Analytica Chimica Acta 1179 (2021) 338836

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Dopant-assisted atmospheric pressure photoionization Orbitrap mass spectrometry – An approach to molecular characterization of lignin oligomers



ANALYTICA CHIMICA ACTA

Dmitry S. Kosyakov^{*}, Ilya I. Pikovskoi, Nikolay V. Ul'yanovskii

Core Facility Center "Arktika", M.V. Lomonosov Northern (Arctic) Federal University, Northern Dvina Emb. 17, Arkhangelsk, 163002, Russia

HIGHLIGHTS

- Acetone, 1,4-dioxane and THF are effective dopants in APPI(+) and
- APPI(-) of lignin.
 Conditions of APPI were optimized (T = 500 °C, dopant flowrate 200 -250 μL min⁻¹).
- Suppression of larger oligomers signals in APPI(+) compared to APPI(-) was observed.
- APPI(-) causes a greater fragmentation of β-O-4 interunit linkages in lignin.
- APPI–Orbitrap HRMS allows detecting up to 3000 wood lignin dimers – decamers.

ARTICLE INFO

Article history: Received 25 January 2021 Received in revised form 19 June 2021 Accepted 4 July 2021 Available online 9 July 2021

Keywords: Lignin Atmospheric pressure photoionization APPI Orbitrap mass spectrometry Dopant

G R A P H I C A L A B S T R A C T



ABSTRACT

Lignin is the second most abundant biopolymer in nature and is considered an important renewable source of aromatic compounds. One of the most promising analytical methods for molecular characterization of lignin is Orbitrap high-resolution mass spectrometry with atmospheric pressure photoionization (APPI), proved itself in the study of lignins of various origins and their depolymerization products. In this work, the photoionization of lignin using acetone, 1,4-dioxane, and THF as solvents for the biopolymer and APPI dopants providing the generation of protonated and deprotonated molecules of lignin oligomers has been studied. The ionization conditions were optimized on the basis of the dependences of the total ion current on temperature and the flow rate of the solution into the ion source. Lignin degradation processes under APPI conditions occur mainly with the cleavage of ether β -O-4 bonds between phenylpropane structural units, demethylation (negative ion mode), as well as the loss of water and formaldehyde (positive ion mode). Negative ion mode APPI provides a higher ionization efficiency in the region of high molecular weights, however, it is characterized by an increased fragmentation of β -O-4 ether bonds compared to APPI(+) leading to a partial depolymerization of lignin in the ion source. The combination of APPI with Orbitrap high-resolution mass spectrometry allows obtaining mass spectra of coniferous and deciduous wood lignins with resolved fine structure and containing signals of up to 3000 oligomers in the mass range of 300-1800 Da. This can be used for comprehensive characterization of lignins at molecular level and tracking changes in biopolymer chemical composition in various processes. © 2021 Elsevier B.V. All rights reserved.

* Corresponding author. E-mail address: d.kosyakov@narfu.ru (D.S. Kosyakov).



1. Introduction

Lignin, constituting up to one third of plant biomass, occupies a special position among biopolymers due to the complexity and lability of its structure, which is formed as a result of enzymatic radical dehydrogenative polymerization of three monolignols (coniferyl, synapyl and *p*-coumaryl alcohols) with the formation of various types of bonds between phenylpropane structural units (Supplementary material, Fig. S1). For this reason, the structure of lignin macromolecules of various plants is still under discussion and its study poses a serious challenge to modern analytical chemistry [1,2].

In recent years, mass spectrometry (along with NMR) has become a most important method for the study of lignin, opening up opportunities for the sequencing of lignin oligomers, which underlies a new field of research - lignomics [3,4]. Given that any isolated lignin preparation consists of thousands of oligomers, including a great number of isobaric compounds, the use of highand ultrahigh-resolution mass analyzers is required to adequately characterize its component composition. In addition to the methods of time-of-flight (TOF) [5] and ion cyclotron resonance (FTICR) [6] mass spectrometry that have been used for a long time, we are talking about mass spectrometry based on an orbital ion trap (Orbitrap MS). The latter has undoubted advantages in the study of lignin, which elemental composition is limited by carbon, hydrogen, and oxygen, due to the availability of equipment, high stability of the mass scale, and the possibility of achieving resolving power >100.000 even on routine benchtop instruments while maintaining a high spectral scan rate [7-9].

