Mini Review

Current Trends in Lignocellulosic Analysis with Chromatography

Fengbo Sun¹ and Qining Sun²*

¹International Center for Bamboo and Rattan, SFA Key Laboratory of Bamboo and Ratten Science and Technology, PR China
²Department of Chemical and Biomolecular Engineering, University of Tennessee, USA

Abstract

The conversion of lignocellulosic biomass into biofuel and biomaterial is promising for the substitution of fossil resources in energy and material applications. Given the complexity of plant cell wall, the main challenge is to obtain lignocelluloses with high yield and purity. For a better understanding of lignocellulosic biomass, chromatography stands out as a powerful separation method that can support the lab directed research and pilot scale production of biomaterial and biochemical. This paper provides a review on the characterization of cellulose, hemicellulose and lignin along with their derivatives and decomposed sugar monomers, in particular their isolation and purification methods using various specific types of chromatography. Methods with various specific types of chromatography. This review also summarizes different chromatographic methods for obtaining the molecular weights of cellulose, hemicellulose and lignin that have been used in recent years, and highlights future opportunities for the application of those biopolymers.

Lignocellulosic Feedstock for Bioenergy and Biomaterial

The rising global demand for energy and material from unstable and expensive petroleum resources plus concerns about the economy and global warming call for the development of renewable energy sources to replace fossil fuels [1,2]. Since the first-generation biofuels produced from sugar, starch, and oil-seed-based feedstocks have generated serious competition and a debate for food resources. Therefore, second-generation biofuels expanded the feedstocks to lignocellulosic biomass, including agricultural residues, agro-industrial wastes, forestry and energy crops, which are abundant, renewable, and cost-effective non-food resources [3]. Certain oils produced by plants and algae can be used directly as fuel or chemically transesterified to biodiesel. Hydrogen gas can be produced by not only photosynthetic algae and cyanobacteria under certain nutrient- and/or oxygen-depleted conditions but also bacteria and archaea utilizing organic substrates under anaerobic conditions. Other biofuels such as bioethanol and biobutanol can also be produced as organic substrates fermented by microbes under anaerobic conditions [1].

First-generation biofuels can impact the environment, the economy, water overconsumption and pollution, deforestation, biodiversity loss, and social conflict [4]. Most authorities such as the EU-Renewable Energy Directive and Food and Agriculture Organization of the United Nations have become aware of these effects and issued regulations on the production of first-generation biofuels [4,5]. To date, one of the most important liquid forms of biofuel from carbohydrates is bioethanol. The United States became the world’s largest producer of ethanol fuel in 2005, the production of which had increased from 1.63 billion gallons in 2000 to 13.2 billion U.S. liquid gallons (49.2 billion liters) in 2010 and subsequently to 13.9 billion U.S. liquid gallons (52.6 billion liters) in 2011 [7]. Moreover, the minimum volumes of different types of biofuels that must be included in the United States’ supply of fuel for transportation by the Renewable Fuel Standard (RFS) is intended to rise to 36 billion gallons by 2022 [5-7]. Recently, technological advances have boosted the production of cellulosic ethanol and ethanol from plant waste (e.g., corn stover) on a commercial scale with the opening of a $275 million, 25-million gallon per year cellulosic ethanol plant in Emmetsburg, Iowa—a joint venture between POET, an American biofuels company, and the Dutch firm Royal DSM [8]. Poet-DSM will produce cellulosic ethanol from corncobs, leaves, husks, and corn stalks harvested by farmers located within a 30- to 40-mile radius of the plant [9].

In addition to the biofuel production, application of biomaterials from lignocellulosic biomass is also essential to the functioning of industrial societies and critical to the development of a sustainable global economy. Although wood and paper products have already played important roles in the evolution of civilization, there is increasing interest in the improvement of the quality and manufacturing efficiency of high performance lignocellulosic composites. As main biopolymers in plant cell wall, cellulose and hemicellulose along with their derivatives such as cellulose esters, silyl celluloses, cellulose sulfonates, aminocelluloses, cellulose nanowhiskers, have been widely and successfully applied to the practical products including novel film, foam, coating systems membranes, pharmaceuticals, cosmetics, and food [10-12]. However, lignin as the second most
abundant biopolymer on earth is still underutilized in related fuel and material projects [13]. Although lignin combustion helps the papermaking chemical recovery process, as a fuel it is very inefficient, processing less than ¼ as much energy per pound as middle distillate fuels [14].

