High Performance Size Exclusion Chromatography of Starch with Dimethyl Sulfoxide as the Mobile Phase*

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Synopsis

Two types of high performance size exclusion chromatographic (SEC) columns were tested for the characterization of starch samples: μBondagel and Aquapore. The mobile phase was dimethyl sulfoxide (DMSO) containing 0.03 *M* sodium nitrate maintained at 80°C. The results indicated that both μBondagel and Aquapore can be used to determine the relative molecular weight of starch. The exclusion limits for these columns exceed ten million. Preliminary results using μStyragel are also reported. Examples of SEC analysis of starch, including starch with anionic groups, are given.

INTRODUCTION

Size exclusion chromatography (SEC) is the most widely used technique to characterize high molecular weight compounds. Its application with carbohydrates using conventional packing materials was reviewed by Churms.[1] Today a wide range of high performance SEC columns is available.[2-4]

Starch analysis, utilizing high performance SEC columns, is still very limited. One of the difficulties is finding a suitable mobile phase to make a stable starch solution. Starches are composed of amylose and amylopectin. Amylose molecules are linear and at high temperatures can be dissolved in water. However, amylopectin molecules are highly branched and may have molecular weights up to several hundred million.[5] Thus, they are very difficult to dissolve in water. Because dimethyl sulfoxide (DMSO) is known to be a good solvent for starch, it may also be a better mobile phase than water for the SEC analysis of starch.

The use of dimethyl sulfoxide (DMSO) or its mixtures for SEC mobile phases has been reported,[6-10] and two of these papers used high performance SEC columns.[6,7] In both cases the largest pore size of the packing material was 1000 Å and the experiments were run at room temperature. At most three columns could be used because of the back pressure caused by the high viscosity of DMSO. Samples were dissolved in hot DMSO then diluted with water until it had the same composition as the mobile phase.

In the present study, newly available large pore-size columns were added to the column set (μBondagel E-High and Aqueous OH-4000). DMSO with 0.03

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M $NaNO_3$ was used as the mobile phase, and the instrument was run at 80°C. The sample was prepared directly in the mobile phase. With this system, the pressure problem was eliminated, the operation was simplified, and the exclusion limit was much higher than those reported previously.

EXPERIMENTAL

A Waters Associates ALC/GPC-150 with model 730 data system was used for all determinations. The experimental conditions are listed in Table I. The Dextran standards were obtained from Pharmacia (Piscataway, NJ) and all starch samples were products of National Starch and Chemical Corporation. DMSO (HPLC grade) was purchased from J. T. Baker or Burdick and Jackson and was used as received.

The mobile phase was prepared by dissolving a suitable amount of sodium nitrate in DMSO to make a 0.03 *M* solution. The mobile phase reservoir was an external 1-gal glass bottle. Sample solutions were prepared by weighing the

TABLE I
Experimental Conditions of Size Exclusion Chromatography of Starch

Column:	μBondagel: Modified silica gel with ether functional groups. Aquapore: Modified silica gel with organic hydroxyl group (Diol column). μStyragel: Styrene-divinyl benzene gel. (A) μBondagel, E-High, E-Linear, E-1000, E-125, 4.6 mm × 25 cm, Waters Associates (Milford, MA) (C) Aquapore OH-4000, OH-500, OH-100, 4.6 mm × 25 cm, Brownlee (Santa Clara, CA) (C) μStyragel 10^5, 7.6 mm × 30 cm, Waters Associates (Milford, MA)
Mobile phase:	Dimethyl sulfoxide with 0.03 *M* $NaNO_3$
Temperature:	80°C
Flow rate:	0.5 m/min for μBondagel and Aquapore column sets 1.0 mL/min for μStyragel column
Detection:	DRI, 128X, scale factor 20
Sample concentration:	0.25 to 0.5%
Injection volume:	100 μL
Calibration standard:	Dextrans

starch directly into a sample vial and diluting to volume with the mobile phase. Sample solutions were heated at 80°C in the injector compartment. Most samples became clear homogeneous solutions in 30 min, but for unmodified starches, dissolution took several days. The solution was injected into the column set through a Rheodyne 7315 prefilter with 2-μm frit.

Operational parameters were not systematically investigated. Concentration effects and the possibiity of shear degradation inside the column were not examined.[11-14] Our goal in this study was to find a set of experimental conditions useful for the relative comparison of different starch samples under the same conditions.

