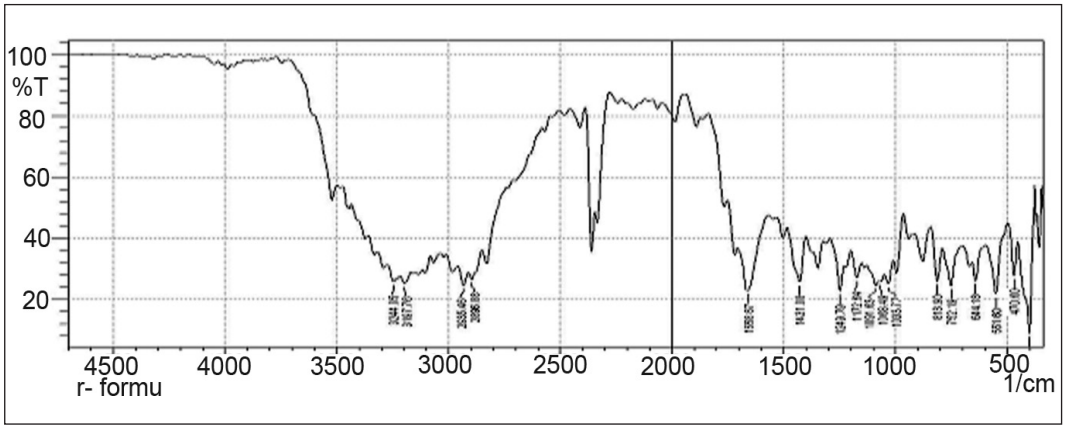

Figure 6: FTIR spectrum of physical mixture (5-fluorouracil, HPMC K4M, ethyl cellulose, and lactose)

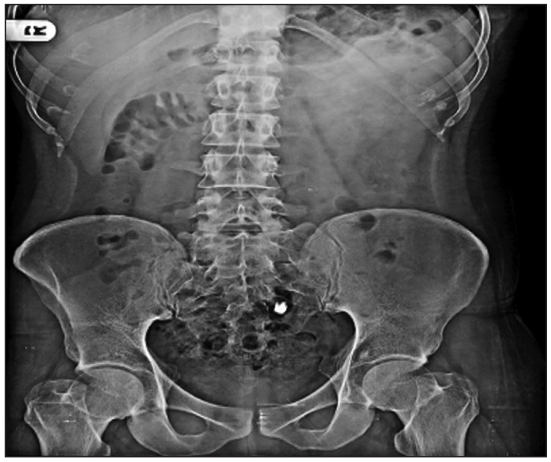

Figure 7: Physical examination of delivery system after 2 h.

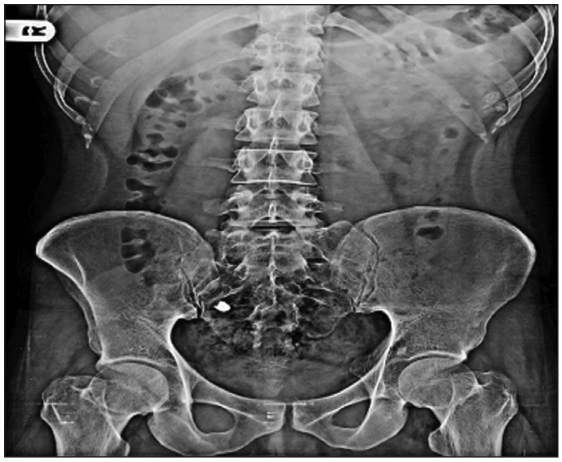

Figure 8: Physical examination of delivery system after 3 h.

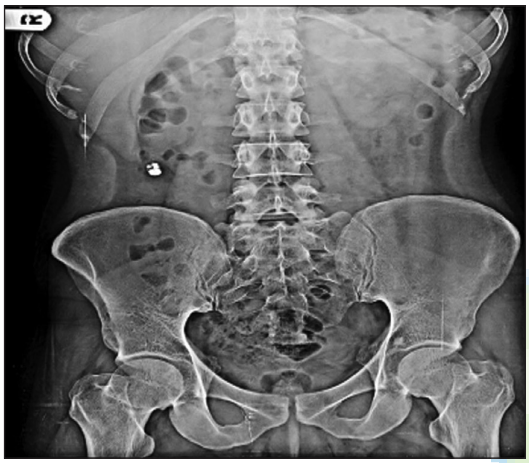

Figure 9: Physical examination of delivery system after 5 h.

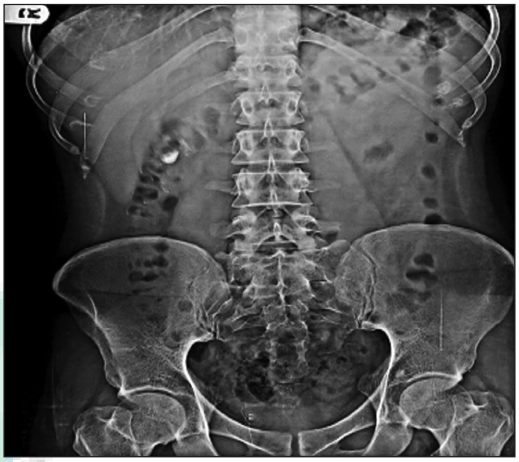

Figure 10: Physical examination of delivery system after 8 h.

Figures 7–10. From the photographs, it was concluded that the dosage form was present in the ileal loop after 2 h. After 3-h interval, the capsule reached the ileocaecal junction. The capsule was present in proximal ascending colon after 5 hand was present at the hepatic flexure of colon after 8 h. From Figure 10, it was clear that the content of the capsule was dispersed in colon. The study revealed the targeting of dosage form and drug release in colon.

CONCLUSION

This study revealed that the modified pulsincap delivery of 5-fluorouracil formulated using sodium starch glycolate, potassium chloride, and HPMC K4M plug was effective in providing controlled zero-order release of 5-fluorouracil after ~5 h lag time. It was also concluded from the results that this modified pulsincap delivery is suitable for the delivery of drug according to daily oscillations of rate limiting enzyme DPD.

ACKNOWLEDGMENTS

The authors thank Shri Sarvajanik Pharmacy College, Mehsana, for providing all other ingredients and required infrastructure for the conduct of this research work.

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How to cite this article: Patel DM, Jani RH, Patel CN. Design and evaluation of colon targeted modified pulsincap delivery of 5-fluorouracil according to circadian rhythm. Int J Pharma Investig 2011;1:172-81.
Source of Support: Nil. **Conflict of Interest:** None declared.