Drug Development and Industrial Pharmacy, 35:224–233, 2009

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040802258854

informa
healthcare

Wax-Matrix Tablet for Time-Dependent Colon-Specific Delivery System of Sophora Flavescens Aiton: Preparation and In Vivo Evaluation

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A wax-matrix time-dependent colon-specific tablet (WM-TDCS) was studied. Wax-matrix tablet core consisting of semi-synthetic glycerides, as a wax polymeric expanding agent, carboxymethyl starch sodium (CMS-Na), and NaCl was prepared, and Sophora flavescens Aiton (ASF, extracts of traditional Chinese medicine) was used as model drug. The wax-matrix ASF tablets core was coated with Eudragit NE 30 D as the inner coating materials and with Opadry OY-P-7171 as the outer coating materials. The in vitro release behaviors of the coated tablets were examined and then in vivo absorption kinetics of the coated tablets in dogs was further investigated. The volume of the tablet core was markedly increased at 37°C because of the expand effect of polymer semi-synthetic glycerides and CMS-Na. The drug release from WM-TDCS was more stable than TDCS in vitro and in vivo. The lag time of ASF release was also controlled by the thickness of the inner coating layer. In vivo evaluation demonstrated that in vivo lag time of absorption was in a good agreement with in vitro lag time of release. ASF wax-matrix tablets coated with Eudragit NE 30 D and Opadry OY-P-7171 using the regular coating technique could be designed to achieve a lag time of 3 h in the small intestinal tract.

Keywords wax-matrix; time-dependent colon-specific delivery; preparation; in vivo evaluation; Sophora flavescens Aiton

INTRODUCTION

Oral colon-specific delivery system is naturally of value for the topical treatment of colon diseases such as ulcerative colitis, Crohn's disease, and colorectal cancer (Chourasia & Jain, 2003; Gazzaniga, Iamartino, Maffione, & Sangall, 1994; Watts & Lllum, 1997), in that high local concentration can be achieved while minimizing side effects. Sophora flavescens Aiton (ASF), a traditional Chinese medicine, have been effective ingredients for the treatment of inflammatory bowel diseases and ulcerative colitis (Li, 2003; Liu, Liu, & Cheng, 2003). As for treating localized colonic diseases, the optimal drug delivery system should selectively deliver the drug to the colon, but not to the upper gastrointestinal tract (GIT). The colonic delivery is also useful in the systemic absorption of drugs like nifedipine, theophylline, and isosorbide. Furthermore, it is found to be a promising site for systemic absorption of peptide and protein because of less hostile environment prevailing in the colon in comparison with stomach and small intestine. Additionally, the colon acts as a highly responsible site to enhance the absorption of poorly absorbable drugs.

Currently, there are a few strategies to achieve colonic specificity, such as bacterially triggered colon-specific delivery system (Kinget, Kalala, Vervoort, & van den Mooter, 1998; Sinha & Kumria, 2001), prodrugs (Carceller et al., 2001; Soodabeh, Jalal, & Abbas, 1999; Tozaki et al., 1999), time-dependent colon-specific delivery system (Conte et al., 1989; Pozzi et al., 1994), pH-dependent colon-specific delivery system (Khan, Prebeg, & Kurjakovic, 1999; Khan, Stedul, & Kurjakovic, 2000; Krogars et al., 2000), pressure-controlled colon-specific delivery system (Cao, Kim, & Lee, 2007; Yang, Chu, & Fix, 2002), and the combination of the methods (Gupta, Beckert, & Price, 2001). The drug release from the colon-specific delivery system was easily affected by the water, food, disease, and the property in GIT which could change the sort of bacterial, the GIT transit time, and the GIT pressure and whereas change the GIT transit time of preparations (Muraoka et al., 1998). The goal of this study was to explore the feasibility of the wax-matrix time-dependent colon-specific delivery system to make the drug release stable. We used semi-synthetic glycerides and carboxymethyl starch sodium (CMS-Na) as volume-expanding agent and disintergrants, achieving the drug release for certain time spans. In addition, NaCl was used as osmotic pressure promoting agent of the tablet cores. The glyceride melts in

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vivo, causing the volume of the tablet core to increase. The wax matrix had the property both of time dependence and of pressure-controlled colon-specific delivery system. These properties enable a more stable release of a wax-matrix time-dependent colon-specific delivery system in vivo than that of a time-dependent colon-specific delivery system.

Furthermore, according to these requirements for being colon-specific, we intended to explore a new wax-matrix time-dependent colon-specific delivery system of ASF. Our wax-matrix time-dependent colon-specific coated tablets consist of a tablet core (containing drug, low-melting point wax, CMS-Na, and NaCl) and an inner coating layer composed of water-insoluble Eudragit® NE 30 D and a water-soluble channeling agent. The outer layer consisted of the pH-sensitive Opadry OY-P-7171. Figure 1 shows the schematic diagram of wax-matrix time-dependent colon-specific tablet (WM-TDCS). The composition and preparation of WM-TDCS are shown in "Preparation of core tablets" of this article. During its transit through the GIT, the wax in the core of WM-TDCS melted, which resulted in the core volume increasing in the stomach. The coating layers remained intact because of the enteric coating layer, but this layer dissolved in the small intestine, and water was imbibed into core. The aqueous environment caused CMS-Na to swell and to constantly form a flowable gel. However, the drug was still not released because only water could go through the pore former. With the swelling of tablet and the higher pressure of GIT, the coating layer was ruptured and the drug was released in a pulsatile manner (Cheng et al., 2004). The drug release from WM-TDCS was more stable than TDCS in vitro and in vivo. WM-TDCS was feasible to release drug with high reproducibility in colon independent of pH and peristalsis.

