DETERMINATION OF WOOD COMPOSITION USING
SOLID-STATE ^{13}C NMR SPECTROSCOPY

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In recent years, solid-state ^{13}C NMR spectroscopy using the technique of cross-polarization (CP) and sample rotation at a magic angle (MAS) has been widely used in the analysis of plant materials, including wood. Knowledge of the composition, structure and behavior of wood components under various conditions is of great importance, since the properties of wood materials depend on this. In this work, differences in the composition of wood belonging to various tree species growing in central Russia (spruce, aspen, birch, oak, linden, pine, poplar and larch) were revealed using CP MAS ^{13}C NMR spectroscopy. The assignment of various peaks in CP MAS ^{13}C NMR spectra to the main components of wood was carried out. It was shown that cellulose is present in its amorphous and crystalline forms, the presence of lignin was unambiguously confirmed by the signals of aromatic carbon atoms, and hemicelluloses were detected by the signals from the carbon atoms of methyl groups of acetylxylose and L-rhamnose. According to the integral intensities, the total ratio of cellulose and hemicelluloses to lignin was determined: the largest amount of lignin was found in coniferous wood (spruce, larch and pine), and the smallest amount of lignin was detected in deciduous species (aspen, oak, linden, birch and poplar).

Keywords: solid-state ^{13}C NMR spectroscopy, CP MAS, wood, cellulose, hemicelluloses, lignin, syringil, guaiacyl

INTRODUCTION

Lignocellulosic biomass is the most common and bio-renewable alternative to oil and other fossil resources, for example, in the case of effective production of fuels and a range of chemical products.[1,2] In addition, it is a raw material that is widely available worldwide.[3] Lignocellulosic biomass is formed from atmospheric carbon dioxide, water and sunlight through photosynthesis, and represents the only stable source of organic matter on the earth. Its main source is wood, along with other non-wood plant sources. Thus, plant materials in general, and wood in particular, have great economic importance.[4]

Woody plants are composed of different types of cells, organized into three main morphological zones: wood (xylem), bark (phloem) and cambium, the area between the wood and the bark where cell division and plant growth take place. The main components of wood are cellulose (45-50%), hemicelluloses (20-25%), lignin (20-30%)

and low molecular weight compounds (extractives), which can be isolated from wood by solvent extraction.[5–7] Cellulose is a linear homopolysaccharide composed of anhydro-D-glucopyranose units linked by β(1-4)-glycosidic bonds, bearing regions of well-organized supramolecular domains (crystalline cellulose) and disordered domains (amorphous cellulose).[8,9] Hemicelluloses are branched and amorphous heteropolysaccharides composed of glucose and other monosaccharides, such as xylose, mannose, galactose and arabinose. Lignin represents a reticulated macromolecule composed of phenylpropane units.[10]

In many cases, wood can be used as such or after suitable chemical modification to produce a product with the desired properties. Besides the usage of wood and wood composite materials in construction and furniture manufacture, it is widely applied as a source of fibers for the production of cellulose and paper, and as a raw

material for the production of chemical products. In order to fully understand the properties of wood and the functional changes that occur as a result of natural or industrial transformation, it is important to acquire knowledge about the structural features of the wood components. This knowledge is especially important in the case of developing new applications for wood and plant materials.

Solid state ^{13}C NMR spectroscopy, using cross-polarization (CP) and magic angle rotation (MAS) techniques, is a powerful experimental method allowing to collect direct information on the chemical structure and composition of various substances and materials.[11,12] The main advantage of this method is the ability to record spectra for solid samples that are insoluble in standard NMR solvents, so this approach significantly expands the range of research objects. General information on recording solid-state ^{13}C NMR spectra is presented in the literature.[13,14] Recently, there has been considerable interest in the use of CP MAS ^{13}C NMR spectroscopy in the analysis of natural organic materials.[15–17]

Solid-state ^{13}C NMR spectroscopy, in our opinion, is a very useful analytical tool for determining the composition of wood, as evidenced by numerous scientific articles published in recent years and devoted to wood and its components, as well as to wood composites and chemical derivatives of wood components. Information on the use of solid-state ^{13}C NMR spectroscopy for the analysis of wood and other lignocellulosic materials can be found in the monograph of Gil *et al.*,[18] which summarizes the results published before 1999. The quantitative determination of the lignin content in oak wood using CP MAS ^{13}C NMR spectroscopy was first carried out in 1992.[19] Also, age and geographic differences,[20] as well as changes occurring in the process of fungal degradation of wood,[21] torrefaction,[22] chemical treatment[23] and charring[24] were investigated. Considering the above-mentioned aspects, in the present study, we investigated interspecies differences in the composition of wood belonging to various tree species, using solid-state ^{13}C NMR spectroscopy.

