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In vitro and *in vivo* evaluation of coated capsules for colonic delivery

Somayeh Handali[a], Eskandar Moghimipour[a], Mohsen Rezaei[b], Maryam Kouchak[a], Zahra Ramezani[a], Farid Abedin Dorkoosh[c,d,*]

[a] Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[b] Department of Toxicology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[c] Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[d] Medical Biomaterial Research Centre (MBRC), Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

The aim of the present study was to design and evaluate the combination of pH-dependent and time-dependent polymers for colon-specific drug delivery using response surface methodology (RSM). Theophylline was selected as a model drug and Eudragit® FS 30D (pH-dependent), Eudragit® RL 30D and Eudragit® RS 30D (as time-dependent) were used as coating polymers. Dissolution test was carried out in different pH media mimicking the transit of capsules from stomach to colon. The morphology of coated capsules was evaluated by scanning electron microscopy (SEM). *In vivo* studies were performed using fluorescent imaging in order to trace the movement of capsules in gastrointestinal (GI) tract. The results of *in vitro* studies showed that optimized formulation had suitable release profile at different pH values, which were in agreement with the predicted value by RSM. The SEM images revealed that surface coated capsules were smooth and also fluorescent imaging indicated that coated capsules disintegrated at the targeted colon region. These findings provide novel insights for the development of enteric coated capsule in order to specify drug delivery to colon.

1. Introduction

Oral dosage forms delivering drugs to the colon have been widely investigated for treatment of bowel diseases such as local inflammation, ulcerative colitis and colon cancer. Colon is a promising site for drug delivery due to bypass the first-pass metabolism in order to increase bioavailability, a longer residence time (up to 5 days), better responsiveness to absorption enhancers of drugs and decreased drug dose and systemic side effects. The colonic mucosa is recognized to facilitate the absorption of drugs which make colon as an ideal site for drug delivery [1]. Moreover, oral dosage forms are the most preferred because of their convenience, non-invasive nature, a greater degree of flexibility in their manufacturing and design as well as minimizing contaminations [1–3]. However, in order to reach successful oral colonic delivery, drug release should be minimal in stomach and small intestine; while, complete release in the colon. Therefore, drugs should be protected from stomach acid and degradation in the upper GI tract [2].

Several approaches have been investigated for oral specific drug delivery to the colon including; time-dependent polymer, pH-sensitive polymer (such as Eudragit, shellac), pressure-dependent systems and microflora-triggered delivery systems [4–6]. Eudragit® FS 30D, an anionic copolymer is consisting of methyl acrylate, methyl methacrylate and methacrylic acid in a ratio of 7:3:1. This polymer is pH-sensitive polymer which is insoluble in acidic media; however, soluble in media with pH 7, owing to methyl methacrylate groups [7]. In comparison with Eudragit® L and Eudragit® S, Eudragit FS 30D retard drug release in the small intestine [8]. Due to similarity of pH between small intestine and the colon, using only pH-dependent polymers is less reliable [9]. For assuring more reproducible drug release in the colon, a combination of a time-and pH-dependent system is suggested [10]. Eudragit® RL and Eudragit® RS as time dependent polymers are acrylic and methacrylic acid esters, respectively. Because of the presence of quaternary ammonium groups, these polymers have some hydrophilic attributes [11]. Eudragit RL and RS are designed to deliver drugs after a particular time period, which is the time required to reach the colon [4]. It is believed that a combination of Eudragit RL and RS with an outer layer of a pH-dependent polymer, Eudragit FS 30D, can be provided as an appropriate targeted drug delivery to the colon. After dissolution of the outer layer, Eudragit RL and RS provide sustained release at pH values typical of the colon [12].

Various coating methods are available including, rolling coating, electrospinning, electrospraying and dip coating [13–16]. X-ray and

* Corresponding author. Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, 14399-56131, Iran.
E-mail address: dorkoosh@tums.ac.ir (F.A. Dorkoosh).

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gamma scintigraphy are used to monitor oral drug delivery systems throughout the gastrointestinal tract [17,18].

In the development of pharmaceutical formulations for sustained release purposes, it is very essential to design an optimum formulation in a short time period and minimum number of experimentations. The response surface methodology (RSM) has been extensively employed for designing of drug delivery systems. Furthermore, it has been reported that RSM is a successful method for optimizing a process when the independent variables have a combined effect on the desired responses [11,19,20]. Central composite design (CCD) is a very common experimental design used in RSM which provides small number of experiments to obtain a predictive model [7].

