In Vitro Evaluation of Calcium Pectinate: A Potential Colon-Specific Drug Delivery Carrier

Abraham Rubinstein,[1,3] Raphael Radai,[1] Miriam Ezra,[1] Seema Pathak,[1] and J. Stefan Rokem[2]

Received January 28, 1992; accepted August 3, 1992

Calcium pectinate (CaP)—the insoluble salt of pectin—can potentially be used as a colon-specific drug delivery system. The use of CaP as a carrier was based on the assumption that, like pectin, it can be decomposed by specific pectinolytic enzymes in the colon but that it retains its integrity in the physiological environment of the small bowel. The biodegradation of the carrier was characterized by monitoring the percent cumulative release of the insoluble drug indomethacin, incorporated into pectin or CaP matrices. Compressed tablets of pectin and indomethacin were analyzed for degradation in the presence of Pectinex 3XL, a typical pectinolytic enzyme mixture, and in the presence of the human colonic bacterium *Bacteroides ovatus*. The degradation of CaP–indomethacin tablets was assessed in the presence of Pectinex 3XL and in rat cecal contents. The release of indomethacin was significantly increased (end-time percentage cumulative release vs control) in the presence of Pectinex 3XL (89 ± 20 vs 16 ± 2 for CaP tablets), *Bacteroides ovatus* (12 and 22 vs 5.2 for pectin tablets), and rat cecal contents (61 ± 16 vs 4.9 ± 1.1 for CaP tablets). The weight loss of tablet mass was significantly higher (end-time dry weight vs control) in the presence of Pectinex 3XL (0 vs 75 ± 6% of initial weight for CaP tablets). These findings indicate the potential of CaP, compressed into tablets with insoluble drug, to serve as a specific drug delivery system to the colon.

KEY WORDS: colon-specific drug delivery; colon; pectin; calcium pectinate; matrix system.

INTRODUCTION

The spatial placement of drugs into selected locations in the alimentary canal is difficult because of physiological constraints, namely, motility and mucus turnover (1). Localizing orally administered drugs in the colon is particularly complicated because it is difficult to predict the exact residence time of solid dosage forms in the stomach and small intestine. The residence time of the drug depends on parameters such as fed/fasted patterns, meal compositions, and intensity of peristalsis (or migrating motor complex) (2,3). As a result, a solid dosage form may stay in the stomach from a few minutes to 8 hr and in the small bowel from 3 to 5 hr (4).

Drugs are commonly delivered to the large bowel by coating them with polymeric substances such as cellulose derivatives (5) or acrylic polymers (6). These polymers protect the drug against the acidic environment of the stomach; they swell while traveling down the intestine. It is postulated that the drug is released from the coated dosage form when it arrives at the distal ileum (5,6). The performance of such colonic delivery systems may be limited by GI motility and pH variations. To overcome these limitations, new delivery systems with alternative drug release mechanism have been suggested (7). Most strategies were based on the assumption that high enzymatic activity of the rich microbial flora in the colon would act as a release trigger.

Typical demonstrations of orally administered specific colonic drug delivery are the various pharmaceutical preparations of 5 amino salicylic acid (5-ASA). Either 5-ASA can be delivered as one of its prodrugs (e.g., sulfasalasine or azodisalicylate: olsalazine), which are specifically azo-reduced by colonic bacteria, locally releasing the desired active moiety 5-ASA (8), or it can be coated in tablet form with a protective polymer (6). Glycoside prodrugs of steroids can be used similarly. Galactose, glucose, or cellobiose, known to serve as substrates for colonic bacteria, have been linked to selected steroid drugs, commonly used in the treatment of inflammatory bowel disease. These carbohydrate carriers were selectively degraded in the cecum of a guinea pig (9,10). In other studies, the dextran ester prodrugs of naproxen and ketoprofen were hydrolyzed in the pig, releasing the parent drug primarily in the cecum and in the colon (11,12). Alternatively, it has been suggested to use a biodegradable polymer that would adhere to the intestinal mucosa, after which a specific release of 5-ASA occurs (13), or a biodegradable carrier with the potential of carrying a number of drug molecules to the colon (14,15).

Pectins, soluble nonstarch polysaccharides, are extracted from plant primary cell walls and used as gelling and thickening agents in food technology. They are predominantly linear polymers of mainly α-(1-4)-linked D-galacturonic acid residues interrupted by 1,2-linked L-rhamnose residues. Depending on the plant source and preparation, they contain varying degrees of methyl ester substituents (16). Pectins are degraded by enzymes from various microbial sources, especially fungi and bacteria, and among the latter, also by human colonic bacteria (17,18). We therefore suggest pectin as a colon specific drug carrier, because of its expected persistence as a solid dosage form in the physiological environment of the stomach and small intestine. Since natural pectin is water soluble, it might not protect its load successfully. However, a better shield could be obtained by reducing the solubility of pectin by forming its calcium salt, calcium pectinate (CaP). The hypothesis that CaP is also degraded by the pectinolytic enzymes of the bacterial flora of the large bowel was tested (a) in buffer solutions containing pectinolytic enzymes, (b) in medium containing *Bacteroides ovatus* of human colon origin, and (c) in buffer solutions containing rat cecal contents. To assess its specific degradability, pectin or CaP was formulated into indomethacin tablets.

