

Hydrodynamic Chromatography of Waxy Maize Starch

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ABSTRACT

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A hydrodynamic column packed with solid beads chemically bonded with *N*-methyl-D-glucamine residues was used with 90% dimethylsulfoxide (DMSO)-H₂O mobile phase as part of a chromatographic system to characterize jet-cooked waxy maize starch. Software calculations based on signals from refractive index and dual-angle light-scattering detectors indicated the column could fractionate molecular weights up to $\approx 5 \times 10^8$. Calculated molecular weight values for the highest molecular weight sample was greatest at the lowest flow rate of 0.1 mL/min. Values of molecular weights and radii of gyration determined by the in-line dual-

angle light-scattering detector were significantly less than those determined with an off-line multiangle light-scattering detector that examined samples that had not traveled through the hydrodynamic column. This work has demonstrated the feasibility of using a hydrodynamic column to characterize waxy maize amylopectins. However, considerations of sample shear sensitivity and questions of in-line light scattering detection show that further efforts are required to develop and optimize a chromatographic system to characterize very high molecular weight amylopectins.

In recent years, high-performance size-exclusion chromatography (HPSEC) has been used to characterize starches, major starch components, and constituents of debranched starches (Kennedy et al 1992, Bradbury and Bello 1993, Fishman et al 1996). One limitation of this technique is that size-exclusion columns do not fractionate the very high molecular weight species that constitute much of the amylopectin component of starches. For example, molecular weights (M_w) of approximately 360×10^6 and radii of gyration (R_g) of approximately 310 nm, obtained from sedimentation field flow fractionation and multiangle laser light-scattering measurements, have been reported recently for potato and waxy maize starches (Hanselmann et al 1996).

The separation of finite size particles by flow through a capillary system is the basis by which a hydrodynamic column fractionates materials. Theoretical considerations of separation by flow have been presented in papers by DiMarzio and Guttman (1970) and by Guttman and DiMarzio (1970). Early applications of hydrodynamic columns to the separation of colloid particles are presented in the papers of Small (1974) and Small and Langhorst (1982). More recent works have tested the potential of hydrodynamic chromatography (HDC) to fractionate polystyrene (Stegman et al 1990) and the water soluble polymers xanthan and hydrolyzed polyacrylamide (Hoagland and Prudhomme 1988). Stegman et al (1993) have proposed a model in which the polymer migration rate is based on the assumption that interstitial channels in a packed column can be represented by a bundle of capillary tubes and have tested this model with some linear random coil polymers. They found that high-resolution separations could be obtained for polymers with molecular weights between $\approx 10^4$ and 5×10^6 with columns packed with 1.5- μ m nonporous particles.

The sensitivity of high-molecular-weight fractions of amylopectins to degradation by shear was one of our major concerns. There is early evidence in the literature that demonstrates that the intrinsic viscosity and molecular weights determined by light

scattering (Banks and Greenwood 1975) of waxy maize starch are sensitive to shear treatment. The problem of degradation of high molecular weight polymers by shear forces in chromatographic columns has been discussed in a review by Barth and Carlin (1984). Thus, even though amylopectin is a compact molecule in solution (Hanselmann et al 1996, Millard et al 1997), we expected shear degradation to be a major limitation on the use of our columns to examine very high molecular weight fractions.

The purpose of this article is to report on the feasibility of using a hydrodynamic column to fractionate processed amylopectins of waxy maize starch and to examine some limitations of such a column. Although two different light-scattering instruments were used for determination of molecular weights, a multi-angle laser light-scattering detector (MALLS) operated in the batch mode and an in-line dual-angle laser light-scattering detector (DALLS), emphasis in this article is on the behavior of the hydrodynamic column.

