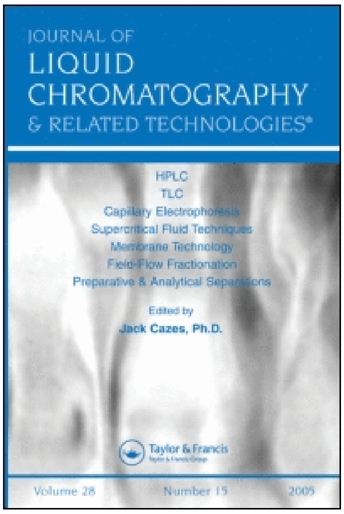

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Gel Permeation Chromatography of Polymers from Wood

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GEL PERMEATION CHROMATOGRAPHY OF POLYMERS FROM WOOD

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ABSTRACT

Polymers from wood, including cellulose via the methylol derivative, are soluble in dimethyl sulfoxide (DMSO). To perform gel permeation chromatography of these high polymers, a packing material was sought which combined adequate pore size with stability in DMSO. Contrary to recommended practice, it was discovered that gel permeation chromatography in DMSO can be performed on prepacked, high-performance columns of μ-Styragel without adverse effects from bead swelling. When using the methylol derivative of cellulose, it is necessary to freeze dry the reaction mixture to obtain a product which gives reproducible results from gel permeation chromatography.

INTRODUCTION

Dimethyl sulfoxide (DMSO) is a good solvent for many polymers including hemicellulose and lignin isolated from wood. However, its use in gel permeation chromatography (GPC) has been limited because of its high viscosity and because of unfavorable interaction with some of the commercially available packing materials such as Styragel (1), Sepharose CL ^{R} (2), etc. It was recently discovered (3) that cellulose could be dissolved rapidly and conveniently in DMSO by addition of formaldehyde or paraformaldehyde (PF) to form a methylol cellulose derivative. This discovery inspired the search for GPC column packing material with which DMSO and formaldehyde would be compatible.

The use of silica for semi-preparative chromatography of methylol cellulose in DMSO has been reported (4). It was our experience (5) that qualitative and preparative chromatography could be performed with Controlled Pore Glass ^{R}, but resolution in the high molecular weight range was inadequate for molecular weight distribution determinations of cellulose. Better resolution was obtained with μ-Bondagel ^{R} which is a bonded silica packing. However, column performance deteriorated rapidly, and higher molecular weight celluloses eluted at or near the void volume. Column deterioration is apparently associated with the products of the cellulose methylolation reaction because subsequent work by the author with dextrans and by other researchers with lignins (6) has shown that dimethyl sulfoxide can be used successfully with μ-Bondagel. The crosslinked polystyrenes (e.g. Styragels) offer the advantages of adequate resolution in the high molecular weight range as well as minimal adsorptive or reverse phase partitioning effects. It was, therefore, considered worthwhile to attempt the use of DMSO with such packing material even though swelling problems could be anticipated.

RESULTS AND DISCUSSION

It was demonstrated on a single Styragel column that the commonly measured parameters for column efficiency using toluene changed markedly as the solvent was changed from tetrahydrofuran (THF) to DMSO (Table 1) and the effective pore volume increased by 50 percent. However, this effect was reversible on returning to THF as solvent. No permanent damage was done by using DMSO. Part of the peak asymmetry is probably due to the viscosity of DMSO and part to solvent/solute/packing interactions caused by the change in dipole moments.

TABLE 1

Performance of 10^5 Styragel 2 by 2 Column Set as Measured with Toluene

Solvent	Peak retention volume	Efficiency	$\underline{a}/A_s^2$
	Ml	Plates/ft	
THF	50.56	1,000	1.00
50 percent DMSO in THF	53.12	700	1.72
DMSO	72.96 73.3	180 170	13.44 19.14
50 percent DMSO in THF	52.8	868	1.78
THF	50.4	917	1.0-1.4

$\underline{a}/A_s^2$ is a measure of peak asymmetry defined as the square of the quotient of the leading side divided by the tailing side measured at 4.4 percent peak height.

Three different four column μ-Styragel sets were investigated: a survey set of 10^3, 10^4, 10^5, and 10^6 Å (I) and two others 10^4, 10^5, 10^6, 10^6 (II), and 10^5, 10^6, 10^6, 10^6 (III). For general purpose work with wood pulps, set II was the most suitable. The data reported in this paper was obtained with this set.

The utility of the procedure for studies of polymers from wood is illustrated with a chromatogram of a low DP oxygen pulp containing UV absorbing residual lignin (Fig. 1). Cellulose and the hemicelluloses give positive peaks in the refractive index detector, while oxymethylenes, water, and excess formaldehyde all give negative peaks in the low molecular weight range.

Because of the effects observed with toluene on Styragel with DMSO, the gel permeation chromatographic performance of μ-Styragel columns was monitored with dextran standards in DMSO without formaldehyde.

