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A novel pH- and time-based multi-unit potential colonic drug delivery system. II. Optimization of multiple response variables

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Abstract

The objective of this work was to optimize a novel potential colonic drug delivery system by using a statistical procedure. Pellets were prepared by powder layering of 5-aminosalicylic acid (5-ASA) on nonpareils (0.5–0.6 mm) in a coating pan. Drug-layered pellets were coated with an inner layer of a combination of Eudragit® RL and RS and an outer layer of Eudragit FS in a fluidized-bed apparatus. Central composite design was used to study the effect of three independent variables. The proportion of the more hydrophilic polymer Eudragit RL had the most significant effect on drug release – higher proportion gave faster release; the amount of inner and outer coat did not have a significant effect on the rate of drug release at either 6 or 12 h in the range studied. A second order polynomial equation was fitted to the data, and the resulting equation was used to predict the responses in the optimal region. An optimized formulation was prepared and evaluated for individual responses. The experimental values of the response variables highly agreed with the predicted values. The results demonstrated the reliability of the model in the preparation of coated pellets having predictable drug release for colonic delivery of 5-ASA.

Keywords: Controlled-release; Colonic delivery; Optimization; 5-aminosalicylic acid; Polymethacrylates; Eudragit

1. Introduction

Colonic drug delivery has gained increased importance not just for the delivery of drugs for the treatment of local diseases of colon but also for its potential for the delivery of proteins and peptides. The colon presents less hostile conditions for drug delivery because of less diversity and intensity of

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enzymatic activities and a near neutral pH (Mrsny, 1992; Ashford and Fell, 1994; Rubinstein, 1995; Van den Mooter and Kinget, 1995; Watts and Illum, 1997).

In a previous study, we developed a novel delivery system for delivering drugs to the colon by combining pH characteristics of polymethacrylates and relatively constant transit time of the small intestine. The delivery system consisted of drug-layered pellets coated with an inner layer of a combination of two pH-independent polymers Eudragit® RL and RS (2:8), and an outer layer of a pH-dependent polymer Eudragit FS. This system demonstrated its potential for colonic delivery by resisting drug release until pH 6.5 and the combination of Eudragit RL and RS proved successful for the sustained delivery of 5-ASA for over 12 h at the expected pH of the colon (Gupta et al., 2000).

The present study dealt with the optimization of the colonic delivery system developed in that study. Several variables usually need to be optimized during optimization of pharmaceutical products. While some of these variables may need to be maximized, others may have to be minimized. In many instances, these responses compete with each other meaning that improving one response may have an opposite effect on another response. For example, in a sustained-release product a positive effect on the release rate at 6 h may have a negative effect on the release rate at 12 h. Hence, all the responses that may affect the quality of product should be taken into consideration. Three approaches have been widely reported to simultaneously optimize multiple response variables. The first approach known as constrained optimization optimizes one response variable while placing constraints on the remaining response variables to keep them within acceptable limits. In the second approach, the contour diagrams of different response variables are superimposed. The last approach utilizes a desirability function that combines the responses into one measurement. The second approach using contour diagrams is probably the easiest to use if the number of response variables is equal to or less than three, and if all the responses are on the same scale on a graph (Hileman et al., 1993; Abu Izza et al., 1996; Bodea and Leucuta, 1997; Zhou et al., 1998).

In this work, central composite design was used to simultaneously study the effect of the three formulation variables of the colonic drug delivery system on two response variables. Central composite design and analysis of response surfaces were used because they are systematic and efficient methods to simultaneously study the effect of multiple variables and to find an optimum formulation (Abu Izza et al., 1996). The three formulation variables studied were the amount of Eudragit FS outer coat, proportion of Eudragit RL in the inner coat, and the amount of Eudragit RL–RS inner coat. The two response variables studied were the amount of drug released in 6 h and the amount released in 12 h. Response surfaces were generated and the formulation was optimized by superimposing the contour plots. The response variable, amount of drug released in 12 h, was maximized while applying a constraint to the model for the other variable, amount of drug released in 6 h.