MATERIALS AND METHODS

Sample Preparation

Amioca starch, a waxy maize starch that contained about 15% moisture when it was used, was purchased from American Maize-Products Company (Hammond, IN). The starch was first dispersed as a 12% (w/w) slurry by stirring in distilled water and then steam-jet cooked in a manual hydroheater (a Venturi device) manufactured by Hydro-Thermal Corp. (Waukesha, WI). The steam pressures were adjusted to obtain three different cooking conditions: 70/65, gentle cook; 70/40, standard cook; and 120/40, severe cook, where the ratios are defined as inlet steam pressure (psig) to steam pressure in the Venturi cooking chamber. The cooked starch was collected in a Dewar flask at approximately 10% solids (the initial slurry became diluted by condensed steam) and was immediately transferred into dimethylsulfoxide (DMSO) to obtain an approximate 1.0% starch solution in 90% DMSO-H₂O (w/w). Sodium azide, 0.2% (w/w), was added to prevent microbial activity and the 1% starch fluid was stored, either at room temperature or in a freezer, until it was diluted and used. Because some samples were repeatedly thawed and refrozen, careful attention was paid to the possibility of molecular weight changes of such samples with time. A MALLS detector was also employed to examine the molecular weight of a waxy maize starch sample freshly prepared and after the sample had undergone six freeze-thaw cycles.

Samples for chromatographic examination were diluted to concentration ranges of 1.0–2.5 mg/mL. The initial 1.0% stock solu-

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tions were weighed into 25-mL glass vials and diluted by weight to desired concentrations with mobile phase. Solutions intended for batch mode examination in the MALLS detector were first diluted to concentrations of about 0.3% and then filtered through 5- or 1- μm polyester membrane filters (Poretics Corp., Livermore, CA). Starch concentrations were obtained by optical rotation using a specific rotation of 190 at 589 nm (Dintzis and Tobin 1969). The initial dilutions were then further diluted by weight to desired concentrations with solvent filtered through a 0.2- μm polyester membrane filter.

Chromatography

The mobile phase was 90% DMSO-10% H₂O (w/w). The DMSO was HPLC grade (Sigma Chemical Co., St. Louis, MO) and was used as supplied. HPLC grade water was prepared by distilling deionized water and filtering it through a 0.4- μm Poretics polyester membrane filter. The mobile phase was deaerated with helium and filtered through a 10- μm stainless steel in-line filter.

The chromatography system consisted of a Spectra System P400 pump and a Spectra System AS3000 autosampler (Thermo Separation Products, Fremont, CA) with 100- μL injection loop. A 5- \times 1-cm guard column and a 25- \times 1-cm fractionation column, Jordi GBR hydrodynamic volume solid bead, were fitted with 10- μm precolumn and 5- μm postcolumn stainless steel frits. These columns were packed with a specially ordered 5–15 μm distribution of particle sizes, estimated 10 μm average, of Jordi-Gel GBR solid beads (Jordi Associates, Inc., Bellingham, MA). These beads, prepared by a proprietary method, were cross-linked with divinylbenzene and chemically bonded with *N*-methyl-D-glucamine through the amine nitrogen to tertiary carbon atoms of polyethylene backbones. Samples were also examined with a mixed bed size-exclusion column: a PL gel, 20 μm Mixed-A column, 30- \times 7.5-cm and a 5- \times 7.5-cm guard column were used (Polymer Laboratories, Amherst, MA). Columns were maintained at 60°C in a Waters microprocessor controlled heating unit.

The in-line detection systems consisted of a DALLS detector that measured scattered light at 15° and 90° from the incident beam and associated software, Version 3.0 (PD2000, Precision Detectors, Amherst, MA) coupled to a Waters 410 differential refractometer (Waters Associates). The light scattering and differential refractive index cells were maintained at 45°C within the refractometer unit. The molecular weights of the starch preparations were also measured in a batch mode with a MALLS detector. These measurements were done at room temperature with a DAWN F instrument (Wyatt Technology Corp., Santa Barbara, CA) that was calibrated with toluene at the 90° scattering angle and normalized with an isotropic “magic glass” cylinder supplied by the manufacturer for calibration of light intensity at other angles. A He-Ne laser light source operating at 632.8 nm was used in both light scattering instruments. PEEK tubing 0.010 i.d. (Upchurch)

was used to carry the samples through the chromatography system. Flow rates of 0.1–0.5 mL/min were used in this study.

