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NO. 251 GPC ANALYSIS OF CELLULOSE

Attached is documentation of a presentation given at the 1986 Pittsburgh Conference held in Atlantic City from March 10 to 14, 1986. The published abstract is included below. Further work is in progress to build on the initial feasibility data described in this paper.

251

NO. 251 GPC ANALYSIS OF CELLULOSE

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Cellulose is the most abundant organic material in the world and is used in the manufacture of paper, cotton products, packaging, insulation, etc. In addition, there are many chemical modifications of cellulose, including its esters (cellulose acetate), ethers (methylcellulose), the nitrated product (nitrocellulose), as well as rayon and cellophane (from cellulose xanthate), all of which are used in a wide variety of products. The use of a renewable natural resource such as cellulose will become even more important in the future as the currently available petroleum supplies are eventually depleted.

Molecular weight distributions (MWD) of cellulose materials are needed in order to make optimum use of this polymer. Since underivatized cellulose could not be dissolved in solvents compatible with gel permeation chromatographic (GPC) systems, GPC analysis of cellulose has been reported to date, with varying success, only on derivatized materials, e.g. acetates, nitrates, carbanilates (via reaction with phenyl isocyanate) and methylol derivatives (via reaction with paraformaldehyde).

In this paper we report a method for the GPC analysis of underivatized cellulose. In order to dissolve underivatized cellulose in a chromatographically acceptable mobile phase, the conditions of dissolution are critical. The sample is first "activated" by water during a process that opens the cellulose internal structure to enhance dissolution. The water is subsequently replaced with a solution of LiCl in N,N-dimethyl acetamide (DMAC) to afford a solution of underivatized cellulose suitable for GPC analysis with DMAC/LiCl as mobile phase. The operational parameters that have been evaluated and will be described include sample preparation procedures, effect of different concentrations of LiCl in the mobile phase, and effect of column temperature.

Any questions regarding this paper should be directed to the author listed at the bottom of this page. Slides are available through Marketing Communications in Milford (X2303) who will order them from the photographer, Kevin Monaghan (617/528-8579).

Slides in Total Package

Slide #	WA#
1- 5	9346-50*
6	5975
7	9351*
8 - 9	9495, 9494
10-18	9352-60*

* Consecutive set of 15 slides, WA#9346-60.

Slide #1 (WA#9346)

Cellulose is the most abundant, renewable, organic raw material in the world. It is renewable because it is obtained from plants and trees and should be readily available as long as we properly manage our use of these resources. Cellulose is not a single, specific molecule but rather a polymeric carbohyrate (polysaccharide) consisting of anhydroglucose units joined by an oxygen linkage to form long molecular chains that are essentially linear and have a repeating unit with molecular weight of 162. Cellulose is used in the manufacture of paper, cotton products, packaging, etc. In addition, derivatized cellulosics (acetates, butyrates, propionates, nitrates) are used in a wide range of applications from consumer goods to explosives. The ability to determine the molecular weight distributions of cellulose is important to assure intelligent use of this material.

Slide #2 (WA#9347)

There has been no report to date of the GPC analysis of underivatized cellulose using the high performance, high efficiency GPC columns that are commercially available today. The molecular weight distribution of cellulose has been determined rather by GPC analysis of derivatized cellulose. The official method of the Technical Association of the Pulp and Paper Industry (TAPPI) requires nitration of cellulose to form a trinitrated derivative which is soluble in tetrahydrofuran (THF) at room temperature and can be analyzed using high performance GPC systems with THF as the mobile phase. Alternatively, reaction of cellulose with phenylisocyanate in pyridine at 80°C generates a tricarbanilide derivative that is soluble in THF. Reaction of cellulose with excess paraformaldehyde in dimethyl sulfoxide (DMSO) at 130°C followed by freeze drying of the reaction mixture affords a uniformly substituted product with a single methylol substitution per glucose unit. This methylol derivative is soluble in DMSO and can be analyzed on polymer-based GPC columns using DMSO as the mobile phase. These derivatization procedures all suffer from the fact that they are inconvenient and time-consuming, requiring from 24 hours to several days (as in the case of freeze drying of methylol derivatives).

Slide #3 (WA#9348)

GPC analysis of underivatized cellulose has been reported to date by dissolving the sample in exotic metal chelate complexing agents such as cadoxen (containing 5-7% CdO in 28% aqueous ethylenediamine) and using the complexing agent as the mobile phase. More recently, it was reported that cellulose does not precipitate when the solution is injected into a GPC system using 0.5N NaOH as mobile phase and it can be separated on low pressure, moderate efficiency columns packed with materials such as Pharmacia's Sepharose® CL (cross-linked agarose gel), Bio-Rad's Bio-Gel® P (polyacrylamide bead), or E. Merck's Fractogel® TSK (hydrophilic vinyl polymer). These columns must be packed by the operator since the materials are available only in bulk form. The low pressure limitations require slow flow rates and long analysis times. A recent report (1984) describes the use of a total of two feet of columns packed with Fractogel® TSK material (~1300 p/ft) in a 90 minute analysis.

Slide #4 (WA#9349)

We will describe here a new method for the GPC analysis of underivatized cellulose using commercially available high performance GPC columns and instrumentation. The cellulose is first activated by swelling in water followed by replacement (exchange) of the water with dry dimethyl acetamide (DMAC). This serves to swell the fibers and activate the pores so that the cellulose can subsequently be dissolved in DMAC containing 6% LiCl. The solution is then diluted by a factor of 12 with DMAC to generate a solution in DMAC/0.5% LiCl, filtered through a 0.5μ Millex® SR disposable filter unit, and injected onto a set of Ultrastyragel® GPC columns using DMAC/0.5% LiCl as the mobile phase. The columns are heated to 80° to reduce the viscosity of the mobile phase and increase column life. Using three columns (30 cm each) at 1 ml/min flow rate generates molecular weight distributions in 40 minutes with efficiencies of 7700 p/ft. Shorter analysis times can easily be obtained by using fewer columns.

Slide #5 (WA#9350)

The instrumentation used for this work consisted of an M6000A Solvent Delivery System with the high sensitivity noise filter, U6K Injector, Column Temperature Control Module, and RI Detector. The experimental parameters that were evaluated during the methods development work included the injection volume, sample concentration, column temperature, and mobile phase composition.