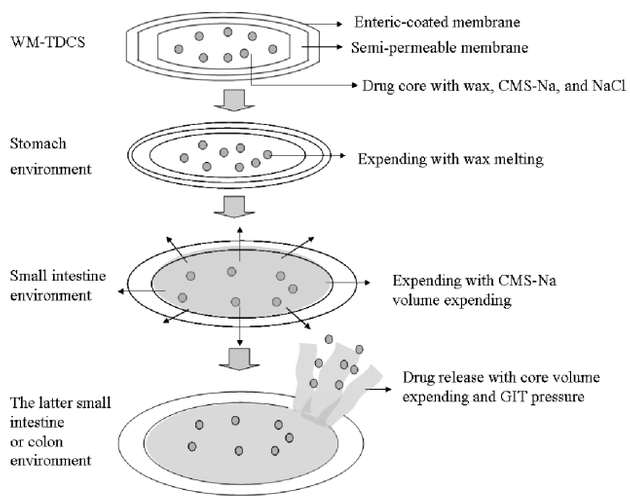

FIGURE 1. The schematic diagram of wax-matrix time-dependent colon-specific tablet (WM-TDCS).

MATERIALS AND METHODS

Materials

The ASF was provided by Ningxia Zijinhua Pharmaceutical Co. Ltd. (ASF, oxymatrine [OM] 68.9%, matrine [M] 11.2%, and other alkaloids, batch No. 060405; Ningxia, China). OM and M was purchased from Ningxia Bo-er-tai-li Pharmaceutical Co. Ltd. (ASF > 98.5%; Ningxia, China). Standard OM, M, and famotidine (internal standard, IS) were supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Theophylline was provided by Shandong Xinhua Pharmaceutical Co. Ltd. (Jinan, China). Semi-synthetic glycerides was purchased from Wuhan he-zhong Medicine and Pharmaceutical Manufacture Co. Ltd. (3436, m.p. 35–37°C, acid number <1.0, iodine number <2.0, saponitication value 220–230, hydroxyl value <60; Wuhan, China). CMS-Na was obtained from Shanghai Yung Zip Pharmaceutical Trading Co. Ltd. (USP-NF CMS-Na; Shanghai, China). Eudragit® NE 30 D was provided by Rohm Co. Ltd. (Shanghai, China). Opadry OY-P-7171 was supplied by Shanghai Colorcon Co. Ltd. (Shanghai, China). Starch, talc, magnesium stearate, and PEG 400 were purchased from Tianjin Kemiou Pharmaceutical Co. Ltd. (Tianjin, China). Sucrose was provided by Tianjin Bodi Chemical Industry Co. Ltd. (Tianjin, China). Lactose and dextrin were supplied by Beijing Yili Chemical Product Co. Ltd. (Beijing, China). Microcrystalline (MCC) cellulose was obtained from Asahi Chemical Industry Co. Ltd. (Avicel PH101; Osaka, Japan). All other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

Apparatus and Chromatographic Conditions

Chromatographic separations were performed by high-performance liquid chromatography (HPLC) system (Kyoto, Japan) that consisted of a LC-10AT pump, a SPD-10A UV detector, and a sample injector. The chromatogram work stations were N2000 in vitro and Ana star in vivo. The analytical column used was Hypersil C_{18} (4.6 mm × 150 mm, 5 μm i.d.) from Dalian Elite Analytical Instruments Co. Ltd. (Dalian, China). The chromatographic analysis was performed at room temperature with a flow rate of 1.0 mL/min. The UV absorbance was monitored at 220 nm. The mobile phase consisted of acetonitrile-5.0 mmol/L acetate sodium (15:85, vol/vol), and the column temperature was maintained at 40°C. About 20 μL of the resulting solution was injected into HPLC system.

Compatibility Study Between ASF and Excipients

To evaluate the compatibility of OM and M and excipients during analysis time, excipients solution or suspension spiked with 10 μmol/L OM and M was analyzed at different times after preparation and at 37°C.

Preparation of Core Tablets

Wet granulation method was utilized to prepare the basic core tablets consisting of 20% (wt/wt) ASF (Yanzhou Pharm., China), 20% (wt/wt) CMS-Na, 20% (wt/wt) wax, 8% (wt/wt) NaCl, 30% (wt/wt) diluents (sucrose: starch/3:1), and 2% (wt/wt) talc. Drug and excipients were sieved (mesh 80) and blended; the mixtures were then granulated with adhesives and dried for 2 h at 40°C. Core tablets with average weight of 310 mg were prepared using a single punch tablet machine (Type-TDP; 1st Pharmaceutical Machine Manufacture Co., Shanghai, China). The diameter of the core tablets was 8.0 mm. The hardness was controlled at 5–6 kg/mm^2. The core compositions were listed in Table 1.

Sucrose, starch, lactose, MCC, and dextrin were selected as diluents. These diluents had significant effect on the hardness of core tablet. These diluents and the different ratio of the diluents were investigated.

The volume was the key of the drug release of time-dependent colon-specific delivery system. Comparing the volume change of the core tablet with and without wax (WM-TDCS and TDCS), formulations R_{14} (with no wax and CMS-Na), R_{11} (with CMS-Na and with no wax), and R_5 (with wax and CMS-Na) were selected as model tablet. The core tablet of R_{14}, R_{11}, and R_5 was placed in the RH 92.5% at 37°C for 8 days. Because of the water solubility of ASF, it was difficult to determine the volume of the tablets. Water-insoluble drug theophylline was selected as model drug. Tablets containing theophylline-replacing ASF were placed in the RH 92.5% at 37°C for 8 days. The growth rate of core tablet volume was described by the following equation. $V_{\text{growth}} = V_{8\text{ days}}/V_{0\text{ day}} \times 100\%$. And the growth rate made by wax was calculated by $V_{\text{wax}} = (V_{R_5} - V_{R_{11}})/V_{R_{11}} \times 100\%$, where V_{R_5} and $V_{R_{11}}$ were the 8th day volume of formulation R_5 and R_{11}.

There were two possible mechanisms contributing to the role of CMS-Na which gave a gel mass or was a disintegrant. The concentration of CMS-Na from 10 to 30% was evaluated.

