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Low-field Benchtop versus High-field NMR For Routine ^{31}P Analysis Of Lignin, A Comparative Study

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Abstract

Over the past decade, advances in lignin characterization methods have led to a better understanding of lignin structure and reactivity. Novel chromatographic and spectrometric techniques, especially NMR, are now essential and routine for lignin analysis. Among these methods, quantitative ^{31}P NMR spectroscopy proved itself as a powerful technique for the qualitative and quantitative analyses of diverse types of hydroxyl groups in lignin. Nevertheless, ^{31}P-NMR spectroscopy is typically accomplished using high-field NMR equipment that necessitates substantial investment and operational cost, limiting its ability to be employed as a routine tool for analysis and quality control in a production environment. In this work ^{31}P-NMR experiments were performed on several lignins, including technical, depolymerized, and modified lignins, using both an 80 MHz benchtop NMR and a 400 MHz high-field conventional NMR. Both NMR spectrometers showed similar results for both non-functionalized and modified lignins. This work demonstrates that low-

field benchtop NMR is an excellent alternative to the conventional high-field NMR equipment to analyze the hydroxyl content of lignins.

Keywords: lignin, benchtop, ^{31}P NMR, hydroxyl groups.

1. Introduction

Lignin is one of the three main components of biomass together with cellulose and hemicellulose. Interest in lignin has been rising during the last decade since it is the most abundant natural source of aromatics on earth and the second largest biomacromolecule after cellulose.(Baral et al., 2019; Liu et al., 2019) Lignin is made of three different monomers (monolignols), namely p-coumaryl, coniferyl and sinapyl alcohols (H, G and S units respectively), that are linked together through ether (*e.g.* β-O-4 and 4-O-5) and carbon-carbon (*e.g.* β-5 and 5-5) linkages resulting from a radical polymerization Figure 1. (Ralph et al., 2019) A growing number of companies are currently commercializing lignin using different extraction and processing technologies such as Kraft, sulfite, and organosolv processing.(Van Nieuwenhove et al., 2020) Indeed, lignin production is growing fast, and this trend is expected to continue for the coming years to overcome the dependence on non-renewable resources and abide by the increasing stricter environmental legislation. (Dessbesell et

al., 2020) Nonetheless, lignin valorization to produce biobased chemicals and materials is still a challenge due to its highly heterogeneous nature. Therefore, universities and research institutes are currently joining forces along with companies to offer lignin-based chemicals and materials as alternatives to fossil-based resources. (Gosselink et al., 2020)

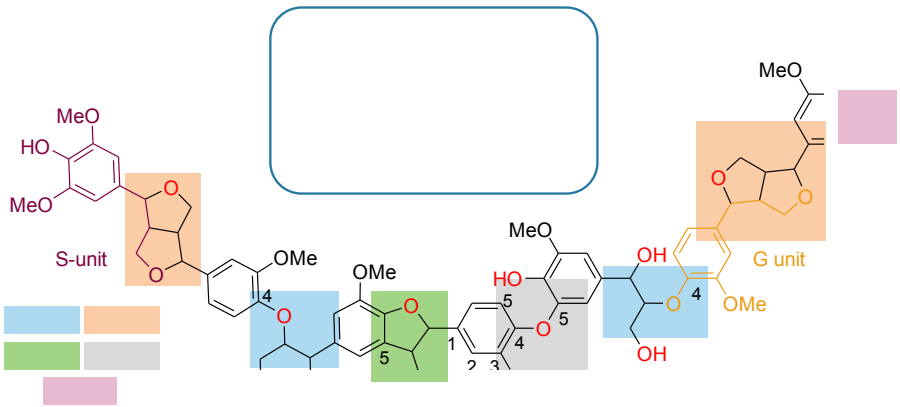

Figure 1 Monolignols of the G, S and H units and a representative structure of lignin with the most common bonds such as β-β, 4-O-5 and β-O-4.

