

Mapping of the Hydrophobic Composition of Lignosulfonates

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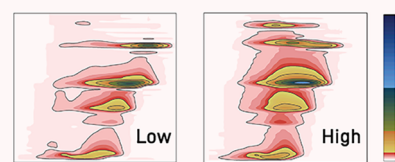


Supporting Information

ABSTRACT: Lignosulfonates are industrial biorefinery products that are characterized by significant variability and heterogeneity in their structural composition. Typically, they exhibit high dispersities in molar mass (molar mass distribution—MMD) and in functionalities (functionality-type distribution—FTD), which crucially affect their material usage. In terms of FTD, state-of-the-art lignin analytics still rely mainly on the determination of functional group contents, which are statistical averages with limited explanatory power. In contrast, our online hydrophobic interaction chromatography—size-exclusion chromatography 2D-LC approach combines the determination of both MMD and FTD in a single measurement to provide a comprehensive picture of the characteristic composition of industrial lignosulfonates—information hitherto inaccessible by state-of-the-art lignin analytics. In this way, the complex interrelationships between these two important structural parameters can be studied in an unprecedented manner. In this study, we reveal the considerable differences in terms of hydrophobic composition and its dispersity present in a range of different industrial lignosulfonates—data desperately needed in tailoring and refining of lignosulfonate composition for material usage.

KEYWORDS: lignosulfonate, lignin, amphiphilicity, hydrophobic interaction chromatography (HIC), size-exclusion chromatography (SEC), two-dimensional liquid chromatography (2D-LC), molecular weight distribution, functionality-type distribution (FTD), charge-to-size ratio, dispersity

online HIC-SEC 2D-LC of lignosulfonates



Dispersity in hydrophobic composition

INTRODUCTION

Lignosulfonates are derivatives of natural lignin polymers. Their common feature is the sulfonation of an otherwise hydrophobic lignin backbone, which allows for water solubility at any pH. Their inherent amphiphilic character—induced by the strongly polar sulfonic acid groups—makes them attractive for any applications exploiting surface-active properties (e.g., dispersing agents, colloidal stabilizers, detergents, surfactants, etc.).^{1–5} Lignosulfonates are important co-products from the sulfite pulping process and, as such, represent technical products with a certain structural diversity. Above all, the later lignosulfonate structure is strongly influenced by the selected plant source material for pulping, as the natural lignin structure varies depending on the respective plant species.⁶ Substantial differences exist particularly between hardwood and softwood species.^{6,7} Besides the variability of native lignins, the scope of structural changes and the extent of sulfonation are strictly regulated by the applied process parameters of the respective pulping plant. Currently, there are several different sulfite pulping processes in use: all are designed for the optimal digestion of the respective plant source material to achieve the desired pulp quality for the later cellulosic product (i.e., pulp, fiber, paper, or tissue). Therefore, process conditions and process intensities can vary widely between different pulp plants. Sulfite pulping processes can be distinguished by the used counter ion (Ca^{2+} , Mg^{2+} , Na^+ , or NH_4^+) and process pH (acidic, neutral, or alkaline), resulting

in grave changes to the pulping reaction mechanisms and, thus, inflicting changes in the native lignin structure.^{2,8,9}

For all these reasons, lignosulfonates range broadly in their structural composition, most notably in molar mass and the degree of sulfonation.^{1,2} Moreover, each lignosulfonate displays a dispersity in these characteristics, thereby constituting a complex polymer mixture with a distribution in size (molar mass distribution—MMD) and in functionality (functionality-type distribution—FTD). Therefore, lignosulfonates are considered as a combination of components with different charge-to-size ratios. Since changes in the inherent amphiphilic character of lignosulfonates have an immediate impact on their performance as surface-active agents, having a clear picture of the hydrophobic composition of lignosulfonates is critically important for the efficient and effective use of these valuable natural products. A comprehensive characterization of complex polymers like lignosulfonates requires multidimensional approaches to unravel the actual structural composition, which is typically accomplished by coupling of liquid

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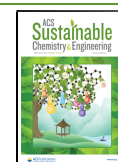


Table 1. List of Lignosulfonate Samples

nr.	abbrev.	wood source	process ion	comment	commercial
1	NSSCEu#1	HW	Na	NSSC eucalyptus	
2	NSSCBe#2	HW	Na	NSSC beech	
3	HWMg#3	HW	Mg	beech	
4	HWNa#4	HW	Na	Sigma-Aldrich (#471011)	yes
5	SWMg#5	SW	Mg		
6	SWMgO ₂ #6	SW	Mg	O ₂ -delignification effluent	
7	SWMg#7	SW	Mg		
8	SWNa#8	SW	Na		yes
9	SWNa#9	SW	Na		yes
10	SWNa#10	SW	Na	Roth (#8999.1)	yes
11	SWCa#11	SW	Ca	Roth (#8208.1)	yes
12	SWNH ₄ #12	SW	NH ₄		yes

chromatography (LC) methods or hyphenation with specific detection systems.^{10–17} For lignosulfonates, determination of the MMD has been routinely implemented by size-exclusion chromatography (SEC), while the FTD is still limited to average values obtained by spectroscopic or wet chemical methods.^{3,18,19} So far, more detailed information on the FTD has only been gathered through preparative sample fractionation using solvents or ultrafiltration and subsequent characterization of the fractions.^{20–23} LC methods that aim to resolve analytically the FTD of lignosulfonates or lignins in general have only recently been pioneered, with hydrophobic interaction chromatography (HIC) being among the most promising approaches.^{24–31} In HIC, the adsorption of solutes to a weakly hydrophobic stationary phase is favored by a high salt concentration in an aqueous mobile phase. Afterward, the elution of solutes is facilitated by a decrease in the salt concentration and/or the addition of modifiers (e.g., alcohols, detergents, and chaotropic salts). In this way, it can also be interpreted as a variation of gradient elution LC, which is a commonly used technique in the multidimensional LC of polymers.^{14,32} In HIC, the separation of lignosulfonates is influenced by both the degree of sulfonation and molar mass. Hence, hyphenation with aqueous SEC seems to be predestined to resolve the MMD and FTD present in lignosulfonates in combined two-dimensional (2D) measurements.²⁹ The resulting 2D plot is a characteristic fingerprint of the lignosulfonate's hydrophobic composition and amphiphilic character.

