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Facile characterization of molecular weight distribution of cellulose by gel permeation chromatography using a dimethyl sulfoxide solution containing 1% EmimOAc

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Abstract Recently, various methods for cellulose dissolution without derivatization pretreatment have garnered attention for measuring the molecular weight of cellulose using gel permeation chromatography (GPC). However, conventional methods require complex pretreatment procedures or use substantial quantities of ionic liquids, which are expensive and exhibit high viscosities. Herein, we report a GPC method for the successful dissolution of cellulose in dimethyl sulfoxide (DMSO) containing 1 wt% 1-ethyl-3-methylimidazolium acetate (EmimOAc). The GPC method is simple, does not require special pretreatment steps, and dissolves cellulose at room temperature. Moreover, as the concentration of the ionic liquid was as low as 1 wt%, the method was robust at low pressures in the GPC system and had

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Research Institute for Sustainable Humanosphere, Kyoto University, Gokasho, Uji, Kyoto 610-0011, Japan low operating costs for the ionic liquid. We demonstrate the applicability of this GPC method to various types of cellulose, including cotton, pulp, and rayon. The GPC profiles of EmimOAc/DMSO were comparable to those of the conventional GPC systems using lithium chloride/dimethyl acetamide in terms of the peak top molecular weight (and shoulder peak in the low-molecular-weight region). Therefore, this GPC method can contribute to the high-throughput evaluation of the molecular weights of native and regenerated cellulose.

Keywords Gel permeation chromatography · Cellulose · Ionic liquid · Mark–Houwink–Sakurada equation · Intrinsic viscosity

Introduction

The molecular weight (MW) of cellulose, which is a linear polymer consisting of β -1,4-linked glucose, varies depending on the natural cellulose type and cellulose products, including paper, pulp, films, and cellulose nanofibers. The determination of MW and degree of polymerization (DP) is important for evaluating physical properties, such as strength and durability, and chemical reactivities in the derivatization and depolymerization of cellulosic materials. The MW of cellulose is commonly measured using gel permeation chromatography (GPC) by derivatizing cellulose into cellulose triphenylcarbamate (CTC) or by directly dissolving cellulose in lithium chloride (LiCl)/dimethyl acetamide (DMAc). The former requires additional derivatization, whereas the latter involves pretreatment, such as solvent replacement. Therefore, an efficient, straightforward, and reliable method to determine the MW of cellulose is crucial.

The MW of cellulose is traditionally measured from the intrinsic viscosity of a dilute solution, as calculated using the Mark-Houwink-Sakurada (MHS) equation and converted into the viscosity-average MW (M_v) and viscosity-average DP (DP_v) (Alexander and Goldschmid 1957; Zhou et al. 2022). The coefficients in the MHS equation vary depending on the type of polymer and solvent used to measure intrinsic viscosity. Even for the same polymer and solvent, the coefficients change when the dispersity (Đ) differs. Generally, the MHS equation is precise only for monodispersed polymers with narrow MW distributions (MWD), not for polydispersed polymers or their mixtures (Manaresi et al. 1988). Koningsveld and Tuijnman emphasized the different MHS equations for polymers with different D values (Koningsveld and Tuijnman 1960). Consequently, the MHS equation was modified by later researchers (Evans et al. 1989). Thus, the D value is important for estimating the MHS equation (Chee 1985). Although M_{y} and DP_{y} can be determined using the intrinsic viscosity, this method does not provide information on the MWD or D. Furthermore, researchers use different coefficients in the MHS equation $([\eta] = K MW^{\alpha})$ for determining the M_v using the intrinsic viscosity, and a complete consensus has not been reached so far. The coefficients of the MHS equation for cellulose using the copper ethylenediamine method have been reported by Klemn et al. (1998) and Łojewski et al. (2010). In particular, the values for constant K were reported as 0.498, 0.395, and 0.334 (Klemm et al. 1998) and the exponential constant α varies from 0.76 to 1 (Łojewski et al. 2010). Thus, the K and α values depend on several parameters of the cellulose, such as the DP range.

Instead of the intrinsic-viscosity method, GPC can be used to directly determine the MW of cellulose dissolved in a solvent. The first successful GPC (Lauriol et al 1987; Pawcenis et al 2015) involved the derivatization of cellulose to increase its solubility in solvents. M_w , M_n , and \tilde{D} (M_w/M_n) can be measured through GPC. This method involves derivatizing cellulose to CTC by reacting it with phenyl isocyanate, solubilizing it in tetrahydrofuran (THF), and measuring the GPC in THF. However, it should be noted that this method carries a risk of losing low molecular weight components during precipitation after the CTC reaction, which can change the molecular weight distribution (Potthast et al. 2015).

