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In vivo evaluation of pH and time-dependent polymers as coating agent for colonic delivery using central composite design

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ABSTRACT

The objective of this study was to evaluate the combination of pH-dependent and time-dependent polymers on drug release in order to optimize coating for colonic delivery. Response surface methodology (RSM) based on central composite design (CCD) was employed for formulation optimization. Theophylline was used as model drug and Eudragit® FS 30D and hydroxypropyl methylcellulose (HPMC) were used as pH-dependent and time-dependent polymer, respectively. Dissolution test was carried out using the release conditions as follow: pH 1.2 for 2 h, pH 6.8 for 2 h, pH 7.4 for 3 h and pH 6.8 for 3 h. Scanning electron microscopy (SEM) was applied to observe the morphology of coated capsules. Drug release was evaluated spectrophotometrically. *In vivo* X-ray imaging study was used to trace the movement and behavior of the capsules in gastrointestinal (GI) tract. The optimized formulation containing 0.5% HPMC and 80% Eudragit FS 30D was prepared according to the software determined levels. There was no drug release for 2 h at pH 1.2 and for 2 h at pH 6.8. Optimum values of drug release were 32.57% and 71.37% at pH 7.4 (7 h) and pH 6.8 (10 h), respectively, which were in agreement with the predicted value by RSM. Surface coated capsules were rougher than gelatin capsules as examined by SEM. X-ray analysis confirmed that coated capsules dissolved at the targeted colon region. The results of this study indicate that the designed system can be potentially used as a carrier for colon delivery of drugs.

1. Introduction

Recently, there has been interest in designing colon-specific drug delivery systems for treatment of colon cancer, irritable bowel syndrome, inflammatory bowel disease (IBD) and infectious diseases. Oral administration of drugs in the form of a colon-specific delivery system would increase drug bioavailability at target site, reduce drug dose and systemic adverse effects [1,2]. However, conventional oral dosage forms are ineffective to deliver drugs to the colon due to their absorption or degradation in the upper gastrointestinal tract [3]. Site-specific targeting of drugs for colon has been employed by several different approaches including; pH-sensitive polymer coatings, time-dependent formulations, microflora-triggered delivery systems, pressure-dependent systems, and prodrugs [4–6]. Eudragit® FS 30D is an anionic copolymer of methyl acrylate, methyl methacrylate, and methacrylic acid [7]. This polymer has been used as pH-sensitive polymer for colon delivery [8]. The gamma scintigraphic studies showed that this polymer is preferable to Eudragit® L and Eudragit®

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S for colon delivery due to more retarding drug release in the small intestine [9]. Nevertheless, because of similarity of pH between small intestine and the colon, pH-dependent systems have unpredictable site-specificity for drug release [10]. This problem could be resolved by combining pH-dependent with time-dependent system in order to ensure drug release under different physiological conditions. The hydroxypropyl methylcellulose (HPMC) is a pH independent polymer that contributes to the delivery of drug in the colon. Due to its swellability in contact with water or biological fluid, would be gradually dissolved upon consumption and release the drug [2,11]. In pharmaceutics, designing extended-release formulations with the minimum number of trials is very essential. Response surface methodology (RSM) is a statistical method for development and optimization of drug delivery systems. The method can determine modeling and analysis interactions between the response and the independent variables [12,13]. Furthermore, it is less time-consuming than other approaches due to decrease of the number of experimental trials [14]. Central composite design (CCD) is a very common experimental design used in RSM that helps to optimize the effective factors with reducing the number of experiments and analyze the interaction between the parameters [12].

Theophylline was chosen as a typical drug in our investigation. It is a Biopharmaceutics Classification System (BCS) Class I drug (high solubility, high permeability). In addition, it has been shown to have a good absorption from the entire gastrointestinal tract [2,15]. The objective of this study was to achieve an optimized release profile for pH and time dependent extended-release of drug from capsule using RSM. Also, X-ray imaging was further used to confirm delivery of drug to the rat colon following oral administration.

