Application of Universal Calibration in Gel Permeation Chromatography for Molecular Weight Determinations of Plant Cell Wall Polymers: Cotton Fiber

Judy D. Timpa

Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 19687, New Orleans, Louisiana 70179

Methodology has been established to determine the molecular weight distribution of the cellulosic composition of cotton fibers without prior extraction or derivatization, processes that can degrade polymers. Dimethylacetamide with lithium chloride is used for dissolution of samples. Commercially available, automated gel permeation chromatography (GPC) instrumentation with incorporation of a viscometer coupled with refractive index detection applies the universal calibration concept. With this configuration, GPC provides both a sensitive monitor of cotton fiber molecular weight distributions and valid molecular weight values. The weight-average molecular weight determined for cotton fiber was 11 000.

Cellulose is the most abundant naturally occurring polymer in plants. Improvements in cellulosic products will be possible only when better basic understanding of the material is acquired. Chemically, cellulose is a polymer consisting of anhydroglucose units linked (β-1,4-D-glycosidic) linearly to form long chains. It is difficult to overstate the importance of cellulose dissolution to both existing and potential applications, which range from the rayon and cellulose film industries to use of cellulose solvents for the characterization of cellulosic materials (Johnson, 1985). Isolation, molecular weight (MW) determination, and fractionation of native cellulose have considerable obstacles because of the great susceptibility of cellulose to hydrolytic and oxidative influences (Marx, 1958). The process of derivatization to facilitate dissolution in organic solvents may expose the cellulose chains to degradation. When cellulose derivatives are used instead of cellulose to determine MW, a further complication is introduced in that the substitution may not be complete or uniform, which influences the solubility of the product (Lindsley and Frank, 1953). Fractional precipitation of the nitrate derivative has been the most widely used method for estimating the chain-length distribution of cellulose because the cellulose degradation is held to a minimum (Timell, 1955; Segal et al., 1970).

Research and development activities devoted to new cellulose solvents worldwide have been especially prolific over the last decade (Johnson, 1985). McCormick (McCormick, 1981; McCormick et al., 1985) determined cellulose solubility in dimethylacetamide with lithium chloride (DMAC/LiCl) for preparation of derivatives. Since dried cellulose does not go into DMAC/LiCl solutions at room temperature, specific dissolution techniques were developed. Not only purified cellulose but ostensibly cellulose from any source, including cotton linters, wood, and paper, was reported to be dissolved in DMAC/LiCl (McCormick et al., 1985). Cotton linters, defined as the short fibers remaining on the seed after ginning, are of lower weight-average MW than cotton fiber (Timpa, 1983). From NMR studies (McCormick et al., 1985), it was determined that the DMAC/LiCl does not react with the cellulose and a true solution is formed. In investigations of solvent systems for spun rayons, Turbak et al. (Turbak et al., 1981; Turbak, 1983) also described cellulose dissolution in DMAC/LiCl. "Activation" of the cellulose, defined as opening up the cellulose structure to the solvent, is necessary for dissolution. This was accomplished by penetration of the cellulose with a polar medium which swells the cellulose to open its structure to permit access by the solvent at ambient temperatures or at temperatures low enough to avoid significant polymer degradation (normally less than 150 °C). Once prepared, cellulose solutions in DMAC/LiCl were reported stable at room temperature for several years even if the container had been repeatedly opened (Turbak, 1983; Turbak et al., 1981). The aging effects of cellulose solutions were monitored in McCormick's study by measurement of the viscosity as a function of time with only a 2–3% loss in viscosity observed after 30 days for a solution maintained at 30 °C (McCormick, 1981; McCormick et al., 1985).

The technique of gel permeation chromatography (GPC) with organic solvents is widely employed for determining the molecular weight distribution (MWD) of polymeric materials. Using wood pulp cellulose solutions in DMAC/LiCl, Ekmanis (1987a) developed a rapid method of MWD characterization by GPC using commercially available high-performance, high-efficiency, cross-linked styrene–divinylbenzene polymer columns with DMAC/LiCl as the mobile phase. Polystyrene standards were used for calibration with logarithm of MW plotted against retention (elution) volume. Ekmanis (1987b) subsequently reported a simpler dissolution ("one pot") procedure.

The conventional calibration method for GPC (log MW vs retention volume) has serious limitations in application to samples different from the polymers employed as standards. Reliable narrow distribution standards are available for only very few polymer materials. Standards of one polymer do not behave in solution in the same manner as another polymer. The calibration problem is so intractable that molecular weight values obtained by GPC are used mostly for comparative purposes among polymers of the same composition, which severely limits applications (Haney, 1985). Grubisic et al. (1967) reported that the hydrodynamic volume (the product of the intrinsic viscosity and the MW) is the most appropriate calibration parameter responsible for GPC retention rather than MW alone. Thus, a universal calibration relationship was linear for a number of different polymers including linear, graft,

This article not subject to U.S. Copyright. Published 1991 by the American Chemical Society

block, and star-shaped because hydrodynamic volume takes into account interactions of all types, even those between polymer and solvent, as well as shapes (coil and rigid rodlike molecules). Attempts have been made to take advantage of the universal calibration concept (Ouano, 1972; Wadsworth et al., 1973). More recently, commercial viscometer detectors with controlled temperature have been introduced to measure the intrinsic viscosity of a polymer as it elutes from GPC columns (Haney, 1985).