The key point in lignin mass spectrometry is the choice of ionization conditions that ensure on the one hand the maximum efficiency of ion generation and, on the other hand, their minimal fragmentation in the ion source. At present, atmospheric pressure ionization is most widely used. Examples of its application for studying various lignins were described in detail in a recent review by Kubatova et al. [10].

Electrospray ionization (ESI), being the most common method for studying biomolecules, is limited in its application to lignin research. Due to the high pK_a values (10–12) of the most common types of phenolic hydroxyl groups in lignin [11,12] and their involvement in intramolecular hydrogen bonds, ESI allows the generation of deprotonated lignin molecules with acceptable efficiency only when using highly alkaline media [13]. This negatively affects the operation of the mass spectrometer, promotes the degradation of lignin in the solution, and at the same time does not allow avoiding unwanted fragmentation in the ion source [8]. Recently proposed alternative methods for the implementation of ESI(+) and ESI(-) based on the formation of lignin adducts with Li^+ and Cl⁻ ions [14,15], showed good results for dimeric compounds, but, in the case of larger oligomers, their effectiveness and the suitability for obtaining tandem mass spectra in a wide mass range remain in doubt. ESI (+) mass spectra of high molecular weight fractions (up to 20 kDa) of lignin were first obtained by Andrianova et al. [16] using 0.1–0.2 M formic acid in aqueous acetonitrile as a solvent and deconvolution of signals of formed multiply charged ions. They adequately reflected the molecular weight distribution of studied lignin preparation, however, despite the high acid concentration, were relatively low in signal intensity.

Atmospheric pressure chemical ionization (APCI) due to its harsh conditions ensures high lignin ionization efficiency. This method was first used to study wheat straw lignin [17] and later to study the mass spectrometric fragmentation of oligolignols [3,4].

Atmospheric pressure photoionization (APPI) first used in lignin mass spectrometry by Banoub et al. [18] showed significantly better results in the detection of both positive and negative ions of lignin oligomers in the molecular weight range < 600 Da and can be considered a milder alternative to APCI. This made it possible to characterize APPI as the most promising method of soft lignin ionization, which has all the advantages of chemical ionization, including excellent response linearity and tolerance to matrix effects [8,19]. A similar conclusion was made by Dier et al. [20] in the study of lignin electrochemical degradation products by FTICR MS. The use of APPI(–) allowed detecting twice as many anions of oligomers in m/z range 100–1000 as compared to ESI and APCI. In the most recent study [21] the application of the APPI(+) with toluene as a dopant allowed obtaining tandem mass spectra of a variety of lignin oligomers isolated from French pine and identifying a wide range of lignin-carbohydrate complexes among them.

Taking into account the good solubility of lignins in highly basic aprotic solvents, especially in the presence of small additives of water [22], it was proposed to use 90% aqueous acetone as a solvent and dopant [8] absorbing UV radiation. Due to its high basicity, acetone promotes the deprotonation of analytes in the gas phase and allows, when using Orbitrap MS, registering signals of several thousands of lignin oligomers in the mass range of 300–1800 Da. This approach has been successfully used to characterize complex mixtures of lignin depolymerization products containing about 7,000 oligomers (from monomers to decamers) with molecular weights up to 1600 Da [23,24], as well as to study the structure of nettle and sedge grass lignins [25,26].

Despite this, there are still no publications aimed at deep studying and optimizing APPI of lignins and their model compounds. The present work aims to fill this gap by developing the methodology for obtaining Orbitrap APPI mass spectra of lignin and their characterization.

