Drug Development and Industrial Pharmacy, 31:127–134, 2005

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1081/DDC-200046990

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Calcium Pectinate Capsules for Colon-Specific Drug Delivery

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ABSTRACT The calcium pectinate (CaP) capsule, a novel, colon-specific delivery system, was designed and developed using 5-fluorouracil (5-FU) as a model drug. Technically, CaP capsules were prepared by dipping a glass or stainless steel rod successively into pectin and calcium chloride solutions, followed by subsequent air-drying and coating. In vitro studies showed that the release of 5-FU from CaP capsules markedly increased in the presence of rat cecal contents, and the release characteristic was mainly associated with some capsule parameters such as calcium content, shell thickness, and coat amount. Gamma scintigraphic studies demonstrated that CaP capsules could pass through the stomach and small intestine intact and could release drug in colon. The 5-FU releasing characteristics acquired both from in vitro biomimic dissolution experiments and from healthy volunteers indicated that the newly developed CaP capsule possessed the ideal colon-specific drug delivery characteristic.

KEYWORDS Colon-specific drug delivery, Calcium pectinate capsule, 5-Fluorouracil, Dissolution, Gamma scintigraphy

INTRODUCTION

Recently, colon-specific drug delivery systems have gained worldwide attention because they could substantially improve the therapeutic efficacy of drugs in treating colon-related diseases, and are particularly advantageous for the delivery and systemic absorption of protein/peptide drugs susceptible to proteinase-initiated degradations in the upper gastrointestinal tract (Ciftci & Groves, 1996; Leopold, 1999; Watts & Illum, 1997; Yang et al., 2002). Many dosage forms such as time- and/or pH-controlled release systems have been examined for possible colon-specific deliveries of drugs. They were found to be not very reliable in terms of "site-specific release," because the drug transit time and pH in the GI tract often change with many factors including age, sex, diet, intestinal motility, disease state, and so forth (Ashford et al., 1993; Qi et al., 2003; Takaya et al., 1995). Another approach for colon-specific targeting purposes has been developed to permit a locally allotted drug release through covalent bond cleavages accomplished by the enzyme originated from the colon microflora. Moreover, some colon-specific degradable materials, such as aromatic azo-polymers and poly- or disaccharide containing insoluble polymers, were used in the enzyme-controlled release system (Mooter et al., 1995; Rubinstein et al., 1992).

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Pectin, widely used in the food industry, is a natural polysaccharide obtained from plant primary cell walls. It consists mainly of linearly connected α-(1-4)-D-galacturonic acid units and their methyl esters. Calcium pectinate (CaP) is a rigid and water-insoluble gel, derived from partial demethylation of pectin through an amidation reaction with DE less than 50% (DE=degree of esterification, expressed by the average number of methoxy groups per galacturonic acid unit), followed by a cross-linkage with calcium ion. It was reported that CaP could be degraded in the colon where a rich microflora was residing (Rubinstein et al., 1993).

Using 5-fluorouracil as a model drug, the present investigation was performed to assess the potentiality of CaP capsules as a colon-specific delivery system. In addition, the colon-specific characteristic of CaP capsules in human volunteers was established by gamma scintigraphic technology. The results are presented in the communication to provide a successful example and helpful advice for designing a colon-specific delivery system.

MATERIALS AND METHODS

Materials

The used materials were: pectin (food grade, methoxyl content of 8.31%, Guyibao Company, Inner Mongolia, China), 5-fluorouracil (5-FU, Nantong Pharmaceutical Factory, Jiangsu, China), polyvinylpyrrolidone (PVP-K30, BASF, Germany), Eudragit L and S (Röhm, Darmstadt, Germany), ethyl cellulose (EC, Shanghai Colorcon Coating Technology Ltd., China); and calcium chloride and arabic gum (Shanghai Chemical Reagent Company, China). $^{99m}TcO_4^-$ solution was kindly supplied by the isotope laboratory of Nanjing Railway Medical College. All other chemicals used in the study were of analytical grade. Wister rats were obtained from the Animal Center of Nanjing Medical University.