2. Materials and methods

HPMC, glyceryl (viscosity of 2% solution in water, 80-120 cP) was obtained from Sigma (Germany). Glyceryl monostearate (GMS) and triethyl citrate (TEC) were of standard pharmaceutical grade and purchased from Sigma (Germany). Eudragit® FS 30D and theophylline were kindly donated by RÖhm GmbH (Darmstadt, Germany) and Dr. ABIDI pharmaceutical Co., Tehran, Iran, respectively. Barium sulphate ($BaSO_4$) was provided by Darou Paksh Pharmaceutical Mfg. Co., Tehran, Iran. Size 9 capsule was optioned from Capsugel (Belgium). Methylene blue and polysorbate 80 were purchased from Merck (Germany).

2.1. Preparation of enteric coated capsules

HPMC was dissolved in glacial acetic acid in different concentrations as indicated in the Table 1. For the preparation of the Eudragit® FS 30 D dispersion, according to RÖhm protocol, 30% of water (377.3 g) was heated to 70–80° C. Polysorbate 80 (33% aqueous solution, 8.8 g) as an emulsifier, TEC (9 g) and GMS (7.2 g) as glidant were added subsequently and stirred for 10 min. The remaining 70% of water was added to GMS emulsion and cooled down to room temperature. Then the suspension was slowly poured into the Eudragit FS 30D dispersion (in different concentrations) under constant mixing.

Gelatin capsules (size 4) were manually filled with theophylline and were coated by dipping once in HPMC solution followed by drying at room temperature. Then capsules were immersed three times in Eudragit FS 30D dispersion. Also, gelatin capsules were filled with methylene blue as an indicator dye. For *in vivo* dissolution study, size 9 capsules were filled manually with $BaSO_4$ and then immersed in solution coating as described above. The schematic of preparation of enteric coated solution and dipping method are shown in Fig. 1.

Table 1
CCD experimental runs and corresponded responses.

Run no.	Independent variables		Dependent variables			
	X1	*X2*	*Y1*	*Y2*	*Y3*	*Y4*
1	2.00	80.00	1.23772	1.23772	10.3995	43.944
2	1.25	60.00	0.5	1.17223	4.2	26.97
3	0.50	80.00	0	0	30.2489	100
4	1.25	60.00	0	0.517354	2.27898	10.01
5	1.25	31.72	3.054	6.33923	72.3445	100
6	1.25	60.00	0	0	6.93517	27.4263
7	1.25	88.28	0	0	2.80943	7.71447
8	2.00	40.00	1.43418	5.16045	94.4794	100
9	0.50	40.00	3.00589	3.00589	63.2482	100
10	1.25	60.00	1.02	2.3	20.3536	23.4512
11	1.25	60.00	0	1.17223	11.9122	39.6726
12	2.31	60.00	1.05	4.44008	71.1657	100
13	0.19	60.00	0.0589391	0.0589391	43.7328	87.6031

2.2. In vitro release study

The *in vitro* dissolution rates of the coated capsules were carried out with a basket method at a 100 rpm rotation speed and 500 ml dissolution medium. For simulating conditions of the GI tract, dissolution tests were employed in media with pH 1.2 (HCl, 0.1 M, simulated gastric fluids) for 2 h. Then capsules were transferred to pH 6.8 phosphate buffer for 2 h (simulated proximal small intestine), for 3 h in pH 7.4 phosphate buffer (simulated postmedian small intestine) and for 3 h in pH 6.8 phosphate buffer (simulated colonic conditions) [2]. The temperature of the medium was set at 37 ± 0.5 °C. For determination of released drug, 5 ml of the mediums were removed and equal volumes of fresh medium were replaced. Then the concentration of released drug was analyzed using a UV spectrophotometer (Biochrom WPA biowave II, England) at 272 nm.

2.3. In vivo X-ray imaging studies

The protocol of the study was performed in accordance with the Declaration of Helsinki as amended in Seoul 2008 for humans, and the European Community guidelines as accepted principles for the use of experimental animals and was approved by Animal Ethics Committee Jundishapur University of Medical Sciences, Ahvaz, Iran (ref no. IR.AJUMS.REC.1395.643). Male, Wistar rats, weighing 250–300 g were fasted for 15 h with free access to water. BaSO4 capsules coated with HPMC and Eudragit FS 30D were administered to rats and X-ray evaluations were carried out at predetermined time intervals (Toshiba, ROTANODE™, Japan). Optimal imaging conditions were achieved with X-ray beams of 50 ms and 55 kVpp.