Slide #6 (WA#5975)

The methods development work was done using a solution of cellulose (8% cellulose, 6% LiCl, 86% DMAC) that had been prepared in Nov., 1980 and was supplied by my coauthor Prof. A. Turbak at Georgia Tech. This cellulose (sulfate pulp) solution was reported to have had an initial dp (degree of polymerization) of ~550 and the dp had dropped only to ~500 on standing for five years. The initial chromatographic methods development work was then done on a single 10^4Å Ultrastyragel® column selected because dp ~500 is equivalent to 81,000 molecular weight which is in the middle of the separation range for this column.

Slide #7 (WA#9351)

The test solution was diluted with DMAC/2% LiCl so that the cellulose concentration was 0.24%. A mobile phase of DMAC/2% LiCl was used in the hope that 6% LiCl would not be required to keep the sample in solution. Hopefully, an even lower amount of LiCl (e.g. 0.5%) would be satisfactory for analysis. An R401 Refractive Index Detector (4X sensitivity) was used to monitor the separation. Injection of 100µl of filtered cellulose solution into the GPC system afforded this chromatogram. A polymer distribution eluted between 6 and 10 minutes, followed by solvent related peaks beyond 10 minutes. There is an unexpected early peak eluting at 5 minutes. The RI response due to cellulose in this mobile phase is rather low and thus results in a chromatogram with significant baseline noise.

Slide #8 (WA#9495)

This is a photograph of Waters™ new high performance Refractive Index Detector (Model 410) that should help with this cellulose analysis since the older R401 does not have enough sensitivity. The temperature controlled optics bench is on the left side of the detector and the electronics with key pad control are on the right side.

Slide #9 (WA#9494)

When the cover is removed from the Model 410 Refractometer, one can get a closer look at the optics compartment which can be temperature controlled from 30-50°C to maximize baseline stability. The optics bench is actually set within the large aluminum tube in a manner similar to the refractometer in a 150C High Temperature GPC System.

Slide #10 (WA#9352)

Substitution of the M410 Refractive Index Detector in place of the R401 Detector (Slide #7) eliminated the baseline noise and improved the chromatogram. The M410 data was obtained using a sensitivity of 32 and scale factor of 5 and resulted in a cellulose peak ~50% greater than at 4X on the R401. The M410 Refractometer was clearly the detector of choice for this analysis and it was used throughout the rest of this work.

Slide #11 (WA#9353)

A series of four injections were made of the test cellulose solution to evaluate the effect of sample concentration on elution volume. The detector response was changed to offset a decrease in sample mass and afford similar chromatographic responses in all four runs. At both injection volumes, decreasing the sample concentration from 0.24% to 0.12% reduced elution time of the apex of the cellulose MWD by ~1.5%, presumably due to reduction or elimination of concentration/viscosity effects. An error of 1.5% in elution time (volume) is equivalent to a 15% error in molecular weight due to the logarithmic nature of a GPC calibration curve and was to be avoided. It was decided to use sample concentrations $\leq$ 0.12% (w/v) and injection volumes of 100µl per column. The M410 detector would then be operated at 32 sensitivity and 10 scale factor to afford a stable baseline. The normal calibration procedure would compensate for the injection volume.

Slide #12 (WA#9354)

In order to evaluate the effect of column temperature as well as % LiCl in the DMAC mobile phase, injections of the cellulose test sample as well as a mixture of two polystyrene standards were performed at each set of conditions. The upper portion of the slide (including the chromatogram) describes the results with 2% LiCl in DMAC as mobile phase. The data at the bottom of the slide (below the baseline of the chromatogram) summarizes the results with 0.5% LiCl in DMAC as mobile phase. The first data was obtained at the 2% LiCl level with columns at 80°C. The vertical arrows designate the elution position of the apex of the cellulose MWD in Chromatogram A as well as the centers of the polystyrene standard peaks (775,000 and 107,000 PS) in Chromatogram B. As the column temperature was reduced to 65°C and finally to 50°C, notice that the cellulose MWD elutes only slightly later as could be attributed to increased coiling of the polymer chains (reduced hydrodynamic volumes) . However, the PS standards shift to significantly longer elution volumes as column temperature is decreased. This implies that the polystyrene standards are exhibiting some adsorption onto the polystyrene gel column and this effect is mimimized with an increase in temperature. This is a recognized phenomenon with solvents such as DMAC and DMF. With a mobile phase of 0.5% LiCl in DMAC, elution of the cellulose MWD is not affected and, again, temperature has little effect on elution. The polystyrene standards, however, elute much earlier with the 0.5% LiCl mobile phase than with 2% LiCl. The effect of column temperature on PS elution is minimal with the 0.5% LiCl mobile phase and can be attributed to temperature effects on hydrodynamic volumes as discussed above. There is probably some reverse-phase adsorption of the PS standards onto the column surface even in the 0.5% LiCl mobile phase. Increasing the LiCl content to 2% serves to make the mobile phase more polar and thus leads to increased retention. Since increased column temperatures had exhibited no detrimental effect on the chromatography and would help to reduce the viscosity of the solvent in the columns, it was decided to operate the columns at 80°C and use a mobile phase of DMAC/0.5% LiCl to minimize adsorption effects with the polystyrene standards that would be used for column calibration.

Slide #13 (WA#9355)

One larger (10^5Å) and one smaller (10^3Å) pore size column were added to the 10^4Å test column to produce a set of three Ultrastyragel® columns that would have a wide range of separation capacity. Calibration with polystyrene standards under conditions developed in the earlier experiments afforded a calibration curve that showed good separation capability up to (and possibly beyond) a 2.88 million molecular weight polystyrene standard.

Slide #14 (WA#9356)

Analysis of the original sulfate pulp test sample using 300µl of 0.1% cellulose generated a chromatogram similar to what had been observed to date. Based on the calibration curve, it was evident that the small peak eluting early in the run was indeed eluting after the exclusion volume and could represent some high molecular weight material that had been generated within the solution during standing for several years. Including this high molecular weight peak in the calculation of molecular weight averages significantly increased the weight-average molecular weight (Mw). Further speculation about the origin of this small peak is not warranted since the original solution had been prepared so long ago and was used only for methods development purposes.