EXPERIMENTAL
Materials and methods

For the study, we used wood of spruce (*Picea abies*), aspen (*Populus tremula*), birch (*Betula pendula* Roth), oak (*Quercus robur*), linden (*Tilia cordata*),

pine (*Pinus sylvestris*), poplar (*Populus nigra*) and Siberian larch (*Larix sibirica* Ledeb.), growing in the forest park zone of Saransk (Republic of Mordovia). The climate in the Republic of Mordovia is moderately continental, the average annual temperature is 4.3 °C, the average annual rainfall is 547 mm.

The procedure for collecting, conditioning and preparing wood samples is outlined below. The collection of samples was carried out from living trees growing in the forest-park zone of Saransk. Preference was given to 60-80 year old trees. For sampling, radial cores were drilled by a Haglof increment borer (d = 5.15 mm, l = 100 mm). Wood was drilled perpendicular to the longitudinal axis of the trunk at a height of about 1 m from the ground surface. Then, the samples were sawn into fragments up to 20 mm long and thoroughly dried at 50 °C to constant weight. Preliminary grinding of the materials to a fraction of 0.5 mm was carried out on a rotary knife mill RM-120 ("Vibrotechnik", St. Petersburg). Then, the shredded wood was transfer to an IZE-14M reconstructed shredder.

Microcrystalline cellulose (MCC-101) of pharmaceutical grade (MingTai Chemical Co., Ltd) and purified hydrolysis lignin of type TU 490822905.002-2017 were used as standards for quantitative measurements.

The ^{13}C solid-state NMR spectra were recorded on a JEOL JNM-ECX400 spectrometer (9.39 T, 100.5 MHz) in the solid phase at room temperature, using the cross-polarization technique (CP/MAS), with a rotation speed of 10 kHz in 4 mm zirconium dioxide rotors.[25] MAS angle was adjusted using a special standard sample of KBr, with rotation at 6 kHz. Adamantane was used to adjust the resolution of the instrument and as a reference sample (Z_0 shim was tuned for signal at δ 29.5 ppm). The magic rotation frequency of the wood samples (MAS) was determined at 10 kHz. All ^{13}C CP/MAS experiments were performed at room temperature with typical parameters ([13]C 90° pulse duration 3.3 us, relaxation delay 7 s, offset 100 ppm, acquisition delay 23.82 us, resolution 19.64 Hz, total scans 1024, acquisition time 0.05091 s, decoupling domain ^{1}H, pulse duration 2.93 us) and RAMP CP techniques. The recording time of CP/MAS ^{13}C NMR spectra was ~2 hours. All the spectra were processed using specific software (Delta 4.3.6 and ACD/NMR Processor Academic Edition, Ver. 12.01).

It is important to prepare wood samples with high homogeneity in terms of particle size, so that the rotor with the sample will be well-centered, to provide the required rotation speed.

RESULTS AND DISCUSSION

For each wood species, we used three representative samples, which were completely identical in terms of their CP MAS ^{13}C NMR spectra. However, the spectra of samples

corresponding to different tree species were significantly divergent from each other. The chemical structure of cellulose, hemicellulose components and lignin are schematically shown in Figure 1.

The obtained CP MAS ^{13}C NMR spectra of the samples of spruce, aspen, birch, larch, oak, linden, pine and poplar wood are shown in Figure 2. The spectra of all the samples contain similar signals, and without quantitative analysis, only insignificant differences in intensity can be noted. In general, two key regions can be distinguished: from 160 to 110 ppm and from 110 to 15 ppm. The first region is characterized by broad signals of low intensity, typical of the aromatic structures of lignin components. The second region includes more narrow and intensive peaks belonging to the carbon atoms of carbohydrate fragments (cellulose and hemicelluloses), which overlap each other strongly due to their chemical similarity.

