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Carbohydrate Research 267 (1995) 271–290

CARBOHYDRATE RESEARCH

Molecular characterization of polysaccharides dissolved in Me_2NAc–LiCl by gel-permeation chromatography ☆

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Received 18 July 1994; accepted 23 September 1994

Abstract

Polysaccharides function in key roles in textiles, biomass, foodstuffs, and plant cell walls, in addition to pharmaceuticals and immunological interactions. Structural information is urgently needed, but the complexity of the polymers, their interactions, and poor solubility have been limiting aspects. The dilute solution behavior of polysaccharides representing typical classes of natural products was investigated via gel-permeation chromatography (GPC) using refractive index and viscometric detectors. The solvent of choice for high-molecular weight cellulose analysis, dimethylacetamide with lithium chloride (Me_2NAc–LiCl), was used for direct polysaccharide dissolution without extraction or derivatization. The utility of this aprotic solvent for effective characterization of carbohydrates was explored. The polysaccharides studied spanned a range of molecular sizes, branching configurations, and linkage types, i.e., celluloses, amyloses, amylopectins, dextrans, pullulans, and curdlan. In addition to molecular weight values and distributions, calculated parameters defining each polysaccharides' dilute solution characteristics (intrinsic viscosity, radius of gyration, Mark–Houwink coefficients) were determined. Comparison between polysaccharides of similar molecular weight differing either in their branching patterns or linkage distribution were made. The calculated values were correlated with theoretical results predicted in the available literature and/or with known results. Relations among polymer linkage type, molecular chain compositions, branching assessments, and solution properties are described.

Keywords: Polysaccharide; Gel-permeation chromatography; *N,N*-Dimethylacetamide–lithium chloride solvent

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Elsevier Science B.V.
SSDI 0008-6215(94)00307-6

1. Introduction

Polysaccharides such as dextrans, pullulans, and cellulose play key roles in the pharmaceutical, food, and textile industries. In addition to traditional uses, they function as plasma volume expanders, low-calorie food ingredients, tablet coatings, chromatography media, cosmetics, and oil-drilling muds [1]. Also, polysaccharides play roles that are essential to all biological systems, although no single common theory has emerged to explain the remarkable complexity and diversity of these molecules [2]. Optimizing functions and understanding biological roles of polysaccharides are directly dependent upon knowledge of composition, structure, molecular weight distribution (MWD), and the extent to which these properties affect polymer behavior. Although the varieties of branched polysaccharides exceed those of linear polysaccharides, it is the linear polysaccharides which are most abundant in nature [1]. Molecular structures of polymers in general, and polysaccharides in particular, govern physical properties. For example, a linear glycan will produce much more viscous solutions than will a branched glycan of the same number of repeating units due to its higher hydrodynamic volume [1]. Functional differences between polysaccharides have been related to chain length distributions; to combinations, arrangements, and interactions of and between the chains; to conformational differences and combinations of linkages; to differences in branching of the molecular chain; or to combinations of these properties.

The characterization of solution properties of polysaccharides, including oligosaccharides, has been an extremely challenging research area. The poor solubility of complex polysaccharides such as cellulose and starch has seriously limited investigations. Isolation and fractionation prior to solubilization has often been necessary, making comparisons of other polysaccharides with the native or starting material difficult. Solution properties of carbohydrates in many cases are of great importance for their biological function with discrete differences observed whether the solutions are dilute, concentrated or exhibit the properties of a gel [3].

The polysaccharide chitin (for which few nondegrading solvents exist) was successfully dissolved in the aprotic solvent *N,N*-dimethylacetamide with lithium chloride Me_2NAc–LiCl) [4,5]. Solubility parameters and physical properties were determined for chitin in Me_2NAc with 5–7% LiCl without degradation of the polymer. Additional solution studies were carried out by light scattering and viscosity measurements of dilute or moderately concentrated solutions of chitin containing 5 or 8% LiCl [6]. Recently, molecular weight distributions of chitin were reported using size-exclusion chromatography (or gel-permeation chromatography, GPC) with Me_2NAc containing 5% LiCl as the eluent [7]. Molecular masses were not reported because of a lack of suitable standards.

The Me_2NAc–LiCl solvent system has been employed to dissolve cellulose [8–11]. The objectives of several groups were to obtain fibers for spinning or derivatization under homogeneous conditions and focused on concentrated solutions of lower molecular weight cellulosic materials. Our laboratory has developed procedures for the dissolution and characterization of high molecular weight (MW) cellulose chains in cotton fiber without the need for prior derivatization, fractionation, or extraction [12]. Solutions of cellulose in the solvent Me_2NAc–0.5%LiCl were analyzed by GPC with dual detection: using a viscometer detector in conjunction with the differential refractometer detector in

Table 1
Branching and linkage of selected polysaccharides

Polysaccharide	Branching and Linkage
Arabinogalactan	branched (1 → 3)-β-D-galactan
Amylopectin	branched (1 → 4)-α-D-glucan
Amylose	linear (1 → 4)-α-D-glucan
Cellulose	linear (1 → 4)-β-D-glucan
Curdlan	linear (1 → 3)-β-D-glucan
Dextran	branched (1 → 6)-α-D-glucan
Pullulan	linear (1 → 6)-α-D-glucan
Chitin	linear (1 → 4)-β-*N*-acetyl-D-glucosaminyl

GPC allows calculations based on the universal calibration concept [13], which relates hydrodynamic volume (log MW × intrinsic viscosity) to elution volume. The hydrodynamic volume takes into account the effective size of the polymer in solution, is linear with respect to elution volume for polymers of different chemical composition, and is particularly useful where standards of the specific polymers being analyzed are not available. Polystyrene standards of sharp MWD, known MW and covering a wide range of MW values were utilized [12]. This methodology was applied for monitoring cell wall polymers during cotton fiber development [14,15] and was subsequently extended to quantify the effects of extrusion processing on starches from corn and wheat [16–18]. Dissolution of the polysaccharides was achieved with 5–8% LiCl in Me_2NAc; solutions were then diluted to 0.5% LiCl–Me_2NAc for GPC analysis. The more dilute (0.5% versus 5–8%) concentration of LiCl in Me_2NAc provides an advantage for more rapid analysis by GPC because of the lower viscosity of the mobile phase while maintaining the polymer in solution. One of the major advantages of utilizing Me_2NAc–LiCl is that the same solvent can be used for dissolving the starting material as well as the processed or derivatized products [9,17,19]. Qualitative assessment of branching in native and processed starch flour samples has been carried out [17,18].

The objective of this study was to investigate the utility of the Me_2NAc–LiCl solvent system for polysaccharide size characterization. This was achieved by determining the dilute solution behavior of representative polysaccharides in Me_2NAc–0.5% LiCl via GPC using universal calibration. Linear and branched polysaccharides of varying linkages covering a range of molecular weight values (Table 1) were evaluated. Calculated molecular parameters are described in this report.

2. Experimental

Materials.—Samples included celluloses (J. T. Baker Chemical Co., cat. nos. 1525-1 and 1528-1); pullulans (Pfanstiehl, cat. nos. 12474 and 12476); curdlan (Wako Chemicals USA); dextran T10, T40, T70, T500, T2000, (Pharmacia, code nos. 17-0250-01, 17-0270-01, 17-0280-01, 17-0320-01, 17-0330-01, respectively); dextran low fraction, high fraction (J. T. Baker, cat. nos. G200-5, G202-05, respectively); dextran industrial grade (Sigma, no. D-5501); arabinogalactan (Atomergic Chemetals Corp., 6030); corn

amylose (Sigma, no. A-7043); potato amylose (Sigma, no. A-0512); corn amylopectin (Sigma, no. A-7780); potato amylopectin (Sigma, no. A-8515). Decalcified chitin was prepared by a standard procedure using EDTA. Materials were *N,N*-dimethylacetamide [1] (Burdick & Jackson), dried with molecular sieves (Baker, activated type 3A); lithium chloride (Baker), oven-dried and stored in a desiccator; 10 mL ReactiVials TM (Pierce); heating block (Pierce); Teflon magnetic stirbars (2.5 cm); Baker 10 extraction apparatus; disposable Teflon filters (Millipore, Millex-SR, and TYPE FH, 0.5 μm); 10 mL glass syringes (Beckton–Dickinson); 4 mL WISP vials with Teflon septa; 50 mL volumetric flasks. Polystyrene standards were from Toyo Soda Manufacturing (types F-288, F-20, F-80, F-10, F-128, F-4, F-40, F-2, A-5000, F-1, with nominal molecular weights of 2.89×10^6, 1.9×10^5, 7.1×10^5, 1.02×10^5, 1.26×10^6, 4.39×10^4, 3.55×10^5, 1.96×10^4, 6.2×10^3, 1.03×10^4, respectively).