Slide #15 (WA#9357)

Fresh solutions of new samples of cellulose were then prepared according to the general procedure described in Slide #4. Cellulose was prepared at 1.2% (w/v) concentration in the DMAC/6% LiCl needed for dissolution. In this way the sample could be diluted with DMAC to afford a 0.1% cellulose solution in DMAC/0.5% LiCl and one would avoid injection of the 6% LiCl solution onto the columns since the effect of such an injection had not yet been evaluated. However, preparation of such a 1.2% cellulose solution presented difficulties in sample handling. The preliminary procedure described on the slide involves adding 120 mg of sample to 10 ml of water in a 15 ml graduated centrifuge tube and shaking (30 min) to swell and activate the cellulose. The mixture was centrifuged (10 min) and the supernatant water was removed with a disposable pipet. The resulting fibers were further compressed with a glass stirring rod, centrifuged (10 min) and the supernatant liquid was removed. DMAC (10 ml) was added, the sample was fluffed up with a spatula and the sample was shaken (15 min) and treated as before to remove the DMAC. Three more exchanges with dry DMAC were done to remove as much water as possible. DMAC/6% LiCl was added to a volume of 10 ml (total) and shaken overnight to dissolve. The solution was diluted with DMAC as indicated, filtered and injected into the GPC system. More work needs to be done to streamline the sample preparation procedure before this can be considered a satisfactory method for analysis of cellulose. More comments on last slide (#18).

Slide #16 (WA#9358)

Using the sample preparation procedure just described, GPC analysis was performed on a fresh sample of softwood sulfite pulp. Molecular weight averages are based on polystyrene standards and, as expected, Mn is most affected by inclusion of different amounts of low molecular weight components in the calculations.

Slide #17 (WA#9359)

Another sample of sulfite pulp was prepared and analyzed by GPC. The MWD of this sulfite pulp (B) can be compared with that of the previous slide. Sulfite pulp B elutes somewhat later and affords lower molecular weight averages.

Slide #18 (WA# 9360)

The basic elements of this cellulose analysis are summarized again. Shorter analysis times are possible by using fewer Ultrastyragel® columns while still generating good separations because of the high efficiencies that these columns provide. The key to this method is the sample preparation procedure used to dissolve the cellulose. Using a more dilute solution in the dissolution step and eliminating the subsequent dilution must be evaluated. Mechanical agitation of mixtures should also help. If necessary, alternate activation procedures (e.g., heating in DMAC) could also be evaluated to build on the initial feasibility data described in this paper and thus develop a practical and convenient GPC analysis for underivatized cellulose. Further work is in progress.

GPC Analysis
of
Cellulose

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9346

Slide #1

GPC Analysis of Derivatized Cellulose

- Nitrate (THF)
- Carbanilate (THF)
- Methylol (DMSO)

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9347

GPC Analysis of Underivatized Cellulose Reported to Date

- Sample - soluble in metal chelate complexes dissolved in aqueous alkaline solutions
- Columns - Sepharose® CL, Bio-Gel® P, Fractogel® TSK
- Mobile Phase - sample dissolution solvent or 0.5N NaOH
- Analysis Time - slow (minimum 90 min.)

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9348

GPC Analysis of Underivatized Cellulose
New Method

- Sample - dissolve in DMAC (6% LiCl) via H_2O and DMAC
- Columns - high efficiency Ultrasytragel® (80°C)
- Mobile Phase - DMAC (0.5% LiCl)
- Analysis Time - 40 min (3 X 30 cm columns @ 7700 p/ft)

Waters
9349

Experimental

Instrumentation: M6000A SDS, U6K injector, column heater, RI detector (401, 410)

Parameters: injection volume (100µl, 50µl per column)
sample concentration (0.24%, 0.12%)
column temperature (80°, 65°, 50°C)
% LiCl in DMAC mobile phase (2%, 0.5%)

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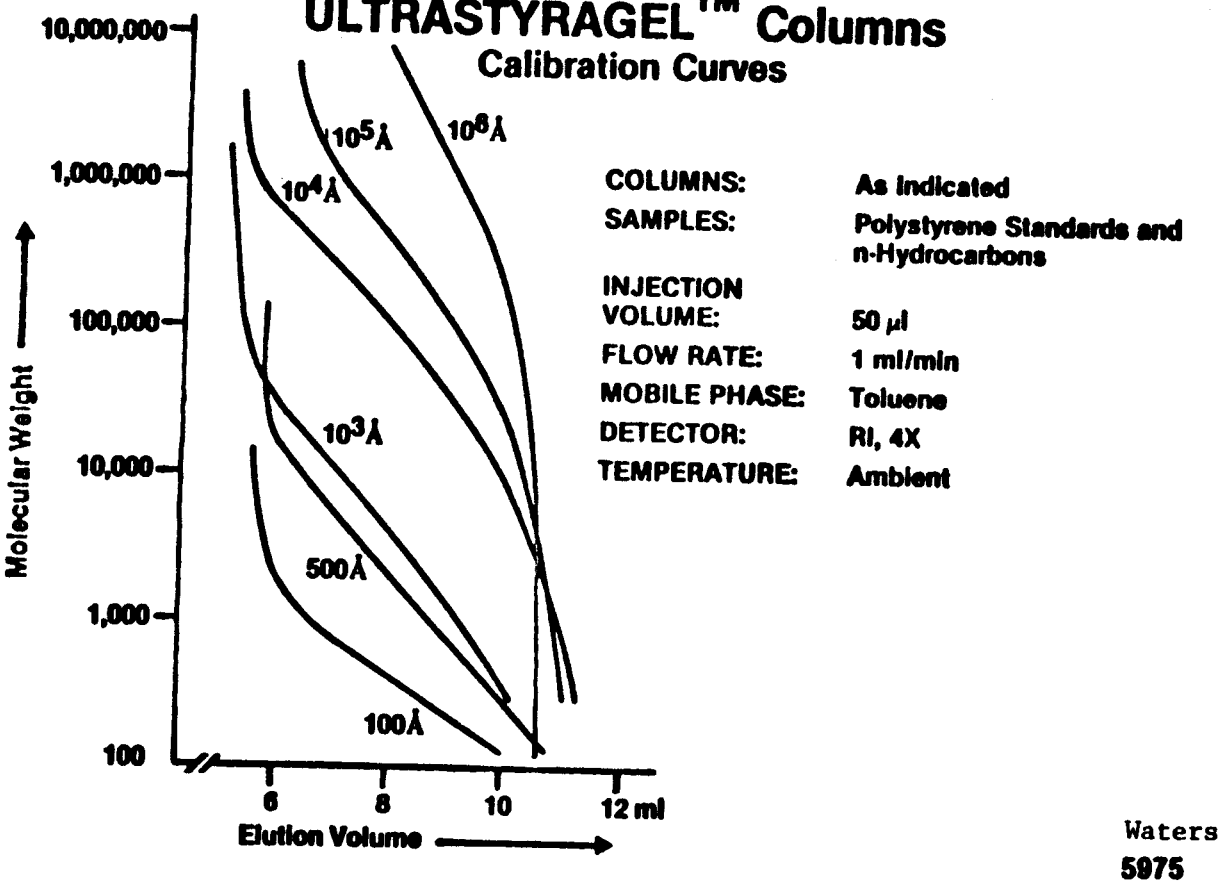
9350


