

Magnesium binding by gum arabic, locust bean gum, and arabinogalactan

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The ability of gum arabic (GA), locust bean gum (LBG), and arabinogalactan (ABO) to complex with magnesium was investigated. Uronic acid presence, total endogenous magnesium, free endogenous magnesium over a pH range 6–8, ability to complex added magnesium, and ability to bind endogenous magnesium after partial digestion were determined. Only GA contained uronic acid, and also contained the most magnesium ($2.08 \pm 0.28 \mu\text{g Mg mg}^{-1}$ fiber). Using size exclusion chromatography to separate free endogenous magnesium from bound endogenous magnesium, all magnesium in the fibers was free. The addition of magnesium as magnesium sulfate to the fibers at pH 7 did not result in magnesium binding. Partial hydrolysis of the fibers with hemicellulase did not result in magnesium binding. From these results, magnesium does not form a stable complex with GA, LBG, or ABO at physiologically relevant pH levels, in the presence of excess amounts of magnesium, or after partial enzymatic hydrolysis.

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INTRODUCTION

An increased intake of dietary fiber has been recommended as part of a diet designed to reduce risk of chronic diseases (American Medical Association, 1989; National Cholesterol Education Program, 1988), and soluble gum supplements have been suggested to reduce hyperglycemia (Tinker & Schneeman, 1989; Edwards *et al.*, 1987; McLean-Ross *et al.*, 1983), and blood lipid levels (Jensen *et al.*, 1993; Haskell *et al.*, 1992; Superko *et al.*, 1988; Anderson *et al.*, 1991; Bell *et al.*, 1989; McLean-Ross *et al.*, 1983; Zavoral *et al.*, 1983; Hashi & Takeshita, 1973). However, whole and purified fibers have been found to bind minerals, including magnesium, *in vitro* and to cause negative mineral balances in humans (Behall *et al.*, 1989; Ha *et al.*, 1989; Laszlo, 1989; Schlemmer, 1989; Behall *et al.*, 1987; Hallfrisch *et al.*, 1987; Lee & Garcia-Lopez, 1985; Mod *et al.*, 1982; Camire & Clydesdale, 1981). The ingestion of a substance capable of binding magnesium and making it unavailable for absorption is undesirable, as hypomagnesemia has been associated with atherosclerosis, congestive heart failure, arrhythmias, acute myocardial infarction, and hypertension (Dyckner & Wester, 1987; Hollifield, 1984; Juan, 1982). Gum arabic (GA), a plant exudate, and locust bean gum (LBG), a seed gum, have

been suggested to reduce blood lipid and glucose levels (Jensen *et al.*, 1993; Edwards *et al.*, 1987; McLean-Ross *et al.*, 1983; Zavoral *et al.*, 1983). Arabinogalactan (ABO), a soluble gum commonly found in grains, has been studied for its ability to reduce serum cholesterol levels in rats (Hashi & Takeshita, 1973). Although there is extensive literature concerning mineral binding to fibers (see reviews by Dreher, 1987; Torre *et al.*, 1991), there is little available information on the binding of magnesium by purified soluble fibers. The purpose of the present work, therefore, was to determine the endogenous magnesium in each fiber, and to assess the ability of each fiber to complex with endogenous magnesium, added magnesium, and endogenous magnesium after partial enzymatic digestion at physiologically relevant pH levels.

MATERIALS AND METHODS

Fiber sources

GA (from *Acacia* sp.), LBG (from *Ceratonia siliqua* seeds), and ABO (from larch wood) were obtained from Sigma Chemical Co. (St Louis, MO). All analyses were conducted with the same lot number for each fiber source.

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Uronic acid presence

Uronic acid presence in 0.08% and 0.32% (w/v) fiber solutions was tested using the carbazole method of Dische (1947). All solutions were made using distilled, deionized water. The percent glucuronic acid in GA was estimated following the procedure of Ha & Thomas (1988).

Total endogenous magnesium measurement

Fiber samples (50 mg) were wet-ashed with nitric acid and perchloric acid by a modification of the method of Neidermeier *et al.* (1971). The fibers and their blanks were diluted with 1% LaCl_3 and analyzed for endogenous magnesium using a Hitachi Model 170-50 atomic absorption spectrophotometer with an air-acetylene flame and hollow Ca/Mg cathode lamp at 285.2 nm. Total endogenous magnesium was calculated from a standard curve of magnesium atomic absorption reference solution (Fisher Scientific Co., Fair Lawn, NJ).