Lignocellulosic biomass is mostly cell wall material mainly composed of three biopolymers: cellulose (35-50%), hemicellulose (20-32%) and lignin (10-35%) [15,16]. The sources can generally be grouped into six categories: energy crops, agricultural residues, logging residues, mill residues, forest resources, and urban waste. Forest-derived resources include forest residues from harvesting or land conversion, unused mills from wood or pulp processing, waste from urban wood, and debris from construction/demolition. The energy crop category includes lignocellulosic crops such as herbaceous switch grass and woody crop poplar [17]. Cellulose is a linear homopolysaccharide composed of β-D-glucopyranosyl units linked by 1→4 glycosidic bonds with cellobiose as the repeating unit [18]. Cellulose also has a strong tendency to form intra- and intermolecular hydrogen bonds that result in unique ultrastructure of native cellulose in plant. Some of these H-bonds contribute to stiffening the straight chain and promoting aggregation in the crystalline structure (highly ordered regions); the others could form less-ordered (amorphous) regions 13 that result in the change of cellulose crystallinity in lignocellulosic materials according to their origin and acquisition process. Crystalline cellulose displays six various allomorphs (I, II, III, IIII, IVI, and VIIII) with the possibility of conversion from one form to another [19]. Hemicellulose is a collective term used to present a family of polysaccharides found in the plant cell wall with various compositions and structures. Different from cellulose, hemicelluloses are composed of combinations of pentose and/or hexoses, such as xylose, arabinose, mannose, galactose, and glucose, with acetylated groups, uronic acid and 4-O-methyl ethers [20,21].

The hemicelluloses are broadly classified into four general classes of polysaccharide types based on structural differences in the cell wall: xylans, mannans, β-glucans with mixed linkages, and xyloglucans. The main hemicelluloses in softwood are galactoglucomannans and arabinogalacturonan while that in hardwood is glucuronoxylan [20,21]. Highly branched structures and acetyl groups on the polymer chain result in a lack of crystalline structure in hemicellulose. The most abundant sugar monomer in the softwood hemicelluloses is mannose; correspondingly, the most abundant sugar monomer in hardwood hemicellulose is xylose. Xylan is a heteropolysaccharide with a homopolymeric backbone chain of 1,4-linked β-D-xylopyranosyl units. The branches of xylan, which may contain arabinose, glucuronic acid, or the 4-O-methyl ether, acetic, ferulic, and p-coumaric acids, vary depending on their origins [22]. For example, poplar normally contains ~20% hemicelluloses in which O-acetylated 4-O-methylglucuronic acid xylan or glucuronoxylan is the main component. Numerous studies revealed that xylans (15.9 to 22.4%) are the major hemicellulose in all the poplar species in their study, followed by mannans (0.9 to 3.4%). 4-O-methyl-glucuronic acid (4-O-MeGlcA; 2.2 to 2.8%), galacturonic acid (2.3 to 2.8%) and minor amounts of glucuronic acid (0.1 to 0.3%) have been identified as the uronic acids present in poplar [23]. Galactoglucomannan is comprised of (1→4)-linked β-D-glucopyranosyl and D-mannopyranosyl units that are partially acetylated at the C2-OH and C3-OH and partly substituted by (1→6)-linked α-D-galactopyranosyl units [24]. Figure 1 summarizes the monosaccharide of several lignocellulosic biomasses from literature [25-30].

Lignin is the second most abundant biomass component and the primary renewable aromatic resource in nature [13]. Lignin fills the spaces between cellulose and hemicelluloses acting as a resin that bonds the lignocelluloses matrix together. Distinctly different from cellulose and hemicelluloses, lignin is one of the most complex natural polymers synthesized by enzymatic dehydrogenative polymerization of 4-hydroxyphenyl propanoid units [31]. Lignin is composed predominantly of three phenylpropane monomers: p-hydroxyphenyl (H, from p-coumaryl alcohol), guaiacyl (G, from coniferyl alcohol), and syringyl (S, from sinapyl alcohol) units [32]. The composition of lignin varies depending on its origins, [33,34] softwood (i.e., gymnosperm, Scots pine) lignin is predominantly derived from G-type monoligol, in contrast, hardwood (i.e., angiosperm, Populus) lignin is mainly derived from G- and S-type monolignols with trace of H-type monolignol [35]. Softwood generally contains about 25-35% of lignin and hardwood 18-25% of lignin. Grass lignin (e.g., switchgrass) is derived mostly from G- and S-type monolignols with significant amount of p-coumaric and ferulic acid that is involved in cross-linking to lignin and hemicelluloses complex [36]. In addition, hemicelluloses are covalently linked by relative hydrophobic lignin and thereby form those cross-links that are also called Lignin-Cellulose Complexes (LCCs) with heterogeneous structures. LCCs, consisted of phenyl glycoside bonds, esters, and benzyl ethers, are presumed to exist in higher molecular weight lignin fractions which are water insoluble. LCCs in hardwood and grass are composed in part from 4-O-methylglucuronoxylan and arabinino-4-O-methylglucuronoxylan, respectively [37-39]. In contrast, carbohydrate portions are mainly composed of galactomannan, arabinino-4-O-methylglucuronoxylan, and arabinoalactan, all of which linked to lignin at benzyl positions for LCCs in softwood [40,41].

