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Quantification and Classification of Carbonyls in Industrial Humins and Lignins by ^{19}F NMR

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Supporting Information

ABSTRACT: Lignin and humins are both (by-)products of biorefining processes aimed at the valorization of lignocellulosic biomass. In order to improve the efficiency of such biorefineries and to develop new valorization pathways for these polymeric materials, detailed insight into their complex chemical structure and functional group distribution is required. Here, we report on the quantification and classification of the ketone and aldehyde carbonyl functional groups contained in these two polymers by ^{19}F NMR. The known method of carbonyl derivatization with 4-(trifluoromethyl)phenylhydrazine to the corresponding hydrazone has been improved and simplified, allowing derivatization directly in an NMR tube, requiring no additional workup before quantification by ^{19}F NMR. Furthermore, the scope of the method was assessed and expanded, with model compound studies, which included monomeric and dimeric compounds as well as synthetic polymers, showing that the carbonyl functions can indeed be reproducibly quantified. Using this model compound library, the carbonyl functional groups in two technical lignins (Indulin Kraft and Alcell) and, for the first time, an industrial humin could be quantified and classified. The industrial humin was found to contain 6.6 wt % of carbonyl functions, with aliphatic and conjugated carbonyls being detected. The relatively high abundance of such functional groups, which are amenable to further chemical modification, provides opportunities for the use of these humin byproducts in various applications, e.g., as materials after derivatization.

KEYWORDS: *Functional group analysis, Carbonyl quantification, Lignin, Humin, Biomass*

INTRODUCTION

The valorization of humins, polymeric carbohydrate-derived byproducts of industrial biorefining processes, is currently receiving more and more attention, for example in materials application[1] or as a potential resource for hydrogen or high-value chemicals production.[2,3] Humins are almost invariably formed during acid-catalyzed (hydrothermal) conversion of the carbohydrate fractions of biomass, e.g., for the production of key renewable platform molecules such as furfural, hydroxymethylfurfural (HMF), and levulinic acid.[4] Even though considerable amounts of these byproducts are or will be produced, options for their valorization are still very limited; new methods for their conversion are nonetheless needed to improve the economic viability of biorefineries. However, humins have a highly complex, heterogeneous structure and can be highly recalcitrant to further chemical valorization. Indeed, the humin chemical structure has not yet been unequivocally established and, similar to the lignins produced by biorefining,[5] will be highly dependent on the biorefining conditions under which they are formed, as well as on isolation/treatment methods. Previous studies provided insight into the structural motifs in synthetic humins, based in particular on IR and solid state NMR studies. For example, Lund et al. studied HMF-derived humins by IR spectroscopy and proposed a conjugated network of C═C bonds and furanic rings decorated with several aldehydes, ketones, and furfuryl alcohols as functional groups.[6] Recently, Vlachos and co-workers[7] followed the structural evolution of humins formed during acid-catalyzed degradation of HMF by, among other methods, FTIR spectroscopy, while also observing different types of carbonyls to be present in the structure. Furthermore, a multitechnique approach on a series of carbohydrate-derived humins, later complemented by 1D and 2D solid state NMR studies of analogous ^{13}C-enriched model humins, allowed for the identification of the various different linkages and functional groups.[8,9] The humins were found to have a furan-rich structure with aliphatic linkers, and a model for the molecular structure of these model humins was proposed. More detailed insight in the humin structure is still required though, both regarding the identification and quantification of interunit linkages, as well as regarding the type and density of functional groups found in the humins. Information on the latter would be required, for

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example, for potential materials applications of humins as they provide a handle for further functionalization. Indeed, the chemistry of ketones or aldehydes allows them to be orthogonally addressed, in the presence of other functional groups, such as alcohols, phenols, acids, etc., allowing selective grafting of the polymer to improve its physical and chemical characteristics. Similar approaches have been described for the preparation of lignin-based polymers[10] and the modification of cellulose[11] and could provide new valorization pathways for humins.

The difficulties faced in determining the structure of humin byproducts are rather similar to the challenges encountered in lignin structure elucidation, for which many different techniques have been developed giving detailed insight into the linkages and functional groups present in this other highly complex, heterogeneous biopolymer.[12] With both conjugated and nonconjugated aldehydes and ketones expected to be the most abundant functional groups in the humin structure, it would be valuable to adapt or translate the analytical methods available for the quantification of such groups in lignins to humin samples. With regards to carbonyl quantification in lignin, the most common approaches involve wet-chemical derivatization prior to analysis,[13,14] such as sodium borohydride reduction followed by UV[15] and GC measurements[16] or oximation prior to back-titration.[17−19] Wet-chemical derivatization followed by quantification by NMR analysis has also been studied; lignin carbonyls are either reacted by trifluoromethylation with trifluoromethyltrimethylsilane[20] or by derivatization with trifluoromethylphenylhydrazine[21] followed by ^{19}F NMR analysis. The reported NMR methods require a laborious workup procedure in order to isolate the ^{19}F-derivatized substrate prior to analysis. Rapid, reliable quantification with as little sample treatment, transfer, or workup as possible is obviously highly desired not only from an analytical precision point of view, but also when large numbers of samples need to be characterized. The latter would be the case in biorefinery operations, where the resulting lignin and humin structure can vary from batch to batch, as a function of feed intake or process conditions.

