Low Swelling, Crosslinked Guar and Its Potential Use as Colon-Specific Drug Carrier

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Purpose. (a) To reduce the swelling properties of guar gum (GG) by crosslinking it with glutaraldehyde (GA), while maintaining its degradation properties in the presence of typical colonic enzymes, (b) to characterize the modified GG and to examine its degradation properties *in vitro* and *in vivo*, and (c) to assess, by drug probes with different water solubilities, the potential of the crosslinked GG to serve as a colon-specific drug carrier.

Methods. GG was crosslinked with increasing amounts of GA under acidic conditions to obtain different products with increasing crosslinking densities. These products were characterized by measuring (a) their swelling properties in simulated gastric and intestinal fluids, (b) their crosslinking densities, (c) the release kinetics of three different drugs: sodium salicylate (SS), indomethacin (Indo) and budesonide (Bud) from the crosslinked products into buffer solutions, with or without a mixture of galactomannanase and α-galactosidase, and (d) their *in vivo* degradation in the cecum of conscious rats with and without antibiotic treatment.

Results. Significant reduction in GG swelling properties, in both simulated gastric and intestinal fluids, was accomplished by its crosslinking with GA. The crosslinking density of the modified GG products was GA concentration-dependent. The release of SS from crosslinked GG discs was completed within 120 minutes. During the same period of time and for more than 10 hours the release of Indo and Bud was negligible. The release rate of the latter two drugs was enhanced when galactomannanase and α-galactosidase were added to the dissolution media. Discs made of the crosslinked GG were implanted in the cecum of rats and their degradation was assessed after 4 days. The extent of degradation was dependent on the amount of GA used for the crosslinking. After 4 days the same discs were recovered intact from rats exposed to antibiotic treatment and from simulated gastric and intestinal fluids.

Conclusions. Reducing the enormous swelling of GG by crosslinking it with GA resulted in a biodegradable hydrogel which was able to retain poorly water soluble drugs, such as Indo and BUD, but not highly water soluble drugs, such as SS, in artificial gastrointestinal fluids. A variety of hydrogels with increasing crosslinking densities were produced and tested for their potential use as colon-specific drug platforms *in vitro* and *in vivo*. Their performance did not depend on creating physical barriers by means of compression.

KEY WORDS: budesonide; colon; colonic delivery; crosslinking; glutaraldehyde; guar gum; hydrogel; indomethacin; sodium salicylate.

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ABBREVIATIONS: Bud, budesonide; GA, glutaraldehyde; GG, guar gum; GI, gastrointestinal; Indo, indomethacin; SS, sodium salicylate; TS, test solution.

INTRODUCTION

Typical polysaccharidase activity in the human colon (1) could potentially be exploited for the specific delivery of drugs via the oral route into this organ. For this purpose, polysaccharide hydrogels, which can be degraded by colonic enzymes, are promising candidates. Enzymatic degradation has been suggested as a superior targeting mechanism to pH dependent carriers (2) because of the shallow pH gradient in the human intestine. Although the epithelium of the small intestine shows some glycosidase activity (3), the major hydrolysis of glycosidic bonds occurs in the colon. Typical colonic enzymes include amylase, pectinase, xylanase, β-D-xylosidase, β-D-galactosidase and β-D-glucosidase. The last three are the most active glycosidases (4).

Various approaches for preparing saccharidic hydrogels as colonic drug carriers have recently been described (5). In a previous study (6), we demonstrated that a chemical modification of guar gum (GG) with borax does not interfere with the ability of GG to be degraded by galactomannanase and α-galactosidase, an observation which can be applied to the design of enzymatically-controlled colon-specific drug carriers. GG is a natural polysaccharide, made of a long 1,4-β-D mannopyranosyl linear backbone (approximately 1,000–1,500 units) to which 1,6-α-D galactopyranosyl residues are attached as single unit side chains (7). GG is widely used as a thickening agent in the food and pharmaceutical industries, both in its native and modified forms (8). As a pharmaceutical adjuvant, it may be used for sustained release (9), or for colon-specific purposes, as has been previously suggested for acetylated galactomannans (10) and GG mixtures with Eudragit (11). The assumption that GG is useful in the area of colon-specific drug delivery stems from its efficient enzymatic degradation in the human large intestine (12,13).