An integrated biorefinery application of the three main biopolymers along with their derivatives and hydrolysates calls for related separation, purification and analytic methods. Chromatographic method is one of the most powerful analytical techniques for the analysis of the type and concentration of carbohydrates and lignin. Thin layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC) are commonly used to separate and identify carbohydrates and lignin, which are separated on the basis of their differential adsorption characteristics, such as their partition coefficients, polarities or sizes, by passing the solution to be analyzed through a
Although the viscometry method provides cellulose DP relatively and gel permeation chromatography (GPC) methods [48]. Commonly used techniques of measuring cellulose DP are viscometry according to the TAPPI T-203 cm-09 method [46,47]. Most holocellulose, and extraction with concentrated sodium hydroxide acid and sodium chlorite and/or peracetic acid to generate the extraction of native biomass, delignification by using glacial acetic cellulose and cellulose derivatives range from 0.75 to 1 [45].

DP can be defined in terms of the number-average DP (DPn), weight-average DP (DPw) and viscosity-average DP (DPv) according to the following equations [45]:

\[
DP_n = \frac{\sum N_i M_i}{\sum N_i}
\]

\[
DP_w = \frac{\sum N_i M_i^2}{\sum N_i M_i}
\]

\[
DP_v = \frac{\sum N_i \eta}{\sum N_i}
\]

where \(N_i\) is the number of moles of a given fraction i with molecular weight \(M_i\), \(M_w\) is the number-average molecular weight, \(M_v\) is the viscosity-average molecular weight, \(M_g\) is the molecular weight of an hydroglucose (162 g/mol), \(\eta\) is viscosity, \(K_m\) is a constant, and the values of \(a\) for cellulose and cellulose derivatives range from 0.75 to 1 [45].

Isolation and purification of cellulose without chain alteration prior to the analysis of cellulose DP normally consist of Soxhlet extraction of native biomass, delignification by using glacial acetic acid and sodium chloride and/or peracetic acid to generate the holocellulose, and extraction with concentrated sodium hydroxide according to the TAPPI T-203 cm-09 method [46,47]. Most commonly used techniques of measuring cellulose DP are viscometry and gel permeation chromatography (GPC) methods [48]. Although the viscometry method provides cellulose DP, relatively quickly and conveniently, it has several limitations, including non-absolute average \(M_v\) values, which depend greatly on solvent and temperature conditions, no clear information concerning the molar mass distribution, and complex metal solutions used with the testing method that can degrade cellulose [49]. In contrast, GPC as a type of size exclusion chromatography (SEC) can provide more detailed information, including \(DP_w\), \(DP_v\) with all three molecular weights (\(M_w, M_v, M_g\)), and the polydispersity index (PDI= \(M_v/M_g\)), which is used to measure the broadness of the molecular weight distribution of a polymer. The relative cellulose molecular weights that GPC provides are based on the molecular weight of well-defined polystyrene standards with varying molecular weights [50]. A list of the DPs of cellulose determined by the nitration method followed by the measurement of viscosity is summarized in Figure 2 [51-55].

