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Preparation and In Vitro Evaluation of pH, Time-Based and Enzyme-Degradable Pellets for Colonic Drug Delivery

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The preparation of pH-dependent, time-based and enzyme degradable pellets was investigated for use as an oral colonic drug delivery system. It was expected that drug would be released immediately once the pellets reached the colon. The pellets were prepared using extrusion-spheronizing equipment and subsequently coated with three layers of three functional polymers by an air-suspension technique. The core consisted of 5-aminosalicylic acid (5-ASA) as a model drug, CaP as an enzyme-degradable material and microcrystalline cellulose (MCC) as an additive. As far as the three coated layers were concerned, the outer layer was coated with Eudragit L30D-55 for protection against gastrointestinal juices, the intermediate layer was coated with ethylcellulose (EC) to inhibit drug release during passage through the small intestine, and the inner film was coated with pectin for swelling and enzyme-degradation, which required a 30, 10, and 12% weight gain, respectively. Several micromeritic properties of the core pellets, including particle size distribution, particle size, degree of circularity, and friability, were evaluated to investigate the effects of the formulations of the cores and preparation conditions. Also, dissolution testing of the cores showed that the presence of calcium pectinate (CaP) markedly increased the drug release rate from the cores, as determined by scanning electron microscopy (SEM). In-vitro release studies indicated that the coated pellets completely protected the drug release in 0.1 mol/L HCl, while the drug release was delayed for 3–4 hr in pH 6.8 PBS. A synergistic effect of enzyme dependence for the coated pellets was seen following removal of the coated layer and during contact with colonic enzymes. Consequently, it was possible to achieve colon-specific drug delivery using this triple-dependence system.

Keywords pH-based; time-based; enzyme-degradable; colonic drug delivery; drug release; CaP

INTRODUCTION

In recent years, oral colonic targeting systems have been regarded as being of increasing importance by pharmaceutical researchers (Krogars, 2000). This dosage form can be used to treat local colonic disease and has shown potential in improving the bioavailability of orally administered peptides and other labile drugs.

Pectin and calcium pectinate (CaP), the poorly water soluble salt of pectin (Rubinstein, 1995), can be widely used as specific drug carriers targeting the colon and exhibiting gelling and enzyme-decomposing properties. Swelling creates a diffusible barrier initially which is degraded by colonic enzymes or bacteria at a later stage (Sinha, 2001). A number of oral systems designed for drug release into the colon using pectin or CaP have recently been reported. Rubinstein (1993) prepared indomethacin and CaP plain matrix tablets in a ratio of 1:10, which delayed the drug release for 8 hr in pH 3.5 buffer solution. Rubinstein (1995) also designed compression coated tablets with CaP to ensure that insulin was released in the colon. Macleod (1999) has investigated the potential of a pectin:chitosan:hydroxypropyl methylcellulose (HPMC) (3:1:1) film for colonic drug delivery following degradation of the coat. Another system, designed by Gazzaniga (1994), to exploit the relatively constant small intestine transit time, consists of cores coated with three polymeric layers: enteric material, HPMC and Eudragit L/S (from the inner layer to the outer). Amylose was applied with EC by Milojevic (1996) to prepare a film for pellets, which could be digested by amylases in the microflora. A new oral timed-release system (El-Gibaly, 2002) which consists of ketoprofen-loaded Zn-pectinate gel (ZPG) microparticles together with pectin/dextran mixtures in a tablet form provided the expected delayed-release sigmoidal patterns with a lag-time of 4.125–4.85 hr. All of these investigations showed that the use of CaP or pectin matrix for colon specific drug delivery is restricted to poor water soluble drugs. For some water soluble drugs, such as insulin, an additional protective coat may be required.

According to previous research, the proportion of CaP and drug could not be less than 10:1 when CaP was employed as an enzyme-degradable material alone to prepare a colonic delivery system (Rubinstein, 1993). Because of the high CaP content required and the poor degree of circularity of CaP pellets, up to the present, there have been no reported investigations of CaP pellets for colonic-specific delivery.

In this paper, the feasibility of preparing 5-ASA-CaP matrix pellets with certain excipients is discussed. The micromeritic properties of the CaP pellets and the drug release profiles

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showed that CaP had an additive effect on colonic delivery systems for poorly water soluble drugs.

Coated pellets for colonic targeting have become the focus of intense research, because they combine the advantages of a high drug content, homogeneous dispersion and easy-controlled drug release.