MATERIALS AND METHODS

All materials and reagents were purchased from Sigma, St. Louis, MO, unless otherwise mentioned in the text. All solvents were analytical or HPLC grade.

[1] The Hebrew University of Jerusalem, School of Pharmacy, P.O. Box 12065, Jerusalem 91120, Israel.

[2] The Hebrew University of Jerusalem, Department of Applied Microbiology, Institute of Microbiology, P.O. Box 1172, Jerusalem 91010, Israel.

[3] To whom correspondence should be addressed.

0724-8741/93/0200-0258$07.00/0

Pectin and Calcium Pectinate Formulations with Indomethacin

Calcium Pectinate Preparation. One hundred milliliters of 4% (w/v) pectin (apple, D.E. 39%, BDH, UK) solution was prepared in double-distilled water. Gentle alkaline deesterification was performed by adjusting the pH of the pectin solution to 8.0 with 0.1 *N* NaOH, followed by stirring at 4°C over 48 hr as described elsewhere (19,20). Deesterification was verified by titrating the residual free carboxyl groups (16). The obtained value was compared to the total galacturonic acid value as determined by the carbazole reaction, using D-galacturonic acid as a standard (21). It was found that the final degree of esterification averaged 16%. After dilution to a final pectin concentration of 1.5% (w/v), the pH was reduced to 4.5 with 0.1 *N* HCl, the solution was heated to 80°C, and 200 mL of 1.32% (w/v) $CaCl_2$ aqueous solution was added (22). The gel thus formed was cooled to room temperature, collected, and mounted in 20-cm dialysis bags (Spectra/por, 6 × 30 mm; MW cutoff, 12,000–14,000; Spectrum, Los Angeles, CA). Each bag was immersed in 2.5 L of double-distilled water, which was stirred overnight at 200 rpm. The viscosity of 1% (w/w) rinsed product mixture with purified water was then measured (Brookfield LVT viscometer, equipped with a small volume adaptor) at 22°C and 5 rpm. A typical value for the CaP was 24.3 ± 2.1 cps (mean of three batches), compared to a viscosity of 0.6 ± 0.5 cps of the 1% (w/w) aqueous pectin solution. The rinsed product was lyophilized, and complete dehydration was validated by no weight loss after further drying. The resulting dry CaP was collected, comminuted to a fine powder, and sealed until further processing. The total amount of calcium prior and after the calcification of the deesterified products was monitored using the atomic absorption method.

Preparation of Pectin or Calcium Pectinate Tablets with Indomethacin. Indomethacin is a water-insoluble drug which we have used as a drug marker in the *in vitro* drug release experiments (see also Ref. 15). Pectin or CaP was sieved (40 mesh) separately and mixed with indomethacin at a concentration of 1 or 10% (w/w), respectively. These mixtures were then pressed into tablets at 7 kgP (Schleunigen-2E/205, Switzerland) using a Perkin Elmer manual press equipped with 10-mm tools. Each tablet weighed 200 mg; thus each pectin or CaP tablet contained 2 or 20 mg indomethacin, respectively.

Degradation of Pectin and Calcium Pectinate Tablets by Pectinolytic Enzymes

The pectin tablets were immersed separately in 25 mL of citrate buffer (pH 3.5) containing a mixture of pectinolytic (polygalacturonase, pectinesterase, pectintranseliminase, and hemicellulase) enzymes [Pectinex 3XL, 12 or 120 ferment depectinization units (FDU)/mL;[4] Novo Industri A/S, Denmark]. Each experiment was performed in a sealed, 50-mL, dark glass bottle kept stationary at 37°C, until no tablet was observed (2–6 hr). Parallel systems containing the same volume of citrate buffer at the same pH value, but without the pectinolytic enzyme, served as controls and were treated identically. The stationary condition was required to avoid drug release as a result of pectin dissolution in the test medium. After gentle shaking to disperse the released indomethacin in the medium, 1-mL samples were withdrawn at predetermined time intervals and assayed spectrophotometrically for indomethacin content. Aliquots of identical fresh medium were added to replace each withdrawn volume. The same experimental protocol was repeated for the CaP tablets with the following changes: (a) a single enzyme concentration was used (120 FDU/mL), (b) the study was performed with gentle stirring (80 rpm in a shaking bath), and (c) the experiments lasted 72 hr. The CaP experiments were performed with gentle shaking, since in preliminary studies we found that under stationary conditions higher concentrations of Pectinex 3XL were required. Each experiment was performed three times.