Our aim was to attempt the online coupling of HIC and aqueous SEC for an in-depth characterization of both the molecular weight distribution and the FTD—and thus the inherent amphiphilic character—of lignosulfonates. This study provides data on the hydrophobic composition of different lignosulfonates in a comparable and reliable way, which is so far missing from the existing literature. Ultimately, we attempted to achieve an in-depth analysis beyond the state-of-the-art characterization of lignosulfonates and to gain knowledge on the complex interplay between functionality and molar mass, which has a crucial impact on the properties of lignosulfonates.

MATERIALS AND METHODS

Lignosulfonates. In total, 12 lignosulfonates were subjected to online 2D-LC measurements (see Table 1). Except for NSSCEu#1 and NSSCBe#2, sample purification was carried out according to Sumerskii et al.³³ using Amberlite XAD-7 (20–60 mesh), a macroporous polyacrylate resin, and Dowex 50WX8, a strongly acidic cation exchange resin. Both resins were obtained from Sigma-Aldrich

and pretreated as described by Sumerskii et al.³³ The purification process removes carbohydrate-derived and inorganic byproducts of the sulfite-spent liquor.

HWMg#3, SWMg#5, and SWMg#7 were isolated from industrial sulfite-spent liquors. SWMgO₂#6 is related to SWMg#5 but was isolated from an oxygen delignification effluent. Although HWNa#4, SWNa#8, SWNa#9, SWNa#10, SWCa#11, and SWNH₄#12 are commercially isolated lignosulfonates, resin extraction was used for further purification (i.e., inorganics, etc.).

NSSCEu#1 and NSSCBe#2 were subjected to ultrafiltration for purification, carried out using a 200 mL ultrafiltration cell (Amicon, model 8200, Merck Millipore, Billerica, U.S.A.) and a Ultracel regenerated cellulose (RC) membrane from Merck Millipore (Billerica, U.S.A.) with a cutoff of 1 kDa (thickness: 230 μm; diameter: 63.5 mm). Filtration was performed in deionized water under nitrogen (2.5–3.0 bar) at room temperature.

First Dimension: HIC. Mobile-phase composition was adopted from Musl et al.²⁹ and Ekeberg et al.,^{28,29} the eluents being 0.5 M ammonium sulfate [(NH₄)₂SO₄] in water (eluent A), ethanol/water (20% v/v; eluent B), and 2-propanol/water (40% v/v; eluent C). Water was purified with a lab water purification system (Milli-Q, Merck). (NH₄)₂SO₄ (extra pure), ammonium hydroxide (20–30%; NH₄OH), and 2-propanol (HPLC grade; I-prop) were purchased from Th. Geyer GmbH (Germany) and used without further purification. Ethanol (absolute, HPLC grade; EtOH) was purchased from Roth GmbH (Germany).

A 0.5 M (NH₄)₂SO₄ solution (eluent A) was prepared by dissolving 66 g of ammonium sulfate in 700 mL of water. A 1 M NH₄OH solution was used to adjust the pH to 10.0, prior to adding water up to 1 L. Eluent B was prepared by mixing 200 mL of ethanol with 800 mL of water (pH 10, set with 1 M NH₄OH). Eluent C was prepared by mixing 400 mL of 2-propanol with 600 mL of water.

Instrument Setup in HIC. The following setup was used in analytical HPLC: an Agilent Technologies 1260 Infinity quaternary pump G1311B; an Agilent Technologies 1260 G1229B autosampler; an Agilent Technologies 1100 G1316A column compartment (temperature 30 °C); RESOURCE ETH, a pre-packed column with a Source 15ETH (6.4 × 30 mm, bed volume 1 mL) (GE Healthcare Europe GmbH, Freiburg, Germany); and an Agilent Technologies 1260 G1315D diode array detector. The signal at 280 nm (4 nm bandwidth; reference wavelength at 360 with 100 nm bandwidth) was used for data evaluation.

The flow rate profile and elution program were adopted from Musl et al.²⁹ (see Table S1). For sample preparation, 35–45 mg of lignosulfonate was dissolved in eluent A, shaken overnight, and finally filtered through a 0.45 μm RC filter. NSSCBe#2 and SWMgO₂#6 were dissolved in 0.1 N sodium hydroxide (NaOH) due to their limited solubility in eluent A. The injection volume was set to 25 μL, that is, an injected mass of 0.875–1.125 mg.

Second Dimension: Aqueous SEC. In SEC, a 0.1 M NaOH solution containing 0.3 M NaCl was used as the mobile phase. For eluent preparation, 4 g of NaOH and 17.5 g of NaCl were dissolved in

1 L of water (Milli-Q). NaOH (pellets, >99.5%) and NaCl (>99.5%) were purchased from Merck (Germany).

Instrumental Setup in SEC. The HPLC system consisted of an Agilent Technologies 1100 isocratic pump G1310A (flow rate: 4 mL/min); a 100 μ L volume transfer valve; a PSS SECurity column compartment (temperature: 35 °C); an MCX HighSpeed column (50 \times 20 mm, 5 μ m, 1×10^3 Å; Polymer Standard Service, PSS GmbH, Mainz, Germany); and an HP1050 variable-wavelength UV detector. For data evaluation, the signal at 280 nm (4 nm bandwidth; reference wavelength at 360 with 100 nm bandwidth) was used.

Polystyrene sulfonate (PSS) sodium salt standards (0.697–679 kDa) were used as reference materials for column calibration (Polymer Standard Service, PSS GmbH, Mainz, Germany). Here, 1.5–2 mg of standard was dissolved in the eluent, shaken, and filtered through a 0.45 μ m RC filter. Then, 100 μ L was injected. The calibration plot is shown in Figure S1.

For lignosulfonates, 2–3 mg of sample was dissolved in 1 mL of eluent, shaken overnight, and filtered through a 0.45 μ m RC filter. Then, 100 μ L was injected. The chromatogram was evaluated between minutes 6 and 13 (corresponding to 24 and 52 mL elution volume, respectively).

For 2D experiments, an eight-port tandem switching valve with two 100 μ L loops for sample transfer to the second dimension was used. For every two minutes, a SEC run was started.

