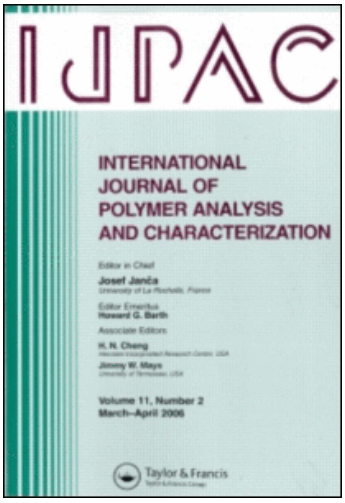

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Gel Permeation Chromatography of Polysaccharides Using Universal Calibration

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Gel permeation chromatography (GPC) has been utilized to characterize a number of representative polysaccharides (i.e., dextrans, pullulans, celluloses, arabinogalactans, amyloses, and amylopectins). *N,N*-dimethylacetamide with lithium chloride, the solvent of choice for high-molecular weight cellulose analysis, readily dissolved all samples and was also used as the mobile phase. GPC allows calculation and comparison of molecular weight averages and distribution, branching and intrinsic viscosity. The concept of universal calibration, which takes into account the hydrodynamic volume of the molecule, was successfully applied to this set of analyses.

KEY WORDS Gel permeation chromatography, polysaccharides, *N,N*-dimethylacetamide/lithium chloride, branching, cellulose, universal calibration

INTRODUCTION

Gel permeation chromatography (GPC) when applied to the characterization of polysaccharides offers a number of distinct advantages. Molecular weight averages and, more importantly, molecular weight distributions can be calculated using this technique. Physical properties are instrumental in the study of polysaccharides, as they are governed by the molecular structure of the polymers. It is well known, for example, that a linear molecule occupies a larger hydrodynamic volume than a branched molecule of equal molecular weight, and thus solutions of the former will be more viscous than those of the latter. In this fashion the intrinsic viscosities of polysaccharides can be directly related to extent of branching.

Polysaccharides, such as dextrans, pullulans, and cellulose, are of great importance in the medical and pharmaceutical fields, as well as in the food, pulp and paper, and textile industries, among others [1] (Table I). They are essential to virtually all biological systems [2]. Optimizing the functions these compounds perform is directly dependent upon knowledge of their structure and molecular weight distribution (MWD), and the extent to which these affect

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TABLE I

Structure and applications of selected polysaccharides.

Polysaccharide	Branching and Linkage	Applications
amylopectin	branched (1→4)-βD-glucan	food product
amylose	linear (1→4)-βD-glucan	food product
arabinogalactan	branched (1→3)-βD-galactan	printing, mining, pharmaceutical, and food industries
cellulose	linear (1→4)-βD-glucan	textile, pulp and paper, and plastics industries
dextran	branched (1→6)-αD-glucan	medical, cosmetics, and photography industries
pullulan	linear (1→6)-αD-glucan	food, printing, plastics, cosmetics, medical, and pharmaceutical industries, chromatographic standards

polymer behavior. This can only be achieved through a technique such as GPC that can quantify these parameters to establish effective comparisons among different polysaccharides.

While a number of polysaccharides are water soluble and can thus be analyzed using aqueous mobile phases, this property is by no means universal. Investigation of a number of important and ubiquitous polysaccharides such as cellulose and starch has been hindered by their poor solubility. Solvents such as dimethylsulfoxide (DMSO), acids (e.g., sulfuric, hydrochloric), bases (e.g., potassium hydroxide), and complexes of nitrogen oxides with different organic solvents have been utilized to effect dissolution of these polysaccharides [3]. However, these solvents suffer from a number of problems, chiefly causing polymer degradation and limited solubility. Isolation and fractionation prior to solubilization is often performed. Thus a solvent which dissolves a wide range of polysaccharides is extremely important in GPC. In view of this, we have used *N,N*-dimethylacetamide/lithium chloride (DMAc/LiCl), the solvent of choice for high-molecular weight cellulose analysis, to dissolve all of the polysaccharides under consideration.