RESULTS AND DISCUSSION

Calibration

Initially dimethyl sulfoxide without electrolyte was used as the mobile phase. With the μBondagel columns, the elution curves for both the T-2000 and T-500 Dextran standards showed two peaks. Such bimodal behavior had been reported earlier[11] and was attributed to interaction between the ionic groups of the Dextran molecules and the packing material. Such ionic interaction can be eliminated by the addition of a low molecular weight electrolyte to the mobile phase. Sodium nitrate at 0.03 M was selected. The results were so favorable that further work to optimize the experimental conditions was not conducted.

Figure 1 shows the typical elution curves of Dextran standards. The large negative peak could be from water. From the retention time values, calibration curves were constructed. Since only five calibration standards were available, several artificial points were added to the calibration table. This allowed the data system to correctly calculate the third-order calibration curve parameters. Figure 2 shows the calibration curves for the three column sets.

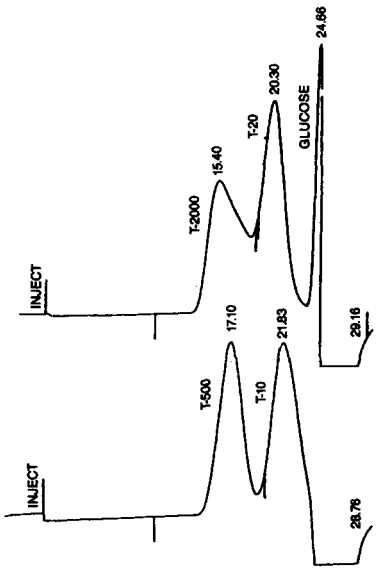

Fig. 1. Elution curves of Dextran standards using the μBondagel-column set.

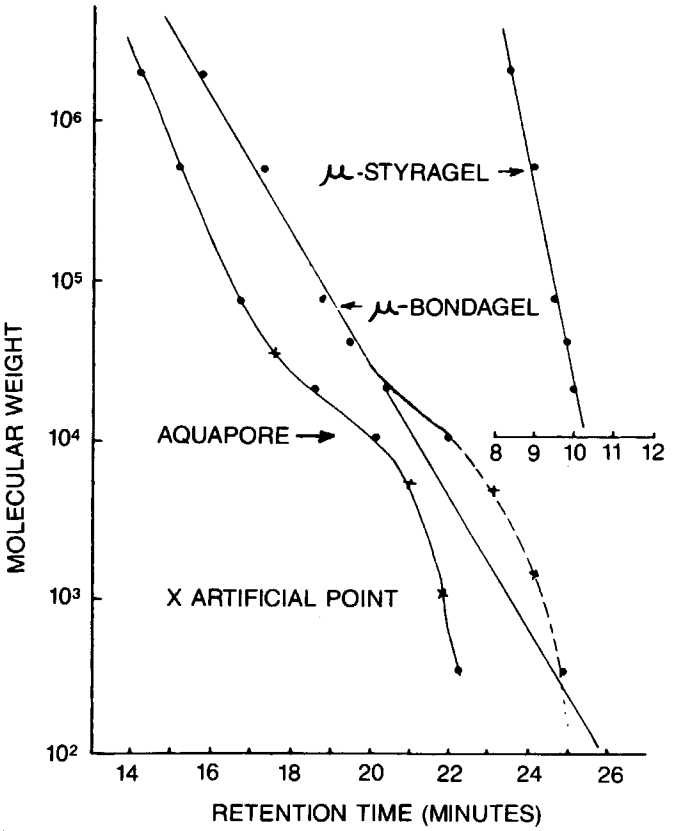

Fig. 2. Calibration curves of μBondagel, Aquapore, and μStyragel columns.

TABLE II
Comparison of Manufacturers and the Experimental Molecular Weight of Dextran Standards

			Experimental			
	By Manufacturer		μBondagel column		Aquapore column	
Dextran	$\overline{M}w$	$\overline{M}n$	$\overline{M}w$	$\overline{M}n$	$\overline{M}w$	$\overline{M}n$
T-500	5.11×10^5	1.92×10^5	5.31×10^5	2.56×10^5	5.03×10^5	2.18×10^5
T-80	7.20×10^4	4.05×10^5	—	—	7.83×10^4	3.70×10^4
T-40	3.95×10^4	2.40×10^4	4.41×10^4	2.45×10^4	—	—
T-10	9.40×10^{3a}	5.50×10^{3a}	8.10×10^3	3.13×10^3	9.14×10^3	3.98×10^3
	1.00×10^{4b}	—	1.06×10^{4c}	4.42×10^{3c}		
T-2000	2×10^6		9.39×10^5	1.94×10^5		

[a] By gel filtration chromatography.
[b] By light scattering.
[c] Calculated from dashed calibration curve of Figure 2.