5-ASA was used as a model drug because there is a therapeutic benefit for the colonic delivery of this drug (Dash and Brittain, 1998). Eudragit RL is more hydrophilic than Eudragit RS, and the release of most drugs is faster from Eudragit RL than from Eudragit RS, hence the pellets can be coated with different combinations of Eudragit RL and RS to provide various degrees of sustained-release of the drug. Eudragit FS30D dissolves at pH 6.8; as the pH in distal ileum is reported to be 7–8, it is expected that Eudragit FS30D will dissolve in that region, and can be used to control the site of release of a pellet system previously coated with an Eudragit RL–RS layer for sustained-release of a drug in the colon (Röhm GmbH and Rohm America, 1999). Pellets were chosen for development because multi-unit delivery systems are statistically more reliable than single-unit delivery systems (Li et al., 1997; Amighi et al., 1998).

2. Materials and methods

2.1. Materials

Mesalazine (5-aminosalicylic acid) was purchased from Chemie-S.p.A, Italy; Eudragit RL30D, RS30D, and FS30D were obtained in-house from Röhm GmbH, Chemische Fabrik, Germany; and the nonpareils were purchased from Hans G. Werner GmbH, Germany. Other excipients used to prepare pellets and for coating were of standard pharmaceutical grade and all chemical reagents used were of analytical grade.

2.2. Preparation and coating of pellets

The preparation and coating of pellets were discussed in detail in a previous publication (Gupta et al., 2001). Briefly, pellets were prepared by powder layering of 5-ASA on nonpareils (nuclei) in a conventional coating pan (Erweka, Germany). Excipients of the powder layering composition were sieved and mixed. Binder solution (aqueous solution of polyvinyl pyrrollidone) was continuously sprayed on the moving nonpareils by means of a peristaltic pump and a spray-nozzle. At fixed intervals, fixed amounts of the powder composition were layered onto the particles. The drug-loaded pellets were dried in an oven at 40°C for 24 h after which sieve analysis was done and the fraction of 0.8–1.0 mm was separated for coating. In order to prevent the batch-to-batch variability of the drug-layered pellets from affecting the different batches of coated pellets, the sieve-cuts of 0.8–1.0 mm from several batches of drug-layered pellets were pooled together and blended and the pellets for coating were taken out from this bulk.

For the inner coat, the pellets were coated with a combination of Eudragit RL–RS in a fluidized-bed coating apparatus (GPCG 1.1, Glatt, Germany). After the coating, the pellets were gently fluidized for about 5 min after which they were cured in an oven for 24 h at 40°C. For the outer coat, the cured pellets containing inner coat of Eudragit RL–RS were further coated with Eudragit FS30D in the fluidized-bed processor. After the coating, the pellets were gently fluidized for about 5 min after which they were again cured in an oven for 24 h at 40°C. To prevent the coated pellets from sticking together, 0.5% Aerosil 200 was added to the finished product after both inner and final coatings.

2.3. Software

Automatic calculations of the drug concentration from UV absorption values were done using UV WinLab® software. Statistica® (Statistica '98 Edition, Kernel release 5.1, Statsoft Inc, USA), a windows-based program was used for generating the experimental design, modeling of response surfaces, and the evaluation of quality of fit of the model.

2.4. Experimental design

Central composite design was used to study the effect of the following three factors at five levels each in the range indicated below:

Amount of Eudragit FS outer coating (X_1): 10–30%

Proportion of Eudragit RL in the inner coating (X_2): 20–80%

Amount of Eudragit RL–RS inner coating (X_3): 2–8%

These three formulation variables were found important for drug release in the previous study and their range was chosen based on the preliminary studies done in our lab and the previous literature reports.

The two responses studied along with their constraint values are listed below:

Amount of drug released in 6 h (Y_1): $50 \leqslant Y_1 \leqslant 65$

Amount of drug released in 12 h (Y_2): $85 \leqslant Y_2 \leqslant 100$

These response variables were chosen because of their bearing on the effectiveness of the delivery system for colonic delivery.

This statistical design provided an empirical second order polynomial equation used for the prediction of the effect of formulation variables on the release characteristics using a smaller number of experimental runs. In this approach, each experimental response Y can be represented by a quadratic equation of the response surface.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3$$

This equation enables the simultaneous investigation of the effect of each factor and their interaction over the experimental responses. The matrix of the experimental plan and the composition of the 16 batches are shown in Table 1.