Tablet Coating

The core tablets were coated in a conventional rotating pan. For the coating process, the rotation speed was adjusted to 36–46 rpm with the coating pan set at an angle of 45° and the nozzle port size of 0.8 mm. Inlet air temperature was 35°C; tablet bed temperature was 25°C; coating solution was sprayed onto the tablets at a flow rate of 0.8 mL/min. The inner coating was Eudragit NE 30 D, which contained PEG 400 as channeling agent and talc (8%, wt/wt) as antiviscosity agent. After coating, the tablets were dried for 12 h at 25°C to remove residual solvent. The outer coating was Opadry OY-P-7171. The weight gains of enteric layer's materials containing Opadry OY-P-7171 and 90% ethanol were 8%. Operating conditions were as follows: the rotation speed of pan, 50 rpm; spray rate, 1.0 mL/min. Coated tablets were dried for 4 h at 25°C.

The weight gain and the concentration of pore-forming agent of the inner coating membrane had a marked effect on drug release. The optimization of weight gain and the concentration of pore-forming agent PEG 400 was conducted. Only the formulation with suitable weight gain and suitable concentration of pore-forming agent could release drug at colon.

Dissolution Test

The behavior of WM-TDCS tablets was evaluated in vitro by dissolution tests (USP 28 ⟨711⟩ dissolution apparatus 2). Place 750 mL of 0.1 mol/L hydrochloric acid in the vessel and assemble the apparatus. Allow the medium to equilibrate at a temperature of 37 ± 0.5°C. Place one tablet in the apparatus, cover the vessel, and operate the apparatus for 2 h at the rate of 75 rpm. After 2 h of operation in 0.1 mol/L HCl, withdraw an aliquot of the fluid and immediately add to the fluid in the vessel 250 mL of 0.20 mol/L tribasic sodium phosphate that was equilibrated to 37 ± 0.5°C. Adjust, if necessary, with 2 mol/L hydrochloric acid or 2 mol/L sodium hydroxide to a pH of 6.8 ± 0.05. Continue to operate the apparatus for 10 h. Withdraw an aliquot of the fluid at 5, 6, 7, 8, 9, 10, and 12 h, the film rupture time, and 10, 20, 30, 45, 60 min after film rupture (Figure 2 shows the release pattern of the WM-TDCS tablets). The release of ASF-coated tablets was determined by HPLC. M being low concentration, the cumulative release percent of OM was investigated.

As known, the pH and peristalsis of GIT would give the effect on the transit of the drug. The pH of the dissolution, the rotation speed of the paddle, and the size of the core tablets were evaluated.

TABLE 1
The Formulations of Core Tablets

Ingredients	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	R_{12}	R_{13}	R_{14}
Wax (%)	20	20	20	20	20	20	20	20	20	10	—	20	20	—
CMS-Na (%)	15	20	25	15	20	25	15	20	25	20	20	—	20	—
NaCl (%)	5	5	5	8	8	8	10	10	10	8	8	—	—	8
Diluents (%)	38	33	28	35	30	25	33	28	23	40	50	58	38	78

Sucrose: starch (3:1) as diluents.

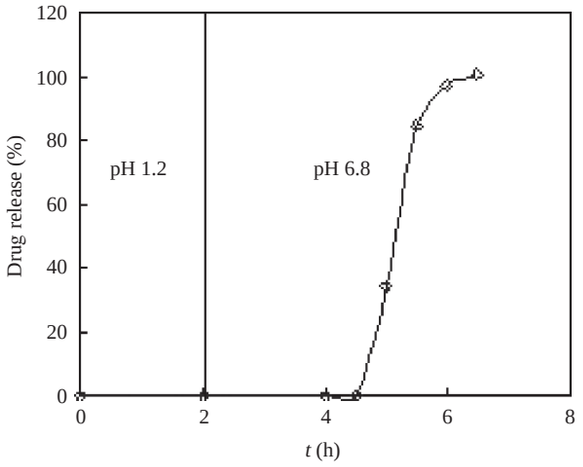

FIGURE 2. Release of Sophora flavescens Aiton (ASF) tablets.

Statistic Analysis

All values were expressed as their $M \pm SE$. One-way analysis of variance test (ANOVA) was performed to check whether there was significant difference among the different formulations and a value of $p < .05$ was statistically significant. Release profiles of formulations were compared using "the fit factors," which included the calculation of similarity factor f_2. The two release profiles were considered to be similar, if f_2 value was 50 (between 50 and 100). For the calculation of f_2 values, only one data more than 85% of the drug release was taken into consideration (Jeffery & Hernry, 1996; Xia & Liu, 2000).

Experimental Protocol In Vivo

All animal studies were performed according to the Guidelines for the Care and Use of Laboratory Animals that was approved by the Committee of Ethics of Animal Experimentation of Shenyang Pharmaceutical University.

Six male beagle dogs were provided by the Animal Center of Shenyang Pharmaceutical University (Shenyang, China). Animals were housed in a room with controlled temperature and humidity and allowed to freely access food and water. Six beagle dogs (weighed 12 ± 2.0 kg) were randomly assigned into three groups, and a crossover 3×3 experiment with a 7-day washout period was conducted on them. Dogs were fasted overnight for 12 h before the administration and then free access to water was allowed. Each dog was orally administered ASF immediate release tablets (reference formulation, formulation 5), ASF TDCS tablet (core tablets, formulation 11 with weight gain 7% of Eudragit NE 30 D containing 1% PEG and 8% of Opadry OY-P-7171), or ASF WM-TDCS tablet (test formulation core tablet, formulation 5 with weight gain 7% of Eudragit NE 30 D containing 1% PEG and 8% of Opadry OY-P-7171), as follows, respectively. Blood samples were collected at predetermined times for each protocol, (a) 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, and 24 h for the reference; (b) 1, 3, 4, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 12; and (c) 24 h for the test. Plasma was immediately obtained by centrifuging the blood samples at 3,000 rpm for 10 min. The plasma samples were stored in a freezer at –20°C until analysis.