The molecular weights of Dextran standards calculated from the calibration curves shown in Figure 2 are given in Table II. Unless specified, the molecular weights were calculated from the solid calibration curve. Except for T-2000 the agreement among the manufacturer's data and the experimental values is good. The supplied number-average molecular weight of T-10 is higher than our experimental value. This could be due to a difference in the end point for low-molecular-weight integration. The discrepancy in the molecular weights of T-2000 from the manufacturer's and the experimental weights will be discussed later.

The study using μStyragel columns is still in progress. The linear calibration (however over a narrow range) seen in Figure 2 is very encouraging.

Exclusion Limit

To find the exclusion limit of an SEC column, a high molecular weight polymer was needed. Amioca is a starch with a molecular weight determined to be approximately ten million.[5] Figures 3 and 4 show the elution curves of amioca with the μBondagel and Aquapore column sets, respectively. The high molecular-weight end of the distribution represents partially excluded polymers. Although the elution curves were not very reproducible among sample solutions prepared on separate occasions, the reproducibility of repeat injections of the same solution was good. Unlike most starch samples, amioca is much more difficult to dissolve in DMSO even at high temperatures. Some samples might not have been true solutions when they were injected, and this could have caused the reproducibility problem. Over a short time period the sample solution did not change significantly; thus no appreciable difference was detected between consecutive injections. However, for different sample solutions at different stages of solvation, different elution profiles were observed.

The peak molecular weights estimated from the calibration curve are 17 million and 60 million for the μBondagel and Aquapore columns, respectively. Conservatively, the exclusion limits for the μBondagel and Aquapore columns are estimated to be about 10 million. Figure 4 shows a broad peak near 31

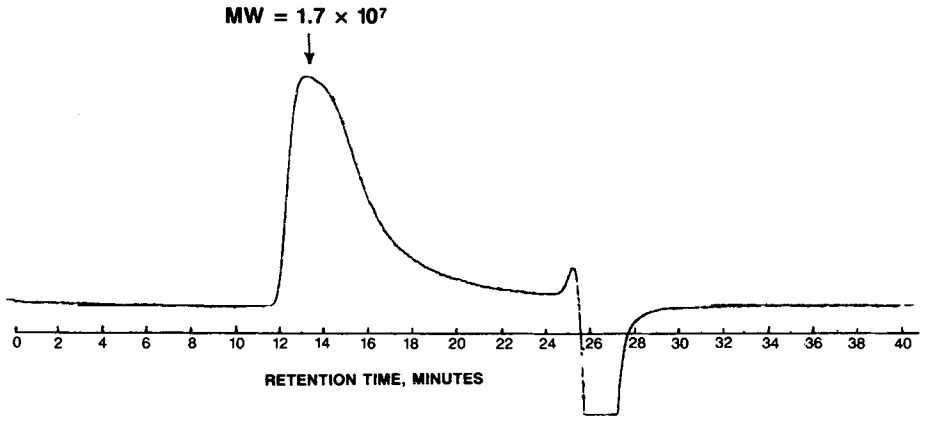

Fig. 3. Elution curve of amioca using the μBondagel-column set.