Free endogenous magnesium measurement

GA and LBG solutions were made at 0.25% (w/v) and arabinogalactan solutions were made at 0.5% (w/v). The fibers were mixed in 0.02 M tris buffer adjusted to pH 6, 7, or 8 with 0.1 N HCl. All fiber solutions were mixed for 2.5–3.0 h. After stirring, 0.5 ml of GA or ABO solution was applied to a Sephadex G-25 Fine column, 2.5 cm \times 14 cm (Pharmacia Fine Chemicals, Uppsala, Sweden). For LBG, 8 ml of LBG solution were centrifuged at 300 rpm for 15 min, and 0.5 ml of the supernatant was pipetted on top of a Sephadex G-25 Fine column (2.5 \times 14 cm). All samples were eluted to 110 ml with tris buffer adjusted with 0.1 N HCl to pH 6, 7, or 8. The void volume, previously determined to be 25 ml with Blue Dextran 2000 (Pharmacia) was collected initially and subsequent fractions were collected in 5 ml increments up to 110 ml. Samples were diluted with LaCl_3 when necessary, and fractions were analyzed for free magnesium by atomic absorption spectrophotometry (AAS). Magnesium calculated from the standard curve of magnesium standards was considered to be free. To determine if pH had an effect on the amount of free magnesium within a fiber, the means of free magnesium at each pH were compared using analysis of variance (SAS Institute Inc., Cary, NC). To determine if any magnesium was bound, the free amount at each pH was compared to the total endogenous amount. No magnesium was considered to be bound if the total of free magnesium eluted from the column was equal to or greater than 100% of the ashed endogenous total magnesium.

Free added magnesium measurement

Fiber solutions were made to the same percent as in free endogenous magnesium experiments. Solutions were made in pH 7 tris buffer, and magnesium sulfate was added to the fiber solution at 100 times the endogenous magnesium amount for LBG and ABO, and 30 times the endogenous magnesium amount for GA. After stirring for 2.5–3.0 h, gel chromatography and AAS were performed in the same manner as for free endogenous measurement, using pH 7 tris buffer as the eluent. Standards for percent recovery of the added magnesium were made by mixing magnesium sulfate in the same amount as the fiber solutions, but no fiber was included in the solution. The magnesium sulfate standards were stirred, subjected to gel chromatography, and measured on the atomic absorption spectrophotometer in the same manner as the fiber solutions. To determine if magnesium was binding to the fiber, mean percent recovery of magnesium from the fiber samples was compared to mean percent recovery of the corresponding magnesium sulfate standard using a *t*-test (SAS Institute).

Enzyme hydrolysis

Fiber solutions were made in 100 ml of distilled, deionized water at 0.25% (w/v) and stirred for 2.5 h. The samples were placed in a 37°C water bath for 30 min, and then hemicellulase (crude from *Aspergillus niger*; Sigma) was added to the solutions in the following amounts: 100 mg hemicellulase for LBG, and 500 mg each for GA and LBG. Aliquots of preliminary samples were injected into a high-performance liquid chromatography (HPLC) system to determine if hydrolysis was progressing (Waters 712 WISP injector, Waters 501 HPLC pump, Waters 410 differential refractometer from Millipore Waters Chromatography Division, Milford, MA; TSK-GEL column from Beckman, Fullerton, CA). Molecular weight and undigested fiber standards were also injected to use as reference points for the hydrolysis (D-9260 and D-1390 Dextran; Sigma). All fibers and standards were filtered through a 0.45 μm filter before injection. LBG was digested for 5.0 min, ABO for 2.5 h, and GA for 3.0 h. Gel chromatography and AAS were performed following the same procedure as for free added magnesium, except that LBG was not centrifuged before applying to the column. To determine if the enzyme itself bound magnesium, a 0.5% (w/v) solution of enzyme was prepared in distilled, deionized water and processed in the same way as the digested fiber samples. To determine the endogenous magnesium content of the enzyme, the enzyme was wet-ashed and the magnesium content measured using AAS. To determine if partial hydrolysis of the fiber caused magnesium to be bound, the mean of free magnesium from the hydrolyzed samples minus the mean of the free magnesium from the enzyme was

compared to the mean of the endogenous magnesium of the fiber using a *t*-test (SAS Institute).