Calculation of Molecular Weights

A specific refractive index increment, dn/dc , of 0.074 (mL/g) was used in our calculations. Values of M_w obtained from MALLS batch mode operation were calculated from Berry plots of the scattered light. The software operations, done so as to minimize the error in M_w , required that one specify the polynomial degree for the best least-squares fit of the extrapolations to zero angle and zero concentration. Minimal errors generally were achieved using polynomials of zero or first degree for extrapolation of concentration and second or third degree for angular dependence.

The software provided with the DALLS unit allowed one to calculate an M_w and R_g value without use of refractive index (RI) information (“batch mode”), or to combine RI information with the light scattering data (“all data mode”), to obtain distribution information on both parameters. After the baselines of the peaks of RI and light scattering data had been adjusted, and a peak integration region set, results for the “all data mode” were considered acceptable when values of calculated dn/dc and concentration agreed within 4% of the injected sample concentration and the accepted dn/dc value 0.074 mL/g. When these conditions were achieved, values of M_w and R_g obtained from both modes were generally in close agreement with each other.

Our initial copy of the software was modified according to manufacturer’s instructions (Precision Detectors, Amherst, MA;

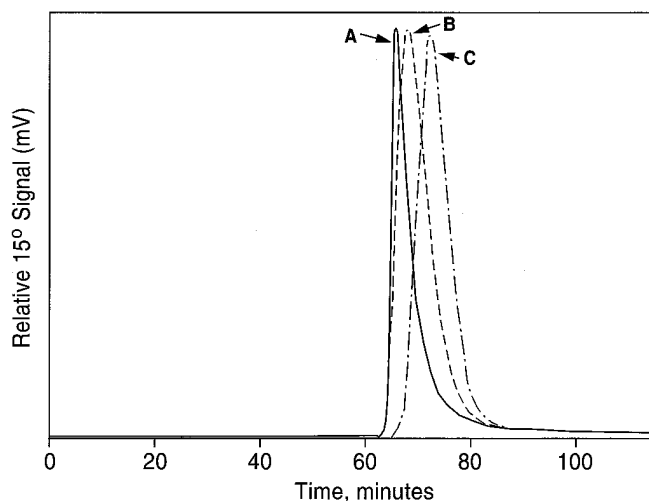


Fig. 1. Elution profiles of jet-cooked waxy maize starch obtained from the hydrodynamic column at a flow rate of 0.1 mL/min. Samples: A, gentle cook; B, standard cook; C, severe cook.

TABLE I
Molecular Weight and Radii of Gyration of Waxy Maize Starch

Type of Measurement	Type of Jet Cook					
	Gentle (Sample A)		Standard (Sample B)		Severe (Sample C)	
	$M_w \times 10^{-6}$	R_g (nm)	$M_w \times 10^{-6}$	R_g (nm)	$M_w \times 10^{-6}$	R_g (nm)
Hydrodynamic with DALLS ^a	224 ± 4 ^b	165 ± 7	48.1 ± 1.4	99.5 ± 10	20.8 ± 1.1	57.7 ± 2.0
MALLS batch mode	412 ± 64 ^c	277 ± 31	93 ± 2.0	138 ± 6	26.5 ± 0.7	91 ± 4
Size exclusion with DALLS			33.4 ± 2.0	80.1 ± 7.7	19.3 ± 1.1	60.4 ± 0.2
	<i>n</i> = 7		<i>n</i> = 7		<i>n</i> = 8	
	<i>n</i> = 2		<i>n</i> = 1		<i>n</i> = 1	
			<i>n</i> = 3		<i>n</i> = 6 ^d	

^a Dual-angle laser light scattering detector.

^b Values are averages ± SD, *n* = number of measurements. Error values are software generated when *n* = 1. R_g values from MALLS are Z averages, from DALLS are wt. averages.