Krogars reported that the core pellets produced with model drug, Eudragit S and citric acid could be coated with enteric polymer to obtain an enteric-specific system. One form of Calcium pectinate gel-coated pellets was investigated by Sriamornsak (1997) to retard the release rate of theophylline. Also Wakerly (1997) has studied a potential colonic delivery system which combined pectin and ethylcellulose as film coating materials. Furthermore, Fu (2000) has designed a pulsatile controlled release system. In this system, the HPMC layer could swell to prevent contacting with water until the outer EC layer had broken down. Recently, pellets coated with pectin in combination with chitosan or calcium has been prepared by Hiorth (2006). Both of the systems had a reduced drug release compared to uncoated pellets and thereby the coated pellets might possibly be used for colon specific drug delivery. According to other associated research, time-controlled pellets with the assistance of enzyme-degradable materials (pectin or CaP mentioned above) may be a more effective colonic drug delivery system. However, it is known that the use of only pH-sensitive polymer or enzyme-sensitive material for colon targeting would not function when the physiological conditions changed or in particular individuals. Therefore, the particles designed for colon targeting should have multiple functions to adapt to changes in the complex colon environment.

In this investigation, a pH and time-controlled system was combined with an enzyme-degradable system to deliver pellets to the colon, where the drug would be released immediately at the target site.

MATERIALS AND METHODS

Materials

The materials used in this paper were as follows: 5-aminosalicylic acid (5-ASA, 99%, Haiqu, China); pectin (Fuda, China); microcrystalline cellulose (MCC, pH 101, ISP, Hong Kong); lactose (Meggle, Germany); hydroxypropyl methycellulose (HPMC, Methocel K4M, Colorcon, UK); sodium dodecyl sulfate (SDS, Yuwang, China); ethylcellulose (EC, Ethocel 10Cp, Dow, UK); triethyl citrate (TEC, Henkel, Germany); Eudragit L30D-55 (Röhm Pharma, Germany); polyethylene glycol (PEG6000, Hoechst, Germany); talc (Yuhang, China). CaP was prepared in our own laboratory. All of other reagents and solvent were of analytical grade.

Preparation of CaP-5-ASA Core Pellets

Core pellets were prepared using extrusion-spheronization equipment (JBZ-300, China). The formulations are shown in Table 1.

TABLE 1
Composition of Core Pellets

Ingredients	Quantities 1	2	3	Function
5-ASA (g)	20	20	20	Active drug
CaP (containing 5 or 15 (Ashford, 1994) or 30 mg/g Ca^{++}) (g)	10	20	30	Enzyme-decomposed material
MCC (pH 101) (g)	60	50	40	Balling material
Lactose (g)	10	10	10	Diluent
0.5% HPMC-water solution (g/mL) (mL)	70	66	52	Binder

5-ASA, CaP, MCC and lactose were thoroughly mixed and an HPMC-water solution was used as a binder for the wet mass. The content of some components was adjusted according to Table 1 to optimize the amount of CaP and Ca^{++} in the formulations.

Then the wet mass was extruded immediately through a screen with a 1 mm mesh size to produce noodle-shaped sticks by an extruder with single horizontal screw. The extruded material was converted into spheres in a spheroniser with the diameter of 30 cm at room temperature, and the obtained pellets were dried in an oven at 40°C for 2 hr. During the extrusion-spheronization process, the effects of technical factors, including extrusion speed, spheronization speed and spheronization time, on the core pellet properties were investigated. Three levels of each factor were examined as follows: extrusion speed (20, 30, 40 rpm), spheronization speed (600, 900, 1200 rpm) and spheronization time (2, 4, 6 min), and several micromeritic properties were employed to evaluate the quality of the core pellets.

Micromeritic Properties of the Core Pellets

Particle size distribution (PSD), particle size (PS) and degree of circularity of the core pellets were directly analyzed using novel software called Drug Particle Size Auto Analysis System (DPAAS). This software could analyze each pellet by calculating the different PS. Around 200 pellets were stuck on a paperboard every time before scanning using an ordinary scanner and the images obtained were digitized and analyzed by the computer program (DPAAS) mentioned above. In this test, 500 pellets of each batch were analyzed. The mean projected area diameter (Heywood diameter, D_H) was used as the particle size, and then the degree of circularity (φ_c) could be obtained using the following equation:

$$D_H = (4A/\pi)^{1/2}, \qquad \varphi_c = \pi D_H / L$$

where, A is the projected area of the pellet; L is the projected circumference of the pellet.

PSD was expressed by the geometric standard deviation (σ_g).

$$\sigma_g = D_{84} / D_{50}$$

where D_{50} is the median diameter; D_{84} is the diameter when the cumulative number of pellets reaches 84%.

Also, the friability of the CaP pellets must be investigated to make sure that cores could be coated without loss of weight. This was measured by comparing the pellets weights before and after a rotating test at 900 rpm for 4 min in a rotating disk device. Before the "weight after test" was obtained, pellets should be sieved and blown to get rid of fines.

$$\text{Friability (\%)} = \frac{(\text{weight before test} - \text{weight after test}) \times 100}{\text{weights before test}}$$

The friability tests were carried out in triplicates for each pellet batch and results averages.

Coating of the Pellets

A swelling layer consisted of pectin and HPMC in a ration of 4:1, and appropriate amounts of SDS, TEC and talc were added as a stabilizer, plasticizer and anti-cohesive agent, respectively. Firstly, the solution of the swelling materials was prepared by dispersing the pectin and HPMC in 95% ethanol and adding water, and then the other excipients were suspended in the system on the next day.