Preparation of Low Methoxyl Pectins (LM-Pectin)

Commercial pectin (methoxyl content of 8.31%) was partly demethylated by the reported ammonia de-esterification protocol (Yamaguchi et al., 1994). Briefly, 30 g of pectin suspended in 100 mL ethanol were stirred with a 25% (v/v) 280-mL ammonia solution for 3 h at room temperature. The addition of 240 mL more ethanol to the suspension afforded the precipitate, which was filtrated and washed with ethanol until free of ammonia, followed by being dried for 1 h at 50°C in an oven.

Analysis of Methoxyl Content of the LM-Pectin

Degree of esterification was expressed as a percentage of the methoxyl group as determined by a titration method (Zhu et al., 2001).

Preparation of CaP Capsules

CaP capsules were prepared by the following procedure. First, 3% (w/v) glycerol and 0.2% (w/v) arabic gum were successively dissolved in 30 mL distilled water. To the obtained solution, LM-pectin was added at a concentration of 10% (w/v) through agitation at 40°C in a water bath. Secondly, a 5.7- or 6-mm-diameter glass or stainless steel rod was dipped into the prepared pectin solution at a depth of about 3 cm for 10–70 s to obtain shells with a desired thickness, followed by inserting it into a 6% (w/v) solution of calcium chloride for about 1 hr at a different temperature (40–60°C) in a water bath. After allowing the rod to be air-dried for about 5 h, capsules that formed were carefully denuded. The shell thickness of the capsule was measured by a micrometer.

The surfaces of these capsules were coated with Eudragit L/S (L:S, 4:1, w/w) as an enteric material by the following procedure. Into the Eudragit L/S solution (5%, w/v) in ethanol, CaP capsules were dipped for coating using a pair of tweezers. Then the coated capsule shells taken out of the solution were dried in the air. To attain the different coat amounts, the coated capsule shells were re-dipped into the Eudragit solution followed by being dried in the air. The coat amounts, indicative of the coat thickness of CaP capsules were quantified in terms of the percentage of capsule weight growth through Eq. 1.

$$\text{Percentage of capsule weight growth (\%)} = (W_1 - W_0)/W_0 \times 100\% \quad (1)$$

where W_1 and W_0 are the weights of the capsule after and before coating.

Finally, the rim of shells was clipped to form the capsule cap with a length of 1 cm and inner diameter

of 6 mm, and the capsule body with a length of 1.7 cm and inner diameter of 5.7 mm.

Analysis of Ca^{2+} Content of the CaP Capsule Shell

A total of 100 mg CaP capsule powder afforded by crushing shell in a mortar was placed in a 50-mL beaker and then digested completely with 4 mL of concentrated HNO_3. The digestion mixture was transferred to a 100-mL flask and diluted by adding an appropriate amount of distilled water. Concentration of the calcium ions was determined by atomic-absorption spectroscopy (AA-670 Shimadzu, Tokyo, Japan).

Preparation of 5-Fluorouracil-Loaded CaP Capsules

The powdered mixture of 5-FU (30 g) and starch (60 g) was kneaded with 5% (w/v) PVP ethanolic solution as the binder. The wet mass was forced through a 40-mesh screen. The granules, after dried at 50°C for 4 h, were sieved through a 40-mesh stainless steel sieve to remove fines.

A given quantity of granules containing 30 mg 5-FU was put into each CaP capsule with the capsule joint sealed carefully with a small amount of an EC solution 5% (w/v, ethanol).