As nature's purest form of cellulose, mature cotton fiber is composed primarily (ca. 95%) of cellulose and has a much higher weight-average MW than wood pulp and celluloses from other sources (Goring and Timell, 1962). Previous MWD determinations of cotton cellulose have been by extensive fractionation and/or derivatization procedures (Marx-Figini, 1982; Segal et al., 1970). Dissolution of cellulose in DMAC/LiCl and subsequent GPC analysis discussed above was conducted with cellulosic materials of lower MW than cotton fiber. That GPC calibration was with polystyrene standards with no compensation for the different solution behavior of cellulose. We investigated direct dissolution of cotton fiber in DMAC/LiCl and analysis by GPC. Our research objective is to evaluate cotton fiber at the polymeric molecular level to gain insight into the process of cellulose biosynthesis. Solubilization of fiber directly without prior extraction or derivatization avoids those procedures that could lead to degradation of the polymeric chains and thus alterations in molecular weight. For comparative purposes, we included evaluation of an extracted cotton fabric and commercial cellulose powders. The universal calibration concept was applied to obtain valid MWD.

MATERIALS AND METHODS

Materials. Samples included cotton fiber from American Upland cotton (*Gossypium hirsutum* L.) variety Texas Marker 1 (TM-1, 98% cellulose); desized, scoured, and bleached 80 × 80 cotton printcloth; and acid-washed cellulose powder (Baker Catalog No. 1525). Materials were DMAC (Burdick & Jackson, dried with molecular sieves, Type 4A, Baker), LiCl (Baker) oven-dried and stored in a desiccator; 10-mL ReactaVials (Pierce); heating block (Pierce); Teflon magnetic stirbars (2.5 cm); Baker 10 extraction apparatus; disposable Teflon filters (Millex SR, Millipore), 10-cm^3 glass syringes (BD); 4-mL WISP vials with Teflon septa; and 50-mL volumetric flasks. Polystyrene standards were from Toyo Soda Manufacturing with nominal molecular weights of 2.89E6, 1.9E5, 7.1E5, 1.02E5, 1.26E6, 4.39E4, 3.55E5, 1.96E4, 6.2E3, and 1.03E4.

Procedure. Cotton fiber and fabric were ground in a Wiley mill to pass a 20-mesh screen. A sample of cotton (0.8–1.2% w/v) was added to 5 mL of DMAC in a 10-mL ReactaVial in a heating block. The temperature was raised to 150 °C and maintained at that temperature with stirring for activation of the cellulose (1–2 h). The mixture was allowed to cool to 100 °C. Dried LiCl (8% w/v) was added. The temperature was lowered to 50 °C and maintained until the cellulose was dissolved (48 h). The vials were shaken for 1–2 h on a laboratory shaker at room temperature and returned to the heating block at 50 °C The solution was quantitatively transferred to a 50-mL volumetric flask and diluted with DMAC. The solution was then filtered through a Teflon solvent-resistant, disposable filter (Millex SR, 0.5 μm, Millipore) prior to injection onto the GPC system. An extraction apparatus (Baker 10) was employed with 10-cm^3 glass syringes fitted onto filters with 4-mL glass vials (WISP, Waters) held in the small volumetric holder. Vacuum pulled samples through. The dissolution of filtered samples was assessed by GPC analysis (area under peaks), and total solids were obtained by drying 1 mL of sample in a vacuum oven at 60 °C for 48 h. It should be noted that the solvent DMAC/LiCl is extremely powerful so glass or solvent-resistant materials were employed throughout sample preparation.

Chromatography. The mobile-phase solvent for GPC was DMAC/0.5% LiCl prepared by raising the temperature of 1000 mL of DMAC to 100 °C and then adding 5 g of LiCl (dried). After the salt was stirred until it dissolved, the solvent was filtered through a Teflon filter (TYPE FH, 0.5 μm, Millipore) with glass filter apparatus.