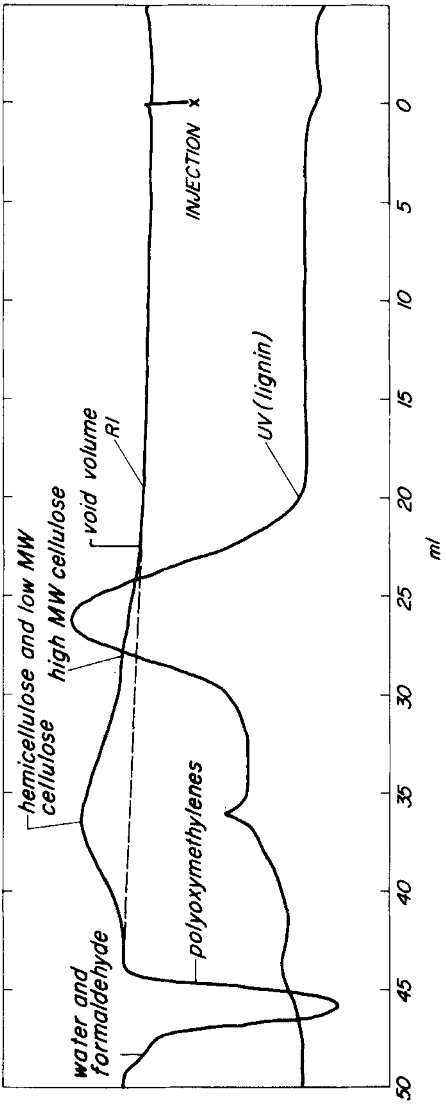

FIGURE 1. GPC trace of the methylol reaction mixture of a degraded oxygen pulp.

A linear calibration curve generated with these standards was closely reproduced after several months of operation. The dextran samples contain a narrow range of molecular weights which broaden the peaks. A low molecular weight impurity in the 10,000 MW dextran and the traces of water generally present in injections of DMSO solutions gave narrow, symmetrical peaks with the differential refractive index detector. The efficiency of the system was calculated to be 5,000 theoretical plates per 30 cm column on the basis of these peaks.

As expected, the dextran calibration curve could not be used directly for methylol cellulose because the dextrans are branched and compact in solution, whereas methylol cellulose is straight chain and has been shown to be extended in DMSO solution (7). The intrinsic viscosities for the dextran standards were determined in DMSO at 30° C so that the universal calibration procedure (8) could be applied. Results are given in Table 2 and the universal calibration curve in Figure 2. The molecular weights of the narrow range dextrans are lower than that applicable to cellulose, so a linear extrapolation is necessary.

The DMSO/PF solvent system, when used to determine molecular weight distributions of wood pulps or other cellulosic materials, has several advantages: the solvent is non-degrading; the methylol derivative does not greatly increase the molecular weight; and the dissolution reaction is rapid and simple. Ideally, the clear derivatization reaction mixture obtained after 4-5 minutes would be injected directly into the chromatograph and the entire analysis would be completed in about an hour.

Gel permeation chromatography of the methylol cellulose reaction mixture--which contains polyoxymethylenes, formaldehyde, traces of water, and other side products--demonstrated that methylol cellulose could be clearly separated from the other reaction products (Fig. 1).

TABLE 2

Molecular Parameters of Dextran Standards

Dextran	$\bar{M}_w$	$\bar{M}_n$	$[\eta]^{H_2O}_{20°}$	Peak M	$[\eta]^{DMSO}_{30°}$ [a]
T 10	9,400	5,500	0.096	8,000	0.140
T 40	39,500	29,500	.21	24,000	.316
T 70	68,500	40,300	.26	35,000	.341
T 500	462,000	168,000	.51	150,000	.748
T 2000	[a] 833,000	---	[a] .64	---	.893

[a] Values from this work. All other values from Pharmacia Fine Chemicals.

However, the location and shape of the cellulose peak was not reproducible from sample to sample and the data on different samples did not correlate reproducibly with independent molecular weight determinations of ICCA pulps (7) by viscosity or light scattering. Also, in some high molecular weight samples, gel formation is indicated as a "prehump" in the GPC curve.

A recent report (9) provides light scattering and viscometric data which indicate that the apparent molecular weight of methylol cellulose changes with time in DMSO/PF solutions. We have also observed that the viscosity of methylol cellulose in the reaction solution was markedly dependent on the conditions of derivatization. These data imply weak crosslinking by oxymethylene chains or other association phenomena. In an experiment without cellulose, it was demonstrated by NMR that polyoxymethylenes could be eliminated by purging the formaldehyde from the reaction solution with dry nitrogen. This technique was not effective in the presence of cellulose.