The objective of this study is to quantify and classify ketone, aldehyde, and enone-type carbonyl groups, in (industrial) humins, in order to better understand the humin structure and mechanism of formation, and to guide future valorization efforts. On the basis of the hydrazine derivatization approach,[21,22] an improved analytical method has been developed to easily identify and quantify the different carbonyl groups. Carbonyl-containing substrates are derivatized directly in an NMR tube, requiring no additional workup, and quantified by ^{19}F NMR. On the basis of the molecular structures previously proposed for humin and lignin materials, a library of model compounds has been built to aid spectral interpretation and validate derivatization efficiency and quantification in more complex substrates. The method is then applied to an actual industrial humin sample, obtained from an biorefinery pilot plant and produced by conversion of fructose in methanol solvent,[1] as well as to two benchmark, industrial lignins (Indulin Kraft and Alcell), for comparison.

■ EXPERIMENTAL SECTION

Materials. All reagents for the derivatization step were obtained commercially: 4-(trifluoromethyl)phenylhydrazine (96%, Sigma-Aldrich, stored dry at 4 °C and used in small batches), 1-methyl-4-(trifluoromethyl)benzene (98%, Sigma-Aldrich), and chromium(III) acetylacetonate (97%, Acros). Deuterated d_6-DMSO solvent was purchased from Buchem. Details about the origin and purity of the model compounds used in this study are reported in Table S1. Kraft lignin Indulin AT was obtained commercially from Meadwestvaco. Alcell organosolv lignin was obtained from Repap Technology, Canada; more details about the lignins are available in the literature.[23] The industrial humin sample was provided by Avantium, The Netherlands, and produced by conversion of fructose in methanol solvent, as previously described.[1] The crude industrial humin was extensively purified by solvent washing to remove residual monomeric 5-hydroxymethylfurfural (HMF) and 5-methoxymethylfurfural (MMF) to <0.5% by weight.[24] The purified industrial humin (PIH) is obtained as a dark colored powder and can be readily handled.

Hydrazine Derivatization of the Model Compounds. In an 4 mL vial, 0.2 mmol of model compound and 10 mg of 1-methyl-4-(trifluoromethyl)benzene (internal standard) were dissolved in 300 μL of d_6-DMSO. At the same time, 0.8 mmol of 4-(trifluoromethyl)-phenylhydrazine was dissolved in 300 μL of d_6-DMSO and added dropwise to the solution of the carbonyl-containing model compound. It is important to note that 4-(trifluoromethyl)phenylhydrazine is unstable in the presence of water and requires storage under cold, dry conditions and under a protective atmosphere in the fridge. The reaction mixture was transferred into a standard NMR tube and placed in an oven at 40 °C for 16 h to ensure complete derivatization. Just prior to analysis, 100 μL of relaxation agent solution (chromium acetylacetonate, 28 mg/mL in d_6-DMSO, 0.008 mmol) was added to the NMR tube after which the sample was homogenized and analyzed immediately.

Hydrazine Derivatization of the Humin and Lignin Samples. Around 150 mg of humin or lignin sample (accurately weighed) and 10 mg of 1-methyl-4-(trifluoromethyl)benzene (internal standard) were dissolved in 500 μL of d_6-DMSO. The mixture was then manually homogenized for about 1 h until there is full dissolution of the polymer. In another vial, 0.8 mmol of 4-(trifluoromethyl)-phenylhydrazine was dissolved in 200 μL of d_6-DMSO and added dropwise to the solution containing the substrate. The mixture was transferred into a standard NMR tube after which the same procedure detailed above for the model compound was followed, with the exception of a reaction time of 24 instead of 16 h.

Nuclear Magnetic Resonance Measurements. All NMR spectra were measured on a Varian 400 MHz NMR spectrometer. The ^{19}F NMR measurements were carried out using a standard fluorine 90° pulse program, a relaxation delay of 3 s, and 256 acquired scans between −50 and −70 ppm. The spectra were processed using MestReNova software; a Bernstein polynomial (order 3) baseline correction was applied, and chemical shifts were referenced to the 1-methyl-4-(trifluoromethyl)benzene signal at −60.90 ppm, prior to integration of the peaks. Details about the ^{1}H, ^{1}H NOE, ^{19}F T1, [^{1}H; ^{1}H] COSY, and [^{1}H; ^{13}C] HSQC measurements are reported in the Supporting Information.