The use of LiCl/DMAc to dissolve cellulose is effective for its GPC because it facilitates the direct dissolution of cellulose without derivatization. This dissolution method was demonstrated in 1980 (McCormick and Lichantowich 1980) and was subsequently applied to GPC (Kennedy et al. 1990). Initially, various methods were employed to dissolve cellulose, such as the activation of cellulose by adding it to DMAc and heating at 150 °C (Striegel and Timpa 1995). Moreover, different procedures, such as solvent exchange at 4 °C with water, methanol, and DMAc, followed by dissolution in LiCl/DMAc at 4 °C (Berggren et al. 2003). As such, the challenges associated with the GPC measurements of cellulose dissolved in LiCl/DMAc have been gradually addressed. In 2015, a comparison of the results of the CTC and LiCl/DMAc methods for six types of cellulose, conducted at eight research institutes worldwide, demonstrated the maturity of the LiCl/DMAc measurement method (Potthast, et al 2015).

Ionic liquids, a class of salts with low melting points that allow them to exist in a liquid state, have been shown to dissolve cellulose. The most common examples of ionic liquids are salts of organic cations and organic-inorganic anions. Ionic liquids containing anions with weak H-bond basicity, such as inorganic [BF₄]⁻, [PF₆]⁻, and [Tf₂N]⁻, are not suitable solvents for cellulose. In contrast, ionic liquids consisting of anions with strong H-bond basicity, such as organic [RCOO]⁻ and X⁻(halogen), have cellulose-dissolving ability (Swatloski et al. 2002; Li et al. 2018). Cellulose can be dissolved at high concentrations in ionic liquids, such as 1-ethyl-3-methylimidazolium acetate (EmimOAc). Previous studies reported the dissolution of cellulose at high concentrations (40% and 28%) in EmimOAc (Endo et al. 2016; Rein et al. 2014). The ternary phase diagram of a three-component system comprising cellulose, EmimOAc, and dimethyl sulfoxide (DMSO) revealed that a solution with a cellulose/EmimOAc ratio of 28/72 remained dissolved, even when diluted with DMSO at various ratios (Le et al. 2014). Since 2011, research has focused on the GPC of cellulose using ionic liquids. Fukaya et al. (2011) groundbreaking work initiated the use of ionic liquids in GPC. However, this technique introduces a challenge owing to the elevated viscosity, which can increase the pressure in the GPC column and decrease the elution flow rate. Low GPC flow rates require several hours to achieve a comprehensive analysis. To overcome this problem, the incorporation of a cosolvent into the cellulose/ ionic liquid dissolution system was proposed. Subsequent methodologies included the dissolution of cellulose in EmimCl/DMSO (1:1) with DMSO as the eluent (Ohno and Miyafuji 2013), utilization of 10-20 wt% EmimOAc/dimethyl formamide as the eluent (Engel et al. 2012), and introduction of 10–20 mg/mL tetrabutylammonium fluoride/DMSO (Rebière et al. 2017). Zhou et al. (2022) developed an eluent comprising 1-butyl-3-methylimidazolium acetate/DMSO/ DMAc (1/1/18) with 5 wt% ionic liquid.

To reduce the pressure in high-performance liquid chromatography (HPLC) systems and the operational costs associated with GPC owing to the use of expensive ionic liquids, the concentration of ionic liquids should be minimized. Cellulose degradation occurs at elevated temperatures in both LiCl/DMAc (Potthast et al. 2002) and ionic liquid (Ikeguchi et al. 2020a, b). Therefore, in GPC, heating to dissolve the cellulose should be avoided. To achieve this objective, a straightforward approach involving reduced solvent immersion is recommended.

In this study, we developed a GPC method for measuring the M_w , M_n , and M_w/M_n of different cellulose types using a small amount of ionic liquid as the

dissolution solvent and GPC eluent. We demonstrated the easy dissolution and accurate measurement of different cellulose types, such as microcrystalline cellulose, regenerated cellulose, wood pulp, and cotton linter. Furthermore, this method can obtain the differences in the MWD of each cellulose type. Considering the MWD, a more accurate relationship between the intrinsic viscosity and DP was proposed.

