Physicochemical Characteristics of Gradual Fractionation Ingredients of Industrial Galactomannan Gums from *Gleditsia microphylla* and *Cyamopsis tetragonoloba*

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Galactomannan in industrial *Gleditsia microphylla* and guar gum was successfully fractionated by gradual precipitation in an aqueous solution with increasing ethanol concentrations. The molecular properties of each fraction were characterized, and the galactomannans were added to photopolymerized hydrogels to test their effects on mechanical properties and swelling capacity. In the series fractions of guar gum, the sample precipitated from 20% EtOH solution had the highest yield, mannose to galactose ratio, and viscosity, and it had a slightly lower molecular weight than that precipitated by 30% EtOH. Correspondingly, the best tensile property of its photopolymerized hydrogel was finally detected. In terms of *G. microphylla* gum, the precipitation in 30% EtOH solution achieved the highest yield, M/G ratio, and molecular weight value, and it exhibited the best rheological property of all the samples. The hydrogel with the addition of this sample also had the best mechanical properties despite its lower hydroscopicity than the blank hydrogel. The unique properties of each fraction could probably lead to their use as biodegradable alternatives in different applications.

Keywords: Gleditsia microphylla gum; Guar gum; Physicochemical characteristics; Fractionation; Rheological and mechanical properties; Hydrogel

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INTRODUCTION

The Leguminosae family is a rich source of galactomannans, which are mainly found in the endosperm of seeds. Galactomannans are neutral, water-soluble polysaccharides with a diverse structure and a wide range of physicochemical properties, resulting in their broad application as thickening, water holding, and stabilizing agents in food products (Brummer *et al.* 2003). Galactomannans are also essential hydrophilic materials with a variety of properties, such as being nontoxic, biodegradable, inexpensive, and readily available. The potential of galactomannans from different sources to be applied as release-controlling agents has been extensively investigated (Varshosaz *et al.* 2006).

In the Leguminosae family, *Gleditsia microphylla* is a medicinal plant widely distributed in China, and it has a high tolerance for environmental conditions.

Galactomannan as a storage polysaccharide exists in the endosperms of the *G. sinensis* seeds, and it consists of β-(1→4) linked mannose backbone substituted by branches of single galactose units with α-(1→6) linkages. Galactomannan from *G. sinensis* has shown excellent behavior as a release-controlling agent in sustained release matrix tablets used for colonic drug delivery (Jian *et al.* 2011a,b; Jiang *et al.* 2011). *G. microphylla* is used currently for food, health care products, and cosmetics, as well as for the treatment of various cancers and heart, vascular, and infectious diseases. Also, its spines contain antimicrobial components, such as flavonoid glycosides, phenols, and amino acids. It has been used to cure swelling, suppuration, and other skin diseases (Ahn 2003). Guar gum is mainly extracted from the seeds of *Cyamopsis tetragonoloba*. It consists of a linear backbone of β-(1→4) linked D-mannopyranose units with randomly attached α-(1→6) linked galactopyranose side chains; its polysaccharide structure is similar to that of *G. sinensis* gum (Maier *et al.* 1993). Because of its low cost and its ability to produce a highly viscous solution even at low concentrations, guar gum has important applications in the food, oil recovery, and personal care industries (Fox 1997). For instance, it has been used to improve shelf-life and prevent creaming or settling in salad dressings, soft drinks, and fruit juices. However, these two types of galactomannans have yet to be adequately exploited.

Hydrogels consist of hydrophilic polymers that form a three-dimensional structure, and they have been widely used in drug-delivery systems, superabsorbents, tissue engineering, and contact lenses (Lee and Mooney 2001; Vermonden *et al.* 2012). However, some challenges must still be overcome in order to develop biomimetic hydrogels for load-bearing soft tissues, such as cartilage, tendon, muscle, and blood vessels. Their poor mechanical strength, toughness, and limited extensibility and recoverability result from their intrinsic structural heterogeneity and/or lack of efficient energy-dissipation mechanisms. Many efforts have been made to develop new polymerization methods for synthesizing strong and flexible hydrogels with novel microstructures and excellent mechanical properties. For example, double-network hydrogels (DN hydrogels)—two cross-linked networks with strong asymmetric structures—have demonstrated enhanced and balanced mechanical properties between strength and toughness by the tuning of inter-intramolecular interactions and structures within and between two networks (Gong and Osada 2010; Gong 2010). Due to the good biocompatibility, flexibility in fabrication, variable composition, and desirable physical characteristics of the two kinds of gums mentioned above, their hydrogels alone or combined with cells could be used in biomedical applications (Slaughter *et al.* 2009; Strehin *et al.* 2010). Nevertheless, some materials lack adequate mechanical properties, tunable structure, and degradability, which can lead to potential immunogenic responses that compromise their utilization as biomaterials (Zhu 2010). Therefore, for these two types of gum, the facile one-pot method described by Chen *et al.* (2013) was adopted to enhance the mechanical properties of the hydrogels for their applications in load-bearing artificial soft tissues.