Although it was found that the modification of GG with borax resulted in a product which could be degraded by the enzyme mixture of galactomannanase and α-galactosidase, the obtained product possessed a higher buffer-uptake capacity, as compared to native GG (6). In the present study glutaraldehyde (GA) was used as a crosslinker in order to decrease the swelling properties of GG. The use of glutaraldehyde to crosslink hydroxyl-containing polymers has been reported in the literature, primarily for the crosslinking of polyvinyl alcohol (14–16). It should be recognized that although GA is toxic, its toxicity could be reduced significantly after its crosslinking (17). The objectives of the present study were: (a) to crosslink GG with GA, (b) to assess the effect of increasing amounts of GA on the crosslinked products by physical characterization, (c) to examine the degradation properties of the crosslinked GG products *in vitro* and *in vivo*, (d) to assess, by different drug probes, whether crosslinked GG can serve as a colon-specific drug carrier.

MATERIALS AND METHODS

Materials

GG was purchased from Aldrich, Milwaukee, WI; GA was purchased from Merck, Darmstadt, Germany; Galactomannanase (from *Aspargillus niger*) was purchased from Fluka

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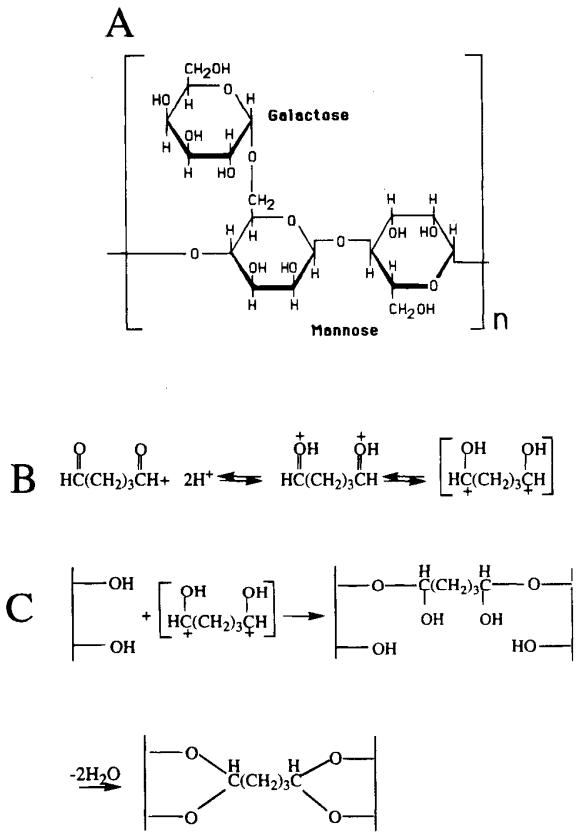
BioChemika, Germany). All other materials and reagents were purchased from Sigma, St. Louis, MO. Solvents were analytical or HPLC grade.

Synthesis of Crosslinked GG Hydrogels

In separate experiments, GG was crosslinked with increasing amounts of GA as follows (Scheme 1): Four g of GG were dispersed for 2 h at 45°C in 800 ml of double-distilled water. Concentrated H_2SO_4 (0.5 ml) was then added, followed by the addition of 1.2, 6, 12, 36, 60 and 84 ml (0.1, 0.5, 1, 3, 5 and 7 equivalents) of glutaraldehyde (25% w/v) solution per half mole of the repeating units in the guar gum respectively. It was assumed that four hydroxyl groups react with a single molecule of GA. The reaction mixture was stirred for 30 minutes and kept in a sealed vessel without stirring for an additional 48 hours at room temperature. The resulting hydrogels (denoted as products GG-0.1, GG-0.5, GG-1, GG-3, GG-5 and GG-7, respective to the equivalents of GA used) were stirred with 5% w/v aqueous solution of $NaHSO_3$ for two hours and then rinsed with distilled water (5 L portions) until no traces of GA could be detected at 235 nm (polymeric GA) and 280 nm (monomeric GA) (Uvikon 930, Kontron Instruments, Switzerland) in the rinsing water (18,19). The crosslinked GG products were either lyophilized (to give approx. 3.5 g of dry powder of each product) or kept hydrated in a gel form. To obtain discs, the reaction mixture was poured into Petri dishes and left without stirring for 48 hours as above. The GG-0.1, GG-0.5, GG-1, GG-3, GG-5 and G-7 wet hydrogels obtained were then cut into discs (12 mm OD, 3–6 mm thick), rinsed with 5% w/v aqueous solution of $NaHSO_3$ as described above and oven-dried (45°C). The crosslinked products were characterized by measuring their equilibrium-weight swelling ratios and their crosslinking densities.