2.4. Scanning electron microscopy (SEM)

The surface characteristics of the coated capsules were evaluated by scanning electron microscopy (SEM) (LEO, 1455VP, Germany). For comparison proposes, the surface of a gelatin capsule was also examined.

2.5. Experimental design

CCD was employed to evaluate the effects of independent variables on the responses and for optimization of the formulations. In this study, independent variables were concentrations of HPMC (X_1) and 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 4 h (Y_2), at

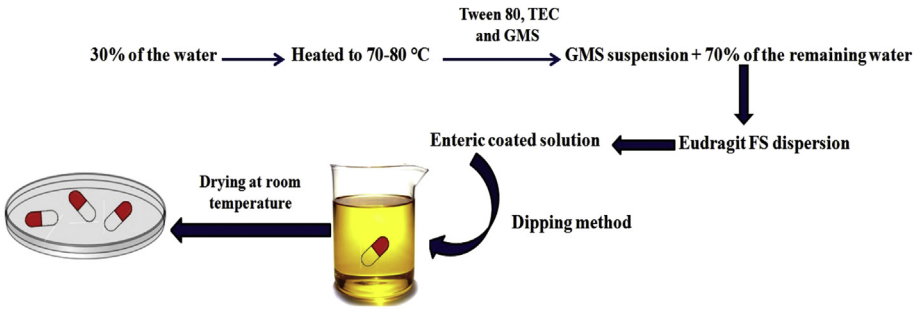

Fig. 1. The schematic of preparation of enteric coated solution and dipping method.

pH 7.4 in 7 h (Y_3) and at pH 6.8 in 10 h (Y_4). Data were fitted by Design-Expert® software (version 7.0.0, stat-Ease, Inc., Minneapolis, MN) and 3D response surfaces were also provided. According to the software, 13 runs were required to develop the appropriate models. Significance differences of the variables of the responses were measured by ANOVA test (p-value < 0.05). Finally, the optimized predicted formulation was prepared and the determined results were compared to the predicted responses.

3. Results and discussion

3.1. Design of the experiment

According to the CCD, the values of independent variables with the observed response are reported in Table 1.

The analysis of variance for drug release in 2 h as the response is shown in Table 2. Based on the results, a quadratic second-order polynomial equation (1) was fitted as below:

$$Y_1 = +13.38036 - 3.60889\,(X_1) - 0.31739\,(X_2) + 0.046824\,(X_1)(X_2) + 0.39096\,(X_1^2) + 1.76545E\text{-}003\,(X_2^2) \quad (1)$$

Where Y_1 is the percentage of drug release at pH 1.2 after 2 h, X_1 and X_2 are the concentration of HPMC and Eudragit FS, respectively. As shown in the Table 2, the model is highly statistically significant ($p < 0.05$) with insignificant lack of fit (F-value = 1.32). The coefficient of determination (R^2) and adjusted R^2 were calculated to be 0.8857 and 0.8041, respectively. The similarity between R^2 and adjusted R^2 demonstrated the efficiency of the model to predict the percent of drug release at pH 1.2 by the optimized method.

The results obtained from the analysis of variance for drug release in 4 h as the response are presented in Table 3 and fitted with a second-order polynomial according to equation (2).

Table 3
The analysis of variances for drug release in 4 h as the response (Y_2).