The first crucial phase towards efficient lignin valorization is the understanding of the reactivity of lignin through in-depth characterization of its structure. Yet, analyzing a highly heterogeneous material that exhibits a complex structure and properties that are highly dependent on the extraction process is challenging. For example, lignin solubility is a critical factor that may hamper its characterization by certain methods such as GPC and NMR.(Meng et al., 2019; Tolbert et al., 2014)

A range of different spectroscopic and chromatographic techniques was developed to elucidate the physicochemical characteristics of lignin. Methods such as GC–MS, ToF-SIMS, near-infrared spectroscopy (NIR), and NMR significantly improved our understanding of lignin.(Hu et al., 2016; Serrano et al., 2018; Stark et al., 2016) Yet, only NMR could offer advanced insights into lignin structure.(Balakshin and Capanema, 2015; Jiang et al., 2018) Amongst the common NMR spectroscopic techniques, ^{31}P-NMR and 2D ^{1}H-^{13}C Heteronuclear Single-Quantum Coherence (HSQC) NMR outperform 1D ^{1}H and ^{13}C NMR when it comes to lignin characterization. ^{31}P-NMR is used to

determine the amount (in mmol/g) of reactive OH groups along with their types (*i.e.*, aliphatic, aromatic condensed and uncondensed, and carboxylic acid) in lignin(Meng et al., 2019)Figure 2. While 2D ^{1}H-^{13}C HSQC is mostly used to study the interlinkages between lignin units and lignin–carbohydrate complexes (LCCs).(Wen et al., 2013; Yuan et al., 2011) However, despite all efforts made by several research groups working on 2D ^{1}H-^{13}C HSQC, there is still a need for a universally accepted standardized protocol (pulse program) to follow while performing HSQC analyses.(Crestini et al., 2011; Talebi Amiri et al., 2019; Van Aelst et al., 2020; Zeng et al., 2013; Zhao et al., 2019; Zijlstra et al., 2019) Accordingly, the comparison of different 2D ^{1}H-^{13}C HSQC spectra of lignin could be difficult or even impossible unless the same pulse programs are used. On the other hand, a standard protocol exists in the case of ^{31}P analysis. This protocol is widely accepted by the scientific community for lignin analysis(Meng et al., 2019) and the characterization of tannins or tricin-related compounds.(Li et al., 2017) This protocol was developed by the group of Argyropoulos in the ’90s based on the phosphitylation of lignin with different phosphorus reagents.(D. S. Argyropoulos, 1994; Granata and Argyropoulos, 1995) The method permits the differentiation and quantification of distinct types of hydroxyl groups, in particular aromatic OHs, which are the pivotal functionalities that influencing lignin reactivity.(Meng et al., 2019) Nevertheless, the required ^{31}P analysis was always done using high-field (HF) NMR equipment that necessitates considerable investment and operational costs which is limited to large research facilities. A minimal spectral resolution is required to distinguish between aliphatic, condensed and uncondensed phenol hydroxyl groups for the correct quantification of the hydroxyl content in lignin (Figure 3). Within this contribution we want to show to the scientific community, both from industrial and academic sides, that low field benchtop NMR provides sufficient spectral resolution for the correct quantification of the different hydroxyl groups of technical, depolymerized and modified lignin’s. This can be an important practical and economic barrier that restrains the use of such characterization techniques in spinoffs and small to medium enterprises (SME) working on the valorization of lignin for routine analyses and quality control in a production setting.

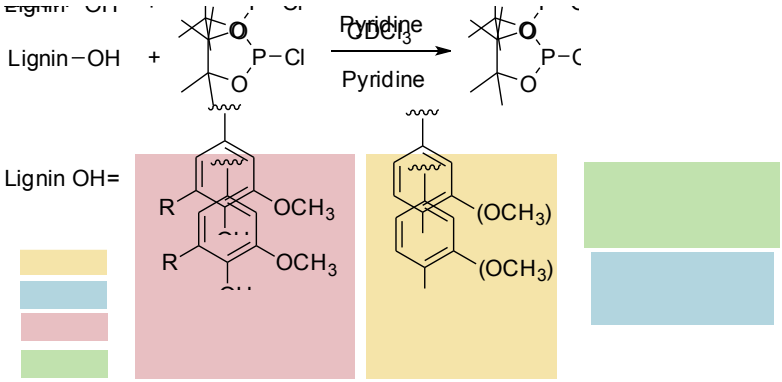

Figure 2Lignin 31P-NMR derivatization with 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP). UnAr: Uncondensed Aromatics, AlOH: Aliphatic OH, 5-S-Ar: 5-substituted aromatics or condensed aromatics, COOH: carboxylic acids.