Materials and methods

Materials

Table 1 lists the 10 cellulose types used in this experiment. Microcrystalline cellulose (Avicel) was purchased from Fluka (PH101; Sigma-Aldrich). Lyocell is a cellulose fiber obtained by dissolving cellulose in N-methylmorpholine N-oxide and was industrialized by Lenzing in Austria. Lyocell were supplied by Fujibow (Tokyo, Japan). Rayon, which is a regenerated cellulose fiber, was supplied by Omikenshi (Japan). Cotton Linters 1 and 2 were supplied by Shandong Silver Hawk (China), who provided data on the viscosity and DP_v. Cotton Linter 3 was supplied by Southern Cellulose (CA, USA). Wood Pulp 1 was supplied by Rayonier (USA). Wood pulp samples 2, 3 and 4 were obtained from Borregaard.

EmimOAc was purchased from Sigma-Aldrich in 1-kg packages and used without purification. GPC grade DMSO, LiCl (anhydrous), and DMAc were purchased from Fujifilm Wako Pure Chemicals

Table 1 List of cellulose materials investigated in the present study

Cellulose	Description		
Avicel	Microcrystalline cellulose, Avicel (R) PH101~50 μm particle size. Supplied by Fluka (Sigma-Aldrich)		
Lyocell	Regenerated cellulose fiber obtained from the NMMO process, 1.4 dtex ¹ , 38 mm, supplied by Fujibo (Japan)		
Rayon	Regenerated cellulose fiber, 1.7 dtex, 5 mm, supplied by Omikenshi (Japan)		
Cotton Linter 1	Cotton linter "PCS500", $DP_v = 592$, supplied by Shandong Silver Hawk (China)		
Cotton Linter 2	Cotton linter "PCS800", $DP_v = 733$, supplied by Shandong Silver Hawk (China)		
Cotton Linter 3	Cotton linter "1215", supplied by Southern Cellulose (USA)		
Pulp 1	Sulfite pulp "Fibenier J", intrinsic viscosity = 3.6 dL/g, supplied by Rayonier		
Pulp 2	Sulfite pulp "Derivat Ultra LV", intrinsic viscosity = 270 mL/g, supplied by Borregaard		
Pulp 3	Sulfite pulp "Derivat V-U", intrinsic viscosity = 357 mL/g, supplied by Borregaard		
Pulp 4	Sulfite pulp "Derivat LV", intrinsic viscosity = 600 mL/g, supplied by Borregaard		

1) dtex: decitex (dtex) is the SI unit for the thickness of fibers and yarns. It is defined as the weight in grams with a length of 10,000 m. Decitex is 1/10 of tex (g/1000 m)

(Osaka, Japan) and used without further purification. Pullulan, a GPC standard, was purchased from Shodex (Tokyo, Japan) using a kit (model no. P-82), containing pullulans with eight different M_w values (736,000, 343,000, 202,000, 110,000, 50,600, 23,000, 9900, and 6600 g/mol (in ascending order)). Glucose was purchased from Fujifilm Wako Pure Chemicals (Osaka, Japan). Cellobiose and cellopentaose with MW values of 180, 342, and 828 g/mol were purchased from Tokyo Kasei Industry (Japan). Cu ethylenediamine (1 M) purchased from Sigma-Aldrich was used for the intrinsic-viscosity measurements.

Cellulose dissolution methods (EmimOAc/DMSO)

The cellulose was heated at 105 °C for 6 h under normal pressure and dried before using in GPC. Initially, 5 mg cellulose was weighed into a sample bottle and added with 50 mg EmimOAc. Although EmimOAc began to penetrate the cellulose, a major portion of the white and unpenetrated cellulose remained, which was easily confirmed with the naked eye. The mixture was stirred with a glass rod (5 mm in diameter) by applying force to promote the penetration of EmimOAc into the cellulose. EmimOAc penetrated the cellulose after stirring with a glass rod until a homogeneous phase was achieved. When the mixture was left at 25 °C for an additional 24 h, cellulose steadily permeated into EmimOAc, forming a single phase. The mechanism of cellulose dissolution by ionic liquids involves the disruption of hydrogen bonds in cellulose by the OAc anions of the ionic liquid (Li et al. 2018). Subsequently, 1.5 g DMSO was added to the mixture of cellulose and EmimOAc and magnetically stirred for 24 h. Finally, 3.5 g DMSO was added to the mixture and stirred for 30 min to obtain a cellulose/EmimOAc/DMSO ratio of 0.1/1/100. A 1 mL aliquot of the resulting solution was filtered through a 13 mm diameter syringe hydrophobic polytetrafluoroethylene filter (Product No. SLPT1345NB; Hawaii Scientific) with a pore size of 0.45 µm. The time required to filter the aliquot differed depending on cellulose content. Good cellulose dissolution was observed, and the 1 mL sample solution was easily filtered using just one syringe filter without the need to change in 1 min.