Source	Sum of Squares	df	Mean Square	F Value	p-value Prop > F
Model	51.03	5	10.21	15.09	0.0012
X_1	11.49	1	11.49	16.99	0.0045
X_2	31.58	1	31.58	46.68	0.0002
X_1X_2	0.21	1	0.21	0.31	0.5946
X_1^2	1.87	1	1.87	2.77	0.1400
X_2^2	6.67	1	6.67	9.86	0.0164
Residual	4.74	7	0.68		
Lack of Fit	1.76	3	0.59	0.79	0.5604
Pure Error	2.98	4	0.47		
Coe Total	55.76	12			
R^2	0.9151				
Adjusted R^2	0.8544				

$$Y_2 = +14.10130 + 0.20847\,(X_1) - 0.37393\,(X_2) - 0.015281\,(X_1)(X_2) + 0.92255\,(X_1^2) + 2.44747E\text{-}003\,(X_2^2) \quad (2)$$

Where Y_2 is the percentage of drug release at pH 6.8 after 4 h, X_1 and X_2 are the concentration of HPMC and Eudragit FS, respectively. The coefficient of determination (R^2) and adjusted R^2 of this model were predicted to be 0.9151 and 0.8544, respectively. The determination coefficient (R^2) demonstrated that 91.51% of the variability in the response could be explained by the model.

According to the results of Table 4, a quadratic second-order polynomial equation was found for drug release in 7 h at pH 7.4 (equation (3)).

$$Y_3 = +216.82586 - 50.23134\,(X_1) - 4.70438\,(X_2) - 0.85134\,(X_1)(X_2) + 43.86994\,(X_1^2) + 0.036852\,(X_2^2) \quad (3)$$

Where Y_3 is the percentage of drug release at pH 7.4 after 7 h, X_1 and

Table 2
The analysis of variances for drug release in 2 h as the response (Y_1).

Source	Sum of Squares	df	Mean Square	F Value	p-value Prop > F
Model	12.77	5	2.55	10.85	0.0034
X_1	0.14	1	0.14	0.61	0.4620
X_2	7.07	1	7.07	30.04	0.0009
X_1X_2	1.97	1	1.97	8.38	0.0231
X_1^2	0.34	1	0.34	1.43	0.2708
X_2^2	3.47	1	3.47	14.74	0.0064
Residual	1.65	7	0.24		
Lack of Fit	0.82	3	0.27	1.32	0.3849
Pure Error	0.83	4	0.21		
Coe Total	14.42	12			
R^2	0.8857				
Adjusted R^2	0.8041				

Table 4
The analysis of variances for drug release in 7 h as the response (Y_3).

Source	Sum of Squares	df	Mean Square	F Value	p-value Prop > F
Model	11943.22	5	2388.64	46.93	<0.0001
X_1	314.73	1	314.73	6.18	0.0418
X_2	5800.54	1	5800.54	113.97	<0.0001
X_1X_2	652.31	1	652.31	12.82	0.0090
X_1^2	4236.15	1	4236.15	83.23	<0.0001
X_2^2	15.11.57	1	1511.57	29.70	0.0010
Residual	356.28	7	50.90		
Lack of Fit	146.51	3	48.84	0.93	
Pure Error	209.77	4	52.44		
Coe Total	12299.50	12			
R^2	0.9710				
Adjusted R^2	0.9503				

X_2 are the concentration of HPMC and Eudragit FS, respectively. The value of R^2 and adjusted R^2 of this model were 0.9710 and 0.9503, respectively. The similarity between their values indicated that there was a good agreement between the experimental and the predicted values obtained from the model. Moreover, the *p*-value of lack of fit was greater than 0.05, which further fortify the reliability of the models. Non-significant lack of fit is good for the model to fit [16].

Table 5 shows the analysis of variance for drug release in 10 h. From the results in the Table 5, a quadratic second-order polynomial model is the best fitted model for drug release with the following equation (4):

Table 5
The analysis of variances for drug release in 10 h as the response (Y_4).

Source	Sum of Squares	df	Mean Square	*F* Value	*p*-value Prop > F
Model	16123.85	5	3204.77	10.60	0.0036
X_1	185.51	1	185.51	0.61	0.4591
X_2	435093	1	4350.93	14.39	0.0068
X_1X_2	785.57	1	785.57	2.60	0.1510
X_1^2	9619.95	1	9619.95	31.82	0.0008
X_2^2	2061.55	1	2061.55	6.82	0.0348
Residual	2116.28	7	302.33		
Lack of Fit	1665.41	3	555.14	4.93	0.0788
Pure Error	450.87	4	112.72		
Coe Total	18140.13	12			
R^2	0.8833				
Adjusted R^2	0.8000				

$$Y_4 = +291.65451 - 115.63992\,(X_1) - 5.16264\,(X_2) - 0.93427\,(X_1)(X_2) + 66.11009\,(X_1^2) + 0.043037\,(X_2^2) \quad (4)$$