The objective of this work was to purify and gradually fractionate the polysaccharides from *G. microphylla* and *Cyamopsis tetragonoloba* and evaluate the properties of the gums, including monosaccharide composition, molecular weight distribution, and rheological properties, *etc*. Furthermore, the corresponding hydrogel products were produced and tested in terms of mechanical properties and swelling behavior.

These tests provide the basis for the relationship between polysaccharide structures and functional properties and for the efficient utilization of these plant gums.

EXPERIMENTAL

Materials

The isolated *Gleditsia microphylla* gum was kindly supplied by Shexian Forestry Bureau in Hebei, China. Food additive grade guar gum was purchased from Feihong Chemical Co., Ltd. (Shijiazhuang, China). All raw materials were oven-dried at 60 °C for 12 h and stored in desiccators until use. Other chemicals and reagents were of analytical grade and used without further purification.

Methods

Purification and gradual fractionation

The purification procedure of the industrial gums was similar to that described by Jian *et al.* (2011a). Briefly, *G. microphylla* or guar gum (5 g) was first dispersed into 100 mL of 80% (v/v) ethanol aqueous solution and boiled for 10 min, and the insoluble substance was collected on a Buchner funnel and washed successively with ethanol, acetone, and ether. The obtained solid material was dissolved in 500 mL of deionized water for 1 h with a magnetic stirrer. The solution was centrifuged for 20 min at 2594 × *g*, and the supernatant was precipitated in two volumes of cold acetone (4 °C). After being re-dissolved in 500 mL of hot water (80 °C), the polymer solution was centrifuged again, and the precipitation of galactomannan was finally achieved by adding the supernatant to ethanol at a proportion of 1:1. The purified gum was collected by centrifugation, dried in a vacuum oven for 36 h, and kept in a desiccator at room temperature before further analysis (Wientjes *et al.* 2000). *G. microphylla* and guar gum samples were labeled as Z and G, respectively. The fractionation was achieved by adding 10%, 20%, 30%, 40%, and 50% (v/v) anhydrous ethanol to the polysaccharide aqueous solution (1%, w/v). Eventually, the five centrifugal precipitations were collected, freeze-dried, and labelled as Z_1 or G_1, Z_2 or G_2, Z_3 or G_3, Z_4 or G_4, and Z_5 or G_5, respectively, according to the different ethanol concentrations. All the experiments were repeated for 3 times at least.

Monosaccharide composition

All polysaccharide fractions (15 mg) were hydrolyzed with 4% H_2SO_4 (w/w) aqueous solution at 121 °C for 1 h. The hydrolysates were filtered and diluted 1:100 in the same solution before chromatography. The monosaccharide components, including galactose, mannose, xylose, glucose, and arabinose, were quantified using a high-performance anion-exchange chromatography (HPAEC) system (Dionex, ICS 3000, Sunnyvale, CA, USA) on a CarboPac PA 20 analytical column (4 × 250 mm) with pulsed-amperometric detection (PAD). The neutral sugars were separated in 18 mM NaOH (carbonate-free and purged with helium) with a post-column addition of 0.3 M NaOH at a rate of 0.5 mL/min. The run time was 45 min, which was followed by a 10-min elution with 0.2 M NaOH to wash the column and then a 15-min elution with 18 mM NaOH to re-equilibrate the column.

Molecular weight distribution

The weight-average molecular weight (M_w), number-average molecular weight (M_n), and polydispersity index D (M_w/M_n) of the samples were analyzed by gel permeation chromatography (GPC) on a PL Aquagel-OH Mixed column (300 × 7.5 mm, Agilent, Santa Clara, USA) with a refractive index detector (RID). The eluent was 0.02 M NaCl in 5 mM sodium phosphate buffer at a pH of 7.5 and with a flow rate of 0.1 mL/min. The samples were dissolved in phosphate buffer at a concentration of 2 mg/mL and passed through a 0.45-μm filter before injection.

To calibrate the column, a monodispersed polysaccharide of known molecular weight (pullulan, peak average molecular weights 783, 12200, 100000, and 1600000; Polymer Laboratories, Ltd., Church Stretton, UK) was used as the standard for calculating the molecular weight of the polysaccharides. The average relative error was found to be 3.8 to 5.6%, with a maximum error of 7.3%. The data was analyzed by Agilent GPC Data Analysis Software version B.01.01 (Santa Clara, USA).