Software PSSWin GPC UniChrom V.8.10 (Polymer Standard Service, PSS GmbH, Mainz, Germany) was used to control the chromatographic system and data acquisition for both single dimensions and their 2D combination.

Nuclear Magnetic Resonance Spectroscopy. All nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance II 400 or a Bruker Avance III HD 400 (resonance frequencies 400.13 MHz for ^1H and 100.63 MHz for ^{13}C) equipped with a 5 mm Observe Broadband probe head or a liquid N_2 -cooled cryoprobe head (Prodigy) with z -gradients at room temperature with standard Bruker pulse programs.

For heteronuclear single quantum coherence (HSQC) experiments, a total of 20–50 mg of the lignosulfonate samples was dissolved in 0.6 mL of dimethyl sulfoxide (DMSO)- d_6 . Chemical shifts were given in parts per million, referenced to residual solvent signals (2.49 ppm for ^1H and 39.6 ppm for ^{13}C). HSQC experiments were acquired in the edited mode with a relaxation delay of 0.5 s using an adiabatic pulse for the inversion of ^{13}C and the GARP sequence for broadband ^{13}C -decoupling, optimized for $^1J_{(\text{CH})} = 145$ Hz. Data processing was performed with Bruker sTopSpin 3.1. Peak assignments were carried out according to the literature.^{34–38} Image post-processing (coloring and sizing) was performed with Adobe Photoshop (Adobe Systems Inc., San José, CA, U.S.A.) for improved clarity.

Molar Mass Determination by SEC–MALS. SEC was carried out on a Dionex UltiMate 3000 with an autosampler, column oven, and UV detector (all Thermo Fisher Scientific, Germany) coupled with an Optilab T-rEX differential refractive index (dRI) detector ($\lambda = 785$ nm) and a Dawn HELEOS II MALS detector with a laser operating at 785 nm, equipped with 18 photodiodes at different measuring angles, every second of them with a narrow band pass filter (± 10 nm) (Wyatt Technology, Santa Barbara, CA, U.S.A.). The analysis parameters were as follows: flow rate (0.5 mL/min); column temperature (35 °C); injection volume (10 μ L); UV detector at 280 nm; and RI detector at 30 °C. Separation was carried out with an Agilent PLgel guard column of 7.5 \times 50 mm and three Agilent PolarGel M columns of 7.5 \times 300 mm (5 μ m particle size) in series. DMSO with 0.5% (w/v) lithium bromide was used as the eluent. Data evaluation was performed with ASTRA software, version 7.3.0 (Thermo Fisher Scientific, Germany). Data processing was carried out as described in Zinovyev et al.³⁹

Samples were dissolved in the SEC eluent (10 mg/mL) at room temperature without requiring derivatization, shaken overnight, and filtered through a 0.45 μ m poly(tetrafluoroethylene) syringe filter before injection.

Functional Group Analysis. Hydroxyl and Carboxyl Groups. Aliphatic hydroxyl, aromatic hydroxyl, and carboxyl group contents were determined by ^{31}P NMR spectroscopy. Sample preparation was adopted from the literature.^{40,41} Specifically, lignosulfonate (30 mg) was dissolved in a 3:1 mixture of N,N -dimethylformamide and pyridine (anhydrous, non-deuterated), phosphitylation reagent (150 μ L of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane), internal standard (4 mg of N -hydroxy-5-norbornene-2,3-dicarboxylic acid imide), and 0.5 mg of NMR relaxation agent, chromium(III) acetylacetonate [$\text{Cr}(\text{acac})_3$]. Spectral evaluation was carried out as described in Korntner et al.⁴⁰

Methoxyl Groups. The methoxyl group content was determined in duplicate according to Sumerskii et al.⁴² In brief, methoxyl groups in the lignin sample were cleaved off using hydroiodic acid and converted into iodomethane (CH_3I), which was then quantified by headspace gas chromatography/mass spectrometry (GC/MS).

Elemental Analysis. The determination of sulfonic acid groups was performed indirectly by elemental analysis at the Department of Geography (Chair of Soil Science) at the Friedrich Schiller University of Jena. Prior to analysis, the samples were thoroughly dried in a vacuum oven at 40 °C and stored under an inert atmosphere. Elemental analysis was conducted as C/N/S analyses on a vario MAX cube elemental analyzer (Elementar Analysensysteme GmbH, Germany).⁴³

Acid Methanolysis. Polysaccharide impurities were determined by acid methanolysis/GC/MS according to literature protocols.⁴⁴ GC/MS analysis was performed on an Agilent 6890N GC and an Agilent 5975B inert XL MSD quadrupole mass selective detector (EI: 70 eV), using an Agilent HP 5MS capillary column (30 m \times 0.25 mm i.d.; 0.25 μ m film thickness) and helium as the carrier gas with a pressure of 0.94 bar, a flow rate of 1.1 mL/min, a split flow rate of 7.5 mL/min, and a split ratio of 7:1.

RESULTS AND DISCUSSION

State-of-the-Art Lignosulfonate Characterization.

The premise of this study was to investigate the compositional differences present in industrial lignosulfonates. For this reason, we compiled a set of lignosulfonates that differ greatly in terms of origin to gain an overview of their prevalent variance in structural composition. Moreover, commercially available lignosulfonates were added to acquire valuable information on current lignosulfonate products.

Commercial lignosulfonate products are often provided without any additional information about the botanical origin or the respective process conditions under which the lignosulfonate is derived. Lignosulfonates are typically provided in their specific salt form, but it remains dubious in some cases (e.g., Sigma-Aldrich and Roth) whether they were really derived from the respective pulping process. Procurement directly from the manufacturer (i.e., a pulp mill) proves to be the better alternative in this respect. In contrast to the applied process conditions, the botanical origin can be ascertained by analytical methods (e.g., HSQC NMR) in retrospect.³⁴ HSQC NMR (see Figure S3) identified NSSCEu#1, NSSCBe#2, HWMg#3, and HWNa#4 as derived from hardwoods due to the dominating presence of syringyl units. SWMg#5 and SWMgO₂#6 showed some minor presence of syringyl units, indicating the proportionate use of hardwoods in pulping. SWNa#8, SWNa#9, SWNa#10, SWCa#11, and SWNH₄#12 exhibited only guaiacyl units and are thus derived from softwoods.

