Eudragit-coated Pectin Microspheres of 5-Fluorouracil for Colon Targeting

Submitted: May 5, 2006; Accepted: August 4, 2006; Published: February 16, 2007

Amol Paharia,[1] Awesh K. Yadav,[1] Gopal Rai,[1] Sunil K. Jain,[2] Shyam S. Pancholi,[3] and Govind P. Agrawal[3]

[1]Department of Pharmaceutical Sciences, Dr. Hari Singh Gour University, Sagar - 470 003 (M.P.), India
[2]SLT Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur - 495 009 (C.G.), India
[3]Shree Dhanvantary Pharmacy College, Kim, District Surat - 94110, Gujrat, India

ABSTRACT

An objective of the present investigation was to prepare and evaluate Eudragit-coated pectin microspheres for colon targeting of 5-fluorouracil (FU). Pectin microspheres were prepared by emulsion dehydration method using different ratios of FU and pectin (1:3 to 1:6), stirring speeds (500-2000 rpm) and emulsifier concentrations (0.75%-1.5% wt/vol). The yield of preparation and the encapsulation efficiencies were high for all pectin microspheres. Microspheres prepared by using drug:polymer ratio 1:4, stirring speed 1000 rpm, and 1.25% wt/vol concentration of emulsifying agent were selected as an optimized formulation. Eudragit-coating of pectin microspheres was performed by oil-in-oil solvent evaporation method using coat:core ratio (5:1). Pectin microspheres and Eudragit-coated pectin microspheres were evaluated for surface morphology, particle size and size distribution, swellability, percentage drug entrapment, and in vitro drug release in simulated gastrointestinal fluids (SGF). The in vitro drug release study of optimized formulation was also performed in simulated colonic fluid in the presence of 2% rat cecal content. Organ distribution study in albino rats was performed to establish the targeting potential of optimized formulation in the colon. The release profile of FU from Eudragit-coated pectin microspheres was pH dependent. In acidic medium, the release rate was much slower; however, the drug was released quickly at pH 7.4. It is concluded from the present investigation that Eudragit-coated pectin microspheres are promising controlled release carriers for colon-targeted delivery of FU.

KEYWORDS: 5-Fluorouracil, pectin, microspheres, Eudragit coating, colon targeting.

INTRODUCTION

Colorectal cancer is the second leading cause of cancer deaths in the United States, and more than 66 000 cases of colon cancer are reported to occur in the Indian subcontinent every year. Conventional cancer chemotherapy is not very effective for treatment of colorectal cancer, as the drug molecule does not reach the target site at therapeutic concentration. Therefore effective treatment of colon cancer by conventional therapy requires relatively large doses to compensate for drug loss during passage through the upper gastrointestinal (GI) tract. These large doses may be associated with undue side effects. This can be overcome by site-specific delivery of the drug molecule to colon. A particular challenge in the pharmaceutical field is the development of a site-specific delivery system that could control delivery time for the release of active ingredient in the lower part of the small intestine, or in the colon. The approaches used in achieving colonic delivery of drugs include the use of prodrugs,[1,2] pH-sensitive polymer coating,[3,4] and time-dependent formulations.[5,6] In addition, the use of biodegradable polymers such as azo-polymer and polysaccharide (eg, pectin and dextrin) for colon targeting are also reported in the literature.[7,8] Among the different approaches to achieve colon-selective drug delivery, the use of polymers, specifically biodegraded by colonic bacteria, holds great promise. The pH-dependent systems exploit the generally accepted view that pH of the human GI tract increases progressively from the stomach (pH 2-3) to the small intestine (pH 6.5-7.0) to the colon (7.0-8.0).[9] Most commonly used pH-dependent coating polymers are methacrylic acid copolymer (ie, Eudragit L100-55, Eudragit L100, and Eudragit S100), which dissolve at pH 5.5, 6.0, and 7.0, respectively.

Pectin is a predominately linear polymer of mainly α-(1-4)-linked D-galacturonic acid residues interrupted by 1, 2-linked L-rhamnase residues. Pectin has a few hundred to about 1000 building blocks per molecule.[3] Because pectin is soluble in water, it is not able to shield its drug load effectively during its passage through the stomach and small intestine. Hydrophilic polymer matrix systems are widely used in oral controlled drug delivery because of their flexibility to obtain a desirable drug release profile, cost-effectiveness, and broad regulatory acceptance.[10-12] The ability of the hydrophilic polymer matrices to release an entrapped drug in aqueous medium and to regulate the release of such drug by control of swelling and cross-linking makes them particularly suitable for controlled-release applications.[11] These matrices can be applied for the release of both hydrophilic and hydrophobic drugs and charged solutes. Recently, many controlled-release formulations based on hydrophilic polymer matrices