^{1}H NMR spectroscopy

The ^{1}H NMR spectra of the obtained fractions Z, Z_2, Z_3, Z_4, G, G_2, and G_3 were recorded with a Bruker DRX-400 spectrometer (Karlsruhe, Germany) at 75.5 MHz. Because of the different viscosities of *G. microphylla* and guar gum, the samples were completely dissolved in D_2O at room temperature (25 °C), employing concentrations of 3.64 mg/mL for Z samples (Z, Z_2, Z_3, and Z_4) and 0.364 mg/mL for G samples (G, G_2, and G_3).

Rheological properties

Rheological tests were carried out on an LVDV-III Ultra Rheometer (Brookfield Engineering Laboratories, Stoughton, MA, USA) fitted with a small sample adapter (spindle no. SC4-31). The viscosity determined in this study indicates apparent viscosity. The gum solutions (1.0% (w/v) for *G. microphylla* gum and 0.5% (w/v) for guar gum) for rheological tests were prepared at room temperature with mechanical stirring for 30 min to achieve complete hydration.

The curves of shear stress as the function of shear rate were determined at 25 °C with Rheocalc V3.2 software (Middleboro, USA), with a test time of 3 min and shear rates ranging from 1.7 to 85 s^{-1}. For evaluating the effect of temperature on viscosity, the tests were carried out on a Brookfield digital Viscometer DV-II+PRO (Brookfield Instruments, Middleboro, USA) using an S34 rotor at an appropriate rotation speed according to the torque (from 20% to 80%) to ensure the accuracy of viscosity. The polysaccharide solutions were subjected to a water bath, with the temperature increasing from 30 °C to 80 °C, and the viscosities were recorded after remaining stable for 10 min.

Hydrogel production and mechanical property analysis

Hydrogel was produced as described by Chen *et al*. (2013). Briefly, *G. microphylla* (100 mg) or guar gum (50 mg) was added to a small tube with 5 mL of H_2O. After the gum was completely dissolved, AM (acrylamide, 900 mg), 2-hydro-4'-(2-hydroxyethoxy)-2-methylpropiophenone (0.0284 g, irgacure 2959, 1 mol% of AM), and N,N'-methylene-bis-acrylamide solution (59 μL, MBA solution of 10 mg/mL, 0.03% of AM) were added into

the solution. The mixture was sealed and stirred in an oil bath at 90 °C until all of the powder was dissolved and a transparent solution was obtained. After degassing cycles with ultrasonic treatment, the resulting homogeneous solution was injected into a mold, cooled to room temperature, and photopolymerized to by irradiation with a UV light (365 nm wavelength) for 3 h. The obtained hydrogel was sealed in a plastic tube and stored at 0 °C for testing.

The mechanical properties of the hydrogels were measured using a universal material testing machine (UTM6503, Shenzhen Suns Technology Stock Co., Ltd., Shenzhen, China) at room temperature. The hydrogel samples were cut into rectangular strips with dimensions of 4 mm in height × 30 mm in length × 8 mm in width, and a filter paper was placed between the clamp and the sample to prevent the wet hydrogel from slipping. Both ends of the sample were clamped and stretched at a constant rate of 30 mm/min, and the specific testing method was preset by the software. Raw data were recorded as force *versus* displacement, and they were converted to stress *versus* strain with respect to the initial sample dimensions (4×8 mm^2). The fracture stress was determined from the stress-strain curves at the breaking points. The tensile hysteresis was analyzed under the same conditions, in which the sample was initially stretched to a predefined strain and immediately unloaded at the same velocity (Yang *et al.* 2013).

For the evaluation of swelling ability, the hydrogels were put in a dryer at 50 °C until their initial mass was constant (M_0). Then, the dry hydrogels were cut into the same size and placed into a beaker, and excess deionized water was added until it was fully absorbed. After using the filter paper to absorb the visible water, the weight of the hydrogel was recorded as M. The swelling ability was calculated according to Eq. 1:

$$\text{Swelling ability (\%)} = (M - M_0)/M_0 \quad (1)$$

RESULTS AND DISCUSSION

Yields and Monosaccharide Composition

Based on the comparison of four gum purification methods by Cunha *et al.* (2007), the present study adopted the purification process from Wientjes *et al.* (2000). This method has wider biological applications, taking into account the purity, thermal stability, rheological parameters, cost, and simplicity of the procedure. After the same purification process, the yields of *G. microphylla* and guar gum were 45% and 55% (the standard deviation was less than 8%), respectively, suggesting the higher purity of guar gum as the mature industrial product. The yields of gradual fractions from these two polysaccharide ingredients are presented in Table 1, and the total recoveries were similar (97.2% for *G. microphylla* gum and 99.5% for guar gum). It has been reported that galactomannan precipitates form in the bulk solution containing >18.6% (v/v) ethanol, but there may have been float and drift owing to the different resources of the ingredients. The data in Table 1 clearly reveal that during the fractionation process of *G. microphylla* gum, the amount of polysaccharide ingredients precipitated in 30% ethanol aqueous solution (Z_3) significantly exceeded other components by more than 70%, similar to the yield (70.5%) of ethanol fractionation (33%) described by Oleynikov *et al* (2010). It is surprising to find that although no significant differences in physicochemical characteristics were detected

between Z_1 and $Z_{2–4}$ as discussed below, only 1.01% of the purified polysaccharides were fractionated in 10% ethanol solution. Correspondingly, the obvious low yield (1.62%) of the polysaccharide ingredients in 50% ethanol solution (Z_5) was entirely caused by its essential properties. Meanwhile, it was surprising to find that arabinose (22.9%), galactose (31.9%), and mannose (39.3%) had almost equal proportions in the Z_5 sample (Table 1), and its negligible mass did not appear to influence the composition of the main monosaccharides in the purified *G. microphylla* gum. In *G. microphylla* samples (except Z_5), galactose (34% to 35%) and mannose (63% to 65%) were the major components, and small amounts of arabinose, glucose, and xylose were also detected. The mannose content had a positive influence on antioxidant activity, while the influence of glucose was negative (Meng *et al.* 2015). In contrast, the guar gum fractions had a lower mannose content (approximately 50%) and higher galactose content (approximately 50%). After the gradual precipitation, the G_2 and G_3 samples occupied more than 95% of the total purified gum, which is close to the galactomannan yield (95.7%) purified by 33.3% ethanol in Jian *et al.* (2014). Similar to the above, the yield of the G_1 sample with almost the same chemical components was only 3.1%, indicating that 10% ethanol aqueous solution could not efficiently fractionate the galactomannan gum. Meanwhile, the polysaccharides (G_4) precipitated at 40% ethanol had small masses, certainly corresponding to their total different ratios of monosaccharide components. Thus, this polysaccharide ingredient could not be called galactomannan and its M/G ratio was not available, because the galactose content could only account for 0.5% of its composition. Such a difference between the yields of the fractions confirmed that the precipitation behavior of galactomannans in polar organic solvents was dependent on the molecular structure, *e.g.*, molecular weight and galactose substitution (Meng *et al.* 2015).

Selected gradual fractionation ingredients (the major proportions of the gum) were detected by NMR technology, and the proton NMR spectra are shown in Fig. 1. The original galactomannans had low peak resolutions and gave a noisy spectrum in the highly viscous solution due to their high molecular weight. The resonances of the anomeric protons were well-separated, and their identifications were self-evident from the known monomeric compositions of the samples (González 1978). The peak resolutions increased with decreasing molecular weight (M_n), and almost no significant difference was detected among the fractionated ingredients, further confirming the data from the sugar analysis.

Table 1. Monosaccharide Yields and Compositions in *G. microphylla* and Guar Gums

Sample	Yield[a]	Monosaccharide Compositions[b]					M/G[c]
		Ara	Gal	Glu	Xly	Man	
Z_1	1.01	0.9	34.5	0.3	0.2	64.1	1.9
Z_2	10.46	0.5	34.8	0.3	0.4	64.1	1.8
Z_3	70.45	0.3	34.3	0.3	Trace	65.1	1.9
Z_4	16.46	0.5	35.1	0.2	0.8	63.4	1.8
Z_5	1.62	22.5	31.9	1.5	4.9	39.3	1.2
Z	--	0.6	35.1	0.3	0.4	63.7	1.8
G_1	3.10	2.9	45.1	0.1	0.6	51.3	1.1
G_2	54.91	3.1	46.2	Trace	0.1	50.6	1.1
G_3	41.16	0.3	49.6	0.1	0.1	49.9	1.0
G_4	0.83	41.0	0.5	1.4	6.9	50.2	96.6
G	--	2.9	47.8	0.1	0.3	48.9	1.0

[a] Weight % of the starting material, with a standard deviation of less than 5%
[b] Ara, arabinose; Gal, galactose; Glc, glucose; Xyl, xylose; Man, mannose. Values are relative wt.% of the total sugar content, and the standard deviations are less than 3%.
[c] M/G, ratio between mannose and galactose.