Preparation of Suspension of Cecal Contents

Fresh cecal contents from nonfasted rats were suspended at 1.25% (v/v, cecal contents/buffer) in phosphate buffer (pH 7.0). The suspension was adjusted to pH 7.0 by bubbling CO_2 prior to filtration through four layers of gauze followed by experimentation as detailed earlier (Krishnaiah et al., 2002; Rubinstein et al., 1993). As the cecum is naturally anaerobic, dissolution in the suspension of rat cecal content was carried out in a nonstop flow of CO_2.

In Vitro Drug Release Experiment

According to China Pharmacopoeia 2000 (C. P.) Method II (Paddle apparatus), one 5-fluorouracil-loaded CaP capsule was tied to the paddle with a cotton thread in each dissolution vessel to prevent flotation. The 5-fluorouracil release experiments were carried out at 37°C separately, with each set of experiments performed in the simulated gastric fluid (250 mL, HCl solution at pH 1.2) (for first 2 h), intestinal fluid (250 mL, phosphate buffer at pH 6.8) (for next 5 h), and the suspension of cecal contents (100 mL, for the coming 17 h). The rotating speed of the paddle was set at 75 rpm, and the sample was taken periodically. Drug concentration of the taken sample was determined after filtration though a 0.45-μm filter and appropriate dilution by UV spectroscopy (UV-260 Shimadzu, Tokyo, Japan) at 266 nm.

In order to describe the kinetics of the drug release from CaP capsules, a drug dissolution test was performed at 37°C for 5 h independently in three media—250 mL of the simulated gastric fluid, 250 mL of pH 6.8 phosphate buffer, and 100 mL of the suspension of cecal contents, while the paddle was stirring at 75 rpm.

Gamma Scintigraphic Studies in Healthy Volunteers

A suitable amount of the tracer $^{99m}TcO_4^-$ was mixed with starch before being passed through a mesh to form granules, followed by being dried immediately at 60°C in an oven. A total of 200 mg of the dried granule was put into each CaP capsule with the joint sealed as mentioned above. Each capsule contained about 1.85 MBq of the tracer $^{99m}TcO_4^-$.

Two healthy male volunteers, ages 20 and 25 and weighing 55 and 65 kg, respectfully, were recruited in the study after providing written, informed consent. Each volunteer ingested one $^{99m}TcO_4^-$/CaP capsule after an overnight fasting. The capsule was imaged using gamma scintigraphy (HPGe-4096, Ortec, Greenville, SC) at the 2nd, 5th, and 23rd hours after ingestion. Two hours after swallowing the capsule, the concentrated solution of $^{99m}TcO_4^-$ (7 MBq) was labeled at the xiphoid position in each volunteer for the purpose of marking the stomach. Subjects had a standard meal after the capsule emptied from the stomach.

RESULTS AND DISCUSSION

Preparation of CaP Capsules

The experiment ascertained the stability of calcium pectinate at low pH, its resistance to extensive hydration in vivo, and its susceptibility-enhancing

effect to large bowel bacterial enzymes, indicating that calcium pectinate thus prepared can be considered as an ideal colon-specific drug-delivery carrier. The degree of esterification (DE), indicated by the percentage of carboxyl groups, is an important criteria to classify pectin. Compared with high methoxyl pectin (HM-pectin), low methoxyl pectin (LM-pectin) possesses better water solubility and can form rigid gels by bonding with calcium ion to cross-link the galacturonic acid chains in the polymer (Grant et al., 1973). In this communication the commercial HM-pectin was demethylated to get LM-pectin by ammonia de-esterification. The methoxyl content of the prepared LM-pectin was 1.98%±1.1% (n=3).

Calcium pectinate, a rigid and water-insoluble gel, was formulated into films, gels, droplets, microspheres, and more often, compressed tablets (Liu et al., 2003). Because of poor film-forming properties and flexibility, the CaP capsule shell was too brittle to be applied as a drug carrier. The present work showed that some substances added in the pectin solution could overcome this limitation. Amphiphilic glycerol used as a plasticizer has better affinity to hydrophilic pectin and hydrophobic calcium pectinate. In addition, the natural polymer arabic gum was used to increase the mucosity of pectin solution and the flexibility of calcium pectinate. Accordingly, the film-forming properties of the gel solution were greatly improved, and eventually, the novel CaP capsule systems for colon-specific drug delivery were prepared.