Low-field (LF) benchtop NMR developments have evolved over the recent decade.(Castaing-Cordier et al., 2021; Giberson et al., 2021; Grootveld et al., 2019) Although the employed magnets and respective fields strengths are much lower than typical high field magnets used in conventional NMR machines, the capital investment, maintenance and support cost are also significantly lower than traditional high-field NMR equipment. Furthermore, low-field benchtop NMR is attractive since for most routine sampling and quality control the resolution and multiplicity of signals are not crucial for the ^{31}P-NMR analysis of lignin and lignin-derived components. What matters in a quantitative ^{31}P-NMR is to have a reliable integration of the different hydroxyl moieties present in lignin, namely, aliphatic OH, aromatic OH (condensed and uncondensed), and carboxylic acids (COOH) Figure 3. Few papers reported in the literature highlight the advantages of using low-field benchtop NMR for lignin characterization(Rönnols et al., 2020) or reaction monitoring,(Kim et al., 2019) but none of which deals with ^{31}P-NMR analysis. In this study, we prove that low-field benchtop NMR can offer a qualitative and quantitative lignin analysis with an accuracy and a precision comparable to that of high-field ^{31}P-NMR techniques but with much lower investment in capital and maintenance cost.

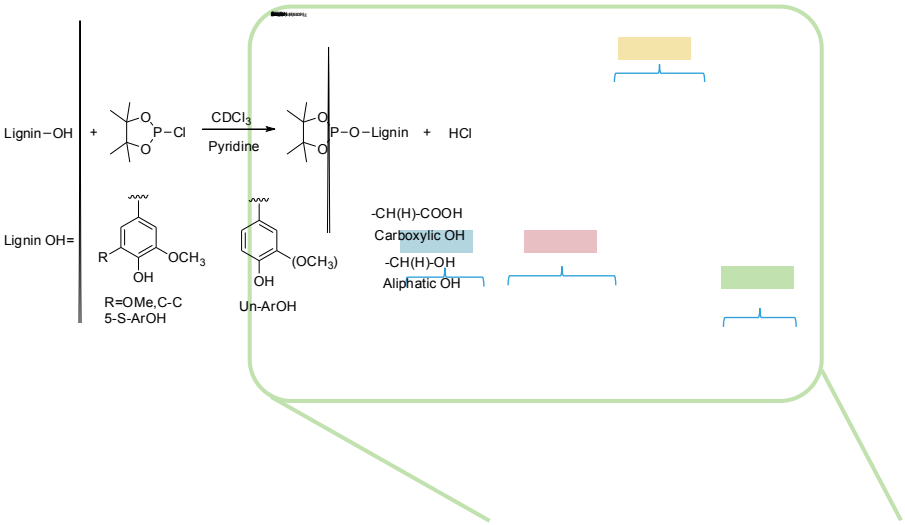

Figure 3 Standard lignin ^{31}P-NMR spectra using TMDP as phosphorylating reagent and NHND as IS.

2. Materials and Methods

Dihydroconiferyl alcohol (**DCA**),(Pepper et al., 1971) dihydrosinapyl alcohol (**DSA**),(Hoover et al., 2013) and propyl guaiacol dimer (**PGD**)(Lahive et al., 2020) were prepared according to existing protocols. Tyrosol, 3-phenyl-1-propanol (**PP**), sebacic acid, phthalic acid, catechol, phenol, propyl guaiacol (**PG**), *N*-Hydroxy-5-norbornene-2,3-dicarboxylic acid imide (**NHND**), 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (**TMDP**), dry pyridine, dry $CDCl_3$, dry DMF, and chromium (III) acetylacetonate (**Cr(acac)$_3$**) were bought from Merck Life Sciences, Across or TCI and used as such without subsequent purification or treatment. Kraft lignin (**KL1**) obtained from softwood was supplied by the LignoBoost plant of RISE (Sweden). **KL2** is Kraft lignin derived from softwood as well. **KL3** is a Kraft lignin derived from hardwood and supplied by Vertoro (The Netherlands). **OS1** is an organosolv lignin extracted from hardwood (beech) provided by Fraunhofer following the procedure described in the literature.(Rossberg et al., 2019) **OS2** is an organosolv lignin obtained from wheat

straw and supplied by Fortum (Finland). **HL1-P** is purified hydrolysis lignin derived from a hardwood mix purified as reported.(Corderi et al., 2021), **EpOS1** was prepared according to described protocol by dissolving lignin in epichlorohydrin in the presence of tetrabutylammonium bromide.(Zhao and Abu-Omar, 2017) **EMK-KL1** was prepared using KL-1 and a lignin modification method described in the literature.(Vendamme et al., 2020) Silylated modified lignin, **Si-EMK-KL1**, was obtained by reacting EMK-KL1 with tert-butyldimethylchlorosilane under basic conditions as described by Habibi et al.(Buono et al., 2016) **Allyl-KL3** was obtained by treating KL3 with allyl bromide under basic conditions as described by Johansson et al.(Jawerth et al., 2016) **LS1**, is a sodium lignosulfonate. **LHO1** and **LHO2** are lignin hydrogenolysis oils produced using lignin first conditions.(Torr et al., 2011; Van den Bosch et al., 2015)