Calculations. The number of millimoles of carbonyl groups per gram of sample corresponds to the millimoles of hydrazone groups per gram of sample and was calculated from the NMR spectra according to eq 1. The commercial 4-(trifluoromethyl)phenylhydrazine reagent contained an impurity (∼3%), giving rise to a low-intensity peak in the ^{19}F NMR spectrum at −59.48 ppm (Figure S22). The amount of this impurity was determined for every batch of hydrazine used. For those samples in which the NMR signal of the impurity overlapped with the hydrazone A_C signal, the latter signal was corrected for the specific impurity concentration in the batch of hydrazine used.

$$\text{mmol carbonyl group/g sample} = \frac{\left[A_C - \left(\frac{A_{H^\circ} \times m_H}{m_{H^\circ}}\right)\right] \times m_{IS}}{A_{IS} \times m_C \times M_{IS} \times P_c} \times 10^3 \quad (1)$$

A_C corresponds to the area of the hydrazone peak; for the humin and lignin samples the integrated area was set from −59.20 to −60.10 ppm. A_{IS} corresponds to the area of the internal standard peak normalized to

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1.00. A_{H° represents the area of the peak at −59.48 ppm in the ^{19}F spectrum of a sample of 4-(trifluoromethyl)phenylhydrazine and 1-methyl-4-(trifluoromethyl)benzene with the area of the internal standard peak normalized to 1.00.

m_{H° corresponds to the mass of hydrazine of the sample containing 4-(trifluoromethyl)phenylhydrazine and 1-methyl-4-(trifluoromethyl)-benzene (mg). m_{IS} is the mass of the internal standard (mg); m_C is the mass of carbonyl compound or biopolymer (mg). m_H corresponds to the mass of hydrazine (mg). M_{IS} is the molar mass of the internal standard (160.14 g/mol). P_c is the purity of the carbonyl compound ($0 < P_c < 1$).

Hydrazone yields were calculated using eq 2.

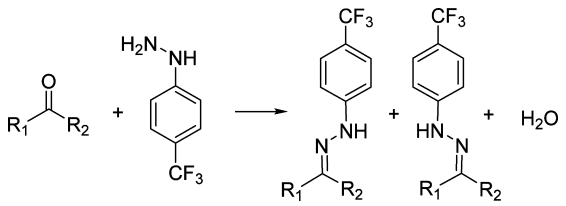
$$\text{hydrazone yield} = \frac{\text{mol carbonyl group/g sample} \times M_C \times 100}{Nb_C} \quad (2)$$

M_C represents the molar mass of the carbonyl compound. Nb_C represents the number of carbonyl functions of the carbonyl compound.

RESULTS AND DISCUSSION

Carbonyl Group Quantification: Method Development. 4-(Trifluoromethyl)phenylhydrazine, as previously employed,[21,22] was used as a carbonyl derivatization agent to give the CF_3-containing hydrazones (Scheme 1), which were quantified by ^{19}F NMR analysis. Contrary to former studies, the method was devised such that the derivatization reaction occurs readily and directly in the NMR tube, requiring no workup or sample isolation procedure prior to NMR analysis. This one-step procedure thus avoids any potential losses or other inaccuracies due to workup or isolation, improving the accuracy of carbonyl quantification. 1-Methyl-4-(trifluoromethyl)-benzene was selected as internal standard for quantification, having a similar chemical shift as the targeted hydrazine and being inert under the applied derivatization conditions. In contrast, the 3-trifluoromethoxybenzoic acid internal standard that has been used previously,[22] was found not to be chemically inert in our hands. The internal standard was also used as a chemical shift reference to calibrate the ^{19}F spectra. The ratio of carbonyl vs hydrazine equivalents was optimized with four equivalents of hydrazine considered optimal in light of convenience and quantification. Unfortunately, no mention was made of the equivalents of hydrazine used for derivatization of model compounds in previously reported protocols.[21,22]

Scheme 1. Hydrazone Formation with 4-(Trifluoromethyl)phenylhydrazine[a]



[a]For nonsymmetrical ketones, both the *E* and *Z* isomers are typically observed.