Scheme 1. A schematic representation of GG (A), isomerization of GA in acidic pH (B) and the typical reaction between GA and two adjacent hydroxyl groups (C).

Swelling Measurements

Swelling properties of the crosslinked products were measured in buffer solutions at different physical states as follows: (a) Products GG-1, GG-3 and GG-5 at their maximum swollen state (right after synthesis and rinsing, prior to drying). These products were placed in simulated USP gastric test solution fluid without pepsin, pH = 1.5 (denoted as Gastric TS) or simulated USP intestinal test solution fluid without pancreatin, pH = 7.4 (denoted as Intestinal TS) (20) until no weight gain could be observed (approx. 3 days). The hydrogels were then blotted dry, weighed and dried at 45°C until no weight loss could be observed (approx. 2 days). Swelling was measured gravimetrically and expressed in percent of wet weight over the dry weight of the polymers. (b) Products GG-1, GG-3 and GG-5 in lyophilized powder state. The powders were sieved through a 40 mesh STM sieve and the fraction of 40\80 mesh was collected. In separate studies 100 mg of each type of powder were dispersed in 20 ml of Gastric TS for 2 hours or Intestinal TS for 4 hours, after which time the swollen powders were blotted dry, weighed, and dried at 45°C until no weight loss could be observed, swelling was measured gravimetrically. (c) Products GG-0.1, GG-3, GG-5 and GG-7 in a film form, cut into 12 mm O.D., 3–6 mm thick discs. In this case the swelling kinetics of the discs was measured by immersing them in Gastric TS or Intestinal TS fluids. At predetermined time intervals the discs were weighed and returned to the buffer media until no additional weight gain was observed. Each study was repeated three times.

Estimation of Crosslinking Density from Swelling Measurements

The volume fraction of the crosslinked GG before swelling, $\nu_{2,r}$, and the volume fraction of the crosslinked GG at equilibrium swelling state, $\nu_{2,s}$, were calculated from the following equations (21):

$$\nu_{2,r} = Vp/Vr \quad (1)$$

$$\nu_{2,s} = Vp/Vs \quad (2)$$

where Vp, Vr, and Vs are the volumes of the polymer in a dry state, relaxed (immediately after the reaction) state, and swollen (equilibrium) state, respectively. Vr was measured gravimetrically by weighing polymer discs (in a relaxed state) outside and inside a water containing picknometer. Vs was measured similarly for swollen discs. Vp was measured for dehydrated (2 days at 45°C, followed by 1 day at 80°C) discs, using a heptane containing pycnometer.

Knowing $\nu_{2,r}$ and $\nu_{2,s}$, the mean molecular weight of the polymer fragments between the crosslinking points (Mc) could be calculated from the following equation (21):

$$\frac{1}{\overline{Mc}} = \frac{2}{\overline{Mn}} - \frac{\frac{\bar{\nu}}{V_1}[\ln(1 - \nu_{2,s}) + \nu_{2,s} + \chi_1\nu_{2,s}^2]}{\nu_{2,r}\left[\left(\frac{\nu_{2,s}}{\nu_{2,r}}\right)^{1/3} - \frac{1}{2}\left(\frac{\nu_{2,s}}{\nu_{2,r}}\right)\right]} \quad (3)$$

where Mn is the number average molecular weight of GG [300,000 as determined by GPC (6)], V_1 is the molar volume of the solvent used (H_2O: 18 cm^3/mole), $\bar{\nu}$ is the specific volume of the bulk polymer at swollen state [0.63 cm^3/g for GG (22)] and χ_1 is the Flory-Huggins polymer-solvent interaction parameter which decreases with an increase in the polymer-solvent interaction. GG is an hydrophilic polymer which forms dispersions in water in concentrations of 0.5% and above. Therefore a χ_1 value of 0.8, similar to polyvinylalcohol (23), was chosen.