2.5. Determination of the response variables

Determination of the response variables was carried out using modified USP XXIII, Method B for enteric-coated products (paddle method, 100 rpm, 37°C). For the *Acid Stage*, 200 mg of pellets ($n = 6$) were added to 700 ml 0.1 N hydrochloric acid and dissolution was done for 2 h. At the end of 2 h, 200 ml of 0.20 M tribasic sodium phosphate was added to all the dissolution vessels and the pH was adjusted to either 6.5 or 7.5 using either 0.1 N sodium hydroxide or 0.1 N hydrochloric acid. Dissolution was continued for another 12 h for the *Buffer Stage*. The dissolution apparatus (DT 80, Erweka, Switzerland) was attached to UV spectrophotometer for automated sampling and online analysis. The release data was plotted against time and both the response variables, the amount of drug released in 6 h (Y_1) and the amount of drug released in 12 h (Y_2) were determined from the graph.

2.6. Model fitting and prediction

A second-order polynomial model was individually fitted to both the response variables using Statistica. Response surfaces that demonstrate the relationship between the response variables and the formulation variables were generated from the fitting. The resulting equations were subjected to lack-of-fit and model simplification at 95% significance level. The optima for individual response variables were located by superimposing the contour plots for all the variables.

3. Results and discussion

3.1. Characterization of coating

Scanning electron micrograph (SEM) pictures of the pellets from all the 16 experimental batches were taken to characterize the coating. The uniformity and homogeneity of both the inner and outer coats can be observed in Fig. 1; it is the

Table 1
Composition of 16 batches prepared using central composite design[a]

Batch no.	Amount of FS coat, X_1 (%)	Proportion of RL, X_2 (%)	Amount of RL–RS coat, X_3 (%)
1	14	32	3
2	14	32	7
3	14	68	3
4	14	68	7
5	26	32	3
6	26	32	7
7	26	68	3
8	26	68	7
9	10	50	5
10	30	50	5
11	20	20	5
12	20	80	5
13	20	50	2
14	20	50	8
15	20	50	5
16	20	50	5

[a] FS – Eudragit FS, RL – Eudragit RL, and RS – Eudragit RS.

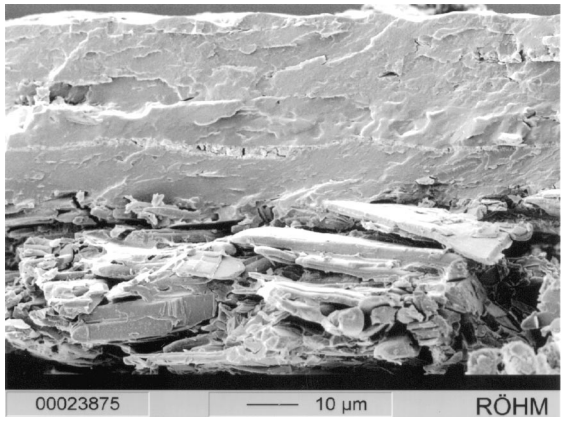

Fig. 1. SEM picture of the coatings (8% inner coating and 30% outer coating) of 5-ASA pellet (magnification 2500 ×).

SEM of a pellet containing 8% inner coating and 30% outer coating. A thin porous layer of Aerosil can be spotted between the inner and outer coating in both the pictures.

3.2. Fitting of data to the model

Dissolution profiles of the 16 formulations prepared using the experimental design are shown in Fig. 2. The model was fitted to the release data simultaneously for both the responses. The quality of fit of the model for Y_1 is shown in Fig. 3 by plotting predicted vs. observed values. The model was acceptable for both the responses as the observed values for the drug release were within $\pm 5\%$ of the predicted values. The significant terms in the model for Y_1 are shown in Fig. 4.