The insoluble layer contained EC with the addition of PEG6000 (plasticizer) and talc (anti-coherent agent). A 4% EC solution was prepared by dissolving it in 95% ethanol, immersing over night, and then adding the other excipients to the solution the next day.

Eudragit L30D-55 (30% (w/w) solid content), which is commonly used to protecting against gastric juice due to sensitivity above pH 5.5, was diluted to give a 10% (w/w) solid content. TEC (plasticizer) and talc (anti-coherent agent) were added to the dispersion to form an enteric coating suspension.

The detailed formulations of the above three layers are listed in Table 2, and the film coating operations were performed in an air-suspension coater with a bottom spray (JHQ-100, China). Known weights of core pellets (50 g) were coated with each dispersion liquid (Table 2) in a designated order until the desired coating level was obtained. During the coating operations, the aqueous dispersions were continuously stirred in order to prevent sedimentation of the insoluble particles. The operating conditions are given in Table 3. After coating, the resultant pellets were dried at 40°C temperature for 4 hr. The weight gains of three layers were then determined as 10% for

TABLE 2
Film Coating Dispersion Composition

Swelling Layer	(% w/w)	Insoluble Layer	(% w/w)	Enteric Layer	(% w/w)
Pectin	4.0	EC	3.0	Eudragit L30D-55	33.3
Methocel K4M	1.0	PEG6000	1.3	TEC	0.5
SDS	1.0	Talc	0.5	Talc	0.5
TEC	0.7	Ethanol	90.4	Water	65.7
Talc	0.5	Water	4.8		
Ethanol	56.3				
Water	37.5				

TABLE 3
Operating Conditions During the Pellet Coating Processes

Operating Parameters	Swelling Layer	Insoluble Layer	Enteric Layer
Pellets loading (g)		50	
Mode of spraying		continuous bottom spray	
Nozzle port size (mm)		1.2	
Inlet air temperature (°C)	45	40	55
Outlet air temperature (°C)	35	30	40
Atomizing air pressure (kg/cm^2)	2.2	2	2
Spray rate (g/min)	2	1.8	1.6
Air flow rate (L/min)	30	32	35

the swelling layer, 12% for the EC layer and 30% for the enteric layer.

The drug contents of the uncoated and coated pellets were then determined by UV-spectroscopy (model 752, China) at 259 nm.

In Vitro Dissolution Studies

The dissolution studies were carried out at 37 ± 0.5°C, in absence or presence of 6 g/L of appendix material of mouse in dissolution medium, using CP2005 Appendix XC, and a No. 2 dissolution apparatus (paddle). Samples of coated pellets ($n = 6$), equivalent to 100 mg 5-ASA, were placed in 900 mL 0.05 M phosphate buffer, pH 6.8. The rotation speed of the paddle was set at 75 rpm.

According to the dissolution profiles of the coated pellets, which were produced for different levels, the optimum level of each factor was determined to allow the design suitable colonic delivery pellets. In this investigation, the content of SDS in the swelling layer, the required weight gain of the swelling layer and EC layer are discussed.

Furthermore, the dissolution profile of the resultant pellets was investigated in various dissolution media to validate the colonic targeting in any altered environment. In this case, three types of dissolution media, i.e., pH 5.5 PBS, pH 5.5 PBS with appendix material and pH 6.8 PBS were employed to check the availability of this triplex-dependence system.

Ultimately, the dissolution testing of the pellets coated with three layers was conducted in 0.1 $mol \cdot L^{-1}$ HCl for 2 hr and then the medium was switched to pH 6.8 PBS with 6 g/L appendix material.

RESULTS AND DISCUSSION

Preparation of Core Pellets by Extrusion-Spheronization

The addition of Cap to the formulations increases the difficulty of forming the required core pellets, so that it is not suitable for a high load of drug (5-ASA). The proportion of 5-ASA in the formulation was determined as 20%. Lactose, employed as a diluent, improved the surface quality of the pellets but, due to its poor performance in the preparation of pellets and disintegration, 10% was selected as the most suitable concentration.

To be suitable for the subsequent coating process, the micromeritic properties of the core pellets should be measured and compared with the standards: The particle size distribution needs to be as narrow as possible (described as σ_g approaching 1) and the diameter should not be more than 0.8 to 1.2 mm; the degree of circularity must exceed 0.8; the friability of the pellets should less than 1%; also a smooth, compact and homogeneous surface is required.

The characteristics of CaP were obviously affected by the amount of Ca^{++} (shown in Table 4): If the ratio of CaP in the formulation was fixed, the degree of circularity of the pellets fell when the content of Ca^{++} in CaP was above 30 mg/g, or less than 5 mg/g. Furthermore, when CaP content was more than 30%, the pellets did not have the required degree of circularity, and the amount of CaP in the formulation had an important effect on the shape of the pellets.