ULTRASTYRAGEL™ Columns
Calibration Curves
10,000,000
1,000,000
100,000
10,000
1,000
100
Molecular Weight
10^5Å
10^6Å
10^4Å
10^3Å
500Å
100Å
6
8
10
12 ml
Elution Volume
COLUMNS: As Indicated
SAMPLES: Polystyrene Standards and n-Hydrocarbons
INJECTION VOLUME: 50 µl
FLOW RATE: 1 ml/min
MOBILE PHASE: Toluene
DETECTOR: RI, 4X
TEMPERATURE: Ambient
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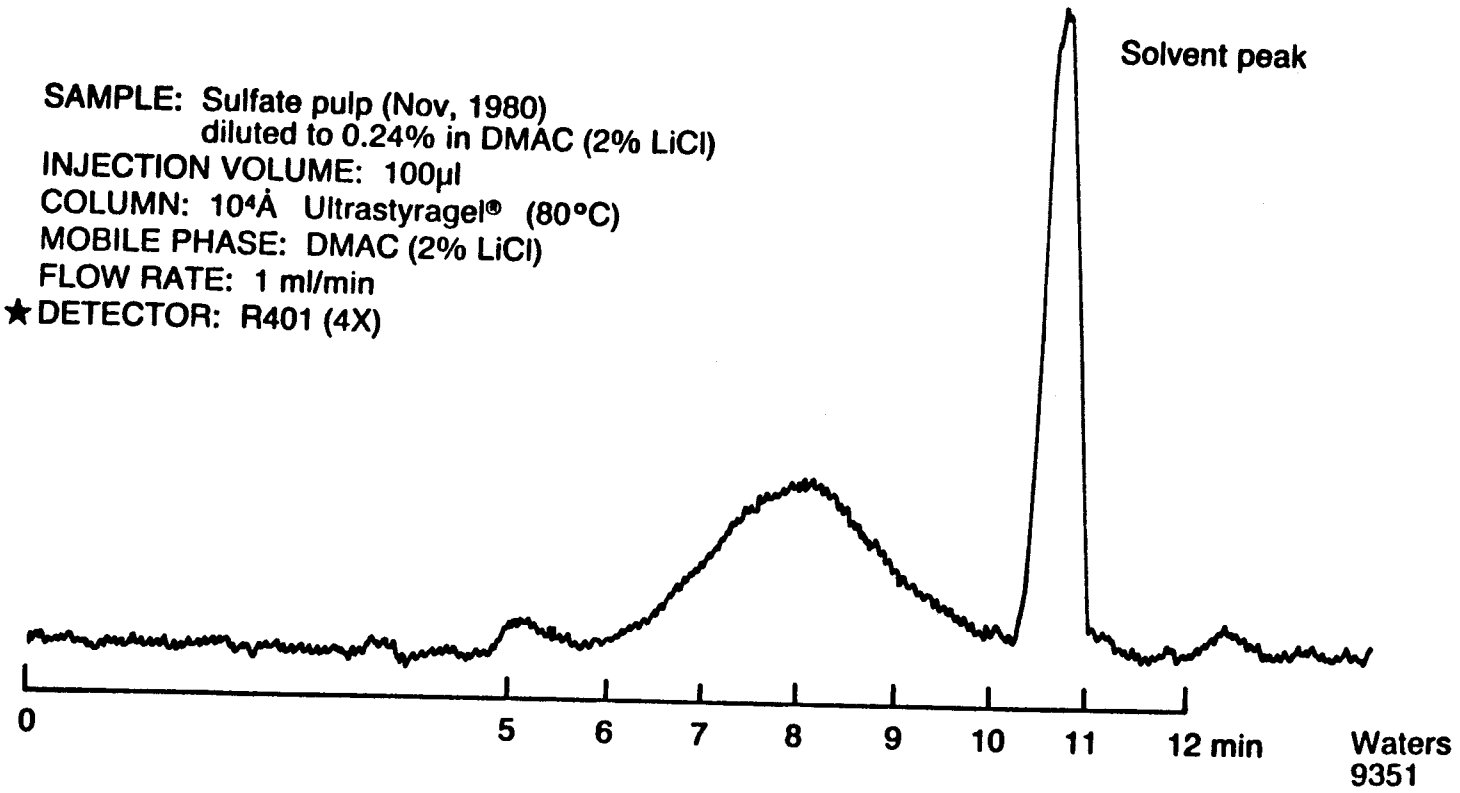
5975