Filtered samples were analyzed on a GPC system consisting of an automatic sampler (Waters WISP) with an HPLC pump (Waters Model 590), pulse dampener (Viscotek), viscometer detector (Viscotek Model 100), and refractive index detector (Waters Model 410). The detectors were connected in series with the RI following the viscometer because of back-pressure limitations on the RI flow cell. The mobile phase of DMAC/0.5% LiCl was pumped through at 1.0 mL/min. Columns were Ultrastyragel 10^3, 10^4, 10^5, 10^6 (Waters) preceded by a guard column (Phenogel, linear, Phenomenex) operated at 80 °C controlled by a column heater (Waters column temperature system). Injection volume was 400 μL on the basis of injection of 100 μL per column. Run time was 60 min. The software package Unical based upon ASYST (Unical, Version 3.02, Viscotek) was used for data acquisition and analysis. The computer system was an IBM compatible computer (Model 200, Dell) with 40MB hard disk, dual floppy disks, 640K RAM, 808287 math coprocessor, color monitor, VGA graphics adaptor with hard-copy output to a dot matrix graphics printer (Model FX850, Epson), 8-pen color plotter (Model Colorpro, Hewlett-Packard), and internal A/D converter. Calibration was with polystyrene standards dissolved and run in DMAC/0.5% LiCl. The data were obtained from two dissolutions per sample with two GPC runs per dissolution.

RESULTS AND DISCUSSION

McCormick developed several methods of dissolution (McCormick, 1981; McCormick et al., 1985). One was suspension of cellulose (~1–3%) in DMAC/3–9% LiCl, followed by heating to 150 °C and cooling to room temperature. A second technique involved swelling the cellulose in water overnight followed by solvent exchange through methanol to DMAC and then adding the cellulose to DMAC/9% LiCl. The swollen cellulose went into solution at room temperature and at concentrations up to 15%. Complete solutions of 1–5% cellulose powders were achieved in less than 1 h, while with 6–15% cellulose it took 24–48 h. Direct application of those procedures in our laboratory was not successful for dissolution of either the native cotton fiber or the extracted cotton fabric. Perhaps this is not surprising since, for example, the use of solubility parameters to explain why a particular solvent dissolves cellulose has not been very fruitful (Johnson, 1985).

In the Ekmanis (1987a) procedure, the cellulose was dissolved in DMAC/LiCl by first swelling the cellulose with water and then solvent exchanging with DMAC four times. The final concentration (0.1% cellulose in DMAC/0.5% LiCl) was compatible with commercial GPC columns. His procedure was based upon Turbak's method, whereby solvent exchange of cellulose following water-swelling procedures was successful to make a solution containing 12% cellulose and 10% LiCl ~4–6 h (Turbak, 1981). They reported that this worked for low molecular weight cellulose, although with higher molecular weight cellulose only 4% solutions were obtained. With cotton fiber samples, we could not successfully achieve acceptable levels of dissolution with this mode of activation. We attempted variations on this procedure but were generally unsuccessful with dissolution of cotton (raw or extracted) by this means. The second procedure developed by Ekmanis (1987b) was also based on a Turbak (1981) process. Cellulose was activated by using hot vapors of DMAC at 150 °C, cooled to about 100 °C when the LiCl was added, and stirred for several hours until the cellulose went into complete solution. It appears that at or near its boiling point the amide has sufficiently high vapor pressure to

enable it to penetrate throughout the fiber and thus swell the fiber (Turbak, 1981). The solutions were reportedly clear. Since it was a "one pot" method, the procedure was attractive for handling large numbers of samples necessary for screening in a physiological study. Direct application of the procedure was not successful for preparation of cotton fiber samples for GPC analysis on a routine, consistent basis; however, the results were the most promising of the literature procedures. In the case of cotton, seemingly minor alterations in a procedure can mean the difference between success and failure in dissolution. Factors important to cellulose dissolution, especially the need to overcome the characteristic hydrogen-bonded association between molecular chains, appear to be extremely important (Johnson, 1985). Cotton cellulose is highly crystalline in addition to possessing high MW.

The strategy that we took to optimize the process of dissolution of cotton fiber was first to evaluate the alterations of conditions with the Ekmanis (Ekmanis, 1987b) one-pot procedure. Then, using those conditions that appeared to give the most complete dissolution, we varied the concentrations of cellulose or LiCl (while holding the other constant) to obtain the most effective combination. Refinement of the methodology was carried out by feedback of the GPC results to recheck conditions. A concentrated solution of cellulose is prepared with subsequent dilution to a concentration compatible with GPC conditions. The critical steps in the procedure were investigated in depth including sample preparation, time of activation, time of stirring for dissolution, means of stirring, concentration of cellulose, and concentration of LiCl.

Specifically, 1.0 or 2.0% (w/v) cellulose was stirred in DMAC in vials in a heating block. The temperature was raised to 150 °C, and the mixture was stirred for an additional period of time for activation. After the mixture cooled to 100 °C, 6 or 8% (w/v) LiCl was added. The mixture was cooled to 50 °C and stirred until the cellulose was dissolved. Final dilution was made in a volumetric flask since it is necessary to know the exact concentration for GPC calculations. The solution was diluted to 0.10 or 0.20% cellulose/(DMAC/0.5% LiCl) for GPC analysis. The results are summarized below.