In Vitro Drug Release Experiment

The drug release from the CaP carrier was closely associated with capsule preparation parameters such as calcium content, shell thickness, and coat amount. Furthermore, CaP capsules with various parameters were prepared to study the relationship between the factor and 5-FU release characters (Figs. 1–3). When the calcium content in capsules increased to 8% (w/w, Ca^{2+}/capsule), the 5-FU release was lowered substantially in the simulated gastric and intestinal fluids, whereas the delivery of this anticancer drug into the suspension of the cecal contents was nearly independent of the concentration of calcium (Fig. 1). Therefore, special care should be taken to optimize the calcium content necessary for a predetermined drug release from CaP capsules. Moreover, we found that the calcium content in shells was strongly influenced by the temperature at a given concentration of Ca^{2+}. This observation could be rationalized by assuming that a higher temperature would promote the cross-links between pectin chains and calcium ions to result in high-calcium shells. Therefore, it was necessary to carefully control the cross-linking temperature, and in the present study pectin was allowed to cross-link with calcium at 60°C with the calcium content in the capsule up to 8%.

Figure 2 illustrates the dependence of 5-FU dissolution on capsule thickness. In all test media, gradual increases in the shell thickness of the capsule resulted in decreased drug releases in a nonquantitative manner. At a shell thickness of 3.0 mm the drug release was unacceptably slow, whereas it dissolved too rapidly at that of 0.7 mm. In the present study, the most suitable shell thickness of capsules was optimized to be 1.7 mm for colon-specific delivery of 5-fluorouracil. Technically, shell thickness of capsules was closely related to the duration of dipping the rod into the pectin solution during the manufacturing process. Our experiment has worked out the suitable dipping time (ca. 40 s), which permitted a shell thickness of 1.7 mm.

Figure 3 shows the effect of the coating amount on the 5-FU release from CaP capsules. The ideal

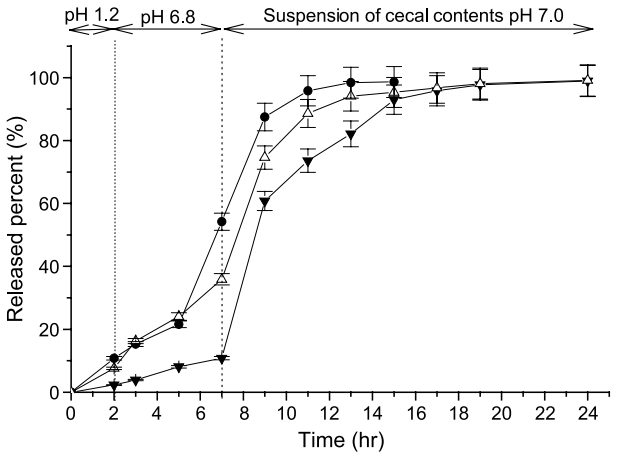

FIGURE 1 **Effect of the calcium content on the release of 5-fluorouracil from CaP capsules (1.7 mm shell thickness, 2% coating amount) determined by the C.P. paddle method. Experimental procedure: 0–2 h (in simulated gastric fluid at pH 1.2), 2–7 h (in simulated intestinal fluid at pH 6.8) and 7–24 h (in the suspension at pH 7.0 of the cecal content fluid). ($\bar{x}$±s, n=6) ▼: 8% calcium content (cross-linking temperature: 60°C); △: 6% calcium content (cross-linking temperature: 50°C); ●: 4% calcium content (cross-linking temperature: 40°C).**