Spectrophotometric Analysis of Indomethacin. Each 1-mL sample was adjusted to a final volume of 10 mL with phosphate buffer, pH 8.0. After the indomethacin was completely dissolved, the absorbance was measured at 320 nm (Uvikon 930, Kontron Instruments, Switzerland); the same medium including the pectinolytic enzyme but without the drug marker was used as a blank. The stability of the indomethacin in the alkaline solutions was ascertained by monitoring their UV shift at 320 nm during the course of the analysis.

Weight Loss of the Tablet Mass. In three experiments three CaP–indomethacin tablets from three batches were incubated with and without Pectinex 3XL (120 FDU). Each tablet was dried and weighed prior to and after 72 hr of incubation.

Rat Cecal Contents Studies

To induce the pectinolytic enzymes postulated to be in the cecum, five Sabra rats (23), weighing 200–300 g and maintained on a normal diet, were intubated with Teflon tubing 3 days before the release experiments were initiated. Each day 1 mL of 2% (w/v) pectin aqueous solution was directly administered to the rats' stomachs through the Teflon tubing, which was then removed. Thirty minutes before the drug release experiments were initiated the rats were sacrificed with an i.p. injection of an overdose of sodium pentobarbital. The ceci were exteriorized, ligated at two ends (2-cm distance), cut loose, and immediately removed from the rat bodies. The cecal bags thus formed were then opened; their contents were individually weighed and then pooled and suspended in phosphate-buffered saline (PBS; pH 7) to give a final cecal dilution of 1.25% (w/v). As the cecum is a naturally anaerobic environment, we maintained anaerobiosis by carrying out this last step under CO_2.

This rat study protocol was approved by the institutional Animal Care and Use Committee of the Hebrew University, Faculty of Medicine and Hadassah Medical Organization.

Indomethacin Release Experiments of Calcium Pectinate Tablets in Rat Cecal Contents. The experiments were performed in 100 mL PBS, pH 7, under a CO_2 atmosphere, at 37°C, over a period of 25 hr, with or without (control) the addition of pooled rat cecal contents, in sealed glass vials

[4] The FDU is determined by measuring the reduction of the viscosity of a solution of pectic acid at pH 3.5 and 20°C.

which were shaken at 80 rpm. One-milliliter samples were withdrawn in duplicate at predetermined time intervals for indomethacin analysis. Each experiment was repeated at least three times. Each time samples were withdrawn, 2 mL of PBS was added back to the system to maintain a constant volume and pH.

Indomethacin HPLC Analysis. One-milliliter samples were acidified with 200 μL 0.4 *N* HCl, and extracted with 1 mL ethyl acetate containing 0.2 mg% flufenamic acid as an internal standard. The mixture was vortexed and then centrifuged for 3 min at 3400 rpm. Five hundred-microliter aliquots of the organic phase were evaporated, and the residue was redissolved in a 50:50 mixture of acetonitrile and phosphate buffer, pH 7.5. Twenty microliters of the solution was injected into the HPLC system (Hewlett Packard 1050 pumping system, Jasco 875 intelligent UV/vis detector, Hewlett Packard 3365 ChemStation data analyzer, and Hewlett Packard analog-digital 35900C Dual Channel Interface Convertor). The wavelength used was 280 nm, and the column was 5-μm, 250 × 4.6-mm RP-18 (LiChroCART 250-4,E. Merck, Germany).

Microbiological Section

Bacteroides ovatus *Batch Culture Experiments. B. ovatus* ATCC 8483, a human colonic anaerobe able to hydrolyze pectin (16), was obtained from the American Type Culture Collection (Rockville, MD).

A modular fermentor (BioFlo. C30, Bench-Top Chemostat, New Brunswick Scientific, NJ) containing 350 mL of minimal liquid medium (MP-7) with 0.5% pectin as the sole carbon source was used (24). The drug release experiments were initiated when the medium inoculated with an overnight growth of *B. ovatus* had reached an optical density of 0.3 at 650 nm (LKB spectrophotometer). The pectin–indomethacin tablet was introduced into the system via a specially fitted sterilizable 316 stainless-steel dissolution basket equipped with a 35-mesh sieve (Fig. 1). The agitation rate of the liquid culture was adjusted prior to the initiation of the dissolution study (200 rpm) and double-checked with electronic tachometer.