Phosphorus NMR sample preparation was done inside a glove box or under nitrogen flow in the fume hood as follows. A stock solution of $CDCl_3$/Pyridine containing an internal standard (IS) and a relaxation reagent was prepared by dissolving 269.57 mg of NHND as internal standard and 24 mg of $Cr(acac)_3$ in a mixture composed of 8 mL of $CDCl_3$ and 12.8 mL of pyridine. About 100 mg of pre-dried molecular sieves (4 Å) were added to the glass container containing the stock solution.
The ^{31}P-NMR sample preparation was done using the following protocol.(Meng et al., 2019) In a 1.5 mL vial, about 30 mg of sample (for lignins, or about 10 mg for model compounds) were added. Afterward, 0.75 mL of the previously prepared stock solution were transferred to the sample's vial with a glass gastight syringe (1000 µl). Then the sample was stirred until complete dissolution. Afterward, 100 µl of phosphorylating agent (TMDP) were added to the vial with a glass gastight syringe (100 µl) and stirred for a few minutes. Finally, all the content of the vial was transferred into the NMR tube. For the analysis of lignosulfonate (LS), $CDCl_3$ was replaced by DMF-*d*7/DMF as reported before.(Stücker et al., 2018)

The benchtop NMR used in this study is an 80 MHz Spinsolve Ultra series magnet from Magritek, equipped with a dual probe for ^{31}P detection. Standard parameters for the ^{31}P lignin analyses experiments were: 512 scans, D1 of 10 s, bandwidth of 154 ppm center frequency at 140 ppm, pulse angle of 90° with standard ^{1}H decoupling.

High field (HF) ^{31}P-NMR spectra were measured on a 400 MHz Varian/Agilent spectrometer equipped with a 5 mm OneNMR probehead and using the s2pul pulse sequence. Standard acquisition parameters were: a 65° pulse of 9.0 µs, a spectral width of 17 kHz, an acquisition time of 1.2 s, a preparation delay of 10 s and 256 accumulations with 256 scans and standard ^{1}H decoupling.

The chemical shift scale (δ) in ppm was always calibrated relative to the peak resulting from the reaction between TMDP and water at 132.2 ppm.

Theoretical OH values were calculated by dividing 1 by the molar mass of each compound and then multiplying by the number of OH specific to the studied lignin model compound.

Where M_w is the molecular weight of a given model compound and represents the number of hydroxyl groups present in the molecule (*e.g.* catechol: 2 aromatic OHs).

Relative error was calculated using the following formula:

1. Results and Discussion

It is well known that the selection of the internal standard (IS) is crucial while performing quantitative analysis. Avoiding any peak overlapping between the IS and any other peaks is one of the main targets.(Zawadzki and Ragauskas, 2001) Furthermore, the stability of the internal standard commonly used in the ^{31}P-NMR analysis of lignin is a very important factor that has been the subject

of several studies.(D. Argyropoulos, 1994; Balakshin and Capanema, 2015; Korntner et al., 2015) The most common internal standards are cholesterol, cyclohexanol, and NHND. Although this last is only stable for 3 h. after derivatization, its signal does not overlap with any OH functionalities of lignin.(Meng et al., 2019)

On the other hand, the phosphorylating reagent, which is a powerful electrophile, reacts with any traces of water, potentially compromising its reactivity with the OH groups in lignin samples or model compounds.(Meng et al., 2019) Consequently, sample preparation was done in a glovebox to avoid any trace of water or oxygen that contributes to the degradation of the phosphorylating reagent. Yet, since the use of a glovebox can increase the overall cost of ^{31}P-NMR analysis and in a tentative to make the procedure attractive for spinoffs and small to medium enterprises (SME), some samples were prepared and analyzed under the fume hood and nitrogen flushing without any significative influence on the outcome of the analysis.