Sample Preparation

To aliquots of 0.5 mL plasma sample, 20 μL of IS solution (50 μg/mL, famotidine in distilled water mixed for 10 s) and 1.5 mL of 6% $HClO_4$ were added and mixed for 1 min, followed by centrifugation at $1{,}000 \times g$ for 10 min. The upper layer was transferred to another 10-mL tube, 400 μL of 20% NaOH was added. A volume of 3 mL of chloroform: isopropanol (98:2) was then added to the solution and shaken on horizontal shaker for 5 min, followed by centrifugation at $1{,}000 \times g$ for 10 min. The organic layer was transferred to another tube, and the solvent was evaporated using a centrifugal vacuum concentrator at 35°C. The residue was then dissolved in 50-μL mobile phase and 20 μL was injected into the HPLC.

Stability Study of Plasma Sample

To evaluate the stability of sample during analysis time and also upon storage for a limited time, normal dog plasma samples spiked with 10 μmol/L of OM and M were analyzed at different times after preparation and at three different temperatures.

Data Analysis In Vivo

c_{max} represents the maximum drug concentration; t_{max} was the time taken to reach peak concentration, and t_{lag} was the lag time of the drug. These values were obtained as directly measured values. The elimination rate constant (K_e) was calculated from the slope of the logarithm plasma concentration to time at the four end points. The areas under the plasma concentration–time curve (AUC_{0-t}) were calculated with the trapezoidal method. The relative bioavailability F (%) was calculated from the equation F (%) = $(AUC_{Test} \cdot Dose_{Ref})/(AUC_{Ref} \cdot Dose_{Test})$.

RESULTS AND DISCUSSIONS

Influence of Diluents

Sucrose, starch, lactose, MCC, and dextrin were selected as diluents. The core tablets consisted of 20% ASF, 20% wax, 20% CMS-Na, 8% sodium chloride, 30% diluents, and 2% talc. Increment of sucrose increased the viscosity of the core tablets and thus resulted in the high hardness of tablets. Whereas other diluents such as starch, lactose, MCC, and dextrin or MCC: starch (1:1) and dextrin: starch (1:1) could not produce enough hardness (Table 2). Although ASF itself had high viscosity, sucrose: starch (3:1) was selected as diluent.

TABLE 2
Effect of Different Diluents on the Hardness of Tablet Core

Formulation	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13
Sucrose (%)					22.5	15			30				
Starch (%)	15	22.5	15	22.5	7.5	15				30			
MCC (%)	15	7.5					15	22.5			30		
Dextrin (%)			15	7.5								30	
Lactose (%)							15	7.5					30
Hardness (kg/mm^2)	—	—	—	—	5.3 ± 0.3	4.6 ± 0.4	2.9 ± 0.5	3.4 ± 0.6	7.6 ± 0.7	—	3.6 ± 0.4	2.4 ± 0.3	—

–, means that the hardness of the tablet was very low.

Compatibility Study Between ASF and Excipients

To evaluate the stability of OM and M during analysis time, excipients solution or suspension spiked with 10 μmol/L OM and M were analyzed at different times after preparation and at 37°C. It was shown that OM and M were stable within 24 h.

Effect of the Amount of Wax on Drug Release

Comparing the volume change of TDCS with and without wax (WM-TDCS and TDCS), formulation with no wax and CMS-Na, formulations R_5 and R_{11}, were selected as model tablet. Water-soluble drug ASF and water-insoluble drug theophylline were selected as model drugs. Because of the water solubility of ASF, it was difficult to determine the volume of the tablets. Tablets containing theophylline-replacing ASF were placed in the RH 92.5% at 37°C for 5 days. It was shown that the volume of tablets R_5 and R_{11} was markedly increased at 37°C, and Figure 3 shows the formulation R_5 with R_{11} before and after placing in the RH 92.5% at 37°C for 5 days. The volume of the core tablets was calculated with $\pi r^2 h$. The growth rate of core volume was determined within 8 days. At the 5th day, the volume of all the formulation was almost unchangeable. The growth rates of core tablet volume of formulation with no wax and CMS-Na, formulations R_{11} and R_5 were 4.91 ± 0.52, 82.9 ± 6.52, and 107.1 ± 8.98% at the 5th day. It proved that the wax could increase the volume of the core tablet by 29.2% than that without wax at 37°C. This was possibly because wax melted and swelled after the test started. It was evident that the drug release rates of R_3, R_6, R_9, R_{10}, and R_{11} were faster than R_5 shown in Figure 4. As the expending property of wax was the key of the studies, the ratio of wax: CMS-Na (1:1) was selected for the formulation of the further studies. In formulations R_3, R_6, and R_9, the ratio of wax: CMS-Na was 4:5. In formulation R_{10} wax was 1:2, and with no wax in R_{11}. Based on these results, the core formulation R_5 was selected as the optimal coating screen for the further studies.

From the drug release of formula R_5, R_{10}, and R_{11} in Figure 4, it was shown that the preparations with no wax had a remarkable burst release. The drug release from the formula R_{11} without wax was rapid and the cumulative release was more than 75% within 10 min. It was obvious that the formulation with wax could retard the drug release compared with the formulation without wax, and the drug release of tablets with 20% wax was complete within 45 min.

