Design, development and optimization of colon targeted drug delivery system for Crohnís disease

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ABSTRACT

The aim of the present investigation was to develop double coated systems comprising of pH independent (Eudragit® RS 100) and pH dependent coatings (Eudragit® S 100) of polymethacrylates for delivery of metronidazole exclusively to the colon. The central composite design was used to optimize independent variables X_1 (coating level of Eudragit® RS 100) and X_2 (coating level of Eudragit® S 100) and study their effect on dependent variables Y_{300} (% drug released in the 5th h) and Y_{480} (% drug released in the 8th h). The results of interactive statistical second order model revealed that lower levels of X_1 and higher levels of X_2 favor the preparation of colon targeted tablets.

Key Words: Double coated systems, Central composite design, pH independent polymer, pH dependent polymer.

INTRODUCTION

Targeting of drugs to the colon is of increasing importance for topical treatment of inflammatory bowel diseases of the colon such as ulcerative colitis and Crohn's disease (CD). CD is a condition in which the wall of the small or large intestine becomes sore, inflamed, and swollen. The inflammation may be "patchy" with segments of healthy tissue between the patches.[1] In children and adults, this causes abdominal pain, diarrhoea, fever, and weight loss.

Metronidazole, a nitroimidazole compound, was first reported to be effective in the treatment of CD by Ursing and Kamme in 1975.[2] Metronidazole was reported to be more effective than sulfasalazine in the treatment of CD.[3] Metronidazole is useful in mild to moderate conditions for healing perianal fistulae in CD.[4] It has high antimicrobial activity against anaerobes, and high concentrations of these microorganisms are present in the ileocolonic region after ileal resection; metronidazole induces changes in fecal flora; bacterial antigens are believed to play a role in CD; and metronidazole may have immunosuppressive activity.[5] Thus, Metronidazole was preferred as a drug of choice in the present study.

Most of the previous colon targeted drug delivery systems have focused on one of the three basic approaches, pH-dependent release, time-dependent release, or bacterial degradation in the distal ileum/colon. Colonic delivery systems based solely on time or pH dependency of release have not been reliable because of the inherent variability of pH and transit times through the upper gastro intestinal tract (GIT).[6-8] Polymers that specifically degrade in the presence of colonic microorganisms have been greatly exploited.[9,10] The activation of systems coated with such polymers depends solely on the presence of microflora in the colon. Metronidazole being an antibiotic itself, bacterial degradation approach cannot be used as the bacteria responsible for the activation of the system will be killed by metronidazole.

The aim of the present investigation was to prepare colon targeted tablets of metronidazole using a combination of time and pH dependent polymethacrylate polymers that offer protection to the drug until it leaves the stomach which is provided by pH dependent polymer, Eudragit® S 100 and major drug release in small intestine is avoided by providing pH independent coating of Eudragit® RS 100. For preparation of colon targeted tablets the core tablets of metronidazole were first coated with pH independent polymer (Eudragit® RS) then with outer layer of a pH dependent polymer (Eudragit® S). Before combining the two coats on a single tablet, each coating system was individually optimized for its effect on drug release. In an earlier study conducted by Akhgari et al.,[11] indomethacin pellets were coated by a single layer of combination of Eudragit® RS, Eudragit® S100 and

Eudragit® L100, wherein 100% drug release was obtained at the end of 12th h. In another study by Nasra et al.,[12] metronidazole colon specific formulations prepared using pectin as a compression coating material, were unable to deliver 100% drug even after 24 h. Topical treatment of CD, for acute therapy, requires immediate treatment as soon as the drug delivery system reaches colon. Thus, colonic systems formulated by these researchers cannot be used as acute therapy for CD.

It is a well established fact that average residence time of a formulation in stomach is 2 h, and that in intestine is 3 h.[9, 11] Thus, average lag time for a formulation to reach colon is taken as 5 h. Since the objective of the study was to formulate a drug delivery system for treatment of acute CD, it was desired to have almost 100% drug release in colon within 3 h after the system reaches colon. It was desirable to have drug release below 10% till 5 h. Thus, essentiality of the study was to protect drug release till 5th h and have near to 100% drug release by 8th h.