Corresponding Author: Govind P. Agrawal, Shree Dhanvantary Pharmacy College, Kim (E), District Surat - 94110, Gujrat, India. Tel: +912621-233345; Fax: +912621-233344; E-mail: gpa1950@yahoo.co.in

have been developed.[12-14] Pectin is a polysaccharide found in the cell walls of plants. It is totally degraded by colonic bacteria but is not digested in the upper GI tract.[9,15] One disadvantage of pectin is its solubility. This drawback can however be adjusted by changing its degree of methoxylation, or by preparing calcium pectinate.[9,15]

The objective of the present investigation was to design a multiparticulate delivery system for site-specific delivery of 5-fluorouracil (FU) using natural polysaccharides (pectin) and pH-sensitive polymer (Eudragit S100) for the treatment of colon cancer. This system is anticipated to protect the drug loss in the upper GI tract, which results from the inherent property of Eudragit S100 (ES), and deliver FU in the colon only. The use of enteric polymers (ES) as protective coating on the microspheres makes them able to release the drug at the particular pH of colonic fluid. A combined mechanism of release is proposed, which combines specific biodegradability of polymer and pH-dependent drug release from the coated microspheres.

Materials and Methods

Chemicals

The drug, 5-fluorouracil (FU) was purchased from M/s Otto Kemi, Mumbai, India. Pectin was obtained from HiMedia Laboratories Ltd, Mumbai, India. Eudragit S100 was procured as a gift sample from Rohm Pharma, Darmstadt, Germany. Span 85, acetone, isooctane, ethanol, hexane, and light liquid paraffin were purchased from Central Drug House Pvt Ltd, Mumbai, India. All other chemicals used were of analytical reagent grade and were used as received. The in vivo study was performed in accordance with the protocol approved by the Institutional Animals Ethical Committee of Dr Hari Singh Gour University, Sagar, India, following the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Preparation of Eudragit-coated Pectin Microspheres

The pectin microspheres were prepared by emulsion dehydration technique.[16] Pectin (3 g) and FU (1 g) were dissolved in 20 mL of distilled water and stirred overnight to solubilize completely. This drug-polymer solution was dispersed in 50 mL isooctane containing 1.25% wt/vol Span 85 and stirred at 1000 rpm continuously to obtain stable water/oil (w/o) emulsion. The solution was rapidly cooled to 15°C and then 50 mL of acetone was added in order to dehydrate the pectin droplets. This system was maintained under mechanical agitation with propeller stirrer at 1000 rpm at 25°C for 30 minutes to allow the complete solvent evaporation. The microspheres were freeze-dried overnight (Heto Drywinner, Birkerod, Denmark) and kept in an airtight container for further studies. Pectin microspheres were prepared using different ratios of FU:pectin (ie, 1:3, 1:4, 1:5, and 1:6).

Pectin microspheres were coated with ES using oil-in-oil solvent evaporation method.[17] Pectin microspheres (50 mg) were dispersed in 10 mL of coating solution prepared by dissolution of 500 mg of ES in ethanol:acetone (2:1) to give 5:1 (coat:core ratio). This organic phase was then poured in 70 mL of light liquid paraffin containing 1% wt/vol Span 85. The system was maintained under agitation speed of 1000 rpm at room temperature for 3 hours to allow for the evaporation of solvent. Finally, the coated microspheres were filtered, washed with n-hexane, and freeze-dried overnight. Microspheres were suspended in the chamber of laser diffraction particle size analyzer (Cilas 1064 L, Orleans, France) containing distilled water, and the particle size and size distribution were determined using the software provided by the manufacturer.

Scanning Electron Microscopy

The shape and surface morphology of pectin microspheres and Eudragit-coated pectin microspheres were investigated using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (Jeol JSM-1600, Tokyo, Japan).

Swellability

A known weight (100 mg) of various FU-loaded pectin microspheres and Eudragit-coated pectin microspheres were placed in enzyme-free simulated intestinal fluid (SIF, KH_2PO_4/NaOH buffer, pH 7.4) and allowed to swell for the required period of time at 37ºC ± 0.5ºC in the dissolution apparatus (United States Pharmacopeia [USP] XXIII, model DT-06, Erweka, Germany). The microspheres were periodically removed and blotted with filter paper; then their change in weight (after correcting for drug loss) was measured until attainment of equilibrium. The swelling ratio (SR) was then calculated using the following formula:

$$SR = \frac{\omega_g - \omega_0}{\omega_o}, \quad (1)$$

where, SR indicates swelling ratio; ω_0, initial weight of microspheres; and ω_g, final weight of microspheres.