It was clearly evident from Figure 5 and Table 2 that the average drug release lag time of drug release from the coated tablets with or without wax was same, but the drug release from the coated tablets with wax was more stable than that without wax. First, ANOVA was performed to check whether there was significant difference among the same formulations, and the value of $p > .05$ (the smallest p value of TDCS with 0.2719 and WM-TDCS with 0.7036) was not statistically significant. No. 6 tablets of TDCS (core tablet R_{11}) and WM-TDCS (core tablet R_5) were placed in the dissolution vessel. The most different release pattern of two

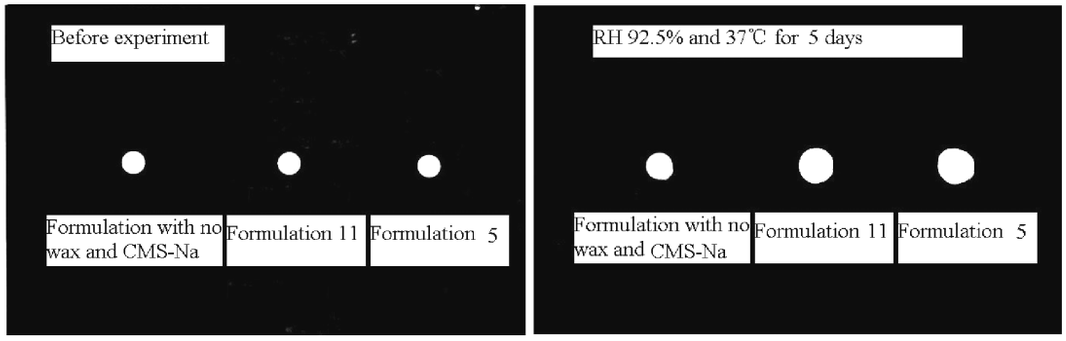

FIGURE 3. Growth rate of core tablet under the relative humidity (RH) 92.5% condition at 37°C.

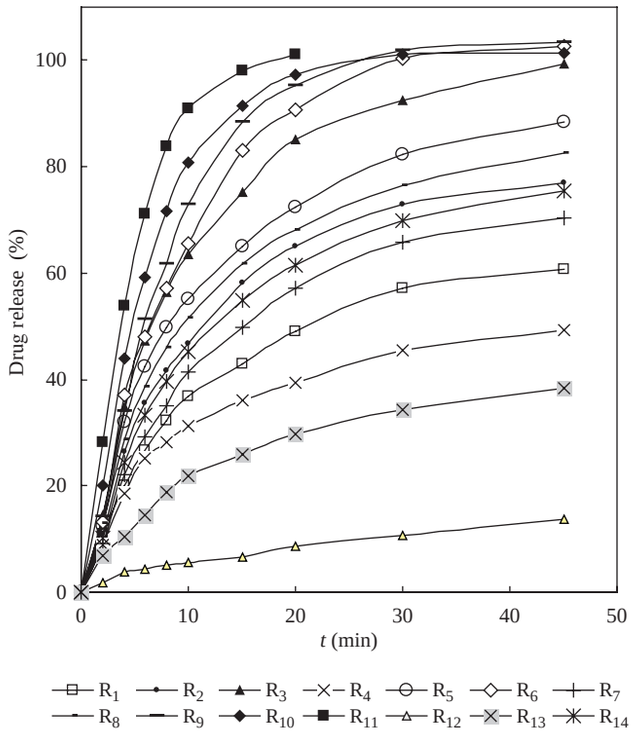

FIGURE 4. Drug release of core tablet at pH 6.8 phosphate buffer (n = 6).

tablets was selected to calculate the f_2. Comparing the release of each other within the same formulation (Table 3), the "similarity factor" f_2 TDCS was 32.6 ± 10.0 and WM-TDCS was 57.4 ± 11.5 by the fit factors method. It suggested that the release of tablets from WM-TDCS was considered to be with similar each other.

It had been demonstrated that polymer with appropriate expanding property could be used as the agents of colon-specific delivery system (Takada, 1997). Because of its low melting point, the potential for wax to be used as a polymeric expanding agent in colon-specific delivery system was obvious.

TABLE 3
The f_2 of the Dissolution Profile of Tablets Between WM-TDCS or Between TDCS by the Fit Factors

	f_2 Compared with No. 6 Tablet						
	1	2	3	4	5	*M*	*SD*
TDCS	36.22	23.12	29.23	48.38	26.29	32.6	10.0
WM-TDCS	43.98	56.51	49.40	64.48	72.64	57.4	11.5

Effect of the Amount of CMS-Na and NaCl on Drug Release

Results for formulas R_{12} and R_{13} showed that the drug release of the tablets without CMS-Na or NaCl was less than the drug release of others, indicating that the combination of CMS-Na and NaCl was important for drug release, with the former exhibiting greater release effect. The erratic and unpredictable drug release influenced by the amount of NaCl could be due to the great osmotic promoting effect (Liu et al., 2007).

There were two possible mechanisms contributing to the role of CMS-Na as shown in Figure 6. It was a swelling polymer which gave a gel mass; with the increase of its concentration up to 30%, the release rate of the drug decreased. Alternatively, it was a disintegrant; by increasing its concentration from 10 to 20% even to 25%, the drug release increased. Taken together, two attributes of CMS-Na were combined together here: a gelling agent and a disintegrant, divided by the concentration of about 30% in our formulation.

Effect of the Weight Gain of the Inner Coating Membrane on Drug Release

The outer coating membranes dissolved in pH 6.8 phosphate buffer although they remained intact in 0.1 mol/L HCl for 2 h.

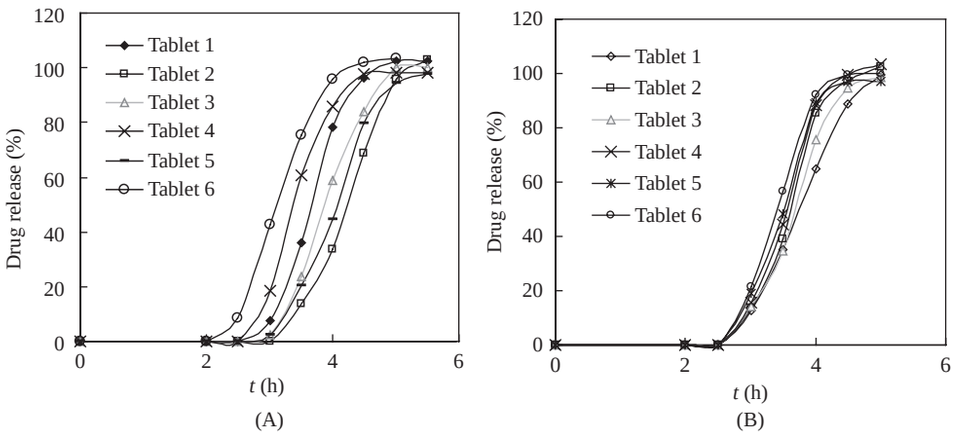

FIGURE 5. Release profiles of time-dependent colon-specific tablet (TDCS) (A, core tablet R_{11}) and wax-matrix time-dependent colon-specific tablet (WM-TDCS) (B, core tablet R_5) no. 6 tablets with inner layer weight gain 4% in pH 6.8 phosphate buffer.