Cellulose dissolution methods (conventional LiCl/ DMAc)

The conventional method for dissolving cellulose in LiCl/DMAc and GPC followed a previously reported procedure (Potthast et al 2015). A household mixer was used in the study. In the mixer, 40 mg cellulose and 40 mL distilled water were added and mixed for 10 s, which was repeated thrice. Subsequently, the mixture was transferred to a 50 mL resin centrifuge tube, centrifuged at 10,000 rpm for 5 min, and decanted to remove the supernatant. Acetone (10 mL) was added to the cellulose in the centrifuge tube, manually shaken, stood for 5 min, centrifuged at 10,000 rpm for 5 min, and decanted to remove the supernatant. This solvent replacement procedure was repeated thrice. Subsequently, a similar solvent replacement step was performed thrice using DMAc. In the original procedure, the number of solvent replacement repetitions was not stated. As such, a single replacement was assumed to be sufficient. Subsequently, 10 mL DMAc was added. The mixture was shaken overnight using a reciprocating shaker at a bath temperature of 25 °C and shaking speed of 50 rpm. The mixture was then centrifuged and decanted. The cellulose was transferred to a 5 mL glass vial, and 2 g of a 9 wt% LiCl/DMAc solution was added. The mixture was stirred with a magnetic stirring bar at 25 °C until the cellulose dissolved. The time required for the cellulose to dissolve after the addition of 2 g of 9 wt% LiCl/DMAc solution varied depending on the cellulose type. Cellulose dissolution was confirmed within 1 h for Avicel and Pulps 1-4. Meanwhile, the dissolution was continued for 24 h for the other cellulose types. Lyocell and Cotton Linters 1-3 completely dissolved after 24 h. Rayon was completely dissolved after 7 d. After dissolving the cellulose, 6 g of DMAc was added to prepare a cellulose dissolution sample with a composition of Cellulose/LiCl/DMAc = 0.5/2.3/100 for the GPC. A 1-mL aliquot of the resulting solution was filtered using a 13 mm diameter hydrophobic polytetrafluoroethylene syringe filter (Product No. SLPT1345NB; Hawaii Scientific) with a pore size of 0.45 µm. Significant cellulose dissolution was observed, and the solution was easily filtered in 1 min using only one filter.

Cellulose moisture content

Information on the moisture content of cellulose is necessary to measure its intrinsic viscosity in a copper ethylenediamine solution. The moisture content was measured using the drying loss measurement method with an infrared heating analyzer. A MS-70 moisture analyzer (A&D, Japan) was used at a heating temperature of 120 °C and a sample size of 1.0 g. Analyses were completed within 5 min.

GPC using EmimOAc/DMSO and LiCl/DMAc

The MW of the cellulose was measured using GPC (Shimadzu HPLC system). A refractive index detector (RID-20A), LC pump (LC-40D), and sample injector (SIL-40C) were used for the analysis, along with Shimadzu LC Solution software. A PLgel 20 µm mixed-A 300-7.5 mm ID column (Agilent) was used with a column oven temperature of 40 °C. Pullulan, glucose, cellobiose, and cellopentaose were used as the standards. For the GPC analysis using EmimOAc/DMSO as the eluent, EmimOAc/DMSO (1/99, w/w) was used at a flow rate of 0.5 mL/min. An injection volume of 100 µL was used for the analysis. EmimOAc (10 g) was mixed with DMSO (990 g), and the mixture was subjected to vacuum degassing for the GPC measurements. The standards were dissolved in DMSO containing 1 wt% EmimOAc (standard/EmimOAc/ DMSO = 0.1/1/100). For the GPC analysis using LiCl/DMAc as the eluent, LiCl/DMAc (1/99, w/w) was used at a flow rate of 0.5 mL/min. LiCl (10 g) was mixed with DMAc (990 g), and the mixture was subjected to vacuum degassing for the GPC measurements. The standards were dissolved in LiCl/DMAc (1/99, w/w). The same column was used for the EmimOAc/DMSO and LiCl/DMAc methods. Solvent replacement in the column was conducted as follows. A 1 wt% LiCl/DMAc solution was passed through a column containing 1 wt% EmimOAc/DMSO. Reverse solvent replacement was performed in the same manner. Three injections for the EmimOAc/DMSO method and two injections for the LiCl/DMAc method were conducted. The average (μ) and standard deviation (σ) values of M_w and M_n were calculated from these N = 3 or N = 2 data points.