3.3. Examination of the equations and coefficients

The equations representing the quantitative effect of the formulation variables at the level of 20% outer coating on the responses Y_1 and Y_2 are shown below:

$$Y_1 = 43.523 + 2.269X_2 - 0.0233X_2^2 - 0.469X_3 - 0.0668X_3^2 + 0.0244(20)X_2 - 0.0209(20)X_3 + 0.0182X_2X_3 - 24.689$$

$$Y_2 = 81.955 + 0.742X_2 - 0.00928X_2^2 - 0.0364X_3 - 0.0422X_3^2 + 0.0113(20)X_2 - 0.0675(20)X_3 + 0.0282X_2X_3 - 6.554.$$

Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor and those with second order

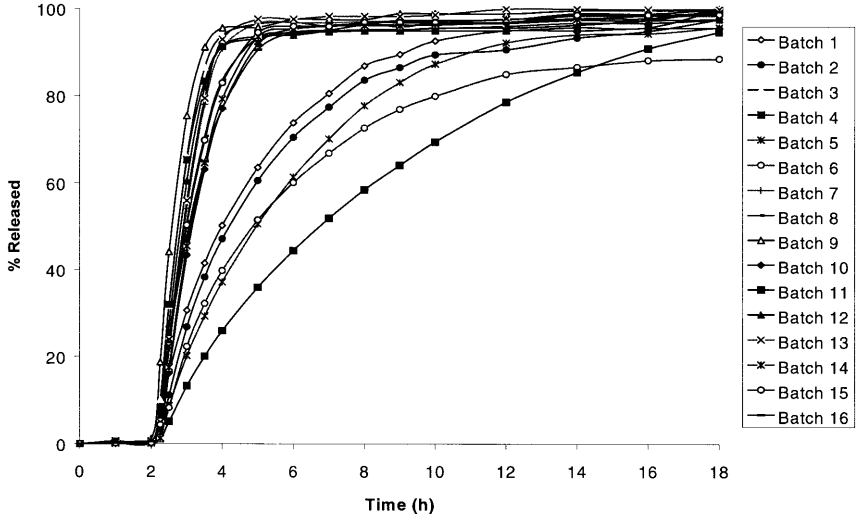

Fig. 2. Release of 5-ASA from the 16 batches of coated pellets prepared using the experimental design. For the composition of batches, refer to Table 1.

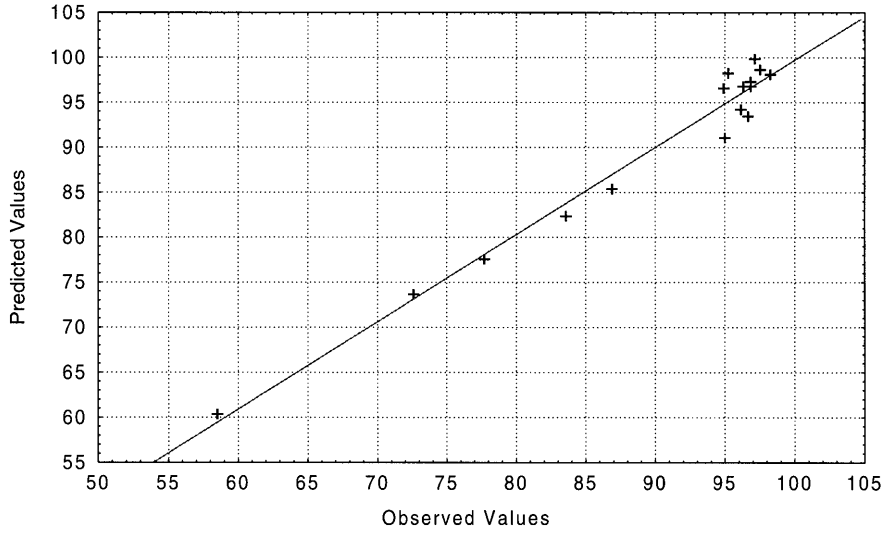

Fig. 3. Predicted and observed values of Y_1 for the 16 batches prepared using experimental design.