In the present study, theophylline, a water soluble molecule was selected as a model drug due to its chemical stability, relatively ease of assay and low cost [11]. The objective of present investigation was to design and develop a controlled release of drug from enteric coated capsule with Eudragit FS (as pH-dependent polymer) and Eudragit RL and RS (as time-dependent polymer) using RSM to achieve a colon targeted delivery. These polymers have previously been employed for colonic delivery by other researchers for coating tablets, granules, pellets and new dosage forms such as microparticles and nanoprticles [3,10,21–23]; but, not for coating of capsules. In addition, we tracked the transportation of enteric coated capsules in rat using fluorescent imaging. To our knowledge, this is the first study to optimize the enteric coated capsules and trace them by fluorescent imaging.

2. Materials and methods

Eudragit® FS 30D, Eudragit RL 30D and RS 30D were kindly donated by RÖhm GmbH (Darmstadt, Germany). Triethyl citrate (TEC), glyceryl monostearate (GMS) and Cy7.5 were obtained from Sigma (Germany). Theophylline was kindly donated by Dr. ABIDI pharmaceutical Co., Tehran, Iran. Polysorbate 80 and methyl red were purchased from Merck (Germany). Wistar rats were provided from the Pasteur Institute of Iran. Size 9 capsule was obtained from Capsugel (Belgium).

2.1. Experimental design

In the study, CCD was used to investigate the effect of independent variables on the responses for optimization of the formulations. Independent variables were the ratio of Eudragit RS:RL (X_1) and the concentration of Eudragit FS 30D (X_2). Dependent variables were the percentage of drug released at pH 1.2 in 2 h (Y_1), at pH 6.8 in 2 h (Y_2), at pH 7.4 in 3 h (Y_3) and at pH 6.8 in 3 h (Y_4). Data were fitted by Design-Expert® software (version 7.0.0, stat-Ease, Inc., Minneapolis, MN). 3D response surfaces were also generated in order to determine the relationship between the responses and each factor. Based on the obtained results, the optimum formulation was prepared and actual results were compared with the responses predicted by software.

2.2. Preparation of enteric coated capsules

For the preparation of the Eudragit® FS 30 D dispersion, polysorbate 80 (33% aqueous solution, 8.8 g), TEC (9 g) and GMS (7.2 g) were added to one-third of water (377.3 g) which was heated to 70–80 °C and stirred for 10 min. Then remaining water was added to GMS emulsion and cooled down to room temperature. The resulting suspension was slowly poured into the Eudragit FS 30D dispersion (in different concentrations as indicated in Table 2) under constant mixing.

For the preparation of the Eudragit RS and RL dispersion, polysorbate 80 (3 g) was dissolved in water and the solution was heated to 70 °C. Then GMS (3 g) was slowly added to the above solution under stirring for 30 min. The solution was allowed to cool at room temperature after then TEC (13 g) was added. Then Eudragit RS and RL were separately mixed (in different ratios as indicated in Table 2) and the pre-dispersion was gradually added to the Eudragit dispersion using magnetic mixer. The dispersion was mixed for about 10 min.

Capsules were filled with theophylline (15 mg) and coated by dipping in Eudragit FS dispersion for 30 s. Then, capsules were dried at room temperature and immersed in Eudragit RS and RL dispersion for another 30 s. After drying at room temperature, weight gain of capsules was also calculated. In addition, capsules (size 4) were filled with methyl red as an indicator dye and for *in vivo* study, size 9 capsules were filled with Cy 7.5 and then immersed in solution coating as described above.

2.3. In vitro drug release study

Dissolution study was performed using basket method at a 100 rpm rotation speed and volume of the dissolution medium was 500 mL. The temperature of the medium was maintained at 37 ± 0.5 °C. The capsules were first kept at pH 1.2 (HCl 0.1 M, simulated gastric fluids) for 2 h. After 2 h, the dissolution media were replaced with phosphate buffer pH 6.8 (simulated proximal small intestine) and dissolution was carried out for 2 h. Then dissolution medium was replaced with pH 7.4 phosphate buffers (simulated postmedian small intestine) and release of drug was measured (for 3 h). After that, dissolution medium was also replaced with pH 6.8 phosphate buffers (simulated colonic conditions) for another 3 h [17]. 5 mL of samples were removed from the dissolution media at a specified time intervals and replaced with same aliquot of fresh medium. The amount of released drug in the dissolution medium was analyzed using a UV spectrophotometer (Biochrom WPA biowave II, England) at 272 nm. The electrolyte composition and ionic strength of phosphate buffer fluid is shown in Table 1.