Where Y_4 is the percentage of drug release at pH 6.8 after 10 h, X_1 and X_2 are concentration of HPMC and Eudragit FS, respectively. Regarding to the results of Table 5, the value of R^2 and adjusted R^2 calculated to be 0.8833 and 0.8, respectively, indicating that this model can explain 88.33% variability in the response.

The 3D response surface plot of the percent of drug release in 2, 4, 7 and 10 h is presented in Fig. 2A–D. As shown, the percent of drug release in pH 1.2 (2 h) and in pH 6.8 (4 h) were decreased by enhancement of the concentration of Eudragit FS 30D and HPMC (Fig. 2A and B). Fig. 2C shows that about 30% drugs are released after 7 h when both the HPMC and Eudragit FS are at lowest and highest level, respectively. Moreover, increasing the Eudragit FS concentration and decreasing the concentration of HPMC lead to enhancement in the percent of released drug at 10 h (Fig. 2D).

3.2. Optimization of drug release and validation of optimized formulation

The dissolution profiles of the optimum formulation and the predicted profile are shown in Table 6 and Fig. 3A and B. It can be seen from the data in the Table 6 that the observed responses were in close with predicted values, which indicated that the optimized preparation conditions were reliable. As shown in Fig. 3A and B, there are no drug and dye released from coated capsules with Eudragit FS 30D and HPMC in pH 1.2 for 2 h (mimicking the acidic