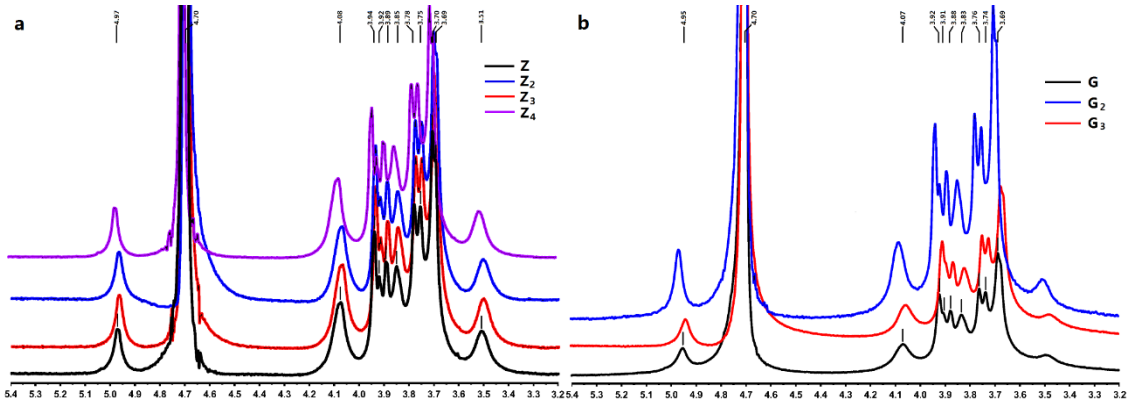

Fig. 1. ^{1}H NMR spectra of the selected Z (a) and G (b) samples

Molecular Weight and Distribution

The molecular weights of these fractions were significantly influenced by ethanol concentration (Table 2). Under different ethanol concentrations, M_w values varied from 4.11×10^4 Da to 5.73×10^7 Da for the Z series and $6.04 \times 1\ 0^6$ Da to 2.73×10^8 Da for the G series. Overall, the molecular weight of the G series was much higher than that of the Z series. The difference in the molecular size of the fractions could be explained as a consequence of molecular structure. Ethanol has a much lower dielectric constant (25) than water (80) at 25 °C (Bilati *et al.* 2005). The dielectric constant of a solvent is closely related to its ability to increase the attractive forces between solute molecules, which eventually initiates precipitation (Gonzales *et al.* 1990). By adding the dehydrating agent (ethanol), the dielectric constants of the polysaccharide solutions became lower, inducing the conformational changes in the polysaccharides and then allowing polymer molecules to

precipitate under different ethanol concentrations. The morphology of precipitates varies with increasing ethanol concentration from random coil conformation to insolubilization with different molecular size and structure (Jian *et al.* 2014). However, the values of molecular weight are supposed to be a little higher due to the formation of aggregations in the purification process as compared with data from other reports (Jian *et al.* 2011a; 2014). It is possible that precipitation with cold acetone promoted aggregate formation by dehydrating the polysaccharide and promoting intra-molecular associations; alternatively, freeze-drying contributed to polysaccharide aggregation.

The distribution patterns of the weight/number-average molecular weights (M_w/M_n) reflected the ethanol concentration (Fig. 2). The $Z_{1–3}$ samples exhibited almost the same distribution regions and adsorption intensities, similar to the M_w and D data (Table 2). Due to the narrowed distribution pattern (2.5), the adsorption peak of the Z_4 sample was shifted to the low-molecular weight region, although the M_w value was only decreased to 6.35×10^6 Da. Furthermore, the distribution curve of the Z_5 sample was mainly concentrated in the range of 0 to 1×10^5 Da with the narrowest polydispersity, corresponding to the lowest M_w and D values. In the guar samples, the distribution curves of the G samples also reflected the differences between the M_w and D values of each fraction.

Both molecular size and mannose to galactose (M/G) ratio affect the rheological properties of galactomannan gum solutions. According to Lazaridou *et al*. (2000), a higher M/G ratio in particular leads to a higher thickening ability. For *G. microphylla* gum, the higher M/G ratio in Z_3 (1.9) compared with Z_2 (1.8) could have produced a thicker polysaccharide solution, although these samples had similar molecular weights. The same phenomenon occurred in G_2 (1.1) and G_3 (1.0) from guar gum. These phenomena were further confirmed by viscosity measurements (discussed below).

