

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

Irit Gliko-Kabir,¹ Boris Yagen,¹ Adel Penhasi,² and Abraham Rubinstein^{1,3}

Received January 27, 1998; accepted March 26, 1998

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.

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

² Perio Products Ltd., P.O. Box 23950, Jerusalem 91237, Israel.

³ To whom correspondence should be addressed. (e-mail: avri@cc.huji.ac.il)

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

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 H₂SO₄ (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 NaHSO₃ 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 NaHSO₃ as described above and oven-dried (45°C). The crosslinked products were characterized by measuring their equilibrium-weight swelling ratios and their crosslinking densities.

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

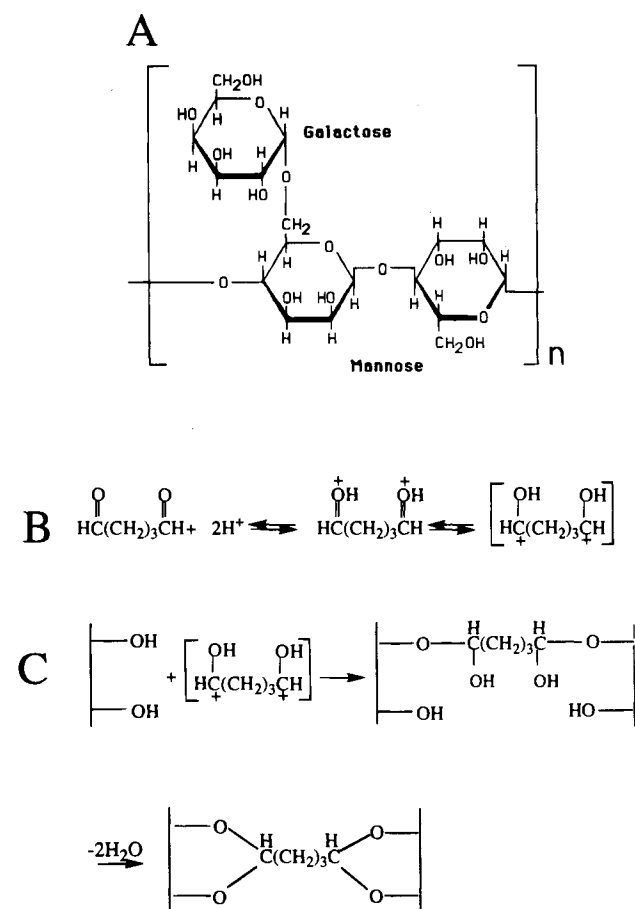
The volume fraction of the crosslinked GG before swelling, $v_{2,r}$, and the volume fraction of the crosslinked GG at equilibrium swelling state, $v_{2,s}$, were calculated from the following equations (21):

$$v_{2,r} = V_p/V_r \quad (1)$$

$$v_{2,s} = V_p/V_s \quad (2)$$

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

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



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).

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{\overline{v}}{V_1} \frac{[\ln(1 - v_{2,s}) + v_{2,s} + \chi_1 v_{2,s}^2]}{v_{2,r} \left[\left(\frac{v_{2,s}}{v_{2,r}} \right)^{1/3} - \frac{1}{2} \left(\frac{v_{2,s}}{v_{2,r}} \right) \right]} \quad (3)$$

where \overline{M}_n 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 : $18 \text{ cm}^3/\text{mole}$), \overline{v} is the specific volume of the bulk polymer at swollen state [$0.63 \text{ cm}^3/\text{g}$ 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):

$$P = \frac{1}{\overline{v} \overline{M}_c} \quad (4)$$

Estimation of the Crosslinking Density from Elasticity Measurements

Specimens (13 mm long \times 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):

$$v/V = \frac{[G^*]_\infty}{A'_\phi K T (v_{2,s})^{2/3}} \quad (5)$$

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 $v_{2,s}$ 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 ($\lambda = 296 \text{ nm}$) after suitable dilution with PBS pH = 6.4. Indo was determined spectrophotometrically ($\lambda = 318 \text{ nm}$) after suitable dilution with PBS pH = 7.4. Bud was determined spectrophotometrically ($\lambda = 247 \text{ nm}$) 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 \times 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