Cellulose Methods Development
R401 Detector



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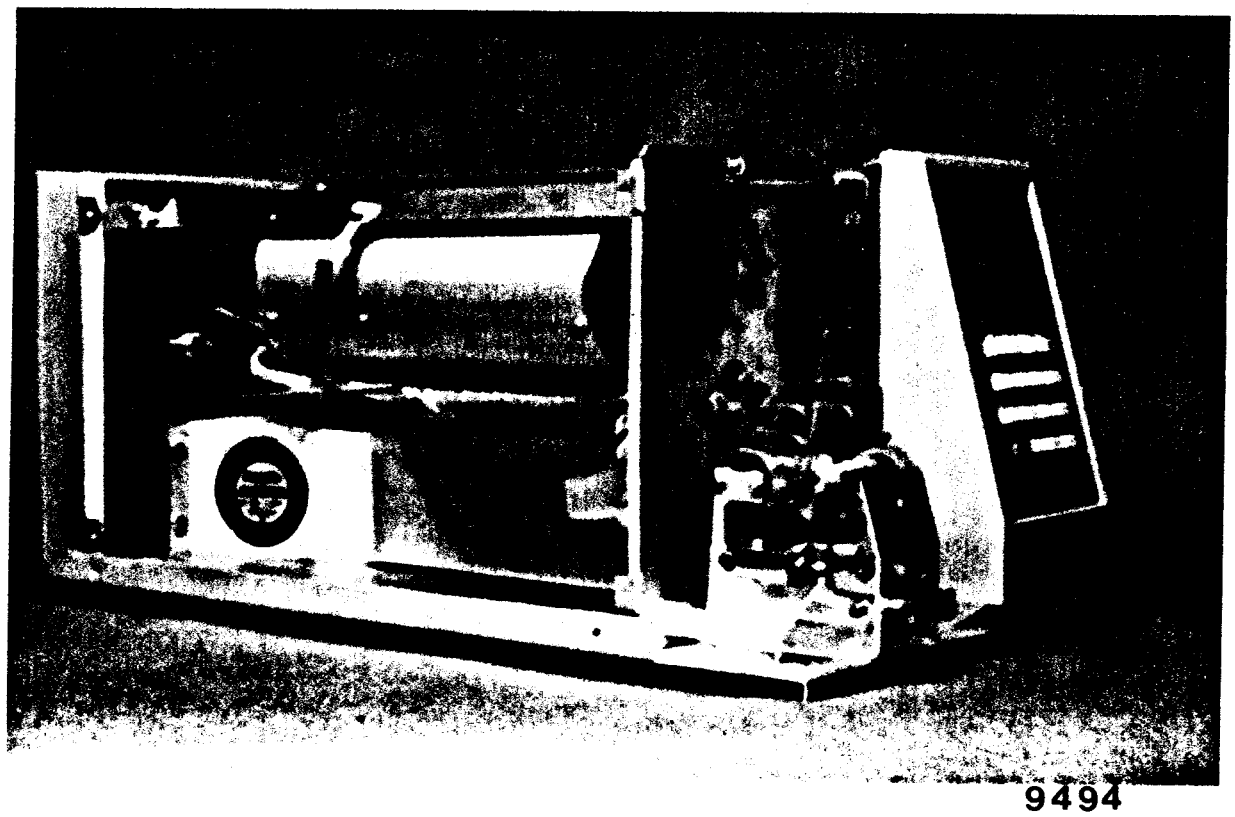
9495

Slide #8



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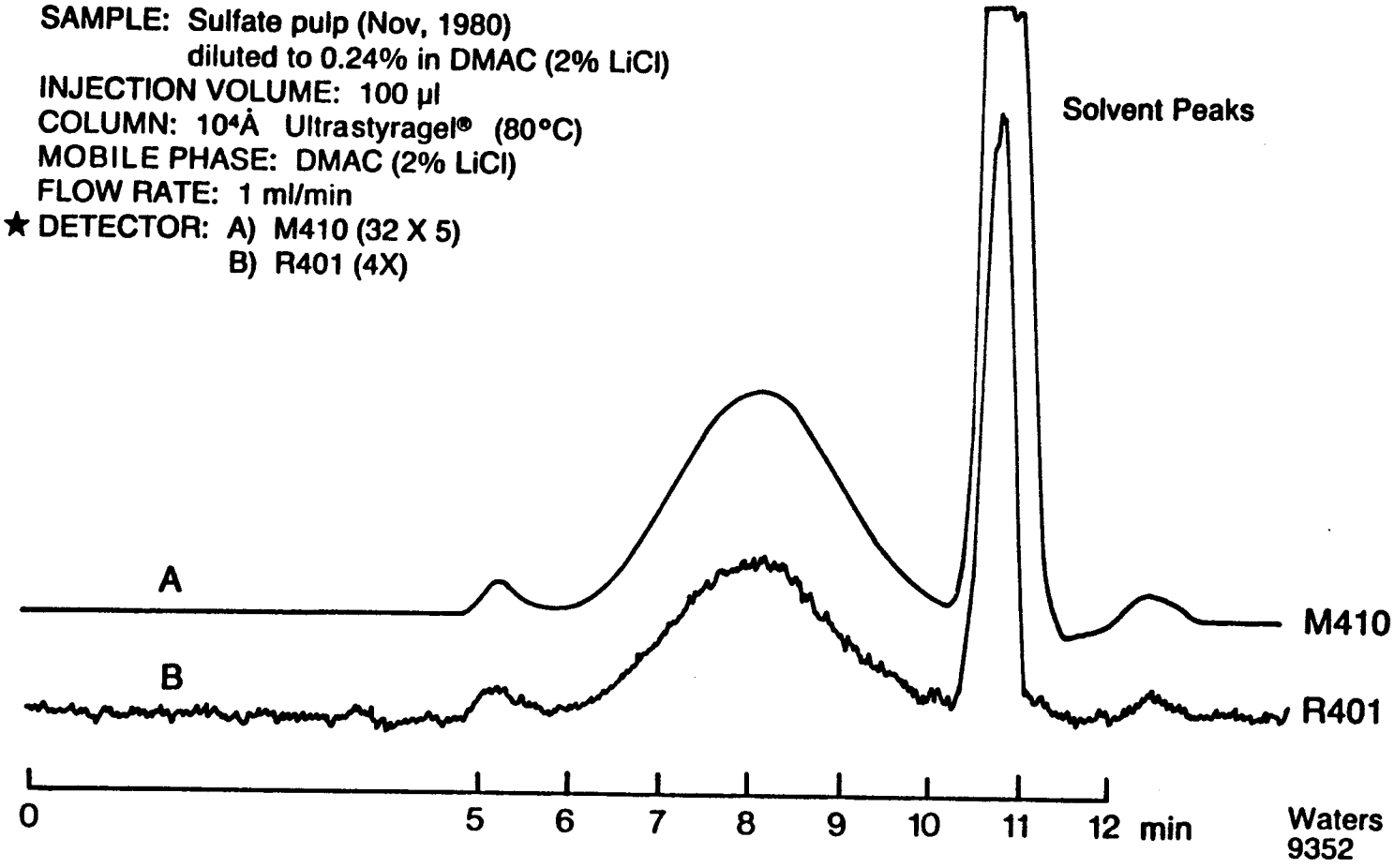
Slide #9