Table 1 shows the assignment of the resonance signals of carbon atoms designated in accordance with the numbering shown in Figure 2. It should be noted that the CP MAS ^{13}C NMR spectroscopy makes it possible to distinguish some chemically equivalent carbon atoms in different conformations of polysaccharide chains, for example, as in the case of amorphous and crystalline cellulose. The most intensive peaks 13 and 14 correspond to the carbon atoms C-2, C-3 and C-5 of glucose; some signals from the propane fragment of lignin are also detected in this region. Two peaks 11 and 12 refer to the C-4 atom of cellulose in the crystalline and amorphous (less ordered structure) forms, respectively. Peak 15 corresponds to the C-6 atom of the glucose moiety, and peak 16 belongs to the C-γ atom of lignin. Peak 17 corresponds to the methoxy groups of the guaiacyl- and syringylpropane moieties. Peak 18 belongs to hemicelluloses and corresponds to the carbon atom CH_3-group of the acetyl fragment of 2-acetyl- or 3-acetylxylose.
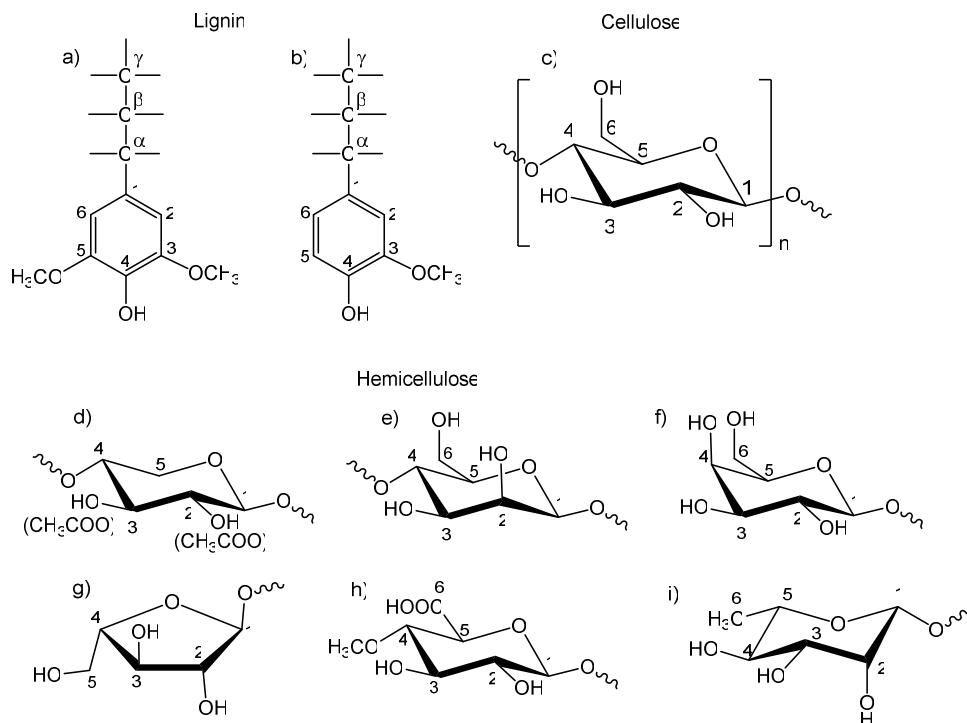