MATERIALS AND METHODS

Metronidazole was obtained as a gift sample from J.B. Chemicals (Ankleshwar, India). Eudragit S 100, RS 100 were generously gifted by Evonik (Darmstadt, Germany). Avicel PH 101 and Aerolac were also obtained as gift samples from FMC Biopolymer (NJ, USA) and Pharmaceutical Coatings Pvt. Ltd. (Mumbai, India) respectively. Tablettose® 100, Cellactose® 80, Flowlac® 100 and Polyvinyl Pyrollidone K 90 (PVP K90) were gifted from Pformulate (FL, USA). Croscarmellose Sodium was obtained as a gift sample from Gujarat Microwax Pvt. Ltd. (Ahmedabad, India). PVP K30 and lactose were purchased from S.D.Fine-Chem Ltd. (Mumbai, India) and CDH (New Delhi, India) respectively. Double distilled water was used throughout the study and all other chemicals used were of analytical reagent grade.

Preparation of core tablets of metronidazole

The method of direct compression has noted advantages over the wet granulation method[13], thus, the attempt was made to prepare core tablets of metronidazole by direct compression initially. Metronidazole (200 mg) was tried to compress with different directly compressible diluents like Avicel® 101, Tablettose® 100, Cellactose® 80, Flowlac® 100 and Aerolac®. Later, wet granulation approach was adopted where PVP K30 (W1) and/or PVP K90 (W2) solution in iso-propyl- alcohol was used as a granulating agent as shown in Table 1. Concentration of PVP was varied from 5 – 15% w/w of total tablet weight. Lactose was used as a diluent and croscarmellose sodium was used as a super disintegrant. The wet mass was forced though 16 # sieve

Table 2: Colon targeted tablets of metronidazole

Eudragit S coated batches						Eudragit RS coated batches					
Polymer soln. concentration	% Weight gain (Coating Level)					Polymer soln. concentration	% Weight gain (Coating Level)				
5%Eudragit S	E1	E2	E3	E4	E5	10%Eudragit RS	RS1	RS2	RS3	RS 4	RS 5
	5	10	15	17.5	20		10	12.5	15	17.5	20
10%Eudragit S	E6	E7	E8	E9	E10						
	5	10	15	17.5	20	15% Eudragit RS	RS6		RS7		RS8
20%Eudragit S	E11	E12	E13	E14	E15		10		12.5	15	
	5	10	15	17.5	20						

Eudragit S and Eudragit RS coated batches (colon targeted tablets)				
Polymer soln. concentration	% Weight gain (Coating Level)			
	Batch RSS1	Batch RSS2	Batch RSS3	Batch RSS4
10% Eudragit RS	10	12.5	15	17.5
10% Eudragit S	20	20	20	20

and the granules so obtained were dried at 40 ± 5 ^{0}C for 45 min in a hot air oven. The dried granules were passed though 20 # sieve and the fines were separated using 40 # sieve to obtain 20-40 # granules. These granules were lubricated with 2% talc and 1% magnesium stearate. The lubricated granules were compressed into tablets using rotary tablet machine (Rimek, Karnavati Engineering Pvt. Ltd.) using 11 mm concave punch.

Preparation of enteric, time coated and colon targeted tablets of metronidazole

In the present study, dip coating method was used to coat the tablets. Enteric coated tablets were prepared by coating the core tablets of metronidazole with pH dependent polymethacrylate, Eudragit® S coating solution. Eudragit® S solution was prepared at three concentration levels 5, 10 and 20% w/v. To prepare Eudragit® S coating solution, Eudragit® S was dissolved in acetone using a magnetic stirrer. After complete solubilization of polymer, castor oil (10% w/w of dry polymer) was added as plasticizer. Talc (0.1% w/v) was added as antiadherant and titanium dioxide (0.05 % w/v) was added as opacifier and the solution was stirred for 15 min. The coated tablets were air dried for 15 min at room temperature after which they were cured for 30 min at 40^{0}C in hot air oven.

Time coated tablets were prepared by coating core tablets with time dependent polymethacrylate, Eudragit® RS. Eudragit® RS solution was tried at two concentration levels viz. 10 and 15% w/v. To prepare Eudragit® RS coating solution Eudragit® RS was dissolved in methylene chloride. The other composition and method of preparation is similar to that of Eudragit® S. Only the amount of castor oil is increased to 15% w/w of dry polymer. Colon targeted tablets were prepared by coating core tablets initially with Eudragit RS. The coat was allowed to dry for 30 min. and then over the coat of Eudragit® RS, a coat of Eudragit® S was provided. The use of polymer concentration and coating level of Eudragit® S, Eudragit® RS and its combination is shown in Table 2.