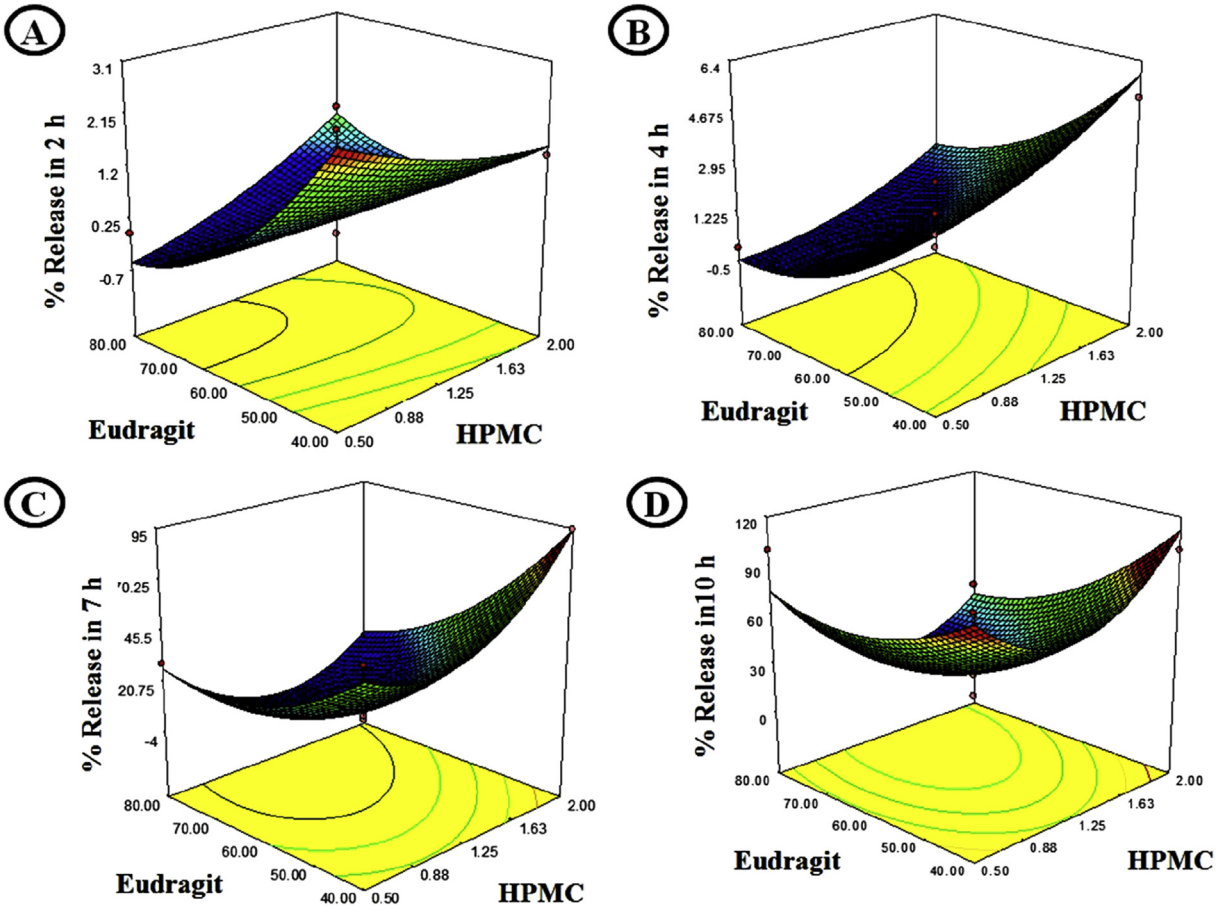

Fig. 2. Response surface plot of the impact of HPMC and Eudragit FS on the drug release in (A): 2 h, (B): 4 h, (C): 7 h and (D): 10 h.

Table 6
Predicted and observed responses of optimum formulation.

Optimized formulation (X_1:X_2)	Response variable	Predicted value	Experimental value	Prediction error (%)
0.5:80	Y_1	0.00	0.00	0.00
	Y_2	0.00	0.00	0.00
	Y_3	28.12	32.57 ± 4.2	15.83
	Y_4	75.42	71.37 ± 6.54	−5.37

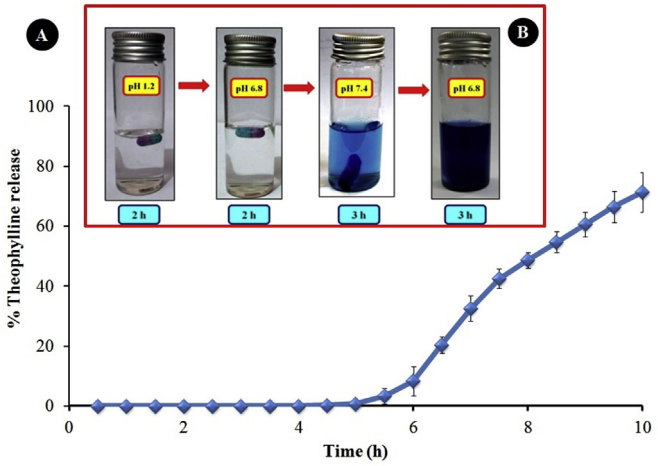

Fig. 3. Dissolution profile of optimized formulation under continuous dissolution based on GI transit time (0–2 h at pH 1.2, 2–4 h at pH 6.8, 4–7 h at pH 7.4 and 7–10 h at pH 6.8).

environment in the stomach) and phosphate buffer (pH 6.8) for 2 h (simulated proximal small intestinal fluids). But, about 32% of the drug was released in phosphate buffer at pH 7.4 (simulated middle and distal small intestinal fluids) at 7 h. It was also observed that a rapid complete release was obtained in phosphate buffer at pH 6.8 at 10 h. Eudragit FS 30D is pH-sensitive and the carboxylic groups are transformed to carboxylate groups at pH above 6.5 and the polymer would be dissolved [7]. But due to similarity of pH between small intestine and colon, the site-specificity of drug release in the colon from pH-dependent systems may not be achieved and predicted properly [10]. It is reported that the mean pH in different parts of small intestine is 6.6, 7.4, and 7.5 for proximal, middle, and distal small intestine, respectively [17] and then drops from 7.5 in the terminal ileum to 6.5 in the ascending colon because of the acidification of the colonic contents via bacterial fermentation [2,18]. Accordingly, for coatings which dissolve at pH 7 the active agent would be released in the ileum rather than the colon. Moreover, drug release in the ileum can result in systemic absorption, leading to unwanted side effects [19]. This problem could be overcome by combining pH-dependent polymers and time-dependent polymers. It means that the system can protect drug until pH 7 while avoiding the complete drug release in the ileum using a time based polymer [20]. HPMC is a semi-synthetic as well as swellable polymer that shows time-dependant release profile. It was previously reported that because of high swellability, presently HPMC contacts with water or biological fluid, dissolves and diffuses quickly into dissolution medium, resulting the diffuse of incorporated drug out of the system [21–23]. The schematic of the delivery system is shown in Fig. 4.