RESULTS AND DISCUSSION

Magnesium binding to both insoluble and soluble fibers has been studied *in vitro*. No studies of magnesium binding to GA were found in the literature. One study of magnesium binding to an ABO isolated from rice was found (Mod *et al.*, 1982). Although no *in vitro* studies of magnesium binding to LBG were found, there was one study of magnesium binding to guar gum, a neutral galactomannan similar to LBG (Camire & Clydesdale, 1981). Laszlo (1989) studied the endogenous magnesium retention by 0.2% solutions of corn bran and soybean hull over the pH range 3–6, and the effect of ionic strength of the fiber solution on magnesium retention. He found that little magnesium was dissociated from the fibers between pH 4.5 and 6.0, while little or no magnesium was retained below pH 3.0; increasing ionic strength of the fiber solutions caused progressively less magnesium to be retained. He concluded that, in the small intestine, all magnesium would be extracted from these fibers due to the high ionic conditions. Lee and Garcia-Lopez (1985) reported that, at pH 6.5, there was no magnesium binding to either a 1% acid detergent fiber solution or a 2% neutral detergent fiber solution. Camire and Clydesdale (1981) studied magnesium binding to both insoluble and soluble fibers. They concluded that there was little magnesium binding to cellulose over the pH range 5–7 with either boiling or toasting, that increasing pH resulted in increased magnesium binding to lignin and wheat bran, and that toasting wheat bran resulted in higher magnesium binding. In addition, these investigators found that 1% solutions of pectin and guar gums to which magnesium was added bound 76% and 34% of the magnesium, respectively. Mod *et al.* (1982) found that ABO isolated from rice bound about 32% of magnesium.

Uronic acid presence

Since neutral polysaccharides have little affinity for alkaline earth metals and anionic polysaccharides have a strong affinity for cations (Rendleman, 1978*b*), knowing if uronic acid groups are present in fibers is important in mineral binding studies. As expected, no detectable uronic acid was present in LBG or ABO at either 0.08% or 0.32% fiber solution concentration. The GA solution turned a purple color after 2 h, indicating the presence of uronic acids. Further analysis revealed that GA was 10.4% glucuronic acid.

Total endogenous magnesium

The mean amounts of total endogenous magnesium determined by wet ashing for each fiber are presented in

Table 1. All fibers contained detectable amounts of magnesium. Of the three fibers, GA had the highest amount of endogenous magnesium, approximately seven times as much as LBG and ABO.

Free endogenous magnesium

Free endogenous magnesium was determined over the pH range 6–8. Table 2 lists the mean free magnesium in each fiber on a weight/weight basis. Within each fiber, the free endogenous magnesium at each pH was not significantly different from that at any other pH ($P > 0.05$). The recovery of magnesium from GA was 100%; both LBG and ABO had $> 100\%$ recovery of total endogenous magnesium, with the exception of LBG at pH 8. Obtaining a measurement of $> 100\%$ total magnesium content was probably due to the different methodologies for measuring magnesium: i.e. wet ashing versus column chromatography and additive error in the free magnesium experiments. These results suggested either that no magnesium was bound by any fiber at any pH level or that any binding present was so weak that the complex was not stable and was easily dissociated. For both LBG and ABO, the mean amounts of free magnesium tended to decrease with increasing pH. This non-significant trend agrees with the generally accepted concept that neutral polysaccharides form weak complexes with cations in non-alkaline media, and only in alkaline solution is there any

Table 1. Total endogenous magnesium content of fibers on a dry weight basis

| Fiber | Magnesium ($\mu\text{g Mg mg}^{-1}$ fiber) |
|-----------------|--|
| Gum arabic | 2.08 ± 0.28 |
| Locust bean gum | 0.30 ± 0.01 |
| Arabinogalactan | 0.27 ± 0.01 |

Values are given as mean \pm standard deviation.