2.4. Scanning electron microscopy (SEM)

Scanning electron microscopy (FESEM, S4160, and Hitachi, Japan) was carried out to characterize the surface of coated and uncoated capsules. The sample was coated with a fine layer of gold to facilitate electricity conduction.

2.5. In vivo studies

The protocol of the study was carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments and was approved by Animal Ethics Committee Jundishapur University of Medical Sciences, Ahvaz, Iran (ref no. IR.AJUMS.REC.1395.643).In order to trace the movement of enteric coated capsule, Cy7.5 was used as an indicator fluorescent dye. Male, Wistar rats, weighing 250–300 g were fasted overnight with free access to water. After fasting, at different time intervals, rats (n = 3) were scanned using small animal imaging (Kodak, Fx Pro, USA) after oral administration of capsules with dosing syringe. Animals were anesthetized before imaging using 4% isoflurane.

Table 1
The electrolyte concentrations and ionic strength of phosphate buffer [24,25].

	Phosphate buffer
Na^+ (mM)	39.5
K^+ (mM)	50
Cl^- (mM)	–
Ca^{2+} (mM)	–
Mg^{2+} (mM)	–
HCO_3^- (mM)	–
HPO_4^{2-} (mM)	39.5
SO_4^{2-} (mM)	–
$H_2PO_4^-$ (mM)	10.5
Osmolality (mOsm/kg)	228
Ionic strength	0.129
Buffer capacity (mmol/L/pH unit)	23.0

Table 2
CCD experimental runs and corresponded responses.

Run	RS:RL	FS (%)	Y1: Release in 2 h at pH 1.2 (%)	Y2: Release in 2 h at pH 6.8 (%)	Y3: Release in 3 h at pH 7.4 (%)	Y4: Release in 3 h at pH 6.8 (%)	Weight gain (%)
1	2.00	80.00	0.23	0.89	8.32	11.02	7
2	4.00	60.00	2.09	0.199	9.27	44.21	6
3	6.83	60.00	0	0	0	1.76	8
4	6.00	80.00	0	0.78	4.98	6.60	9
5	4.00	60.00	2.6	5.01	6.27	29	6
6	4.00	60.00	2.08	4.44	13.99	26.69	5
7	6.00	40.00	0.84	1.62	8.75	15.03	6
8	1.17	60.00	0.91	11.98	12.49	22.31	5
9	4.00	31.72	5.42	10.98	23.55	30.09	5
10	4.00	60.00	1.49	5.02	14.32	25.18	5
11	4.00	60.00	2.28	5.42	17.40	30.42	6
12	4.00	88.28	0	15.98	21.98	25.46	8
13	2.00	40.00	7.32	50.21	70.24	100.89	5

The excitation and emission wavelengths were set at 670 and 790 nm, respectively.

2.6. Statistical analysis

All the experiments were carried out at least in triplicate. The results were expressed as mean ± SD and statistical evaluation of data was performed using one-way analysis of variance (ANOVA). A difference of $p < 0.05$ was considered statistically significant.

3. Results and discussion

RSM is widely used in the development and optimization of drug delivery systems. The method requires minimum runs and time which is more effective and cost-effective than conventional methods [26]. In the present study, CCD was employed to investigate the effect of independent variables on the drug release from capsules at different time intervals. The values of independent variables with the observed response in 13 suggested formulations based on CDD design are shown in Table 2.

Table 3 shows analysis of variance for drug release in 2 h as the response. According to the results, a cubic model is the best fitted model for drug release in 2 h with the following equation (1):

$$Y_1 = +2.11–0.32\ (X_1)\text{-}1.92\ (X_2)+1.56\ (X_1)(X_2)\text{-}0.70\ (X_1^2)+0.43\ (X_2^2)\text{-}0.064\ (X_1^2)(X_2)\text{-}1.35(X^1)(X_2^2) \quad (1)$$

Where Y_1 is the percentage of drug release at pH 1.2 after 2 h, X_1 and X_2 are the ratio of Eudragit RS:RL and concentration of Eudragit FS, respectively. It is clear from Table 3 that the model is significant ($p < 0.05$) with insignificant lack of fit (F-value = 3.28). The coefficient of determination (R^2) and adjusted R^2 were calculated to be 0.97 and 0.95, respectively. The proximity between R^2 and adjusted R^2 confirmed the efficiency of the model to predict the percent of drug release at pH 1.2.