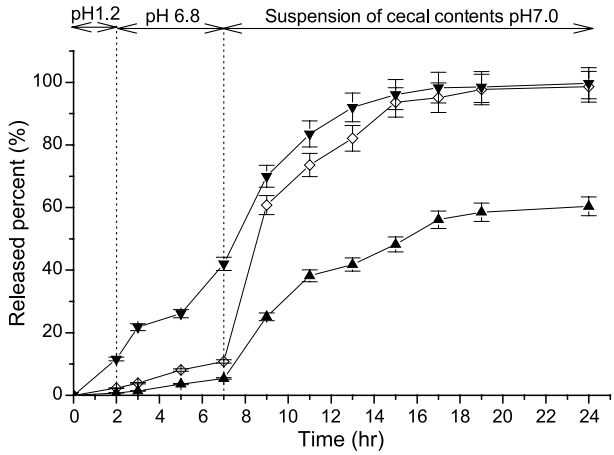

FIGURE 2 **Effect of the shell thickness of capsule on the release of 5-fluorouracil from CaP capsules (8% calcium content, 2% coating amount) experimented at the thicknesses of 3.0 (▲) (dipping time: 70 s), 1.7 (◇) (dipping time: 40 s) and 0.7 (▼) (dipping time: 10 s) mm. The experimental procedure was the same as highlighted in Fig. 1. ($\bar{x}$ ± s, n = 6).**

percentage was ascertained to be at 2%, but the drug release was reduced significantly in the three dissolution media if the coat amount went up to 5%. It was obvious that an excess of coating decreased the drug release from the capsule.

The results indicate that the CaP capsule made following the above optimized parameters (a calcium content of 8%, a shell thickness of 1.7 mm, and a coating amount of 2%) could provide a predetermined drug release and was chosen as the optimal one for further study. The 5-FU release profile of the optimal CaP capsules is presented in Fig. 4. Less than 15% of the drug was released from the capsules in the

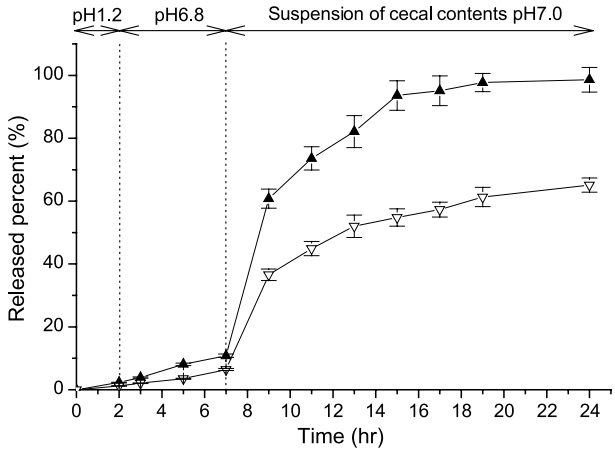

FIGURE 3 **Effect of the coating amount on the release of 5-fluorouracil from CaP capsules (8% calcium content, 1.7 mm shell thickness), compared between the coating amounts of 5% (▽) and 2% (▲). The experimental procedure was the same as highlighted in Fig. 1. ($\bar{x}$ ± s, n = 6).**

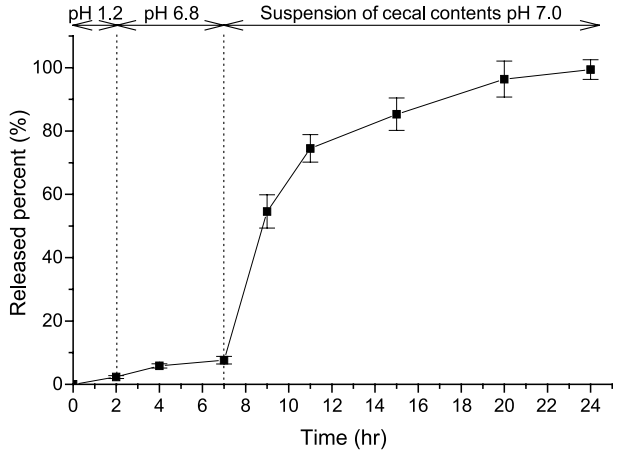

FIGURE 4 **Release of 5-fluorouracil from CaP capsules determined by the C.P. paddle method. The experimental procedure was the same as highlighted in Fig. 1. ($\bar{x}$ ± s, n = 6).**