B. ovatus grows optimally at pH 6.2, close to the natural pH of the human colon. The pH of the growth medium was maintained at 6.2 by the addition of 1 *N* NaOH through a pH-controlled pump (Modcon, Haifa, Israel). Throughout the experiment anaerobiosis was maintained by bubbling CO_2 at a rate of 0.2 vol of CO_2/vol of broth/min. Samples (3–5 mL) were withdrawn at predetermined time intervals through the sampling port. Control experiments were performed using the same setup without bacteria but with all other parameters equal. Each experiment lasted 4 hr due to bacterial viability limitation, i.e., the logarithmic growth phase could be maintained over 4 hr only.

The spectrophotometric analysis of the indomethacin was performed as described above.

Statistical Analysis

To analyze the significance of the differences in the amounts of indomethacin released in the probe systems vs the controls, a one-way paired *t* test was done at each time point. Use of a paired *t* test was necessary because we wished to compare the amounts of drug released from a given batch of carrier matrices at each time point simultaneously for the experimental and control groups. A two-tailed *t* test was unnecessary because the specific degradation of pectin and calcium pectinate was always expected to result in an increase in the release of the drug in the presence of the pectinolytic enzymes, *B. ovatus,* and the rat cecal contents. A difference was considered to be statistically significant when the *P* value was less than 0.01.

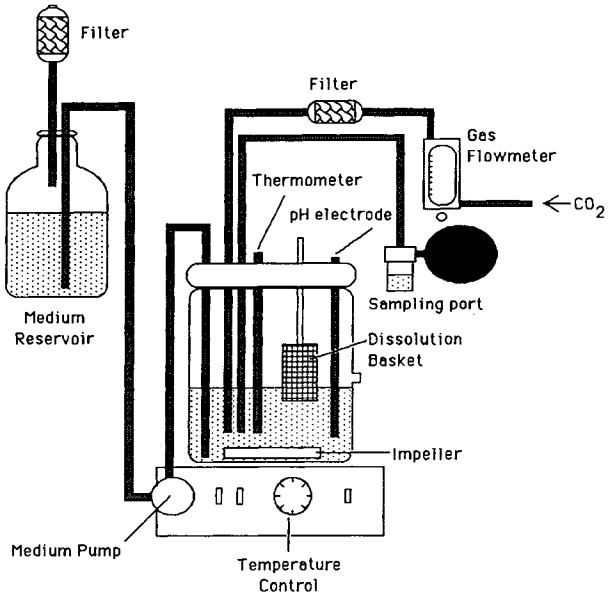

Fig. 1. Schematic presentation of the modular fermentor used as a batch culture system for the drug release experiments in the presence of *B. ovatus*, under anaerobic conditions.

RESULTS

Three methods were used to evaluate the potentials of pectin and CaP to serve as specific drug delivery carriers to the colon. The enzymatic experiments performed with compressed pectin–indomethacin tablets are summarized in Fig. 2. The degradation of the pectin tablets was assessed by measuring the percentage cumulative release of indomethacin in citrate buffer, pH 3.5, in the presence or absence (control) of the pectinolytic enzyme mixture Pectinex 3 XL. The experiments lasted without stirring until the tablet in the presence of the enzymes had completely disappeared, yielding full release of their drug load. Figure 2 also shows that over a period of 6 hr, there was no drug released in the control systems (no enzymes). The release rate in the presence of 120 FDU/mL was higher and the release process was completed within 2 hr, compared with 8 hr, when 12 FDU/mL was used. The higher concentration of the enzyme resulted in a higher release rate and, hence, faster completion of the release process (2 hr for 120 FDU/mL and 8 hr for 12 FDU/mL).

The results of drug release from the pectin tablets in the presence of the anaerobic bacterium *B. ovatus* are shown in Fig. 3. The percentage cumulative drug release values at the end of each experiment were 12.1 and 21.6% for two independent experiments, compared with only 5.2% for the con-

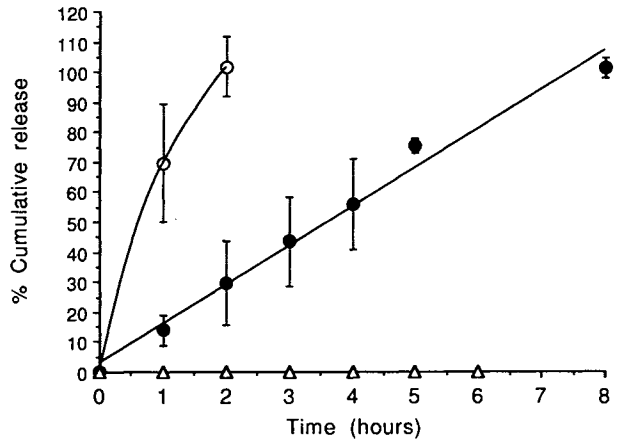

Fig. 2. Percentage cumulative amounts of indomethacin released from pectin tablets, with the pectinolytic enzymes mixture Pectinex 3XL in citrate buffer, pH 3.5, at the following concentrations: 120 FDU/mL (open circles), 12 FDU/mL (filled circles), and without enzymes (open triangles). Data shown are the mean of three sets of experiments ± SD.

trol (two experiments). The initial release of indomethacin into the media can be explained by diffusion from the surface of the tablets. The matrix erosion as indicated by the cumulative drug release observations was verified by weighing the pectin tablets after drying them at the end of each experiment (data not shown).