^c Multiangle laser light scattering detector.

^d Flow rate of 0.35 mL/min; all others 0.1 mL/min.

Norman Ford, *personal communication*) to permit calculation of large R_g values.

Pre-sheared vs. Not Pre-sheared Sample

An attempt was made to test for suspected shear effects on sample molecular weight. A sample of waxy maize starch was jet-cooked under standard conditions and made to a concentration of 1.5 mg/mL (0.15%) in a solvent of 90% DMSO-H₂O containing 0.2% NaN₃. This sample was placed in a stainless steel chamber and with the use of dry nitrogen gas pressure forced through a hydrodynamic column. The pressure was adjusted to obtain a flow rate of 0.10 mL/min through the column at room temperature. However, with time, the flow rate decreased and it was necessary to increase the gas pressure to recover a flow rate of 0.1 mL/min. The first 20 mL of fluid was discarded to avoid dilution of the sample with mobile phase. The eluted starch fluid was a light orange-pink color. The attempt to pre-shear the jet-cooked sample was repeated: the sample was diluted with mobile phase to a concentration of 1.5 mg/mL starch and 0.03% NaN₃ and the column was maintained at 60°C. This time the eluant was colorless. However, the starch fluid again was not able to maintain a constant flow rate at a given pressure. Thus, the pre-sheared samples were exposed to average estimated flow rates of about 0.07–0.05 mL/min.

RESULTS AND DISCUSSION

The mobile phase, 90% DMSO-H₂O, was chosen because it is a good solvent for starch components (Jackson 1991). Starch components are stable in this solvent over long periods of time (Bradbury and Bello 1993) and both the hydrodynamic and size exclusion columns were compatible with this mobile phase. We did not use mainly aqueous mobile phases because we did not find a formulation for which both hydrodynamic column performance and starch stability were acceptable. It often required 24–48 hr, or more, for the hydrodynamic column to reestablish stability after flow rates were changed. No changes in M_w values were detected as a result of sample freeze-thaw cycling or storage time in the frozen or liquid state.

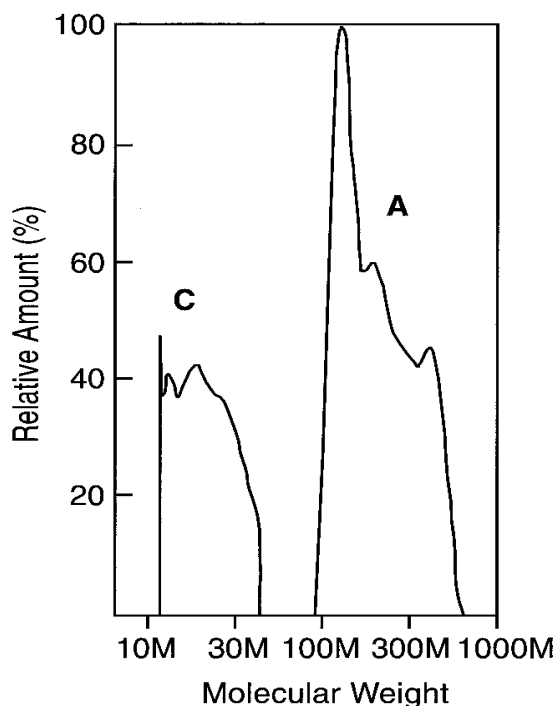


Fig. 2. Examples of molecular weight distributions obtained for samples A and C from the hydrodynamic column, 0.1 mL/min.

Two results are clearly indicated in the data of Table I. The hydrodynamic column was able to fractionate the extremely high molecular weights of waxy maize starch. The two measurement systems, MALLS vs. DALLS with the hydrodynamic column, yielded different molecular weight and radii of gyration values. However, the two software programs calculate different R_g averages. With MALLS, the Z average is calculated. With DALLS “all data mode”, weight average is calculated. In “batch mode”, Z-average is calculated. We note that, within experimental error, the MALLS and DALLS calculated values for a standard sample of dextran agreed with the manufacturer’s M_w value of 4.9×10^6 (data not shown).