Quantitative ^{19}F NMR analysis typically requires a large number of scans and long relaxation times to ensure good signal-to-noise ratios and full relaxation of the fluorine nuclei, respectively. Consequently, long measurement times are needed for quantification, at least 2 h with d1 = 25 s and 256 scans, for example. The acquisition time can, however, be considerably shortened by adding a relaxation agent such as chromium acetylacetonate prior to analysis. Conveniently, with 0.008 mmoles of $Cr(acac)_3$ T1 relaxation times of the fluorine nuclei decreased about 10-fold (Table S2). Thus, a delay time of 3 s (>17 times T1) was sufficient to record quantitative ^{19}F spectra, leading to a measurement time of about 17 min. Importantly, the chromium salt was found not to affect the stability of the hydrazine, nor did its addition lead to the formation of any unexpected compounds, even after 24 h of contact time (Table S3). With the sample being directly prepared in the NMR tube, hydrazone formation could be conveniently followed by ^1H NMR as a function of time, as illustrated in Figure S1 for the reaction of 5-methylfuran-2-carbaldehyde with 2 equiv of hydrazine showing the disappearance of the aldehyde peak. The aldehyde region of the ^1H spectrum and the ^{19}F spectrum after derivatization of 5-methylfuran-2-carbaldehyde are shown in Figure 1a,b. The ^1H spectrum shows that the aldehyde proton at 9.47 ppm is absent after derivatization, with two new peaks having appeared at 10.65 and 9.84 ppm. The two products associated with these peaks were separated and isolated and, on the basis of their ^1H NOE, [^1H; ^1H] COSY, and [^1H; ^{13}C] HSQC spectra, identified as the *trans*-hydrazone (*E*) and *cis*-hydrazone (*Z*) stereoisomers, respectively (see Supporting Information section C). The two corresponding signals in the ^{19}F NMR spectrum were observed at −59.46 (*E*) and −59.66 (*Z*) ppm (Figure 1b). The *E* isomer is usually the more stable one and indeed was found to be the major isomer present in this case. As expected, these two stereoisomers were observed for nearly all of the nonsymmetrically substituted carbonyl compounds we tested (Supporting Information section D). Surprisingly, the for-

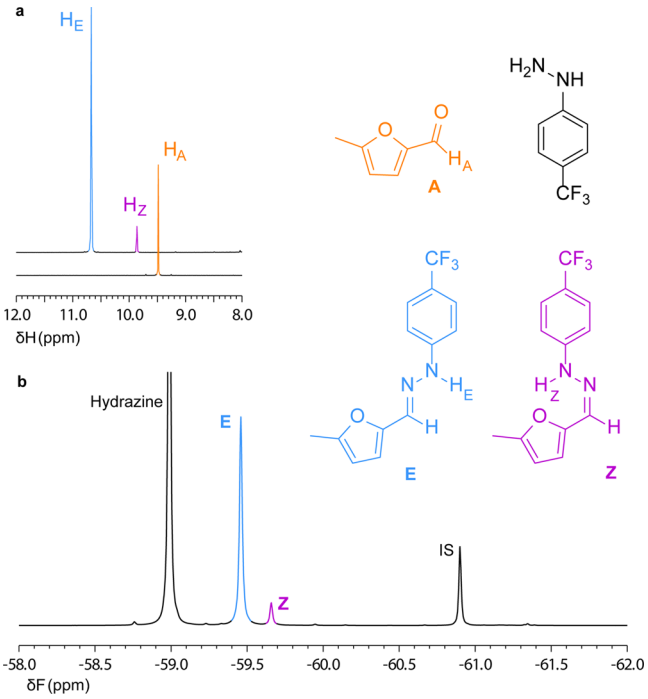


Figure 1. (a) Partial ^1H spectrum of the reaction mixture of 5-methylfuran-2-carbaldehyde and 4-(trifluoromethyl)phenylhydrazine (top) superimposed with the spectrum of 5-methylfuran-2-carbaldehyde (bottom). (b) ^{19}F NMR spectra of the reaction mixture of 5-methylfuran-2-carbaldehyde and 4-(trifluoromethyl)phenylhydrazine obtained after the standard derivatization protocol.

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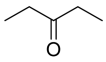
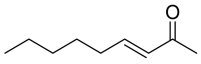
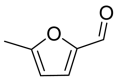
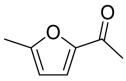
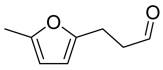
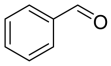
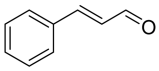
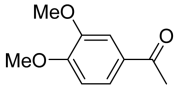
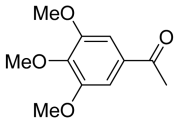
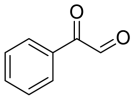
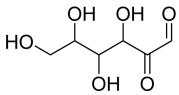
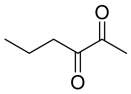
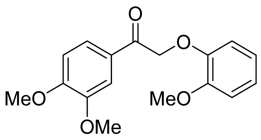
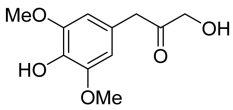
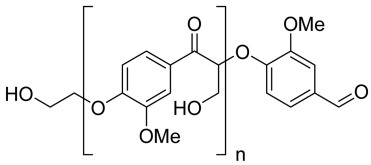
Table 1. Quantification of Various Carbonyl-Containing Model Compounds by Derivatization with 4-(Trifluoromethyl)phenylhydrazine and Analysis by Quantitative ^{19}F NMR[c]