Table 3
Analysis of variances for drug release in 2 h (at pH 1.2) as the response (Y_1).

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	57.21	7	8.17	34.86	0.0006
X_1	0.41	1	0.41	1.77	0.2412
X_2	14.74	1	14.74	62.85	0.0005
X_1X_2	9.75	1	9.75	41.58	0.0013
X_1^2	3.41	1	3.41	14.55	0.0124
X_2^2	1.28	1	1.28	5.47	0.0665
$X_1^2X_2$	8.26	1	8.26	0.03	0.8585
$X_1X_2^2$	3.67	1	3.67	15.65	0.0108
X_1^3	0.00	0			
X_2^3	0.00	0			
Residual	1.17	5	0.23		
Lack of Fit	0.53	1	0.53	3.28	0.1444
Pure Error	0.64	4	0.16		
Cor Total	58.38	12			
R^2	0.97				
Adj R^2	0.95				

Table 4
Analysis of variances for drug release in 2 h (at pH 6.8) as the response (Y_2).

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	2124.63	7	303.52	33.58	0.0007
X_1	71.84	1	71.84	7.95	0.0371
X_2	12.46	1	12.46	1.38	0.2933
X_1X_2	587.48	1	587.48	64.99	0.0005
X_1^2	25.01	1	25.01	2.77	0.1572
X_2^2	221.39	1	221.39	24.49	0.0043
$X_1^2X_2$	409.30	1	409.30	45.28	0.0011
$X_1X_2^2$	125.96	1	125.96	13.93	0.0135
X_1^3	0.00	0			
X_2^3	0.00	0			
Residual	45.20	5	9.04		
Lack of Fit	26.46	1	26.46	5.65	0.0763
Pure Error	18.74	4	4.69		
Cor Total	2169.83	12			
R^2	0.97				
Adj R^2	0.95				

The analysis of variance for drug release in 2 h (as the response) is shown in Table 4. Based on the results, a cubic model is the best fitted model for drug release in 4 h with the following equation (2):

$$Y_2 = +4.02–4.24(X_1)\ +1.76(X_2)\ +12.12(X_1)(X_2)\ +1.90(X_1^2)\ +5.64(X_2^2)\ -14.31(X_1^2)(X_2)\text{ - }7.94(X_1)(X_2^2) \quad (2)$$

Where Y_2 is the percentage of drug release at pH 6.8 after 2 h, X_1 and X_2 are the ratio of Eudragit RS:RL and concentration of Eudragit FS, respectively. As shown in Table 4, lack of fit of the model is not significant ($F = 5.65$, $p > 0.05$), which comfirm the reliability of the model. The coefficient of determination (R^2) and adjusted R^2 of this model were predicted to be 0.97 and 0.95, respectively. The similarity between R^2 and adjusted R^2 demonstrated the efficiency of the model to predict the response by the optimized method. Moreover, these results indicated that this model can explain 97% variability in the response.

The results obtained from the analysis of variance for drug release in 3 h at pH 7.4 (as the response) are presented in Table 5 and fitted with a cubic model according to equation (3):

$$Y_3 = +12.25–4.42\ (X_1)\text{-}0.56\ (X_2)+14.54(X_1)(X_2)\text{-}0.86(X_1^2)+7.40(X_2^2)\text{-}15.87(X_1^2)(X_2)\ -11.79(X_1)(X_2^2) \quad (3)$$

Table 5
Analysis of variances for drug release in 3 h (at pH 7.4) as the response (Y_3).