First, the reliability of the low field benchtop for ^{31}P-NMR analysis of lignin was checked using some selected lignin model compounds Figure 4. These compounds were chosen in a way to cover different classes of hydroxyl groups so that one can have a better assessment of the accuracy of ^{31}P-NMR analysis using a low field magnet. Then, the results were compared with the calculated theoretical values (in mmol/g).

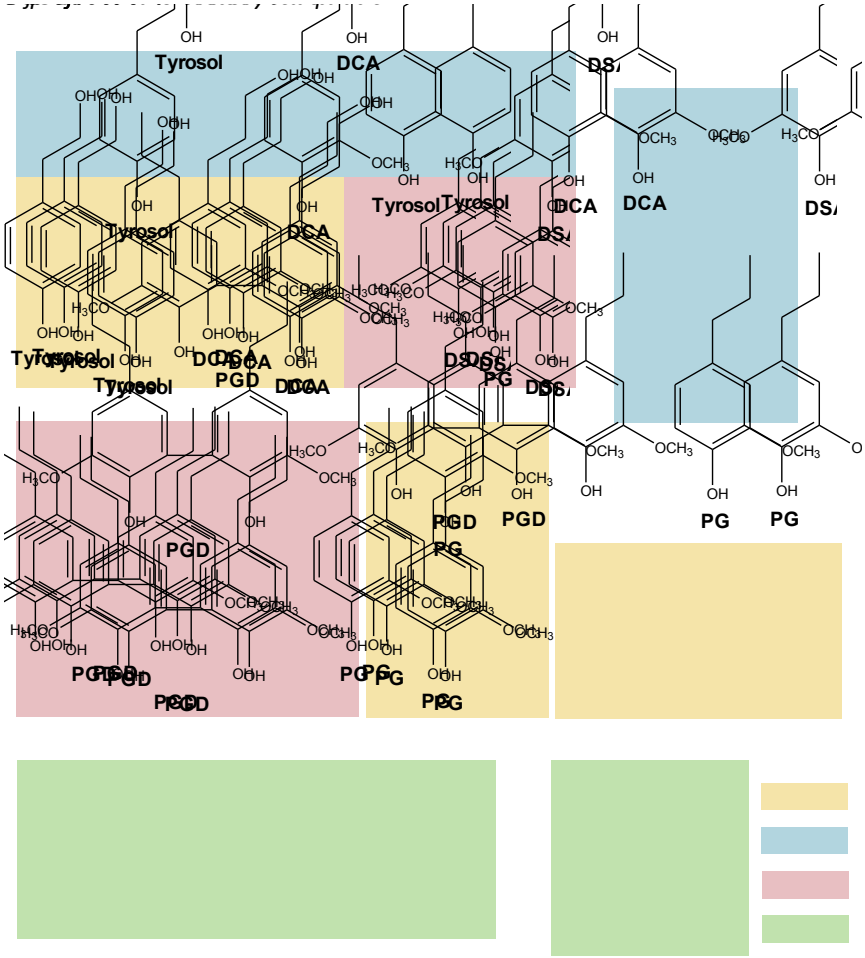

Figure 4 Model compounds employed concerning the hydroxyl group types they represent. UnAr: Uncondensed Aromatics, AlOH: Aliphatic OH, 5-S-Ar: 5-substituted aromatics or condensed aromatics, COOH: carboxylic acids.

3-phenyl-1-propanol (**PP**) was arbitrarily selected to optimize the parameters of the benchtop NMR for the ^{31}P-NMR experiments, especially to get to know the minimum scans needed to have a proper integral value (obtained vs theoretical). It was found that 256 scans were adequate for the model compounds to have a suitable integration with no significant difference from 512 scans Figure 5. Thus, reducing experimental time and lowering the time-lapse between sample preparation and spectra acquisition. An overestimation of the experimental OH values versus the theoretical was observed in all cases (between 2% and 5%), from the first scan to scan 512. A constant integration value was observed from 200 scans onwards. Only when 100 scans or less are used a deviation of 5% was found, which might be because of a bad signal-to-noise ratio. Upon scans accumulation, the

difference was reduced with a factor of two and stayed stable up to 512 scans (Table S1). A D1 of 10s was found to be the right one also for the LF magnet.