Universal calibration as applied to GPC takes into account the behavior of the polymer in solution, thereby providing accurate MW values [4]. Linear calibration curves are obtained employing this method, which describes the relationship between logarithm of the hydrodynamic volume (log MW[η]) and retention volume. It avoids the problem of limited availability of polymer standards having identical chemical structure and narrow MWD at discrete levels of molecular weight over the range of MW of the unknown polymers. It was thus possible to use well-characterized polystyrene (PS) standards of narrow polydispersity and wide MW range [5]. This methodology has been applied in our laboratory for monitoring cell-wall polymers during cotton fiber development [6,7], and was subsequently extended to quantify the effects of extrusion processing on starches from corn and wheat [8–10]. PS standards were also employed in the calculations for the samples described in this paper.

In this work, GPC of polysaccharides, dissolved in DMAc/0.5% LiCl, has been used to characterize different classes of polysaccharides using universal calibration.

EXPERIMENT

Materials

All polysaccharides analyzed are commercially available, and were used without further purification. Samples included celluloses (J. T. Baker, Phillipsburg, NJ, 1525-1 and 1528-1); pullulans (Pfanstiehl, Waukegan, IL, 12474 and 12476); dextran T10, T40, T70, T500, T2000, (Pharmacia, Uppsala, Sweden, 17-0250-01, 17-0270-01, 17-0280-01, 17-0320-01, 17-0330-01, respectively); dextran low fraction, high fraction (J. T. Baker, G200-5, G202-05, respectively); dextran industrial grade (Sigma, St. Louis, MO, D-5501); arabinogalactan (Atomergic Chemetals, Farmingdale, NY, 6030); corn amylose (Sigma, A-7043); potato amylose (Sigma, A-0512); corn amylopectin (Sigma, A-7780); potato amylopectin (Sigma, A-8515). Polystyrene standards were from Toyo Soda Manufacturing (Tokyo, Japan), 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 (g/mol), and injection concentrations (mg/mL) shown in parenthesis: 2.89×10^6 (0.3), 1.9×10^5 (0.5), 7.1×10^5 (0.4), 1.02×10^5 (0.7), 1.26×10^6 (0.4), 4.39×10^4 (0.8), 3.55×10^5 (0.7), 1.96×10^4 (1.0), 6.2×10^3 (1.3), and 1.03×10^4 (1.2), respectively. The injections concentrations were varied by MW to give equivalent RI detector response, according to standard practice. The solvent was *N,N*-dimethylacetamide (Burdick & Jackson, Muskegon, IL), dried with molecular sieves (Baker, activated type 3A). Lithium chloride (Baker) was oven-dried and stored in a desiccator. The following equipment was used: 10-mL ReactiVials (Pierce, Rockford, IL); heating block (Pierce); Teflon magnetic stirbars (2.5 cm); Baker 10 extraction apparatus; disposable Teflon filters (Millipore, Bedford, MA, Millex-SR and type FH, 0.5 μm); 10 cm^3 glass syringes (BD); 4-mL WISP vials with Teflon septa.

Sample Preparation

Samples were dissolved generally following the procedure reported for cotton [5]. A 30-mg quantity of polysaccharide was added to 5-mL DMAc in 10-mL ReactiVials 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. Dried LiCl, 0.250 g, 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 samples were stirred at this temperature overnight. The solutions were quantitatively transferred to 50-mL volumetric flasks and diluted to volume with DMAc. They were then filtered through a solvent-resistant Teflon disposable filter (0.5 μm). An extraction apparatus was employed with 10-cm^3 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 DMAc/0.5% LiCl.

Chromatography

The mobile-phase/solvent for GPC was DMAc/0.5% LiCl prepared by raising the temperature of 1 L of DMAc 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 [5]. Filtered samples were analyzed on a GPC system consisting of an automated sampler (Waters WISP, Milford, MA) with an HPLC pump (Waters Model 590), pulse dampener (Viscotek, Houston, TX), 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 with the refractive index detector last due to back-pressure considerations. The columns configuration consisted of three 10μm mixed-B columns (Burdick & Jackson/Polymer Laboratories) with a linear range of MW of 500–10,000,000 g/mol, 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, V. 2.70). Universal calibration was determined with polystyrene standards dissolved directly in DMAc/0.5% LiCl. The universal calibration curve was 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.