Table 2. Molecular Weight and Distributions of *G. microphylla*, Guar Gum, and the Gradual Fractions

Concentration of Ethanol	*G. microphylla* Gum			Guar Gum		
	M_w (Da)	M_n (Da)	D (M_w/ M_n)	M_w (Da)	M_n (Da)	D (M_w/ M_n)
Purified Gums	5.73×10^7	5.24×10^6	10.9	2.13×10^8	1.25×10^7	17.0
10%	5.15×10^7	4.86×10^6	10.6	2.41×10^8	1.05×10^7	23.0
20%	5.17×10^7	5.06×10^6	10.2	1.92×10^8	8.36×10^6	20.5
30%	8.07×10^7	6.10×10^6	13.2	2.63×10^8	1.20×10^7	22.0
40%	6.35×10^6	2.59×10^6	2.5	6.04×10^6	3.00×10^5	20.1
50%	4.11×10^4	3.64×10^4	1.1	--	--	--

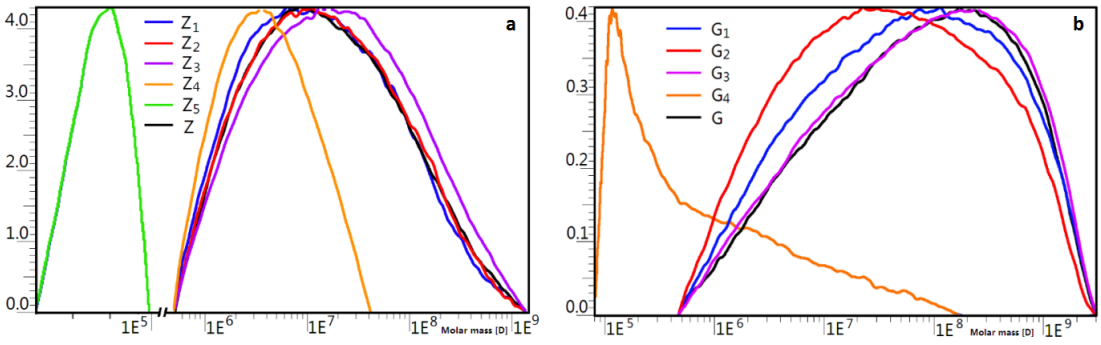

Fig. 2. Molecular weight distribution curves of the Z (a) and G (b) samples

Rheological Properties

Results of rheological measurements for *G. microphylla* solution (1%, w/v) and guar gum solution (0.5%, w/v) concentration are shown in Fig. 3. The Ostwald-de Waele model or Power-law model ($\sigma = k \cdot \gamma^n$) was applied, where the parameters *k* and *n* are related to the consistency index ($Pa \cdot s^n$) and flow behavior index (dimensionless), respectively. The values of *n* for the Z and Z_3 solutions were 0.82 and 0.78, respectively, and 0.42 and 0.52 for the G and G_3 solutions, respectively. Therefore, the whole purified gum and the fractionated ingredient precipitated from the 30% (v/v) ethanol aqueous solution had different rheological properties.

The viscosity of the all samples decreased with increasing shear rate, indicating their pseudoplastic nature. This result revealed that the galactomannan polysaccharides had interlacing molecular interactions between the polymer chains, and the long chains of the galactomannan molecules tended to have a coiling structure at lower energy levels, resulting in interlocking between polymer chains.

Additional intermolecular bridging may occur through simple hydrogen bonds. Furthermore, deformation may result from successive increments of force as shear was imparted. The continued shear breaks of the linkages could lead to a drop in apparent viscosity (Singh *et al.* 2009). The viscosity at the weak shear rate was related to the concentration of the product (Morris and Taylor 1982); the viscosity at high shear rates corresponds to the viscosity of the product that is important during many processing operations, *e.g.*, when it is pumped in a machine. Shear-thinning behavior was clearly exhibited by the gum solutions.

The viscosity values for the G and G_3 solutions at a shear rate of 5 s^{-1} were 467.90 and 359.92 mPa·s, respectively, and these values decreased to 215.25 and 197.96 mPa·s, respectively, at a shear rate of 85 s^{-1}. Based on this data, it was concluded that the rheological characteristics of galactomannan gum were profoundly affected by its molecular weight if the fundamental chemical components were similar.

The effect of temperature on the viscosity of 1.0% (w/v) *G. microphylla* and guar gum solutions was studied from 30 to 80 °C (Fig. 4). As temperature increased, the viscosity of the fully hydrated gum solutions decreased by approximately 85% for the Z samples (Z, Z_2, and Z_3) and 75% for the G samples (G, G_2, and G_3). These results indicated that the inter-chain interactions established by the galactomannan chains were less resistant to temperature.

Higher temperatures produced higher kinetic energy of molecules, which could diminish molecular interactions such as hydrogen bonds, allowing greater fluidity. In addition, prolonged heat may induce changes in the molecular structure. The reduction of gum viscosity with temperature might be caused by irreversible changes in molecular conformation.