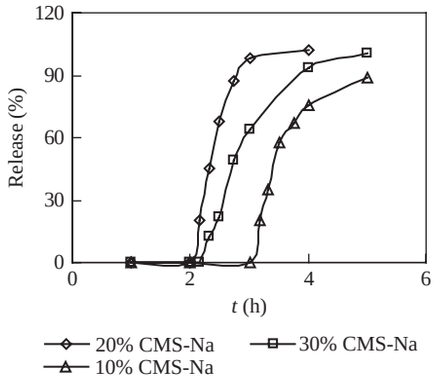

FIGURE 6. Effect of carboxymethyl starch sodium (CMS-Na) amount on drug release in pH 6.8 phosphate buffer (n = 6).

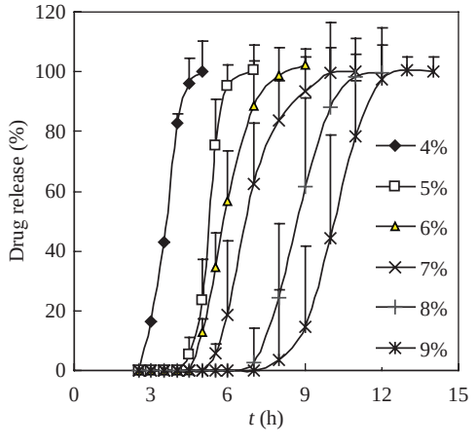

FIGURE 7. Effect of weight gain on drug release of coated tablet at 0.1 mol/L HCl for 2 h and pH 6.8 phosphate buffer for 12 h (n = 6).

It was clearly evident from Figure 7 that the weight gain of the inner coating membranes had a marked effect on drug release; the greater the weight gain, the longer the lag time of drug release. In addition, further weight gain of Eudragit NE 30 D membrane would result in no drug release.

Effect of the Concentration of Pore-Forming Agent on Drug Release

In initial trials, core tablets were coated with Eudragit NE 30 D with no pore-forming agent and enteric coating. However, when the weight gain of the inner coating membrane reached 7%, there was no drug release till the 24th hour. Based on this, it was necessary to add an appropriate amount of water-soluble pore-forming agent. When the amount of PEG 400 added into the membranes was increased, the lag time of drug release decreased. When the amount of PEG 400 reached 2%, the weight gain of the inner coating membrane must be increased to 12% to achieve 3 h of lag time in pH 6.8 phosphate buffer (Figure 8). Finally, 1% PEG 400 was selected.

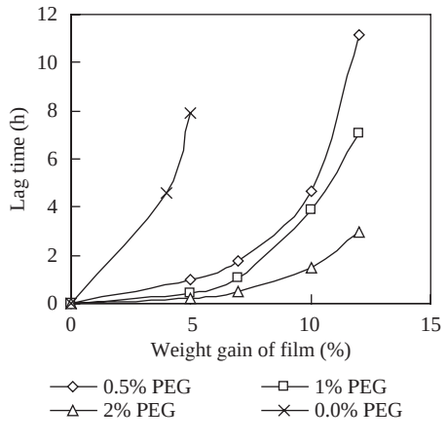

FIGURE 8. The effect of pore-former on film rupture time in pH 6.8 phosphate buffer.

Effect of other Pharmaceutical Factors on Drug Release

The effect of the rotation speed of the paddles at pH 6.8 phosphate buffer was also studied. For coated formulation R_5, a speed of 75 rpm had no significantly different effect on drug release lag time compared with a speed of 50, 100, and 150 rpm. Only after the member was abrupt, drug release was slightly increased as a function of the rotation speed but no significant difference was found.

Release profiles under different dissolution media (distilled water, pH 6.8, 7.0, 7.4, and 7.8 phosphate buffer) were also evaluated. It showed that the drug release lag time and drug dissolution were independent of the dissolution media. So the coating material, Eudragit NE 30 D, was pH-independent.

Besides, the thermal treatment time after coating Eudragit NE 30 D, size of the core tablets and coating in different seasons were also studied. It was evident that if the thermal treatment time was more than 12 h at 25°C, the drug release lag time was unchangeable. For the WM-TDCS, the temperature of the thermal treatment must be less than 25°C for the low-melting point wax. The size of the core tablets (diameter 6.0, 7.0, 8.5, and 9.0 mm) and coating in different seasons (spring, summer, autumn, and winter) had no effect on drug release (Figure 9).