Comparison of sample preparation was made among native fiber, chopped fibers, and Wiley-milled samples. Incomplete and inconsistent dissolution indicated that grinding in the Wiley mill was necessary. This is standard practice in preparation of cotton fiber samples in many analyses to minimize surface area variations (Newman et al., 1953).

Using exposure to hot DMAC vapors as the mode of activation as in the second Ekmanis (1987b) procedure, we tested exposures for different periods of time. Stirring fibers in hot DMAC vapors at 150 °C in the small vials in a heating block was carried out for 0.5–3 h. Cotton required a longer time of activation than lower MW celluloses, with 1–2 h being most effective. We observed that effective dissolution was achieved with elevation of temperature of the material stirring in an open vial until the temperature was reached. Apparently this allows any moisture in the cotton to be driven off, but the system is still maintained below the boiling point of DMAC (166 °C). The vials were capped after reaching 150 °C to allow the exposure to hot DMAC vapors. Stirring is critical in this process because of the heterogeneity (fiber/solvent) of the mixture. We employed different kinds of magnetic stirring bars and concluded that the most agitation was obtained with a 2.5-cm stir bar. As to time required for dissolutions, the length of time of stirring at 50 °C was varied from 18 to 96 h. Our results indicate that 48 h at 50 °C after addition of the LiCl is adequate for dissolution of cotton cellulose in DMAC/LiCl. A period of 1–2 h of shaking on a laboratory shaker improved the procedure. Not surprisingly, cotton fiber required a longer time of activation and had a slower rate of dissolution than reported for wood pulp (Ekmanis, 1987a,b), probably due to the higher molecular weight and greater crystallinity of the cotton fiber cellulose. As pointed out by both McCormick (McCormick, 1981; McCormick et al., 1985) and Turbak et al. (1981), only the rate of dissolution appears to change with the different type of cellulose source due to the molecular weight, crystallinity, and lignin content. Activation for the disruption of hydrogen bonding of cellulose (by swelling in polar or hydrogen-bonding solvents or by heating) appears to be crucial in dissolution of cellulose in DMAC/LiCl.

In the initial dissolution stage, concentrations ranging from 0.5 to 2.5% cellulose were evaluated against amounts of LiCl varying from 4 to 9%. Thus, final concentrations ranged from 0.05 to 0.25% cellulose in DMAC/0.5% LiCl. The 0.5% LiCl is compatible with the mobile phase in the GPC operation. Specifically, the concentration of cellulose was varied from 1 to 2.5% initially in 0.2% increments for 6% LiCl. Tests were run for 0.5–2.2% cellulose initially with 0.1% increments with 8% LiCl. For 4, 6, 7, 8, and 9% LiCl initially, the concentrations of cellulose evaluated at 1 and 2%. The concentrations and dilutions involved adjustments in order that the solutions were not so viscous that filtration was impossible while peaks were discernible by both detectors upon GPC analysis. Minimization of amount of solvent (DMAC) required for preparation of large numbers of samples in addition to the physical problem of adequate stirring of heterogeneous mixtures for extended periods of time must be considered. When dissolution occurs, the solutions are clear.

We obtained the best results with an initial concentration of 0.8–1.2% cellulose in DMAC with 8% LiCl. The samples were diluted to a final concentration of 0.08–0.12% cellulose/(DMAC/0.5% LiCl). Higher molecular weight cotton samples were more completely dissolved at lower concentrations (data not shown). We found that 8% LiCl in DMAC was necessary for dissolution of cotton cellulose. McCormick et al. (1985) reported concentrations of greater than 6% LiCl in DMAC were required for complete dissolution of the lower MW celluloses used in his study. Apparently, a critical number of complexed sites are required for dissolution. This observation supports the mechanism of dissolution that involves hydrogen bonding of the hydroxyl protons of cellulose with the chloride ion, which is in turn associated with the Li^+ (DMAC) macrocation complex (McCormick et al., 1985).

For GPC analysis, Ekmanis (1987a) employed three GPC columns operated at 80 °C with flow rate of 1 mL/min with RI detector. The advantage of faster flow rate with corresponding decrease in analysis time for the GPC operation at elevated temperature was adopted in our work. Because of the broader MW range of cotton, we included four GPC columns to obtain maximum resolution. The salt (LiCl) is necessary in the mobile phase to be compatible with the solvent in which the sample is dissolved. If the salt were not included, the potential for precipitation of the samples on the columns would exist.