Sample preparation.—Samples were dissolved generally following the procedure reported for cotton [12]. Polysaccharide (30 mg) was added to 5 mL Me_2NAc in 10 mL ReactiVials TM with a conical magnetic stirrer in a heating block. The temperature was raised to 150°C and maintained with stirring for 1 h. The mixture was allowed to cool to 100°C, and 0.250 g of dried LiCl was added. The vials were shaken by hand and returned to the heating block, where the mixture was maintained with stirring at 100°C for 1 h. The temperature of the block was lowered to 50°C, and the samples were stirred at this temperature overnight. The solutions were quantitatively transferred to 50-mL volumetric flasks and diluted to volume with Me_2NAc. They were then filtered through a solvent-resistant Teflon disposable filter. An extraction apparatus was employed with 10-mL glass syringes fitted onto filters with 4-mL glass vials held in the small volumetric holder. The final concentration of each polysaccharide was 0.6 mg/mL in Me_2NAc–0.5% LiCl.

Chromatography.—The mobile-phase/solvent for GPC was Me_2NAc–0.5% LiCl prepared by raising the temperature of 1 L of Me_2NAc to 100°C and then adding 5 g of dried LiCl. The salt was stirred until it dissolved, and the solvent was filtered through a Teflon filter with a glass filter apparatus [12]. Filtered samples were analyzed on a GPC system consisting of an automated sampler (Waters WISP) with an HPLC pump (Waters Model 590), pulse dampener (Viscotek), viscometer detector (Viscotek Model 100), and refractive index detector (Waters Model 410), at a flow rate of 1.0 mL/min. The detectors were connected in series. The column configuration consisted of three 10 μm Mixed-B columns (Burdick & Jackson/Polymer Laboratories) preceded by a guard column (Burdick & Jackson/Polymer Laboratories). The system was operated at 80°C, with temperature controlled by a column heater (Waters column temperature system). Injection volumes were 100 and 150 μL with a run time of 34 min per sample. Data acquisition and calculations were performed using the software package TriSEC GPC (Viscotek, Version 2.22). Universal calibration was determined with polystyrene standards dissolved directly in Me_2NAc–0.5% LiCl. The universal calibration curve was

[1] *N,N*-Dimethylacetamide (Me_2NAc) is an exceptional contact hazard that may be harmful if inhaled or absorbed through skin and may be fatal to embryonic life in pregnant females (Baker Chemical Co. *N,N*-dimethylacetamide, Material Safety Data Sheet, 1985, D5784-01; pp 1–4).

linear as a logarithmic function of the product of the intrinsic viscosity times molecular weight versus retention volume using a third-order fit. Data were obtained from two dissolutions per sample with two GPC runs per dissolution.

3. Results and discussion

The Me_2NAc–LiCl solvent system for polysaccharides.—As predicted from previous reports [4,19], Me_2NAc–LiCl effectively dissolved each of the polysaccharides selected for evaluation. GPC analyses were conducted, and average molecular weight values for the whole polymer, as well as molecular weight distributions, were obtained. The following parameters were determined for the samples analyzed: Weight-average molecular weight (M_w), number-average molecular weight (M_n), and molecular weight polydispersity ratio (M_w/M_n). By incorporating a viscometer detector in the GPC analysis, additional parameters to help define solution behaviour such as weight-average intrinsic viscosity ($[\eta]_w$), weight-average radius of gyration (Rg_w), and Mark–Houwink constants (a and log K) were measured (Table 2). Comparison of the calculated values for M_w with those provided by the suppliers indicates excellent agreement (with the exception of curdlan which is discussed below) (Table 2). From polydispersity ratios, it

Table 2
Calculated solution parameters for polysaccharides in Me_2NAc–LiCl

Sample	M_w (Supplied)	M_w (Cald)	M_n (Cald)	M_w/M_n	$[\eta]_w$ (dL/g)	Rg_w (nm)	Mark–Houwink		λ (Avg.)
							a	log K	
Amylopectin (Potato)	N/A [a]	1.2×10^6	5.8×10^4	21.4	0.59	25.7	0.4	−2.7	0.01
Amylopectin (Corn)	N/A	2.1×10^7	6.1×10^4	349.0	0.40	33.0	0.2	−1.9	0.05
Amylose (Potato)	N/A	4.9×10^5	5.0×10^4	9.8	0.83	17.7	0.9	−5.1	0.02
Amylose (Corn)	N/A	6.2×10^5	8.6×10^4	7.2	0.65	19.2	0.7	−4.0	0.01
Arabinogalactan	8.0×10^4	8.4×10^4	3.2×10^4	2.6	0.06	5.2	−0.1	−0.9	0.61
Cellulose 4	1.8×10^5 [b]	1.8×10^5	2.7×10^4	6.8	1.04	14.0	1.0	−5.1	0.00
Cellulose 5	3.2×10^5 [b]	3.3×10^5	4.2×10^4	7.9	0.71	16.2	0.7	−4.0	0.00
Curdlan	8.1×10^4	4.5×10^5	3.7×10^4	11.9	3.62	34.5	0.8	−3.7	0.00
Dextran T10	1.0×10^4	1.9×10^4	1.7×10^4	1.1	0.15	4.5	1.1	−5.5	0.00
Dextran T40	4.4×10^4	4.7×10^4	3.6×10^4	1.3	0.29	7.3	1.8	−8.8	0.44
Dextran T70	7.0×10^4	7.6×10^4	4.8×10^4	1.6	0.40	9.4	1.4	−7.4	0.40
Dextran LF [c]	$6.0–9.0\times10^4$	8.4×10^4	6.4×10^4	1.3	0.38	10.0	1.1	−5.8	0.08
Dextran HF [d]	$2.0–3.0\times10^5$	2.5×10^5	5.6×10^4	4.4	0.64	16.2	0.5	−3.0	0.00
Dextran T500	5.0×10^5	5.5×10^5	6.8×10^4	7.9	0.84	21.0	0.4	−2.3	0.00
Dextran T2000	2.0×10^6	1.9×10^6	5.4×10^4	36.2	0.78	26.1	0.4	−2.5	0.00
Dextran IG [e]	$5.0–40.0\times10^6$	5.1×10^6	4.8×10^4	104.9	0.14	21.1	0.4	−3.6	0.56
Pullulan 1	1.0×10^5	1.1×10^5	3.5×10^4	3.0	0.69	12.3	1.1	−5.6	0.02
Pullulan 3	3.0×10^5	3.1×10^5	3.4×10^4	9.3	0.89	14.5	0.8	−4.3	0.00
Chitin, Decalcified	N/A	5.4×10^6	1.8×10^4	360.6	0.16	12.5	0.6	−4.0	0.82

[a] N/A, Not available.
[b] Values from McCormick et al. [9].
[c] LF, Low Fraction.
[d] HF, High Fraction.
[e] IG, Industrial Grade.

is evident that these polysaccharides ranged from narrow MWDs for some of the lower MW dextrans to an extremely broad MWD for corn amylopectin. For copolymers and when polymers are not homogeneous, this limits application of theories of polymer solution (as most equations have been derived for polymers with consistent repeat units) [20,21], although useful qualitative information can be obtained by viscosity measurements [1,3].

Many properties of a particular polymer depend not only on the solvent but also on the temperature at which measurements are taken [20,21]. By employing the Me_2NAc–LiCl solvent system, which is effective for a range of naturally occurring polymers, and by holding column temperatures constant (80°C) for all separations, comparisons could be made more readily. For each polymer–solvent system with specific constants, the Mark–Houwink relationship via the intrinsic viscosity provides an index of random coil dimensions and, hence, of chain conformation [1]. Values for $[\eta]_w$ were less than or equal to ~ 1.0, the exception being arabinogalactan, whose extremely low intrinsic viscosity value is most likely due to the extensive branching present in this polysaccharide (see *Comparison of polysaccharides: arabinogalactan vs. dextran LF*, below) (Table 2). Mark–Houwink constants were in accordance with previously reported ranges for polysaccharides in mostly aqueous solvents [20,21]. The presence of branching was related to decreases in the value for a. In addition, branching assessments were conducted based upon Zimm–Stockmeyer equations [20]: average branching number (Bn), and average branching frequency (λ) were determined (Table 2). Generally, the branched polymers (see Table 1) had lower $[\eta]_w$ and Rg_w values, as expected due to branched molecules occupying a smaller hydrodynamic volume in solution than linear molecules of the same MW [20,21]. When comparing measured solution behavior to typical characteristics of polymers in dilute solution, results support the observation that Me_2NAc–LiCl is a thermodynamically favorable (''good'') solvent, i.e., one that effectively dissolves associated segments found within a coil, thus lowering the coil density and increasing the viscosity [20,21].

Cellulose.—Cellulose, the most abundant biopolymer in the world, is present in all higher plants, and exists in its purest naturally occurring form in cotton fiber. Its characterization is of particular interest to the textile, pulp and paper, and plastics industries. It consists of anhydroglucose units linked linearly to form long chains [a linear (1 → 4)-β-D-glucan]. Unlike other polysaccharides (e.g., high-MW dextrans), it shows no variability in structure over its entire MW range. Differences in types of cellulose from different sources stem from differing MWDs as well as supramolecular structure, particularly crystallinity. As mentioned above, characterization of cellulose from cotton fiber from a variety of sources and at several stages of cell wall development has been carried out in this laboratory. The celluloses analyzed in this study were commercially available powders. The broad MWDs and low MW ranges are in direct contrast to the high MW secondary wall component of native cotton fiber [12]. Cellulose 5 was taken as a linear polymer reference in the branching assessments.