Table 3: Formulation and Evaluation of CCD batches

Batch No.	Coded Values		Response	
	X_1	X_2	Y_{300} ± SD	Y_{480} ± SD
RSSD1	-1	-1	20.74 ± 2.45	100 ± 0.27
RSSD2	-1	1	9.52 ± 1.56	100 ± 0.72
RSSD3	1	-1	6.27 ± 1.75	80.52 ± 3.5
RSSD4	1	1	6.14 ± 1.96	78 ± 3.87
RSSD5	-1.41	0	12.38 ± 1.55	100 ± 0.27
RSSD6	1.41	0	5.88 ± 1.74	73.68 ± 3.70
RSSD7	0	-1.41	5.56 ± 1.04	97.92 ± 1.38
RSSD8	0	1.41	6.66 ± 1.49	84.87 ± 4.36
RSSD9	0	0	6.52 ± 1.09	85.39 ± 3.69
RSSD10	0	0	7.38 ± 1.27	88.37 ± 3.95
RSSD11	0	0	7.26 ± 1.53	85.17 ± 4.53
RSSD12	0	0	8.57 ± 1.58	87.65 ± 1.17
RSSD13	0	0	8.53 ± 1.12	89.77 ± 4.11
Independent variables		**Coded values**	**Actual values**	
X_1 – Coating level of Eudragit RS 100 (%)			**X_1**	**X_2**
X_2 – Coating level of Eudragit S 100 (%)		-1.41	11.9875	13.975
Dependent variables		-1	12.5	15
Y_{300} - % drug released in the fifth hour		0	13.75	17.5
Y_{480} - % drug released in the eighth hour		1	15	20
		1.41	15.5125	21.025

In vitro drug release studies

In vitro drug release studies were carried out using USP XXIII dissolution test apparatus Type II, paddle apparatus (100 rpm, 37± 0.5 ^{0}C). *In vitro* release study for enteric coated tablets was carried out by keeping the tablets for 2 h in 0.1 N HCl (900 ml), simulated gastric fluid (SGF). The dissolution medium was then replaced with pH 7.4 phosphate buffer solution (900 ml), simulated intestinal fluid (SIF), and tested for 3 h. The time dependent coated tablets were evaluated by exposing them to 900 ml SIF for 3 h which was later replaced by pH 6.8 phosphate buffer solution (900 ml), simulated colonic fluid (SCF), and tested for release for 3 h. Colon targeted tablets containing enteric and time dependent coats were evaluated by keeping them in 900 ml SGF for 2 h, which was then replaced with 900 ml SIF wherein it was kept for 3 h and lastly SIF was replaced with 900 ml SCF wherein it was kept for 3 h. The drug release at different time intervals was analyzed by UV double beam spectrophotometer (Electrolab TDT-06 T) at 276.5 nm in SGF, 319.4 nm in SIF and 320.4 nm in SCF.

Experimental design

To determine the influence of formulation variables on colon targeting a central composite design was selected. The independent variables used in the experimental design were coating level of Eudragit RS 100 (X_1) and coating level of Eudragit S 100 (X_2). The % drug released in the 5th h, when the tablet is expected to reach the colon (Y_{300}) and % drug released in the 8th h, when the tablet is in the colon (Y_{480}) were selected as dependent variables. The independent and dependent variables along with actual and coded values are summarized in Table 3.

RESULTS AND DISCUSSION

Optimization of core tablets

Metronidazole is not directly compressible, so an attempt was made to compress it using different directly compressible diluents like Avicel 101®, Tablettose 100®, Cellactose 80®, Flowlac 100® and Aerolac® were unable to give sufficient hardness to the tablets. Even on increasing the amounts of directly compressible diluents and concentration of binder (PVP K30/K90), tablets with sufficient strength and hardness could not be obtained. Thus, direct compression method was replaced with wet granulation.