Therefore, the gradual precipitation of *G. microphylla* gum efficiently fractionated the polysaccharide ingredients dependent on the biomolecule structure without significantly changing the rheological properties. However, almost half of the guar gum (G_3, 41.2%) was fractionated with a lower viscosity, which could lead to completely different applications.

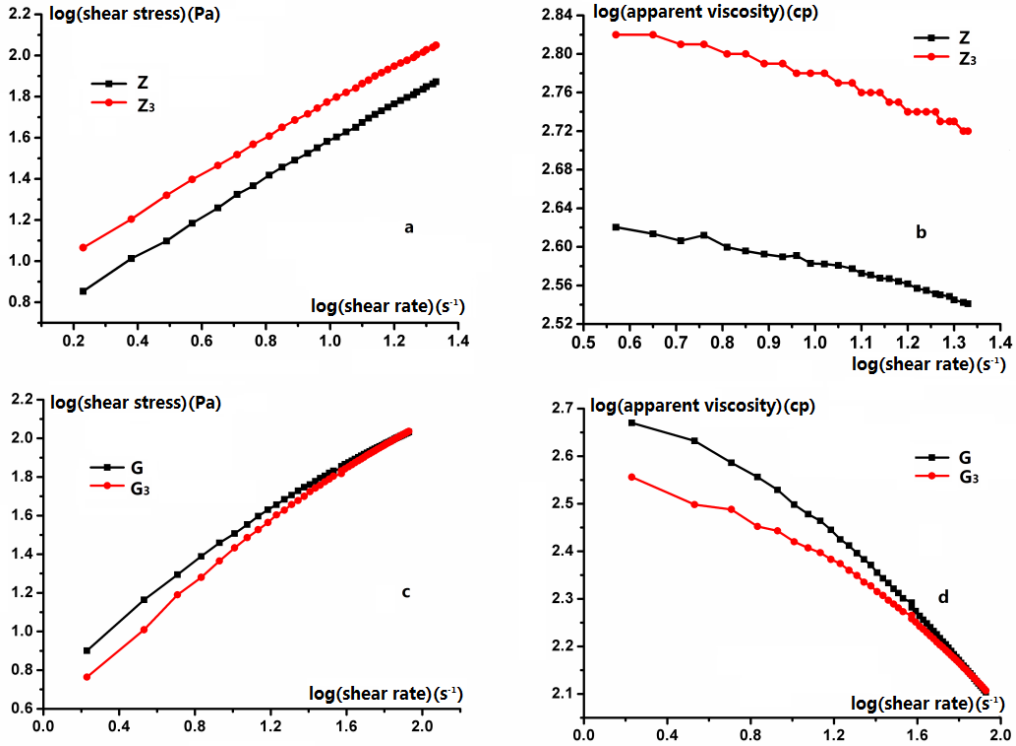

Fig. 3. Flow curves (a, c) and apparent viscosity curves (b, d) of 1% (w/v) *G. microphylla* gum (a, b) and guar gum (c, d) at 25 °C

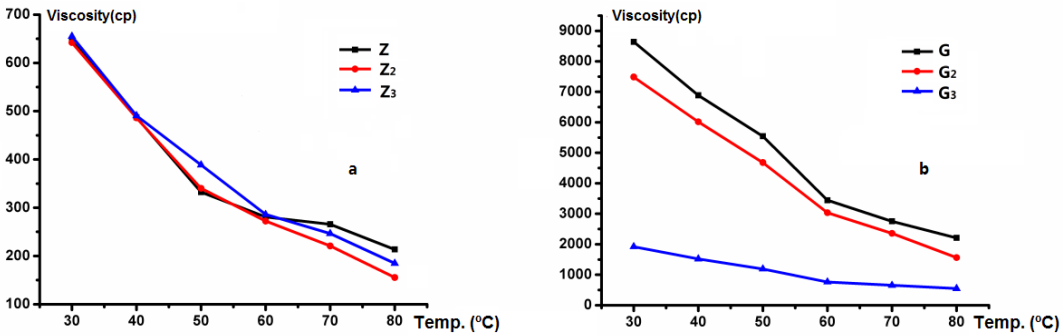

Fig. 4. Temperature dependence of 1.0% (w/v) solution viscosity for G. microphylla and guar gum solutions