Percentage Drug Entrapment

The microspheres (100 mg) were digested in 10 mL of pectinase solution (4% wt/wt) for 12 hours. The digested homogenate was centrifuged (Remi, Mumbai, India) at 3000 rpm for 5 minutes, and the supernatant was assayed for FU content using high-performance liquid chromatography (HPLC) method. The HPLC system (LC-10AT VP, Shimadzu liquid chromatograph, Shimadzu, Kyoto, Japan) consisted of SPD-M10A VP Shimadzu diode array detector, and a Luna 5μ C_{18} column (250 × 4.60 mm) was used for the analysis of drug. The system was operated by Class M10A (Shimadzu, Kyoto, Japan). The mobile phase was composed of acetonitrile/acetate buffer pH 4.4, (ratio 15:85). At a flow rate of 0.8 mL/min, FU was detected at 260 nm, with a detection limit of ~20 ng. A linear curve was constructed between the peak area and concentration and the equation of line was obtained, which is $y = 0.1078x + 0.0226$ with correlation coefficient of 0.9999. The linearity was observed in the range between 0.1 and 40 μg/mL. Validation and calibrations were performed before and during analysis.

In Vitro Drug Release Studies in Simulated Gastrointestinal Fluids

Eudragit-coated pectin microspheres and uncoated pectin microspheres were evaluated for the in vitro drug release in simulated GI fluids (SGF). The drug dissolution test of microspheres was performed by the paddle method (model DT-06, Erweka, Darmstadt, Germany) specified in USP XXIII. Microspheres (100 mg) were weighed accurately and gently spread over the surface of 500 mL of dissolution medium (SGF).[18] The content was rotated at 100 rpm at 37°C ± 0.5°C. Perfect sink conditions prevailed during the drug dissolution study period. The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time intervals. The pH of the dissolution medium was kept 1.2 for 2 hours using 0.1 N HCl. Then KH_2PO_4 (1.7 g) and $Na_2HPO_4.2H_2O$ (2.2 g) were added to the dissolution medium, adjusting the pH to 4.5 with 1.0 M NaOH, and the release rate study was continued for an additional 2 hours. After 4 hours, the pH of the dissolution medium was adjusted to 7.4 with 0.1 N NaOH and maintained up to 24 hours.[19] The samples were withdrawn from the dissolution medium at various time intervals using a pipette fitted with a microfilter. The rate of FU release was analyzed using HPLC method. The receptor volume was maintained constant by replacing equivalent amount of SGF. The concentration of FU in the samples was calculated based on average calibration curves (n = 6). All dissolution studies were performed in triplicate.

In Vitro Drug Release Study in the Presence of Rat Cecal Content

Rat cecal content was prepared by the method reported by Van den Mooter et al.[20] Four albino rats, (Sprague-Dawley strain) of uniform body weight (150-200 g) with no prior drug treatment, were used for all the present in vivo studies; they were weighed, maintained on normal diet, and administered 1 mL of 2% dispersion of pectin/ES in water, and this treatment was continued for 7 days for polymer induction to animals. Thirty minutes before starting the study, each rat was humanely killed and the abdomen was opened. The cecal were traced, legated at both ends, dissected, and immediately transferred into phosphate buffered saline (PBS) pH 6.8, which was previously bubbled with CO_2. The cecal bag was opened; the contents were weighed, homogenized, and then suspended in PBS (pH 7.4) to give the desired concentration (2%) of cecal content, which was used as simulated colonic fluid. The suspension was filtered through cotton wool and ultrasonicated for 10 minutes in an ice bath at 40% voltage frequency using a probe sonicator (Soniweld, Imeco Ultrasonics, Mumbai, India) at 4°C to disrupt the bacterial cells. After sonication, the mixture was centrifuged (Remi) at 2000 rpm for 20 minutes.

Microspheres (100 mg) were placed in 200 mL of dissolution media (PBS, pH 7.4) containing 2% wt/vol rat cecal content. The experiment was performed with continuous CO_2 supply into the dissolution medium. At different time intervals, the samples were withdrawn and replaced with fresh PBS. The experiment was continued up to 24 hours. The withdrawn samples were pipetted into a series of 10-mL volumetric flasks, and volumes were made up to the mark with PBS and centrifuged. The supernatant was filtered through 0.45-μm membrane filter (Millipore Corp, Billerica, MA) and the filtrate analyzed for FU content at 260 nm using HPLC method. All the experiments were performed in triplicate.

Organ Distribution Study

Albino rats were kept in well-spaced ventilated cages and maintained on healthy and fixed diet (Bengal gram soaked in water). These animals were divided into 3 groups of 6 rats, each. The first group served as control. The second group received 2 mg FU (Biochem Pharma, Mumbai, India). Animals of the third group were given Eudragit-coated pectin microspheres containing equivalent amount of drug. The formulations were orally administered in suspension form followed by sufficient volume of drinking water. After 2, 4, 6, and 8 hours, the rats were humanely killed. Stomach, small intestine, and colon were isolated. These organs were homogenized by Micro Tissue Homogenizer (Mac, Mumbai, India) along with a small amount of PBS (pH 7.4); 1 mL of acetonitrile was added to homogenate and kept for 30 minutes.