Table 2. Mean free endogenous magnesium of fibers at pH levels 6–8

| Fiber | pH | Free Mg (mg Mg mg^{-1} fiber) |
|-----------------|----|--|
| Gum arabic | 6 | $2.17 \pm 0.03\text{a}^2$ |
| | 7 | $2.12 \pm 0.11\text{a}$ |
| | 8 | $2.02 \pm 0.08\text{a}$ |
| Locust bean gum | 6 | $0.33 \pm 0.05\text{b}$ |
| | 7 | $0.32 \pm 0.04\text{b}$ |
| | 8 | $0.24 \pm 0.04\text{b}$ |
| Arabinogalactan | 6 | $0.31 \pm 0.02\text{c}$ |
| | 7 | $0.31 \pm 0.01\text{c}$ |
| | 8 | $0.30 \pm 0.03\text{c}$ |

Values are given as mean \pm standard deviation. All means represent two replicates with triplicate analyses in each replicate. Values within a fiber with the same superscript letter are not significantly different at $P < 0.05$ by analysis of variance.

great affinity between cation and polysaccharide (Rendleman, 1978a; Burger & Nagy, 1990). However, as the gastrointestinal tract is only slightly alkaline, there is no physiological relevance in determining complexing in an alkaline environment.

Binding of added magnesium

The amount of magnesium added to the fibers and corresponding standards and the mean percent recovery for each is presented in Table 3. For each fiber at pH 7, there were no significant differences in the percent recovery of magnesium from the fibers when compared to the magnesium sulfate standards. As with free endogenous magnesium, free magnesium recovery was greater than 100%. It is possible that the additive error in the measurement of added magnesium experiments is greater than in free endogenous magnesium measurements, since the amount of magnesium in the magnesium sulfate standards (and corresponding fiber solutions) necessitated further dilution in some of the eluted 5 ml fractions. Based on these results, addition of 30 or 100 times the endogenous magnesium did not result in magnesium binding by the fibers.

Effect of enzyme digestion

GA, LBG, and larch ABO are at least partially digested by various human colonic bacteria species (Salyers *et al.*, 1977, 1981; Wyatt *et al.*, 1986). With digestion, the original polysaccharides present become a new mixture of polysaccharides and oligosaccharides, possibly with different cation binding abilities from the original polysaccharides. The effect of partial digestion on the binding ability of the three fibers is given in Table 4. The endogenous magnesium in the hemicellulase, measured by wet ashing, was $0.034 \pm 0.001 \mu\text{g Mg mg}^{-1}$ hemicellulase. The free magnesium, separated from the

Table 4. Free magnesium in fibers after enzymatic digestion

| Fiber | Free Mg (mg Mg mg ⁻¹ fiber) |
|-----------------|--|
| Gum arabic | 2.12 ± 0.05^a |
| Locust bean gum | 0.31 ± 0.02^a |
| Arabinogalactan | 0.32 ± 0.02^b |

Values are given as mean \pm standard deviation. All means represent two replicates with triplicate analysis in each replicate.

^aNot significantly different than total endogenous magnesium at $P < 0.05$.

^bSignificantly different from total endogenous magnesium at $P < 0.05$ by *t*-test.

enzyme by column chromatography, was $0.054 \pm 0.010 \mu\text{g Mg mg}^{-1}$ hemicellulase. Free magnesium determined by chromatography was greater than total magnesium determined by ashing; therefore, magnesium was assumed not to be bound to the enzyme.

Since the mean amount of free magnesium recovered from the digested fiber was greater than the total endogenous amount of magnesium and the enzyme did not bind to magnesium, the amount of magnesium contributed by the enzyme was subtracted from the total free amount of magnesium separated from the digested fiber. After this adjustment, the amounts of free

Table 3. Recovery of added magnesium from fibers and standards

| Fiber/standard | Mg (mg) | Fiber (mg) | Recovery of Mg (%) |
|--------------------------|---------|------------|--------------------|
| Gum arabic | 15.0 | 250 | 104.6 ± 1.9^a |
| Gum arabic standard | 15.0 | — | 102.6 ± 4.6^a |
| Locust bean gum | 7.5 | 250 | 105.1 ± 3.2^b |
| Locust bean gum standard | 7.5 | — | 109.0 ± 2.4^b |
| Arabinogalactan | 15.0 | 500 | 107.7 ± 3.4^c |
| Arabinogalactan standard | 15.0 | — | 102.6 ± 4.6^c |

Values are given as mean \pm standard deviation. All means represent two replicates with triplicate analysis in each replicate. Endogenous magnesium contained in each fiber was included in calculating percent recovery by analysis of variance. Values with the same superscript are not significantly different at $P < 0.05$ by analysis of variance.