Figure 4 shows the results obtained from CaP tablets which were examined with and without the enzyme mixture. When incubated in citrate buffer (pH 3.5) without enzyme, the CaP tablets retained most of the drug, and after 72 hr, only 16.2 ± 2.2% of the drug was released. In the presence of the enzyme mixture the entire drug content was released within 6 hr, following a linear release kinetics (Fig. 4, bottom). Here the tablets' degradation was also verified by weight loss of the tablet mass. After 6 hr no tablets were left in the presence of the enzyme mixture and the percentage cumulative drug release was 89.0 ± 20.0. Since in the presence of enzymes the release of indomethacin was completed within 6 hr, data are presented for both probe and control experiments at that specific time point. However, the CaP

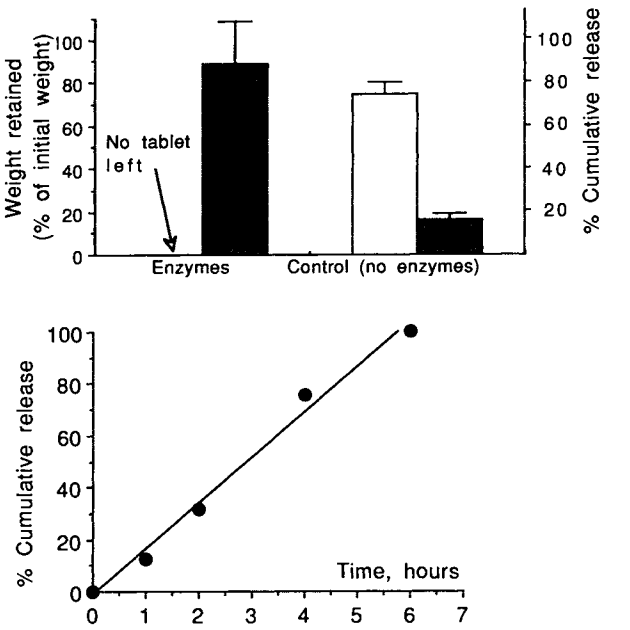

Fig. 4. Weight loss of CaP tablet mass (white columns) after 6 hr of incubation with (left) and without (right) 120 FDU/mL Pectinex 3XL, and the resulting total percentage cumulative amounts of indomethacin released (hatched columns) after 6 hr (n = 3 ± SD). The plot at the bottom is a typical indomethacin release profile from the CaP tablet showing the linearity of the drug release in the presence of the enzymes mixture.

tablets did not erode even after 72 hr without the enzyme mixture.

Finally, the degradation of CaP–indomethacin tablets in the presence of rat cecal contents was analyzed after enzyme induction by gastric intubation of pectin solutions. The indomethacin release profiles are summarized in Fig. 5. In every case, the drug levels were significantly higher in the presence of rat cecal contents than in the control systems. At the end of the experiment (after 24 hr), the percentage cumulative release in the control medium was 4.9 ± 1.1, com-

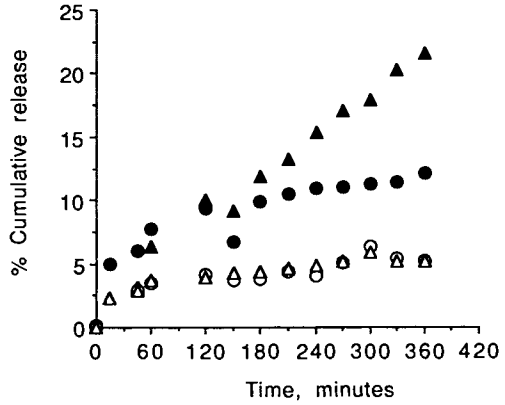

Fig. 3. Data from two experiments showing percentage cumulative amounts of indomethacin released from pectin tablets in batch culture medium in the presence of *B. ovatus* (filled circles and triangles) or without bacteria (open circles and triangles), at pH 6.2.