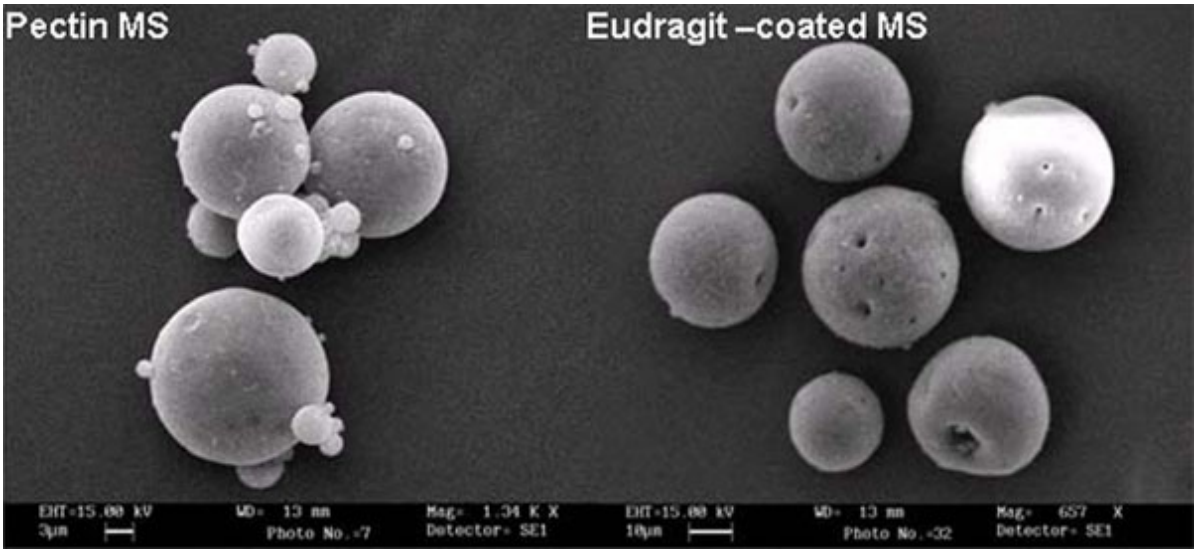

Figure 1. Scanning electron photomicrographs of (A) Pectin microspheres (original magnification ×422) and (B) Eudragit-coated pectin microsphere (original magnification ×3.06K). MS indicates microspheres.

Contents were centrifuged and the supernatant liquid was separated. After appropriate dilution of supernatants, the drug content was determined by HPLC method. The drug content in different parts of the GI tract at different time intervals was calculated.

Statistical Analysis

The mean percentage of FU released in SGF (at different pH) and in presence of 2% cecal content of rats from both pectin microspheres and Eudragit-coated pectin microspheres was prepared by using various drug:polymer ratios and compared. The Student *t* test was used to find the statistical significance. A value of *P* less than .05 was considered statistically significant.

RESULTS AND DISCUSSION

Preparation of Eudragit-coated Pectin Microspheres

Pectin microspheres of FU were successfully prepared by emulsion dehydration technique. Uniform, surface cross-linked, and almost spherical microspheres were obtained as shown in scanning electron photomicrographs (Figure 1A). The pectin microspheres were coated with Eudragit S100 by oil-in-oil solvent evaporation method, using coat:core ratio 5:1. The coated microspheres were found to be of spherical shape as observed in SEM photomicrographs (Figure 1B). The method was optimized using different stirring rate and emulsifier concentration to produce microspheres of small size and narrow size distribution, high drug loading efficiency, and controlled drug release at the colonic pH.

The mean diameter of pectin microspheres varied from 25.11 ± 2.5 μm to 29.47 ± 2.0 μm with varying pectin concentration from 3% wt/vol to 6% wt/vol. The percentage drug entrapment was found to be 70% ± 5% in all the microsphere formulations. The highest drug loading efficiency was found with 3% pectin (Table 1). A higher concentration of polymer produced a more viscous dispersion, which formed larger droplets and consequently larger microspheres as reported by Pongpaibul et al.[21] In the study of effect of emulsifier concentration on formation of microspheres, the mean diameter of microspheres was found to vary from 30.30 ± 2.4 μm to 24.70 ± 2.2 μm on varying emulsifier concentration (Span 85) from 0.75% wt/vol to 1.5% wt/vol for pectin microspheres. Increased surfactant concentration led to the formation of particles with a lower mean geometric diameter. Increasing Span 85 concentration

Table 1. Effect of Fabrication Variables on the Particle Size and Percentage Drug Entrapment of Various Pectin Microspheres*