An example of the ability of the hydrodynamic column to fractionate the jet-cooked samples at a flow rate of 0.1 mL/min is presented in Fig. 1. These are typical elution patterns of separate runs generated from the 15° signals of the DALLS detector and have been scaled by the software to provide approximately equal peak heights. We note that the slopes of the initially eluted samples follow the order A (gentle jet-cook) > B (standard jet-cook) > C (severe jet-cook). The elution times of the peaks, A, B, and C, were 65.5, 70.4, and 74.7 min, respectively. Examples of molecular weight distributions calculated by the PD2000 software are displayed in Fig. 2 for samples A and C. The molecular weight vs. elution time plot calculated for the gently jet-cooked sample is presented in Fig. 3. The result of the calculation is that molecular weights of up to about 500 million were observed by the detectors.

Some influences of flow rates on M_w and R_g are summarized in Table II. We note that for both columns, hydrodynamic and size exclusion, R_g was independent of flow rate for all three samples. With the hydrodynamic column, M_w of the highest molecular

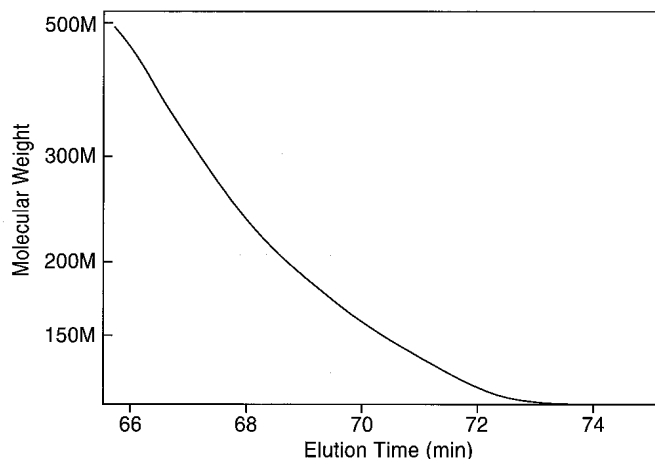


Fig. 3. An example of molecular weight versus elution time from the hydrodynamic column for sample A, 0.1 mL/min.

TABLE II
Effects of Flow Rate on Molecular Weight and Radius of Gyration

Sample	Flow Rate (mL/min)	$M_w \times 10^{-6}$	n	R_g (nm)
Hydrodynamic column				
A, Gentle jet cook	0.1	224.1 ± 4.1^a	7	164.8 ± 7.0
	0.2	193.2 ± 2.2	5	166.1 ± 8.4
	0.3	122.6 ± 1.3	4	162.5 ± 3.1
B, Standard jet cook	0.1	48.1 ± 1.4	7	99.5 ± 10.1
	0.2	48.4 ± 1.1	3	89.5 ± 1.6
	0.3	35.3 ± 1.5	3	93.8 ± 2
C, Severe jet cook	0.1	20.8 ± 1.1	8	57.7 ± 2.0
	0.2	19.8 ± 0.6	3	55.5 ± 0.8
Size-exclusion column				
C, Severe jet cook	0.35	19.3 ± 1.0	6	60.4 ± 0.2
	0.50	19.5 ± 0.5	2	60.3 ± 0.2

^a Values are averages \pm standard deviations.

weight sample (A) was sensitive to the flow increases; M_w of the standard jet-cook (B) was stable between 0.1 and 0.2 mL/min flow, but decreased at a flow rate of 0.3 mL/min; and M_w of sample C was the same at 0.1 and 0.2 mL/min flow rates. It is tempting to infer that these results represent evidence of shear degradation caused by increased flow rates that would subject the amylopectin molecules to increased shear forces. However, it is puzzling that R_g was not sensitive to flow rates.