3.3. In vivo release study

In vivo X-ray imaging study was employed in rats in order to trace the movement and behavior of the capsule in GI tract. X-ray Technique is inexpensive, simple and supplies simultaneous visualization of both capsule and the GI tract using contrast agents [24]. The results of X-ray imaging study are shown in Fig. 5. It presents the capsule remained intact in the stomach, confirming *in vivo* efficiency of the gastro-resistant feature of Eudragit FS 30D. It was also found that when the capsule reached and stayed in the small bowel, no significant difference was observed in the integrity of the capsule in stomach and small intestine, consequently indicating intactness of the capsule in small intestine. Reduction in size of capsule indicated that the capsules were broken down and released the drug in colon. The results are in accordance with the fact that the optimized formulation could be targeted specifically to the colon, without any premature drug release in the stomach and small intestine.

3.4. Scanning electron microscopy (SEM)

SEM image of the surface of gelatin capsules, capsules coated with HPMC, capsules coated with Eudragit FS 30D and capsules coated with HPMC and Eudragit FS 30D are shown in Fig. 6. The difference between surfaces is clearly visible. Gelatin capsules surfaces are smooth (Fig. 6A), whereas a rough surface can be observed on the coated capsules (Fig. 6B–D). Moreover, no cracks were observed. According to obtained results from extended release profiles, dipping method may provide a simple, rapid and suitable technique for coating capsule at laboratory scale.

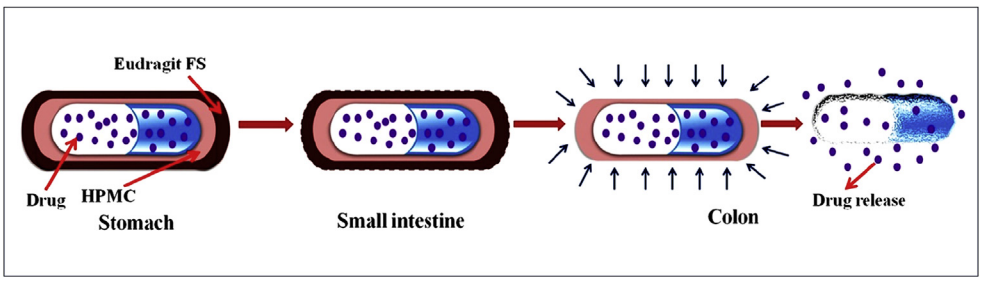

Fig. 4. The scheme of colon-specific targeting of the coated capsule with HPMC and Eudragit FS 30D.