Nitration was carried out in a mixture of \(\text{HNO}_3\):\(\text{H}_2\text{PO}_4\):\(\text{P}_2\text{O}_5\) (64:26:10, w/w), and the final product was then used for a nitrogen assay and for the viscosity measurement after fractionation. The specific viscosity of each fraction was calculated from the measurement of the efflux times at decreasing concentrations in a viscometer at 25°C. The intrinsic viscosity was calculated by extrapolation to zero of a plot of specific viscosity over concentration. The average molecular weight of each fraction was estimated by the Mark-Houwink equation [56]. Thus, the distribution pattern of the DP of the cellulose could be determined. This method does not require pre-isolation of cellulose through holocellulose pulping and the base-catalyzed hydrolysis of the hemicellulose [50]. However, the nitration method is rarely used because of uncertainty arising from the possible acid hydrolysis of the cellulose chain during derivatization along with the instability of the derivative. Nitric and phosphoric acids are very dramatic, resulting in the over-hydrolysis of cellulose chains, which has been confirmed by several studies that have shown a significant reduction in the DP of cotton and aspen cellulose by the nitration method [48-50].

To date, cellulose tricarbanilate (CTC) has been a commonly used cellulose derivative for DP measurement by GPC [57]. Cellulose tricarbanilate is commonly performed by the reaction of cellulose with phenyl isocyanate in pyridine as the solvent. The unreacted phenyl isocyanate is quenched by methanol that is then added to the mixture. Afterwards, the mixture is poured into a 3:7 water-methanol mixture to precipitate the cellulose tricarbanilate. The derivatized cellulose is finally purified and dissolved in tetrahydrofuran (THF) for GPC measurement [47,57]. When CTC facilitates the study of cellulose DP by GPC, it has several advantages, including complete substitution, no de-polymerization during derivatization, the stability of the derivative, and solubility and stability in the THF [58]. However, this testing method is only applied to the pure cellulose isolated from samples that are native biomass and biomass after various treatments. Figure 3 summarizes the DP values of several native cellulose samples based on the viscometry technique, [59] which is more adequate for analyzing cellulose DP because of the complexity of the lignocellulosic biomass [59-63]. Cellulose DP ranges from around 1,500 to 4,500, depending on the various origins of biomass such as hardwoods (e.g., poplar and aspen) with a cellulose DP of 3,500 and 4,500, and agricultural residues, which vary within a range of 1,800 to 4,000.

Furthermore, for the molecular weight analysis without cellulose derivatization there are two basic types of direct SEC
molecular weight analysis employing novel solvent mixture of dimethylformamide (DMF) containing 10-20% (v/v) 1-ethyl-3-methylimidazolium acetate (EMIM Ac) has also been developed as cellulose solvent and eluent that eliminates time intensive sample preparation and allows to measure larger sample numbers necessary for in-depth understanding of enzymatic cellulose hydrolysis. This mixture dissolves cellulose and elutes cellulose from the GPC column to evaluate three commonly used cellulose types Avicel, α-cellulose and Sigmacell 101, showing great advantages, such as no requirement of any prior cellulose swelling, activation, or derivatization, reducing the sample preparation time from several days to a few hours [73]. This method can potentially be extended for the measurement of lignin and wood, similar to earlier approaches working with ionic liquids [74,75]. Moreover, in order to obtain the molecular weight distributions of the eluted cellulose, either the system of the column set will be calibrated or the use of a molecular weight sensitive detector will be required. The simplest and widely accepted way to perform the column calibration is to use a relative calibration based on a set of well characterized polymer standards with a narrow distribution. Therefore, poly (ethylene oxides), dextran, and polystyrene standards are widely applied for the organic SEC analysis [76,77].

Fractional purification and DP of hemicelluloses

The isolation of hemicellulose typically begins with a dignified holocellulose followed by an aqueous alkaline extraction [80]. In order to determine the detailed structure without tedious wet-chemistry fractional precipitation, the extracted hemicelluloses can be further fractionated and purified by using chromatographic techniques, including the size-exclusion chromatography and anion-exchange chromatography. Alumina, carbon, cellulose, diethylaminemethyl (DEAE) cellulose, and ion-exchange resins are the common column packings to fractionate the hemicelluloses, of which DEAE derivatized cellulose has been successful used to fractionate the water- and alkali-soluble hemicelluloses from dewaxed sugarcane bagasse [81]. Compared with cellulose, hemicelluloses are more easily fractionated by SEC according to molar mass or more correctly according to hydrodynamic volume due to their good solubility either in aqueous and aprotic organic solvents. Most hemicelluloses have relatively low molecular weight with a DP no more than 400-500. However, several drawbacks, such as the aggressive property to destroy the packing of the column and the time-consuming preparation, hindered the further use for the undissolved cellulose with longer molecular chain length [64,65]. For the SEC in aprotic solvents, DMAc/LiCl was widely used as the solvent of choice for direct analysis of cellulose in the study of wood pulps of different origin [66]. However, studies found that DMAc/LiCl caused (1) aggregation of cellulose in the solution, which is dependent on the concentration of cellulose or LiCl; (2) incomplete dissolution of certain cellulose samples, such as tunicate cellulose and soft wood bleached kraft pulps; and (3) detrimental degradation of cellulose upon heating during dissolution [67,68]. The application of lithium chloride/1,3-dimethyl-2-imidazolidinone (LiCl/DIM) has exhibited great advantages over DMAc/LiCl, such as stability of cellulose and solubility of tunicate cellulose and kraft pulps [68-72].