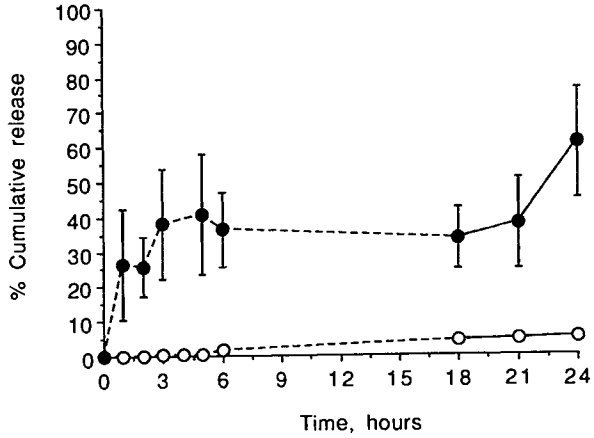

Fig. 5. Percentage cumulative amounts of indomethacin released from CaP tablets in PBS medium, pH 7.0, with (filled circles) and without (open circles) rat cecal content. Data are the mean of three experiments ± SD.

pared with 60.8 ± 15.7 obtained in the presence of rat cecal contents.

DISCUSSION

Pectin is biodegradable in the human colon by bacteria containing pectinolytic enzymes (18,25), and therefore it could serve for colon-specific drug delivery by using tablets of pectin and the poorly water-soluble drug indomethacin. Based on the assumption that the appearance of insoluble drug in the outer medium is caused mostly by matrix erosion, the tablets were shown to be biodegradable. With the pectinolytic enzyme mixture Pectinex 3XL, at a concentration of 120 FDU/mL under stationary conditions, the drug was completely released in 2 hr. In the absence of enzyme, there was no degradation and no drug released into the dissolution medium (Fig. 2). At the lower enzyme concentration of 12 FDU/mL, the rate of drug release was lower and linear. This second drug release profile included sufficient data to demonstrate that the indomethacin release kinetics is pseudozero, typical for the erosion of such matrix systems (26). When analyzed in the presence of *B. ovatus,* enhanced degradation of the pectin tablets and drug release was observed over a period of 6 hr. However, the total cumulative indomethacin release in the presence of *B. ovatus* was much lower than that in the presence of the enzyme mixture Pectinex 3XL. This difference can be explained by low enzyme activity as a result of the low bacterial biomass concentration (OD_{650} of 0.3).

Specific biodegradability is not sufficient for creating a drug carrier that can be targeted to the colon. The drug carrier must be designed to protect its drug load in the physiological environment of the stomach and in most of the small intestine. However, pectin is a soluble polysaccharide and its solubility must be decreased. Therefore, we prepared calcium pectinate (CaP), the calcium salt of the pectin, thus reducing its solubility. Ca^{2+} ions cause pectin solutions to gel, forming an "egg box" configuration (27), with a consequent sharp decline in the solubility of the pectin. Ideally, the biodegradability of the pectin would be maintained in spite of the reduction of its solubility. As shown in Fig. 5, indomethacin release was enhanced by the presence of rat cecal contents in the PBS buffer. This is similar to the results obtained with tablets formulated of indomethacin and cross-linked chondroitin sulfate, as reported previously (15). However, the indomethacin was released more slowly from the cross-linked chondroitin sulfate matrices than from the CaP matrices. In the case of cross-linked chondroitin sulfate tablets in the presence of rat cecal contents, there was no significant drug release up to 6–9 hr of incubation (15), whereas for CaP tablets, a significant enhancement in drug release was observed immediately (Fig. 5).

The use of a highly soluble drug in a matrix system would make it hard to distinguish between simple diffusion of the drug and erosion of the drug carrier. Therefore, in order to analyze the specific degradation of a drug carrier, it was necessary to use a water-insoluble drug model such as indomethacin. Although three pH values were used (3.5, 6.2, and 7), indomethacin is insoluble in pH 3.5 and 6.2 and sparingly soluble at pH 7 (the last two values are similar to the physiological pH of the human colon).

Since the cumulative drug levels in the presence of rat cecal contents were significantly higher than in the control systems (Fig. 5), we assume that CaP is biodegradable in the rat cecum, presumably by pectinolytic enzymes of bacterial origin. This assumption is supported by the observation that CaP is biodegradable by a mixture of pectinolytic enzymes Pectinex 3XL (120 FDU) (Fig. 4). In the presence of the enzymes, the indomethacin was completely released from the CaP tablets within 6 hr, while in the control systems only 16.2 ± 2.2% of the drug was released over 72 hr and the drug release observations were verified by the weight loss of the tablet mass (Fig. 4). This shows that CaP tablets, although enzymatically degraded by the pectinolytic enzymes mixture, might be more stable in the physiological environment of the upper alimentary canal, as a result of its lower solubility, thus serving as colonic delivery system. It is worth noting that in the presence of rat cecal contents, two patterns of release kinetics can be observed. The first, which lasts 6 hr, is the result of biodegradation of CaP tablets (as compared to the control system). The second is presumably a result of a drug diffusion from the tablets debris, once the tablets were broken down. Since at pH 7, indomethacin is more soluble than at pH 6.2 or 3.5, the increase in the exposed surface area is probably the cause for the second elevation in cumulative drug release.