TABLE 4
The Effect of the Ca^{++} Content of CaP and CaP in Pellets on Their Circularity

No.	Content of Ca^{++} in CaP (mg/g)	Content of CaP in Pellets (%,w/w)	Degree of Circularity ± SD ($n = 500$)
1	5	10	0.715 ± 0.087
2	5	20	0.583 ± 0.073
3	5	30	0.435 ± 0.064
4	15	10	0.890 ± 0.088
5	15	20	0.885 ± 0.069
6	15	30	0.728 ± 0.066
7	30	10	0.841 ± 0.076
8	30	20	0.836 ± 0.090
9	30	30	0.703 ± 0.059
p value			0.000**

SD refers to the standard deviations.
**Significant difference at the 99% level of confidence.

According to Table 4, a 3-dimensional (3D) contour plot was obtained to describe the effects of the content of Ca^{++} and CaP on the circularity of the pellets, which could also be used for selecting the optimal formulation. All the spots inside the region with the darkest color in Figure 1 represent pellets with a suitable degree of circularity under given content values of Ca^{++} and CaP. Since a greater enzyme-degradable capability depended on a higher content of CaP, there is a maximum CaP content for the pellets, which is in the region mentioned above.

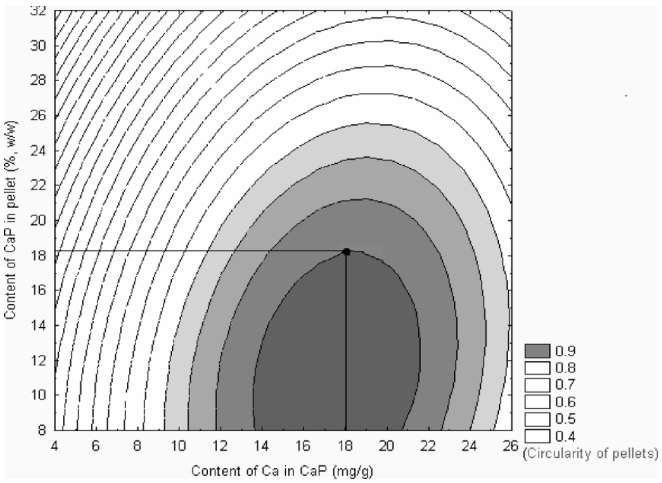

FIGURE 1. 3D Contour plot to describe the effects of the Ca^{++} and CaP content on the circularity of the pellets.

The selected figure corresponded to 18% CaP in the pellets and 18 mg/g Ca^{++} in the CaP, which was also chosen as the optimum formulation.

During the preparation of the core pellets using extrusion-spheronization, it was also found that incompact pellets could be obtained when the content of Ca^{++} reached 30 mg/g, and a fine powder tended to be produced during the spheronizing process, which resulted in a rough surface and unsuitable friability. A reasonable explanation for this is that the viscosity of the pellet material was reduced due to the rise in calcified pectin. However, if the content of Ca^{++} was only 5 mg/g, the wet mass would be too viscous to be spheronized due to the excess pure pectin, and the particle size exhibited a very broad distribution.

If the content of CaP was not more than 20%, pellets exhibited good sphericity. However, when the content of CaP reached 30%, the pellets became brittle and friable. Furthermore, they also exhibited a very poor sphericity and a wide PSD. With the selected content of Ca^{++} (18 mg/g) and CaP (18%), the micromeritic properties of the CaP-pellets were good enough to survive the subsequent coating operation.

Effects of Technical Factors on the Properties of the Core Pellets

With regard to the preparation technology, the process of preparing the wet mass should be prolonged compared with common pellets to make sure that the binder solution has completely infiltrated into the CaP, pectin and other additives for improving the blending uniformity of the wet mass. Having a wet mass of suitable viscosity will help in the preparation of pellets by extrusion-spheronization. Since some pure pectin is viscous in the wet stage, unless the concentration of HPMC is 0.5% or less in the binder solution, the extruded material will stick together when rolling in the spheronizer. The optimal proportion of dry substances to binder solution is 3:2 (w/w).

The effects of the three main technical factors on the properties of the core pellets are shown in Table 5. According to this, the micromeritic properties are markedly affected by technical factors, so that the optimal level of each factor needs to be determined.

In this test, the most appropriate extrusion speed was selected as 30 rpm, because core pellets with better micromeritic properties were obtained under this condition. A higher screw speed (40 rpm) generates heat in the system and it results in the evaporation of water from the wet mass and the lower water content of the wet mass reduces the degree of circularity (Ponto, 1993) and increases friability, and also the generated heat affects the drug stability. However, the too low a screw speed (20 rpm) would not only require a longer operating time, but also produce poor circularity and a coarse surface.