Dextran.—Fig. 1 shows the MWDs of dextran samples overlaid for comparison; the dextrans designated ''T'' are shown to have a narrow, defined weight-average molecular weight range [22]. Dextran Low Fraction (LF), High Fraction (HF), and Industrial Grade (IG) samples are also included in the plot. The lower MW dextrans (T10, T40, T70, and

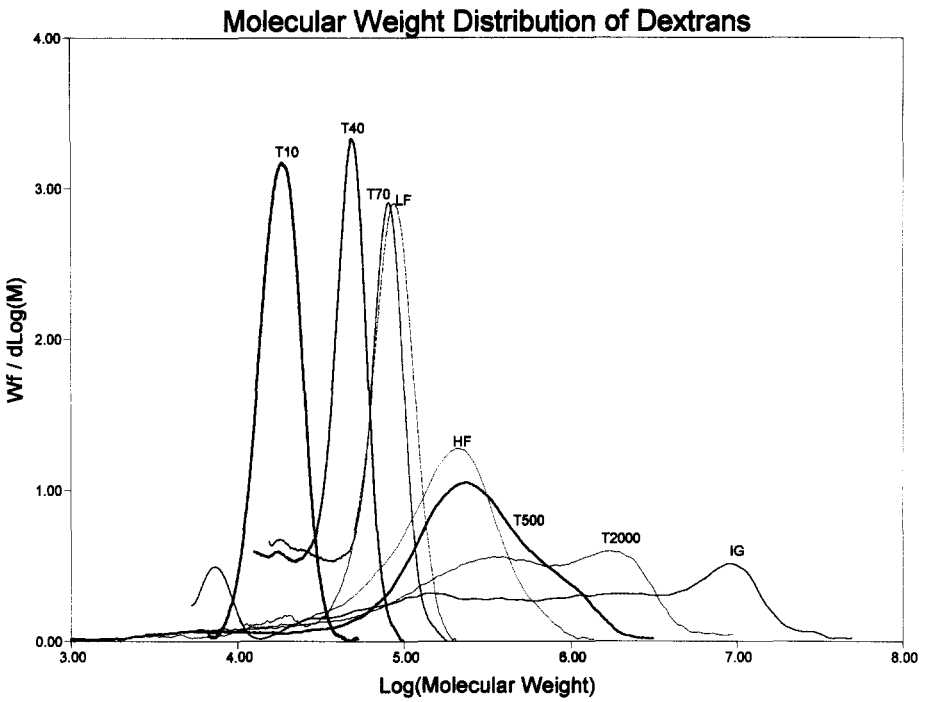


Fig. 1. Relationship between molecular weight distributions (MWD) of defined molecular weight "T" dextrans, dextran fractions, and industrial grade dextran.

LF) had fairly narrow MWDs, which was also indicated by the polydispersity ratios (~ 1.1) in Table 2. Increasing M_w of the dextrans corresponded to an increase in Rg_w and $[\eta]_w$. Intrinsic viscosity was found to be more correlated to Rg_w ($r = 0.958$) than to M_w ($r = 0.667$), a relationship which holds for other compounds as well [25] (Table 2). Dextran IG data was not included in these calculations, as the chromatography of this sample proved difficult to reproduce due to its extremely high MW and broad MWD. It has been reported [23] from solution behavior that dextrans of lower MW possess a near-linear structure and that branching in dextrans diminishes rapidly as MW values approach or become less than 10^4. In this study, the greater extent of branching in higher MW dextrans is reflected in the value of the Mark–Houwink constant a, which falls below 0.5 when $M_w = \sim 2.5 \times 10^5$ (Table 2). It is evident from the plots in Fig. 1 that the MWDs of the higher MW dextran polymers are not narrow, and moreover seem to broaden with an increase in M_w. For example, the MWD for T2000 spans several million daltons.

The Mark–Houwink relationship for near-linear dextrans ($M_w \leq \sim 2.5 \times 10^5$) in Me_2NAc–LiCl can be fitted to the following equation:

$$[\eta]_w = 1.7 \times 10^{-7} M_w^{1.3} \quad (1)$$

The equation fails above the mentioned molecular weight probably due to unpredictable branching behavior of the dextrans. In Table 3, the values for the Mark–Houwink

Table 3
Calculated and predicted a, $[\eta]_w$ of dextrans and pullulans

Sample	Calculated [a] a	Calculated [a] $[\eta]_w$ (dL/g)	Predicted [b] $[\eta]_w$ (dL/g)	Granath [c] $[\eta]_w$ (dL/g)	Wales [d] $[\eta]_w$ (dL/g)
Dextran T10	1.1	0.15	0.03	0.12	0.10
Dextran T40	1.8	0.29	0.25	0.22	0.27
Dextran T70	1.6	0.40	0.38	0.27	0.37
Dextran LF	1.1	0.38	0.37–0.64	0.25–0.30	0.33–0.44
Dextran HF	0.5	0.64	N/A [e]	0.42–0.51	0.75–0.99
Dextran T500	0.4	0.84	N/A	N/A	N/A
Dextran T2000	0.4	0.78	N/A	N/A	N/A
Dextran IG	0.4	0.14	N/A	N/A	N/A
Pullulan 1	1.1	0.69	0.46 [f]		
Pullulan 3	0.8	0.89	0.95 [f]		

[a] From GPC analysis
[b] Applying Eq. (1) from text.
[c] (Ref. [1]; H_2O, 20°C, $a = 0.43$, $K = 2.23 \times 10^{-3}$).
[d] (Ref. [24]; H_2O, 25°C, $a = 0.675$, $K = 1.99 \times 10^{-4}$).
[e] N/A, Equation not applicable.
[f] (Ref. [27]; H_2O, 25°C, $a = 0.658$, $K = 2.36 \times 10^{-4}$).

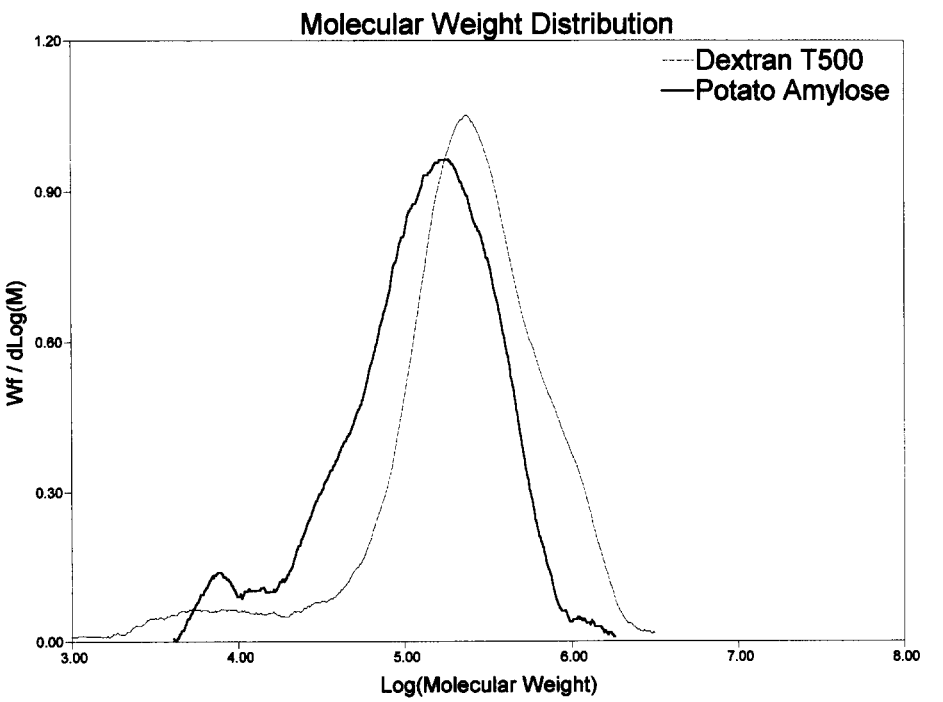
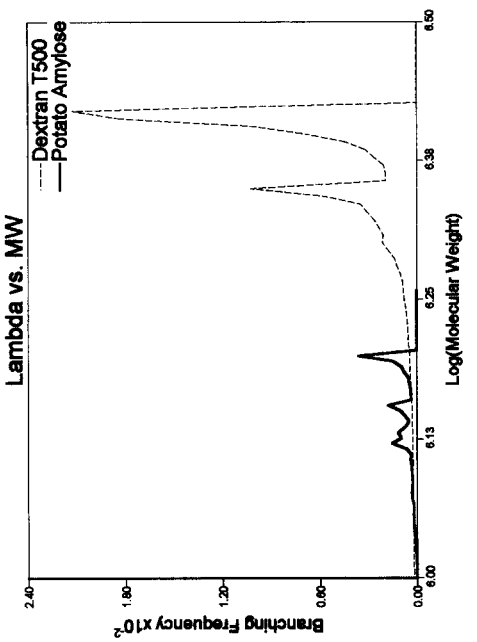
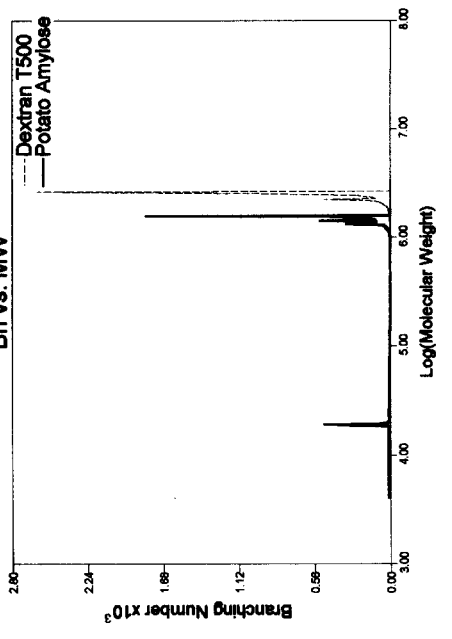
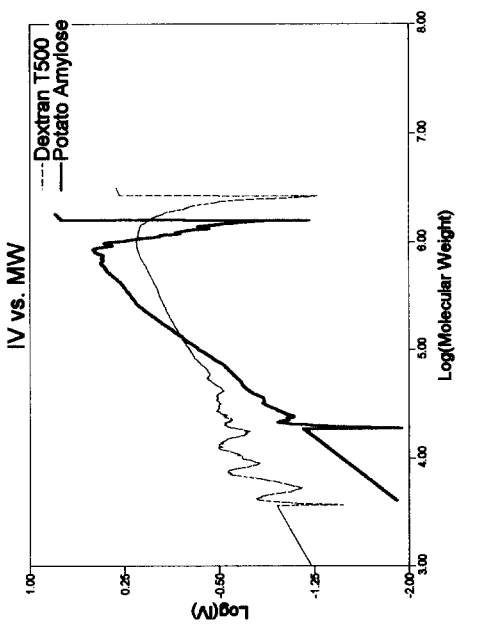
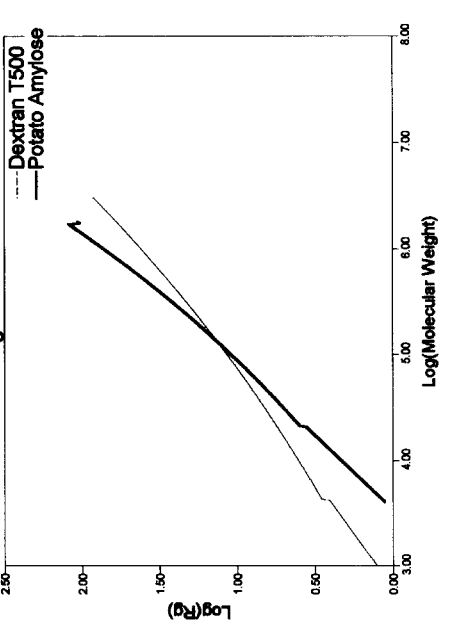