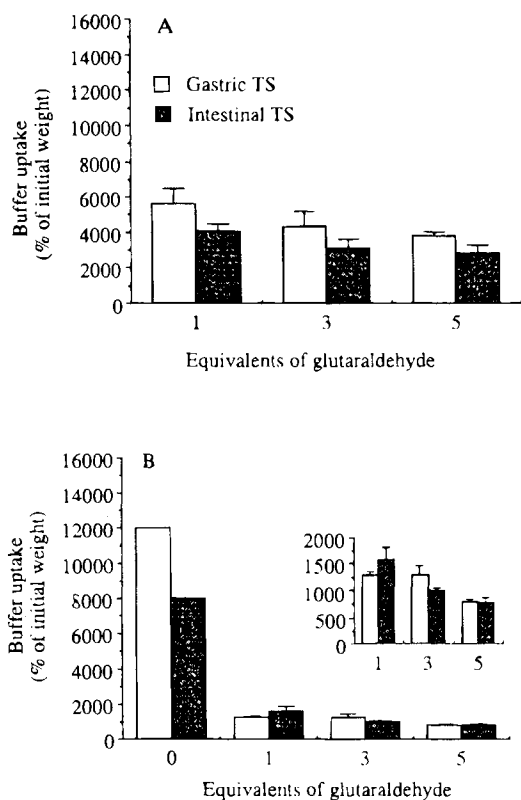


Fig. 1. Buffer (Gastric TS, 2 hours or Intestinal TS, 4 hours) uptake of GG crosslinked with increasing amounts of GA in a swollen state [A] and in a powder form after lyophilization [B]. Expanded scale of the results obtained for the crosslinked products is included for the powder form. Numbers at the x-axis are the equivalents of GA used; GG-0 is native GG. Shown are the mean values \pm 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 \bar{M}_c (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 (ν/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

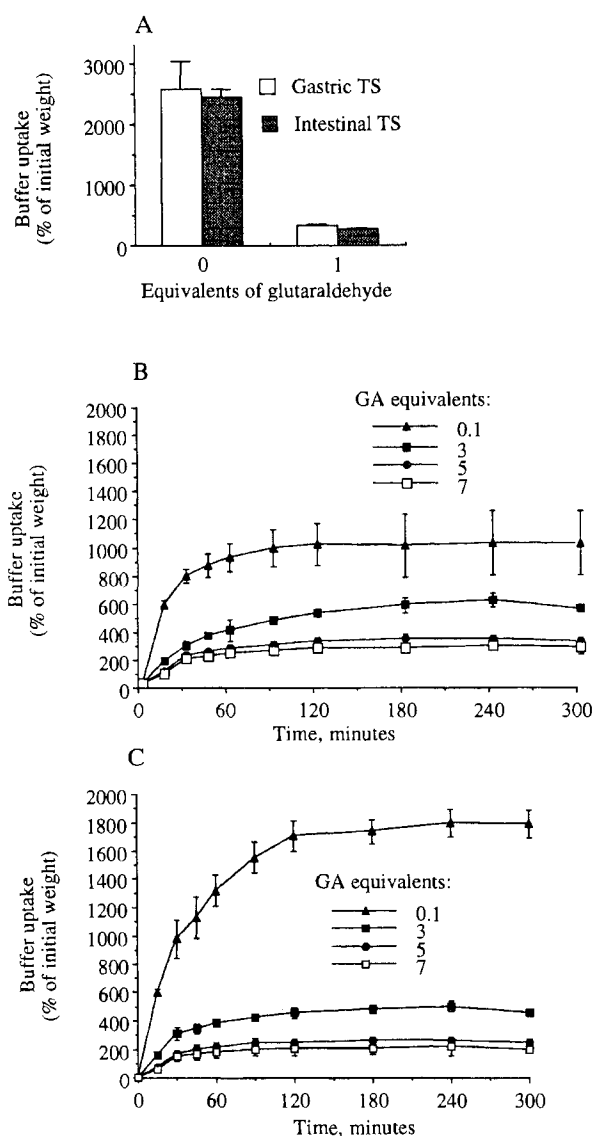


Fig. 2. The reduction in the swelling properties of GG in a film form as a result of its reaction with 1 equivalent of GA (A) and the swelling kinetics of crosslinked GG discs in Gastric TS (B) or Intestinal TS (C) buffers of the following products: GG-0.1, GG-3, GG-5 and GG-7. Shown are the mean values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