The disk speed plays a major role in influencing the physical characteristics (e.g., particle size, rigidity, degree of circularity, yield, bulk density, etc) of the product (Yang, 2005). A higher disk speed (e.g. 1200 rpm) could increase the moisture loss and reduce the plasticity of the wet particles, consequently, some of the particles would exhibit deformed shapes of cylinders and dumbbells. On the other hand, a lower disk speed (600 rpm) could not provide sufficient shear force so that the particles might not be rounded off into spheres and easily adhere to one another. In this study, a 900 rpm rotating speed was chosen as a suitable disk speed for preparation of the desired CaP-pellets.

TABLE 5
The Effects of Technical Factors on Micromeritic Properties of Pellets

Technical Factors	Parameter Levels	Average Particle Size (mm) (n = 500)	σ_g	Degree of Circularity (n = 500)	Friability (%) ± *SD* (n = 3)
Screw speed (rpm)	20	1.098 ± 0.046	1.101 ± 0.041	0.878 ± 0.046	0.423 ± 0.039
	30	1.076 ± 0.055	1.080 ± 0.037	0.885 ± 0.028	0.452 ± 0.048
	40	0.996 ± 0.036	1.092 ± 0.043	0.854 ± 0.028	0.787 ± 0.067
	p value	—	—	—	0.000**
Disk speed (rpm)	600	1.209 ± 0.053	1.171 ± 0.040	0.837 ± 0.031	0.512 ± 0.050
	900	1.076 ± 0.055	1.080 ± 0.037	0.885 ± 0.028	0.450 ± 0.048
	1200	1.043 ± 0.041	1.137 ± 0.021	0.807 ± 0.026	0.638 ± 0.061
	p value	0.015*	0.043*	0.039*	0.012*
Spheronizing time (min)	2	1.114 ± 0.066	1.158 ± 0.028	0.826 ± 0.031	1.114 ± 0.099
	4	1.076 ± 0.055	1.080 ± 0.037	0.885 ± 0.028	0.448 ± 0.048
	6	1.098 ± 0.064	1.079 ± 0.024	0.900 ± 0.022	0.323 ± 0.033
	p value	—	0.028*	0.035*	0.000**

SD refers to the standard deviations.
**Significant difference at the 99% level of confidence.
*Significant difference at the 95% level of confidence.
—No Significant difference.

The spheronization time was another important factor affecting the properties of the pellets. As the spheronization time increased, the pellets became more spherical and compact, whereas, the drug release rate from the pellets was reduced. Thus, 4 min was selected as the optimal spheronization time, and it should not be longer than 6 min. As the major factors influencing the shape of the pellets, the spheronization speed and residence time always acted in conjunction with each other (Parikh, 1997).

Comparison of Dissolution Behavior of the Different Core Pellets

The drug release rate from CaP pellets was significantly faster than that from pellets without CaP in PBS medium (see Figure 2), and the drug release rate could be improved in presence of an enzyme medium. The drug (5-ASA) could be released completely within 0.5 hr in PBS containing 6 g/L mouse appendix material (Figure 2), and the release rate was faster than that in the absence of enzyme medium. The addition of mouse appendix material here was to simulate the colonic environment. It was demonstrated that the enzyme-degradability of CaP actually took place, so that 18% Cap in the pellets could have an additional effect on the release. Based on the cores, pH-dependant and time-dependant materials could be coated to prepare a multi-layer colonic drug delivery system.

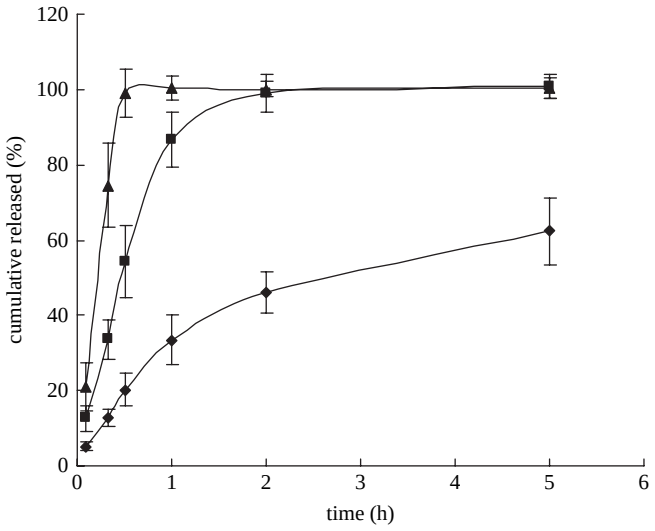

FIGURE 2. 5-ASA released peofiles from various core pellets. (▲) CaP pellets in pH 6.8 PBS containing 6 g/L appendix material of mouse. (■) CaP pellets in pH 6.8 PBS. (♦) pellets without CaP in pH 6.8 PBS.

The SEM microphotographs show that the interior of the pellets without CaP (Figure 3a) was very compact except for a few small holes, while that of the CaP-pellets (Figure 3b) contained a lot of holes and channels, which made the pellets less compact. The difference in the internal structures between the pellets with and without CaP might be the reason for their different drug release rates, which could deduce the drug release mechanism of the CaP-pellets.