The initial batches prepared by wet granulation contained metronidazole (200 mg), lactose and 5% PVP K30 (Table 1). But these tablets had insufficient hardness. In order to increase hardness amount of PVP K30 was also increased to 15%, but sufficient hardness could not be obtained. Thus, PVP K30 was replaced with PVP K90. When PVP K30 was replaced with PVP K90 the hardness obtained was tremendously high, about 9 kg/cm^2 and the disintegration time was also increased to 30 min. PVP K 90 formed a cohesive gel with iso-propyl-alcohol which was responsible for higher hardness and higher disintegration time. As, PVP K30 produced lower hardness (< 4 kg/cm^2) and PVP K90 produced higher hardness (> 9 kg/cm^2), a 50:50 combination of PVP K30:PVP K90 at 5% concentration was utilized as shown in Batch WF (Table 1). The tablets had a hardness of 6 kg/cm^2 and disintegration time was 4 min. The aim of the present work was to prepare colon targeted tablets of metronidazole using double coating technique. Thus, initially enteric coat of Eudragit S was optimized and later the time dependent coat of Eudragit® RS was optimized. Both the coats, of Eudragit® S and RS, were optimized for polymer concentration and coating level (coating thickness).

Optimization of enteric coat (Eudragit S)

Three different strengths of coating solution were prepared for Eudragit® S, 5%, 10% and 20%. Each strength solution was coated at five different coating levels, 5, 10, 15, 17.5 and 20% as shown in Table 2. The tablets coated using Eudragit® S at 5% concentration (E1 to E5), lacked viscosity resulting in the formation of poor non elastic films which ultimately was responsible for premature drug release in SGF. At 10% polymer concentration only batch E10 (20% coating level) was able to prevent drug release for 2 h. With a view to reduce coating thickness, a higher polymer concentration of 20% was tried. Batches E11 to E13 failed to prevent premature drug release. Only two batches, Batch E14 and E15 at 17.5% and 20% coating levels respectively prevented the drug release in SGF. After 2 h when SGF was replaced with SIF almost 100% drug was released between 4th and 5th h. This indicated that at higher coating levels polymer dissolution was slower. On increasing the polymer concentration from 10% (batch E10) to 20% (batch E15) at similar coating level of 20% the drug release was prolonged due to higher amount of Eudragit® S which

forms more elastic and tortuous films. The dissolution data of the formulations clearly demonstrated that the solubility of the films obtained using Eudragit S 100 was strongly dependent on polymer concentrations and coating levels. Eudragit® S which consists of carboxylic group ionizes at neutral pH.[14] Therefore, drug release from formulations was rapid after 2 h when SGF was replaced with SIF due to polymer dissolution at higher pH.

Optimization of time dependent coat (Eudragit RS)

Since 5% polymer concentration and 5% coating level both failed to prevent drug release in Eudragit® S, optimization using Eudragit® RS was initiated at 10% polymer concentration and 10% coating level. Batches prepared at different polymer concentrations and coating levels of Eudragit RS are shown in Table 2. The batches which exhibited less than 10% release at the end of 3 h in SIF, which was considered as intestinal emptying time, were considered to be the promising batches. At higher polymer concentration of 15% the drug release was slower and surprisingly the sustained effect was found even at lower coating level of 10% (batch RS6), 100% drug did not release even after 8 h. The results might be attributed to the pH independent characteristic of Eudragit® RS which kept the coat intact.

Eudragit® RS contains quaternary ammonium groups in their chemical structure which play an important role in controlling drug release because they relate to water uptake followed by swelling of Eudragit® RS.[15] The active ingredients are gradually dissolved by penetration of dissolution media since release is primarily diffusion controlled.[16] The release rate was slower at higher coating levels because of the increased diffusion path-length and tortuosity at higher coating levels. Moreover, the coating layer of all the tablets containing Eudragit® RS did not disintegrate at the end of the dissolution study indicating an apparent intactness of the coat. The drug released from the openings of the coating layer that were found at the end of dissolution study. From the batches RS1 to RS8, batch RS4 and RS5 coated using 10% Eudragit® RS solution at 17.5% and 20% coating levels respectively were considered as promising batches as the drug release was below 10% in SIF, while other batches gave faster or slower release.