Validation of Analysis Method In Vivo

Calibration curves for the plasma assay developed with peak–area ratio (Y) of OM or M to IS versus drug concentration (c) were found to be linear over the concentration range of 0.5–50 μmol/L using weighted least square method, and the weight was $1/c$. The linear regression equation of the calibration curve was $Y = 0.0204 \times c - 0.003$ (r = .9998) for OM and $Y = 0.0165 \times c - 0.024$ (r = .9991) for M. The limit of quantification (LOQ) for both OM and M was 0.5 μmol/L. The precision and accuracy of the method were examined by adding known amounts of OM and M to dog blank plasma at concentrations of 1, 5, and 40 μmol/L. For intraday precision and accuracy, three replicate quality control samples at each concentration were analyzed on the same

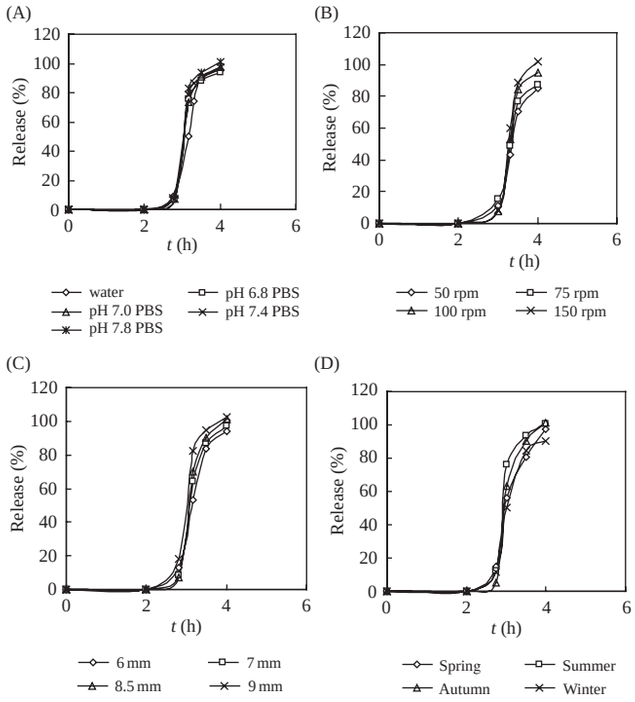

FIGURE 9. Effect of pH (A), rotation speed (B), size (C), and different season (D) on drug release (n = 6).

day. The interday precision and accuracy were evaluated on three different days. The results indicated that the intraday and interday precisions were within 15% for OM and M, which indicated that the methods were reproducible. The extraction recoveries under the liquid–liquid extraction conditions were 85.9 ± 8.9, 71.0 ± 4.6, and 72.7 ± 1.2% (n = 3) at concentrations of 1, 5, and 40 μmol/L, respectively. Therefore, the criteria of precision, accuracy, and recovery for analyzing biological samples were satisfied in the developed analytical method. Representative chromatograms of OM and M are showed in Figure 10.

Stability Study of OM and M in Plasma Samples

The stabilities of OM and M in plasma (10 μmol/L) at different temperatures were evaluated. A decrease of about 0.5% ASF in plasma samples after 24 h at room temperature was observed. After 24 h at 37°C, about 1.0% of ASF degraded. The frozen samples were very stable, which were stored at −20°C for 1 month or treated after three freeze and thaw cycles. OM and M in plasma sample were stable.

Changes in the Plasma Level of OM and M

The changes in the plasma drug level for 24 h after administration were shown in Figure 11. In addition, pharmacokinetic parameters were calculated from the plasma levels, as were shown in Tables 4 and 5. It showed that the drug release from TDCS and WM-TDCS had a lag time of about 3.5 h, thus the formulation could release the ASF in colon.

Comparison of Pharmacokinetics Study

$t_{1/2}$ of ASF in vivo was 3.3 ± 0.9 h indicating rapid elimination in the plasma. The lag times of the ASF release from TDCS tablet and WM-TDCS tablets were 3.7 ± 0.6 and 3.2 ± 0.3 h, respectively. This lag time allows the release of drug in colon for the topical treatment of diseases. Comparatively, more stable release patterns both in vitro and in vivo were achieved in WM-TDCS tablet preparations.

Furthermore, the t_{lag} of WM-TDCS tablet administrated to dog was 3.2 ± 0.3, more stable than tablet with TDCS in vivo. It was shown in Table 6 that WM-TDCS was feasible to release drug with high reproducibility. The *RSD* of lag times of TDCS tablet and WM-TDCS tablet were 16.4 and 8.1. The *RSD* of lag time of WM-TDCS was within 10%.

The metabolite of OM was M and the metabolism of OM increased significantly as the microbial population multiplied (Wang et al., 2005). Therefore, it was suggested that the metabolism should extend duration of the therapeutic effect of OM.

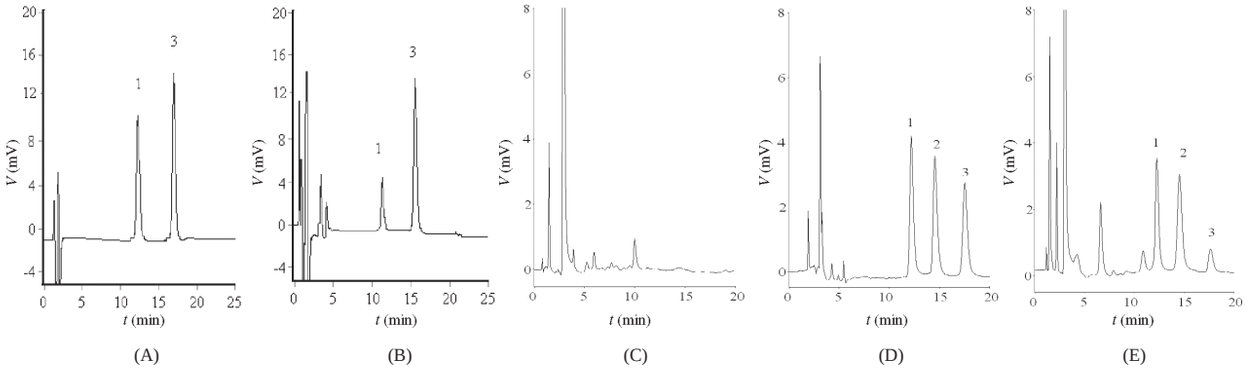

FIGURE 10. Representative chromatograms of matrine (M) and oxymatrine (OM) in dissolution test and M, OM, and internal standard (IS) in dog plasma. (A) standard of OM and M in distilled water; (B) the dissolution of ASF tablet in 4 h; (C) blank dog plasma; (D) blank dog plasma spiked with IS, OM, and M; (E) a plasma sample collected at 4 h after administration of AFS tablet in dog. 1, M; 2, IS; 3, OM.