The difference in the ability of our two columns to fractionate a high molecular weight sample is illustrated in Fig. 4. Part A of the figure shows the molecular weight versus elution time of the standard jet-cooked sample (sample B) as determined by passage through the hydrodynamic column. Part B of the figure presents one result of the same experiment performed with the size exclusion column. We note that with the size exclusion column, M_w apparently increases slightly with time for about 4 min before it begins to decrease. The plot in part B of the figure demonstrates that this size exclusion column is unable to properly fractionate the high molecular species in the sample.

The DALLS determined values of M_w (46×10^6) and R_g (76 nm) of the sample that had been jet-cooked under standard conditions and forced through a hydrodynamic column with dry nitrogen pressure were the same as those of the sample not pre-sheared. We suspect the appearance of color in the initial pre-sheared sample might be caused by a Maillard type reaction between some remaining small percentage of underivatized amine groups on the particles of the column packing. We do not know why sample that contained less NaN_3 and was forced through the column at 60°C was colorless. Thus, we did not detect shear degradation of waxy maize amylopectin that had passed initially through a column at 0.15% concentration at flow rates estimated to average < 0.07 mL/min. Further experiments, consisting of

examination with MALLS batch mode detection of samples forced through a hydrodynamic column at suitable flow rates, are needed to demonstrate the dependence of expected shear degradation as functions of sample molecular weight and flow rate through the column. We believe the decreases in M_w as a function of flow rate, Table II, are likely to be caused by shear degradation and/or flow rates that exceed the ability of the column to maintain proper hydrodynamic flow characteristics for a given range of molecular weights and sizes.

Reasons are unclear for the differences in M_w and R_g values determined by MALLS instrumentation, for samples that have not gone through a hydrodynamic column, versus DALLS instrumentation, for samples that have gone through a hydrodynamic column. Although it may be tempting to attribute this result mainly to differences in instrumentation and software approaches between detection based on two angles versus 14 to 16 angles, one should not rule out the possibility that a small percentage of "aggregates" in the jet-cooked samples may remain in aliquots of sample that have not gone through a hydrodynamic column. Further experiments using MALLS to examine samples forced through a hydrodynamic column would be helpful in addressing this situation.

CONCLUSIONS

This work has demonstrated the ability of a hydrodynamic column to fractionate waxy maize amylopectins of molecular weights at least up to 100 million. The usefulness of this system is limited, in part, by the shear sensitivity of the material flowing through the column, and because of this, low flow rates with subsequent long run times were needed for investigation of extremely high molecular weights. Optimization of such columns and chromatographic conditions would undoubtedly lead to significant improvements in resolution and possibly a significant reduction in run times. There is good reason to expect that further efforts could develop hydrodynamic chromatography into a useful method for characterization of very high molecular weight starch components.

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LITERATURE CITED

- Banks, W., and Greenwood, C. T. 1975. *Starch and Its Components*. Halsted Press: New York.
- Barth, H. G., and Carlin, F. J., Jr. 1984. A review of polymer shear degradation in size-exclusion chromatography. *J. Liquid Chromatogr.* 7:1717-1738.
- Bradbury, A. G. W., and Bello, A. B. 1993. Determination of molecular size distribution of starch and debranched starch by a single procedure using high-performance size-exclusion chromatography. *Cereal Chem.* 70:543-547.
- DiMarzio, E. A., and Guttman, C. M. 1970. Separation by flow. *Macromolecules* 3:131-146.
- Dintzis, F. R., and Tobin R. 1969. Optical rotation of some α -1, 4-linked glucopyranosides in the system H_2O -DMSO. *Biopolymers* 7:581-593.
- Fishman, M. L., Rodriguez, L., and Chau, H. K. 1996. Molar masses and sizes of starches by high-performance size-exclusion chromatography with on-line multi-angle laser light scattering detection. *J. Agric. Food Chem.* 44:3182-3188.
- Guttman, C. M., and DiMarzio, E. A. 1970. Separation by flow. II. Application to gel permeation chromatography. *Macromolecules* 3:681-691.
- Hanselmann, R., Burchard, W., Ehrat, M., and Widmer, H. M. 1996. Structural properties of fractionated starch polymers and their dependence on the dissolution process. *Macromolecules* 29:3277-3282.
- Hoagland, D. A., and Prudhomme, R. K. 1988. Analysis of water-soluble polymers using hydrodynamic chromatography. *J. Appl. Polym. Sci.* 36:935-955.
- Jackson, D. S. 1991. Solubility behavior of granular corn starches in methyl sulfoxide (DMSO) as measured by high performance size ex-