simulated gastric and intestinal fluids. However, over 90% of the 5-FU could be released from capsules in the suspension (pH 7.0) of the rat cecal contents. The results showed that CaP capsules hardly broke down in the physiological environment of the stomach and the small intestine, and consequently could protect the drug from being released completely. However, in the suspension of the cecal contents, the drug release markedly increased, suggesting that the CaP capsules were degraded in the presence of rat cecal contents where various microorganisms reside.

The 5-fluorouracil-loaded CaP capsules were tested further by comparing different drug releases

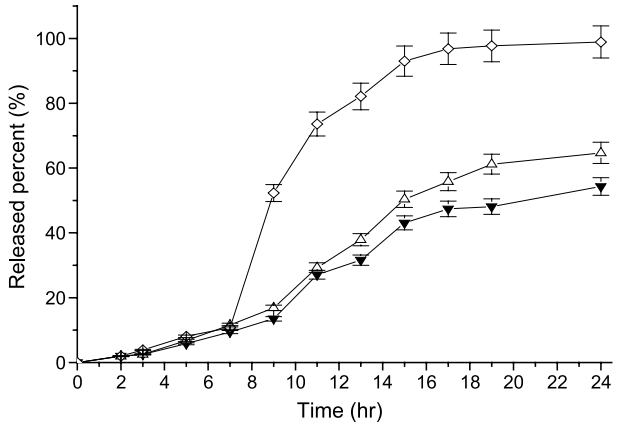

FIGURE 5 **Effect of the dissolution condition on the release of 5-fluorouracil from CaP capsules. ▼: in simulated gastric fluid in the first 2 hours, and then in simulated intestinal fluid from 2nd through 24th hours; △: in simulated gastric fluid in the first 2 hours, then in simulated intestinal fluid from 2nd through 7th hours, and finally in the suspension (bubbling O_2 throughout dissolution test) of the rat cecal content from 7th through 24th hours; ◇: the same as "△" except for continuously bubbling CO_2 instead of O_2. ($\bar{x}$ ± s, n = 6).**

successively in simulated gastric and intestinal fluids and in the differently treated suspensions of the rat cecal content. Additional drug releasing tests were performed in the suspension of the rat cecal content with bubbling O_2 or CO_2. As shown in Fig. 5, the dissolution amount of 5-FU in the two fluids was almost the same in the first 7 hours. Moreover, only 54.3% and 65.4% of the total 5-FU in capsules could be released, respectively, in the simulated intestinal fluid (without adding rat cecal content) and in that mixed with the content and oxygen gas throughout the dissolution experiment. However, 96.8% of the encapsulated 5-FU was released into the suspension (continuously bubbled with CO2) of the rat cecal content. This observation could be explained by assuming that anaerobic bacteria(um) in the rat cecal content could produce enzyme(s) playing key role(s) in the degradation of the calcium pectinate. The