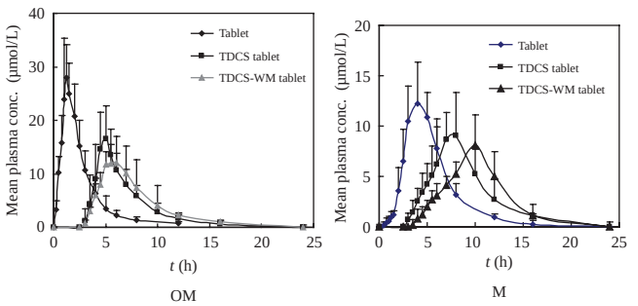

FIGURE 11. Mean plasma concentration time curves of oxymatrine (OM) and matrine (M) following oral administration for Sophora flavescens Aiton (ASF) tablet, ASF time-dependent colon-specific tablet (TDCS) tablet, and ASF wax-matrix time-dependent colon-specific tablet (WM-TDCS) tablet of 30 mg/kg to beagle dogs. Each value represents the $M \pm SD$ ($n = 6$).

CONCLUSIONS

A new wax-matrix time-dependent colon-specific delivery system of ASF was developed based on both the volume expanding of low-melting point wax and the time-dependent colon-specific delivery system. Together with the low-melting point wax expanding, the higher pressure of colon could also control the drug release. The paddle agitation speed had no effect on drug release in vitro. The in vitro and in vivo evaluation indicated that WM-TDCS was feasible to release drug with high reproducibility in colon independent of pH. Comparing the TDCS and other time-controlled release system (Halsas, Penttinen, Veski, Jurjenson, & Marvola, 2001; Rao & Ritschel, 1992), the lag time of release and drug release was highly stable.

The use of semi-synthetic glycerides, low-melting point wax as a polymeric expanding agent, combined with CMS-Na in the formulation of the tablets, increased markedly the volume of the core tablet after contact with the release medium for certain time, which resulted in enough expanding pressure (Yonezawa, Ishida, & Sunada, 2005) for the rupture of the tablet coating.

It has been well documented that gastric emptying of a preparation varies. In contrast, the transit time in small intestine for the preparation is surprisingly constant at 3 ± 1 h and appears to be independent of the types of the preparations whether subjects are fed or not (Halsas, Hietala, Veski, Jurjenson, & Marvola, 1999; Halsas et al., 2001). Based on the aforementioned understandings, we designed the enteric time-dependent colon-specific drug delivery system to achieve a considerable drug release after the preparation's arrival at colon.

Water uptake through numerous micro-channels caused the expansion of the disintegrating agent within the tablet core, resulting in a gradual build-up of pressure within the coated tablets. Once the inside pressure exceeded the critical disrupted value that the coating layer resisted most, the coating layer would be ruptured and the drug was released in a manner of burst, hence the delaying effect of drug release from the coated tablets (Fan, Wei, Yan, Chen, & Li, 2001).

TABLE 4
Pharmacokinetic Parameters of OM for ASF Tablet, ASF TDCS Tablet, and ASF WM-TDCS Tablet in Beagle Dogs After a Single Oral Administration Dose of ASF 30 mg/kg ($M \pm SD$, $n = 6$)

Parameter	t_{max} (h)	c_{max} (μmol/L)	AUC_{0-t} (μmol·h/L)	t_{lag} (h)	K_e (1/h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (μmol·h/L)	MRT (h)	F (%)
ASF tablet	1.2 ± 0.1	29.8 ± 7.3	78.6 ± 25.8	—	0.234 ± 0.109	3.3 ± 0.9	78.6 ± 25.8	2.8 ± 0.3	—
TDCS tablet	4.7 ± 0.4	19.5 ± 4.9	68.9 ± 18.8	3.7 ± 0.6	0.256 ± 0.094	3.0 ± 0.9	68.9 ± 18.8	7.0 ± 1.1	89.0 ± 8.8
WM-TDCS tablet	5.2 ± 0.5	14.9 ± 3.7	74.1 ± 23.5	3.2 ± 0.3	—	—		8.3 ± 1.3	95.1 ± 11.2

TABLE 5
Pharmacokinetic Parameters of M for ASF Tablet, ASF TDCS Tablet, and ASF WM-TDCS Tablet in Beagle Dogs After a Single Oral Administration Dose of ASF 30 mg/kg ($M \pm SD$, $n = 6$)

Parameter	t_{max} (h)	c_{max} (μmol /L)	AUC_{0-t} (μmol·h/L)	t_{lag} (h)	K_e (1/h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (μmol·h/L)	MRT (h)	F (%)
ASF tablet	4.0 ± 1.1	13.3 ± 2.9	63.3 ± 10.7	—	0.326 ± 0.120	2.4 ± 1.0	63.3 ± 10.7	5.2 ± 0.4	—
TDCS tablet	7.7 ± 1.4	12.1 ± 2.4	60.9 ± 17.7	3.7 ± 0.7	—	—	60.9 ± 17.7	8.9 ± 1.3	95.1 ± 13.2
WM-TDCS tablet	10.3 ± 0.8	8.9 ± 2.2	56.6 ± 10.0	4.2 ± 0.5	—	—	57.8 ± 11.0	10.5 ± 0.7	89.8 ± 10.2

TABLE 6
t_{lag} of OM for ASF TDCS Tablet and ASF WM-TDCS Tablet in Beagle Dogs After a Single Oral Administration Dose of ASF 30 mg/kg (n = 6)

	t_{lag} (h)								
	1	2	3	4	5	6	*M*	*SD*	*RSD* (%)
TDCS tablet	3.0	4.0	4.5	4.0	3.0	3.5	3.7	0.6	16.4
WM-TDCS tablet	3.0	3.0	3.5	3.0	3.0	3.5	3.2	0.3	8.1

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