Figure 1: Chemical structure of wood components: fragments of lignin – syringil (a) and guaiacyl (b); cellulose – glucose (c); fragments of hemicelluloses – xylose and acetylated form (d), mannose (e), galactose (f), L-arabinose (g), methylglucuronic acid (h), L-rhamnose (i); (Note: the numbering of atoms corresponds to the numbering given in Table 1)
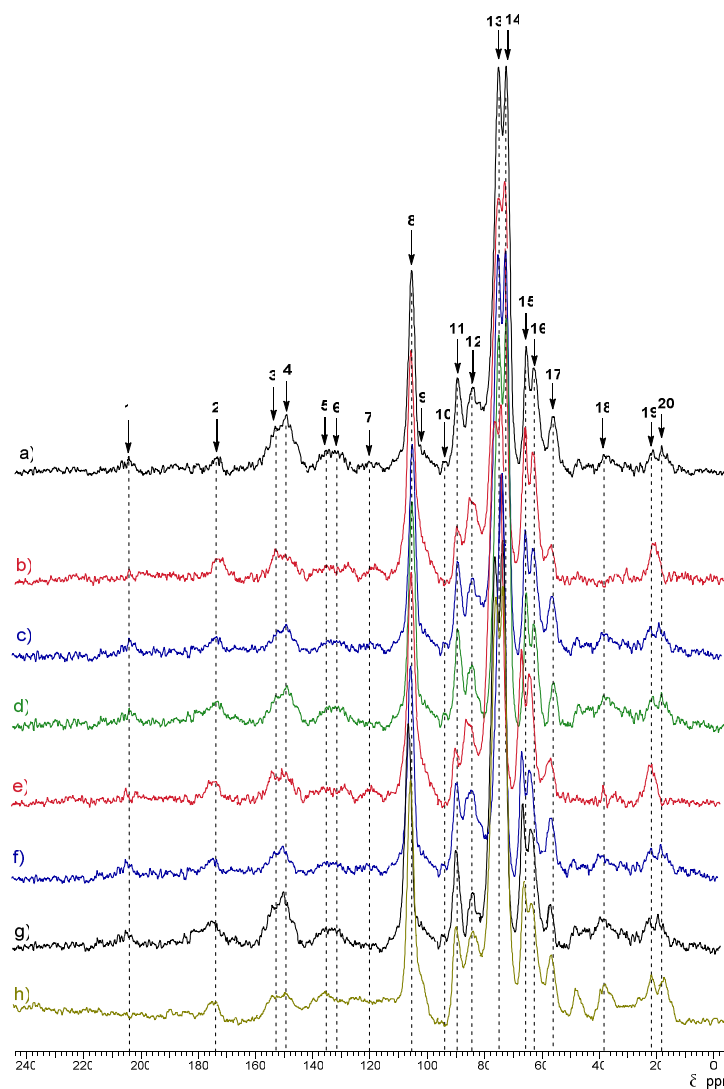
Figure 2: CP MAS ^{13}C NMR spectra of wood samples: spruce (a), aspen (b), birch (c), larch (d), oak (e), linden (f), pine (g) and poplar (h)

Peak 8 corresponds to the C-1 atom of cellulose, and the overlapping signal 9, located in a stronger field, corresponds to a similar atom of hemicelluloses (102 ppm). Undoubtedly, two signals can be attributed to hemicelluloses: peaks 19 and 20, corresponding to the CH_3 group of L-rhamnose. For the assignment of the signals belonging to hemicelluloses, we relied on a previously published study.[26] The low-field peak 1 with extremely low intensity corresponds to the carbon atom of carbonyl groups belonging to the structural fragments of cinnamaldehyde, benzaldehyde and propanone. Peak 2 belongs to the carbon atoms of the carboxyl groups of D-galacturonic and 4-O-methyl-D-glucuronic acids.

Three different types of carbon atoms of aromatic units constituting the lignin lattice are recognized by three groups of signals in the range of 160–105 ppm. Peaks 3 and 4 correspond to oxygenated carbon atoms ($C_{arom.}$–OH and $C_{arom.}$–OCH$_3$). Peaks 5 and 6 belong to the quaternary atoms and peak 7 corresponds to the methine atom of the phenyl fragment of lignin. The assignment of the signals from the lignin carbon atoms was carried out on the basis of the data reported by Holtman *et al.*[27]

Table 1 shows the assignment of the signals observed in CP MAS ^{13}C NMR spectra to carbon atoms of wood components.

