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Size exclusion chromatography of lignin: The mechanistic aspects and elimination of undesired secondary interactions

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ABSTRACT

Characterization of lignin and its degradation products, more specifically determination of their molecular weight (MW) distribution, is essential for assessment and applications of these potentially renewable phenolics. Several representative gel filtration and gel permeation systems were evaluated in this work focusing on understanding of undesired secondary non-SEC interactions while utilizing four sets of commercially available polymeric standards as well as low-MW lignin model compounds including diarene standards synthesized in-house. The gel permeation column with a nonpolar highly cross-linked porous polystyrene/divinylbenzene-based stationary phase provided the most effective separation by MW for both low and high MW model compounds. Notably, the column with a higher pore and lower particle size provided a better resolution towards polymeric standards, even though the particle size effect was downplayed in the earlier SEC studies of lignin. For two other evaluated gel filtration and gel permeation columns, the separation was strongly affected by functionalities of the analytes and correlated with the compounds' pK_a rather than MW. We showed that the separation on the stationary phases featuring polar hydroxyl groups led to specific column-analyte secondary interactions, perhaps based on their hydrogen bonding with lignin. Further, the SEC column evaluation yielded similar results with two sets of chemically different standards. This setup may be used as a general approach to selecting an applicable column for lignin SEC analysis. We confirmed the obtained results with a different independent method implementing a novel approach for lignin number-average MW (M_n) calculation based on laser desorption/ionization time-of-flight mass spectrometry (LDI-TOF-MS) data. The determined M_n corroborated the SEC results.

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1. Introduction

Lignin, one of the most abundant biomacromolecules, occurs in plant cell walls [1] and contributes up to 20 and 30% of the dry weight of hardwood and softwood, respectively [2,3]. As of 2017, paper and pulp industries have produced over 70 million tons of lignin as a by-product annually [4]. Being a renewable material, lignin is a potential source of liquid fuel and valuable phenolic chemicals [5–7]. To transform lignin into these commercially

beneficial substances, various thermal, catalytic and biological degradation processes are being tested. However, evaluation of lignin degradation efficiency is challenging due to the lack of a reliable and accurate method for lignin and its degradation product characterization, particularly, accurate molecular weight (MW) determination [8–11].

Both alkali and Indulin ATTM (softwood kraft) lignins were selected as target analytes in this study as they are among the most abundant waste products of the pulp and paper industry, with millions of tons being generated every year [12].

Currently, the most common method for lignin MW determination is size exclusion chromatography (SEC) with either organic (gel permeation chromatography, GPC) or aqueous (gel filtration chromatography, GFC) mobile phases [7,10,13]. Yet, it has been reported that the observed MW values strongly depend on the SEC analysis setup, lignin pretreatment and data handling [14,15]. The

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Table 1
Indulin AT lignin molecular weight determined by size exclusion chromatography (SEC) reported in literature.

M_n	M_w	PDI	Method			Reference (year)
			Stationary Phase	Mobile Phase	Standards	
530	4290	8.1	GFC			
1900	6900	3.6	Hydroxylated methacrylate (TSKgel GMPWxl)	Aqueous sol. 0.5 M NaOH	PSS Na ^a	(2016) [18]
2600	5500	2.1	NR ^b	Aqueous-based	PSS Na	(2013) ^c
			Sulfonated styrene-divinylbenzene copolymer-network	Aqueous sol. 0.1 M NaOH	PSS Na	(2014) [17]
			GPC			
656	6549	10.0	Cross-linked styrene-divinylbenzene copolymer (Styragel)	Tetrahydrofuran	PS ^d	(2016) [19]
1580	6060	3.8	NR ^b	Lithium chloride/dimethylacetamide	Pullulan	(2011) [16]

^a PSS Na: sodium polystyrene sulfonate.^b Analysis details were not reported.^c MeadWestvaco Indulin AT™ lignin used in this study. Its properties were provided by the supplier.^d PS: polystyrene.

uncertainty in the reported MW values (see Table 1 for Indulin AT lignin) [16–22], with the number-average (M_n) and weight-average (M_w) values varying within a range of 530–2600 Da and 3500–6549 Da, respectively, stresses the challenge in lignin MW characterization, thus hindering a progress in the development of an effective approach to lignin degradation.