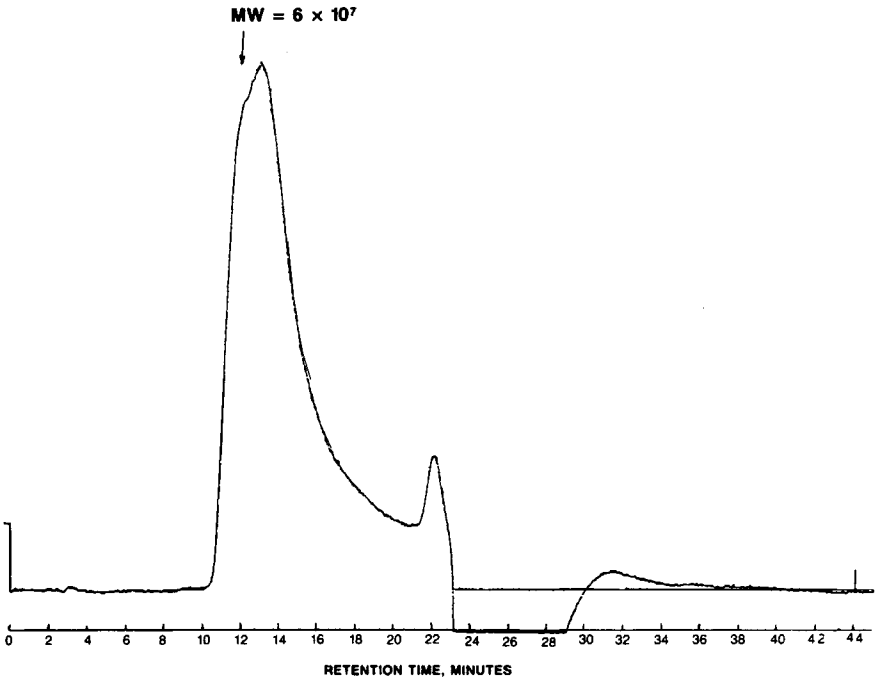

Fig. 4. Elution curve of amioca using the Aquapore-column set.

minutes, which could be the impurities in the sample that interacted with the Aquapore packing.

Dextran T-2000 is known to be a branched polymer having a wide molecular weight distribution. Compared to the linear polymers of the same weight, branched polymers are smaller in molecular size. Because SEC separates polymers according to their molecular size, the molecular weights determined by SEC are always smaller than the actual values (from nonuniversal calibration curve). Because of this, the SEC system can only determine the relative molecular size of starch. With the addition of a light scattering detector or viscometer detector, the absolute molecular weights of starch could be determined. However, relative molecular weight distribution comparisons among samples are desirable from a quality control point of view.

Starch Analysis

Figures 5 to 8 are the elution curves of several starch samples. All samples elute within the exclusion limit. Many starch samples gave multiple peaks, as seen in Figures 6 and 7. Similar elution profiles for degraded starches were also observed by other investigators.[15,16] Because this kind of elution pattern occurs most often with medium and low molecular weight samples, most likely it is not caused by the viscosity effect of the sample solution.[17] However, it may arise from the branch structure of the starch molecules. Different branches have different branch lengths, which on degradation elute at different retention times.

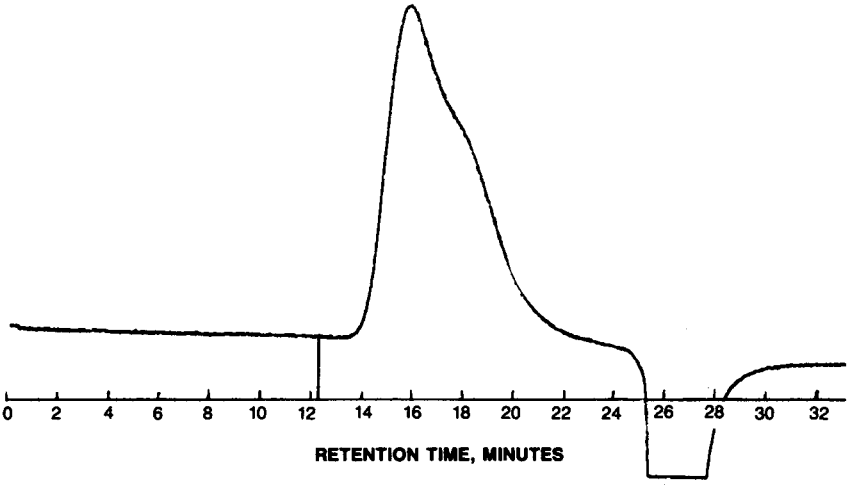

Fig. 5. SEC analysis of acid-converted potato starch using the μBondagel column set.