When the viscometer is coupled with a concentration detector (usually refractive index, RI), two plots are obtained which provide information for determining

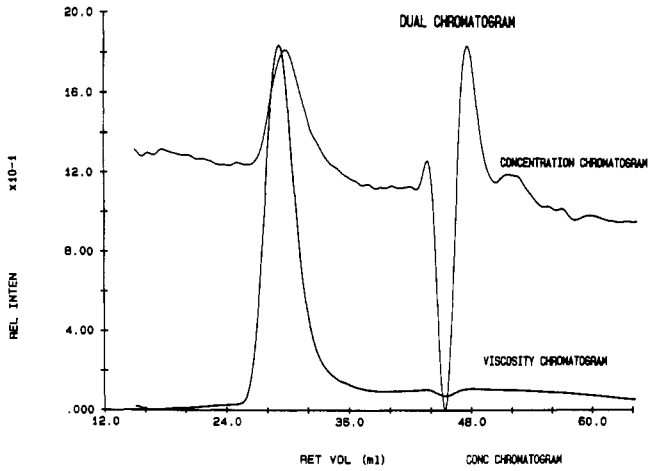

Figure 1. Detector outputs from viscometer and differential refractometer for raw cotton fiber sample dissolved in (DMAC/0.5% LiCl). Concentration was 1.2 mg/mL cellulose. Injection was 400 μL. Sensitivity for viscometer was 1-V output with RI on 256 attenuation. Run time was 65 min. Relative intensity as the Y axis is scaled to the maximum and minimum of the viscometer output. The RI minimum is matched to the viscometer minimum and scaled from there. Ret vol is the retention volume in milliliters. Flow rate was 1 mL/min.

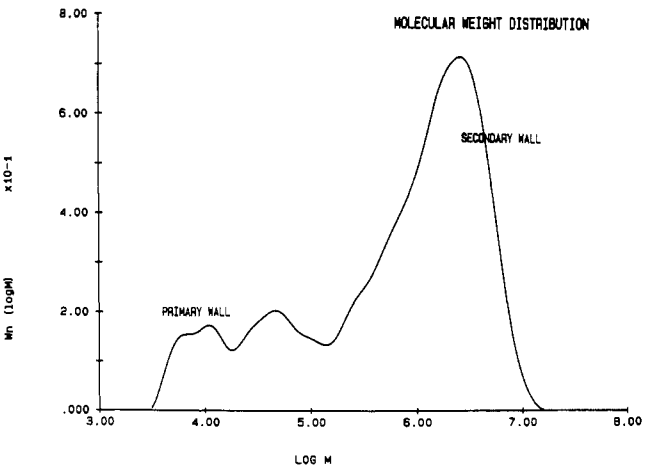

Figure 2. Molecular weight distribution for cotton fiber sample calculated from Figure 1. The intrinsic viscosity [η] is determined from viscosity/concentration at a specific retention volume. Log M is then obtained from calibration of log [η]M vs retention volume.

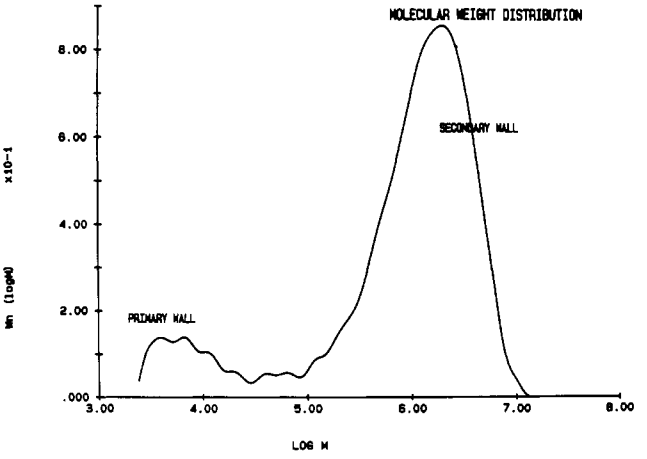

Figure 3. Molecular weight distribution for cotton printcloth, desized, scoured, and bleached. The intrinsic viscosity [η] is determined from viscosity/concentration at a specific retention volume. Log M is then obtained from calibration of log [η]M vs retention volume.

MWDs for polymers of interest based upon well-characterized standards with narrow MWDs used to prepare a universal calibration (Haney, 1985). For cellulose, standards having narrow MWDs are not commercially available and are extremely difficult to prepare by classical fractionation methods. Commercially available, narrow, well-characterized polystyrene fractions were used for calibration over the range of MW from 10^3 to 10^4. Molecular weights were the nominal weight-average MWs from the supplier. The calibration curve determined for this GPC configuration/system as a plot of the logarithm of the product of intrinsic viscosity times molecular weight versus retention volume was linear and first order. The GPC system had a viscometer and a RI detector in series. Concentrations ranged from 0.3 mg/mL for high MW standards to 1.5 mg/mL for lower standards. The Mark–Houwink constants obtained for polystyrene in DMAC/LiCl (0.5%) at 80 °C were 0.642 for α with log k = −3.761.

Representative curves of the two detector outputs are given in Figure 1 for the cotton fiber sample. The peaks of interest are of retention volumes from 24 to 44 mL approximately. The large solvent/air peak was observed beginning at about 44 mL. Any moisture present shows up markedly on RI just past the solvent/air peak. Variations in this negative peak are also observed because of the presence of the salt in the sample solution and in the mobile phase (Max Haney, personal communication).