RESULTS AND DISCUSSION

The DMAc/LiCl solvent system has been employed to dissolve cellulose [11,12]. Our laboratory has developed procedures for the dissolution and characterization of high MW cellulose in cotton fiber without the need for prior extraction, derivatization, or fractionation [5]. Intrinsic viscosity provides an index of random coil dimensions and chain conformation through the Mark-Houwink equation [1]. All polysaccharides were dissolved without difficulty. Comparison of observed solution behavior presented here to that characteristic of polymers in dilute solutions leads to the conclusion that DMAc/LiCl is a good solvent for polysaccharides. A major advantage gained by employing this solvent system is that it can be used both for dissolving the starting material as well as the processed or derivatized products [3,8,11]. Therefore the solvent used for dissolution is the same as the mobile phase being utilized in the GPC analysis, and the polymer properties will not vary as a function of solvent.

In utilizing GPC to analyze dilute solutions of the polysaccharides (Table I) dissolved in DMAc/LiCl, molecular weight averages (M_w and M_n), polydispersity ratio (PD = M_w/M_n), and weight-average intrinsic viscosity ($[\eta]_w$) were calculated.

Even though the dextrans are generally described as being branched glucans, it has been reported that lower molecular weight (MW) dextrans possess a near-linear structure [13]. Previously reported results from our group support this conclusion and extend the range of near-linearity to $M_w \leq \sim 2.5 \times 10^5$ [14]. The importance of this fact will become evident in the discussion to follow. A Mark-Houwink equation for near-linear dextrans dissolved in DMAc/LiCl was derived, with the values $a = 1.3$ and $K = 1.7 \times 10^{-7}$ [14].

Because a linear molecule will occupy a larger hydrodynamic volume than a branched molecule of equal molecular weight, the higher coil density of the latter will be reflected in lower $[\eta]$ values, and deviations from linearity will be observed in the dependence of log $[\eta]_w$ vs. log M_w [15,16]. Archetypal behavior can be observed when comparing arabinogalactan with dextran LF. The first is a highly branched galactan consisting of two

main fractions. Approximately 10% of the molecular weight fraction of arabinogalactan is located below MW = 20,000 g/mol and comprises the smaller of the two main fractions, with M_w ~ 14,000 g/mol. The larger fraction comprises the other approximately 90% of the molecular weight fraction, with M_w ~ 90,000 g/mol. The overall M_w of this polysaccharide is 8.4×10^4 g/mol. Dextran LF is a near-linear dextran (see definition above) with an M_w equivalent to that of arabinogalactan (Table II). Even though the values for M_w for these two saccharides are nearly identical, large differences in their solution behavior can be noted, as exemplified by comparing their weight-average intrinsic viscosities (Fig. 1): $[\eta]_w$ for arabinogalactan is 0.06 dL/g, while for dextran LF it is 0.38 dL/g. These results are in accordance with the large amount of branching present in arabinogalactan as opposed to the dextran. The positive and negative spikes observed at both the high and low end of Figures 1 and 2 are due mainly to the low signal/noise (S/N) at the ends of the distributions. The linear line of the plots at the low MW end is an extrapolated line. For accurate comparison, the portion of the plot between log (MW) ~4.5 and log (MW) ~5.2 should be considered.

A further example of the influence of branching of the main polysaccharide chain on solution behavior is given by contrasting potato amylose with dextran T500. The former is a linear glucan while the latter is branched (Table I). Their M_w values are fairly close (<15% difference), that of dextran T500 being slightly higher. Although the weight-average intrinsic viscosities of these polysaccharides are nearly identical, the high-MW portion of the dextran, where the majority of the branching is believed to occur in dextrans [14], possesses a lower $[\eta]_w$ than the amylose, which is considered linear throughout its entire MWD (Fig. 2). The drop at the high MW end of the plot is partly due to the low S/N mentioned earlier, but may also be caused by the presence of some highly branched structures of high MW. For accurate comparison, the portion of the plot between log (MW) ~4.3 and log (MW) ~5.8 should be considered.