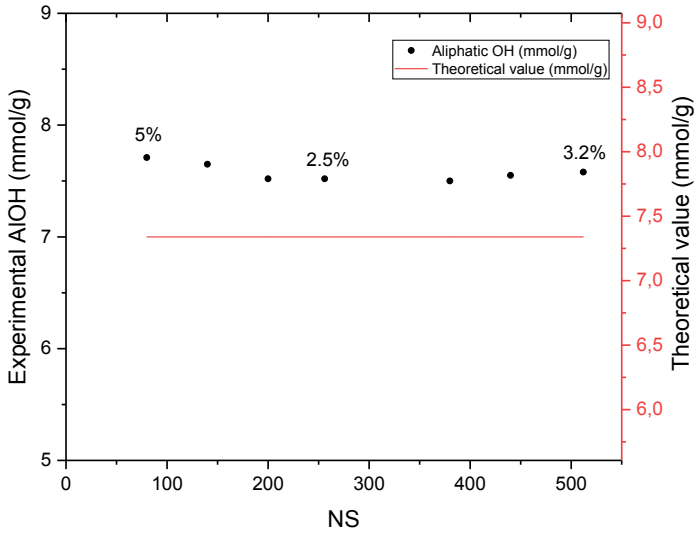

Figure 5 Quantification of OH value (mmol/g) versus the number of scans (NS) used for the 80MHz benchtop NMR PP analysis. Relative errors are included for 80, 256 and 512 scans.

Once the number of scans was optimized on **PP**, these were applied to analyze the other lignin model compounds. The results obtained when ^{31}P analyses were performed on the benchtop NMR are shown in Figure 6. The percentage of error was quite constant and independent of the hydroxyl group type (Figure 6, Table S2) which implies that the measured hydroxyl values are in agreement with those obtained by calculation regardless of the OH type. In every case, an overestimation of around 2 to 4% was obtained, which is an acceptable margin of error within the limits of a typical NMR analysis and the multistep protocol for the sample preparation. This permits us to conclude that ^{31}P-NMR analysis performed using an 80 MHz benchtop NMR offers a good alternative for hydroxyl group quantification in the lignin models with reference to classical high-field NMR.

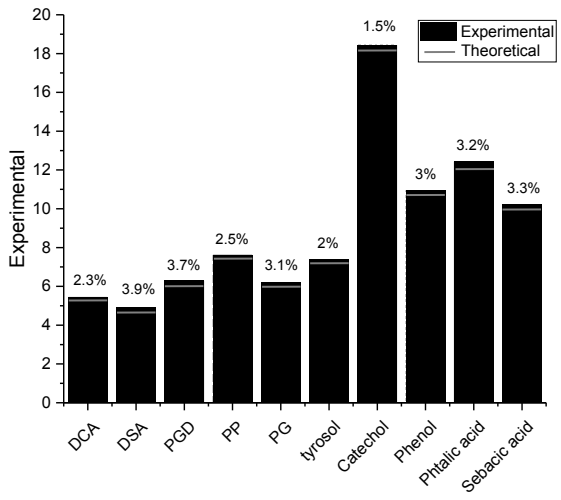

Figure 6 OH values (mmol/g) of model compounds as obtained experimentally by 31P analyses performed on a 80 MHz benchtop NMR and as calculated theoretically

After testing the reliability of the ^{31}P analysis on lignin model compounds using a low field magnet, some lignins with different botanical and process origins were analyzed using both high field (HF) NMR and low field (LF) benchtop NMR to allow a comparison. These lignin compounds can be classified into three distinct categories:

1) Technical (or polymeric) lignins that include: Kraft lignin (KL), lignosulfonate (LS), hydrolysis lignin (HL), organosolv lignin (OS) and soda lignin (SL). It is to be noted that in this work two KL, two OS, a (purified) HL and a LS were used.

2) Lignin hydrogenolysis oils (LHOs) including **LHO1** and **LHO2** which are metal-catalyzed depolymerized low molecular weight lignin oils obtained under lignin first conditions(Abu-Omar et al., 2021) using a Palladium and Ruthenium catalyst, respectively.

3) Modified lignins that refer to lignins whose structure has been modified under a certain reaction process like allylation, epoxidation and silylation, respectively **allyl-KL3**, **EpOS1**, **Si-EMK-KL1**, as well as **EMK-KL1** which is a solvent extracted KL.

As per the case of lignin model compounds, here lignin **KL1** was used to determine the suitable amount of scans required to get reliable analysis results. Based on the OH values obtained after given scans (from 1 to 1024) it was concluded that for lignins 512 scans were sufficient to get a suitable signal-to-noise ratio and therefore reliable OH values (See SI, Table S3).