The MWD determined from this dual chromatogram for the sample of cotton fiber is given in Figure 2. Cotton fibers are very long (>2.5 cm) single cells that differentiate from the epidermal layer of the developing cotton seed. Elongation of the primary cell wall occurs for a period of 3 weeks after anthesis, followed by deposition of a thick cellulose secondary cell wall. The location of the secondary wall and primary wall peaks were identified from other work in progress on the development of the cotton fiber (Timpa and Triplett, 1989). Fiber taken from bolls over the period of 8 days postanthesis (DPA) until 60 DPA was analyzed. The primary wall is the major component in the early stages of development, while the secondary wall begins rapid accumulation at 18–21 DPA. The major contribution to the cellulosic content of the cotton fiber comes from the secondary wall, which is the major peak identified in the figure. The location of the primary wall peak is at much lower molecular weight than the secondary wall peak, in agreement with previous results. Historically, values for MW for cotton cellulose have been reported as degree of polymerization (DP), which is a measure of repeating anhydroglucose units. Hessler et al. (1948) reported DP of 10 650 for secondary wall and DP of 5950 for primary wall by measuring the viscosity of cellulose nitrate in butyl acetate. Marx-Figini (1982) reported DP of 14 000 for secondary wall also obtained from the nitrate derivative. In this study, we obtained a DP of 9600 for the secondary wall of TM-1.

In Figure 3, the MWD of a sample of cotton printcloth is presented. Again, the major portion of the cellulosic composition arises from the secondary wall at a high MW. In contrast, the MWD of the cellulose powder shown in Figure 4 indicates discrete peaks at several locations at lower MWs. Overlays of the MWD from different dissolutions of the same sample indicated 5–10% reproducibility.

Cotton fabric samples have been included throughout our methods development to ascertain whether or not the small (<2% in this case) amount of pectins and waxes present on native cotton fiber would interfere with the dissolution process. The reports of Turbak et al. (1981)

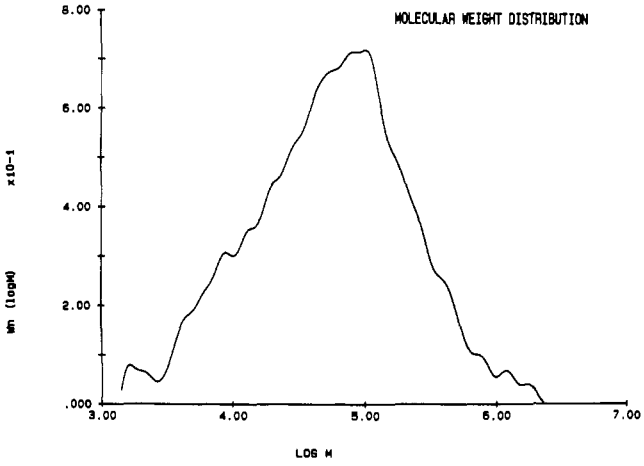

Figure 4. Molecular weight distribution for acid-washed cellulose powder. The intrinsic viscosity $[\eta]$ is determined from viscosity/concentration at a specific retention volume. Log M is then obtained from calibration of log $[\eta]M$ versus retention volume.

and McCormick (1981) anticipate that it would not. Unidentified minor peaks, which are probably these components, appear after the solvent peak for native cotton. Further study is planned for definitive identification. Similar behavior with respect to extent and ease of dissolution was observed for both the native and the extracted celluloses for sample preparation, activation time, sample concentration, and levels of lithium chloride. Our experience has been that if the dissolution is complete, the solutions are clear with no particles or haze present. The only visible difference between the fiber and fabric samples was the slight amber hue of the fiber samples. Obviously, the fabric had been cleaned by the customary textile procedures of desizing, scouring, and bleaching, producing clear, colorless solutions.

This is the first time that the universal calibration concept with the viscometer detector has been applied to cotton cellulose samples. The requisite of a calibration standard for GPC is that fractions with a narrow MWD be available over a wide MW range. For cellulose and many other polymers, calibration standards of known molecular weight and low polydispersity are not available. Calibration should be valid for any polymer regardless of its chemical nature or morphological structure, should be universal, and thus should be characteristic for any given set of columns and elution solvent at a given temperature. In this case we analyzed cellulosic materials, but any other polymeric species present are legitimately characterized because of the universal calibration aspect. With this technique, a valid MWD for the polymer composition is obtained even though the structural/chemical identification is not available.