The degradation of both pectin and CaP tablets was very efficient with Pectinex 3XL (Figs. 2 and 4) relative to the biodegradation process in the presence of *B. ovatus* grown at pH 6.2, a pH considered to be in the range of the human colon (pH 5.5–7.0) (Fig. 3) and rat cecal contents (Fig. 5). The lower efficiencies of the latter two systems are due to the relatively low enzyme levels in our rat cecum model and the bacteria, compared with Pectinex 3XL, an enzyme mixture not found naturally in the gut. This is a problem which must be thought of if rational design of colon-specific delivery system is considered. The long residence time of large bowel content is a possible compensation for its relatively low enzyme activity in terms of biodegradable solid dosage forms.

The above results show that it may be possible to use tablets formulated of drug and CaP as colon-specific delivery systems. However, it is as yet unclear how similar the microbial populations of the rat cecum and the human colon are, although some studies indicate similar levels of β-glucosidases in both species (28). The formulation technique presented here may be useful in the local treatment of a variety of large bowel diseases. Drugs such as steroids or salicylate derivatives such as 5-amino salicylic acid, used for the treatment of inflammatory bowel diseases, or pivanerium bromide (29), a drug for the local treatment of irritable bowel syndrome, may be incorporated into CaP formulations. Most previous carriers were prodrugs used to deliver individual drug molecules to the colon. Saffran and co-workers (14) made the first attempt to develop a microbially controlled drug delivery system to the colon, in which the drug itself is delivered, and not its prodrug, by using azopolymers to coat insulin and lysine–vasopressin. If, in fact, protein drugs are less susceptible to proteolytic degradation in the colon than in the stomach (30), then CaP could serve as a carrier for these drugs as well.

ACKNOWLEDGMENTS

This work was supported by the Robert Szold Institute for Applied Science of the PEF-Israel Endowment Funds Inc. The results reported here are included in the dissertation projects of Raphael Radai and Miriam Ezra as partial fulfillment of their Ph.D. and M.Sc. degree requirements at the Hebrew University of Jerusalem. The study has been presented in part at the 18 International Symposium on Controlled Release of Bioactive Materials, 1991.