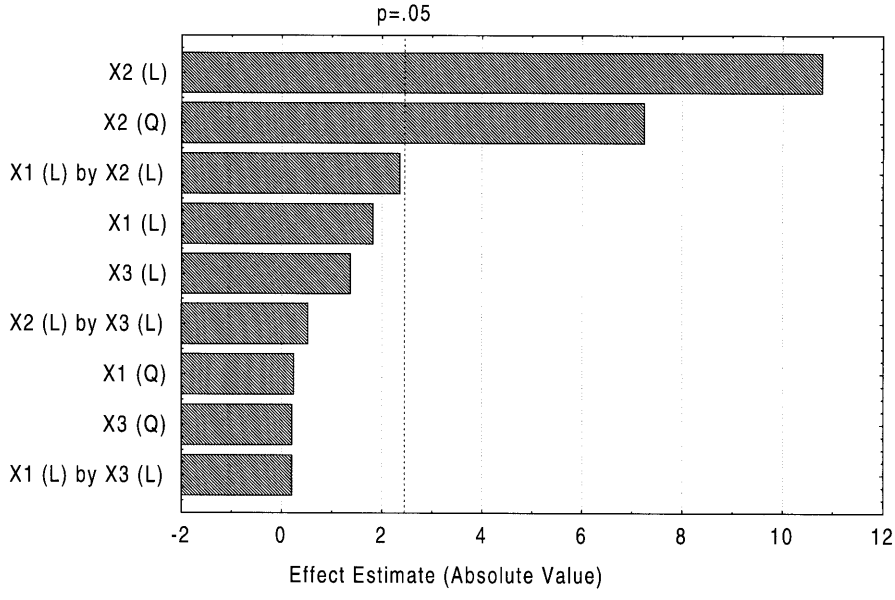

Fig. 4. Significant terms in the model for Y_1.

terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Positive sign in front of the terms indicates synergistic effect while negative sign indicates antagonistic effect upon the factors.

It can be concluded from the equations that X_2 (proportion of Eudragit RL in the inner coat) had the largest synergistic effect on both the responses. The effect of the quadratic term of X_2 was also significant. The effects of X_1 (amount of outer coat), X_3 (amount of inner coat), and the interaction among the factors were statistically insignificant.

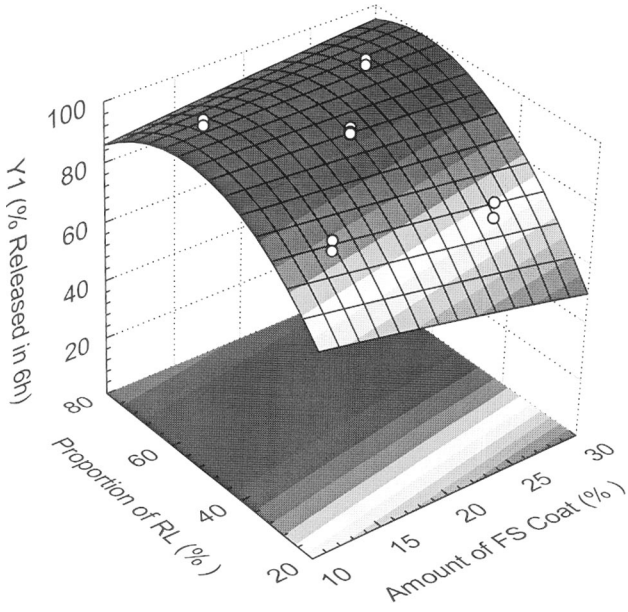

Fig. 5. Effect of the proportion of Eudragit RL (RL) and the amount of Eudragit FS (FS) coat on Y_1.

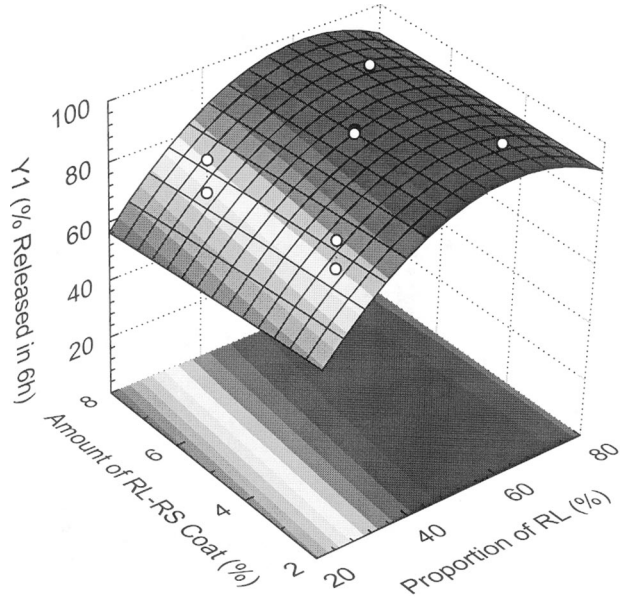

Fig. 6. Effect of the proportion of Eudragit RL (RL) and the amount of Eudragit RL–RS (RL–RS) coat on Y_1.