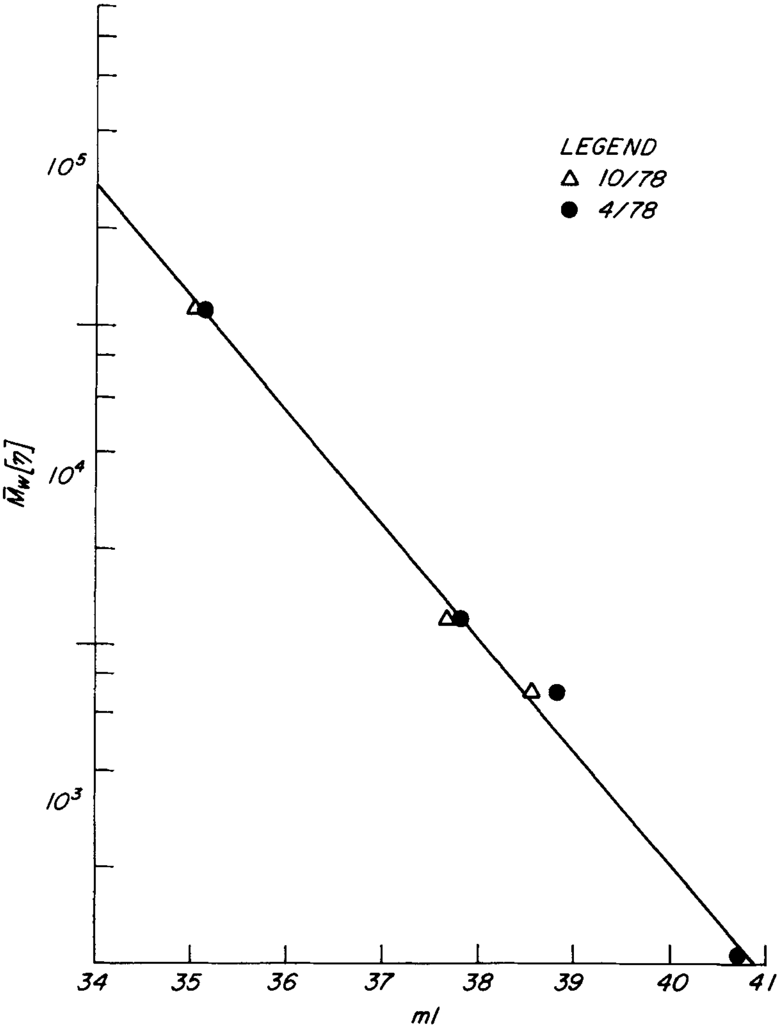

FIGURE 2
Universal calibration curve of dextrans in DMSO on 10^6, 10^6, 10^5, 10^4 Å μ-Styragel.

Freeze drying of the methylol cellulose reaction mixture is reported (10) to be an effective way to obtain a uniformly substituted product with a methylol substitution of 1 per glucose monomer. Freeze drying of DMSO is a slow process (3-5 days) and must be done carefully with external cooling to about 0° C, otherwise all of the methylol groups will be removed and the product will no longer be soluble in DMSO. This additional processing eliminates much of the advantage of the rapid solubilization of cellulose in DMSO/PF. However the technique does show promise for molecular weight distribution of DMSO soluble polymers such as dextrans, hemicelluloses, and lignin--and for cellulose providing freeze drying is used.

EXPERIMENTAL

Gel permeation chromatography was performed on four column sets of μ-Styragel as listed in the text. With a flow rate of 0.5 ml dimethyl sulfoxide per minute, the back pressure developed was about 600 psi. The flow could be increased to 1.0 ml/min with a pressure of less than 1000 psi, but a slight loss of resolution was observed. Cellulose concentrations of 0.2-0.5 percent were used with injections of 100-200 μl. A Waters model 300 chromatograph modified for micro colums and equipped with a U6K injector was used.

Dimethyl sulfoxide (99.9 pct) was purchased from Burdick and Jackson, or was recovered as the center cut of vacuum distillation after drying over calcium hydride. Paraformaldehyde which decomposed completely at less than 135° C was purchased from Tridom Chemical Inc. Dextran standards were purchased from Pharmacia. Methylol cellulose was prepared from wood pulps or cotton by the procedure of Nicholson and Johnson (11).

The dry, fluffed pulp, 50 mg, was placed in a 30 ml weighing bottle with outside ground glass cover. DMSO, 10 ml, was added and a uniform suspension was obtained with vigorous magnetic stirring. The mixture was placed in a 130°C oil bath and paraformaldehyde, 300 mg, was added with continuous stirring. Within 4 minutes, the paraformaldehyde had decomposed and a clear solution was obtained. Although the chromatogram shown in Figure 1 was obtained by direct analysis of a solution prepared as described here, more reproducible results are obtained by first freeze-drying this solution and analyzing a dilute solution of the freeze-dried product.

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Mention of brand names is for information only and does not imply endorsement by the USDA.

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