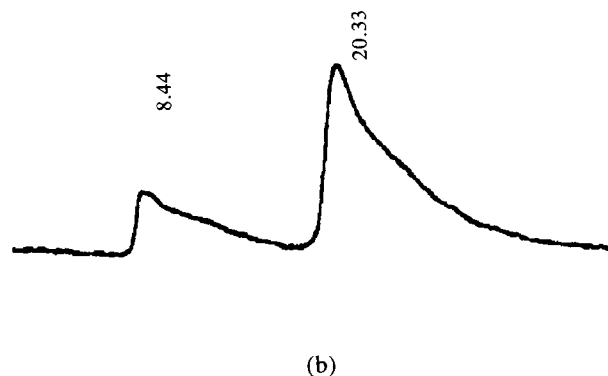
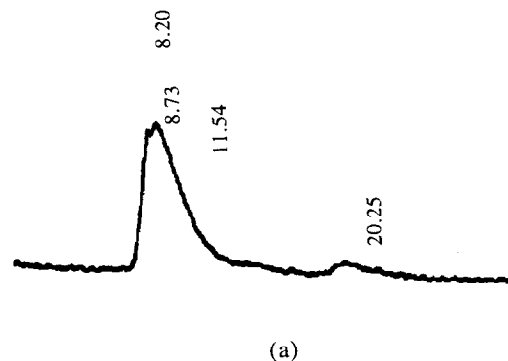


Fig. 1. HPLC retention times (min) for (a) undigested 0.25% gum arabic standard, and (b) 0.25% gum arabic after 3 h 0.5% hemicellulase digestion.

magnesium from the digested GA and LBG were not different ($P > 0.05$) from the total endogenous amount, and the free magnesium from digested ABO was significantly higher than the total endogenous amount ($P < 0.05$). Therefore, partial enzymatic digestion of the fiber did not increase their ability to complex with magnesium.

The HPLC chromatographs and retention times of the digested fibers and corresponding undigested standards are shown in Figs 1–3. Partially digested fibers had either a shorter and broader peak than the corresponding undigested standard, indicating that some of the fiber has been partially digested (Fig. 1), or peaks whose retention time had shifted towards that of the smaller molecular weight standards, indicating partial digestion. The chromatographs and retention times of the molecular weight standards and the hemicellulase are given in Figs 4 and 5.

From the results of previous studies, it seems that different fibers have different abilities to bind magnesium, and where magnesium binding is present, pH and ionic strength influence the extent of that binding. Camire and Clydesdale (1981) found guar gum, a neutral galactomannan similar to LBG, had the ability to bind about one-third of the magnesium added. There are at least two explanations of their results. Since their technique used an ultrafiltration membrane to separate

the guar gum from solution, it is possible that the guar gum was fouling the membrane, preventing free magnesium from passing through, thereby producing an artificially high bound magnesium value. Another possible explanation for the difference in binding between the neutral galactomannans has been discussed by Schlemmer (1989), who, unlike Camire and Clydesdale (1981), found no binding of calcium to guar gum. Schlemmer (1989) postulated that the higher ionic strengths of his fiber solutions were responsible for differences in his results and those of Camire and Clydesdale (1981), who used water as the solvent. The fiber solutions in the current study were stirred and eluted at higher ionic strengths than those at which the solutions of Camire and Clydesdale (1981) were stirred. The ABO studied by Mod *et al.* (1982) had the ability to bind magnesium. Their ABO solutions were made and dialyzed in deionized water, so the comments by Schlemmer (1989) relating to ionic strength may also explain the difference between the results from Mod *et al.* (1982) and the current study. It is also possible that the complexes formed were unstable and could have been displaced had the fiber been passed through a Sephadex column.