HSQC NMR spectra also function as fingerprints to identify similar lignosulfonates. There are striking similarities in HSQC spectra between some commercial samples (SWNa#10, SWCa#11, and SWNH₄#12), despite their individual designation (i.e., ions). We therefore suspect a common origin of

Table 2. Functional Group Contents and Relative Hydrophobicity of the Studied Lignosulfonates

nr.	sample	SEC–MALS	HS–GC	EA	³¹ P NMR			HIC
		M_w (Da)	OCH ₃ (mmol/g)	SO ₃ H (mmol/g)	aliph. OH (mmol/g)	arom. OH (mmol/g)	carboxyl (mmol/g)	
1	NSSCEu#1	3 670	2.15	1.11	4.38	1.45	0.39	0.05
2	NSSCBe#2	8 650	1.63	1.07	2.93	0.63	0.28	0.09
3	HWMg#3	12 930	5.34	1.47	1.79	2.75	0.22	0.61
4	HWN#4	25 140	4.90	1.86	2.18	2.28	0.18	0.40
5	SWMg#5	39 720	3.88	1.78	2.98	1.83	0.23	0.46
6	SWMgO ₂ #6	64 380	2.64	1.51	2.22	0.74	0.47	0.35
7	SWMg#7	40 020	3.84	1.94	2.09	1.94	0.12	0.52
8	SWNa#8	44 700	3.53	1.95	1.92	2.42	0.30	0.62
9	SWNa#9	54 400	3.51	1.87	1.76	2.29	0.31	0.63
10	SWNa#10	57 280	3.73	1.67	1.98	2.02	0.19	0.62
11	SWCa#11	50 920	3.73	1.70	2.24	2.01	0.19	0.59
12	SWNH ₄ #12	44 040	3.72	1.65	2.19	1.99	0.18	0.60

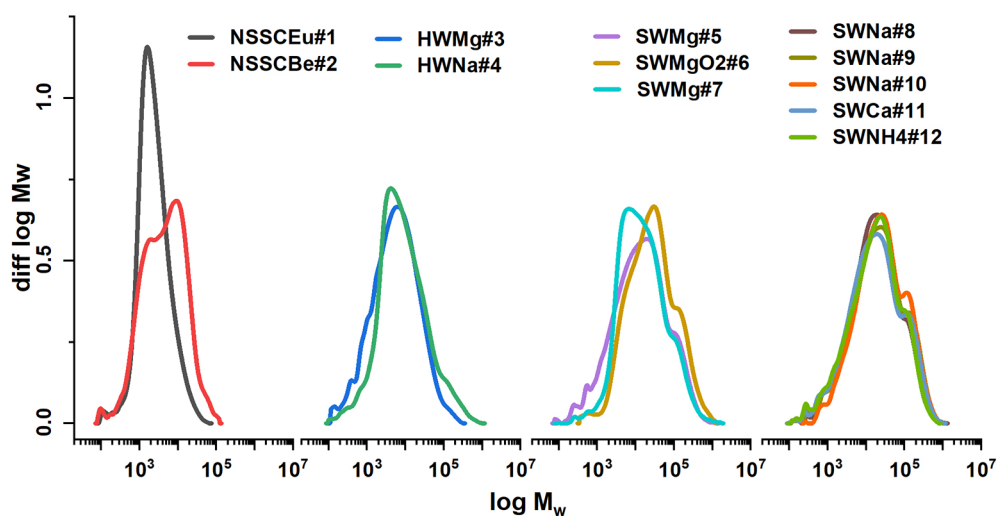


Figure 1. Normalized MMDs of lignosulfonate samples determined by SEC–MALS. Significant differences in the MMD and functional group composition—due to their botanical and/or pulping origin—allowed for classification into four groups.

these lignosulfonates with subsequent refining of their respective ions. Our hypothesis is also supported by the additional analytical data we gathered (see below). HSQC spectra also revealed high amounts of xylans present in NSSC samples (NSSCEu#1 and NSSCBe#2), as a result of ultrafiltration for sample purification. In a follow-up, acid methanolysis of NSSCEu#1 and NSSCBe#2 confirmed the extremely high amounts of carbohydrates (490 and 550 μg per mg, respectively), which accounted for 49 and 55% of the total sample mass, respectively (Figure S2). For this reason, their functional group contents and molar mass data should be treated with great caution. Besides polysaccharide impurities, some samples contained certain amounts of fatty acids (NSSCEu#1, NSSCBe#2, SWMgO₂#6, SWNa#8, and SWNa#9). The aliphatic region (top right corner) also showed some variation, but assignments proved to be unfeasible, as little to nothing is known from the literature about this region for lipophilic lignin impurities.⁴⁵

The MMD and its statistical moments (i.e., number-average molar mass M_n , weight-average molar mass M_w , etc.) exert a significant influence on the physical properties of a given polymer. Hence, molar mass determination by SEC has become a standard analytical method in polymer characterization. In general, the studied hardwood lignosulfonates (i.e., NSSCEu#1, NSSCBe#2, HWMg#3, and HWNa#4) showed

relatively low M_w values ranging between 3.6 and 25.1 kDa compared to softwood lignosulfonates (39.7–64.4 kDa; see Tables 2 or S2), which is in good agreement with the literature.^{46,47} In part, this is related to the shoulder of the high-molar mass range that all softwood lignosulfonates exhibit (see Figure 1). Notably, SWMgO₂#6 (i.e., oxygen delignification effluent related to SWMg#5) showed an elevated shoulder and, thus, increased M_w compared to its liquor counterpart SWMg#5. Dispersity (D) values ranged between 2.4 and 11.4 with a median value of 7.0. A comparison based on the applied (stated) process ion revealed only small differences in M_w for softwood sodium (Na) lignosulfonates (i.e., SWNa#8, SWNa#9, and SWNa#10) but a significantly lower M_w for the hardwood Na lignosulfonate (HWNa#4), with NSSC lignosulfonates showing even lower M_w values. Furthermore, the NSSC lignosulfonate from eucalyptus (NSSCEu#1) showed a more uniform distribution compared to the bimodal distributions of NSSC and Mg lignosulfonates from beech (NSSCBe#2 and HWMg#3; see Figure 1). Likewise, softwood magnesium (Mg) lignosulfonates showed comparable M_w values (i.e., SWMg#5 and SWMg#7)—although their distributions differed to a certain extent (see Figure 1)—whereas hardwood Mg lignosulfonates showed lower M_w values (HWMg#3). Commercially available softwood calcium (Ca; SWCa#11) and ammonium (NH₄; SWNH₄#12) lignosulfo-