2. Experimental

2.1. Objects of the study and their characterization

Six monomeric phenols of the guaiacyl and syringyl types (isoeugenol, IE; coniferyl aldehyde, CA; ferulic acid, FA; acetovanillone, AV; vanillin, VA; and syringaldehyde, SA) modeling the structural units of coniferous and deciduous lignins with different functional groups as well as four dimeric compounds with ligninspecific types of bonds between structural units - guaiacylglycerol- β -guaiacyl ether, GGGE (ether β -O-4 bond); secoisolariciresinol, SILR (alkyl-alkyl β - β bond); dihydrodehydrodiisoeugenol, DDDI (alkyl-aryl β-5 and ether β-O-4 bonds in phenylcoumarantype structure); and pinoresinol, PR (alkyl-alkyl β - β and ether α - $O-\gamma$ bonds in pinoresinol-type structure) – were used as model objects in the study (Fig. 1). DDDI was synthesized from isoeugenol in accordance with the well-known procedure [27]. Its purity (>98%) was confirmed by HPLC and mass spectrometry. All other compounds were purchased from Sigma-Aldrich (Steinheim, Germany) and had a purity of at least 98%.

The preparations of softwood and hardwood dioxane lignins (SDL and HDL) were isolated from spruce (*Picea Abies*) and birch (*Betula Pendula*) wood, respectively, according to the Pepper's [28] method by extraction of wood flour with a mixture of dioxane with water (9:1) under inert atmosphere in the presence of 0.1 M HCl followed by purification by reprecipitation from dioxane to diethyl ether. The full procedure for the isolation and purification of lignin was described in detail earlier [29]. The yields of SDL and HDL preparations were 14% and 12% of the mass of oven-dried wood, respectively. The choice of the Pepper's method involved the mild conditions for lignin isolation with minimal structural changes while maintaining a high yield and representativeness of the sample. This makes dioxane lignin preparations suitable for structural studies and determines their widespread use in lignin research [30].



Fig. 1. Structural formulae and monoisotopic molecular masses of lignin model compounds.

The elemental compositions of the lignin preparations were determined by the catalytic combustion method on a EuroEA-3000 CHNS-analyzer (EuroVector, Pavia, Italy). The obtained results were as follows: C - 63.4%, H - 6.5%, O - 30.1% for SDL and C - 59.9%, H - 6.9%, O - 33.2% for HDL. They correspond to the SDL and HDL gross formulas $C_{10}H_{12.2}O_{3.6}$ and $C_{10}H_{13.8}O_{4.2}$, respectively, recalculated for guaiacylpropane (C_{10}) structural unit.

Weight-average (M_w) and number-average (M_n) molecular weights of SDL and HDL were determined by size-exclusion chromatography on an HPLC system LC-20 Prominence (Shimadzu, Kyoto, Japan) consisted of LC-20AD pump, vacuum degasser, SIL-20A autosampler, STO-20A column thermostat and SPD-20 spectrophotometric detector (detection wavelength was 275 nm). Separation was achieved at 40 °C on an MCX column, 300 \times 8 mm, pore size 1000 Å (PSS, Mainz, Germany). Aqueous solution of sodium hydroxide (0.1 M) was used as a sample solvent and mobile

phase. Calibration of HPLC system was performed using monodisperse sodium polystyrene sulfonate standards (PSS, Mainz, Germany) in the molecular weight range 300–680,000 Da. According to the analyses results, M_w and M_n values were 8.2 and 1.4 kDa, respectively, for SDL and 4.5 and 0.8 kDa, respectively, for HDL.

2.2. Solvents and dopants

High-purity grade acetone, 1,4-dioxane and tetrahydrofuran (Komponent-Reaktiv, S.-Petersburg, Russia) with the addition of 10% Milli-Q water (w/w) were used as solvents, mobile phases and APPI dopants for model compounds and lignin preparations. Their choice was due to the high dissolving power with respect to lignin and the ionization energies (9.7, 9.2, and 9.4 eV, respectively) lying below the quanta energy of the krypton lamp UV radiation. In the

experiments without dopant HPLC gradient grade methanol and acetonitrile (Khimmed, Moscow, Russia) were used.