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	3458.52	7	494.07	10.97	0.0090
X_1	78.06	1	78.06	1.73	0.2451
X^2	1.24	1	1.24	0.028	0.8746
X_1X_2	845.22	1	845.22	18.77	0.0075
X_1^2	5.17	1	5.17	0.11	0.7486
X_2^2	380.79	1	380.79	8.46	0.0335
$X_1^2X_2$	503.52	1	503.52	11.18	0.0205
$X1X_2^2$	277.94	1	277.94	6.17	0.0555
X_1^3	0.00	0			
X_2^3	0.00	0			
Residual	225.17	5	45.03		
Lack of Fit	146.77	1	146.77	7.49	0.0521
Pure Error	78.40	4	19.60		
Cor Total	3683.69	12			
R^2	0.93				
Adj R^2	0.85				

Table 6
Analysis of variances for drug release in 3 h (at pH 6.8) as the response (Y_4).

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	6642.09	7	948.87	7.98	0.0181
X_1	211.15	1	211.15	1.78	0.2401
X_2	10.75	1	10.75	0.090	0.7758
X_1X_2	1657.95	1	1657.95	13.94	0.0135
X_1^2	264.27	1	264.27	2.22	0.1962
X_2^2	20.30	1	20.30	0.17	0.6966
$X_1^2X_2$	1052.22	1	1052.22	8.85	0.0310
$X^1X_2^2$	468.35	1	468.35	3.94	0.1039
X_1^3	0.00	0			
X_2^3	0.00	0			
Residual	594.47	5	118.89		
Lack of Fit	363.35	1	363.35	6.29	0.0662
Pure Error	231.13	4	57.78		
Cor Total	7236.57	12			
R^2	0.91				
Adj R^2	0.80				

Where Y_3 is the percentage of drug release at pH 7.4 after 3 h, X_1 and X_2 are the ratio of Eudragit RS:RL and concentration of Eudragit FS, respectively. According to the results, the *p*-value of model is less than 0.05 which indicate the model is highly statistically significant. The value of R^2 and adjusted R^2 of this model were 0.93 and 0.85, respectively. The closeness between R^2 and adjusted R^2 confirmed the efficiency of the model to predict drug release in 7 h.

The analysis of variance for drug release in 3 h (as the response) is presented in Table 6. According to the results, a cubic model is the best fitted model for drug release in 10 h with the following equation (4):

$$Y_4 = +31.10–7.27(X_1)\text{-}1.64(X_2)+20.36(X_1)(X_2)\text{-}6.16(X_1^2)$$

Table 7
Predicted and observed responses of optimum formulation.

Independent variable	Optimized amount	Dependent variable	Predicted amount	Observed amount	Prediction error (%)
X_1	2.56	Y_1	0.00	0.00	0.00
X_2	50.03	Y_2	0.00	0.00	0.00
		Y_3	28.51	25.21	−11.56
		Y_4	50.32	50.78	0.92

$$+1.71(X_2^2)\text{-}22.94\ (X_1^2)(X_2)\text{-}15.30(X_1)(X_2^2) \quad (4)$$

Where Y_4 is the percentage of drug release at pH 6.8 after 3 h, X_1 and X_2 are the ratio of Eudragit RS:RL and concentration of Eudragit FS, respectively. Regarding Table 6, the lack of fit the obtained model is not significant (*F*-value = 6.29; $p > 0.05$). The value of R^2 and adjusted R^2 calculated to be 0.91 and 0.80, respectively, confirming the good correlation between the response and the selected variables.

The 3D response surface plot of the percentage of drug release in 2 h (at pH 1.2), 2 h (at pH 6.8), 3 h (at pH 7.4) and 3 h (at pH 6.8) are shown in Fig. 1A–D. As can be seen from Fig. 1A and B, the percentage of drug released at pH 1.2 (2 h) and pH 6.8 (2 h) were reduced by increasing the concentration of Eudragit FS 30D and the ratio of Eudragit RS:RL. While, it was found that the percentage of drug released in 3 h at pH 7.4 and in 3 h at pH 6.8 increased by decreasing the concentration of Eudragit FS 30D and the ratio of Eudragit RS:RL (Fig. 1C and D).