Upon analyzing technical lignins with both high field NMR and low field benchtop NMR, it was seen that the total OH values differed by 2 to 6% between both instruments except for LS, for which the deviation was 10% Figure 7. Particularly, an overestimation of the total OH value was found with the benchtop NMR analysis, in every case except for LS, following the trend observed for model compounds. These close results obtained with the two NMR instruments are quite impressive considering the difference in magnetic field strength and the complexity of the sample preparation involving several steps and a sample reactivity depending on time. To assess the precision and repeatability of the experiments on the benchtop NMR, samples **KL1** and **OS1** were analyzed three times. Standard deviations of total OHs value were 0.15 and 0.04 mmol/g respectively (Figure 7, Table S4) which shows an exceptional reproducibility of the ^{31}P-NMR analyses. In addition, ^{31}P-NMR experiments of **KL1** and **OS1** were performed with the sample preparation under the fume hood and not inside the glovebox. In those cases, an underestimation of 5% and 1% of total OH value was respectively observed to HF magnet (Table S4). Comparing those results to the average value for the experiments done in the LF NMR, and sample preparation inside the glove box, the underestimation was for both lignin samples, **KL1** and **OS1**, 4%. These results demonstrated that even for sample preparation with standard laboratory facilities the total OH values obtained in the LF magnet are valid.

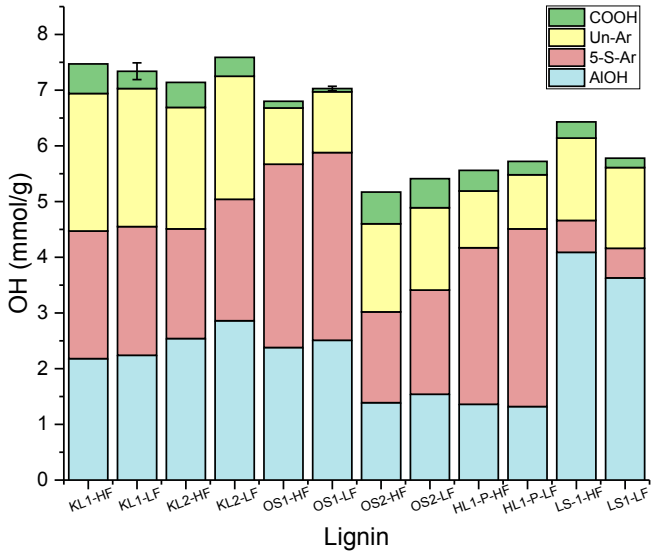

Figure 7 Comparison between the OH values of technical lignins using HF magnet vs LF magnet. KL1-LF and OS1-LH include error bars on the total OH value that were obtained upon the analyses of three replicates. UnAr: Uncondensed Aromatics, AlOH: Aliphatic OH, 5-S

When analyzing lignin hydrogenolysis oils, **LHO1** and **LHO2** with the LF benchtop NMR, the results were even closer to the values obtained using an HF NMR spectrometer compared to the results obtained for the technical lignins. Indeed, the relative deviation was around 2-3%, with a similar overestimation of the OH values when using the LF benchtop NMR (Figure 8, SI Table S5). The smaller deviation between the LF and HF NMR results for the LHOs could be due to the lower heterogeneity of LHO compared to technical polymeric lignins, which results in sharper signals and therefore an easier integration of the peaks of the hydroxyl groups.

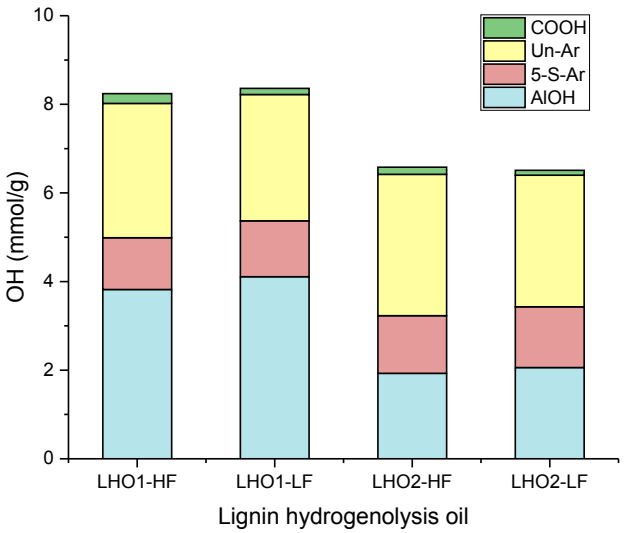

Figure 8 Comparison OH values of lignin hydrogenolysis oils using HF magnet vs LF magnet. UnAr: Uncondensed Aromatics, AlOH: Aliphatic OH, 5-S-Ar: 5-substituted aromatics or condensed aromatics, COOH: carboxylic acids