Table I. The Volume Fraction $\nu_{2,s}$ (After Swelling), the Mean Molecular Weight of the Polymer Fragments Between the Crosslinking Points (\bar{M}_c), 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	$\nu_{2,s}$	\bar{M}_c (g/mole)	$P \times 10^4$ (mole/cm ³)
GG-0.5	0.003 ± 0.0006	$25,208 \pm 3040$	0.64
GG-1	0.006 ± 0.0002	$14,231 \pm 539$	1.12
GG-3	0.010 ± 0.002	$9,774 \pm 1340$	1.68
GG-5	0.012 ± 0.002	$5,088 \pm 582$	3.24

Note: Shown are the mean values \pm 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) $\times 10^4$
GG-0.1	0.004 \pm 0.0008	0.19 \pm 0.001
GG-0.5	0.02 \pm 0.0005	0.71 \pm 0.03
GG-1	0.08 \pm 0.015	2.7 \pm 0.03

Note: Shown are the mean values \pm 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,

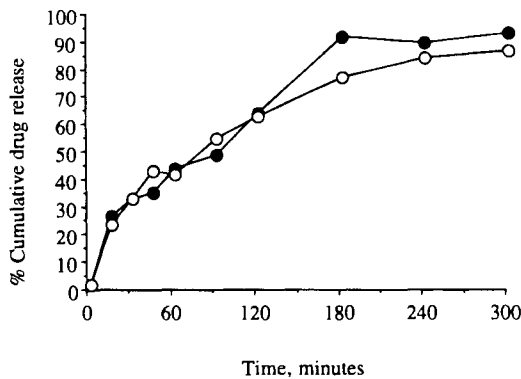


Fig. 3. SS release kinetics from discs made of GG-3 (closed circles) and GG-7 (open circles) into PBS pH = 6.4.

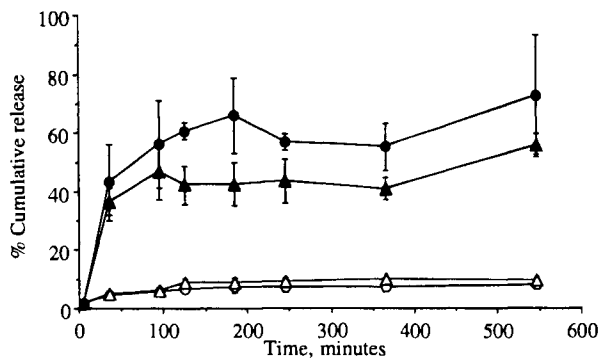


Fig. 4. Indo release kinetics from discs made of GG-0.1 and GG-1 in PBS pH = 6.4, with (closed circles and triangles) and without (open circles and triangles) enzyme mixtures. Shown are the mean values of three different measurements \pm S.D.

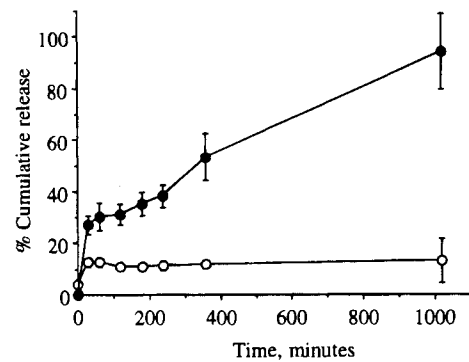


Fig. 5. Bud release kinetics from discs made of GG-0.5 in PBS pH = 6.4, with (closed circles) and without (open circles) enzymes mixture. Shown are the mean values of three different measurements \pm S.D.

to 13.0 \pm 5.3 and 7.0 \pm 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 \pm 5.3	0
GG-1	100	7.0 \pm 2.7	0
GG-3	38.5 \pm 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) \pm S.D. ($n = 3$ rats).