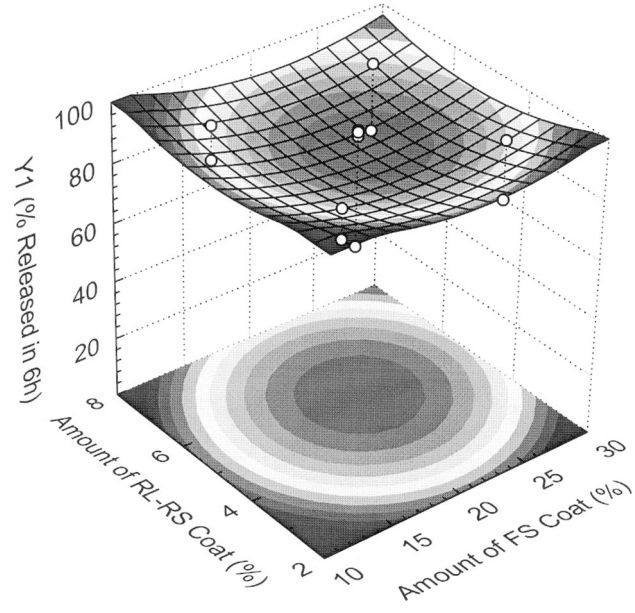

Fig. 7. Effect of the amount of Eudragit FS (FS) coat and amount of Eudragit RL–RS (RL–RS) coat on Y_1.

3.4. Analysis of response surfaces and fitted data

All the 16 batches prepared using statistical design showed that the integrity of enteric coating was evident because of less than one percent drug release in pH 1.2 dissolution media. Moreover, all the batches released less than 1% drug in pH 6.5 dissolution media indicating that even 10% Eudragit FS coating can be potentially used for delivering majority of the drug to the colon. This is significant because, if the objective of colonic delivery can be achieved with a lower coating level of the polymer, it leads to lower cost, reduction in processing time, and lower weight and smaller size of the final dosage form.

Three dimensional response surfaces depicting the effects of three formulation variables X_1, X_2, and X_3 on the response variable Y_1 are shown in Figs. 5–7. The formulation variables had a similar effect on Y_2, however, the effect on Y_2 was less pronounced as compared to the effect on Y_1. The rate of release of 5-ASA increased with an increase in the proportion of Eudragit RL in the inner layer. Eudragit RL and Eudragit RS are water-insoluble, diffusion-release polymers over

Table 2
Composition of optimum formulation (A) and two other random formulations (B and C)[a]

Formulation	Amount of FS Coat, X_1 (%)	Proportion of RL, X_2 (%)	Amount of RL–RS Coat, X_3 (%)
A	20	21	3
B	20	40	6
C	20	25	4

[a] FS – Eudragit FS, RL – Eudragit RL, and RS – Eudragit RS.

the entire pH range. Eudragit RL has more hydrophilic quaternary ammonium groups than Eudragit RS; this leads to higher hydration and increased permeability of the coating. Higher permeability of the coating because of higher proportion of Eudragit RL results in faster drug release.

The effect of the amount of Eudragit FS coat in the range studied was not statistically significant. As can be seen from the release curves in Fig. 2, the formulations containing higher amount of Eudragit FS coating released the drug after a lag time compared to the formulations that have lower amount of Eudragit FS coating. However, because of faster ionization of the carboxyl groups of Eudragit FS at pH 7.5, the lag times are too small (15–30 min) to make the effect of Eudragit FS statistically significant.