The drug release mechanism for the CaP-pellets can be summarized as follows: during preparation of the wet mass, the pure pectin in the material absorbed water and swelled. After agglomeration, lots of small pathways were formed during the evaporation of water in the drying stage, and the dissolution medium passed easily into the interior of the pellets, so that the drug could be released immediately. Furthermore, the addition of CaP could reduce the amount of disintegrant needed, which would have some disadvantages for the pellets, such as friability and a poor circularity.

Coating Process of the Pellets

The coating formulations and technical parameters had marked effects on the quality of the three layers. The components of the three coating formulations differed slightly: TEC, employed as a plasticizer, could obviously improve the continuity of the swelling layer and enteric layer, which would prevent drug leakage. PEG6000, which was more suitable for the EC layer, has a similar effect to TEC. The addition of talc

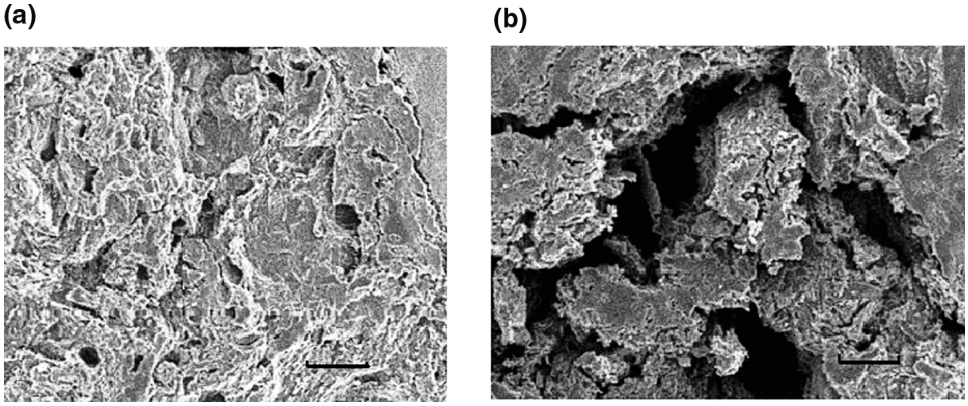

FIGURE 3. SEM microphotograph of a cross-section of the pellet (a) without CaP and (b) with CaP (bar = 100 μm).

could effectively reduce conglutination of the pellets during the coating process. Furthermore, the proportion of ethanol in the dispersion medium should be adjusted according to different coating materials, so that an optimal coating effect could be achieved. SDS could obviously influence the drug release rate from coated pellets, and this is discussed below.

Since coating the outer layer was more difficult than the inner layer, some measures were adopted to improve the continuity of the layers during the coating process, such as: increasing the air temperature/air flow rate and reducing the spray rate. The formulations and technical parameters for the three layers proved suitable for processing in an air-suspension coater, and enabled us to achieve a continuous polymeric layer of the desired thickness, with satisfactory properties in terms of appearance, homogeneity and mechanical resistance.

Comparison of the Dissolution Behavior of the Coated Pellets

In this investigation, the traditional pulsatile dosage form was improved by substituting HPMC or other swelling materials with pectin, which had the same enzyme-decomposing action as CaP in the cores. The drug release mechanism was designed with the following in mind: When the coated pellets passed through the stomach under the protection of the enteric coat and entered the small intestine, the enteric coat dissolved, and the water (enteric juice) then penetrated gradually through the insoluble layer (second layer, i.e., EC film) and the inner pectin layer swelled. The drug was not released until EC layer was forced to break up. At the target site, the CaP and pectin loaded in the core pellets were degradable by colonic enzymes, thereby accelerating drug release.

The addition of SDS to the swelling layer might help to increase the hydrophilicity of the swelling layer so as to accelerate water permeability and drug release after the EC layer breaks up. The effect of the SDS concentration on the dissolution behavior is shown in Figure 4. When the required weight gain of the swelling layer, EC layer and enteric layer were fixed at 12%, 10% and 30% respectively, adding SDS to the swelling coating suspension would obviously improve the drug release rate. According to Figure 4, without SDS, drug release was not complete in 9 hr, however, when the content of SDS was increased to 1%, over 90% of the 5-ASA was released within 6 hr. However, it shows that continuously increasing the concentration of SDS does not increase the drug release rate obviously.

The effects of the swelling layer with different weight gains on the dissolution behavior are shown in Figure 5. When other conditions were fixed, an 8% weight gain by the swelling layer made it difficult to break up the EC layer and the cumulative release did not exceed 10% in 10 hr. However, a swelling layer with a 16% weight gain easily broke up the EC layer and the lag-time was shortened. So, the weight gain of the swelling layer should be tightly controlled.