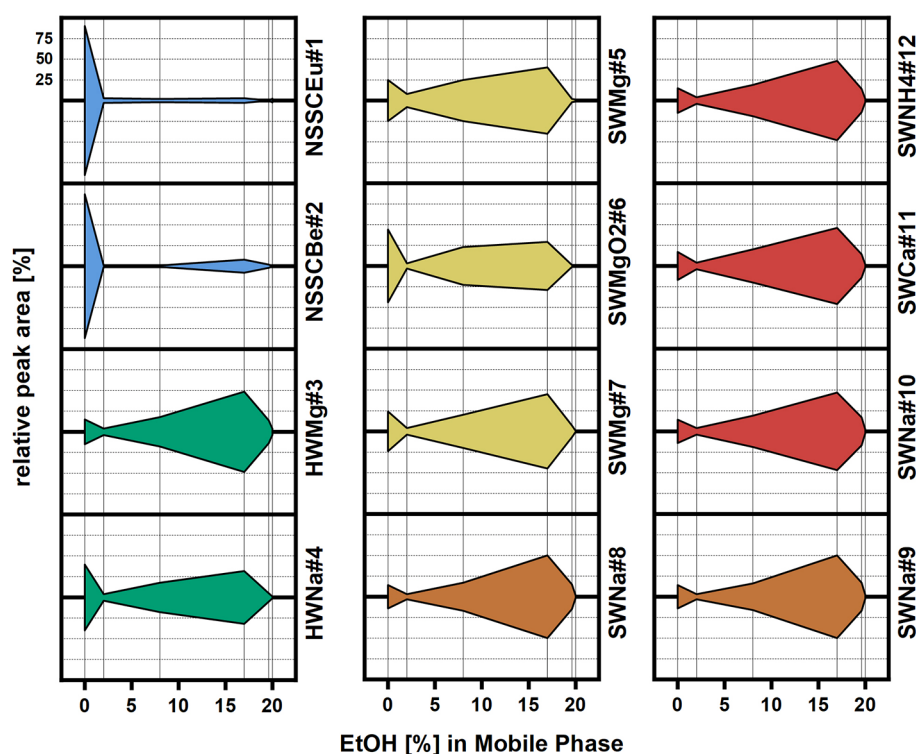


Figure 2. Relative peak areas of the studied lignosulfonates in HIC (based on UV detection in the second dimension) plotted against the EtOH content in the mobile phase. The considerable differences in their elution profiles (i.e., their hydrophobicity) allowed for a classification (indicated by color coding), similar to the combined effort of state-of-the-art methods.

nates showed comparable M_w values to those of softwood Na lignosulfonates, but again, these lignosulfonates may share a common origin with SWNa#10, since their MMDs also exhibit striking similarities (see Figure 1).

The determination of the methoxy (OCH_3) group content essentially verified the botanical assignments made from HSQC: hardwood lignosulfonates showed a considerably higher OCH_3 group content (4.90–5.34 mmol/g) than softwood lignosulfonates (3.51–3.84 mmol/g) due to the presence of two OCH_3 groups per syringyl unit (see Table 2). NSSC lignosulfonates showed a significantly lower OCH_3 group content (1.63–2.15 mmol/g), attributable to their contamination with sugars. SWMgO₂#6 (i.e., oxygen delignification effluent related to SWMg#5) showed a lower OCH_3 group content than its liquor counterpart SWMg#5 (2.64 and 3.88 mmol/g, respectively).

The sulfonic acid (SO_3H) group content exerts a great influence on the polarity of a given lignosulfonate polymer. During pulping, a surge in the SO_3H group content renders the otherwise hydrophobic lignin backbone increasingly hydrophilic until it becomes water-soluble. Moreover, the ratio between the SO_3H group content and molar mass defines the degree of amphiphilicity, which is a key parameter in surface chemistry. For standard lignosulfonates, SO_3H group contents varied between 1.65 and 1.95 mmol/g (see Table 2), which is within the range reported in the literature.^{46,48} NSSC lignosulfonates showed lower contents of 1.07–1.11 mmol/g; however, their content may actually be higher due to their contamination with xylans. HWMg#3 also showed a lower SO_3H group content of 1.39 mmol/g. Again, SWMgO₂#6 (i.e., oxygen delignification effluent related to SWMg#5) showed a lower SO_3H group content than its liquor counterpart SWMg#5 (1.51 and 1.78 mmol/g, respectively).

Also, the natural lignins underlying the lignosulfonates naturally boast a range of functional groups (i.e., hydroxy and carboxyl groups) that may undergo changes in their content during pulping. Of course, these functionalities affect the polarity of a lignosulfonate polymer and, thus, represent an important influence on its solubility. For standard lignosulfonates, the aliphatic hydroxy group contents varied between 1.76 and 2.24 mmol/g, whereas the aromatic hydroxy group contents varied between 1.83 and 2.42 mmol/g (see Table 2). However, certain samples showed considerable deviations: HWMg#3 showed a high aromatic hydroxy group content of 2.75 mmol/g, whereas SWMg#5 showed a high aliphatic hydroxy group content of 2.98 mmol/g. Moreover, SWMgO₂#6 (from the oxygen delignification effluent) showed a very low aromatic hydroxy group content of 0.74 mmol/g. Carboxyl group contents ranged between 0.12 and 0.31 mmol/g with a median of 0.19 mmol/g. Again, SWMgO₂#6 deviated and showed a high content of 0.47 mmol/g. In the case of NSSC lignosulfonates (NSSCEu#1 and NSSCBe#2), the results should be treated with great caution due to their contamination with xylans. Hence, their aliphatic hydroxyl content is certainly overestimated, whereas their aromatic hydroxyl content is underestimated.