2.3. Mass spectrometry

Mass spectra were recorded on a Q Exactive Plus mass spectrometer (Thermo Scientific, Waltham, USA) with a quadrupole mass filter and an orbital ion trap mass analyzer with resolving power 70,000 (FWHM, at m/z 200). The instrument was equipped with Ion Max ion source in APPI configuration. A krypton gas discharge lamp with a MgF₂ optical window and quantum energy of 10.0 (10.6) eV was used as a radiation source. Mass scale calibration was carried out daily using a Pierce standard mixture (Thermo Scientific, Waltham, USA) according to manufacturer's recommendations to ensure long-term mass accuracy < 3 ppm. In order to eliminate the solvent signals and minimize ion source contamination the flow injection was used. The sample solution $(5 \,\mu L)$ with a concentration of 50–100 $\mu g \,m L^{-1}$ was introduced into the ion source with the solvent (dopant) flow using an LC-30 Nexera HPLC system (Shimadzu, Kyoto, Japan) consisting of vacuum degasser, LC-30AD pump and SIL-30AC autosampler. Each sample injection was carried out in at least three replicates with further averaging the peak intensities. In all experiments the following ion source parameters optimized in preliminary tests were used: desolvation capillary temperature - 250 °C, S-lens RF voltage -55 arb. units, curtain gas (nitrogen) flow -2 arb. units, sheath and auxiliary gas (nitrogen) pressure - 25 and 5 psi, respectively. The full spectrum scanning mode with registering signals of both positive and negative ions was used in m/z ranges 100-400 and 300-2000 for model compounds and lignin preparations, respectively. The results of at least 10 scans were averaged with further subtracting the background signals of the solvent flow before and after sample zone. The peak picking procedure was performed using the relative intensity threshold value of 0.1% corresponding to the dynamic range of an Orbitrap mass analyzer (5000:1). Mass spectrometer control, data collection and primary processing were performed using Xcalibur software (Thermo Scientific, Waltham, USA). The elemental compositions of lignin oligomers were determined on the basis of accurate mass data applying the following constrains: minimum degree of unsaturation (ring and double bond equivalent, RDB) – 8; maximum number of C, H, and O atoms -100, 200 and 50, respectively; m/z range 300–1000. Home-made Microsoft Excel based software was used to filter data and build van Krevelen diagrams.

3. Results and discussion

3.1. APPI mechanism and dopant effect

Generally, the formation of analyte ions in an APPI ion source can occur via mechanisms of no-discharge APCI (release of ions formed in solution into the gas phase due to the rapid vaporization of solvent molecules) [31], dopant-free photoionization upon absorption of UV radiation directly by analyte molecules or solvent clusters, and doped photoionization upon interaction of the analytes with ionized solvent (dopant) molecules having an ionization energy below the energy of the quanta of UV radiation source. Due to the low content of strongly acidic or easily protonated groups, the first mechanism hardly makes any significant contribution to lignin ionization — in the absence of UV radiation the signal intensities of both model compounds and lignin preparations decrease by about two orders of magnitude both in APPI (+) and in APPI (-) modes. To assess the role of dopant-free photoionization, we compared the signal intensities of four model compounds, as well as low molecular weight methanol soluble fractions (~10%) of lignin preparations in methanol, acetonitrile-water, and 1,4dioxane-water binary mixtures (9:1). The obtained results (Fig. 2) demonstrate that the absorption of UV radiation directly by the components of methanolic and acetonitrilic solutions leads to the generation of significant quantities of both positive and negative analyte ions, while the efficiency of this APPI mechanism is an order of magnitude inferior comparing to the processes involving dopant. An exception is the structures containing carbonyl and carboxyl groups (vanillin, ferulic acid) in the APPI (+) mode, providing signals of protonated molecules in acetonitrile-water medium, comparable in intensity to those obtained using dopant. It is worth noting that, when implementing dopant-free APPI (in a medium of methanol and aqueous acetonitrile), radical cations (molecular ions) formed during direct interaction of analytes with electromagnetic radiation guanta were not observed in the mass spectra of model compounds and lignins. This indicates the predominant occurrence of the secondary proton exchange reactions with solvent molecules (S) according to the known scheme [32,33]:

 $M + h\nu \rightarrow [M]^{+\bullet} + e^{-}$

 $[M]^{+\bullet} + S \rightarrow [M+H]^{+} + [S-H]^{\bullet}$

An alternative pathway is photoionization of clusters of methanol or acetonitrile molecules (including those involving water) with a reduced ionization energy (<10 eV) [34], leading to the formation of both proton donor and acceptor reactive particles (including $O_2^{-\bullet}$), interacting with analyte molecules. This mechanism also rationalizes the formation of deprotonated molecules $[M - H]^-$ observed in the APPI mass spectra along with $[M+H]^+$ ions. Another important mechanism of $[M - H]^-$ formation may involve protolytic dissociation of phenol molecules as a result of photoexcitation, leading to an increase in the acidity of the phenolic group by 3–9 orders of magnitude [35].