Controversy has existed for many years over the "true" MW of native cotton fiber (Goring and Timell, 1962; Hessler et al., 1948; Marx-Figini, 1982; Wadsworth et al., 1973). The values for the weight-average MWs and DPs for the three samples are given in Table I. Reproducibility within 10% was obtained for values of weight-average MWs for separate dissolutions of the same sample. Solutions of samples appeared stable for at least 30 days after preparation. The values we have given in Table I fall within the upper range of predictions. Previous work has been done with fractionation and/or derivatization, with potential loss of MW species as well as chain cleavage to obtain mostly average values and ranges of DP. Pascu postulated in 1947 that, as a consequence of common procedure adhered to by most investigators, native cellulose is unintentionally degraded to about DP 3000, which is then reported as the average MW of cellulose. Although these two samples of cotton fiber and cotton fabric are not known to be related, the smoother MWD of fabric possibly reflects the processing history. The cleanup process of the fabric has probably removed some lower MW species or perhaps the treatment caused chain scission, both of which would alter the average value. The MW of the cellulose powder is 16% greater in this determination than the value obtained in McCormick's study (McCormick et al., 1985). Since we have no certainty that the samples were from the same source or lot, comparisons are limited.

Table I. Molecular Weights of Cellulose Samples Dissolved in DMAC/LiCl Determined by Gel Permeation Chromatography via Universal Calibration (Log $[\eta]$MW vs Retention Volume)

sample	wt av MW[a]	wt av DP[b]
cotton fiber	1 830 000	11 300
cotton cloth	1 310 000	8 100
cellulose powder	211 000	1 300

[a] Molecular weight. [b] Degree of polymerization, where the MW of the repeat unit is 162.

In chromatography in general, dual-detector analysis can improve quantitative accuracy and yield enhanced sensitivity. A suitable two-channel data acquisition and analysis system is extremely useful for deriving maximum value from the resulting data. In this GPC configuration, the sensitivities of the two detectors are directly opposite. The viscometer detector gives a much better qualitative indication of the presence of high MW species in a sample than the RI detector. Presence of high MW polymer is barely discernible from noise in the RI trace, while a substantial peak is obtained from the viscometer detector (Styring et al., 1987). This became immediately obvious in our early investigation in analysis of commercial cellulose powders compared to cotton fiber and fabric samples. The secondary wall peak of cotton fiber samples is at a high MW. Thus, despite the increased difficulty in dissolution of cotton samples of higher MW, greater sensitivity to the presence of high MW species is obtained fortuitously. Adjustment of concentration levels becomes necessary for effective analysis.

We previously reported that variety and growth conditions affect the weight-average MWs of cotton fibers. Those values were determined by conventional calibration with polystyrene (Timpa and Wanjura, 1989; Timpa and Ramey, 1989). Comparison of average MW values obtained from the universal calibration according to the procedure reported here are of the same order of magnitude, although the exact values are different (data not shown). The dual-detector GPC system with the universal calibration gives much greater definition of the distribution of chain lengths than seen with the RI detector solely and conventional calibration. A limitation to advances in fundamental research in cellulose biosynthesis has been characterization of the product. Prior to the development of this method, cellulose first had to be subjected to time-consuming derivatization to form soluble derivatives for GPC analysis. Evaluation of the methodology for dissolution of cotton fiber in DMAC/LiCl involved balancing multiple factors. The dissolution was primary, of course, requiring study of activation as well as concentration of cellulose versus concentration of LiCl. The amount of cellulose in solution must be known exactly for MWD calculations and must be dilute enough to be filtered but concentrated enough to be detected. We have determined conditions for dissolution of cellulose within a single vial without sample transfer except for final dilution and

filtration. This allows for efficient processing of multiple samples of polymer material. Since cotton fiber, which has the highest MW of native cellulosic materials (Goring and Timell, 1962), can be dissolved by this procedure, extension of the methodology to other cellulosic materials should be straightforward.

In summary, the solvent DMAC/LiCl, which had previously been employed to dissolve cellulose from other sources and the short fibers of low MW cotton linters, has now been used successfully to dissolve cotton fibers for examination by GPC for molecular characterization. The feasibility of obtaining valid MWDs of cellulose polymers with polystyrene standards has been demonstrated by employing the universal calibration concept. Differences in polymeric composition of cotton fiber can now begin to be addressed with respect to development and related to physical properties.

SAFETY

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, 1985).

ACKNOWLEDGMENT

I gratefully acknowledge the contribution of sample materials from B. A. Triplett (USDA–ARS, Southern Regional Research Center, New Orleans, LA). I appreciate the helpful discussions with J. L. Ekmanis (Waters Chromatography, Milford, MA), C. L. McCormick and R. Hester (University of Southern Mississippi, Hattiesburg, MS), and A. Turbak (Georgia Technical University).

LITERATURE CITED

Baker Chemical Co. N,N-Dimethylacetamide, Material Safety Data Sheet, 1985, D5784-01; pp 1–4.

Ekmanis, J. L. Gel Permeation Chromatographic Analysis of Cellulose. *Am. Lab. News* **1987a**, Jan/Feb, 10–11.

Ekmanis, J. L. Sample Preparation for High Performance GPC Analysis of Cellulose. *Abstracts*, The Pittsburgh Conference and Exposition, Atlantic City, NJ, 1987b; Abstract 783.