Intrinsic viscosity

The intrinsic viscosity of the cellulose was measured according to the ASTM D1795-13 standard test method. Air-dried cellulose with an equilibrium moisture content was used without further drying. The weight of the air-dried cellulose was corrected for moisture content to obtain the weight of the ovendried cellulose. The cellulose concentration in the copper ethylenediamine solution was calculated from the dry cellulose weight. Although ASTM D1795-13 uses a single-point method for measuring intrinsic viscosity to estimate time savings, we used Huggins and Mead-Fuoss plots (Zhou et al. 2022), which measures viscosity at 2-4 concentration levels, to accurately evaluate intrinsic viscosity. Subsequently, 0.01-0.08 g cellulose (0.5-4.0 g/L) was weighed and placed in a 100 mL Erlenmeyer flask; 10 mL distilled water was added with a measuring pipette. Cellulose was magnetically stirred for 10 min and then soaked in distilled water. During stirring, 10 mL of a 1 M copper ethylenediamine solution was gradually added for approximately 1 min, and the cellulose was dissolved by stirring for 30 min to 4 h until the intrinsic viscosity was measured. Cellulose concentration was calculated in g/dL. The flow times of the pure solvent (t_0) and solution (t) were measured using an Ostwald viscometer with a capillary diameter of approximately 0.5 mm. Relative viscosity (η_r) and specific viscosity (η_{sp}) were calculated as $\eta_r = \eta/\eta_0 = t/t_0$ and $\eta_{sp} = \eta_r - 1$, respectively. The reduced viscosity (η_{sp}/c) was plotted against concentration (c), that is, the Huggins plot. The inherent viscosity $(\ln(\eta_r)/c)$ was plotted against concentration (c), that is, the Mead-Fuoss plot. The intrinsic viscosity $[\eta]$ was then calculated using the Huggins and Mead-Fuoss plots. A mixture of 10 mL distilled water and 10 mL copper ethylenediamine solution without cellulose was used as the blank solution. The temperature for the viscosity measurements was adjusted to 25 ± 0.1 °C.

Results and Discussion

Evaluation of cellulose dissolution in 1 wt% EmimOAc in DMSO

Different cellulose types (Table 1) were dissolved in DMSO containing 1% EmimOAc to obtain a cellulose/EmimOAc/DMSO solution with a ratio of 0.1/1/100 (Fig. 1). Figure 2 shows the polarizing microscopy images of Pulp 1 and the cellulose solution after dissolution (cellulose/EmimOAc/ DMSO=0.1/1/100). Cellulose I and II regions disappeared for Avicel, Lyocell, Cotton Linter 1, and Pulp 1 in EmimOAc and DMSO, confirming their dissolution (Fig. 2 and S1). Cellulose was dissolved in EmimOAc (cellulose/EmimOAc=28/72) in different amounts of DMSO to form a ternary cellulose/EmimOAc/DMSO mixture (Le et al. 2014). The cellulose solution obtained using this procedure is in the cellulose-soluble region.



Fig. 1 Photograph of the cellulose dissolved in EmimOAc: **a** dried cellulose (Avicel; 5 mg) and EmimOAc (50 mg) at 0 h and **b** solution after complete dissolution (Avicel/EmimOAc/DMSO=0.1/1/100)

Accuracy of the calibration curve

Pullulan, glucose, cellobiose, and cellopentaose standards were dissolved in DMSO with 1 wt% EmimOAc (standard/EmimOAc/DMSO=0.1/1/100). These standards were analyzed by GPC, and the retention times and common logarithms of M_w ($Log_{10}M_w$) were plotted, as shown in Fig. 3.