Table 1
Assignment of signals in CP MAS ^{13}C NMR spectra

| Signal number	Chemical shift (ppm)	Polymer main assignments	Carbon atom
1	200–210	Hemicelluloses	COOH
2	170–180	Lignin	C=O
3	146–148	Lignin	C-3, C-5 (syringil), C-3 (guaiacyl)
4	144–145	Lignin	C-4
5	135–137	Lignin	C-1 (syringil)
6	133–135	Lignin	C-1 (guaiacyl), C-4
7	118–124	Lignin	C-2, C-6
8	105–106	Cellulose	C-1 (acetal)
9	101–103	Hemicelluloses	C-1 (acetal)
10	93–95	Hemicelluloses	C-1 (hemiacetal)
11	89–91	Cellulose	C-4 (crystalline)
12	84–86	Cellulose	C-4 (amorphous)
13	76–77	Cellulose, hemicelluloses, lignin	C-2,3,5 (cellulose and hemicelluloses), C-α (lignin)
14	74–75	Cellulose, hemicelluloses, lignin	C-2,3,5 (cellulose and hemicelluloses), C-α в β–O-4, C-γ в β-β
15	64–65	Cellulose, hemicelluloses	C-6
16	62–63	Lignin	C-γ, C-γ в β–O-4, C-5 (xylose)
17	55–57	Lignin	OCH$_3$
18	38–40	Hemicelluloses	CH$_3$ (acetylxylose)
19	21–22	Hemicelluloses	C-6 (rhamnose)
20	18–20	Hemicelluloses	C-6 (rhamnose)
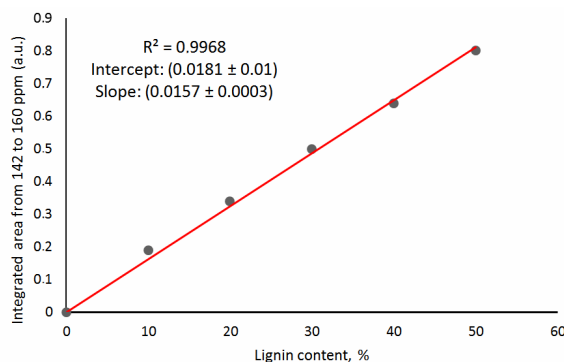
Figure 3: Calibration curve correlating the lignin contents with the area of the signal due to aromatic carbon atoms in the ^{13}C NMR spectra obtained by integration of the spectral window from 142 to 160 ppm

For all the wood samples, the ratio of lignin to the total amount of polysaccharides (cellulose and hemicelluloses) was determined. The determination of lignin was carried out by the integral intensities of peaks 3 and 4, and the determination of polysaccharides was based on the intensity of peaks 8 and 9.

The calibration curve of the dependence of the lignin content (in % by weight) on the intensity of the signals of aromatic carbon atoms was plotted for determining the lignin content (Fig. 3). To construct the calibration curve, the CP/MAS ^{13}C NMR spectra were obtained for five samples with the following composition: hydrolysis lignin (10, 20, 30, 40 and 50% by weight) and microcrystalline cellulose (90, 80, 70, 60, 50% by weight), respectively. The intensity of the spectral range 100–112 ppm was equated to 1, and then the intensity of the spectral range 142–160 ppm was determined.

^{13}C NMR spectra of the mixture of cellulose (50%) and lignin (50%), microcrystalline cellulose and hydrolysis lignin are presented in Figure 4. The spectral range of the aromatic carbon atoms of lignin (area 1) and the spectral

range of the acetal carbon atoms of cellulose (area 2) are marked.