Cellulose Methods Development
R401 vs M410 Detector

SAMPLE: Sulfate pulp (Nov, 1980)
diluted to 0.24% in DMAC (2% LiCl)
INJECTION VOLUME: 100 µl
COLUMN: 10^4Å Ultrastyragel® (80°C)
MOBILE PHASE: DMAC (2% LiCl)
FLOW RATE: 1 ml/min
★ DETECTOR: A) M410 (32 X 5)
B) R401 (4X)



Cellulose Methods Development

Injection Volume and Sample Concentration

INJECTION VOLUME:	100 µl	50 µl	100 µl	50 µl
SAMPLE CONCENTRATION:	0.24%	0.24%	0.12%	0.12%
SAMPLE MASS:	0.24mg	0.12mg	0.12mg	0.06mg
410 (SENSITIVITY X SF):	32 X 5	32 X 10	32 X 10	32 X 20
"401 EQUIVALENT":	4X	2X	2X	1X
CELLULOSE MWD (peak):	8.18 Min	8.04 Min	8.04 Min	7.92 Min

SAMPLE: Sulfate pulp (Nov, 1980)
COLUMN: 10^4Å Ultrastyragel® (80°C)
MOBILE PHASE: DMAC (2% LiCl)
FLOW RATE: 1 ml/min

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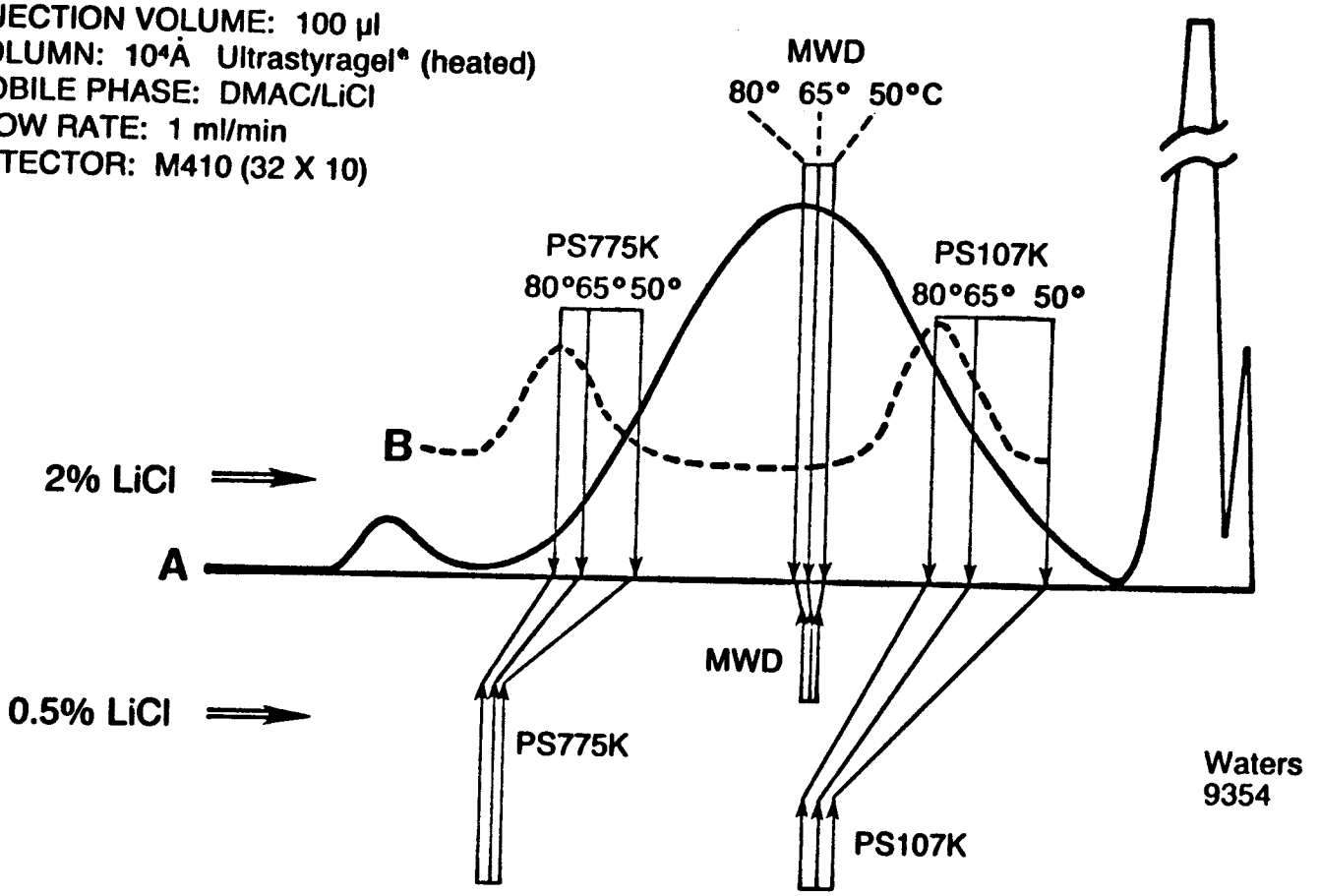
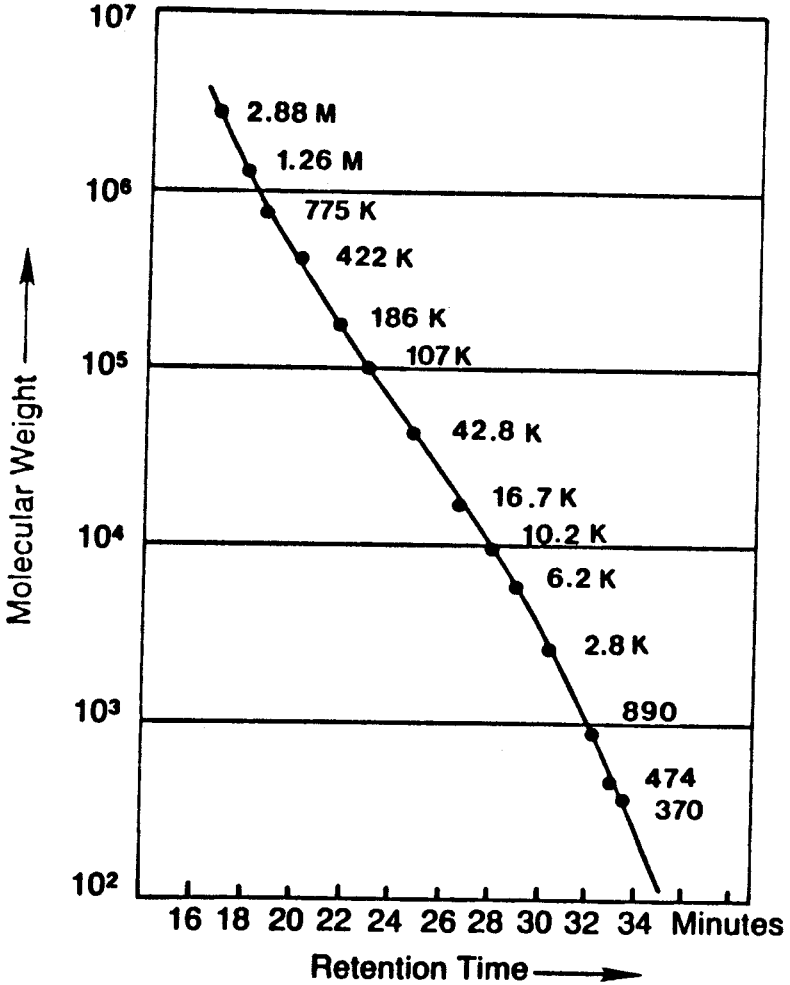
9353