Pullulan is the most frequently used standard for the GPC of cellulose, and the linearity of its calibration curves has been previously reported (Patkar et al. 2016). Commercial pullulan with the M_w range of 6,600–736,000 g/mol and eight samples of different MWs was used in the study. Similar linearity was observed in our measurements. The monomers and pentamers of glucose were aligned on the same linear calibration curve as that of pullulan under GPC



Fig. 3 Calibration curve prepared with pullulan standards and cello-oligosaccharides for GPC with EmimOAc/DMSO



Fig. 2 Polarizing microscopy images of the cellulose **a** before and **b** after dissolution in EmimOAc/ DMSO (cellulose/EmimOAc/DSMO=0.1/1/100)

conditions. The permeation limit and exclusion limit are observed, where smaller or larger molecules are not separated by their molecular size in general GPC (Skoog et al. 1997). In our study, the permeation limit was not observed because the interval from an M_w of 720,000 (DP_w: weight-average degree of polymerization = 4545) to that of 6600 ($DP_w = 40$), and from a DP of 1 to 5 were on the same linear calibration curve. Previous studies have reported the creation of calibration curves for GPC by combining standard pullulans with monosaccharides and oligosaccharides. Ikeguchi et al. (2020a, b) demonstrated the elution of standard pullulan, glucose, maltose, maltotriose, and maltoheptaose on a GPC column (Shodex SB-806 M HO) at different retention times. In another study by the same authors (Ikeguchi et al. 2020a, b), a linear calibration curve was proposed using eight standard pullulans and maltoheptaose (1153) at nine points. Patkar et al. (2016) proposed a linear calibration curve at 10 points using 10 standard pullulans, including two (180 and 667) and eight standards above 6100. They used the same column (PLgel 20 µL Mixed-A) as that in our experiments. In particular, the Shodex and PLgel columns used in their study and ours, respectively, are mixed columns consisting of gels with different pore sizes that provided a wide range of separable MWs. The PLgel column was specified in the catalog to separate MWs from 2000 to 40,000,000. However, based on the experimental results, we confirmed separation in the M_w range of 180-736,000. Despite subtle differences in the chemical structures of pullulan, glucose, and oligoglucose, as suggested in previous studies, we verified that the calibration curve was aligned as a straight line. However, the detailed reason for this separation, even for MWs below 2000, remains unclear. It is speculated that the glucose molecules are recognized by the gel to have a MW larger than that calculated from the atomic number owing to the $1,4-\beta$ linkage in the glucopyranose ring and presence of three hydroxyl groups. The chromatograms of all standard samples are shown in Fig. 4.

In a typical commercially available GPC data analysis program, various calibration curves, such as linear, cubic, and quintic equations, can be used. Calibration curves often employ an S-shaped calibration curve to account for the upper and lower size exclusion limits, and often use a cubic equation. In this study, we used a linear calibration curve (Eq. 1) because the eight



Fig. 4 Chromatograms of pullulan standards (M_w 736,000–6600) and cello-oligosaccharides with EmimOAc/DMSO

pullulan standards and three oligosaccharides fall in a single line:

$$\log(Mw) = a rt + b \tag{1}$$

GPC of cellulose

Ten types of cellulose (Table 1) were successfully dissolved in DMSO with 1 wt% EmimOAc (cellulose/ EmimOAc/DMSO=0.1/1/100) and accurately separated by GPC. The constructed MWD from the chromatogram and calibration curve are shown in Fig. 5. Figure 5a-c shows the results for Avicel, Lyocell, Rayon, Cotton Linter, and Pulp, respectively. Avicel exhibits a shoulder peak in the lower-MW region, as noted in the GPC measurements (Denilson et al. 2021). This shoulder peak represents the low-MW cellulose or hemicellulose containing xylose or mannose during the extraction of crystalline cellulose through acid treatment (Denilson et al. 2021). Shoulder peaks are also observed in Lyocell, Rayon, and the pulps. The Cotton Linter curve has small shoulder peaks. The magnitudes of the shoulder peaks differed for different cellulose types. The M_w , M_n , and M_w/M_n values obtained from the GPC measurements and their standard deviations are listed in Table 2 and S1.



Fig. 5 MWD measured by the EmimOAc/DMSO method of the cellulose samples: a Avicel, Lyocell, and Cotton Linter 1; b Rayon and Cotton Linter 2 and 3; and c Wood Pulps (1–4)

Table 2 $\,M_w,\,M_n,\,M_w^{\prime}\!/M_n$ and intrinsic viscosity $[\eta]$ values measured by the EmimOAc/DMSO method