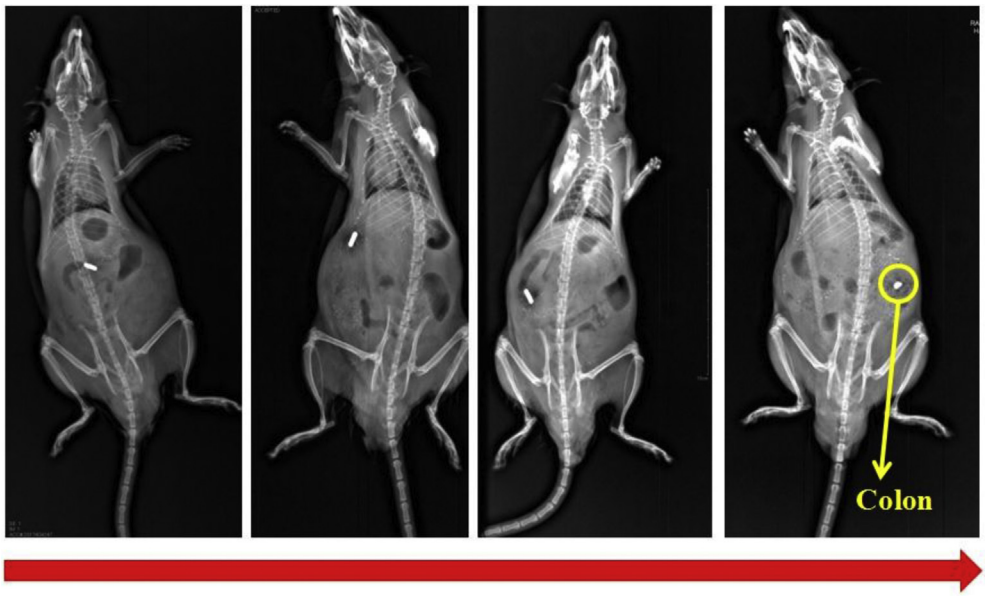

Fig. 5. X-ray images of the GI of a rat, showing the movement of coated capsule from the stomach to the colon (left to right after 2, 4, 7 and 10 h).

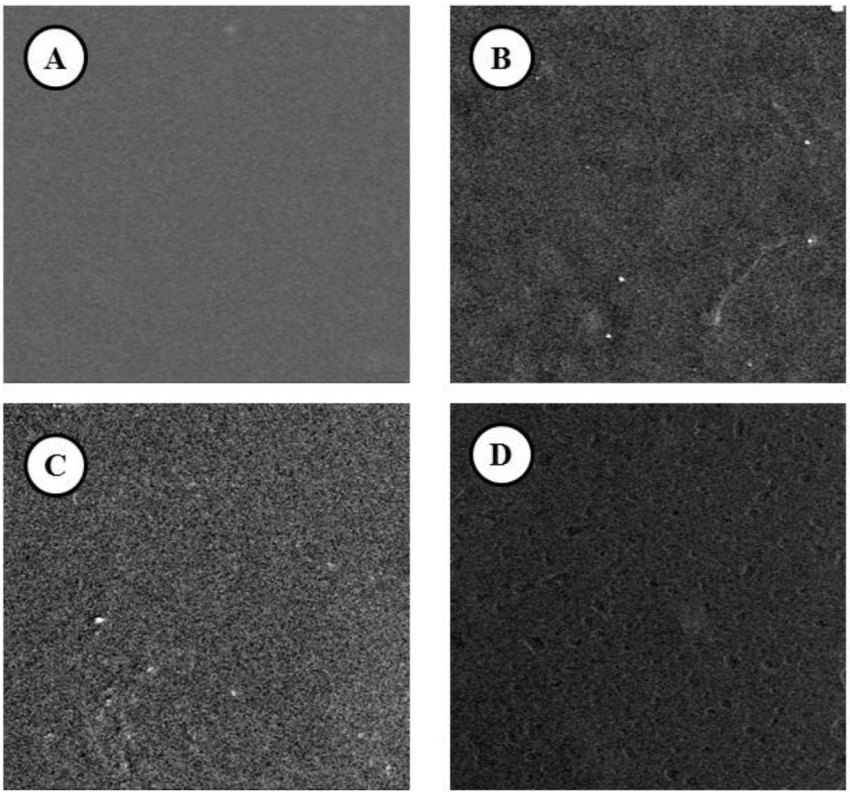

Fig. 6. SEM image of the surface of A) gelatin capsule, B) capsule coated with HPMC, C) capsule coated with Eudragit FS 30D and D) capsule coated with HPMC and Eudragit FS 30D.

4. Conclusion

The results of the present study indicated that RSM and CCD can be successfully used for development of coating formulations based on Eudragit FS 30D and HPMC to acquire appropriate colon delivery system. Release profiles and responses of the optimized formulation showed a closer characteristic to predicted responses. Dissolution studies of capsules in the media with different pH showed that the combination of pH-dependent polymers with time-based polymers could be more advantageous for designing of controlled release formulations.

Conflict of interest

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

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