The use of aprotic solvents with a low ionization energy in mixtures with water as dopants expectedly leads to higher efficiency of generation of many positively and negatively charged particles of various types [36,37] under the action of Kr lamp radiation and, as a result, protonation/deprotonation of analyte molecules. For all the studied model compounds, the efficiency of APPI (-) turned out to be significantly (up to 2 orders of magnitude) higher compared to APPI (+) due to the presence in their structure of functional groups with protolytic properties and high proton affinity of dopants. It is worth noting that this pattern was not observed for lignin preparations demonstrated comparable values of the total ion current (TIC) in both negative and positive ion modes. This can be explained by the lower availability of phenolic hydroxyl groups in lignin macromolecules for interactions with the dopant, as well as their involvement in the formation of intramolecular hydrogen bonds obstructing protolytic dissociation.

3.2. APPI parameters optimization and dopants comparison

The key parameters affecting the ionization efficiency in APPI are the temperature and mobile phase flow rate. Taking into account the differences noted above in the behavior of model compounds and lignin preparations under APPI conditions, these parameters were optimized using SDL and HDL preparations.

The obtained dependences of the temperature of the ion source on the total ion current (Fig. 3) showed an increase in the efficiency of lignin ionization up to an order of magnitude with an increase in temperature in the range 300–500 °C for all three dopants used in both the APPI (+) and APPI (-). Further heating to 550 °C did not lead to a significant increase in TIC and, in some cases, caused its decrease due to the development of the in-source lignin decomposition processes.

Since the efficiency of dopant assisted APPI depends on the rate of proton transfer between the analyte and dopant (solvent), the



Fig. 2. The comparison of total ion current values for model compounds and methanol soluble fraction of lignins under conditions of APPI with different solvents/dopants.

intensity of the signals of protonated/deprotonated analyte molecules is significantly affected by the concentration of reagents in the gas phase and, therefore, the amount of dopant fed to the ion source. This factor is responsible for the observed close to linear growth in the TIC value of lignin with an increase in the mobile phase flow rate up to 200 μ L min⁻¹ (Fig. 4). Regardless of the dopant used, at high flow rates (>200–250 μ L min⁻¹) a substantial decrease in the ionization efficiency was observed. A similar effect has been described earlier in the literature being associated with the ion recombination and the clustering of dopant molecules resulting in a decrease in their ability to participate in proton transfer reactions [38,39].

Thus, the optimal conditions for aprotic solvent doped APPI are as follows: temperature of 500 °C and a mobile phase flow rate of 200–250 μ L min⁻¹, providing maximum signal levels for both coniferous and deciduous lignins.

Comparison of the lignin TIC values obtained under optimal conditions using the three dopants (Fig. 4) demonstrated that acetone, 1,4-dioxane, and THF are characterized by comparable efficiency in generating both positive and negative ions of lignin oligomers. Thus, the maximum difference in signal intensities for APPI (+) does not exceed $3 \times$, and in the case of APPI (-) it becomes very insignificant (less than two-fold). Acetone and THF slightly outperform 1,4-dioxane. A similar pattern is observed for most of the model compounds (Fig. S2), although the difference in signal intensities during their ionization with different dopants increases up to an order of magnitude due to the presence of only certain specific functional groups in the structure of monomers and

dimers, in contrast to polyfunctional macromolecules. In this case, in terms of effectiveness, the dopants are interchanged and can be located in the following sequences: 1,4-dioxane > THF ~ acetone (positive ion mode) and 1,4-dioxane > THF > acetone (negative ion mode).