Fig. 2. Comparison of solution parameters of potato amylose and dextran T500 in Me_2NAc–LiCl. (A) Molecular weight distribution (MWD). (B) log–log plot of intrinsic viscosity ($[\eta]$) vs. molecular weight (MW). (C) log–log plot of radius of gyration (Rg) vs. molecular weight. (D) Plot of branching frequency (λ) vs. log molecular weight. (E) Plot of branching number (Bn) vs. log molecular weight.


Lambda vs. MW
Dextran T500
Potato Amylose
Branching Frequency x10-2
Log(Molecular Weight)
2.40
1.80
1.20
0.60
0.00
6.00
6.13
6.25
6.38
6.50


Bn vs. MW
Dextran T500
Potato Amylose
Branching Number x10-3
Log(Molecular Weight)
2.80
2.24
1.68
1.12
0.56
0.00
3.00
4.00
5.00
6.00
7.00
8.00


IV vs. MW
Dextran T500
Potato Amylose
Log(IV)
Log(Molecular Weight)
1.00
0.25
-0.50
-1.25
-2.00
3.00
4.00
5.00
6.00
7.00
8.00


Rg vs. MW
Dextran T500
Potato Amylose
Log(Rg)
Log(Molecular Weight)
2.50
2.00
1.50
1.00
0.50
0.00
3.00
4.00
5.00
6.00
7.00
8.00

constant a and intrinsic viscosity calculated from GPC analyses are compared to $[\eta]_w$ values calculated using Eq. (1). These results are in agreement with calculations based on Wales' constants for hypothetical linear dextrans, with the findings using water as the solvent [23,24], and also with published results for other polysaccharides [25].

Pullulan.—Pullulans are linear molecules similar in structure to dextrans, both being (1 → 6)-α-D-glucans [27]. In addition to commercial uses, pullulans are widely used as standards for aqueous GPC. The a values for the linear pullulans calculated in this study were similar to those of the near-linear dextrans and the celluloses (Table 2). Comparison of Mark–Houwink constants calculated for the pullulans in Me_2NAC–LiCl with those reported for water as a solvent ($[\eta] = 2.36 \times 10^{-4}\ M^{0.658}$) [27] indicate similar behavior in both solvents as expected for a linear random coil in solution. The intrinsic viscosities of the pullulans in Me_2NAc–LiCl calculated by GPC agree reasonably well with those predicted using the literature constants [27], especially considering the difference in solvents and temperatures (80°C vs. 25°C) (See Table 3).

Comparison of polysaccharides. Amylose vs. Dextran T500.—Solution behavior of matched pairs of polysaccharides in Me_2NAc–LiCl was investigated. The linear (1 → 4)-α-D-glucan amylose was compared to a branched (1 → 6)-α-D-glucan (Dextran T500)

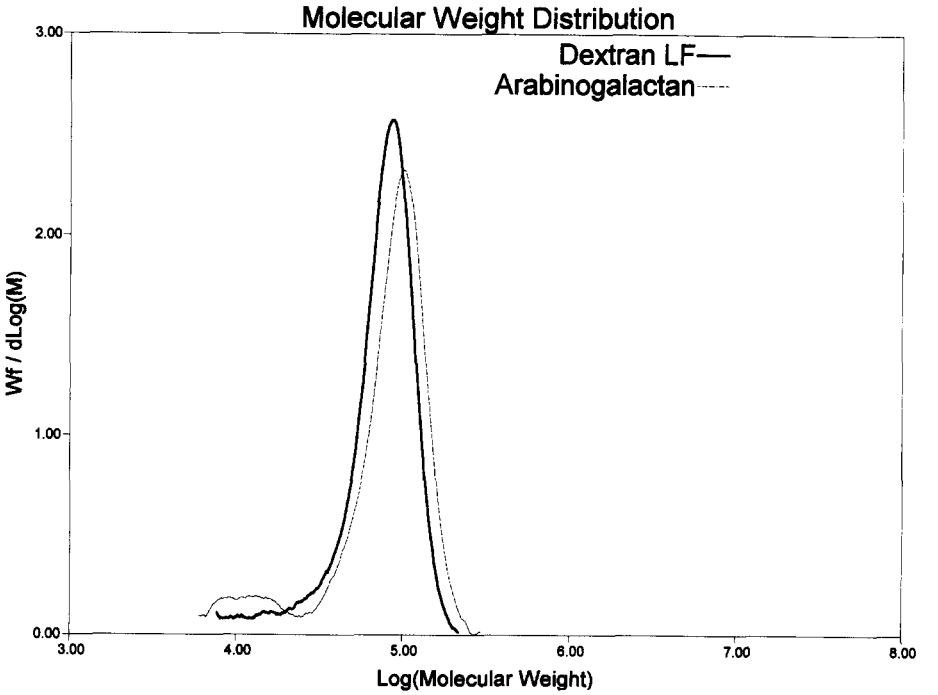
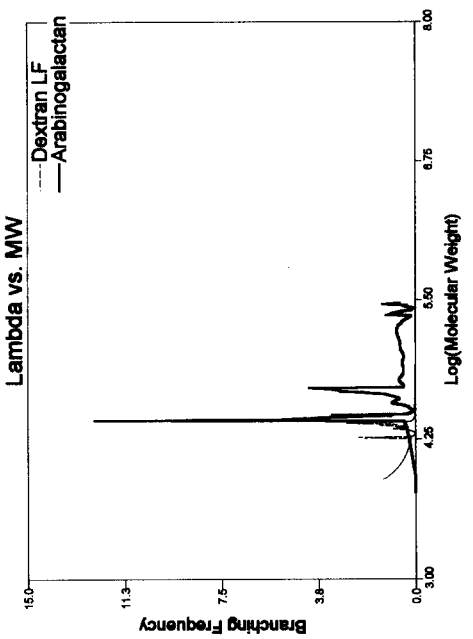
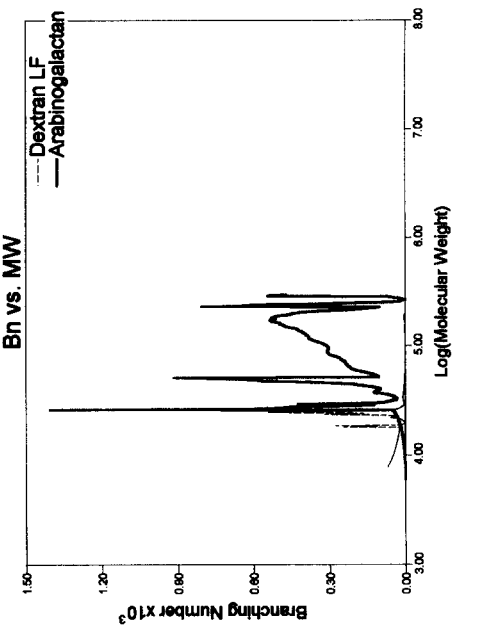
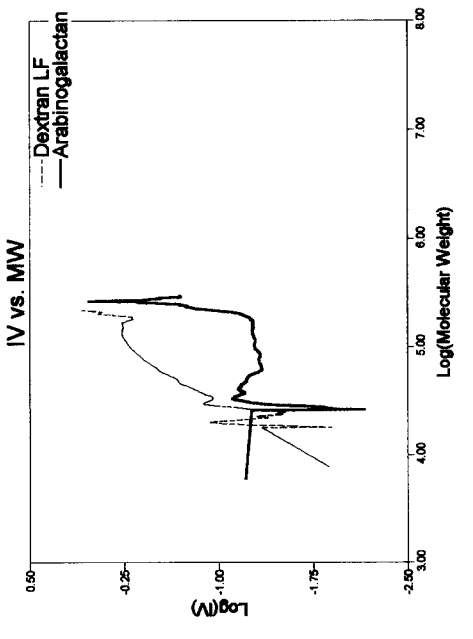
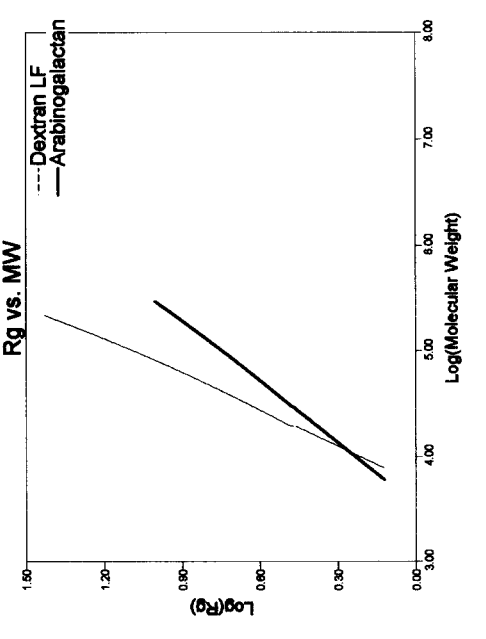