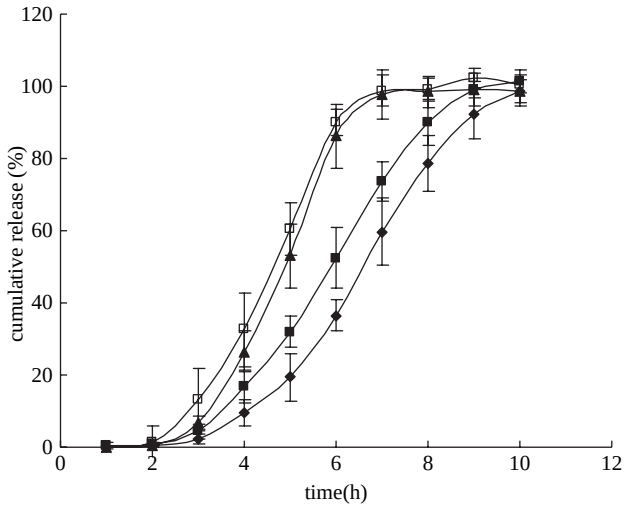

FIGURE 4. 5-ASA release profiles from coated pellets with different content of SDS (dissolution medium is pH 6.8 PBS). (□) 0% SDS. (■) 0.5% SDS. (▲) 1% SDS. (□) 1.5% SDS.

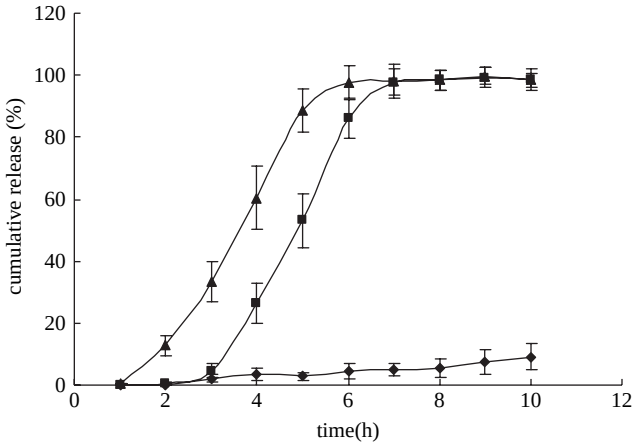

FIGURE 5. Effect of increased weight of swelling layer on drug release behavior. (□) 8% weigh gain. (■) 12% weight gain. (▲) 16% weight gain.

Figure 6 shows the effect of different weight gains of the EC layer on drug release behavior. It was found that the EC layer was destroyed easily when the weight gain was only 7%, and when it increased up to 13%, the EC layer became difficult to break up and there was almost no drug releasing in 10 hr. According to these tests, 10% was chosen as optimal weight gain of the EC layer in order to obtain an appropriate lag-time and release rate.

The required weight gains of the swelling layer and the EC layer were closely linked. The EC layer influenced the lag-time directly, while swelling layer had a less direct effect. When the weight gain of the EC layer increased, the weight gain of the swelling layer must also increase to make sure that the EC layer breaks up at the designed time. So, a large number of

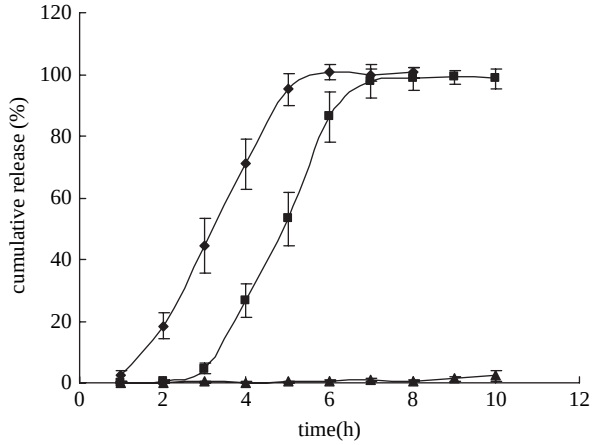

FIGURE 6. Effect of increased weight of EC layer on drug release behavior. (□) 7% weight gain. (■) 10% weight gain. (▲) 13% weight gain.

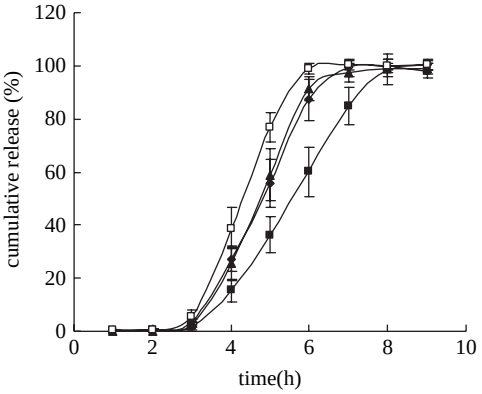

FIGURE 8. Drug release behavior of three layers coated pellets in different dissolution medium. (□) pH 6.8 PBS with 6 g/L appendix material of mouse. (▲) simple pH 6.8 PBS. (□) pH 5.5 PBS 6 g/L appendix material of mouse. (■) pH 5.5 PBS.