In addition, some modified lignins were measured with both LF and HF magnets. The influence of the chemical modifications on the hydroxyl groups quantification using the LF benchtop NMR with reference to the HF NMR is represented in Figure 9. In the case of **EMK-KL1** which is modified lignin resulting from the fractionation of **KL1** using ethyl methyl ketone (EMK), the results show a 7.3% overestimation of the total OH content in the case of the LF NMR in comparison with the HF NMR. Reactions to realize allylated Kraft lignin (**allyl-KL3**) and epoxidized organosolv lignin (**EpOS1**) are more reactive towards aromatic OHs and carboxylic moieties than to aliphatic OHs. Therefore, the ^{31}P analysis in both HF and LF magnets did not show any aromatic unit and only the HF spectrometer showed a small COOH peak. The overestimation of the total OH content (mostly aliphatic OHs) was 14.6% and 16% respectively. In the case of silylated Kraft lignin (**Si-EMK-KL1**), the modification was effective on both aromatic and aliphatic OH moieties. Indeed, a difference of 25% was obtained on aromatic OHs, 18% on aliphatic OHs and 27.5% overall. Another ^{31}P NMR observation for modified lignins using LF benchtop NMR is that when the total OH value is low (≈ 1 mmol/g), the relative deviation from the HF NMR results for OH group quantification is high (up to 27.5%) (Figure 9, SI

Table S6). Still, the results obtained with the benchtop LF NMR are good enough to be able to assess and optimize some lignin modifications taken into consideration the easiness and low-cost of the analysis method.

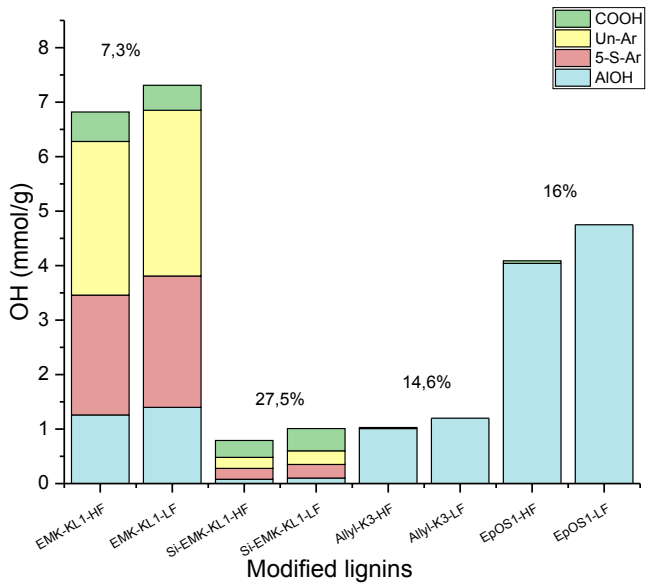

Figure 9 Comparison of OH values of certain modified lignins using a HF magnet vs a LF magnet. UnAr: Uncondensed Aromatics, AlOH: Aliphatic OH, 5-S-Ar: 5-substituted aromatics or condensed aromatics, COOH: carboxylic acids. Relative errors are included between t

2. Conclusion

The effectiveness of LF benchtop NMR for the quantification of OH groups in lignin models and different lignin types using ^{31}P-NMR has been assessed. As compared to conventional 400 MHz HF NMR, no significant differences were observed in total OH for the model compounds, technical lignins, and lignin hydrogenolysis oils. In case of modified lignins the error found was above 10%. These results highlight the capabilities of a benchtop NMR as a routine analytical and quality control tool as it offers quantification of hydroxyl groups within short measurement times (90-180 min), using low sample amounts (±30 mg) along with a reasonable investment in infrastructure and maintenance.

CRediT authorship contribution statement

Jaime Gracia-Vitoria: Conceptualization, Methodology, Analysis, Writing. **Maarten Rubens:** Conceptualization, Methodology, Writing. **Elias Feghali:** Writing - review & editing. **Peter Adriaensens:** Analysis, Writing – review. **Karolien Vanbroekhoven:** Writing - review & editing. **Richard Vendamme:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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