Fig. 3. Comparison of solution parameters of arabinogalactan and dextran LF in Me_2NAc–LiCl. (A) Molecular weight distribution (MWD).(B) log–log plot of intrinsic viscosity ($[\eta]$) vs. molecular weight (MW).(C) log–log plot of radius of gyration (Rg) vs. molecular weight. (D) Plot of branching frequency (λ) vs. log molecular weight. (E) Plot of branching number (Bn) vs. log molecular weight.


Lambda vs. MW
Dextran LF
Arabinogalactan
Branching Frequency
Log(Molecular Weight)


Bn vs. MW
Dextran LF
Arabinogalactan
Branching Number x10³
Log(Molecular Weight)


IV vs. MW
Dextran LF
Arabinogalactan
Log(IV)
Log(Molecular Weight)


Rg vs. MW
Dextran LF
Arabinogalactan
Log(Rg)
Log(Molecular Weight)

with approximately equivalent MW (Fig. 2). Differences in the MWDs are obvious from Fig. 2A although the M_w values are fairly close. We observed that the $[\eta]_w$ and Rg_w of potato amylose were greater than those of Dextran T500, when comparing the high-MW portion of the latter, where the majority of the branching is believed to occur in dextrans (Table 2 and Fig. 2B–E). Branching in the dextran causes the molecule to be smaller (higher coil density) than a linear molecule of the same molecular weight. Therefore, the hydrodynamic volume, $[\eta]$, and Rg will be lower, and the dependence of $\log[\eta]_w$ vs. $\log M_w$ will deviate from linear behavior [20,28]. For the dextran, the branching frequency increased as the MW increased as discussed above (Fig. 2C).

Arabinogalactan vs. Dextran LF.—Arabinogalactans (AGs) are of two varieties, Type I and Type II. The former are found only in pectins and are composed of branched (1 → 4)-β-D-galactan chains. The latter consist of a highly branched (1 → 3)-β-D-galactopyranosyl backbone, with a number of branched (1 → 6)-β-D-galactan side chains connected to it [1,29]. Type II is found in high concentration in the heartwood of many species of larch and consists mainly of two fractions with molecular weights of ~ 16 000 and 100 000, or 18 000 and 78 000, depending on the species of wood from which the AGs are obtained [1]. The arabinogalactan analyzed by our group was a Type II AG

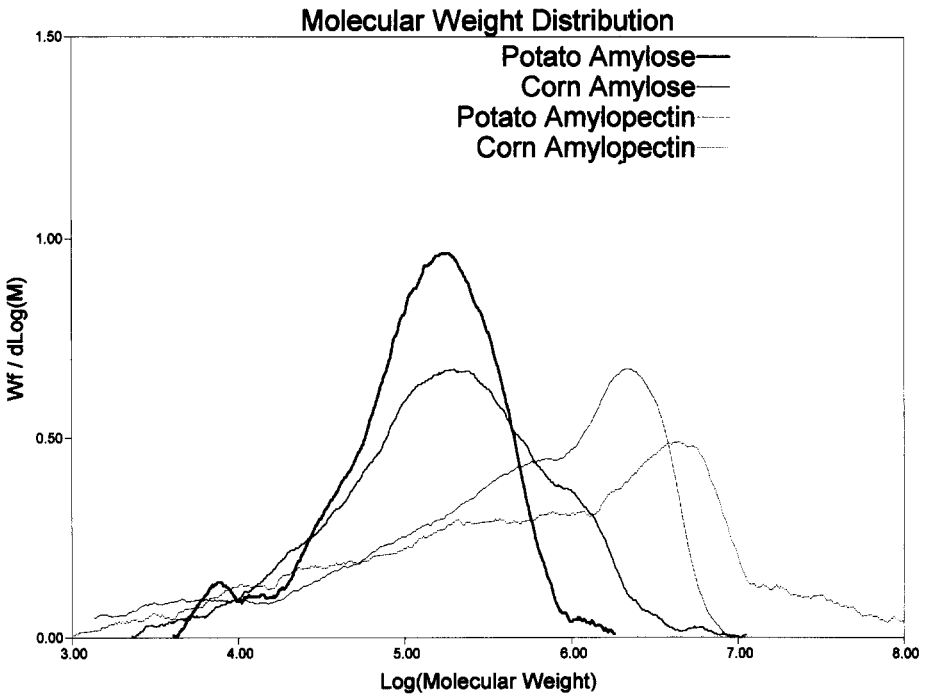
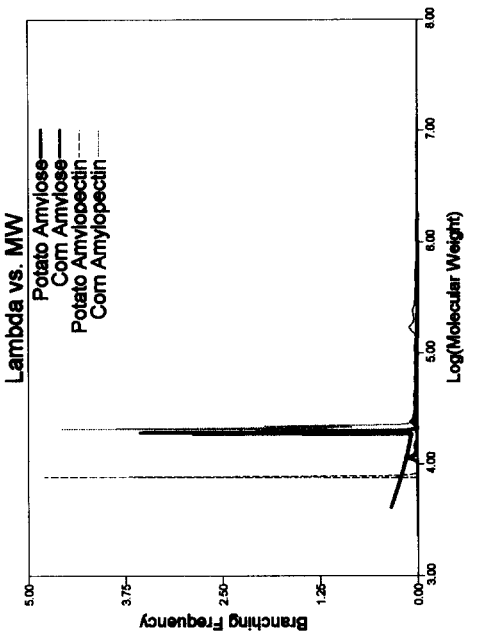
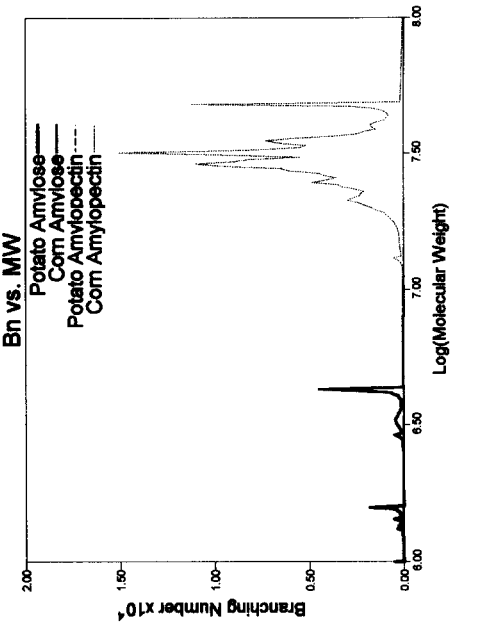
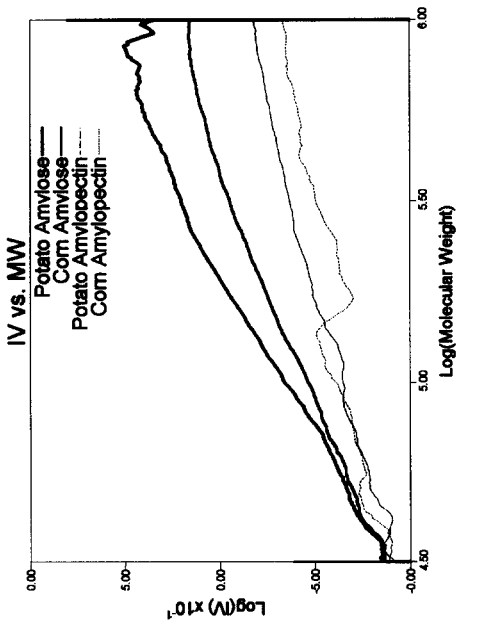
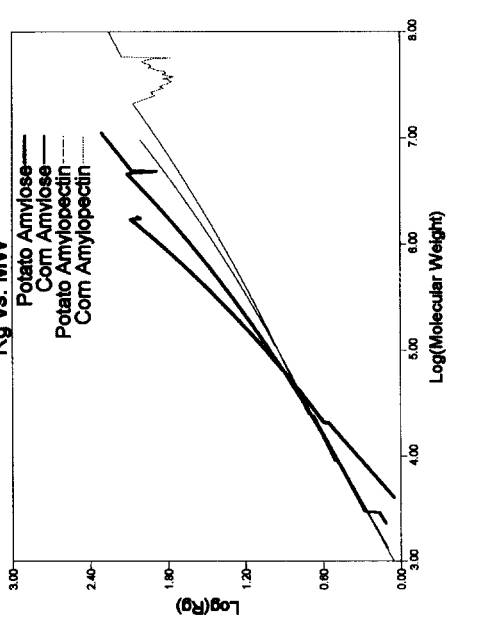