To study the effect of the lignin molecular weight on the efficiency of doped APPI, the previously proposed approach based on comparing the signal intensities of a number of oligomers differing only in the degree of polymerization [8] was used. A number of dimers - octamers with the high peak intensities in mass spectra differing in the number of typical guaiacylpropane units with the elemental compositions of C₁₀H₁₀O₃ (178.0632 Da) or C₁₀H₁₂O₄ (196.0741 Da) were selected as test components in SDL and HDL. The obtained results (Fig. S3) were in a good agreement with the presented above data on the effect of the dopant nature on TIC values of lignin preparations and demonstrate an insignificant redistribution of the efficiency of the dopants with an increase in the degree of polymerization of lignin oligomers. It is noteworthy that discrimination (suppression) of signals of larger oligomers in APPI (+) mode compared to APPI (-) was observed for both studied lignins. Thus, the ratio of the intensities of the octamer and dimer peaks in the APPI (+) turned out to be an order of magnitude lower than in the negative ion mode, which suggests an obvious advantage of APPI (-) in the study of high molecular weight fractions of lignin.

When comparing dopants, in addition to the efficiency of lignin ionization, an important factor deals with the level of their own background signals in the mass spectra. The latter can affect the



Fig. 3. Effect of APPI ion source temperature on total ion current of HDL and SDL lignin preparations in negative and positive ion modes using acetone, 1,4-dioxane and THF as dopants.



Fig. 4. Effect of solution (dopant) flowrate on total ion current of HDL and SDL lignin preparations in negative and positive ion modes using acetone, 1,4-dioxane and THF as dopants.

sensitivity of the Orbitrap MS analysis and create difficulties in characterizing the chemical composition of lignins. In this respect, regardless of the m/z range and the ionization mode, 1,4-dioxane has an obvious advantage (Fig. S4) – the TIC value for this solvent is 1.5–5.5-fold lower than that of acetone. It should be noted that intense dopant signals are observed for m/z < 300, while in the region most important for the study of lignins (m/z 300–2000) the contribution of the dopant to the TIC of lignin samples is insignificant (Fig. S5).

3.3. Stability of lignin oligomers under APPI conditions

Taking into account the high lability of lignins under atmospheric pressure ionization conditions, an important factor in optimizing mass spectrometric methods for studying a biopolymer and interpreting the obtained mass spectra is the knowledge of the degradation of structural units of macromolecules and the bonds between them in an ion source. Analysis of the mass spectra of monomeric compounds in the negative ion mode under the established optimal APPI conditions showed a high stability of all studied phenols - the signals of deprotonated molecules dominated in the obtained mass spectra. The main fragmentation pathway involved demethylation with the loss of a methyl radical or methane molecule (Table S1), while the degree of fragmentation was practically independent of the dopant used. Deprotonated isoeugenol exhibited the greatest tendency to fragment in the ion source, the relative intensities of the $[M - CH_3]^-$ and $[M - CH_4]^$ signals reach 12 and 23%, respectively. A completely different pattern characterised APPI (+) mode, when the loss of water, formaldehyde, and CO by carbonyl-containing compounds as well as elimination of CO₂ from ferulic acid were observed (Table S2). The latter is the least stable compound for which the signal of the protonated molecule was not dominant in the mass spectrum. Unlike APPI (-), in the positive ion mode in-source fragmentation is highly dependent on the dopant nature. Thus, only the use of 1,4dioxane led to the elimination of ethylene from the propane chain of isoeugenol, while acetone and THF promoted degradation of coniferyl aldehyde and acetovanillone, respectively.

Comparison of mass spectra of dimeric model compounds obtained using three dopants in negative and positive ion modes (Tables 1 and 2) made it possible to evaluate the effect of ionization conditions on the possibility of partial depolymerization of lignin in the ion source. At first glance, APPI (-) has an undoubted advantage, allowing one to obtain intense signals of [M – H]⁻ ions, while protonated molecules of the analytes under study were generated with high efficiency under APPI (+) conditions only for DDDI. However, in the case of such important structures for lignins of woody plants as GGGE and PR, fragmentation in the positive ion mode proceeded to a greater extent with the elimination of water and formaldehyde, as well as water and CO, respectively, without cleavage the bonds between aromatic monomers. Thus, when acetone and 1,4-dioxane doped APPI (+) is used, the most common β -O-4 ether bond undergoes fragmentation by ~ 20%, while under APPI (-) conditions the total signal intensities of dimeric and monomeric species in the GGGE mass spectra were approximately the same (Fig. S6). SILR is the only model dimer that was highly stable under APPI (–) conditions. Its alkyl-alkyl β - β bond does not undergo cleavage at all, while the signals of monomeric fragments dominated in the mass spectra of this compound obtained in the positive ion mode. Considering the need to minimize the cleavage of bonds between the structural units of lignin macromolecules, the least preferred dopant is THF, which generally ensured harsh ionization conditions compared to acetone and 1,4-dioxane and caused a significantly greater degree of dimer degradation (Fig. S6). It should be noted that the rupture of interunit bonds in lignin macromolecules is not a feature of APPI alone, but is observed on a comparable scale even when using such a mild ionization method as electrospray [8]. Nevertheless, consideration of the effects described above makes it possible to explain the absence of signals of high molecular weight fractions (>2 kDa) in the mass spectra of lignins, which was repeatedly described in the literature, and also to suggest the presence of a certain specificity in the molecular weight distribution and chemical composition of oligomers, detected in lignin preparations in APPI (+) and APPI (-) modes.