Model compound		Hydrazone yield (%)
Octanal		87.4 ± 1.2
Pentan-3-one		100.3 ± 1.2
trans-Non-3-en-2-one		100.9
5-Methylfuran-2-carbaldehyde		106.1 ± 0.8
1-(5-Methylfuran-2-yl)ethan-1-one		96.5 ± 2.9
3-(5-Methylfuran-2-yl)propanal		103.5 ± 0.8
Benzaldehyde		101.5 ± 1.4
trans-Cinnamaldehyde		101.2 ± 2.5
1-(3,4-Dimethoxyphenyl)ethan-1-one		100.9[a]
1-(3,4,5-Trimethoxyphenyl)ethan-1-one		101.7 ± 4.1
2-Oxo-2-phenylacetaldehyde		98.3[a,b]
3,4,5,6-Tetrahydroxy-2-oxohexanal		94.7[a,b]
Hexane-2,3-dione		86.0[a,b]
1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)ethan-1-one		99.5
1-Hydroxy-3-(4-hydroxy-3,5-dimethoxyphenyl)propan-2-one		100.1[a]
Oxidized β-O-4 G-polymer		83.1

[a]Reaction time: 24 h. [b]100% hydrazone yield equates to both carbonyls being fully functionalized. [c]The hydrazone yields were quantified after derivatization with 4 equiv of 4-(trifluoromethyl)phenylhydrazine at 40 °C during 16 h.

mation of the *Z* isomer was not reported in the previous studies on hydrazine derivatization of lignin model compounds.[21,22]

Evaluation of the Method for Carbonyl Group Quantification and Signal Assignment. The efficiency of derivatization with the modified method was evaluated using a library of model compounds varying in the nature of the contained carbonyl group. The hydrazone yields calculated from the ^{19}F NMR spectra are given in Table 1. Excellent

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reproducibility and full functionalization were seen for aliphatic, furanic, and benzylic carbonyl-containing model compounds. Octanal proved an exception, with derivatization not being complete even after long contact times of up to 48 h. As no evidence was seen in the ^{1}H or ^{19}F NMR spectra for the formation of any products other than the hydrazones for this substrate, the origin of the lower yields is presently not clear and requires further study; it should be noted though that such long chain aliphatic aldehydes are not expected to be present in the humin or lignin samples. The hydrazone yields determined for two furanic compounds (5-methylfuran-2-carbaldehyde and 3-(5-methylfuran-2-yl)propanal) slightly overestimated the actual carbonyl content. Again, the spectra obtained for these furanics were clean and showed no changes over time (up to 48 h), and no undesired products (e.g., from furan ring opening) could be detected. Furthermore, quantification of a mixture of two compounds (5-methylfuran-2-carbaldehyde and pentan-3-one) also proceeded efficiently (Table S3). In addition to the monomeric carbonyl-containing model compounds, dimeric and polymeric lignin model compounds were also investigated, i.e., 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)ethan-1-one and a synthetic polymer prepared by oxidation of a β-O-4 G-polymer.[25] Quantitative derivatization was observed for the simple dimeric model compound; derivatization of the oxidized β-O-4 polymer did not go to full completion, but a good hydrazone yield was still obtained. This latter result is illustrative of the inherent challenges associated with quantitatively addressing all functional groups in a complex substrate, such as this synthetic polymer. Neither longer reaction times or larger amounts of hydrazine led to an improvement in the yield (Table S3).

The ^{19}F chemical shifts observed for the different hydrazones are schematically given in Figure 2, categorized as aldehyde versus ketone and aliphatic versus conjugated. The chemical shift distribution shows no clear distinction between aldehyde and ketone derivatives. However, conjugated and non-conjugated carbonyls can be differentiated, with conjugated carbonyls generally being found more downfield. Aliphatic carbonyls were observed between −59.3 and −59.5 ppm, whereas the conjugated ones gave signals between −59.4 and −59.8 ppm (Table S4). It is important to notice that the aliphatic carbonyl signals that cause the limited overlap with the conjugated region are mainly the *Z*-hydrazones that are formed as minor products in most cases.

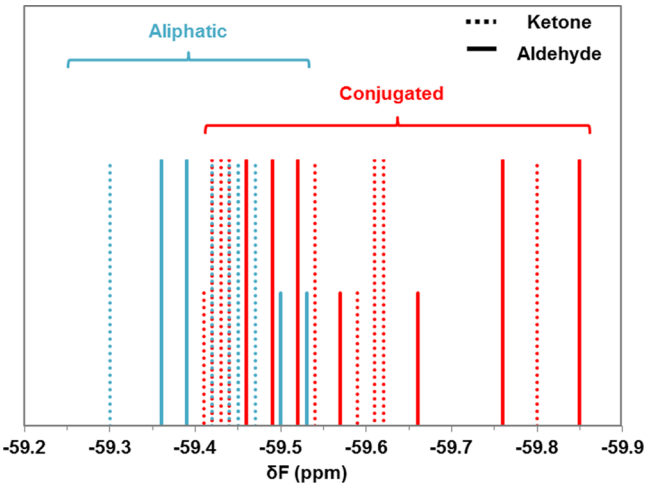

Figure 2. ^{19}F chemical shifts of (*E*, *Z*)-hydrazones obtained after reaction of the library of carbonyl-containing model compounds with 4-(trifluoromethyl)phenylhydrazine. The shorter lines correspond to the *Z*-hydrazones and the longer ones to the *E*-hydrazones, respectively.