Table 2 shows the content of lignin and polysaccharides in the samples of spruce, aspen, birch and larch, calculated on the basis of CP MAS ^{13}C NMR spectra, excluding other components. The spectra of three samples were recorded for each tree species. The values of the integral intensities for the signals in each spectrum were measured by an automatic method using standard algorithms for integrating isolated and combined signals presented in the Delta 4.3.6 software package.
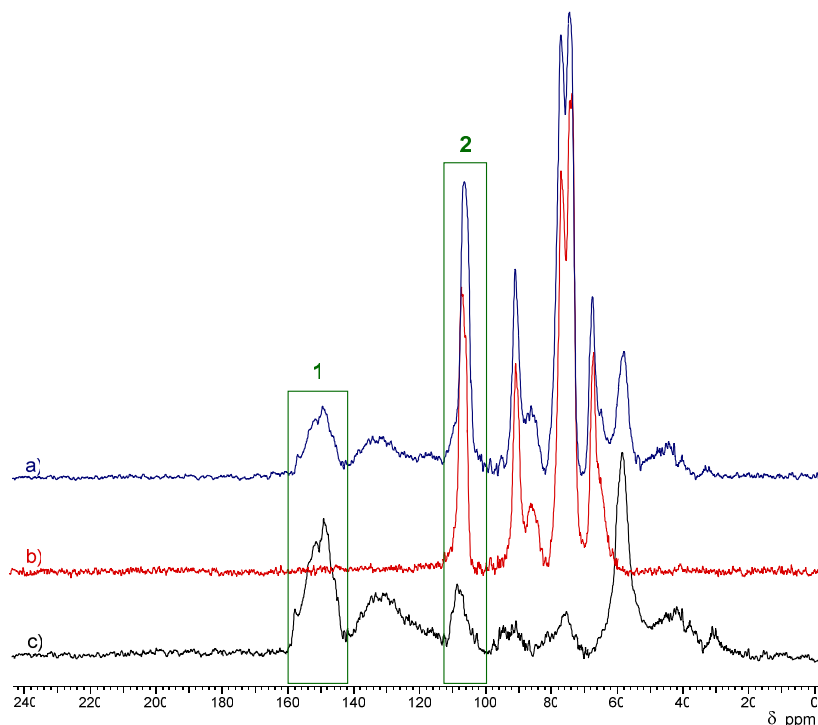

Figure 4: CP MAS ^{13}C NMR spectra of the mixture of cellulose (50%) and lignin (50%) (a), microcrystalline cellulose (b) and hydrolysis lignin (c)

Table 2
Calculated content of lignin and polysaccharides in wood samples

| Sample | Lignin, % | | Polysaccharides, % | | |
| | NMR | Lit. | NMR total | Lit. | |
				Cellulose	Hemicelluloses
Spruce	30.2	27.9[28]	69.8	45.5[28]	22.9[28]
Aspen	23.0	19.5[29]	77.0	52.7[29]	21.7[29]
Birch	22.0	22.4[30]	78.0	42,6[30]	26,7[30]
Larch	29.1	29.2[31]	70.9	37.5[31]	28.4[31]
Oak	24.1	24.1[28]	75.9	40.4[28]	35.9[28]
Linden	22.6	28.3[32]	77.4	46.8[32]	24.3[32]
Pine	20.7	20[28]	79.3	42.0–50.0[28]	24.0–27.0[28]
Poplar	17.0	15.5–16.3[28]	83.0	50.8–53.3[28]	26.2–28.7[28]

As can be seen from Table 2, the highest content of lignin was detected in conifers (spruce, larch), and the lowest content was established for deciduous species (aspen and birch), and these findings agree with those reported in related literature.[28] Thus, solid-state ^{13}C NMR spectroscopy using the technique of cross-polarization and sample rotation at a magic angle (CP MAS) can be successfully used for determining both the qualitative and quantitative

composition of wood. The described method requires a small amount of substance (approximately 100 mg), and only shredding the sample is necessary as a preliminary preparation. The recording time of CP MAS ^{13}C NMR spectra was ~2 hours. Thus, solid-state ^{13}C NMR spectroscopy is an efficient alternative to most analytical methods for determining the composition of wood.

CONCLUSION

The chemical composition of spruce (*Picea abies*), aspen (*Populus tremula*), birch (*Betula pendula* Roth), oak (*Quercus robur*), linden (*Tilia cordata*), pine (*Pinus sylvestris*), poplar (*Populus nigra*) and Siberian larch (*Larix sibirica* Ledeb.), growing in central Russia, was analyzed using CP MAS NMR ^{13}C spectroscopy. The assignment of signals in the spectra was carried out, and the main component composition of wood was determined. The content of lignin was detected on the basis of the integral intensities of the signals corresponding to aromatic carbon atoms, and the total content of polysaccharides (cellulose and hemicelluloses) was determined by the intensities of acetal carbon atoms. The obtained results are in good agreement with the available published data. It has been found that cellulose is represented by crystalline and amorphous modifications, the lignin content increases from deciduous to coniferous tree species, and hemicelluloses are present in all the samples. Thus, CP MAS ^{13}C NMR spectroscopy is a convenient tool for studying the components and chemical composition of wood.

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