Fig. 4. Comparison of solution parameters of amyloses (corn and potato) and amylopectins (corn and potato) in Me_2NAc–LiCl. (A) Molecular weight distribution (MWD).(B) log–log plot of intrinsic viscosity ($[\eta]$) vs. molecular weight. (C) log–log plot of radius of gyration (Rg) vs. molecular weight. (D) Plot of branching frequency (λ) vs. log molecular weight. (E) Plot of branching number (Bn) vs. log molecular weight.


Lambda vs. MW
Potato Amylose
Corn Amylose
Potato Amylopectin
Corn Amylopectin
Branching Frequency
Log(Molecular Weight)


Bn vs. MW
Potato Amylose
Corn Amylose
Potato Amylopectin
Corn Amylopectin
Branching Number x10⁴
Log(Molecular Weight)


IV vs. MW
Potato Amylose
Corn Amylose
Potato Amylopectin
Corn Amylopectin
Log(IV) x10¹
Log(Molecular Weight)


Rg vs. MW
Potato Amylose
Corn Amylose
Potato Amylopectin
Corn Amylopectin
Log(Rg)
Log(Molecular Weight)

from wood larch. Its MWD shows two distinct fractions with $M_w \sim 14\,000$ and 90 000 (Fig. 3A), in good agreement with the literature reports. When comparing this arabinogalactan with a near-linear dextran, Dextran LF, which has an equivalent M_w (Table 2), the extensive branching of arabinogalactan is evident in the extremely high values for Bn (~ 300) and λ (0.61). Hence, low values for a, $[\eta]_w$, and Rg are expected. We calculated $a = -0.1$ and $[\eta]_w = 0.06$ dL/g. The Rg_w of arabinogalactan was 5.2 nm, nearly half as large as that of Dextran LF (10.0 nm). The similarities in MW of the two samples can be seen in the MWDs, compared in Fig. 3A. Insights into differences in the solution behavior of these molecules are facilitated by closer inspection of plots of the $[\eta]$, Rg, Bn, and λ versus MW (Fig. 3B–3E). Significant differences over the entire MW range are evident with lower $[\eta]$ and Rg for the arabinogalactan than for the dextran. In addition, the Bn increased with increasing MW, although the λ values were greater throughout the distribution for the arabinogalactan compared to the dextran. The utility of the GPC technique for providing multiple profiles of various important solution behavior properties is demonstrated from this type of evaluation.

Amylopectin vs. amylose. — Regardless of the source, amylopectin had a higher M_w than amylose (Table 2). From the overlays of MWDs in Fig. 4A, the more complicated

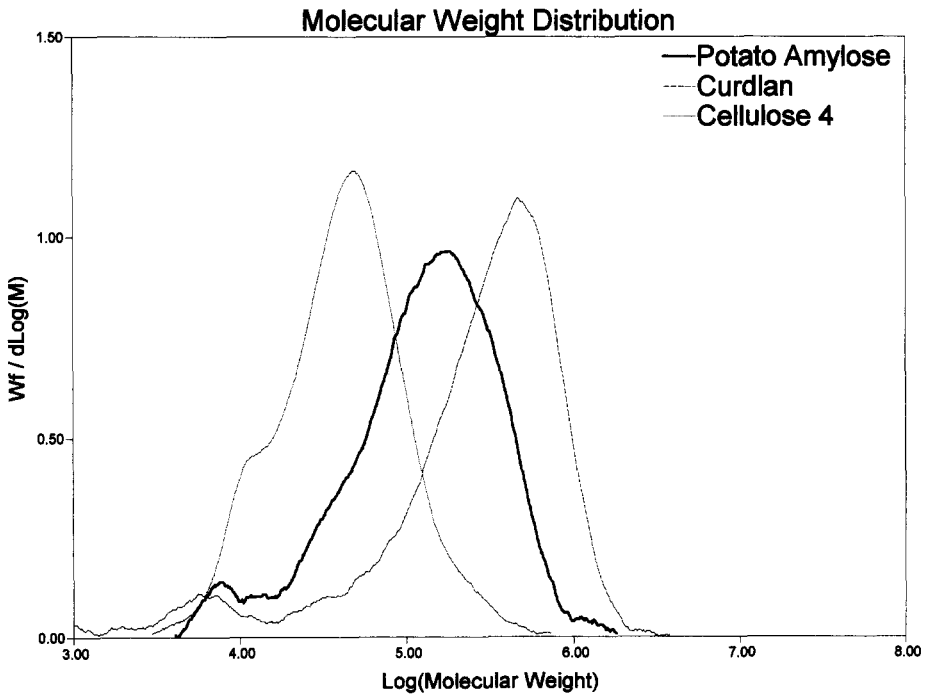
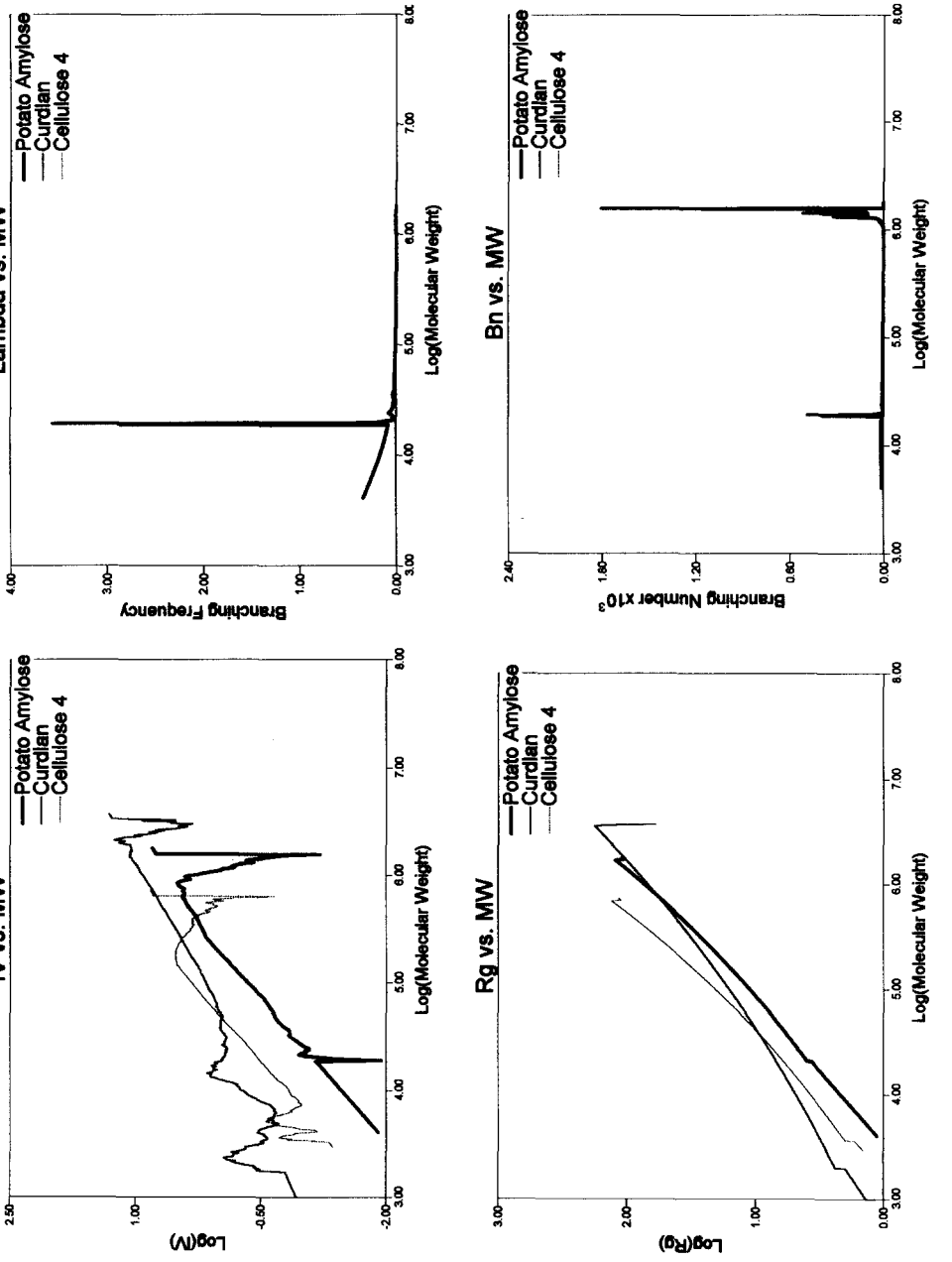


Fig. 5. Comparison of solution parameters of curdlan, potato amylose, and cellulose 4 in Me_2NAc–LiCl.(A) Molecular weight distribution (MWD). (B) log–log plot of intrinsic viscosity ($[\eta]$) vs. molecular weight. (C) log–log plot of radius of gyration (Rg) vs. molecular weight. (D) Plot of branching frequency (λ) vs. log molecular weight. (E) Plot of branching number (Bn) vs. log molecular weight.