In brief, viscometry and GPC are the two commonly used techniques to measure DP of cellulose, by which the viscosity average DP (DPv), the number average (DPn) and weight average DP (DPw) can be provided respectively. However, given the complex structure of lignocellulosic materials, the future DP measurement techniques need to meet requirements that can both fractionate biomass so that cellulose can be extracted, dissolved, and analyzed for DP values without derivatization, and minimize alteration of the native structure of cellulose [78,79].

Lignin isolation and molecular weight analysis

Characterization of lignin molecular weight has been studied using a pool of instruments, including vapor pressure osmometry (VPO), ultra filtration, light scattering (static and dynamic), mass...
To achieve quantitative saccharification of wood that is also used for fermentation and dissolution of cellulose and lignin, one of the best methods is the use of diluted acids to release most of the sugar residues. This method facilitates the separation of lignin intact, since too high temperatures resulted in the side reaction of the cell-wall structure, leading to slower hydrolysis rates. Equipment maintenance due to the corrosive problem by strong acids was another issue, as salts formed during the neutralization step along with high cost of equipment maintenance. Derivatization of sugars after produced from biomass through an acidic hydrolysis pathway, including neutralization of sugar-containing hydrolysate, filtration of formed salt if mineral acids were used as catalysts, and/or evaporation of organic acid if used. Thereafter, the monomeric sugars can be separated to different sugar fractions with the aid of chromatographic techniques, in which different types of anionic or cationic exchanger resins facilitate the separation. Cellulose hydrolysis with concentrated mineral acids generates abundance of monomeric sugars that are more sensitive to further reactions.

The most abundant sugars in nature consist of D-glucose, D-fructose, D-galactose, and D-mannose (hexoses), as well as D-xylose, L-arabinose, and D-ribose (pentoses). Sugar production from acid and enzymatic hydrolysis has been investigated for centuries [2]. There are several steps required to obtain pure sugars after produced from biomass through an acidic hydrolysis pathway, including neutralization of sugar-containing hydrolysate, filtration of formed salt if mineral acids were used as catalysts, and/or evaporation of organic acid if used. Thereafter, the monomeric sugars can be separated to different sugar fractions with the aid of chromatographic techniques, in which different types of anionic or cationic exchanger resins facilitate the separation. Cellulose hydrolysis with concentrated mineral acids generates abundance of monomeric sugars that are more sensitive to further reactions.

Carbohydrate Analysis and Sugar Monomers Separation

The most abundant sugars in nature consist of D-glucose, D-fructose, D-galactose, and D-mannose (hexoses), as well as D-xylose, L-arabinose, and D-ribose (pentoses) [96]. Sugar production from acid and enzymatic hydrolysis has been investigated for centuries [2]. There are several steps required to obtain pure sugars after produced from biomass through an acidic hydrolysis pathway, including neutralization of sugar-containing hydrolysate, filtration of formed salt if mineral acids were used as catalysts, and/or evaporation of organic acid if used. Thereafter, the monomeric sugars can be separated to different sugar fractions with the aid of chromatographic techniques, in which different types of anionic or cationic exchanger resins facilitate the separation. Cellulose hydrolysis with concentrated mineral acids generates abundance of monomeric sugars that are more sensitive to further reactions.