As humins (and lignins) are known to also contain various other functional groups in addition to carbonyls, the reactivity of the hydrazine derivatization agent with other functional groups was also investigated. To this extent, carboxylic-acid-, ester-, ether-, alcohol-, phenol-, acetal-, and lactone-containing compounds were subjected to the standard hydrazine derivatization conditions (Supporting Information section E). No hydrazone related peaks were observed in the ^{1}H and ^{19}F NMR spectra of any of the reaction mixtures. Expectedly, monomeric reducing sugars such as glucose, fructose, xylose, and galactose did react with the hydrazine, but glycosides (e.g., α-methylglucoside) showed no reactivity (see SI). Given that (monomeric) reducing sugars might be contained in the humin and lignin[23] samples, their reactivity with hydrazine needs to be considered during carbonyl group quantification.

Determination of Carbonyl Groups in Industrial Humin and Lignins by ^{19}F NMR. Our optimized derivatization protocol was then applied to industrial humin and lignin samples; in previous reports, the exact procedures used for the model compound studies differed from the ones applied to the actual lignin samples.[21,22] Conveniently, the purified industrial humin (PIH) sample was, in contrast to some synthetic humins previously investigated,[3,26] fully soluble in DMSO, allowing for the one-step derivatization and quantification method to be applied. A kinetic study with ^{19}F spectra recorded after 16, 24, or 48 h of derivatization showed that the spectra did not change any more after 24 h of contact time (Table S6). The amount of biopolymer analyte was also optimized and set to ∼150 mg in order to obtain a spectrum with a good resolution and signal-to-noise ratio using the same amount of moles of 4-(trifluoromethyl)phenylhydrazine as in the model compound study. In addition, this amount also resulted in an appropriate intensity ratio between the hydrazone and internal standard peaks, which is essential for accurate quantification of the carbonyl groups. Furthermore, reaction with higher amounts of 4-(trifluoromethyl)-phenylhydrazine did not change the results (Table S6).

The ^{19}F NMR spectrum of the purified industrial humin sample after derivatization, shown in Figure 3, revealed both aliphatic and conjugated carbonyl signals to be present. The aliphatic signals were of lower intensity, and two peaks could be observed in the aliphatic region at −59.33 and −59.25 ppm. Conjugated carbonyls, characterized by a relatively sharp (multicomponent) peak at −59.50 ppm, were found to be most abundant. The quantification revealed a total carbonyl group content of about 2.35 mmol per gram of humin, which corresponds to 6.6 wt % of the humin (Table 2). This relatively high abundance of carbonyl functions is also apparent from the ^{1}H NMR spectrum of the purified industrial humin, displaying relatively strong aldehyde signals between 9 and 10 ppm. A detailed structure analysis study of the PIH also shows that monomeric reducing sugars and structurally incorporated sugar(-like) components are present in the sample.[24] As mentioned before, monomeric reducing sugars might contribute to the broad peak at −59.50 ppm and thus to an overestimation of the carbonyl content determined by ^{19}F NMR. On the basis of a separate analysis of the amount of

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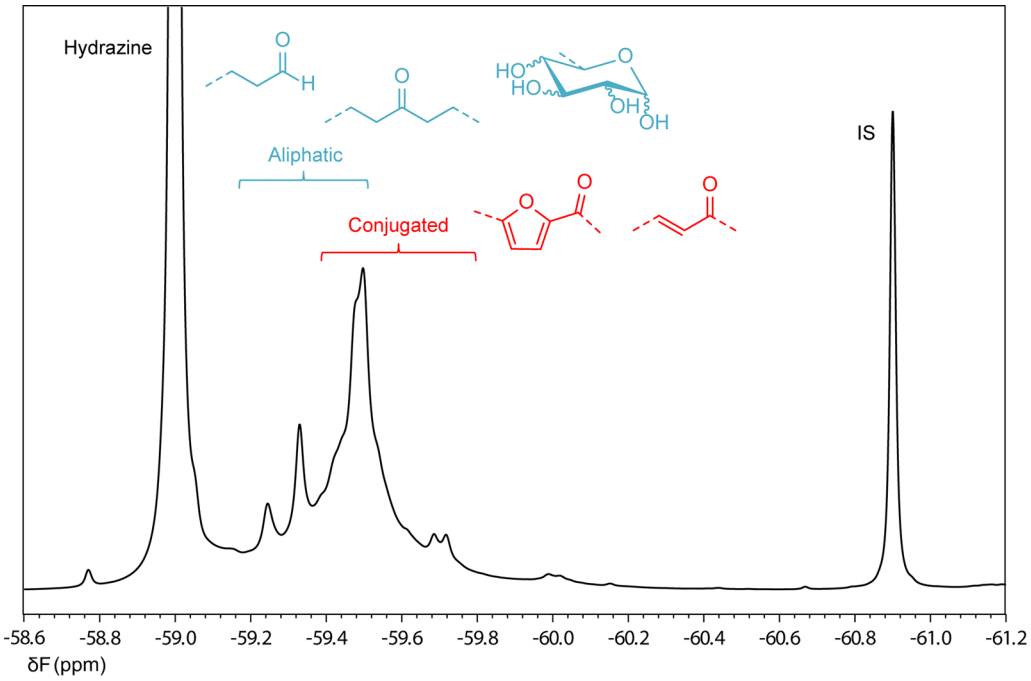