3.1. Optimization and validation of model

In order to confirm the validity of the optimization of formulation, capsules were coated with the predicted levels of pH and time-

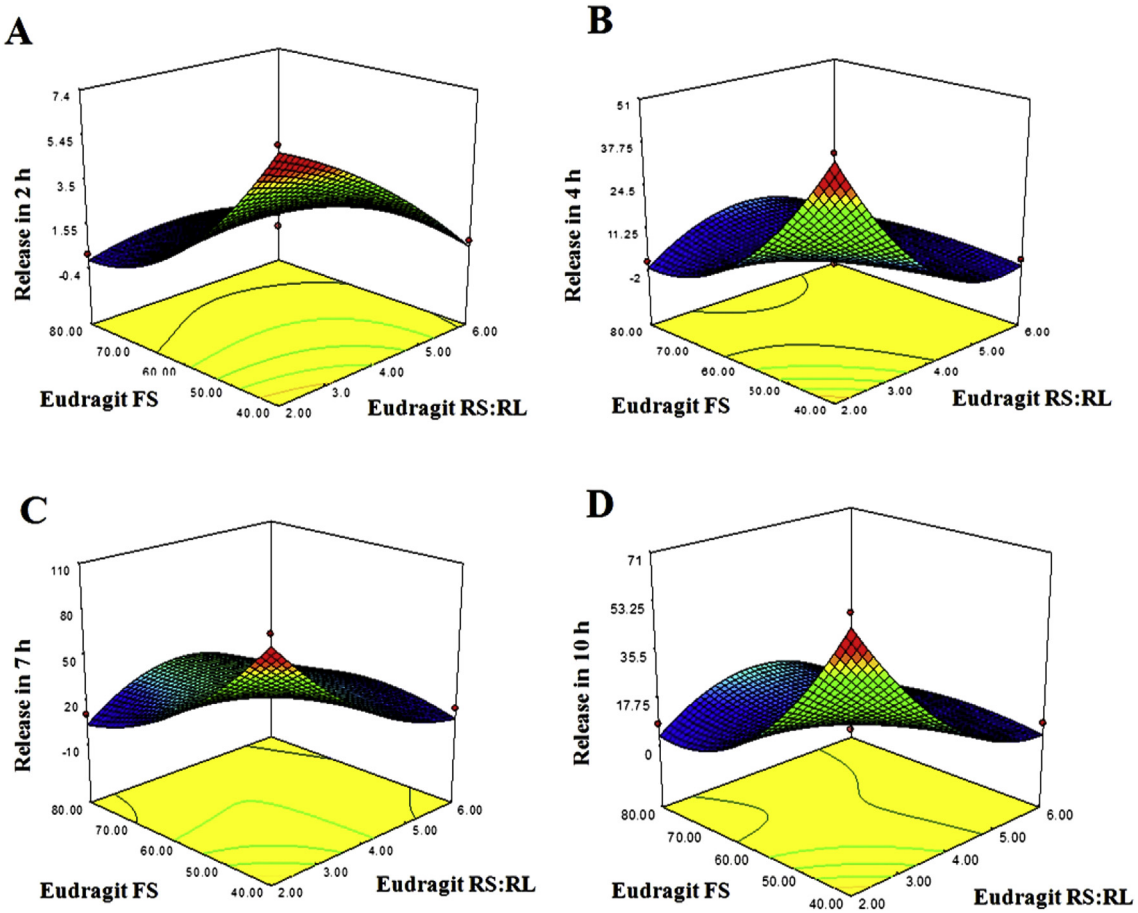

Fig. 1. Response surface plot of the impact of Eudragit FS and Eudragit RS:RL on the drug release in (A): 2 h, (B): 4 h, (C): 7 h and (D): 10 h.

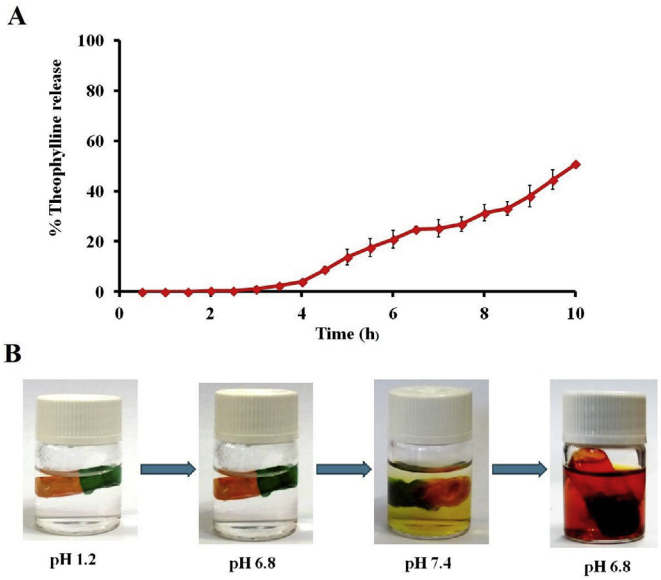

Fig. 2. Release profiles of A) theophylline and B) methyl red under continuous dissolution based on GI transit time (0–2 h at pH 1.2, 2–4 h at pH 6.8, 4–7 h at pH 7.4 and 7–10 h at pH 6.8). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