Goring, D. A. I.; Timell, T. E. Molecular Weight of Native Celluloses. *Tappi* **1962**, *45*, 454–460.

Grubisic, A.; Rempp, P.; Benoit, H. A. Universal Calibration for Gel Permeation Chromatography. *Polym. Lett.* **1967**, *5*, 753–759.

Haney, M. A. A. New Differential Viscometer. *Am. Lab.* **1985**, *17*, 116–126.

Hessler, L. E.; Merola, G. V.; Berkley, E. E. Degree of Polymerization of Cellulose in Cotton Fibers. *Text. Res. J.* **1948**, *18*, 628–634.

Johnson, D. C. Solvents for Cellulose. In *Cellulose Chemistry and Its Applications*; Nevell, T. P., Zeronian, S. H., Eds.; Horwood Wiley: New York, 1985; pp 181–222.

Lindsley, C. H.; Frank, M. B. Intrinsic Viscosity of Nitrocellulose Related to Degree of Nitration. *Ind. Eng. Chem.* **1953**, *45*, 2491–2497.

Marx, M. Molecular weight determinations of fiber and wood celluloses. *J. Polym. Sci.* **1958**, *30*, 119–130.

Marx-Figini, M. The Control of Molecular Weight and Molecular-Weight Distribution in the Biogenesis of Cellulose. In *Cellulose and Other Natural Polymer Systems: Biogenesis, Structure, and Degradation*; Brown, R. M., Ed.; Plenum Press: New York, 1982; pp 243–271.

McCormick, C. L. Novel Cellulose Solutions. U.S. Patent 4,278,790, 1981.

McCormick, C. L.; Callais, P. A.; Hutchinson, B. H. Solution Studies of Cellulose in Lithium Chloride and N,N-Dimethylacetamide. *Macromolecules* **1985**, *18*, 2394–2401.

Newman, S.; Loeb, L.; Conrad, C. M. Viscosity, Sedimentation, Diffusion, and Osmotic Behavior of Long-Chain Nitrocellulose Molecules. *J. Polym. Sci.* **1953**, *10*, 463–487.

Ouano, A. C. Gel-Permeation Chromatography. VII. Molecular Weight Detection of GPC Effluents. *J. Polym. Sci., Part A-1* **1972**, *10*, 2169–2180.

Pascu, E. Cellulose Studies. IX. The Molecular Structure of Cellulose and Starch. *Text. Res. J.* **1947**, *17*, 405–418.

Segal, L.; Timpa, J. D.; Wadsworth, J. I. Gel Permeation Chromatography and Cellulose. I. Effect of Degree of Nitration of Cellulose on Molecular Weight Distribution Data. *J. Polym. Sci., Part A-1*, **1970**, *8*, 25–35.

Styring, M. G.; Armonas, J. E.; Hamielec, A. E. An Experimental Evaluation of a New Commercial Viscometer Detector for Size Exclusion Chromatography (SEC) Using Linear and Branched Polymers. *J. Liq. Chromatogr.* **1987**, *10*, 783–804.

Timell, T. E. Some Factors Influencing the Results Obtained by Fractional Precipitation of Cellulose Nitrates. *Sven. Papperstidn.* **1955**, 58, 234–239.

Timpa, J. D. Cotton Processing and Utilization. In *Handbook of Processing and Utilization in Agriculture, Vol. II: Part 2 Plant Products*; Wolf, I. A., Ed.; CRC Press: Boca Raton, FL, 1983; pp 541–574.

Timpa, J. D.; Ramey, H. H. Molecular Characterization of Three Cotton varieties. *Text. Res. J.* **1989**, *59*, 661–664.

Timpa, J. D.; Triplett, B. A. Molecular Characterization of Developing Cotton Fiber. *Proceedings of the Beltwide Cotton Conferences*, Nashville, TN; National Cotton Council: Memphis, TN, 1989; p 636.

Timpa, J. D.; Wanjura, D. F. Environmental Stress Responses in Molecular Parameters of Cotton Cellulose. In *Cellulose and Wood: Chemistry and Technology*; Schuerch, C., Ed.; Wiley: New York, 1989; pp 1145–1155.

Turbak, A. F. Newer Cellulose Solvent Systems. In *Wood and Agricultural Residues*; Soltes, E. J., Ed.; Academic Press: New York, 1983; pp 87–99.

Turbak, A. F.; El-Kafrawy, A.; Snyder, F. W.; Auerbach, A. B. Solvent System for Cellulose. U.S. Patent 4,302,252, 1981.

Wadsworth, J. I.; Segal, L.; Timpa, J. D. Average Degree of Polymerization of Cellulose by GPC without Viscosity Measurements. In *Polymer Molecular Weight Methods*; Advances in Chemistry Series 125; American Chemical Society: Washington, DC, 1973; pp 178–186.

Received for review March 12, 1990. Revised manuscript received September 4, 1990. Accepted September 24, 1990. 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 U.S. Department of Agriculture over others not mentioned.