TABLE II

Calculated molecular weight averages and intrinsic viscosity of polysaccharides dissolved in DMAc/LiCl.

Sample ID	M_w (Supplied)	M_w[a]	M_n[a]	PD[b]	$[\eta]_w$ (dL/g)
Amylopectin (Potato)	N/A[c]	1.2×10^6	5.8×10^4	21	0.59
Amylopectin (Corn)	N/A	2.1×10^7	6.1×10^4	3.5×10^2	0.40
Amylose (Potato)	N/A	4.9×10^5	5.0×10^4	9.8	0.83
Amylose (Corn)	N/A	6.2×10^5	8.6×10^4	7.2	0.65
Arabinogalactan	8.0×10^4	8.4×10^4	3.2×10^4	2.6	0.06
Cellulose 4	1.8×10^5[d]	1.8×10^5	2.7×10^4	6.8	1.04
Cellulose 5	3.2×10^5[d]	3.3×10^5	4.2×10^4	7.9	0.71
Dextran T10	1.0×10^4	1.9×10^4	1.7×10^4	1.1	0.15
Dextran T40	4.4×10^4	4.7×10^4	3.6×10^4	1.3	0.29
Dextran T70	7.0×10^4	7.6×10^4	4.8×10^4	1.6	0.40
Dextran LF[e]	$6.0 - 9.0 \times 10^4$	8.4×10^4	6.4×10^4	1.3	0.38
Dextran HF[f]	$2.0 - 3.0 \times 10^5$	2.5×10^5	5.6×10^4	4.4	0.64
Dextran T500	5.0×10^5	5.5×10^5	6.8×10^4	7.9	0.84
Dextran T2000	2.0×10^6	1.9×10^6	5.4×10^4	36	0.78
Dextran IG[g]	$5.0 - 40.0 \times 10^6$	5.1×10^6	4.8×10^4	1.0×10^2	0.14
Pullulan 1	1.0×10^5	1.1×10^5	3.5×10^4	3.0	0.69
Pullulan 3	3.0×10^5	3.1×10^5	3.4×10^4	9.3	0.89

[a]Values calculated using GPC. [b]PD = M_w/M_n. [c]N/A = Value not available.
[d]Values from ref. [11]. [e]LF = low fraction. [f]HF = high fraction. [g]IG = industrial grade.

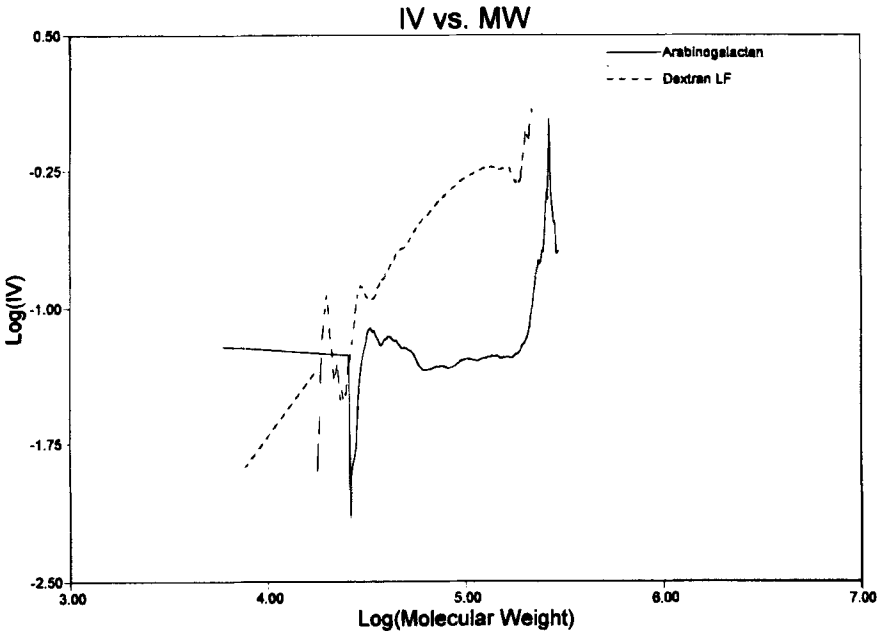

FIGURE 1 Log-log plot of intrinsic viscosity vs. molecular weight of arabinogalactan vs. dextran LF.