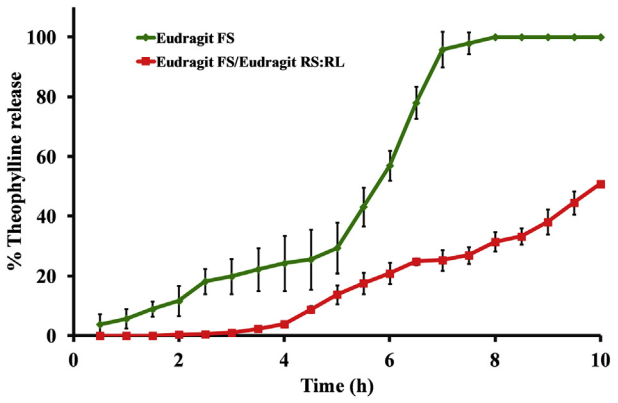

Fig. 3. *In vitro* drug release profiles from enteric coated capsules with Eudragit FS and enteric coated capsules with Eudragit FS and Eudragit RS:RL (0–2 h at pH 1.2, 2–4 h at pH 6.8, 4–7 h at pH 7.4 and 7–10 h at pH 6.8).

depended polymers. As shown in Table 7, observed responses were in agreement with predicted values, confirming that the RSM method was reliable for optimizing extended release. The release profile of optimum formulation in media with different pH is also presented in Fig. 2A and B. No drug or dye were released from coated capsules at pH 1.2 (simulated the acidic environment in the stomach) and at pH 6.8 (simulated proximal small intestinal fluids). The capsules stared to release the drug or dye (about 25%) at pH 7.4 (simulated middle and distal small intestinal fluids) and 50% of the drug was released at pH 6.8 (simulated colonic conditions). Eudragit FS 30D is a pH-sensitive polymer that at pH above 6.5, their carboxylic groups are transformed to carboxylate groups which leads to the dissolution of the polymer [27]. However, due to similarity of pH between small intestine and colon, using only pH-dependent polymers is not very reliable [7]. Previous studies have reported that the pH in different parts of small intestine is 6.6, 7.4, and 7.5 for proximal, middle, and distal small intestine, respectively [28] and then falls from 7.5 in the terminal ileum to 6.5 in the ascending colon due to accumulation of short-chain fatty acids resulting from bacterial fermentation activities [17,29,30]. A colonic delivery system should release a little drug in the small intestine and release a great amount of the drug in the colon [7]. As shown in Fig. 3, when Eudragit FS is used as a coating, it led to release a greater amount of the drug in ileum. However, by employing the pH and time-dependent polymer, the rate of drug release was decreased in about 25%. This can be explained by the fact that Eudragit FS is an anionic polymer containing carboxyl groups that completely ionize in neutral to alkaline medium. However, Eudragit RL and RS are water swellable polymers which are dissolved by penetrating dissolution media and resulting the release of drug out of the capsules [27,31]. According to the obtained results, this delivery system was capable of retarding drug release in the small intestine until the capsules reached the colon. The present results are in agreement with Sharma et al. findings which showed that the coating combination of hydroxypropyl cellulose (as time-dependent) and Eudragit S100 (as pH-dependent) polymers had prevented the drug release in the upper part of GI tract [32]. Patel et al. used the combination of hydroxypropyl methylcellulose and Eudragit L100 as time and pH-dependent polymer, respectively for colon delivery of mesalamine. The authors observed that this system was effectively retarded the drug release in small intestine and delivered the drug to the colon [33]. Akhgari et al. also reported that Eudragit S100 and Eudragit L100 as pH-dependent polymers and Eudragit RS as a time-dependent polymer exhibited little release before entering the colon [10].

Weight gain of capsules of optimal formulation was also 6%.