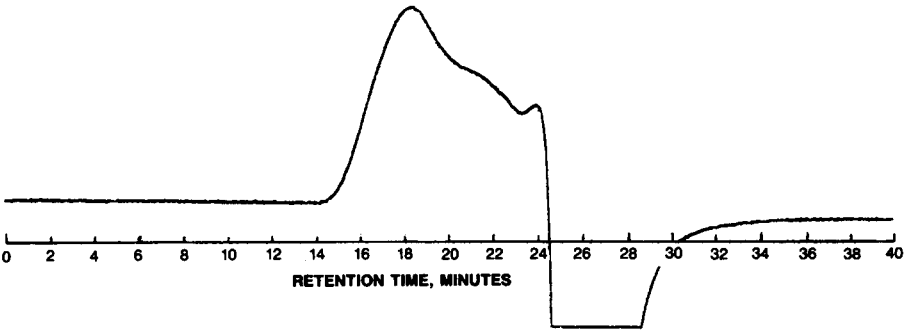

Fig. 6. SEC analysis of enzyme-converted potato starch using the μBondagel column set.

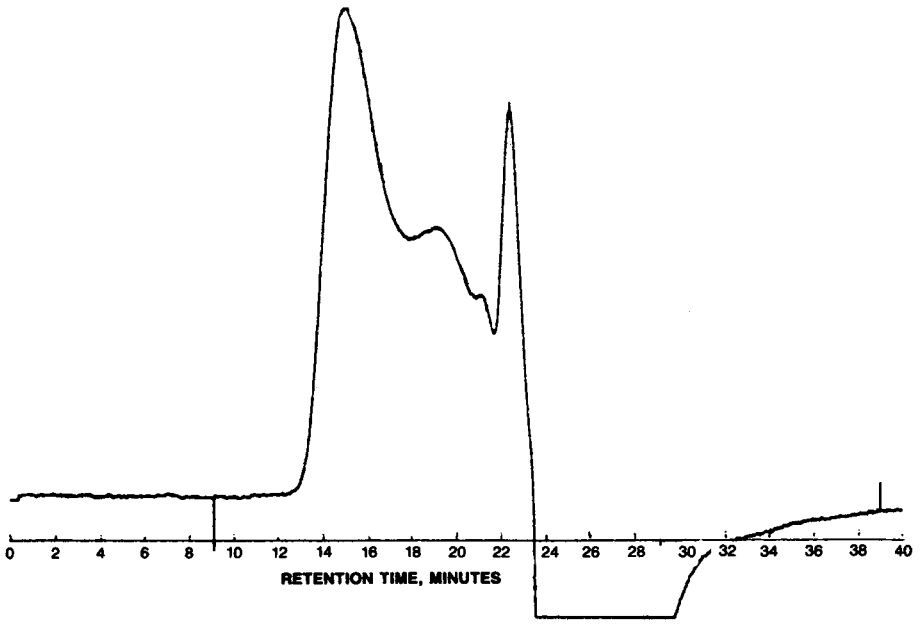

Fig. 7. SEC analysis of potato Dextrin using the Aquapore-column set.

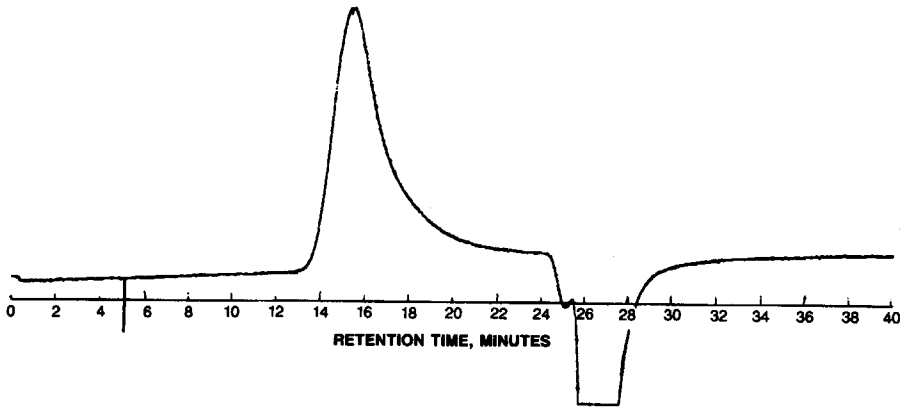

Fig. 8. SEC analysis of acid converted amioca using the μBondagel-column set.

Most starches contain some fat, which can be removed by solvent extraction. Table III shows the results of corn starch before and after fat extraction. The presence of fat does not affect the molecular weight determination, which is an indication that fat does not associate with the starch molecules in the sample solution. The number-average and the weight-average molecular weights given in Table III are only used to compare the relative molecular weights of the same types of starch. For example, in the comparison of the molecular weights of two acid converted corn starches under different conditions, the results will be valid. But in the comparison of acid converted corn starch and acid converted potato starch, the results could be misleading.