Cellulose	M_w	M _n	M_w/M_n	η (dL/g)
Avicel	77,400	11,600	6.6	1.02
Lyocell	380,000	31,100	12.2	3.34
Rayon	139,000	23,400	6.0	1.53
Cotton Linter 1	317,000	59,500	5.3	3.64
Cotton Linter 2	397,000	72,700	5.5	4.79
Cotton Linter 3	404,000	65,000	6.2	4.42
Pulp 1	315,000	39,000	8.1	3.46
Pulp 2	174,000	19,100	9.1	2.43
Pulp 3	268,000	26,900	10.0	3.16
Pulp 4	524,000	30,400	17.3	5.57

 M_w weight-averaged molecular weight M_n number-averaged molecular weight

Comparison between the MW parameters obtained using the EmimOAc/DMSO and LiCl/DMAc methods

GPC measurements were performed using the conventional LiCl/DMAc method. Ten types of cellulose (Table 1) were dissolved in LiCl/DMAc. All cellulose samples were readily dissolved and analyzed (Table S2 and Fig. 6). The measured M_w , M_n , and M_w/M_n values obtained using the EmimOAc/DMSO and LiCl/DMAc methods and their standard deviations are listed in Tables S1 and S2. The M_w and M_n values for the two methods were compared

(Fig. 7 and S3). The M_w and M_n values obtained using the two methods were also compared by plotting them on horizontal and vertical axes (Figure S4 and S5). The MWD for all cellulose types obtained using the two methods were directly compared, as shown in Fig. 8 (Avicel) and S6 (other celluloses). The MWDs obtained by the EmimOAc/DMSO and LiCl/DMAc methods were comparable for all samples (Fig. 8 and S6). Shoulder peaks in the low-MW region are observed for Avicel, Lyocell, Rayon, and Pulps 1-4 using both methods. However, for Pulps 2 and 4, the intensity of the low-MW peak slightly varies, which can be ascribed to the hemicellulose contents. The M_n values obtained using the EmimOAc/DMSO method tended to be smaller than those obtained using the LiCl/DMAc method (Figure S3 and S5). As the M_n values decreased based on the MWD data, stronger peak intensities in the low-MW region are obtained (Fig. 8 and S6). The MWD and M_w values obtained using the EmimOAc/ DMSO method are comparable to those obtained using the conventional LiCl/DMAc method. In particular, the M_n and M_w/M_n values demonstrate a moderate level of concordance.

In this study, both the EmimOAc/DMSO and conventional LiCl/DMAc methods used standard materials, such as pullulan and sugars, to measure the MW parameters. GPC can measure absolute MWs by applying multiangle light scattering (MALS) method (Potthast, et al 2015).



Fig. 6 MWD measured by the LiCl/DMAc method of the cellulose samples: **a** Avicel, Lyocell, and Cotton Linter 1; **b** Rayon and Cotton Linter 2 and 3; and **c** Wood Pulps (1–4)



Fig. 7 Comparison between the M_w obtained by the EmimOAc/DMSO and LiCl/DMAc method. The error bars represent $\mu\pm\sigma$

Relationship between intrinsic viscosity, M_w , and M_w/M_n

The intrinsic viscosity of the cellulose was determined using the ASTM standard. The Huggins and Mead–Fuoss plots for the 10 cellulose types are shown in Figs. 9 and S2. The results of the intrinsic-viscosity measurements for the various cellulose types are listed in Table 2. The measured intrinsic



Fig. 8 Comparison of MWD of Avicel as measured by the EmimOAc/DMSO and LiCl/DMAc methods

viscosities of Pulp 1–4 are consistent with those provided by the cellulose supplier.

Table 2 presents the M_w and intrinsic viscosity measured by GPC. These values are plotted in Fig. 10. All the samples exhibited a consistent trend in the curve, with Lyocell showing a slightly lower intrinsic viscosity. This conforms to the MHS equation, which represents the relationship between the MW and intrinsic viscosity.



Fig. 9 Intrinsic viscosity $[\eta]$ of the **a** Cotton Linter 3 and **b** Pulp 1. c: concentration



Fig. 10 Relationship between $\rm M_w$ and intrinsic viscosity of the cellulose

The MHS equation is widely used to describe the relationship between the intrinsic viscosity and MW:

$$[\eta] = KMW^{\alpha} \tag{2}$$

As discussed in the Introduction, the MHS equation is precise only for monodispersed polymers. Koningsveld and Tuijnman conducted a mathematical analysis of a disperse polymer and suggested that the intrinsic viscosity can be expressed in terms of M_w and M_w/M_n for a polymer with a log-normal number distribution (Koningsveld and Tuijnman 1960), as shown in Eq. 3. Manaresi et al. (1988) proposed the use of M_w , M_w/M_n and M_z/M_w to express the intrinsic viscosities of polymers with different shape distributions (Manaresi et al. 1988), as shown in Eq. 4. Examples of the monodispersed and log-normal number distributions with narrow (M_w/M_n =1.13) and moderate D values (M_w/M_n =2.0) are shown in Figure S8.