3.2. Morphological properties

The surface morphology of uncoated capsules, coated capsules with Eudragit FS and coated with Eudragit FS and Eudragit RS:RL are displayed in Fig. 4A–C. The surfaces of gelatin capsules are smooth (Fig. 4A). As shown in Fig B and C, the surfaces of coated capsule by Eudragit FS and combination of Eudragit FS and Eudragit RS:RL were also smooth and no pores or cracks were observed, confirming the well controlled coating process. The results of SEM indicated a uniform coating around the capsule. These findings indicated that dipping method can provide a simple and rapid method for coating capsules at laboratory scale as well as with high efficacy.

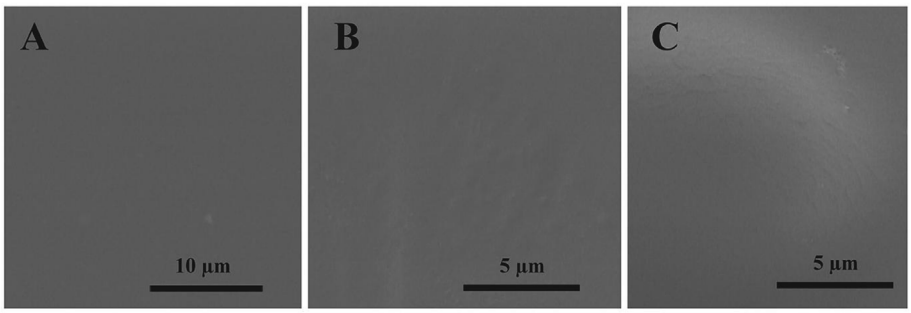

Fig. 4. SEM image of the surface of A) gelatin capsules, B) coated capsule with Eudragit FS and C) coated capsule with Eudragit FS and Eudragit RS:RL.

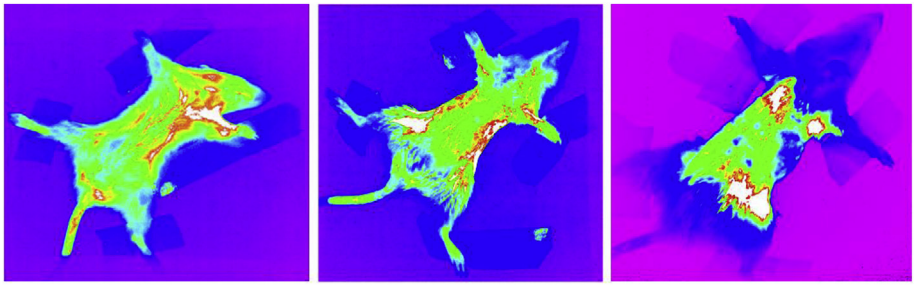

Fig. 5. Fluorescent images of the movement of enteric coated capsule from the stomach to the colon (left to right after 0–4 h: stomach and proximal small intestinal, 7 h: middle and distal small intestinal and 10 h: colon).

3.3. *In vivo* studies

In the study, Cy 7.5 was used as fluorescent dye to trace the movement of the enteric coated capsule with Eudragit FS and Eudragit RS:RL in GI tract. No release was observed at 2 and 4 h after oral administration, which correlated well to the *in vitro* release studies. As shown in Fig. 5, it is clear that capsules were broken down and released the fluorescent dye in the colon. These findings were further indicated that the optimized formulation could provide a targeted drug delivery to the colon. The objective of applying combining pH-dependent polymers and time-dependent polymers was to avoid the complete drug release in the ileum and provide sustained release at pH values typical of the colon [12].

4. Conclusion

According to the obtained results, the coated capsules with Eudragit FS at concentration of 50.03% and Eudragit RS:RL in the ratio of 2.56 exhibited suitable release *in vitro*. The capsules started to release the drug (about 25.21%) at pH 7.4 (simulated middle and distal small intestinal fluids) and 50.78% of the drug was released at pH 6.8 (simulated colonic conditions). The surfaces of coated capsule were smooth and no pores were observed which confirm the well controlled coating process. In addition, *in vivo* results showed that optimized formulation could deliver the maximum amount of fluorescent dye to the colon. The obtained findings indicate that the RSM can be employed successfully to design of colon drug delivery systems with reducing the number of experimental trials and cost of formulation development.

Conflicts of interest

The authors report no conflict of interests and are responsible for the content and writing of this article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jddst.2018.07.027.

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