Double coated tablets of metronidazole

From the individual results of coating studies using Eudragit® RS and Eudragit® S it was found that 10% polymer concentration of Eudragit® RS and Eudragit® S was sufficient for protecting drug release in SGF and SIF. It was speculated that Eudragit® S at 20% coating level would be necessary to prevent drug release in gastric pH. Thus, only coating levels of Eudragit® RS was varied. In all batches (batch RSS1 to RSS4) drug release was negligible in SGF (Figure 1). In SIF drug release was below 10% in all the batches except batch RSS1. Thus, batch RSS2, RSS3 and RSS4 can be considered as promising batches. When these double coated formulations were exposed to SIF Eudragit S coat dissolved completely and when Eudragit RS coat came in contact with the dissolution medium, coat of Eudragit RS swelled and after a lag phase of 5 h the active principle, metronidazole, releases.

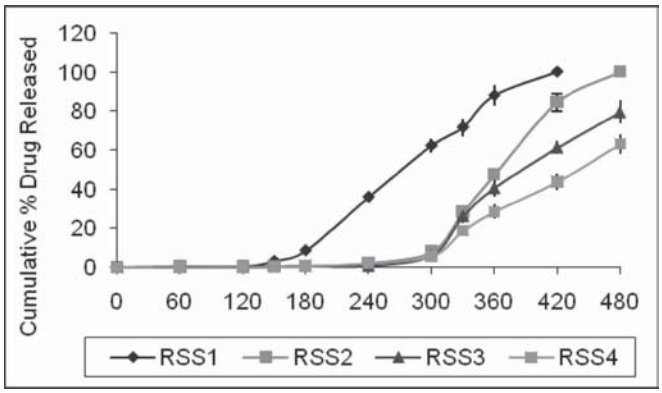

Figure 1: Comparative release profiles of preliminary batches of colon targeted tablets, the values represented mean ± S.D (n=3).

Experimental design

The effect of independent variables viz. coating level of Eudragit RS® 100 (X_1) and coating level of Eudragit® S 100 (X_2) was studied on dependent variables Y_{300} (% drug released in the fifth hour) and Y_{480} (% drug released in the eighth hour). The response layout and release profiles of central composite design batches are shown in Table 3 and Figure 2 respectively, which clearly indicates that both the independent variables are dependent on Y_{300} and Y_{480} as they show distinct variation among the thirteen batches. An interactive statistical second-order complete model (Equation 1) was generated to evaluate the responses (Y_{300} and Y_{480})

Table 1: Core tablets of metronidazole

Ingredients	W1	W2	WF
Metronidazole	200	200	200
Lactose	128	128	128
PVP* in iso-propyl-alcohol (5%)	20	20	(10+10) 20
Cross carmellose sodium (10%)	40	40	40
Talc (2%)	8	8	8
Magnesium stearate (1%)	4	4	4
Total	400	400	400

The weights showed above correspond to values in mg/tablet.

* Batch W1 contained PVP K30, W2 contained PVP K90 and WF contained 50:50 ratio of PVP K30 : PVP K90.

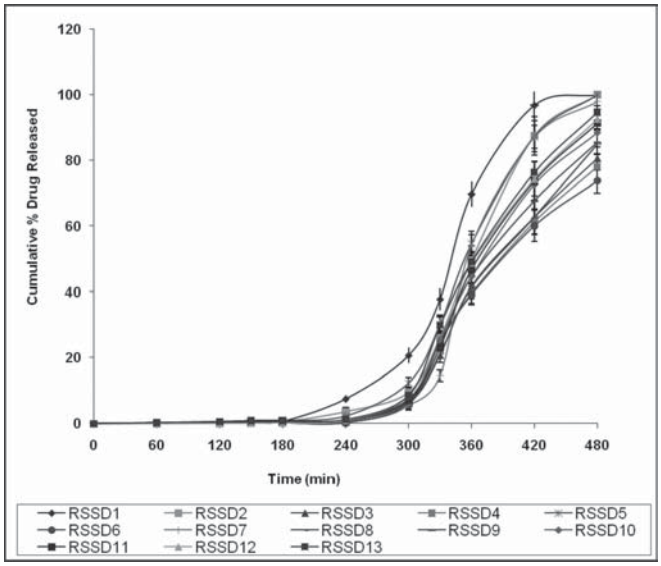

Figure 2: Comparative release of all the factorial design batches, the values represented mean ± S.D (n=3).