Cellulose Methods Development
Column Temperature and % LiCl in Mobile Phase

SAMPLE: A Sulfate pulp (Nov, 1980), 0.12%
B PS standards
INJECTION VOLUME: 100 µl
COLUMN: 10^4Å Ultrastyragel® (heated)
MOBILE PHASE: DMAC/LiCl
FLOW RATE: 1 ml/min
DETECTOR: M410 (32 X 10)



GPC Calibration Curve



COLUMNS: Ultrastyragel®
10^3, 10^4, 10^5 Å (80°C)
SAMPLES: Polystyrene Standards
INJECTION VOLUME: 300 µl
MOBILE PHASE: DMAC (0.5% LiCl)
FLOW RATE: 1 ml/min
DETECTOR: M410

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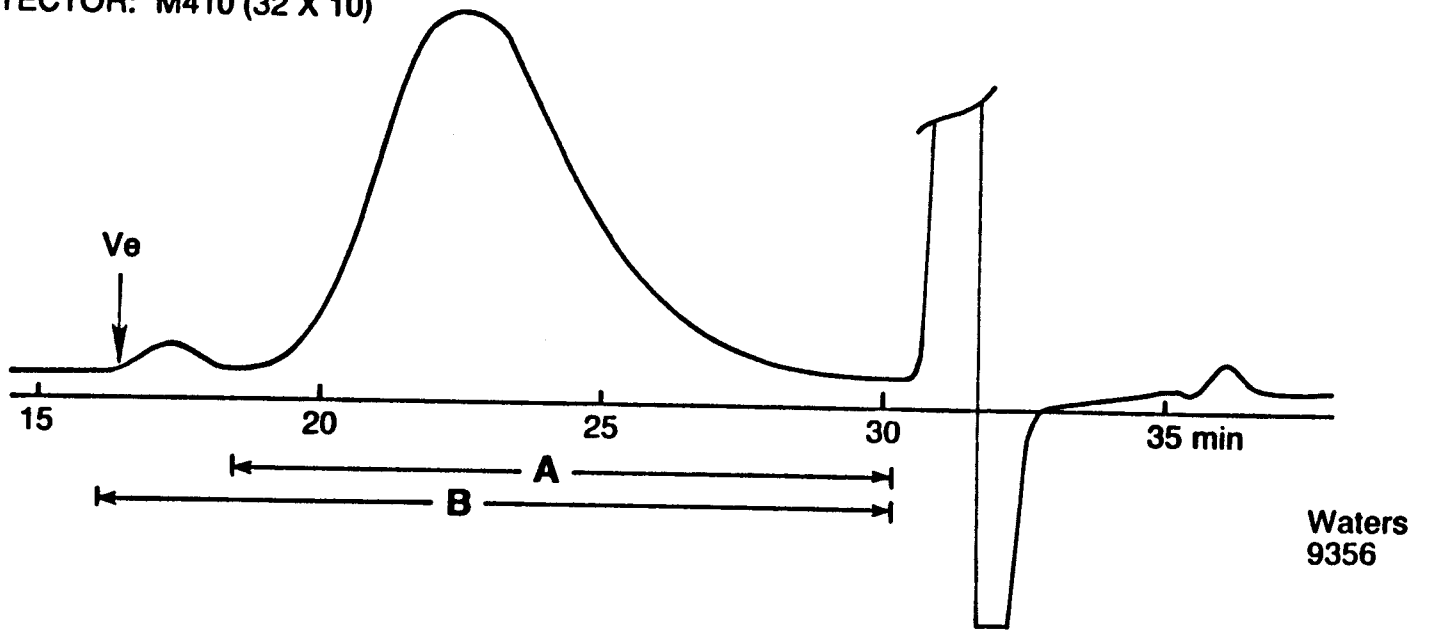
9355

GPC Analysis of Cellulose
Sulfate Pulp

SAMPLE: Sulfate pulp (Nov, 1980)
INJECTION VOLUME: 300 µl (0.1% solution)
COLUMNS: 10^3, 10^4, 10^5Å Ultrastyragel® (80°C)
MOBILE PHASE: DMAC (0.5% LiCl)
FLOW RATE: 1 ml/min
DETECTOR: M410 (32 X 10)

	$\overline{M}w$	$\overline{M}n$
A	131,900	64,700
B	175,200	66,300

(based on PS standards)



Cellulose Sample Preparation

- H_2O activation (120 mg/10 ml H_2O)
- DMAC exchange (4 X 10ml DMAC)
- Add DMAC (with 6% LiCl) to 10 ml volume (1.2% cellulose)
- Dilute to 0.1% cellulose (and 0.5% LiCl) with DMAC
- Filter (0.5 µ teflon membrane)