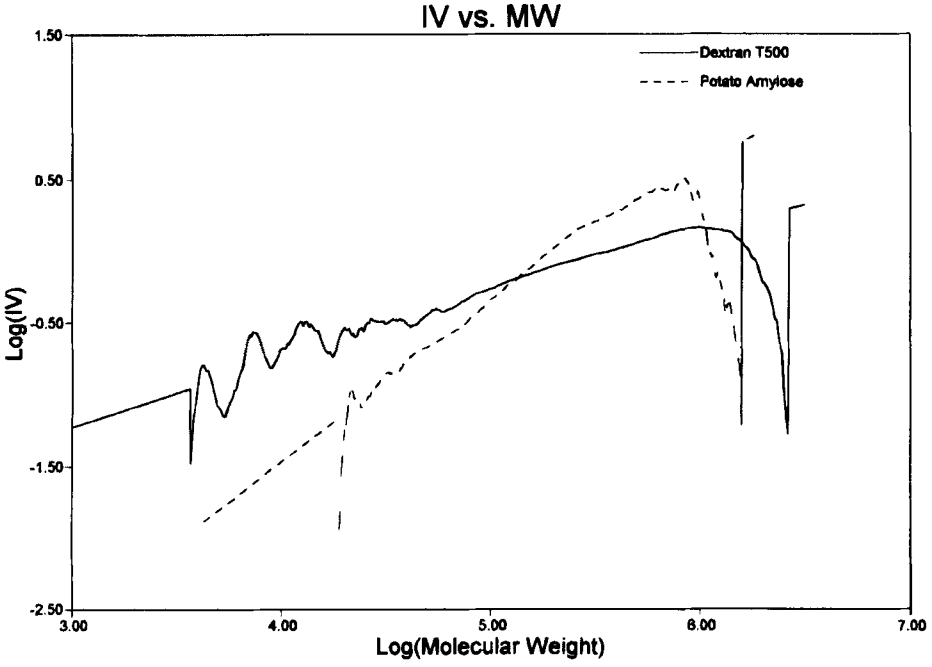

FIGURE 2 Log-log of intrinsic viscosity vs. molecular weight of dextran T500 vs. potato amylose.

When comparing the two major components of starch, amylose and amylopectin, to each other, the second had a higher M_w regardless of the source (corn or potato). As expected, the branched amylopectins did have a lower $[\eta]_w$ than the amyloses (Table II).

Even though pullulan is a linear polysaccharide, branching frequency and branching number calculations show it as possessing a small degree of branching, when compared to the linear cellulose 5 samples (data not shown). While it is impossible from this study to conclude with certainty the reason for these results, we postulate what we believe to be a likely cause. The structure of pullulan consists of repeating units of maltotriose (α-D-(1→4) linkages) joined by α-D-(1→6) linkages in a step-wise manner. In addition, pullulan contains ~6.6% of a maltotetraose subunit [17]. It should not be concluded that the maltotetraose units are arranged in such a fashion as to cause short-chain branching, as Catley and Whelan[18] showed that the tetramer is linked exclusively through its ends in pullulan. We propose that it is possible that the combination of different repeating units, linkage types, and the step-wise arrangement of the molecule all contribute to the calculated branching results.

CONCLUSIONS

GPC with differential viscometer and refractive index detectors is an effective means of characterizing polysaccharides dissolved in DMAc/LiCl. Applying the concept of universal calibration based on polystyrene standards afforded M_w values comparable to those supplied with the samples. The parameters determined were related to branched versus linear composition of the polymers within the limits of the determinations. Finally, DMAc/LiCl seems to behave as a thermodynamically good solvent for polysaccharides, one in which dilute solution behavior can be readily studied.

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Notes

Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the United States Department of Agriculture over others not mentioned.

Safety Considerations

N,N-dimethylacetamide 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 C. *N,N*-dimethylacetamide, Material Safety Data Sheet, 1985, D5784-01; pp. 1–4).

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