In sum, based on standard lignin analytics, the 12 investigated lignosulfonates can be classified into four distinct groups: (1) NSSC lignosulfonates (low molar mass, low sulfonation, and high content of xylans; NSSCEu#1 and NSSCBe#2); (2) hardwood lignosulfonates (low to medium molar mass and low to average sulfonation; HWMg#3 and HWNa#4); (3) Mg softwood lignosulfonates (medium molar mass and average sulfonation; SWMg#5 and SWMg#7); and (4) Na and other commercial softwood lignosulfonates (high

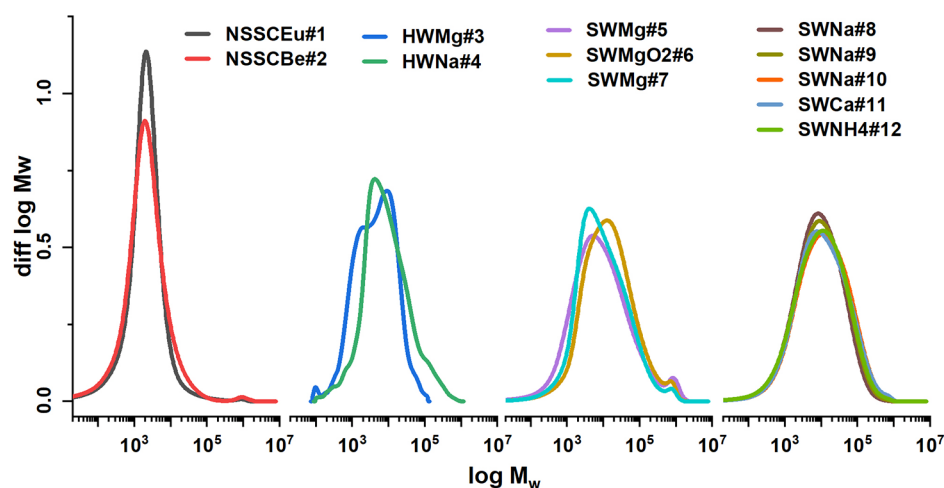


Figure 3. Normalized MMDs of lignosulfonate samples determined by aqueous high-speed SEC. The obtained MMDs are in acceptable agreement with the results from SEC–MALS, despite some forfeit in resolution.

molar mass and average sulfonation; SWNa#8, SWNa#9, SWNa#10, SWCa#11, and SWNH₄#12).

Lignosulfonate Characterization by HIC. Choosing appropriate adsorption conditions in HIC is a delicate task. The ionic strength of the selected eluents must be strong enough to cause adsorption of the analytes but is limited by their precipitation limit upon dissolution. In this study, we attempted to establish conditions that allowed the analysis of a broad range of lignosulfonates while retaining a maximum of sample adsorption. However, NSSCEu#1, NSSCBe#2, and SWMgO₂#6 demonstrated difficulties during dissolution and filtration; thus, the recorded data may represent only a part of the actual sample. For lignosulfonates, the precipitation limit in salt solutions depends mainly on molar mass, polarity, and the sample composition.^{49–53} In NSSCEu#1 and NSSCBe#2, HSQC revealed contamination of the sample with xylans, which may be responsible for the observed problems; their comparably low degree of sulfonation may be an additional factor. For SWMgO₂#6, precipitation upon dissolution may have occurred due to its high molar mass values combined with a comparably lower degree of sulfonation, resulting in increased aggregation.

Unfortunately, the recorded HIC chromatograms in the first dimension were distorted to some degree by UV signal saturation due to the high injected mass needed in 2D separations (see Figures S4 and S5). For this reason, we decided to integrate the respective regions on the 2D plot (see Figure 2). For comparison, the elution profiles can also be converted to a dimensionless factor (i.e., relative hydrophobicity I_{hyd}) with values between 0 and 1 (i.e., low and high hydrophobicity, respectively).⁵³ The respective I_{hyd} values are shown in Table 2. First, the selected eluent [0.5 M (NH₄)₂SO₄] allowed for a degree of absorption of 56.4–86.1% [$\Sigma(\text{peak area } 2-6)$] with a median of 83.8%, except for NSSCEu#1 and NSSCBe#2, which showed only 9–12% adsorption. In part, their low molar mass may be responsible for this “top-heavy” elution profile with low adsorption and, thus, a very low I_{hyd} of 0.05 and 0.09, respectively. For NSSCBe#2, using 0.1 M NaOH for dissolution may have weakened the adsorption conditions. In any case, NSSC lignosulfonates exhibit a notable difference in their hydrophobicity compared to other lignosulfonates. Next, certain samples with medium to low molar mass showed a more

balanced profile with maxima in the center of the chromatogram with an I_{hyd} of 0.35–0.52 (HWNa#4, SWMg#5, SWMgO₂#6, and SWMg#7). Interestingly, SWMg#5 exhibited lower hydrophobicity than SWMg#7, despite its lower average degree of sulfonation. SWMgO₂#6 may also show some distortion in the elution profile due to the use of 0.1 M NaOH in dissolution and, thus, was much less hydrophobic than its liquor counterpart SWMg#5, despite its higher molar mass. Finally, most of the softwood lignosulfonates with a high molar mass showed a “tail-heavy” elution profile with a considerable increase in I_{hyd} of 0.59–0.63 (SWNa#8, SWNa#9, SWNa#10, SWCa#11, and SWNH₄#12). The impact of increasing the molar mass—causing a shift to “tail-heavy” elution profiles—is in good agreement with previous experiments in the literature.²⁹ In contrast, the impact of the average degree of sulfonation remains uncertain, as no clear relationship with the elution profiles was observed. In part, this may be explained by the small differences in sulfonation between the samples (1.39–1.94 mmol/g). However, it may also be an indication that sum parameters, such as the average degree of sulfonation, are insufficient to describe the behavior of structurally complex lignosulfonates.

HIC as a stand-alone technique measures a global parameter: amphiphilicity; hence, it reflects the sum of the structural composition of a given sample. For this reason, the HIC data essentially mirror our findings, gained from state-of-the-art lignin analytics but in just one single measurement. Moreover, HIC not only captured familiarities between lignosulfonates needed for classification; it was also able to resolve subtle differences between quite similar lignosulfonates (SWMg#5 vs SWMgO₂#6; SWMg#5 vs SWMg#7; SWNa#8 vs SWNa#9; or SWNa#10 vs SWCa#11 vs SWNH₄#12). Therefore, HIC can be regarded as a novel and useful tool for lignosulfonate characterization.

Lignosulfonate Characterization by High-Speed SEC.