	Pectin Microspheres		
Serial No.	Fabrication Variables	Mean Diameter (μm)	Percentage Drug Entrapment
1. Polymer amount (Drug:polymer)	1:3	25.11 ± 2.5	74.85 ± 2.4
	1:4	26.64 ± 1.6	70.85 ± 1.0
	1:5	29.17 ± 1.8	64.55 ± 1.2
	1:6	29.47 ± 2.0	65.95 ± 3.0
2. Surfactant concentration (wt/vol)	0.75	30.30 ± 2.4	69.15 ± 1.5
	1.00	28.77 ± 1.2	69.35 ± 1.1
	1.25	25.24 ± 0.9	70.65 ± 1.8
	1.50	24.70 ± 2.2	73.35 ± 2.2
3. Stirring speed (rpm)	500	31.83 ± 1.2	69.15 ± 1.2
	1000	27.71 ± 2.0	70.15 ± 1.0
	1500	27.30 ± 1.6	72.95 ± 2.2
	2000	25.11 ± 2.1	73.35 ± 2.7

*Values are average of 3 readings ± standard deviation.

from 0.75% to 1.50% wt/vol led to stabilization of the emulsion droplets avoiding their coalescence, resulting in smaller microspheres.[22] The drug loading efficiency varied from 69.15% ± 1.5% to 73.35% ± 2.2% with varying emulsifier concentration from 0.75% to 1.5% during preparation of pectin microspheres (Table 1). The mean diameter of pectin microspheres decreased from 31.83 ± 1.2 μm to 25.11 ± 2.1 μm with increasing agitation speed of the mechanical stirrer from 500 rpm to 2000 rpm. This result was expected because high stirring rates provide the shearing force needed to separate the oil phase into smaller globules.[23] The stirring speed of 1000 rpm was found to be optimum for pectin microspheres, as the drug loading efficiency was 70.15% ± 1.0% at this speed (Table 1). High stirring speed produced an irregular shape of microspheres but a slightly increased entrapment efficacy was found. Stirring time of 30 minutes was found to be optimum for pectin microspheres because at this time period, the microsphere size was low with good drug loading efficiency of 71.60% ± 3.2% (data not shown). Swellability of different microspheres was determined. No significant swelling was observed with Eudragit-coated pectin microspheres as compared with pectin microspheres (Table 2), thus ensuring better resistance of Eudragit-coated microspheres in the upper GI tract to swelling and preventing subsequent drug release at the nontarget site.

In Vitro Drug Release Studies in Simulated Gastrointestinal Fluids

In vitro FU release study of pectin microspheres and Eudragit-coated pectin microspheres was performed in pH progression medium at 37ºC ± 0.5ºC. The results showed that the rate of release of FU from pectin microspheres was mainly influenced by polymer concentration. FU release from pectin microspheres (PMS) in SGF followed the order PMS-P1> PMS-P2 > PMS-P3> PMS-P4 (Figure 2). The initial higher release of FU from microspheres might have resulted from the dissolution of drug crystals on the surface of microspheres. The cumulative percentage drug release from Eudragit-coated pectin microspheres showed the desired rate, as there was no measurable drug release observed up to 2 hours in SGF (pH 1.2), while at pH 4.5, the FU release was quite insignificant (<2%) up to 4 hours. FU release from

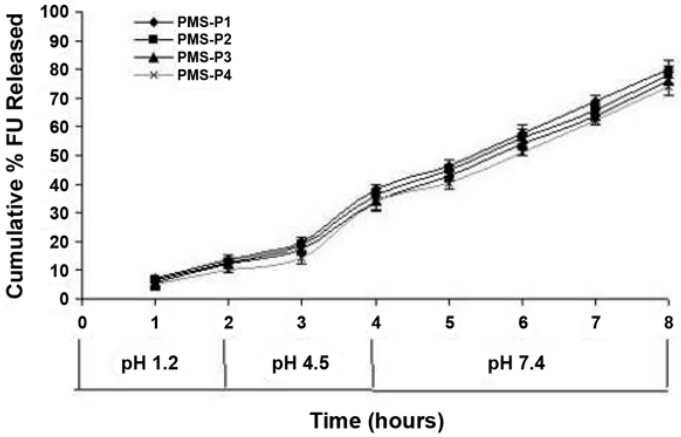

Figure 2. Percentage cumulative in vitro FU release from pectin microspheres containing different drug:pectin ratios (1:3 to 1:6) in simulated gastrointestinal fluids of different pH. Values are average of 3 readings ± standard deviation. FU indicates 5-fluorouracil; PMS, pectin microspheres.

Eudragit-coated pectin microspheres in SGF followed the order EMS-P1> EMS-P2 > EMS-P3> EMS-P4 (Figure 3).

In Vitro Drug Release Study in the Presence of Rat Cecal Content

The in vitro release of FU from pectin microspheres and Eudragit-coated microspheres in presence of 2% rat cecal content in simulated colonic fluid showed faster drug release at different time periods when compared with release study without rat cecal content (Figures 4 and 5). This finding could be attributed to the various anaerobic bacteria present in cecal content and responsible for digestion/degradation of pectin in order to release drug from microspheres. Figure 6 shows the SEM photomicrograph of Eudragit-coated pectin microspheres (EMS-P4) after the in vitro release study in simulated colonic fluid.