The effect of the amount of Eudragit RL–RS inner coating in the range of 2–8% was also statistically insignificant. This gives more flexibility to the formulators as they can chose the minimum amount of coating from this range that gives them reproducible coating of the batches. While the SEM pictures of all the 16 batches of experimental design revealed a uniform Eudragit RL–RS coating, in general, a 2% coating level is considered too small to provide homogenous and uniform coating, primarily because of the relatively short time spent by the charge load in the fluidizing chamber. On the other hand, very high coating levels lead to longer processing time and escalation of cost. In order to strike a balance between the coating uniformity of the batch and the processing time, the formulator may decide to chose 3–5% inner coating to ensure the uniformity of coating in a batch without appreciably increasing the processing time.

It must be mentioned that in the present study, the effect of the thickness of inner Eudragit RL–RS coat was insignificant probably because of the narrow range (2–8%) of coating level studied. At higher coating levels, the effect might be more pronounced.

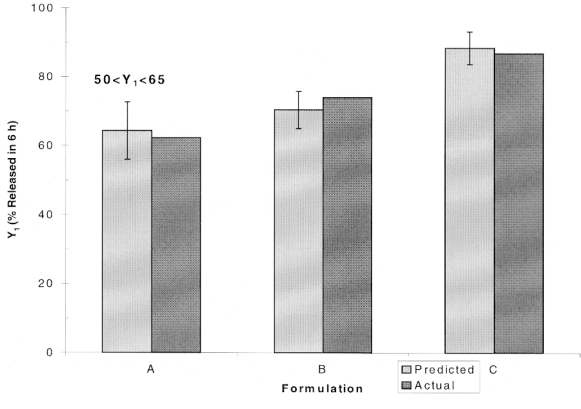

Fig. 8. Predicted and actual values of Y_1 for optimized formulation (A) and two other random formulations (B and C). The bars on the predicted values represent 95% confidence interval.

3.5. Optimization of the formulation

The values of the constraints were decided after careful consideration of the transit time of dosage forms through the gastrointestinal (GI) tract, especially the residence time in the colon. Since the outer coat of the colonic delivery system used in this study is pH-dependent and not time-dependent, the variability in time for the colonic arrival of the delivery system will not significantly affect the effectiveness of the system and is hence, not important for the purpose of formulation optimization. Compared to the other regions of GI tract, the mean colonic residence time is highly variable and has been reported to range from

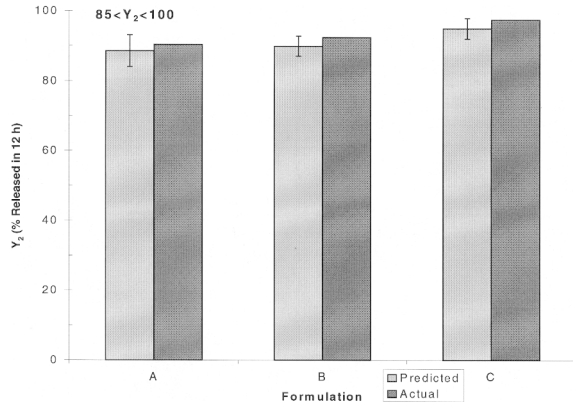

Fig. 9. Predicted and actual values of Y_2 for optimized formulation (A) and two other random formulations (B and C). The bars on the predicted values represent 95% confidence interval.

10–36 h (Mrsny, 1992). However, since the main function of the colon is absorption of water, the viscous consistency of colonic contents increases appreciably as one moves down the colon; this may impede the drug release from a dosage form as time progresses. Hence, a time value of 12 h was considered reasonable for 85–100% removal of drug from the delivery system in the colon. A value of 50–65% for the amount of drug released in 6 h (Y_1) combined with the value of 85–100% for the amount of drug released in 12 h (Y_2) would ensure sustained and complete release of drug in the colon.

Optimization was performed for the response Y_1 and Y_2 by applying constraints on both the responses. Optimization was performed by superimposing the contour plots of the two responses and locating the area of interest (optimal surface) common to both the plots. Since the optimal area was small, only one formulation (A) was chosen in the center of the area. Two additional random formulations (B and C) were chosen in the experimental matrix to determine the validity of the model generated using the study. The compositions of the formulations A, B, and C are shown in Table 2.