In 2D-LC, eluent compatibility between the first and second dimensions is an absolute prerequisite. For this reason, we implemented an aqueous SEC system complementary to HIC. However, transfer from HIC to SEC proved to be challenging, as the salt gradient in the HIC eluent initially showed a strong influence on elution times in SEC. Therefore, the ionic strength of the SEC eluent (0.1 M NaOH + 0.3 M NaCl) was increased to compensate for this effect. However, using a high

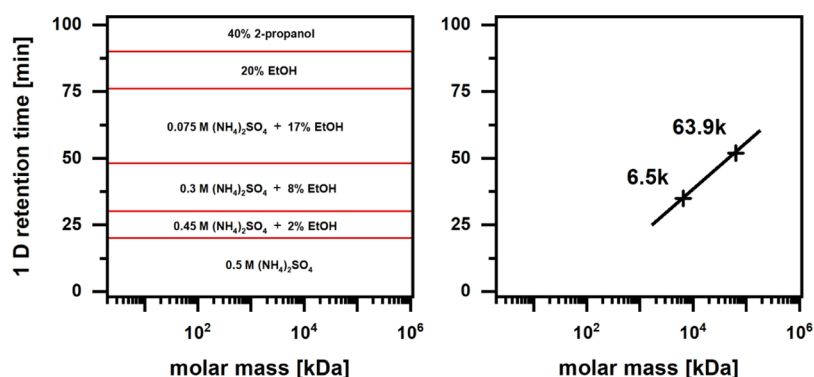


Figure 4. Sections of the gradient program used in HIC on the 2D plot (left) and line of constant charge-to-size ratio for 6.5 and 63.9 kDa PSS standards in HIC-SEC (right).

salt content in the mobile phase rendered UV detection the only viable option, as other detection systems are highly sensitive to changes in the mobile-phase composition (e.g., RI) or restricted to volatile buffer systems (e.g., evaporative light scattering).

Molar mass data were obtained by calibration with PSS standards; hence, the determined MMDs demonstrated a small shift toward the low-molar mass range compared to SEC-MALS. Of course, one high-speed SEC column also cannot provide the same separation efficiency as three analytical SEC columns in series; hence, high-molar mass shoulders are missing from the MMDs (see Figure 3). Despite somewhat lacking the resolution known from its 1D parent, high-speed SEC proved to be suitable for application in the 2D characterization of lignosulfonates, as the obtained MMDs are in good acceptable agreement with the ones obtained by SEC-MALS.

Online 2D HIC-SEC LC. Online coupling of HIC with SEC appears to be a natural fit due to the impact of molar mass on the elution profiles in HIC. The resulting 2D plot reveals the differences in hydrophobicity or polarity present within certain molar mass ranges.²⁹ Thereby, important information on the complex distributions of functionalities within lignosulfonates can be derived, which is otherwise not accessible.

Coloring of the 2D plots was carried out scrupulously, taking guidelines from the literature into consideration,⁵⁴ to guarantee undistorted and accurate data representation. For coloring, a “batlow” palette was adjusted to enhance the visibility of lower levels. Contour levels were kept constant for all 2D plots to ensure comparability between samples. However, normalization of the plots by sample concentration is not applicable in 2D measurements; therefore, intensity fluctuations between samples may be observed. Moreover, due to the differences in the solvent composition of the first and second dimensions—and the additional necessary gradient used in HIC (see Figure 4)—strong solvent signals in the 2D evaluation were unavoidable. However, the optimized sample concentration and flow rates allowed the recognition of sample components below 2 wt % of the total sample.

In the 2D plots, the impact of molar mass on the elution in HIC became very clear, as the peaks shifted to the upper right corner with increasing elution times (see Figure 5; big pictures in Figure S6). This is particularly evident for most of the softwood lignosulfonates (SWNa#8, SWNa#9, SWNa#10, SWCa#11, and SWNH₄#12) but also for hardwood lignosulfonates with a higher molar mass (HWN#4). However, the

peaks typically showed a broad distribution in molar mass, indicating differences in hydrophobicity within certain molar mass ranges and, thus, a distribution in their functionality. In contrast to molar mass, the degree of sulfonation decreases with increasing elution time in HIC.²⁹ Therefore, within a certain molar mass range, peak areas on the top are supposed to exhibit a lower degree of sulfonation than those on the bottom of the 2D plot. Moreover, within a certain HIC fraction, the degree of sulfonation is also supposed to increase with molar mass to reach the respective charge-to-size ratio necessary for elution. In this context, lines that describe a constant size-to-charge ratio would be inclined to the right in the 2D plots. Therefore, the more a 2D plot is tilted toward the center, the higher the dispersity ought to be in its FTD. Musl et al.²⁹ determined the elution behavior of certain PSS standards in HIC: an elution line of constant charge-size-ratio can be drawn on the 2D plot for the fully sulfonated PSS standards (see Figure 4). It has not yet been investigated where these lines of constant charge-to-size ratio run for lignosulfonates. At this point, it remains unclear whether they show the same slope for different lignosulfonates. Despite the current lack of knowledge, the 2D plots allow a quick assessment of the nature of a lignosulfonate under investigation, especially since the observed differences can be quite drastic.

The commercial softwood lignosulfonates (SWNa#8, SWNa#9, SWNa#10, SWCa#11, and SWNH₄#12) showed only subtle differences in their 2D profile. Again, this can be considered an indication of a common origin or at least of the fact that—regardless of which ion is stated for them—they possibly originate from Na sulfite processes with comparable process conditions. In general, the Mg softwood lignosulfonates (SWMg#5, SWMgO₂#6, and SWMg#7) showed similar but less-hydrophobic 2D profiles compared to the commercial Na softwood lignosulfonates. In particular, SWMg#5 and SWMgO₂#6 stand out due to their lack of highly hydrophobic fractions, revealing a comparably higher degree of sulfonation in the high-molar mass range for those samples, despite boasting a lower degree of sulfonation on average (see Table 2). This case highlights how HIC-SEC 2D-LC is still able to differentiate lignosulfonates when standard lignin analytics can no longer do so, thereby exposing its great potential in lignosulfonate characterization. Hardwood Na lignosulfonates (HWN#4) also showed a similar but much less-hydrophobic 2D profile compared to softwood lignosulfonates. However, hardwood lignosulfonates with lower molar masses showed a significantly different 2D profile: molar mass peaks are situated straight on the top of each other instead of a shift to the right,