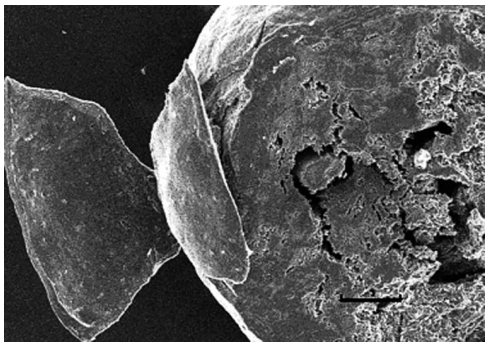

FIGURE 7. SEM microphotograph of a cross section of the CaP-pellet with swelling layer and EC layer (bar = 100 μm).

tests need to be carried out to select the most suitable weight gains for the two layers.

The SEM microphotographs (Figure 7) show that the swelling layer and the EC layer are both relatively compact and continuous to the CaP cores, owing to the addition of plasticizer to the coating suspension and the application of a bottom-spray coater. This was essential to obtain a better time-controlled effect.

Then pulsatile pellets were coated with Eudragit L30D-55 (30% weight gain) as the enteric layer, so that the colonic delivery of the pellets could be obtained. Since some diseases change the condition of the intestine, such as reducing the pH value and the enzymatic activity, the dissolution behavior of these pellets were investigated in different dissolution media (Figure 8). pH 6.8 PBS, pH 5.5 PBS with 6 g/L appendix material and pH 5.5 PBS were employed to simulate the conditions of a loss of enzymatic activity, a lower pH value and both cases in the colon, respectively. It was shown that this drug delivery system could delay the drug release by more than 3 hr, and then the 5-ASA was released completely over a certain period in the four kinds of dissolution media, which was suitable for in-vitro colon-specific drug delivery.

First of all, the enteric layer protected the coated pellets from gastric juice during transit through the stomach. As soon as the pellets entered the small intestine, the enteric layer immediately dissolved due to the pH in the small intestine, and then the coated EC layer and pectin layers were able to begin their colon-targeting depending on the time-based and enzyme-degradable effects. In any of the four media, liquid penetrated through EC layer to swell the inner pectin layer (the swelling time was time-dependent) and drug was released when the EC layer was forced to break up. In the media with appendix material, a synergistic enzyme effect increased the release rate of drug at the target site. This shows that this dosage form is applicable under the conditions mentioned above.

Figure 9 shows that almost no drug was released in 0.1 $mol \cdot L^{-1}$ HCl medium over 2 hr, and no more than 5% 5-ASA was released over the first 3 hr after the dissolution medium was transferred to pH 6.8 PBS with appendix material. Thereafter, 5-ASA was released completely in pH 6.8 PBS with 6 g/L appendix material over 3 hr. The weight gains of enteric layer, EC layer and swelling layer were $29.53 \pm 0.77\%$, $10.03 \pm 0.30\%$ and $12.40 \pm 0.31\%$, respectively. This shows that this dosage form is able to deliver the drug to the targeted colonic region.

CONCLUSION

The pH, time- and enzyme-based 5-ASA colonic targeting pellets were prepared by the extrusion-spheronizing and air-suspension techniques.

The 5-ASA-CaP core pellets improved the drug release rate and possessed enzyme degradability. The micromeritic properties of the core pellets were influenced by the content of CaP and Ca^{++} as well as three technical factors (screw speed, disk speed and rotating time).

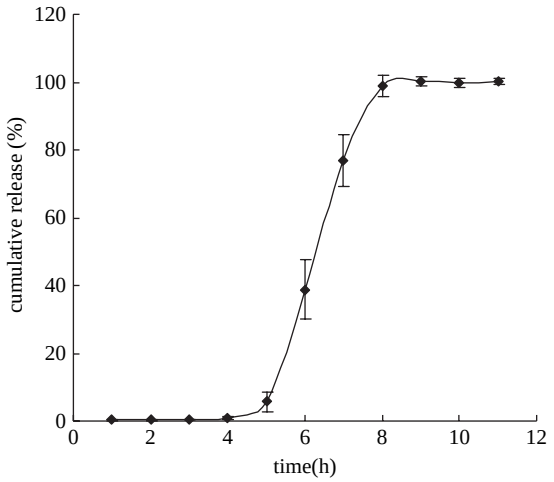

FIGURE 9. Drug release behavior of three layers coated pellets in 0.1 mol/L HCl for 2 hr and then transforming the medium into pH 6.8 PBS with 6 g/L appendix material.

With regard to the coated pellets, the addition of SDS improved the drug release rate, and the weight gains of the swelling layer, EC layer and enteric layer had a marked effect on the drug release behavior of the pellets. The drug release curve shows that coated pellets could be successfully delivered to the colon.

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