Given the neutral nature of LBG and ABO, the low fiber solution concentrations utilized, and the nature of the magnesium ion, it is not surprising that endogenous magnesium binding was not found. Some binding was expected when excess amounts of magnesium were added, but any binding that might have been measured in an equilibrium system must have been unstable, as

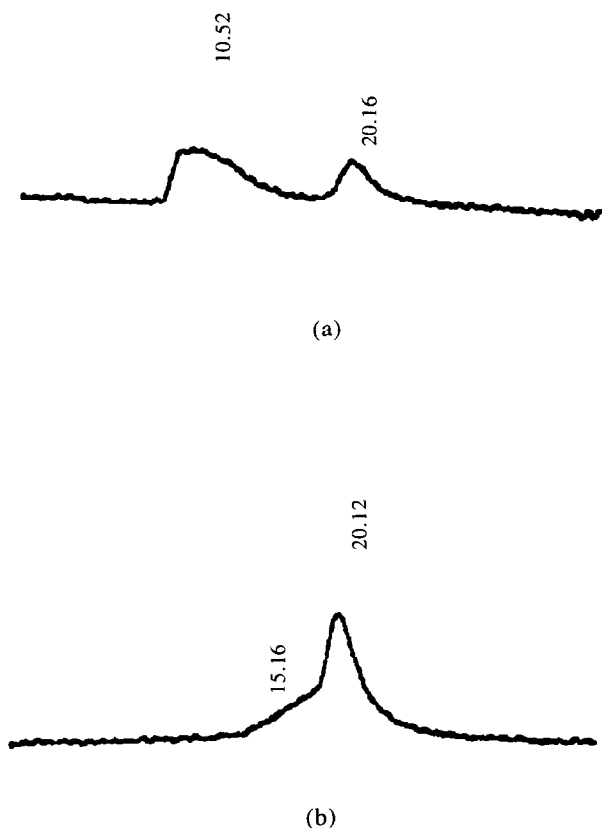


Fig. 2. HPLC retention times (min) for (a) undigested 0.25% locust bean gum standard, and (b) 0.25% locust bean gum after 6 min digestion with 0.1% hemicellulase.

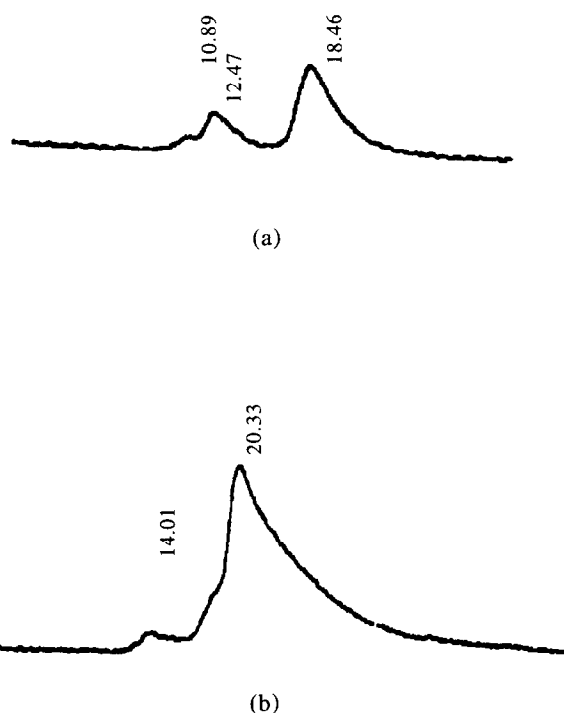


Fig. 3. HPLC retention times (min) for (a) undigested 0.25% arabinogalactan standard, and (b) 0.25% arabinogalactan after 2.5 h digestion with 0.5% hemicellulase.