The crosslinking density, *P*, was calculated from the following equation (21):

$$P = \frac{1}{\bar{\nu}\,\overline{Mc}} \quad (4)$$

Estimation of the Crosslinking Density from Elasticity Measurements

Specimens (13 mm long × 4 mm wide) of the various crosslinked products were soaked in distilled water at 37°C until equilibrium and their modulus of elasticity $[G^*]_\infty$ could be measured (Instron, Mini, model 44, Buckinghamshire, U.K.). The effective network density was calculated using the following equation (24):

$$v/V = \frac{[G^*]_\infty}{A'_\phi KT(\nu_{2,s})^{2/3}} \quad (5)$$

where v/V is the effective network density or the molar concentration of the elastic (effective) chains in 1 cm^3 of polymer, A'_ϕ is the structure factor (in the case of highly swollen network, selected for our case, $A'_\phi = 1 - 2/\phi$), ϕ is the crosslinking functionality (in our case, for glutaraldehyde, $\phi = 4$), K is the Boltzman constant, and $\nu_{2,s}$ is the volume fraction of the crosslinked GG at equilibrium swelling state, calculated from *Equation 2*.

Drug Loading of the Hydrogels

Discs taken from the GG-3 and GG-7 products (for sodium salicylate, denoted as SS), GG-0.1 and GG-1 products (for indomethacin, denoted as Indo) and GG-0.5 (for budesonide, denoted as Bud) immediately after completion of their synthesis, as described above, were oven-dried (45°C, overnight). The discs were then immersed in the drug solutions as follows: SS: the discs were immersed in 10 ml of 10 mg/ml SS in water overnight; Indo: the discs were immersed in 10 ml of 0.8 mg/ml Indo in PBS pH = 7.8 overnight; Bud: the discs were immersed in 10 ml of 0.1 mg/ml Bud in water:ethanol, 5:1 v:v overnight. The recovered drug-loaded discs were oven-dried at 45°C for 24 h. Drug excess (assumed to be on the surface of the discs) was removed by rinsing the discs three times with 10 ml portions of PBS pH = 6.4. The amount drug loaded was 357, 146 and 65 mg/g dry polymer for sodium salicylate, indomethacin and budesonide respectively.

Drug Release Studies

The release kinetics of SS (from GG-3, GG-5 and GG-7), Indo (from GG-0.1 and GG-1) and Bud (from GG-0.5) were studied in 10 ml of PBS pH = 6.4 (0.2 M), or 10 ml of a mixture of 0.175 U/ml of galactomannanase and 0.033 U/ml of α-galactosidase (from *E. coli*) in the same buffer solution (except for the SS, which was studied in buffer solution pH = 6.4 only). The studies were conducted separately in sealed glass beakers mounted in a shaking (100 rpm) bath at 37°C. Samples (200 μl) of SS were withdrawn at 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 minutes and centrifuged with the supernatant collected for drug analysis. The same procedure was repeated for the Indo and Bud sampling with the time intervals for withdrawal at: 0, 30, 60, 90, 120, 180, 240, and 360 minutes. Withdrawal volumes were replenished with an equal volume of fresh dissolution medium.

At the end of the release studies, each disc residue was digested with an excess of galactomannanase and α-galactosidase mixture (3.3 and 17.5 U/ml, respectively) and the residual drug left in the discs was determined to verify the observed value of the total amount of drug released.

Drug Assays

SS in the withdrawn samples was determined spectrophotometrically (λ = 296 nm) after suitable dilution with PBS pH = 6.4. Indo was determined spectrophotometrically (λ = 318 nm) after suitable dilution with PBS pH = 7.4. Bud was determined spectrophotometrically (λ = 247 nm) after suitable dilution with a water:ethanol (52:48 v:v) mixture. When samples taken from enzyme-containing dissolution media were measured, enzyme solutions (containing equal concentrations and handled similarly) served as blank solutions.