the disc preparation prevented the hydrogels from returning to their original volume (swollen state, Figure 1A). Thus, the drying introduced irreversible changes to the hydrogels. For example product GG-5 swelled 28-fold at equilibrium, 7.8-fold in lyophilized form, and only 2.7-fold in a dry disc form (Figure 1 and Figure 2). A similar trend in equilibrium swelling was reported by Peppas and Korsmeyer who crosslinked poly (vinyl alcohol) with GA. They concluded that such a pattern is a characteristic of physical crosslinking rather than a change in the crystalline structure (26). This means that physical entanglements in the polymer network are responsible for the changes in the degree of swelling of the various crosslinked GG products. The lyophilized GG-5 swelled 3.6-fold less than the same product in the swollen state. That is, physical entanglements occurred even in the lyophilized product, although lyophilization is supposed to keep the network structure of the polymer as intact as possible. The order of magnitude decrease in the equilibrium swelling of GG-5 in the disc form compared with the swollen state, and also compared with the 1.9-fold reduction observed in GA crosslinked PVA (26), may be explained by the much higher molecular weight of GG and the fact that it is a branched polysaccharide.

The non-ionic nature of crosslinked GG causes the buffer uptake rates of products GG-3, GG-5, and GG-7 to be similar (Figure 2). The swelling rate of GG-0.1, however, was significantly lower in Gastric TS, probably because in this loosely bound product it is easier for GA to continue to react in an acidic environment (Scheme 1).

The degree of crosslinking of the GG hydrogels was assessed from swelling and modulus of elasticity calculations. The number average molecular weight between two adjacent crosslinking points, \overline{M}_c , of the crosslinked products was calculated according to Bray and Merrill (27), as used by Peppas and Merrill (21). Table I shows that increasing amounts of GA in the products caused a decrease in the \overline{M}_c values, as would be expected from Flory's theory (28). That is, an increase in the concentration of the crosslinker in the reaction mixture resulted in a densely crosslinked product (lower \overline{M}_c , higher p and higher $v_{2,s}$ values). The degree of crosslinking was also derived from the modulus of elasticity and the effective network density (v/V) measurements. As shown in Table II increase in the amount of GA used in the reaction resulted in increase in the mechanical strength and effective network density for the various crosslinked products.

The fact that high amounts of GA (more than 1 equivalent) were required for the crosslinking reaction suggests that the crosslinking efficiency was low. This could be attributed to (a) low reactivity of the GG hydroxyl groups as a result of the limited water solubility of the polymer, (b) GA polymerization during the crosslinking process and (c) possible masking effect of the hexose units of the branched polymer.

Figure 3 demonstrates that, due to rapid drug diffusion through its network, crosslinked GG cannot be used for the specific delivery of highly water-soluble drugs, such as SS, into the colon. Total drug release from the most dense products GG-3 and GG-7, was accomplished within 180 minutes. In terms of GI transit time, this is barely sufficient for the polymer to arrive at the mid ileum. The fast release of SS is a result of the relatively rapid swelling kinetics of the two crosslinked GG products tested, as shown in Figure 2, a phenomenon which allows a highly water-soluble drug to easily leach out of the

carrier. The release kinetics of two poorly water-soluble drug probes, Indo and Bud, in the presence or absence of specific enzymes, was therefore evaluated. The enhanced drug release kinetics of the two drugs in the presence of the enzyme mixture, as demonstrated in Figure 4 and Figure 5, indicate that (a) the ability of GG to be degraded by specific enzymes of the colon was maintained despite its modification with GA, (b) cross-linked GG can potentially be used as a colon-specific carrier only for poorly-water-soluble drugs. The ability of the cross-linked GG to be degraded by the enzymes could be explained by the assumption that after crosslinking, the polymeric network was composed of two parts, an unchanged GG section and a crosslinked section, mixed together randomly. McCleary (29), who investigated the enzymatic degradation mechanism of carob-gum galactomannans, suggested that enzymes cleave the polymer at a distance of three hexose units from the branching point. Assuming that this is the situation with crosslinked guar, the mean distance between the crosslinking points of product GG-0.5 is $25208/160 = 157$ sugar units (160 is the molecular weight of a single hexose unit, and 25208 is the calculated \overline{M}_c value for GG-0.5, Table I). It is speculated that this distance is large enough for the enzyme to penetrate into the network and cleave the GG section.