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9357

GPC Analysis of Cellulose

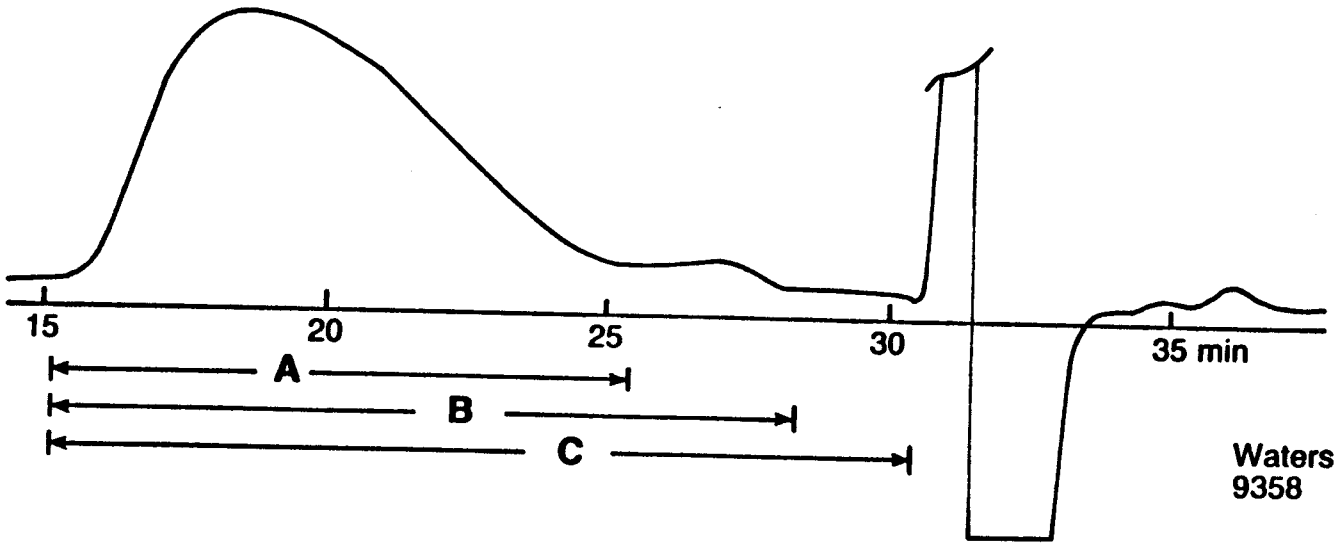
Softwood Sulfite Pulp

(Rayon Grade)

SAMPLE: Softwood sulfite pulp
(ICCA-4)
INJECTION
VOLUME: 300 µl (0.1% solution)
COLUMNS: 10^3, 10^4, 10^5Å Ultrastyragel® (80°C)
MOBILE
PHASE: DMAC (0.5% LiCl)
FLOW RATE: 1 ml/min
DETECTOR: M410 (32 X 10)

	$\overline{Mw}$	$\overline{Mn}$
A	834,900	265,400
B	795,700	158,800
C	790,000	131,900

(based on PS standards)

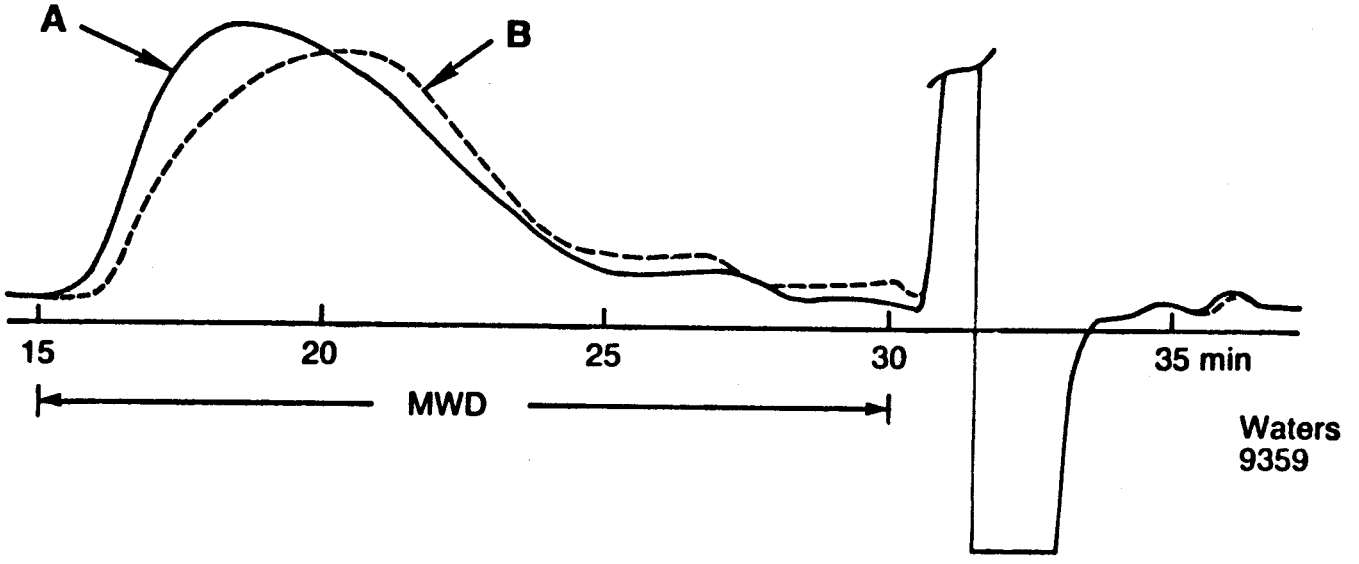


GPC Analysis of Cellulose

SAMPLES: A) Softwood sulfite pulp (rayon grade; ICCA-4)
B) Sulfite pulp (cellophane pulp; ICCA-5)
INJECTION VOLUME: 300 µl (0.1% solution)
COLUMNS: 10^3, 10^4, 10^5Å Ultrastyragel® (80°C)
MOBILE PHASE: DMAC (0.5% LiCl)
FLOW RATE: 1 ml/min
DETECTOR: M410 (32 X 10)

	$\overline{Mw}$	$\overline{Mn}$
A	790,000	131,900
B	555,700	76,900

(based on PS standards)



GPC Analysis of Underivatized Cellulose Summary

- Sample - dissolve in DMAC (6% LiCl) via H_2O and DMAC
- Columns - high efficiency Ultrastyragel® (80°C)
- Mobile Phase - DMAC (0.5% LiCl)
- Analysis Time - 40 min (3 X 30 cm columns @ 7700 p/ft)

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