In Vivo Degradation Analysis

Pre-weighed discs (8 mm OD × 2 mm thick) of GG-0.5, GG-1 and GG-3 were hydrated over 2 hours in PBS pH = 7.4 and tested for biodegradation in the cecum of Sabra rats using a previously described method (25). Briefly, the discs were mounted in gauze bags which were individually implanted in the cecum by attaching them to the organ wall of an anesthetized rat (a single disc/rat) with 3/0 silk sutures. The rats were allowed to recover and kept on a normal diet for 4 days, after which they were sacrificed and the bags opened. In the bags where disc residues were found, residues were collected and dried until no further weight loss was observed. Sabra rats which were treated with an antibiotic cocktail (300 ml of intra-cecal administration of ampicillin 250 mg/ml, chloramphenicol 0.5 mg/ml and cefazolin 250 mg/ml) were used as controls. In the "antibiotics" study discs of the same size were weighed and implanted. The residues were then dried and weighed after 4 days. The antibiotic cocktail was also added to the drinking water of the control rats for the 4 days of the study. A parallel *in vitro* control study was performed by soaking discs of the same crosslinked products and similar sizes in PBS, pH = 7.4 for 4 days.

RESULTS

The reduction in swelling properties of GG as a result of its crosslinking with GA is shown in Figure 1. Swelling was

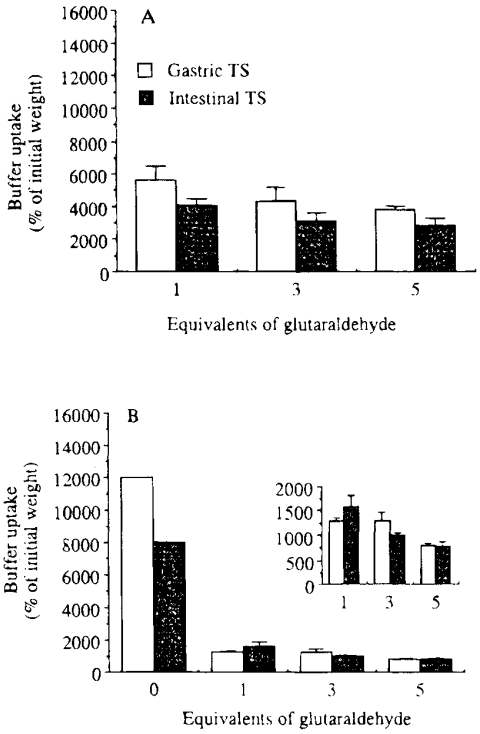


Fig. 1. Buffer (Gastric TS, 2 hours or Intestinal TS, 4 hours) uptake of GG crosslinked with increasing amounts of GA in a swollen state [A] and in a powder form after lyophilization [B]. Expanded scale of the results obtained for the crosslinked products is included for the powder form. Numbers at the x-axis are the equivalents of GA used; GG-0 is native GG. Shown are the mean values ± S.D., n = at least three different batches.

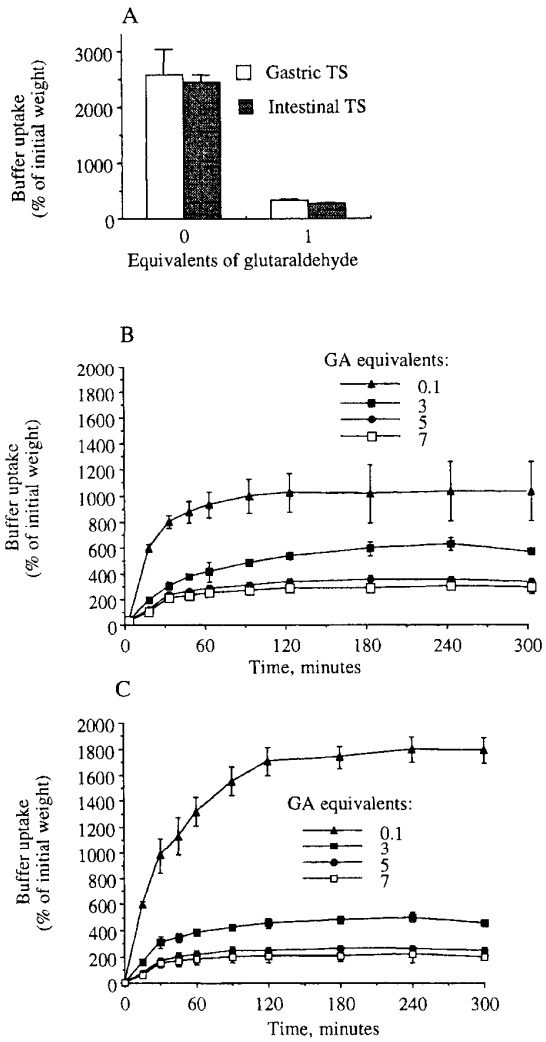


Fig. 2. The reduction in the swelling properties of GG in a film form as a result of its reaction with 1 equivalent of GA (A) and the swelling kinetics of crosslinked GG discs in Gastric TS (B) or Intestinal TS (C) buffers of the following products: GG-0.1, GG-3, GG-5 and GG-7. Shown are the mean values ± S.D. n = at least three different batches.