Discs of products GG-0.5, GG-1 and GG-3 were also analyzed for their enzymatic degradation properties in the cecum of the rat. One group of rats was kept on a normal diet, while the control group was treated with antibiotics to reduce its cecal microbial flora. Table III shows that out of the three products used in this section of the study, GG-3, which was crosslinked with the largest amount of GA, was not completely degraded four days after implantation. The other two products, GG-0.1 and GG-0.5, which were crosslinked with lower amounts of GA, were degraded completely. This, together with the observation that in positive control studies (Table III) the crosslinked products were not degraded over four days, indicates that degradation of crosslinked GG in the rat cecum (Figures 4 and 5) results from enzymes of bacterial origin.

CONCLUSIONS

In this study we reduced the swelling properties of GG by crosslinking it with increasing amounts of GA. The crosslinked products retained the ability of GG to be degraded *in vitro* by a mixture of galactomannanase and α -galactosidase. This was verified by drug release studies, which showed that crosslinked GG can retain a poorly water-soluble drug load, such as Bud and Indo, but not highly water soluble drugs such as SS. This, together with the observation that crosslinked GG discs are degradable *in vivo* in the cecum of the conscious rat, suggest that crosslinked GG with GA can potentially be used for the specific delivery of poorly water-soluble drugs to the colon. Compared to plain GG tablets (30), this new colonic delivery system is unique in its ability to function specifically without relying on compression coats or compressed cores.

ACKNOWLEDGMENTS

The results reported here are included in the dissertation project of Irit Gliko-Kabir as partial fulfillment of her PhD degree requirements at The Hebrew University of Jerusalem. The study was presented in part at the 3rd Jerusalem Conference

of Pharmaceutical Sciences and Clinical Pharmacology, 1996 and the 24th Intern. Symp. Controlled Release of Bioactive Materials, Stockholm, Sweden, 1997.