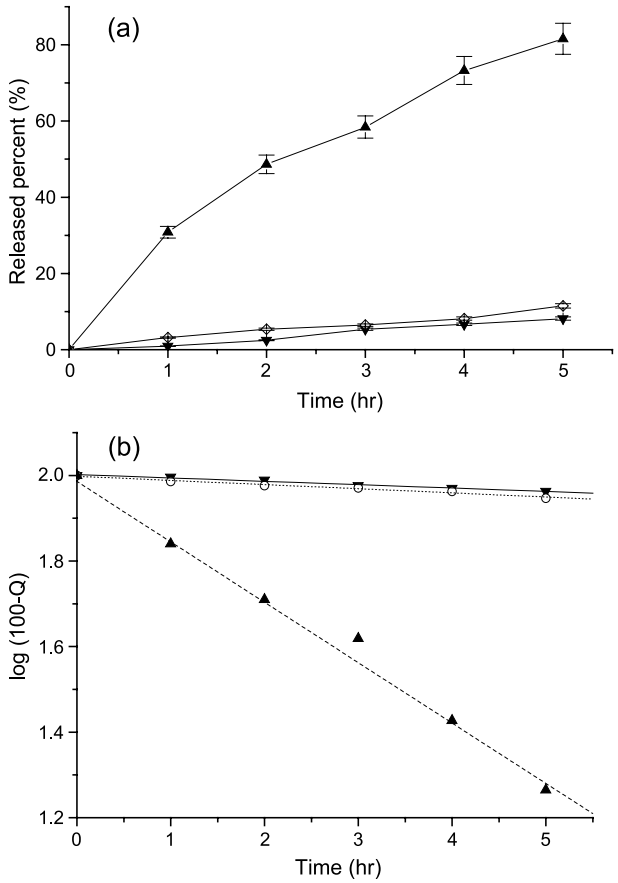

FIGURE 6 **Dissolution and correlation in 5-fluorouracil release from CaP capsules in different media. (a) the 5-hour release comparison among in the simulated gastric (▼) and intestinal (◇) fluids, and the suspension (▲) of the rat cecal content. (b) First order plots for 5-fluorouracil release in the three media. ($\bar{x} \pm s$, n=6).**

TABLE 1 **5-Fluorouracil Releasing Parameters in Different Dissolution Media According to the First Order Model**

Dissolution media	Intercept	K	r
Simulated gastric fluid	2.002	0.0079	−0.9913
Simulated intestinal fluid	1.998	0.0097	−0.9877
Suspension of cecal content	2.001	0.1430	−0.9958

K is the release rate constant for the first order, *r* is the correlation coefficient.

present finding that microorganisms in the cecal content can trigger the degradation of the calcium pectinate is fairly helpful in designing drug delivery systems with a pectin-based framework.

Technically, the 5-FU release from the calcium pectinate capsule can be described by the first-order model with obvious correlation (Desai et al., 1966). The computer package Microsoft Excel 2000 was applied for the linear parameter estimation.

$$\log(100 - Q_t) = a + k\,t \qquad (2)$$

where Q_t is the cumulative drug released (%) in time t, k is the release rate constant for first-order, and a is the intercept of the line. The dissolution data in different media were plotted in accordance with the first-order Eq. 2 (Fig. 6). As shown in the figure and in Table 1, the dissolution data observed in different media showed a discernible relationship with $r > 0.98$. However, the release rate in simulated gastric ($k = 0.0079$) and intestinal ($k = 0.0097$) fluids was slower than that in the suspension ($k = 0.143$) of the cecal content, indicating that the microorganism in the cecal content was helpful in degrading the calcium pectinate. This microbial function happens to allow a better colon-specific delivery of CaP capsules.

Gamma Scintigraphic Studies in Volunteers

Gamma scintigraphy is a reliable tool for evaluating the in vivo performance of a dosage form in the different regions of gastrointestinal tract (Wilding et al., 1991). The results from the gamma scintigraphic studies on CaP capsules for colon-specific delivery are shown in Fig. 7. The bright spots on the top of gamma scintigraphs recorded at the 5th and 23rd