evaluated by measuring buffer (Gastric TS and Intestinal TS) uptake. The GA concentration-dependent reduction in buffer uptake was much more marked in the swollen (Figure 1A) and lyophilized (Figure 1B) states. Also, crosslinking diminished the effect of pH on the swelling properties of GG (Figure 1B).

The reduction in swelling properties of GG in a film form as a result of its crosslinking with GA and the swelling kinetics of films made of four crosslinked GG products in Gastric TS and Intestinal TS are shown in Figure 2. Apart from the observation that the higher the crosslinking, the lower the buffer uptake (Figure 2B and C), it can be seen that in all cases the swelling reached equilibrium within 90 minutes. The GA concentration dependent changes in $\overline{Mc}$ (the mean molecular weight of the polymer fragment between crosslinking points), the crosslinking density (p), the modulus of elasticity [G*] and the effective network density (v/V) are summarized in Table I and Table II.

Figure 3 shows that the release of the soluble drug marker SS out of discs made of two highly crosslinked products (GG-3 and GG-7) was relatively rapid, complying similar kinetic profiles. Indo release kinetics from discs made of two low crosslinked products, GG-0.1 and GG-1, in buffer solutions with and without guar-hydrolyzing enzymes, are shown in Figure 4. While almost no drug was released in the buffer solutions due to the low water-solubility of Indo, the addition of enzymes accelerated the release kinetics of the drug, with the total amount

Table I. The Volume Fraction $\nu_{2,s}$ (After Swelling), the Mean Molecular Weight of the Polymer Fragments Between the Crosslinking Points ($\overline{Mc}$), as Calculated from *Equation 3*, and the Crosslinking Density P as Calculated from *Equation 4* of the Four Crosslinked GG Products: GG-0.5, GG-1, GG-3, and GG-5

Product	$\nu_{2,s}$	$\overline{Mc}$ (g/mole)	$P \times 10^4$ (mole/cm^3)
GG-0.5	0.003 ± 0.0006	25,208 ± 3040	0.64
GG-1	0.006 ± 0.0002	14,231 ± 539	1.12
GG-3	0.010 ± 0.002	9,774 ± 1340	1.68
GG-5	0.012 ± 0.002	5,088 ± 582	3.24

Note: Shown are the mean values ± S.D., n = 10 measurements.

Table II. The Modulus of Elasticity of the Four Crosslinked GG Products: GG-0.1, GG-0.5, GG-1, and GG-3, and Their Effective Network Density (v/V) as Calculated from *Equation 5*

Product	[G*] (MPa)	(v/V) × 10^4
GG-0.1	0.004 ± 0.0008	0.19 ± 0.001
GG-0.5	0.02 ± 0.0005	0.71 ± 0.03
GG-1	0.08 ± 0.015	2.7 ± 0.03

Note: Shown are the mean values ± S.D., n = 10 measurements.

of Indo released increased by 9.7-fold and 6.7-fold for the GG-0.1 and GG-1, respectively. Similar results were obtained for Bud. Its release kinetics from discs made of GG-0.5, with and without enzyme mixture, are shown in Figure 5. In the presence of enzyme mixture the total Bud released was 7.3-fold greater than the total drug release in the buffer solution without enzymes.