Polysaccharides with anionic groups can also be run under these experimental conditions. Figure 9 is the elution curve of a starch sample containing carboxylate groups.

Recently, two articles on the SEC of starches with similar conditions to this study were reported. Kabayashi et al.[16] used a μBondagel-DMSO system to determine the ratio of amylose and amylopectin in starch. However, this system may not be suitable for molecular weight determinations because of

TABLE III
SEC Analysis of Acid-Converted Corn (μBondagel-Column Set)

Sample	$\overline{M}w$	$\overline{M}n$	Dispersity
Acid-Converted Corn			
1	1.12×10^6	1.16×10^5	9.65
2	1.20×10^6	1.50×10^5	8.00
3	1.16×10^6	1.48×10^5	7.84
4	1.15×10^6	1.26×10^5	9.13
Acid-Converted Corn, Fat Extracted[a]			
1′	1.16×10^6	1.21×10^5	9.59
2′	1.21×10^6	1.26×10^5	9.60
3′	1.16×10^6	1.38×10^5	8.41
4′	1.17×10^6	1.27×10^5	9.21

[a] Method B-18, Standard Analytical Methods of the Member Companies of the Corn Industries Research Foundation, Inc., 6th Edition.

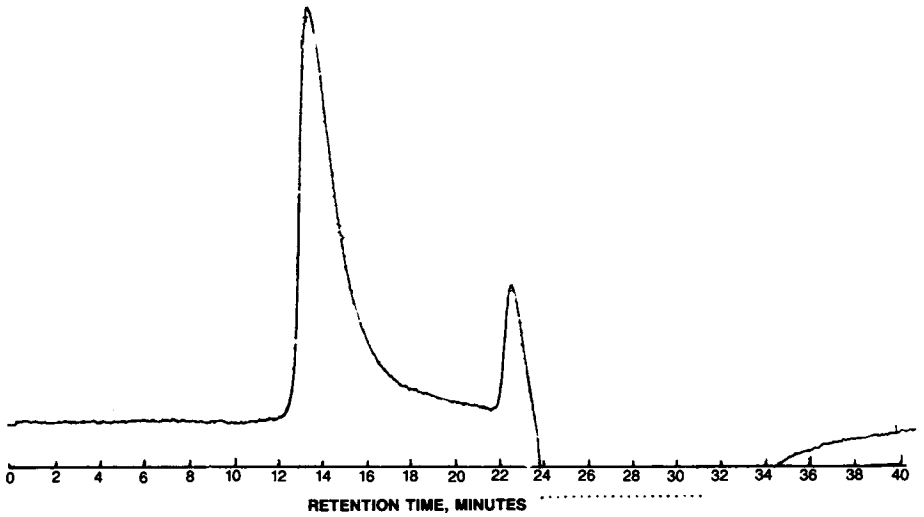

Fig. 9. SEC analysis of modified-starch containing carboxylate groups using the Aquapore-column set.

the bimodal elution curve of Dextran standards that were observed. Another SEC system was reported by Salamis and Rinaudo.[18] Merck diol (same functionality as Aquapore) columns with porosities of 10,000 Å and 1000 Å were used as packings. The mobile phase was DMSO in the presence of 15% (V/V) methanol and 0.5 *M* ammonium acetate run at 60°C. These operating conditions were necessary to reduce the sample adsorption by the column packing material. The adsorption of Dextran by the diol column was slight, but starch adsorption increased dramatically depending on the type of starch, particularly the amylopectin content. However, with our operating parameters, we did not observe any significant differences in the elution behavior of the Dextran or starch samples.

The three types of packing tested in this study can also be used for SEC with an organic mobile phase. In organic-phase SEC, the exclusion limit of μStyragel can be up to 40 million (polystyrene) which is higher than the eight million obtained with μBondagel and Aquapore columns. Helm and Young[15] recently utilized an organic-phase SEC system (polystyrene-divinyl benzene packing, THF as mobile phase) to characterize starches after the conversion of starches to corresponding tricarbanilate derivatives. The chemical modification is probably unnecessary except to increase detection limit when using a UV detector. In the past year, we have been using μStyragel columns and DMSO-0.3 *M* sodium nitrate mobile phase to analyze starch samples. Satisfactory results were obtained and will be reported in the future.

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