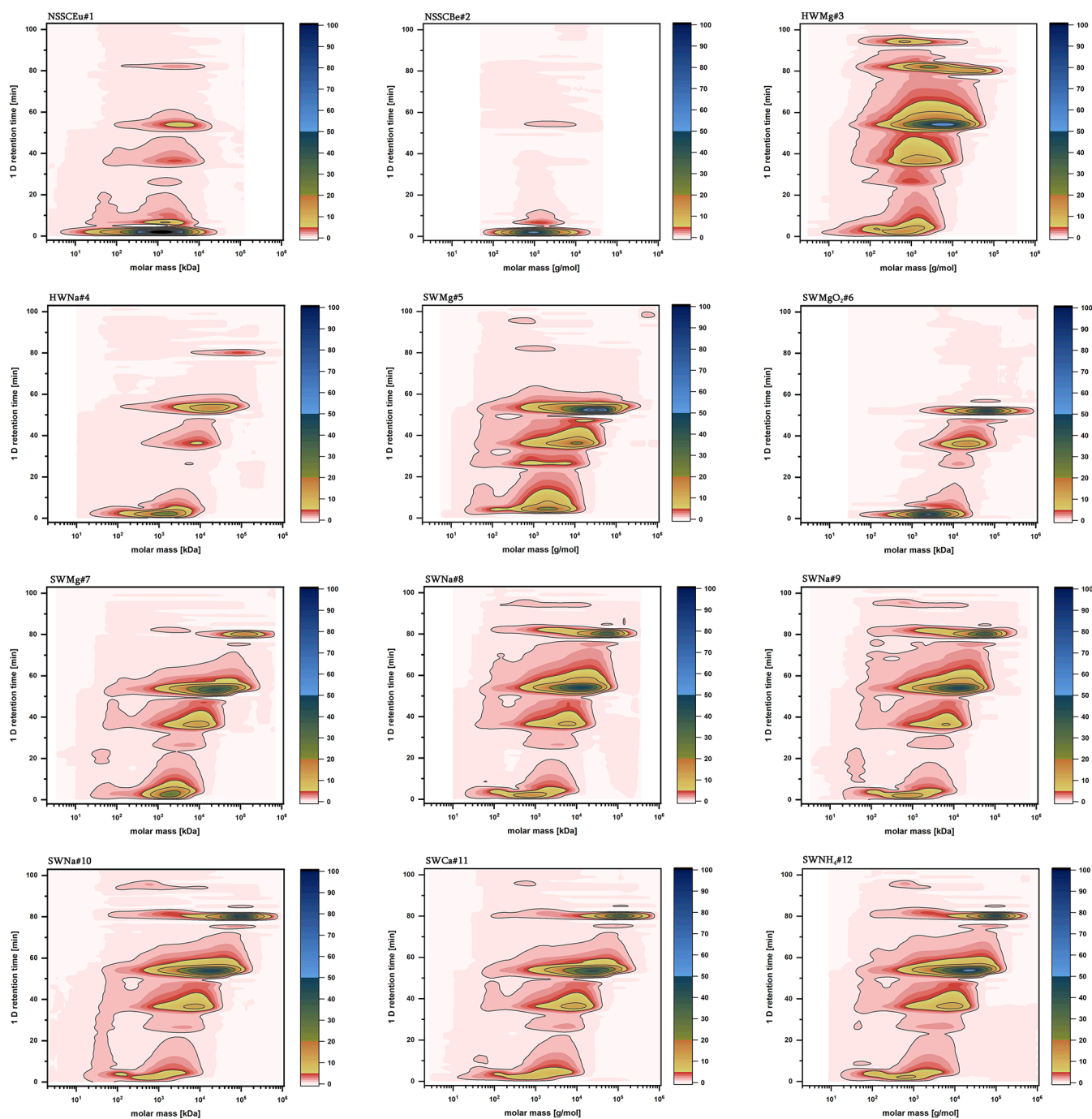


Figure 5. 2D plots from online coupling of HIC and aqueous SEC. An increase in retention time in the first dimension (HIC) is related to an increase in molar mass (shift of peaks toward the upper right corner) and decrease in functionality.²⁹ Hence, dispersity in the hydrophobic composition is indicated by a shift toward the center.

which indicates a high dispersity in the FTD. Presumably, the low-molar mass range is generally characterized by a higher dispersity in the FTD. Influences from the applied pulping process and other structural effects may not be ruled out completely, especially since NSSC (NSSCEu#1 and NSSCBe#2) appears to be quite different from the Mg hardwood lignosulfonate (HWMg#3). In the case of NSSC lignosulfonates (NSSCEu#1 and NSSCBe#2), a certain amount of dispersity in the FTD is still present, even though the majority of the sample is not adsorbed. As discussed earlier, difficulty during dissolution and filtration allowed only a part of the sample to be analyzed; thus, dispersity in the FTD may be

greater than expected. Among all lignosulfonates, HWMg#3 stands out due to its distinctive 2D profile; its low average degree of sulfonation appears to manifest itself in a broad dispersity in the FTD with considerable amounts of hydrophobic fractions in the low-molar mass range.

CONCLUSIONS

Overall, HIC–SEC 2D-LC is a proven, powerful analytical tool that can be used for fingerprinting—similar to HSQC NMR—and for in-depth characterization of the structural composition of lignosulfonates. In terms of FTD, state-of-the-art lignin analytics rely exclusively on the determination of average

functional group contents, unaware of the underlying distribution. In contrast, HIC–SEC 2D-LC is capable of providing otherwise inaccessible compositional information on a deep structural level. Of course, there is still much work to be done to fully exploit the information that 2D LC potentially offers, but it already allows us to accurately depict the characteristic structural composition of lignosulfonates—information desperately needed to tailor and refine their composition for material usage. In sum, the use of HIC–SEC 2D-LC is the first step toward a more comprehensive understanding of the complex structural composition of lignosulfonates.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssuschemeng.1c06469>.

HIC elution program, calibration plot (SEC), molar mass data, hemicellulose composition, 2D NMR, HIC chromatograms, rel. peak area graphs (HIC), and 2D plots (HIC–SEC) (PDF)

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Notes

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