Organ Distribution Study

Formulations PMS-P4 and EMS-P4 were selected on the basis of in vitro release performance for further in vivo study. Organ distribution study of optimized formulations was

Table 2. Degree of Swelling of Various Pectin Microspheres and Eudragit-coated Pectin Microspheres*

	Pectin Microspheres		Eudragit-coated Microspheres	
Serial No.	Formulation Code (Drug:Polymer)	Degree of Swelling	Formulation Code (Drug:Polymer)	Degree of Swelling
1	PMS-P1 (1:3)	0.88 ± 0.06	EMS-P1 (1:3)	0.04 ± 0.01
2	PMS-P2 (1:4)	1.21 ± 0.12	EMS-P2 (1:4)	0.13 ± 0.03
3	PMS-P3 (1:5)	1.27 ± 0.16	EMS-P3 (1:5)	0.16 ± 0.04
4	PMS-P4 (1:6)	1.31 ± 0.15	EMS-P4 (1:6)	0.18 ± 0.02

*PMS indicates pectin microspheres; EMS, Eudragit-coated pectin microspheres. Values are average of 3 readings ± standard deviation.

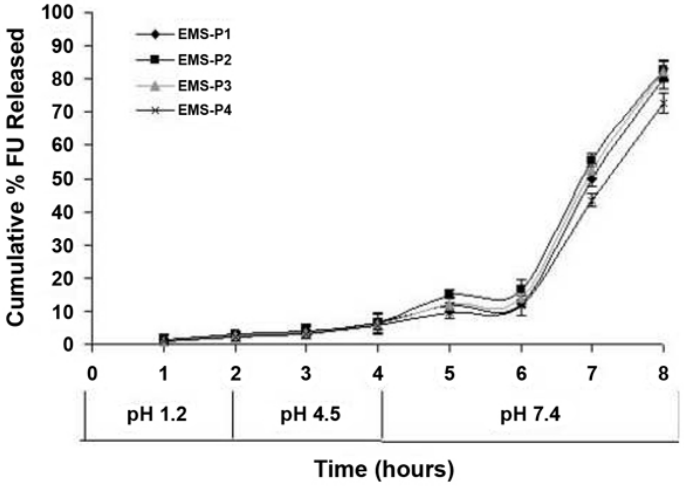

Figure 3. Percentage cumulative in vitro FU release from different Eudragit-coated pectin microspheres in simulated gastrointestinal fluids of different pH. Values are average of 3 readings ± standard deviation. FU indicates 5-fluorouracil; EMS, Eudragit-coated pectin microspheres.

performed in albino rats in order to establish its targeting potential in colon. The results indicated that maximum (66.7% ± 3.2%) concentration of FU was observed after 2 hours in stomach following oral administration of plain FU and in subsequent hours; much less drug reached the small intestine, and no drug was found in colon. Only 24.7% ± 1.4% of total drug load of conventional dosage form reached the colon after 8 hours. The pectin microspheres were found to release relatively better than plain drug but they also released maximum 66.3% ± 2.0% drug in the small intestine.

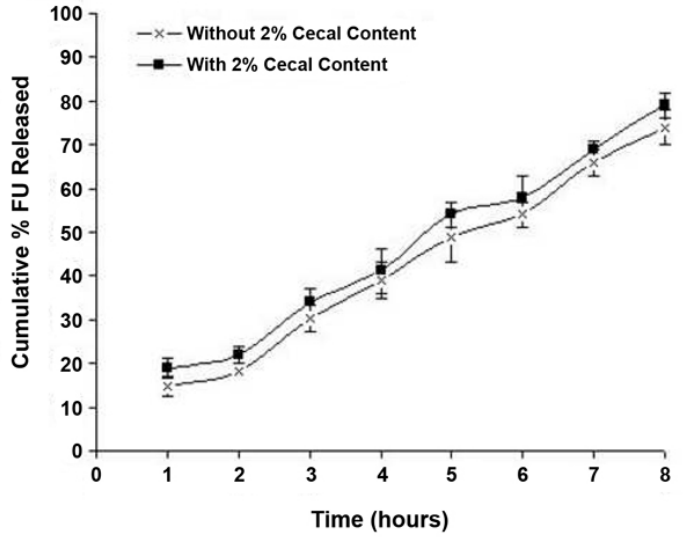

Figure 4. Effect of cecal content on percentage FU release from (PMS-P4) pectin microspheres in simulated colonic fluid (pH 7.4) with and without 2% cecal content. Values are average of 3 readings ± standard deviation. FU indicates 5-fluorouracil; PMS, pectin microspheres.

