Journal of Drug Delivery Science and Technology 43 (2018) 50–56

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

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

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ARTICLE INFO

Article history:
Received 2 August 2017
Received in revised form
30 August 2017
Accepted 9 September 2017
Available online 14 September 2017

Keywords:
Response surface methodology
Colon delivery
Hydroxypropyl methylcellulose
Eudragit® FS 30 D
X-ray imaging

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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https://doi.org/10.1016/j.jddst.2017.09.010
1773-2247/

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