The predicted and actual values of the two responses for formulations A, B, and C are shown in Figs. 8 and 9. Since all the observed values for dissolution were within 95% confidence level of the predicted values, it was concluded that the optimal surface was chosen correctly and that the model has satisfactory predictive power.

4. Conclusions

A statistical model was developed by simultaneously studying the effect of various formulation factors using experimental design to predict the release properties of the drug from the delivery system. The amount of fast-release polymer, Eudragit RL, in the inner layer had the most significant effect on the rate of drug release. An optimum formulation for colonic delivery, with respect to the release rate of the drug, was found. The optimum formulation (dissolution at pH 7.5: 50–65% in 6 h and $>85\%$ in 12 h) was comprised of 5-ASA pellets having 3% inner coat of a combination of Eudragit RL and Eudragit RS, 21% Eudragit RL in the inner coat, and 20% Eudragit FS in the outer coat. The observed values of drug release were close to the predicted values confirming the predictive value of the model. Formulators may decide to make changes by changing the constraints for the responses depending on the therapeutic moiety being formulated and the desired therapeutic objective.

References

Abu Izza, K., Garcia Contreras, L., Lu, D.R., 1996. Preparation and evaluation of zidovidune-loaded sustained-release microspheres. 2. Optimization of multiple response variables. J. Pharm. Sci. 85 (6), 572–576.

Amighi, K., Timmermans, J., Puigdevall, J., Baltes, E., Moes, A.J., 1998. Peroral sustained-release film-coated pellets as a means to overcome physicochemical and biological drug-related problems. I. *In vitro* development and evaluation. Drug Dev. Ind. Pharm. 24 (6), 509–515.

Ashford, M., Fell, J.T., 1994. Targeting drugs to the colon: delivery systems for oral administration. J. Drug Targeting 2, 241–258.

Bodea, A., Leucuta, S.E., 1997. Optimization of propranolol hydrochloride sustained release pellets using a factorial design. Int. J. Pharm. 154, 49–57.

Dash, A.K., Brittain, H.G., 1998. Mesalamine. In: Brittain, H.G. (Ed.), Analytical Profiles of Drug Substances and Excipients, vol. 25. Academic Press, New York, pp. 209–242.

Gupta, V.K., Beckert, T.E., Price, J.C., 2000. Development of a novel multi-particulate colonic delivery system using multi-functional coatings of aqueous polymethacrylates, In: Third World Meeting (APV/APGI) on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, 4–6 April, Berlin, Germany.

Gupta, V.K., Beckert, T.E., Price, J.C., 2001. A novel pH- and time-based multi-unit potential colonic drug delivery system. I. Development, Int. J. Pharm., 213, 83–91.

Hileman, G.A., Goskonda, S.R., Spalitto, A.J., Upadrashta, S.M., 1993. Response surface optimization of high dose pellets by extrusion and spheronization. Int. J. Pharm. 100, 71–79.

Li, S.P., Feld, K.M., Kowarski, C.R., 1997. The effect of polymer coating systems on the preparation, tableting, and dissolution properties of sustained-release drug pellets. Drug Dev. Ind. Pharm. 23 (7), 623–631.

Mrsny, R.J., 1992. Drug absorption in the colon: a critical review. In: Friend, D.R. (Ed.), Oral Colon-Specific Drug Delivery. CRC Press, Boca Raton, pp. 1–44.

Röhm GmbH and Rohm America, 1999, *Eudragit* literature.

Rubinstein, A., 1995. Approaches and opportunities in colon-specific drug delivery. Crit. Rev. Ther. Drug Carrier Sys. 12 (2&3), 101–149.

Van den Mooter, G., Kinget, R., 1995. Oral colon-specific drug delivery: a review. Drug Delivery 2, 81–93.

Watts, P.J., Illum, L., 1997. Colonic drug delivery. Drug Dev. Ind. Pharm. 23 (9), 893–913.

Zhou, F., Vervaet, C., Massart, D.L., Massart, B., Remon, J.P., 1998. Optimization of the processing of matrix pellets based on the combination of waxes and starch using experimental design. Drug Dev. Ind. Pharm. 24 (4), 353–358.