Table 1: Composition and DP of Hemicellulose in lignocellulosic feedstocks.

| Biomass   | Hemicellulose | Sugar residues | Molar ratio | DP     |
|-----------|---------------|----------------|-------------|--------|
| Aspen     | Glucuronoxylan| 4-O-MeGlcA:Xyl | 9:100       | 101-122|
| Birch     | Glucuronoxylan| 4-O-MeGlcA:Xyl | 5:100       | 101-122|
| Poplar    | Glucuronoxylan| 4-O-MeGlcA:Xyl | 12.5~13.8:100 | 50-300|
| Larch     | Arabinogluconoxylan | Ara:4-O-MeGlcA:Xyl | 10:12:100 | 107-145|
| Spruce    | Arabinogluconoxylan | Ara:4-O-MeGlcA:Xyl | 6:13:100 | 107-145|
| Pine      | Arabinogluconoxylan | Ara:4-O-MeGlcA:Xyl | 10:16:100 | 107-145|

Carbohydrate composition of the hemicellulose

The analysis of carbohydrate and Klasson lignin is a two-step method to saccharify biomass as follows: acid hydrolysis with 72 wt% sulfuric acid at 30°C for 1 h, followed by a secondary hydrolysis using 4 wt% sulfuric acid at 121°C for 1 h in autoclave. Oligosaccharides were formed at the first stage, whereas at the second stage, monomeric sugars were obtained as products. Alternatively the second hydrolysis can also be performed with 1 wt% sulfuric acid to produce xylose and arabinose, which are more sensitive to further reactions.

With the development of technology, current bio-processes for producing lignocellulosic ethanol are typically divided into several steps: size reduction of biomass, pretreatment, enzymatic cellulose hydrolysis (saccharification), fermentation, and distillation [98], of which various sugar monomers are generated after the enzymatic hydrolysis of cellulose and hemicelluloses. Methodologies for analysis and separation of woody and non-woody sugars have undergone rapid advances in recent years. The older methods used to determine carbohydrate composition of wood and pulp were paper and thin-layer chromatography, which were initially replaced by gas chromatography–mass spectrometry (GC-MS) following derivatization, and later by cation-exchange HPLC coupled with refractive index detection. Recently anion-exchange HPLC with pulsed amperometric detection (PAD) has been found to provide rapid and versatile methods for carbohydrate analysis with great advantages of high sensibility, selectivity and specificity, and easier sample preparation [43,99]. PAD is based on the oxidation of the carbohydrates in multistep potential waveforms applied to Au electrodes in miniature flow-through cells. The separation of sugar monomers is based on the weakly acidic properties of carbohydrates (Table 2) in alkaline solutions. Neutral and acidic carbohydrates are partially or completely ionized at high pH and retained on the column. This technique is also applicable to monosaccharides, disaccharides and oligosaccharides. Furthermore, an improved separation method of carbohydrates utilizing a carbonate-modified anion exchange column was also developed to give an improved separation of monosaccharide with higher accuracy, shorter instrument analysis time, and minimal sample preparation [100]. In this study, a commercially available anion-exchange column that is modified with carbonate prior to analysis and pulsed amperometric detection was developed to alleviate stability concerns common to HPACC separations. The modified column is capable of providing near-baseline resolution of monomeric sugars and sucrose (with isotropic elution) in approximately 5 minutes. Cellobiose and maltose can also be accommodated with a minimal increase in run time.

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Table 2: Dissociation Constants of Some Common Carbohydrates (in water at 25°C).

| Sugar       | pKa   |
|-------------|-------|
| Fructose    | 12.03 |
| Mannose     | 12.08 |
| Xylose      | 12.15 |
| Glucose     | 12.28 |
| Galactose   | 12.39 |
| Dulcitol    | 13.43 |
| Sorbitol    | 13.60 |

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Conclusion and Outlook

This review has outlined various chromatographic methods for the molecular weight determination of lignocellulosic biopolymers and fractionation of carbohydrate hydrolysates. The key parameter in the methods to determine molecular weight of cellulose and lignin is that the isolation and derivatization/solution techniques must not significantly alter their native properties. GPC is one of the best techniques to use for detailed analysis on the molecular weight and/or DP of cellulose, hemicellulose and lignin because it allows analyzing the distribution of the molecular weights and acquiring more insights on the nature of the biopolymer chains. In an effort to resolve the main limitation of all methods- biopolymer solubility, new solvents, like the ionic liquids that were introduced recently in cellulose and lignin chemistry, are also of great interest in the analytical field but still with limited level of application in SEC. Comparative studies of different methods for determining molecular weight and sugar fractionation are still rare. Besides, developing a molecular weight measurement technique that can minimize alteration of the native structure of cellulose and lignin is a major future need in the field of biofuel and biomaterial.

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