$Y = b_0 + b_1X_1 + b_2X_2 + b_1^2X_1^2 + b_2^2X_2^2 + b_{12}X_1X_2$ …….....(1)

Where, Y = the measured response

b_0 = Intercept

Xi = the level of i^{th} factor

bi, bij, bijk = the estimated coefficients

The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low value to its high value. The interaction terms (X_1X_2) show how the response, Y_{300} and Y_{480}, changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate nonlinearity. The statistical analysis of the factorial design batches was performed by multiple linear regression analysis using Microsoft Excel®. The values of the regression coefficients for Y_{300} and Y_{480} are shown in Table 4. The

Table 4: Regression statistics table

Regression Statistics		
	Y_{300}	Y_{480}
Multiple R	0.862755	0.972269
R Square	0.744346	0.945307
Adjusted R Square	0.561737	0.906242
Standard Error	2.716163	2.674183
Observations	13	13

fitted equations relating the response Y_{300} and Y_{480} to the transformed factor are shown in Equation 2 and Equation 3 respectively.

$Y_{300} = 7.64 - 3.38X_1 - 1.22X_2 + 1.51X_1^2 - 0.005X_2^2 + 2.77X_1X_2$. ..... (2)

$Y_{480} = 87.26 - 9.85X_1 - 2.62X_2 - 0.08X_1^2 + 2.20X_2^2 - 0.63X_1X_2$ ……..….. (3)

Negative signs of X_1 and X_2 in Equation 2 and 3 indicate that the drug release in inversely proportional to coating thickness of both the polymers.

Percentage drug released in the fifth hour (Y_{300}) for the 13 batches show wide variation in the response ranging from a minimum 5.56% to a maximum of 20%. This large difference between the minimum and maximum value indicates that the coating thickness of the topmost coat of the formulation, Eudragit® S (X_2), plays an important role till the formulation is in small intestine (Y_{300}). As, the coating thickness of Eudragit® S increases the drug release is also controlled at intestinal pH.

The variation in release profile of 13 batches for drug release at eighth hour (Y_{480}), was also found on the higher side. The response ranged from minimum of 73.68% to maximum of 100% drug release. When the formulation reaches the colon the topmost coat of Eudragit® S has already dissolved and the Eudragit RS is exposed to the intestinal fluid. So, the drug release in colonic fluid is controlled by coating thickness of the inner coat of Eudragit® RS (X_1) only. On comparing equation 2 with equation 3, in equation 3 the difference between the coefficients of X_1 and X_2 is large, and coefficient of X_1 is on the higher side which clearly indicates that the effect of X_1 plays a dominant role for formulation to reach the colon.

From ANOVA study (Table 5) it was found that Significance F value for Y_{300} is 0.04 and that Y_{480} is 0.0002 which indicates that the effect of X_1 and X_2 on Y_{480} is

Table 5: ANOVA table

ANOVA of Y_{300}					
	df	SS	MS	F	Significance F
Regression	5	150.3602	30.07204	4.076158998	0.04707352
Residual	7	51.6428	7.377543		
Total	12	202.003			
ANOVA of Y_{480}					
	df	SS	MS	F	Significance F
Regression	5	865.2263	173.0453	24.19787	0.000278185
Residual	7	50.05881	7.151259		
Total	12	915.2851			

significantly different and one of the two factors played a more important role on drug release at 8th h. Figures 3 and 4 shows the plot of coating level of Eudragit® RS 100 (X_1) and coating level of Eudragit® S 100 (X_2) versus Y_{300} and Y_{480}, respectively. The plot was drawn using Sigma Plot® software (Jandel Scientific Software, San Rafael, CA). Figure 3 demonstrates that Y_{300} is affected by X_1 and X_2 equally with slightly more significance towards X_2, whereas Figure 4 demonstrates that effect of X_1 is more significant for Y_{480}.