Figure 3. ^{19}F spectrum of the purified industrial humin derivatized with 4-(trifluoromethyl)phenylhydrazine.

Table 2. Carbonyl Group Quantification in Humin and Lignins by ^{19}F NMR[a]

polymer	mmol function/g of polymer	wt %	CO per C_{900}[b]
humin	2.35	6.6	
Indulin Kraft lignin	0.60	1.7	10.8
Alcell lignin	1.17	3.3	20.9

[a]The carbonyl contents were quantified after derivatization of around 150 mg of polymer with 0.8 mmol of 4-(trifluoromethyl)-phenylhydrazine at 40 °C for 24 h. [b]Expressed as mole per 100 C_9 units, as defined previously.[21] The values were calculated using the C_9 formula reported by Milne et al.[28]

monomeric sugars, this contribution was estimated to be in the range from 0.05 to 0.30 mmol per gram of humin (Table S8).

The high proportion of conjugated carbonyl groups detected by ^{19}F NMR is in agreement with previously proposed structural features of (model) humins, which are indeed suggested to contain enone and more highly conjugated carbonyl functions.[6,9,27] From the ^{19}F spectrum, it can be estimated that roughly 1/3 of the carbonyls present in the structure of PIH are aliphatic. Aliphatic fragments containing carbonyl functions were previously observed in the DE-DQSQ NMR spectrum of a synthetic ^{13}C-labeled humins.[9] These might result from the chemical incorporation of levulinic acid or from sugar dehydration and HMF rehydration intermediates.[9] Similar saturated aliphatic ketones were also suggested on the basis of the FTIR spectra of HMF-derived synthetic humins.[7] With regard to the dicarbonyl units included in some DHH-based humin models,[6,27] our results cannot confirm or rule out the presence of saturated aliphatic keto-aldehydes or diketones in the PIH structure. Indeed, the hexane-2,3-dione model compound gives signals that overlap with the broad peak at −59.50 ppm in the humin spectrum (Figure S58). The peaks corresponding to the dicarbonyl moiety in 2-oxo-2-phenyl-acetaldehyde around −59.80 ppm are not observed in the PIH (Figure S52), while the signals seen for the dicarbonyl sugar around −59.76 ppm (Figure S55) do share some resemblance with the two relatively sharp signals around −59.70 ppm seen for the PIH. These three examples do show that the ^{19}F hydrazine peak position is rather sensitive to the substitution pattern of the vicinal dione; the study of a more extensive model dione compound set is hampered by the limited commercial availability of such compounds, however. In any case, it should be kept in mind that the structure of the purified industrial humin used here is expected to differ considerably from the previously studied model humins, as a result of the (severity of the) process conditions under which they have been obtained, precluding direct comparison. To the best of our knowledge, no estimation of the quantity of carbonyls in a humin is available in the literature, either for synthetic or industrial samples.

Finally, our ^{19}F method was applied to two commercial lignins (Indulin Kraft and Alcell). The latter one has previously been characterized by ^{19}F after hydrazine derivatization, thus allowing for a direct comparison. The amounts of carbonyl groups are reported in Table 2, expressed in millimoles of carbonyl functional groups per gram of lignin. Interestingly, the amount of carbonyl groups quantified in the Alcell lignin was about 2 times higher than that in the Indulin Kraft one. This might in part be due to the presence of furfural units in the structure of the Alcell lignin, as recently observed by HSQC NMR.[23] For the Alcell lignin, a slightly higher value is found than that previously reported (2.94 wt %) after hydrazine derivatization with the method that included a workup procedure.[21] Notably, the values obtained by hydrazine derivatization are significantly different from those determined by other analytical techniques such as trifluoromethyltrimethylsilane derivatization, oximation, quantitative ^{13}C NMR, or sodium borohydride reduction (Table S7). For example, between 10 and 29 mmol of carbonyl functions per 100 C_9 units were detected in the Alcell lignin with these other techniques.[19−21,28,29] The contribution of carbohydrates to the carbonyl content of the lignins is probably limited. Indeed, the carbohydrates quantified in the lignin (1.4 and 0.2 wt % in Kraft