REFERENCES

1. C.-M. Lehr, F. G. P. Poelma, H. E. Junginger, and J. J. Tukker. An estimate of turnover time of intestinal mucus gel layer in the rat *in situ* loop. *Int. J. Pharm.* 70:235–240 (1991).
2. J. N. Hunt and M. T. Knox. A relation between the chain length of fatty acids and the slowing of gastric emptying. *J. Physiol. (London)* 194:327–336 (1968).
3. J. H. Meyer, J. B. Dressman, A. Fink, and G. L. Amidon. Effect of size and density on canine gastric emptying of non digestible solids. *Gastroenterology* 89:805–813 (1985).
4. S. S. Davis, J. G. Hardy, and J. W. Fara. Transit of pharmaceutical dosage forms through the small intestine. *Gut* 27:886–892 (1986).
5. D. S. Levine, V. A. Raisys, and V. Ainardi. Coating of oral beclomethasone dipropionate capsules with cellulose acetate phthalate enhances delivery of topically active antiinflammatory drug to the terminal ileum. *Gastroenterology* 92:1037–1044 (1987).
6. S. N. Rasmussen, S. Rondesen, E. F. Hvidberg, S. Honore Hansen, V. Binder, S. Halskov, and H. Flachs. 5-Aminosalicylic acid in a slow-release preparation: Bioavailability, plasma level, and excretion in humans. *Gastroenterology* 83:1062–1070 (1982).
7. A. Rubinstein. Microbially controlled drug delivery to the colon. *Biopharm. Drug Dispos.* 11:465–475 (1990).
8. U. Klotz. Clinical pharmacokinetics of sulphasalazine, its metabolites and other prodrugs of 5-aminosalicylic acid. *Clin. Pharmacokin.* 10:285–302 (1985).
9. D. R. Friend and G. W. Chang. Drug glycosides: Potential prodrugs for colon-specific drug delivery. *J. Med. Chem.* 28:51–57 (1985).
10. T. N. Tozer, J. Rigod, A. D. McLeod, R. Gungon, M. K. Hoag, and D. R. Friend. Colon-specific delivery of dexamethasone from a glucoside prodrug in the guinea pig. *Pharm. Res.* 8:445–454 (1991).
11. C. Larsen, E. Harboe, M. Johansen, and H. P. Olsen. Macromolecular prodrugs. XVI. Colon-targeted delivery—comparison of the rate of release of naproxen from dextran ester prodrugs in homogenates of various segments of the pig gastrointestinal tract. *Pharm. Res.* 6:995–999 (1989).
12. C. Larsen and B. H. Jensen. Stability of ketoprofen-dextran ester prodrugs in homogenates of various segments of the pig GI tract. *Acta Pharm. Nord.* 3:41–44 (1991).
13. P. Kopeckova and J. Kopecek. Release of 5-aminosalicylic acid from bioadhesive N-(2-hydroxypropyl)methacrylamide copolymers by azoreductases *in vitro*. *Makromol. Chem.* 191:2037–2045 (1990).
14. M. Saffran, G. S. Kumar, C. Savariar, J. C. Burnham, F. Williams, and D. C. Neckers. A new approach to the oral administration of insulin and other peptide drugs. *Science* 233:1081–1084 (1986).
15. A. Rubinstein, D. Nakar, and A. Sintov. Colonic drug delivery: Enhanced release of indomethacin from cross-linked chondroitin matrix in rat cecal content. *Pharm. Res.* 9:276–278 (1992).
16. G. A. Towle and O. Christensen. Pectin. In R. L. Whistler and J. N. BeMiller (eds.), *Industrial Gums, Polysaccharidesm and Their Derivatives,* Academic Press, New York, 1973, pp. 429–461.
17. A. A. Salyers, J. R. Vercellotti, S. E. H. West, and T. D. Wilkins. Fermentation of mucin and plant polysaccharides by strains of *Bacteroides* from the human colon. *Appl. Environ. Microbiol.* 33:319–322 (1977).
18. S. C. Werch and A. C. Ivy. On the fate of ingested pectin. *Am. J. Digest. Dis.* 8:101–105 (1941).
19. J. F. Thibault and M. Rinaudo. Interactions of mono- and divalent counterions with alkali- and enzyme-deesterified pectins in salt-free solutions. *Biopolymers* 24:2131–2143 (1985).
20. R. P. Graham and A. D. Shepherd. Pilot plant production of low-methoxyl pectin from citrus peel. *Agr. Food Chem.* 1:993–1001 (1953).
21. T. Bitter and H. M. Muir. A modified uronic acid carbazole reaction. *Anal. Biochem.* 4:330–334 (1962).
22. D. A. Powell, E. R. Morris, M. J. Gidley, and D. A. Rees. Conformation and interactions of pectins. II. Influence of residue sequence on chain association in calcium pectate gels. *J. Mol. Biol.* 155:517–531 (1982).
23. I. Lutsky, F. Aizer, and N. Mor. The Sabra rat: Definition of a laboratory animal. *Isr. J. Med. Sci.* 20:603–612 (1984).
24. L. Hankin and D. Sands. In M. L. Speck (ed.), *Compendium of Methods for Microbiological Examination of Foods,* APHA Intersociety/Agency Committee on Microbiological Methods for Foods, 1976, pp. 208–214.
25. A. A. Salyers and J. A. Z. Leedle. Carbohydrate metabolism in the human colon. In D. J. Hentges (ed.), *Human Intestinal Microflora in Health and Disease,* Academic Press, New York, 1983, pp. 129–146.
26. J. Heller. Use of polymers in controlled release of active agents. In J. R. Robinson and V. H. Lee (eds.), *Controlled Drug Delivery Fundamentals and Applications,* Marcel Dekker, New York, 1987, pp. 179–212.
27. E. R. Morris, D. A. Powell, M. J. Gidley, and D. A. Rees. Conformation and interactions of pectins. I. Polymorphism between gel and solid states of calcium polygalacturonate. *J. Mol. Biol.* 155:507–516 (1982).
28. G. Hawksworth, B. S. Drasar, and M. J. Hill. Intestinal bacteria and the hydrolysis of glycosidic bonds. *J. Med. Microbiol.* 4:451–459 (1971).
29. S. Passaretti, M. Sorghi, E. Colombo, G. Mazzotti, A. Tittobello, and M. Guslandi. Motor effects of locally administered pinaverium bromide in the sigmoid tract of patients with irritable bowel syndrome. *Int. J. Clin. Phamracol. Ther. Tox.* 27:47–50 (1989).
30. P. Kopeckova, M. A. Longer, J. F. Woodley, R. Duncan, and J. Kopecek. Release of p-nitroaniline from oligopeptide side chains attached to N-(2-hydroxypropyl)methacrylamide copolymers during incubation with rat intestinal brush border enzymes. *Makromol. Chem. Rapid Commun.* 12:101–106 (1991).