Eudragit S did not play a major role in drug release in the intestine as the coat dissolves at intestinal pH. The small change in coating level of Eudragit® S does not affect drug release to a higher extent when the tablet is kept in SCF. Batch RSSD3 having 15% Eudragit® S weight gain and RSSD4 with 20% Eudragit S weight gain showed about 77% and 80% drug release respectively at the end of third hour in SCF. Batch RSSD3 and RSSD6 with 15 and 15.5125% coating level showed only 80 and 73% drug release respectively after staying for 3 h in

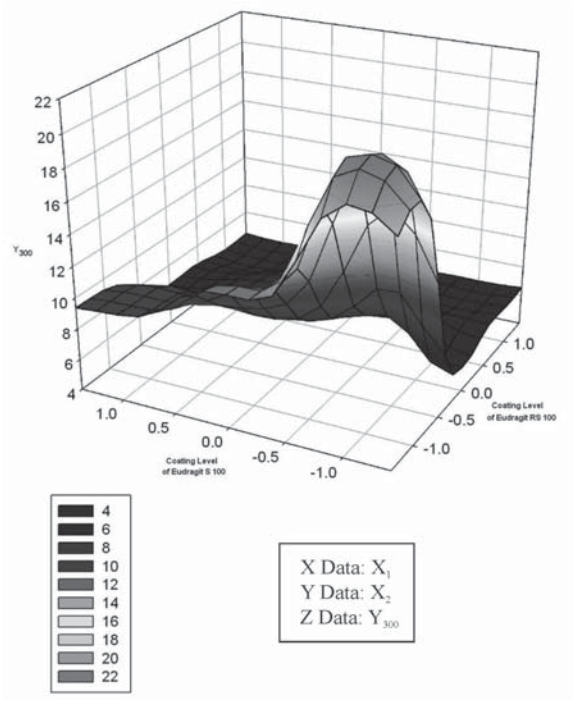

Figure 3: Response Surface Plot for amount of drug released in 300 min (Y_{300})

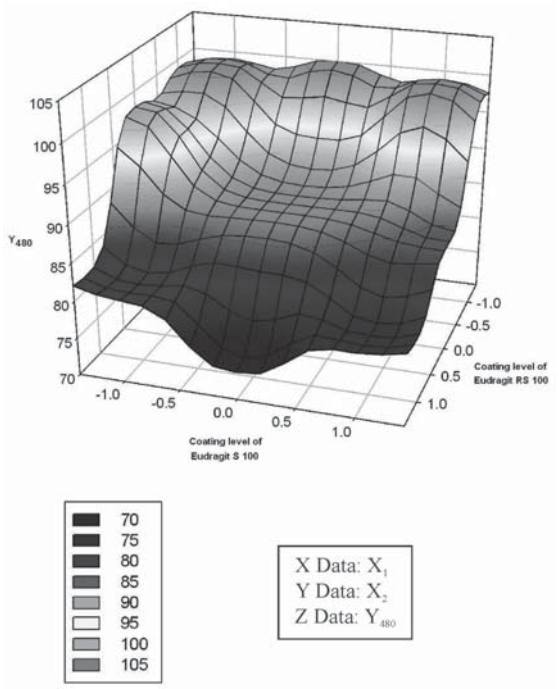

Figure 4: Response Surface Plot for amount of drug released in 480 min (Y_{480})

SCF. This clearly indicates that even small change in coating level of Eudragit® RS drug release was sustained to a higher extent, after drug passes the small intestine. Thus, X_1 is more dominant factor than X_2 in case of Y_{480}. Thus, from above release studies and statistical analysis it can be concluded that lower levels of X_1 and higher levels of X_2 favor the preparation of colon targeted tablets of metronidazole.

Selection of best batches

Best batch was selected on the basis of following criteria:

1) Less than 10% release in SGF and

2) 100% release within 3 hours after the tablet reaches colon

Batch RSSD1 failed to prevent drug release in SGF and SIF as about 20% drug was released at the end of 5 h. All other batches showed less than 10% release at the end of 5 h which is shown in the Figure 2. In most of the batches majority of the drug release is found in the colon. Batch RSSD2, RSSD5 and RSSD7 are the only batches which showed 100% drug release in SCF within 3 h after the tablet reaches the colon. Of these batches batch RSSD2 can be considered as best batch since about 9.5% drug released till 5th h and 100% drug released within 8 h. All the 5 replicates showed good correlation in drug release with 84 – 90% drug release at the end of 8th h.

CONCLUSION

Metronidazole colon targeted tablets prepared in the present work by double coating method consisted of inner coat of Eudragit® RS and outer coat of Eudragit® S. Optimization of coating levels of both the coats using central composite design reveals that polymer concentration and coating level of both the coats play a significant role in drug release property of which coating level of Eudragit® RS was more significant after the tablet reaches colon. Thus, proposed system may be successfully used for colon targeting of metronidazole.

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