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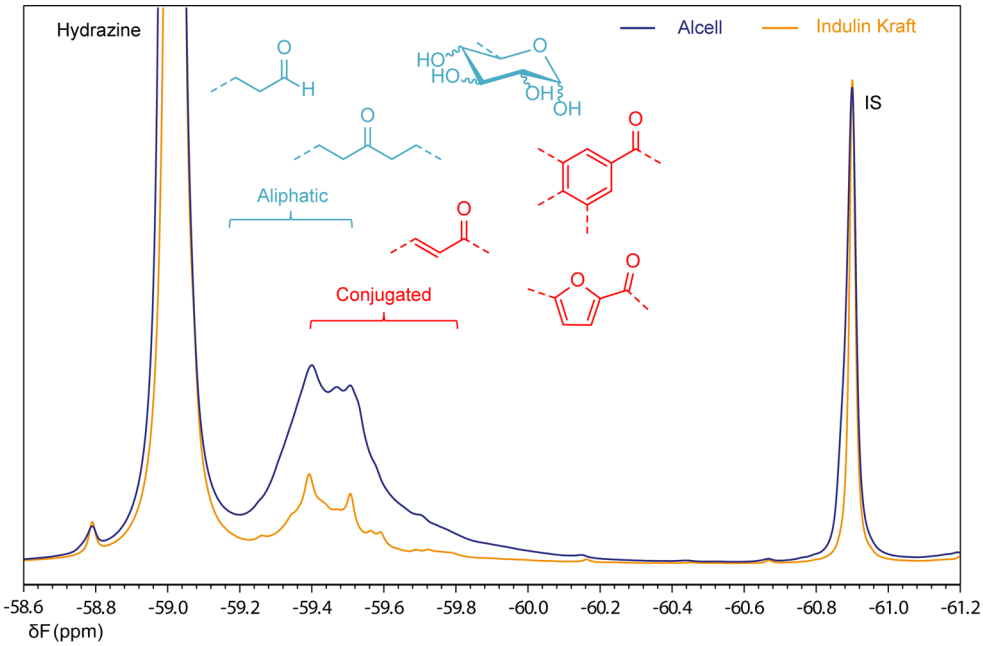

Figure 4. ^{19}F spectra of the Indulin Kraft lignin and Alcell lignin derivatized with 4-(trifluoromethyl)phenylhydrazine.

and Alcell, respectively) might be mainly engaged in the lignin−carbohydrate complex (LCC) or become trapped during the lignin precipitation.[23,30]

The ^{19}F NMR spectra of the derivatized Indulin Kraft and Alcell lignins show two distinct, sharp peaks at −59.40 and −59.51 ppm (Figure 4). S-units substituted with carbonyls have indeed been previously identified by HSQC NMR,[23] and might correspond to the peak observed at −59.51 ppm on the basis of the chemical shift of 1-(3,4,5-trimethoxyphenyl)ethan-1-one (Figure S49). Furthermore, the ^{19}F spectrum of the Alcell lignin is broader than the Kraft one, showing an additional third maximum at −59.46 ppm which might correspond to the chemical shift observed for 5-methylfuran-2-carbaldehyde (Figure S8), consistent with the presence of the previously identified furfural units, as already mentioned above.

CONCLUSIONS

The amount and type of carbonyl functional groups in complex humin and lignin biopolymers has been quantified by derivatization with 4-(trifluoromethyl)phenyl hydrazine followed by quantitative ^{19}F NMR analysis. Improvements were made to the reported method, with derivatization now carried out directly in the NMR tube limiting the number of sample handling steps. Quantification of the carbonyl content in model compounds in monomeric, dimeric, and polymeric forms proved reproducible. It is furthermore shown that only aldehyde, ketone, and enone-like carbonyl functions are selectively converted to hydrazone compounds, with carboxylic acid, ester, ether, enol ether, alcohol, phenol, acetal, and lactone functional groups being left untouched. Such selective functionalization is of prime importance for the characterization of complex, multifunctional biopolymers such as the ones studied here. Both the *E*- and *Z*-hydrazone stereoisomers were observed with nonsymmetric substrates, with the former being the major product. The formation of the *Z* isomer was surprisingly not mentioned in previous hydrazine derivatization studies. On the basis of a prepared library of carbonyl-containing model compounds, aliphatic and conjugated carbonyl groups could be differentiated by the ^{19}F chemical shift of the corresponding hydrazones.

Characterization of two technical lignins has been conducted to benchmark our simplified method and to compare the characterization data obtained for the ^{19}F method with other techniques. For the first time, the amount of carbonyl groups in a humin sample was determined and found to be considerable at 6.6 wt %, with both aliphatic and conjugated carbonyls being detected. The relatively high abundance of carbonyl functional groups in the humin makes them a potential target for chemical functionalization through grafting pathways, for example. Further studies into the chemical structure of this complex biorefinery byproduct are now required to confirm and/or correlate the results obtained by ^{19}F NMR with other techniques.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.6b02292.

Methods and experimental and NMR data (PDF)

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