The *in vivo* degradation of discs made of the crosslinked products GG-0.5, GG-1 and GG-3 in the cecum of the rat, with and without antibiotic treatment, is summarized in Table III, which shows a complete degradation of GG-0.5 and GG-1 discs, and partial degradation of the GG-3 discs. Dosing the rats with antibiotics caused a significant decrease in the implanted discs' degradation (from 100% without antibiotics,

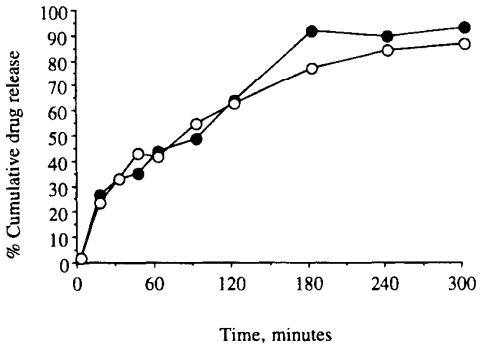


Fig. 3. SS release kinetics from discs made of GG-3 (closed circles) and GG-7 (open circles) into PBS pH = 6.4.

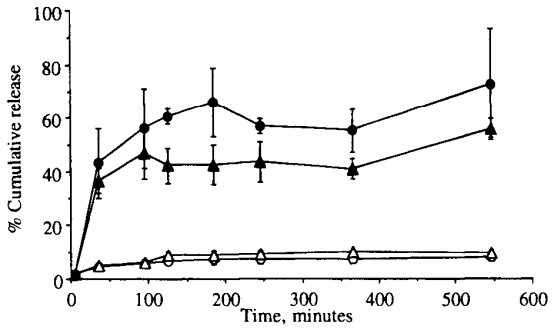


Fig. 4. Indo release kinetics from discs made of GG-0.1 and GG-1 in PBS pH = 6.4, with (closed circles and triangles) and without (open circles and triangles) enzyme mixtures. Shown are the mean values of three different measurements ± S.D.

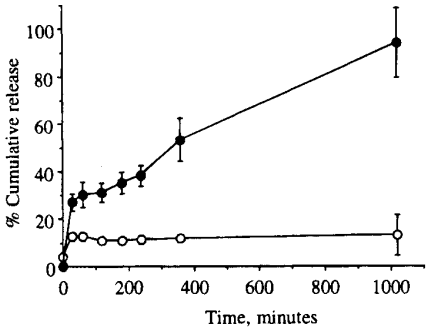


Fig. 5. Bud release kinetics from discs made of GG-0.5 in PBS pH = 6.4, with (closed circles) and without (open circles) enzymes mixture. Shown are the mean values of three different measurements ± S.D.

to 13.0 ± 5.3 and 7.0 ± 2.7% with antibiotics for GG-0.5 and GG-1, respectively. The GG-3 disc did not degrade at all). Table III also shows that the same discs were resistant in a pH = 6.4 buffer solution over 4 days.

DISCUSSION

GG is degraded by colonic bacteria (4,6,13). However, its enormous swelling is a drawback in its use as a microbially controlled colon-specific delivery system because of the possible risk that an entrapped drug will leak out prior to arriving at the colon. In the present study, glutaraldehyde (GA) was used as a crosslinker to decrease GG swelling properties. The reaction with GA takes place with hydroxyl groups of the galactose or mannose subunits of GG (Scheme 1), typical of the GA reaction with polyols at acidic conditions (14–16).

The swelling properties of the resulting crosslinked GG products were studied in buffer solutions at three different physical forms: swollen state; dried discs; and lyophilized form. The latter was performed to account for intrinsic swelling characteristics of GG and its crosslinked derivative. In such a physical state, the difference between native GG and the crosslinked product is most profound due to the reduced number of entanglements which may restrict the equilibrium degree of swelling that occurs upon reconstitution. As expected, the higher the amount of GA, the lower the buffer uptake observed, indicating an increase in the crosslinking density (Figure 1 and Figure 2). Figure 2 clearly shows that the drying procedure involved in

Table III. The Degradation of Discs Made of GG-0.5, GG-1, and GG-3 in the Cecum of the Rat, Exposed (+) and Not Exposed (−) to Antibiotic Treatment, and in PBS pH = 6.4 (Not Implanted), as Detected 4 Days After Implantation

Product	Weight loss − antibiotic (%)	Weight loss + antibiotics (%)	Weight loss in buffer (%)
GG-0.5	100	13.0 ± 5.3	0
GG-1	100	7.0 ± 2.7	0
GG-3	38.5 ± 11.5	0	0

Note: Results are presented as a fraction of weight loss during the study, expressed in % of initial weight. Shown are mean values (for the degraded products) ± S.D. (n = 3 rats).

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