Lambda vs. MW
Potato Amylose
Curdlan
Cellulose 4
Branching Frequency
Log(Molecular Weight)
Bn vs. MW
Potato Amylose
Curdlan
Cellulose 4
Branching Number x10^3
Log(Molecular Weight)
IV vs. MW
Potato Amylose
Curdlan
Cellulose 4
Log(IV)
Log(Molecular Weight)
Rg vs. MW
Potato Amylose
Curdlan
Cellulose 4
Log(Rg)
Log(Molecular Weight)

(more distinct) components of the amylopectin samples can be noted compared to the broad distributions of the amylose samples. The amylose samples from corn and potato were more comparable to each other than the two amylopectin samples. As expected, branching of amylopectins produced lower $[\eta]$ with larger Rg. The larger Rg values are accounted for by the much higher M_w values of the amylopectins. Branching effects can also be seen in the $[\eta]$ plots over the entire MW range (Fig. 4B) where the amylopectin curves are displaced to a lower $[\eta]$. That branching markedly affects the Mark–Houwink constant a is verified here (Table 2 and Figs. 4D and E). This GPC technique has been used to investigate the mechanism of extrusion-induced starch fragmentation in corn and wheat [17,18]. Fragmentation was most significant for the high MW amylopectin.

Curdlan and chitin.—The calculated M_w of curdlan ($\sim$ 440 000 Da), a linear (1 $\rightarrow$ 3)-β-D-glucan, was much higher than the supplied value ($\sim$ 81 000 Da). This anomalous behavior was evaluated by repeated dissolutions with the same results. The dissolution procedure was altered in both the order of addition of the salt (before or after the addition of curdlan) and in duration. This did not have a noticeable effect on results. Literature reports of X-Ray diffraction studies and density measurements have shown that this polysaccharide possesses a low crystallinity ($\sim$ 30%) and adopts a triple-helical structure in the formed gel network [30–32] Curdlan's behavior in Me_2NAc–LiCl solutions may potentially resemble its properties as a gel even though the solutions are dilute. We cannot confirm this at the present time, but do believe it warrants further research. One fact that becomes apparent from the experiments with curdlan is that discretion should be exercised when using polysaccharides as standards in GPC. For comparison, the high $[\eta]_w$ and Rg_w values of curdlan are contrasted with those of potato amylose, a linear polysaccharide of somewhat higher molecular weight than curdlan, as well as with a linear cellulose sample (Figs. 5A–E).

Decalcified chitin analyzed in this study displayed multiple peaks over the molecular range (Fig. 6). The higher MW components were less resolved than the two distinct lower MW components. Multiple peaks in isolated chitin have been previously reported [7]. The value for the Mark–Houwink constant a obtained in this evaluation using 0.5% LiCl in Me_2NAc was 0.57 (Table 2) which compares closely to values of 0.69 [6] and 0.71 [33] reported for chitin in 5% LiCl–Me_2NAc. To reiterate, the lower concentration of LiCl facilitates GPC analysis because the lower viscosity of the mobile phase allows higher flow rates leading to faster time for complete elution. The MWD for chitin was difficult to reproduce, which would tend to support previous observations that changes, as measured by the ultraviolet absorption spectra, occur with time in chitin solutions in Me_2NAc/LiCl [34]. More extensive study of this important polysaccharide appears warranted.

Branching and universal calibration.—A final note should be made concerning branching. Calculations are made according to the Zimm–Stockmayer theory [20,21], and the assumptions involved therein must be considered. The first assumption made is that branch points are random and not clustered. Secondly, the equations involved apply only to molecules with long-chain branching when the length of the side chain is comparable to that of the main chain or, in the absence of the latter, when random dendritic branching occurs. For short-branching, or a combination of long- and short-branching, other sets of equations should be used. Thirdly, the relation for g, the ratio of

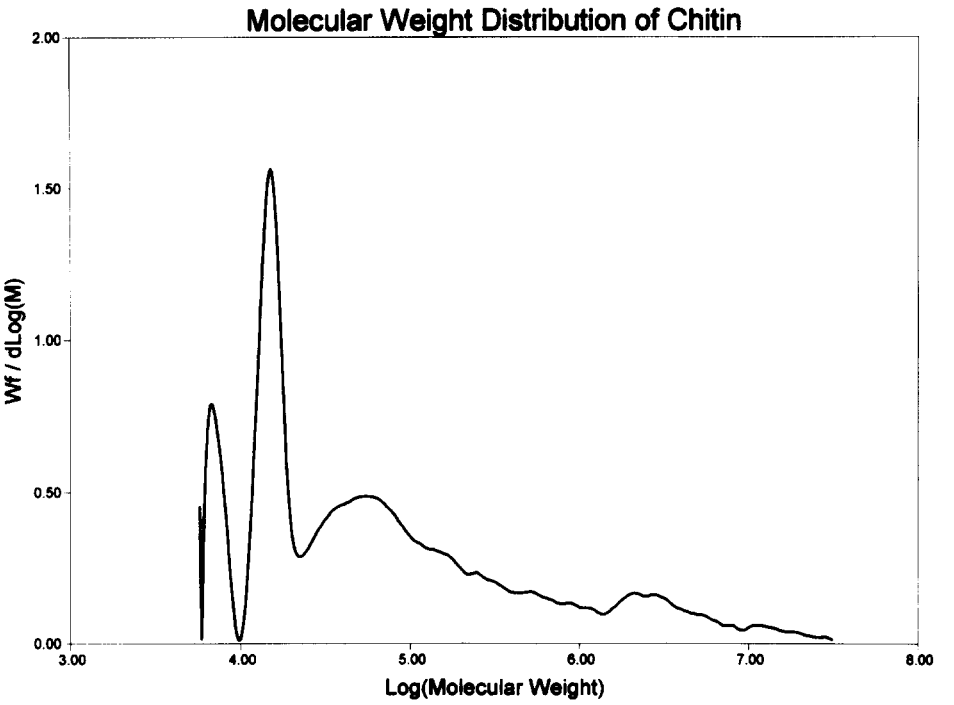


Fig. 6. Molecular weight distribution (MWD) of decalcified chitin.

the mean-square radii, was derived for a theta (θ) solvent [28,35], not a good solvent such as Me_2NAc–LiCl. At θ conditions as the solvent becomes poorer, there are no polymer–solvent interactions, and deviations from ideal conditions disappear [20,21]. A θ solvent is defined as one in which at normal temperatures (0°C–40°C) the exponent a has a value of 0.5; the temperature at which $a = 0.5$ is called the θ temperature. Coil density decreases in a good solvent due to chain stiffening and expansion of the coil. The exponent a in good solvents will not be 0.5, but a larger value such as 0.6 to 0.8, or even 1 or 2 for polymers with bulky chains. An example of where these factors can become important occurs when performing branching calculations on "lower" molecular weight molecules with short-chain branches, e.g., near-linear dextrans. On the other hand, GPC is the only current analytical technique that can provide information on the variation of long branching with MW without fractionation of the polymer. Reports for polyethylene indicate that measurements of long-chain branch frequency rank polymers correctly and probably provide values within a factor of 2 of the "true" value [35]. It can be seen from results obtained in this study that the branching assessments, in conjunction with viscosity measurements, have accurately correlated with the branched or linear nature of the polysaccharides evaluated.