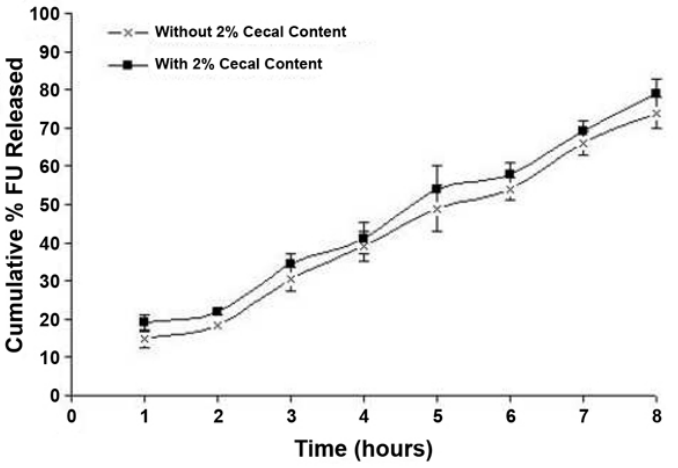

Figure 5. Effect of cecal content on percentage FU release from Eudragit-coated pectin microspheres (EMS-P4) in simulated colonic fluid (pH 7.4) with and without 2% cecal content. Values are average of 3 readings ± standard deviation. FU indicates 5-fluorouracil; EMS, Eudragit-coated pectin microspheres.

The Eudragit-coated pectin microspheres were observed relatively intact in the upper part of the GI tract. Approximately 2% to 3% of total drug load was released during its transit through the upper GI tract (2-6 hours) because of the leaching process. After 6 to 8 hours, the maximum percentage of drug was observed in the colon, and no drug was found in the stomach and small intestine (Figure 7). This release pattern of FU from pectin microspheres in colon could be due to the swelling property of the polysaccharide (pectin), so the appropriate amount of drug could not reach in the colon. The release pattern of FU from Eudragit-coated pectin microspheres can be understood by the protective nature of Eudragit coating delaying/minimizing drug release in upper

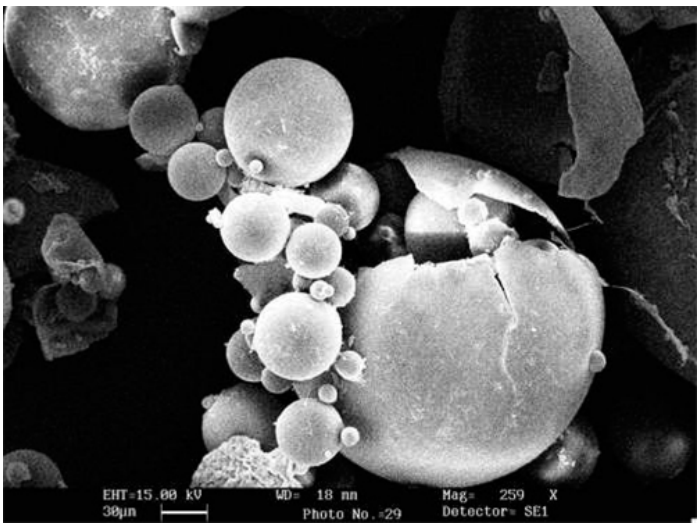

Figure 6. Scanning electron photomicrograph of Eudragit-coated pectin microspheres (EMS-P4) after in vitro release study in simulated colonic fluid.

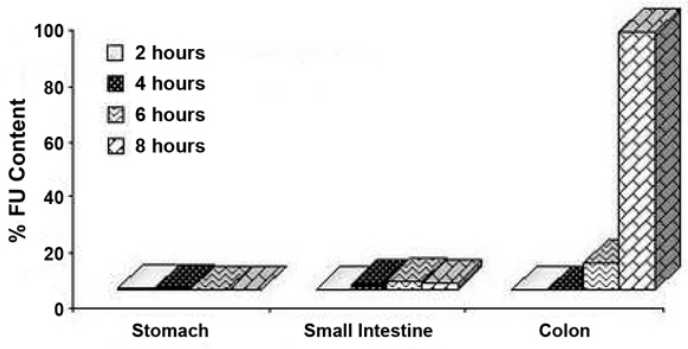

Figure 7. FU content in isolated organs of albino rat after oral administration of Eudragit-coated pectin microspheres (EMS-P4). FU indicates 5-fluorouracil.

GI tract. The drug FU was released from Eudragit-coated pectin microspheres only after reaching the colon owing to the dissolution of Eudragit-coated pectin microspheres in colonic pH and reaction with microbial flora residing in the colon.

CONCLUSION

The designed site-specific delivery of 5-FU from the system may reduce the side effects of the drug caused by its absorption from the upper part of the GI tract when given in conventional dosage forms such as tablets and capsules. The experimental results demonstrated that Eudragit-coated pectin microspheres have the potential to be used as a drug carrier for an effective colon-targeted delivery system.