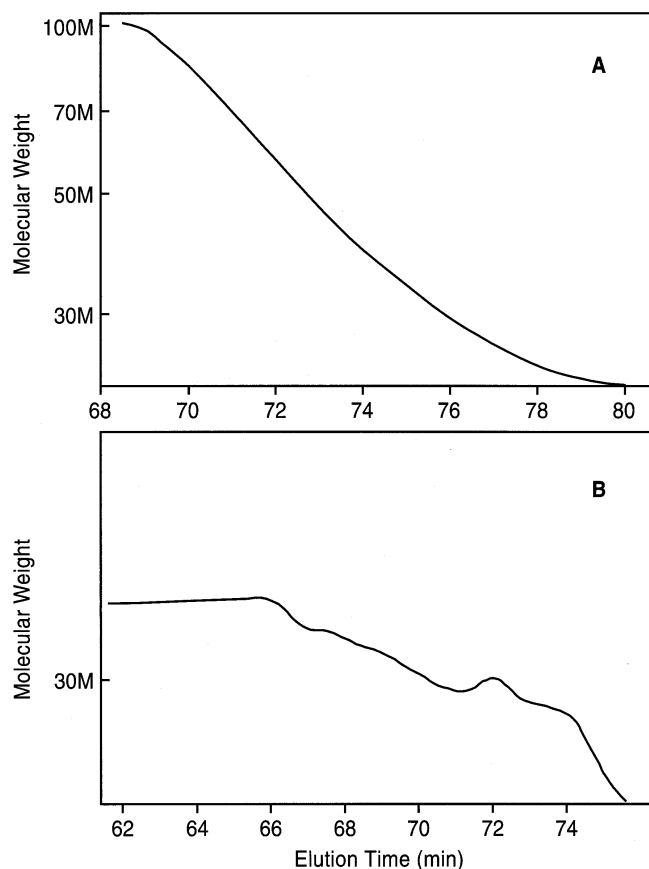


Fig. 4. Comparison of molecular weight versus elution time profiles for sample B at a flow rate of 0.1 mL/min: profile A, hydrodynamic column, profile B, size exclusion column.

- clusion chromatography. *Starch/Staerke* 43:422-427.
- Kennedy, J. F., Rivera, Z. S., Lloyd, L. L., and Warner, F. P. 1992. Fractionation of starch amylopectin and amylose by high performance gel filtration chromatography. *Starch/Staerke* 44:53-55.
- Millard, M. M., Dintzis, F. R., Willett, J. L., and Klavons, J. A. 1997. Light scattering molecular weights and intrinsic viscosities of processed waxy maize starch in 90% DMSO-H₂O. *Cereal Chem.* 74:687-691
- Small, H. 1974. Hydrodynamic chromatography. A technique for size analysis of colloidal particles. *J. Colloid Interface Sci.* 48:147-161.
- Small, H., and Langhorst, M. A. 1982. Hydrodynamic chromatography. *Analytical Chem.* 54:892A-894A, 896A-898A.
- Stegman, G., Oostervink, R., Kraak, J. C., Poppe, H., and Unger, K. K. 1990. Hydrodynamic chromatography of macromolecules on small spherical non-porous silica particles. *J. Chromatogr.* 506:547-561.
- Stegman, G., Kraak, J. C., and Poppe, H. 1993. Hydrodynamic chromatography of polymers in packed columns. *J. Chromatogr.* 657:283-303.

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