The universal calibration concept with GPC provides accurate MW values because the behavior of the polymer in solution is taken into account [13]. The problem of limited availability of polymer standards having identical chemical structure and narrow

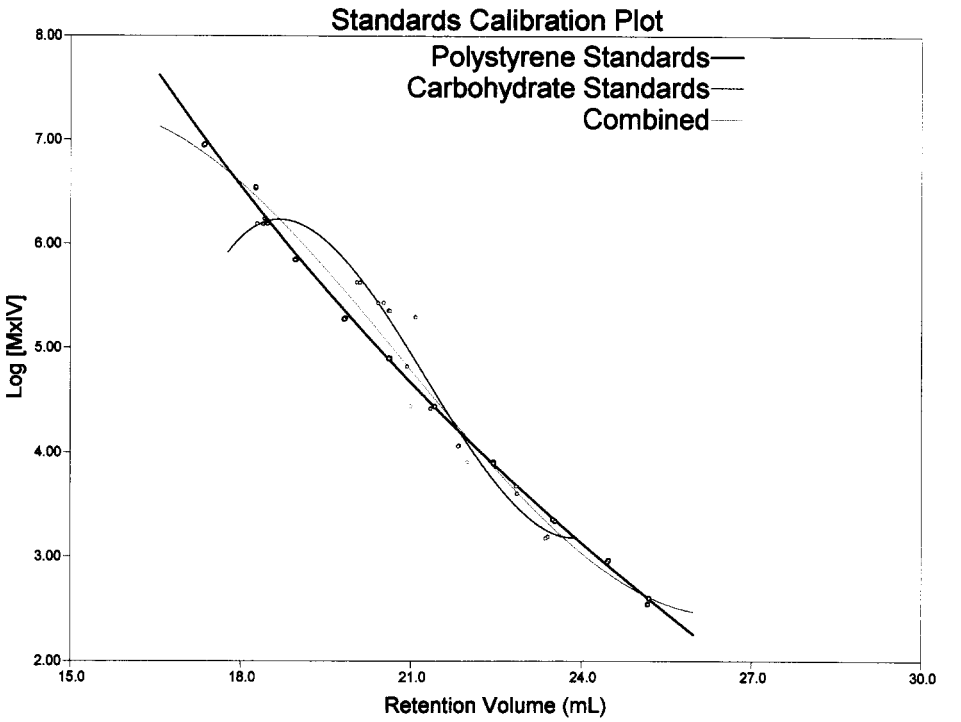


Fig. 7. Universal calibration curves derived from polystyrene standards, carbohydrate standards, and a combination of both.

MWDs at discrete levels of molecular weight for the range of MW for unknown polymers is avoided [12,13]. In Fig. 7, the calibration curve (which describes the relationship between hydrodynamic volume (log $MW \times [\eta]$) and retention volume) based upon polystyrene standards is shown. It was employed in the calculations for the samples described herein. For comparison, a calibration based upon the polysaccharides for which MW data was available was also prepared (Fig. 7). The range of MW for the polysaccharides was not as great as with the polystyrene standards. The polysaccharides were incorporated with the polystyrene standards into a composite curve as shown verifying the universal calibration concept. The most significant difference was the limited range of MW standards available for the polysaccharides which should be considered when characterizing unknown materials of higher or lower MW.

4. Conclusions

Me_2NAc–LiCl is an effective solvent for polysaccharides differing in linkage type, extent of branching and molecular weight. GPC with viscometer and refractive index detectors is an effective technique for determining molecular characteristics of the polysaccharides dissolved in Me_2NAc–LiCl, particularly over the range of the MWD.

Using a universal calibration based upon polystyrene standards, MW values comparable to those supplied with the carbohydrates were obtained. Curdlan was the sole exception of the classes evaluated in this study. MWD and polydispersity ratios demonstrated the broadness or narrowness of the distribution of polymer chain lengths. Behavior in dilute solution as measured by intrinsic viscosity ($[\eta]_w$), radius of gyration (Rg_w), Mark–Houwink constants (a and log K), average branching number (Bn), and average branching frequency (λ) reflected branched versus linear composition within the limits of the determinations. Specifically, linear and branched molecules of virtually equal average molecular weights were contrasted; lower $[\eta]_w$ and Rg_w were determined for the branched polymer as would be predicted for a higher coil density. For the dextrans analyzed, Mark–Houwink constants were derived relating $[\eta]_w$ and M_w in this solvent. From the dilute solution behavior measured in this study, Me_2NAc–LiCl seems to behave as a thermodynamically good solvent for polysaccharides. Universal calibration curves based upon polystyrene standards, carbohydrate standards, and a composite of both differed primarily because of the range of MWs available for the carbohydrates.

Potential utility lies in using GPC analysis of unknown polysaccharides dissolved in Me_2NAc–LiCl to determine molecular characteristics. Evidence for linear or branched chains can be obtained by this technique.

Acknowledgements

We thank Dr. Richard Cole (University of New Orleans) for his valuable advice and partial sponsorship of this work under USDA, ARS Cooperative Agreement 58-6435-2-110. We also thank Drs. Margaret Clarke and Les Edye (Sugar Processing Research Institute) for the gift of the dextrans, Drs. Bruce Wasserman and Mary Politz (Rutgers University) for the amyloses and amylopectins, and Dr. Barbara Triplett (SRRC) for helpful discussions. We thank Dr. John Vercellotti (SRRC) for preparation of the chitin sample.

References

[1] R.L. Whistler and J.N. BeMiller (Eds.), *Industrial Gums*, 3rd ed., Academic Press, San Diego, 1993.
[2] A. Varki, *Glycobiology*, 3 (1993) 97–130.
[3] D.A. Rees, E.R. Morris, D. Thom, and J.K. Madden, in G.O. Aspinall (Ed.), *The Polysaccharides*, Vol. 1, Academic Press, New York, 1982, 195–290.
[4] P.A. Austin, in J.P. Zikakis (Ed.), *Chitin, Chitosan, and Related Enzymes*, Academic Press, New York, 1984, pp 227–237.
[5] P.A. Austin, *Methods Enzymol.*, 161 (1988) 403–407.
[6] M. Terbojevich, C. Carraro, and A. Cosani, *Carbohydr. Res.*, 180 (1988) 73–86.
[7] M. Hasegawa, A. Isogai, and F. Onabe, *J. Chromatogr.*, (1993) 334–337.
[8] C.L. McCormick, U.S. Pat. 4 278 790 (1981).
[9] C.L. McCormick, P.A. Callais, and B.H. Hutchinson, *Macromol.*, 18 (1985) 2394–2401.
[10] A.F. Turbak, A. El-Kafrawy, F.W. Snyder, and A.B. Auerbach, U.S. Patent 4 302 252 (1981).
[11] A.F. Turbak, in E.J. Soltes (Ed.), *Wood and Agricultural Residues*, Academic Press, New York, 1983, 87–99.

[12] J.D. Timpa, *J. Agric Food Chem*, 39 (1991) 270–275.
[13] A. Grubisic, P. Rempp, and H.A. Benoit, *Polym. Lett.*, 5 (1967) 753–759.
[14] J.D. Timpa, *ACS Symp. Ser.*, 521 (1993) 302–313.
[15] J.D. Timpa and B.A. Triplett, *Planta*, 189 (1993) 101–108.
[16] B.P. Wasserman and J.D. Timpa, *Starch / Starke*, 43 (1991) 389–392.
[17] M.L. Politz, J.D. Timpa, and B.P. Wasserman, *Cereal Chem.*, in press.
[18] M.L. Politz, J.D. Timpa, A.R. White, and B.P. Wasserman, *Carbohydr. Polym*, 24 (1994) 91–99.
[19] T.R. Dawsey and C.L. McCormick, *JMS-Rev. Macromol. Chem. Phys.*, C30 (1990) 405–440.
[20] B. Vollmert, *Polymer Chemistry*, Springer-Verlag, New York, 1973, 513–529.
[21] P.J. Flory, *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, N.Y., 1953, 610–612 and 622–626.
[22] Pharmacia Biotech, Products Catalog, 1994.
[23] K. Gekko, *Makromol. Chem.*, 148 (1971) 229–238.
[24] A.N. DeBelder, in [1], pp 399–425.
[25] M.L. Fishman, D.T. Gillespie and B. Levaj, *ACS Symp. Ser.* 521, (1993) 314–325.
[26] M.L. Fishman, D.T. Gillespie, S.M. Sondey and R.A. Barford, *J. Agric. Food Chem.*, 37 (1989) 584–591.
[27] Y. Tsujisaka and M. Mitsuhashi, in R.L. Whistler and J.N. BeMiller (Eds.), *Industrial Gums*, 3rd ed., Academic Press, San Diego, 1993, pp 447–460.
[28] L. Vilenchik and R. Ayotte, *J. App. Polym. Sci., & App. Polym. Symp.*, 51 (1992) 73–90.
[29] N.C. Carpita and D.M. Gibeaut, *Plant J.*, 3 (1993) 1–30.
[30] R.H. Marchessault and Y. Deslandes, *Carbohydr. Res.*, 75 (1979) 231–242.
[31] Y. Deslandes, R.H. Marchessault, and A. Sarko, *Macromol.*, 13 (1980) 1466–1471.
[32] C.T. Chuah, A. Sarko, Y. Deslandes, and R.H. Marchessault, *Macromol.*, 16 (1983) 1375–1382.
[33] K. Shimahara and Y. Takiguchi, *Methods Enzymol.*, 161, (1988) 417–423.
[34] J.E. Castle, J.R. Deschamps, and K. Tice, in J.P. Zikakis (Ed.), *Chitin, Chitosan, and Related Enzymes*, Academic Press, 1984, pp 273–288.
[35] S. Pang and A. Rudin, *ACS Symp. Ser.*, 521 (1993) 254–269.