ACKNOWLEDGMENTS

One of the authors, Mr Amol Paharia, acknowledges the financial support received from University Grants Commission, New Delhi, India. The authors gratefully acknowledge Head, Department Pharmaceutical Science, Dr. Hari Singh Gour University, Sagar, India, for providing the necessary facilities to carry out the research work. The authors also wish to thank Dr Rajesh Pathania, All India Institute of Medical Sciences, New Delhi, India, for providing SEM facility.

REFERENCES

1. Riley SA, Turnberg LA. Sulphasalazine and aminosalicylate in the treatment of inflammatory bowl disease. *Q J Med.* 1990;75: 561–562.

2. Bartalsky A. Salicylazobenzoic acid in ulcerative colitis. *Lancet.* 1982;319:960–964.

3. Ashford M, Fell J, Attwood D, Sharma H, Woodhead P. In vitro investigation into the suitability of pH dependent polymer for colonic targeting. *Int J Pharm.* 1993;95:193–199.

4. Marvola M, Nykanen P, Rautio S, Isonen N, Autere AM. Enteric polymer as binder and coating material in multiple unit site-specific drug delivery systems. *Eur J Pharm Sci.* 1999;7:259–267.

5. Gazzaniga A, Busetti C, Sangali ME, Giordana ME. Time-dependent oral delivery system for colonic targeting system for the colon targeting. *STP Pharma Sci.* 1995;5:83–88.

6. Gazzaniga A, Iamartino P, Maffione G, Sangal ME. Oral delayed release system system for colonic specific delivery. *Int J Pharm.* 1994;108:77–83.

7. Hovgaard L, Brondsted H. Dextran hydrogels for colon-specific drug delivery. *J Control Release.* 1995;36:159–166.

8. Watts PJ. Lllum L. Colonic drug delivery. *Drug Dev Ind Pharm.* 1997;23:893–913.

9. Ashford M, Fell T. Targeting drugs to colon: delivery system for oral administration. *J Drug Target.* 1994;2:241–258.

10. Kudela V. Hydrogels. In: Mark HF, Bikales N, Overberger CG, Menges G, Kroschwitz JI, eds. *Encyclopedia of Polymer Science and Engineering.* vol. 7. New York, NY: John Wiley & Sons; 1987:703–807.

11. Graham NB, McNeill ME. Hydrogels for controlled drug delivery. *Biomaterials.* 1984;5:27–36.

12. Tiwari SB, Murthy TK, Pai MR, Mehta PR, Chowdary PB. Controlled release formulation of tramadol hydrochloride using hydrophilic and hydrophobic matrix system. *AAPS PharmSciTech.* 2003;4:E31.

13. Krusteva S, Lambov N, Velinov G. Pharmaceutical investigation of a bioerodible nystatin system. *Pharmazie.* 1990;45:195–197.

14. Naggar VF, El-Khawas M, Ismail FA, Boraie NA. Pectin, a possible matrix for oral sustained release preparations of water-soluble drugs. *STP Pharma Sci.* 1992;2:227–234.

15. Rubinstein A, Radai R, Ezra M, Pathak S, Rokem JM. In vitro evaluation of calcium pectinate: a potential colon-specific drug delivery carrier. *Pharm Res.* 1993;10:258–263.

16. Esposito E, Cortesi R, Luca G, Nastruzzi C. Pectin based microspheres: a preformulatory study. *Ann N Y Acad Sci.* 2001;944: 160–179.

17. Lorenzo-Lamosa ML, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Design of microencapsulated chitosan microspheres for colonic drug delivery. *J Control Release.* 1998;52:109–118.

18. Indian Pharmacopoeia. *Disintegration and Dissolution*. Delhi, India: Controller of Publication - Ministry of Health and Family Welfare; 1996: A80–A86.

19. Rai G, Jain SK, Agrawal S, Pancholi SS, Agrawal GP. Chitosan hydrochloride based microspheres of albendazole for colonic drug delivery. *Pharmazie.* 2005;60:131–134.

20. Van den Mooter G, Samyn C, Kinget R. The relation between swelling properties and enzymatic degradation of azo polymers designed for colon-specific drug delivery. *Pharm Res.* 1994;11:1737–1741.

21. Pongpaibul Y, Price JC, Whitworth CW. Preparation and evaluation of controlled release indomethacin microspheres. *Drug Dev Ind Pharm.* 1984;410:1597–1616.

22. Maia JL, Santana MHA, Re MI. The effect of some processing conditions on the characteristics of biodegradable microspheres obtained by an emulsion solvent evaporation process. *Braz J Chem Eng.* 2004;21:1–12.

23. Yang Q, Owusu-Ababio G. Biodegradable progesterone microsphere delivery system for osteoporosis therapy. *Drug Dev Ind Pharm.* 2000;26:61–70.