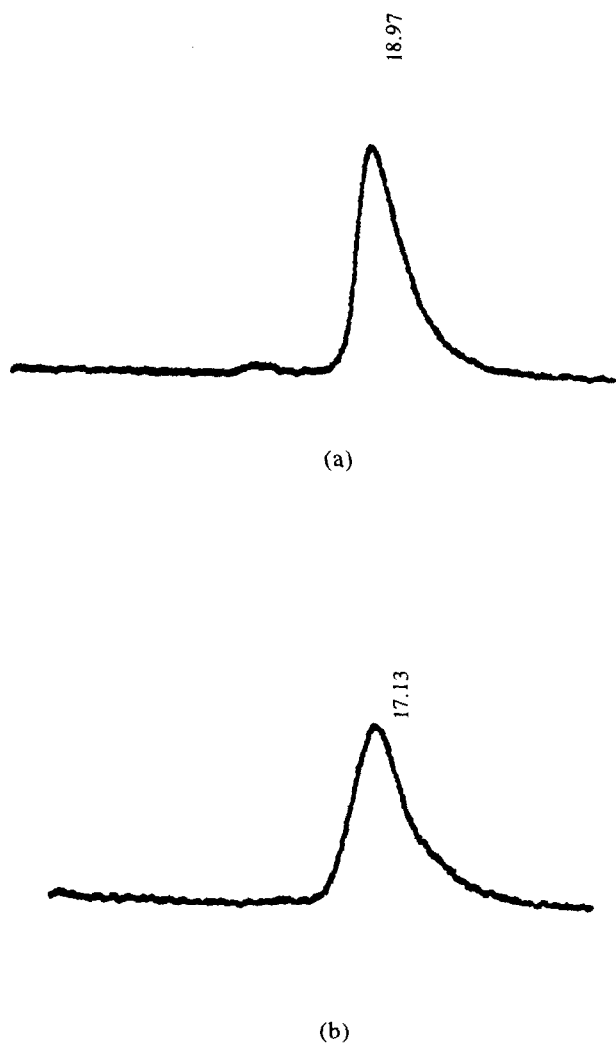


Fig. 4. HPLC retention times (min) for (a) 0.1% D-9260 Dextran standard, average molecular weight 8800, and (b) 0.1% D-1390 Dextran standard, average molecular weight 66300.

passage through the Sephadex column (a non-equilibrium system) produced a magnesium-free fiber. This lack of binding with the acidic polysaccharide GA was unexpected. These results may reflect the low fiber solution concentrations, the small size of the magnesium ion, and/or competition from other cationic species.

From these experiments, magnesium does not form stable complexes with acidic GA or the neutral polysaccharides LBG and ABO between pH 6 and 8, in the presence of added magnesium, or with enzymatic digestion. The levels at which these fibers are present in food would not cause magnesium to become unavailable for absorption in the gastrointestinal tract. Although ingestion of these soluble fibers for pharmacological purposes would be at higher levels than those used in these experiments, this still would probably not cause magnesium to become unavailable for absorption due to the high ionic environment of the stomach, the low affinity of magnesium for polysaccharides, and competition by other cations for binding sites.

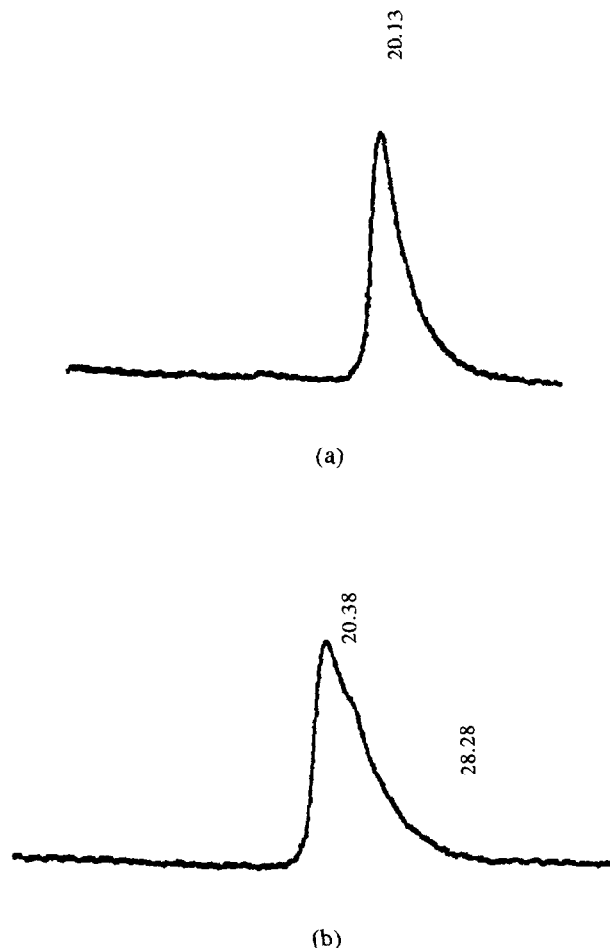


Fig. 5. HPLC retention times (min) for (a) 0.1% hemicellulase standard, and (b) 0.5% hemicellulase standard.

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