REFERENCES

- G. Hawksworth, B. S. Drasar, and M. J. Hill. Intestinal bacteria and the hydrolysis of glycosidic bonds. *J. Med. Microbiol.* **4**:451–459 (1971).
- M. Ashford, J. T. Fell, D. Attwood, H. Sharma, and P. J. Woodhead. An *in vivo* investigation into the suitability of pH dependent polymers for colonic targeting. *Int. J. Pharm.* **95**:193–199 (1993).
- J. Conchie and D. C. Macdonald. Glycosidases in the mammalian alimentary canal. *Nature* **184**:1233 (1959).
- H. N. Englyst, S. Hay, and G. T. Macfarlane. Polysaccharide breakdown by mixed populations of human fecal bacteria. *FEMS Microbiol. Ecol.* **95**:163–171 (1987).
- A. Rubinstein. Approaches and opportunities in colon-specific drug delivery. *Crit. Rev. Ther. Drug Carr. Sys.* **12**:101–149 (1995).
- A. Rubinstein and I. Gliko-Kabir. Synthesis and swelling dependent enzymatic degradation of borax modified guar gum for colonic delivery purposes. *s.t.p. Pharm. Sci.* **5**:41–46 (1995).
- H. Neukom. Galactomannans: properties and applications. *Lebensm.-Wiss. u.-Technol.* **22**:41–45 (1993).
- A. A. Lawrence. *Natural Gums For Edible Purposes*, Noyes Data Corporation, N.J., 1976, pp. 17–55.
- S. K. Baveja, K. V. R. Ranga Rao, J. Arora, N. K. Mathur, and V. K. Vinayak. Chemical investigation of some galactomannan gums as matrix tablets for sustained drug delivery. *Ind. J. Chem.* **30**:133–137 (1991).
- A. W. Sarlikiotis and K. H. Bauer. Synthese und untersuchung von polyurethanen mit galactomannan-segmenten als hilfsstoffe zur freisetzung von peptid-arzneistoffen im dickdarm. *Pharm. Ind.* **54**:873–880 (1992).
- K. O. R. Lehmann and K. D. Dreher. Methacrylate-galactomannan coating for colon-specific drug delivery. *Proceed. Int. Symp. Contr. Rel. Bioact. Mater.* **18**:331–332 (1991).
- J. Tomlin, N. W. Read, C. A. Edwards, and B. I. Duerden. The degradation of guar gum by a faecal incubation system. *Br. J. Nutr.* **55**:481–486 (1986).
- G. T. Macfarlane, S. Hay, S. Macfarlane, and G. R. Gibson. Effect of different carbohydrates on growth, polysaccharidase and glycosidaseproduction by *Bacteroides ovatus*, in batch and continuous culture. *J. App. Bacteriol.* **68**:179–187 (1990).
- C. T. Reinhart and N. A. Peppas. Solute diffusion in swollen membranes. Part II. Influence of crosslinking on diffusive properties. *J. Membrane Sci.* **18**:227–239 (1984).
- B. C. Thanoo, M. C. Sunny, and A. Jayakrishnan. Controlled release of oral drugs from cross-linked polyvinyl alcohol microspheres. *J. Pharm. Pharmacol.* **45**:16–20 (1993).
- C.-J. Kim and P. I. Lee. Composite poly(vinyl alcohol) beads for controlled drug delivery. *Pharm. Res.* **9**:10–16 (1992).
- M. Grimm, M. Grabenwoger, E. Eybl, A. Moritz, P. Bock, M. M. Muller, and E. Wolner. Improved biocompatibility of biprosthetic heart valves by L-glutamic treatment. *J. Card. Surg.* **7**:58–64 (1992).
- J. Kawahara, T. Ohmori, T. Ohkubo, S. Hattori, and M. Kawamura. The structure of glutaraldehyde in aqueous solution determined by ultraviolet absorption and light scattering. *Anal. Biochem.* **201**:94–98 (1992).
- A. Ya. Sorokin, V. A. Kuznetsova, F. O. Pozdnyakova, L. E. Mal'tseva, M. M. Onopleva, N. A. Domnicheva, and N. O. Shemyakina. Effect of the quality of glutaraldehyde on the properties of crosslinked polyvinyl alcohol. *Zhurnal Prikladnoi Khimii*, **62**:665–669 (1989).
- U.S.P. XXIII, United States Pharmacopeial Convention (1995) Rockville, MD, pp. 2053.
- N. A. Peppas and E. W. Merrill. Development of semicrystalline poly(vinyl alcohol) hydrogels for biomedical applications. *J. Biomed. Mater. Res.* **11**:423–434 (1977).
- W. R. Sharman, E. L. Richards, and G. N. Malcolm. Hydrodynamic properties of aqueous solutions of galactomannans. *Biopolymers* **17**:2817–2833 (1978).
- P. Munk. *Introduction to Macromolecular Science*, New York, John Wiley & Sons, Chapter 4, 67–98 (1989).
- J. E. Mark. Experimental determinations of crosslink densities. *Rubber Chem. Tech.* **55**:762–768 (1982).
- J. Kopecek, P. Kopeckova, H. Brondsted, R. Rathi, R. Rihova, P.-Y. Yeh, and K. Ikesue. Polymers for colon-specific drug delivery. *J. Contr. Rel.* **19**:121–130 (1992).
- R. W. Kormsmeier and N. A. Peppas. Effect of the morphology of hydrophilic polymeric matrices on the diffusion and release of water soluble drugs. *J. Memb. Sci.* **9**:211–227 (1981).
- J. C. Bray and E. W. Merrill. Poly(vinyl alcohol) hydrogels. Formation by electron beam irradiation of aqueous solutions and subsequent crystallization. *J. Appl. Polymer Sci.* **17**:3779–3794 (1973).
- P. J. Flory. *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, N.Y., Chapters 11 and 13, 347–398, 541–593 (1953).
- B. V. McCleary, E. Nurthen, F. R. Taravel, and J. -P. Joseleau. Characterization of the oligosaccharides produced on hydrolysis of galactomannan with β -D-mannanase. *Carbohydr. Res.* **118**:91–109 (1983).
- D. Wong, S. Larabee, K. Clifford, J. Tremblay, and D. R. Friend. USP dissolution apparatus III (reciprocating cylinder) for screening of guar-based colonic delivery formulations. *J. Contr. Rel.* **47**:173–179 (1997).