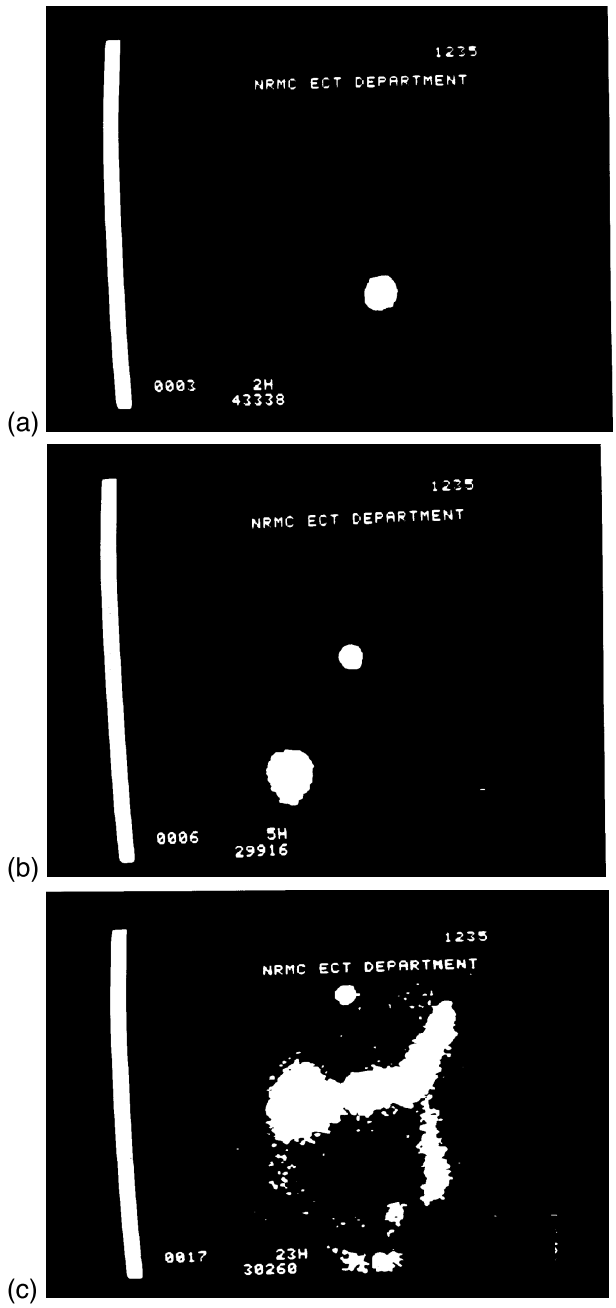

FIGURE 7 **Scintigraphic images of a radiolabelled CaP capsule in the volunteer via oral administration with tracer release from CaP capsule in stomach at the 2nd (a), 5th (b) and 23rd hours (c, complete degradation of CaP capsule and distribution of tracer throughout the entire colon).**

hours were the marks radiolabeled in the position of the human xiphoid, while the 2nd-hour scintigraph was not marked. Scintigraphs indicated the presence of a small amount of tracer released from the CaP capsule in the stomach and small intestine (Fig. 7a,b). This observation paralleled the percentage (about 15%) of the drug released from the CaP capsule in the simulated gastric and intestinal fluids. Furthermore, the tracer was found to distribute extensively throughout the whole colon (Fig. 7c), due to an efficient degradation of calcium pectinate, accomplished presumably by the enzyme produced by anaerobic bacteria(um) in the rat cecal content. The results clearly indicate that the capsule also possessed a better in vivo colon-specific delivery.

CONCLUSION

In this study, we have successfully prepared CaP capsules by an easily repeatable protocol. In vitro studies demonstrated that a small amount of 5-FU was released from CaP capsules in the simulated gastric and intestinal fluids. However, the drug release was markedly increased in the presence of rat cecal contents. The in vivo tests by gamma scintigraphy indicated that the CaP capsules remained nearly intact in the stomach and small intestine before degradation by microorganisms in the region and subsequent release of the encapsulated drug upon reaching the colon. Hence, the prepared CaP capsules may be used as a satisfactory carrier for colon-specific drug delivery purposes.

ACKNOWLEDGMENT

This work was supported by Natural Science Foundation of China (30171104).

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