Hydrogel Production Properties

The tensile properties of the *G. microphylla* and guar gum photopolymerized hydrogels are listed in Table 3, and the blank was the gel with no polysaccharide. The mechanical properties varied, presumably because of the differences in polysaccharide structure and gel formation. With the addition of polysaccharides, the mechanical properties of hydrogels were not significantly improved, from which it can be inferred that the polysaccharides don't alter the main structure of the blank hydrogels. Hydrogels with the addition of Z_3 and G_3 exhibited the highest tensile strength compared with other hydrogels in the respective series, which corresponded to the high molecular weights of these two galactomannan gradients. As previously reported, the polysaccharides with high molecular weight could be connected together through hydroxyl groups; however, the low-molecular weight polysaccharides could get more stacking than the impact of linking (Tiwari *et al.* 2009). During the loading-unloading tests, a large hysteresis loop was observed in the first loading-unloading cycle (Fig. 5). After the immediate second cycle, the hysteresis became much smaller. The four loading-unloading cycles for G_4 (data not shown) indicated that the second, third, and fourth curves were almost concordant. In the latter half of the first cycle, the curve was close to the other curves, suggesting that they had almost the same force. In the first cycle, the structure of the polymer network was not organized. After the first tensile force, the polymer had a regular three-dimensional structure. The impact of force on the second, third, and fourth cycles were similar. Thus, a larger hysteresis loop was observed in the first cycle. The swelling ability—calculated by $(M-M_0)/M_0$)—reflected the amount of water that could be absorbed by the hydrogels (Table 3).

Table 3. Tensile and Swelling Properties of *G. microphylla* and Guar Gum Hydrogels (All standard deviations were less than 5%.)

Sample	Strain (%)	Swelling Ability, $(M-M_0)/M_0$ (%)
Blank	10.14	15.89
Z	9.26	13.31
Z_2	10.10	11.57
Z_3	12.19	13.19
Z_4	10.79	13.30
G	10.69	12.55
G_2	10.85	12.88
G_3	11.13	12.63

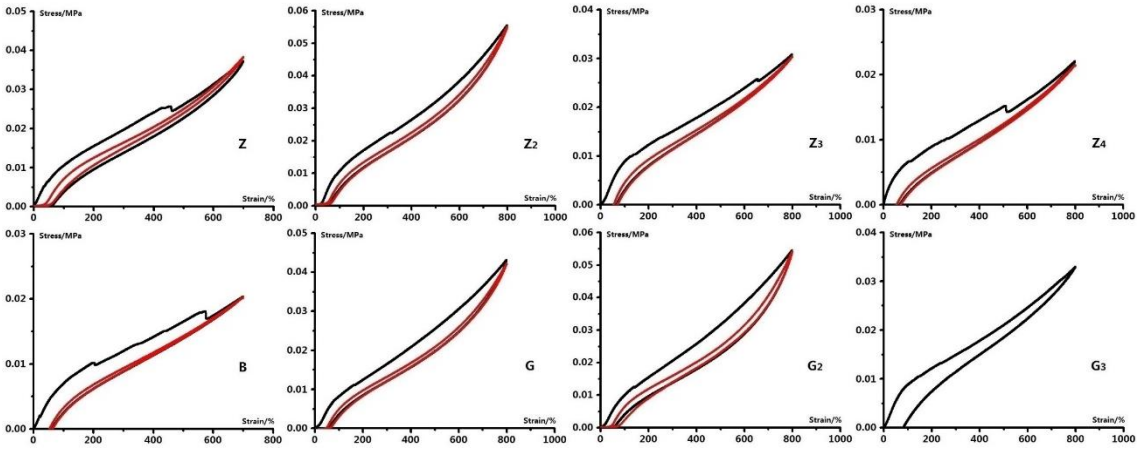

Fig. 5. Hysteresis loops of hydrogels prepared from fractionated *G. microphylla* and guar gum

The blank sample had the best swelling ability because galactomannans of high molecular weight filled the spaces between the cross-linked macromolecules, although more hydroxyl groups were introduced from polysaccharides. Despite the slight difference, the *G. microphylla* gum hydrogels showed better swelling ability than those obtained from guar gum, which was related to the sugar content rather than the molecular weight.

CONCLUSIONS

1. The guar gum G_2 sample precipitated in 20% EtOH solution had the highest yield, M/G ratio, and rheological property, and it had a slightly lower molecular weight than G_3. Hence, the best tensile property was detected in the hydrogel containing the G_3 galactomannan gradient. For *G. microphylla* gum, the 30% EtOH precipitate (Z_3) possessed the highest yield, M/G ratio, molecular weight, and rheological property among all the samples. The Z_3 hydrogel also had the best mechanical properties, despite its lower hydroscopicity than the blank hydrogel.

2. The yields and the qualities of *G. microphylla* and guar gum ingredients varied with differing ethanol concentrations of the polysaccharide solutions, including monosaccharide composition, molecular distribution, and rheological properties.

3. Galactomannans were successfully fractionated by the gradual precipitation of *G. microphylla* and guar gum in EtOH solutions. The unique properties of each fraction could potentially suit different applications, including biodegradable materials.

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