Accurate MW determination may address several notable inconsistencies in lignin characterization. Some of the studies suggest a high polydispersity index (PDI) defined as an M_w/M_n ratio being as high as 8 or even 10, while the supplier provided the values of 1900 Da and 6900 Da for M_n and M_w , respectively, resulting in a lower PDI of 3.6 (Table 1). In contrast, the other application of GFC with an aqueous-based mobile phase suggested a low heterogeneity of Indulin AT lignin with a PDI of 2.1 [17]. One of the pioneering studies characterized Indulin AT lignin using vapor pressure osmometry and sedimentation equilibrium having reported the values of 1600 Da and 3500 Da for M_n and M_w , respectively [22]. Thus, lignin's MW is likely sample-dependent and may vary from one batch to another, so a comprehensive characterization of a representative sample from every batch of lignin is essential, even for lignin of the same type.

The main uncertainty of SEC when analyzing lignin and its decomposition products is that the separation is not driven exclusively by size exclusion but also by other interactions occurring due to lignin's heteropolymer nature and variety of its functional groups [7–9]. This problem may be amplified when the polymeric standards different from lignin chemical structure are used, e.g., polystyrenes (PS). Such standards cannot emulate the non-size-specific interactions and thus may result in a misleading SEC calibration, yet they are frequently used as 'universal' standards.

In order to minimize the uncertainty of SEC when analyzing lignin, it is essential to elucidate the nature of unwanted secondary interactions and justify the choice of the stationary phase and calibration standards employed. This would lead to a proper selection of such analysis conditions that selectively promote the size-driven separation mechanism and reliable calibration. Similar studies focusing on the non-SEC interactions were conducted for proteins [23] and polysaccharides [24] but not for lignin. Lambrecht et al. discussed the impact of co-solvents in extraction media when separating proteins and suggested an optimal medium for analysis of various protein mixtures, avoiding any non-SEC effects [23]. He et al. stressed the effects of stationary phase material and pore size on the efficiency of size-based separation of polysaccharides in reversed-phase high performance liquid chromatography (RP HPLC) [24].

Unlike those polymeric analytes, a well-defined series of standards is not available for lignin. This highly heteropolymeric matrix features a rather different suite of unwanted secondary column

interactions, whose detailed analysis is yet to be provided. An additional poorly explored aspect of lignin SEC is the influence of pore and particle size arising from two conflicting effects, thermodynamic and kinetic, that may lead to opposite trends in separation [25].

One of the approaches to overcome the uncertainty in SEC results was the application of a multi-angle laser light scattering detector in combination with SEC to verify MW [26–31], however, the resulting MW values might be skewed by polymer association and interference due to lignin's fluorescence [32,33]. Another problem is the low lignin solubility in a vast majority of solvents. To address this limitation, lignin is often acetylated [15], yet acetylation may contribute to the undesired polymer MW increase, which depends on the number of hydroxyl groups subjected to acetylation, and may differ for various samples [33]. Lange et al. suggested introducing an additional abundance-weighted direct correction factors to account for the system-inherent bias [33]. This factor accounted for both the sample type and derivatization characteristics and resulted in lower MW values. The precision and accuracy of this corrected GPC-based determination of acetylated and aceto-brominated lignin M_n was assessed with an independent (nuclear magnetic resonance) NMR-based M_n determination.

Another common method for lignin characterization is mass spectrometry (MS) with matrix-assisted laser desorption/ionization (MALDI). Whereas MALDI MS was widely employed for structural characterization of lignin and mainly its degradation products [34–41], only a few studies focused on the determination of lignin MW. Yet suppression of the ionization of high MW species may take place thus skewing the obtained MW distribution [39,42]. Fractionation of lignin by SEC prior to MALDI MS was demonstrated to improve the mass spectrum quality [43]. Altogether, MALDI MS may serve as an independent and complementary method to SEC for the determination of lignin MW distribution.

In the present study, we evaluated the SEC application to lignin with a focus on undesired secondary non-SEC interactions using a broad range of standards of high and low MW including those featuring lignin functionalities. We demonstrated the feasibility of size-based separation and accurate MW determination using the standards of varied chemical structure. The determined average MW of lignin utilizing the optimal separation system was compared to that obtained by laser desorption/ionization time-of-flight mass spectrometry (LDI-TOF-MS) with no matrix used. To enable lignin MW determination with LDI MS, a NIST approach was adopted and successfully applied. Furthermore, we investigated the effect of commonly used lignin acetylation on its MW and elution profile on the GFC and GPC stationary phases underlining the limitations of using lignin acetylation.

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