$$[\eta] = K' M_{w}^{a} (M_{w}/M_{n})^{0.5a(a-1)}$$
(3)

$$\left[\eta\right] = K' M_{\rm w}^{\rm a} \left(M_{\rm w}/M_{\rm n}\right)^{\rm b} \left(M_{\rm z}/M_{\rm w}\right)^{\rm c} \tag{4}$$

where a is a coefficient of the viscosity relation: $[\eta] = K MW^a$; b and c are parameters determined by data fitting; and M_z is the Z-average MW. The rest of the variables are as follow:

$$\begin{split} M_n &= \frac{\Sigma(m_i \; n_i)}{\Sigma(n_i)} \quad \text{number} - \text{averaged MW} \\ M_w &= \frac{\Sigma(m_i^2 \; n_i)}{\Sigma(m_i \; n_i)} \quad \text{weight} - \text{averaged MW} \end{split}$$

$$M_{z} = \frac{\Sigma(m_{i}^{3} n_{i})}{\Sigma(m_{i}^{2} n_{i})} \quad Z - averaged MW$$

We did not use the Koningsveld equation because the MWD does not always follow a log-normal number distribution in actual polymers. In particular, the results for Avicel, Lyocell, Rayon, and Pulps 1-4 with shoulder peaks in the low-MW region are far from the log-normal number distribution. Meanwhile, Manaresi et al.'s formula is complex and impractical owing to the inclusion of M_{z} . Performing data fitting with only approximately 10 data points, as in the current experiment, and adding two terms, such as (M_w/M_p) and (M_z/M_p) M_w), to Manaresi et al.'s equation may lead to overfitting. Based on the results of these two formulas, incorporating the M_w/M_n term into the traditional MHS formula to use both the M_w and (M_w/M_n) terms are effective, particularly for samples with a broad MWD or different distributions. Therefore, we attempted to fit the following equation (Eq. 5).

$$[\eta] = K M_w^a (M_w/M_n)^b$$
(5)

where the K, a, and b values were obtained by the multiple regression analysis using the experimental data of $[\eta]$, M_w , and M_w/M_n , as shown in Table 2. The details of the multiple regression analysis are provided in the supporting information.

 $a = 0.891, b = -0.062, K = 5.06 \times 10^{-5}$

$$\left[\eta\right] = K' M_{\rm w}^{\rm a} \left(M_{\rm w}/M_{\rm n}\right)^{\rm b} \left(M_{\rm z}/M_{\rm w}\right)^{\rm c} \tag{6}$$

We successfully predicted the intrinsic viscosity of cellulose samples with wide MWD and various shapes using a simple calculation formula (Eq. 6). As shown in Fig. 11, the measured and predicted intrinsic viscosity are close to the diagonal line, indicating good accuracy in predicting the measured intrinsic viscosity.

Conclusion

We developed a novel preparation method of a cellulose solution without heating using minimal amounts of ionic liquids. Various cellulose types were dissolved in EmimOAc/DMSO=1/100 (w/w) at room temperature. Using this dissolution method, a new GPC method was developed for cellulose preparation. To date, various GPC methods using ionic liquids have been reported; however, the ionic liquid/ cosolvent ratio is at least 5/95. In contrast to previous methods, our new GPC system can reduce such ratio to 1/99, which lowers the amount of expensive



Fig. 11 Measured and predicted intrinsic viscosities $[\eta]$ obtained using the modified MHS equation (Eq. 6)

ionic liquid used and reduces the pressure damage to the GPC column owing to viscosity changes of the eluent.

Comparable MWD and Mw values were obtained using the EmimOAc/DMSO and LiCl/DMAc methods. The bimodal shoulder peak was reproducible. The magnitude of the shoulder peak obtained using the EmimOAc/DMSO method was larger than that obtained using the conventional LiCl/DMSO method. Therefore, the intrinsic viscosity of cellulose with different MWDs can be calculated more accurately by incorporating the results of our GPC measurements, specifically the M_w and M_w/M_n values, into an adapted MHS equation that includes an additional M_w/M_n term.

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Declarations

Conflict of interest The authors declare no competing interests.

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