3.4. APPI mass spectra of lignins

Mass spectra of coniferous and deciduous lignin preparations (Fig. S7) are highly complex and contain up to 1700 and 3000 peaks of lignin oligomers (up to decamers) with relative intensities > 1%and >0.1%, respectively (Table 3). Noteworthy is the significant gain of APPI (-) over APPI (+) both in the range of molecular weights (1.8 vs 1.4 kDa) and in the number (up to 2-fold) of detected oligomers. The apparent contradiction with the data presented in the previous paragraph on the greater stability of β -O-4 ether bonds under positive ion mode ionization conditions may be rationalized by a rapid decrease in the efficiency of lignin protonation with an increase in the degree of polymerization, which is the reason for the above-noted suppression of signals from the highest molecular weight fractions of lignin when APPI (+) is used (Fig. S3). According to the number of detectable oligomers, THF stands out among the studied dopants, demonstrating not so significant difference in positive and negative ion modes in the case of HDL. In the case of the SDL preparation, APPI (+) gives even more peaks with I > 1%. Despite this, the number-average molecular weights of lignins obtained from the mass spectra turned out to be close for all three

Table 1

Negative ion mode	APPI-Orbitrap mas	s spectra of mode	l dimeric compoun	ds obtained with	different dopants.

Compound	Ion	m/z	Error, ppm	Relative intensity (%) obtained with dopant		t
				Acetone	1,4-Dioxane	THF
GGGE	$[M - H]^{-}$	319.1187	-0.1	35	36	26
	[M-H-H ₂ O-CH ₂ O] ⁻	271.0976	-0.1	100	100	100
	$[M - C_9 H_{13} O_3]^-$	151.0400	-0.3	15	72	86
	$[M - C_9 H_{15} O_3]^-$	149.0244	-0.3	5	22	31
	$[M - C_{12}H_{15}O_3]^-$	121.0269	-0.2	6	22	22
SILR	$[M - H]^{-}$	361.1657	0.2	100	100	100
	[M-H-CH ₄] ⁻	345.1346	0.7	15	6	8
	[M-H-CH ₃ OH] ⁻	329.1395	0.3	12	7	10
DDDI	$[M - H]^{-}$	327.1601	-0.2	100	100	100
	$[M-H-CH_4]^-$	311.1291	0.6	22	5	8
	$[M - C_2 H_7]^-$	297.1134	0.7	4	2	6
PR	$[M - H]^{-}$	358.1346	0.8	50	100	31
	$[M-H-CH_4]^-$	341.1031	0.2	72	13	25
	$[M - C_8 H_{11} O_3]^-$	203.0714	0.4	10	8	20
	$[M - C_{10}H_{13}O_3]^-$	177.0556	-0.6	18	7	15
	$[M - C_{12}H_{15}O_3]^-$	151.0400	-0.2	100	71	100

Table 2

Positive ion mode APPI-Orbitrap mass spectra of model dimeric compounds obtained with different dopants.

Compound	lon	m/z	Error, ppm	Relative intensity (%) obtained with dopant		nt
				Acetone	1,4-Dioxane	THF
GGGE	[M+H] ⁺	321.1333	-0.3	0	0	0
	$[M + H - H_2O]^+$	303.1221	-1.9	8	19	4
	$[M + H - 2H_2O]^+$	285.1116	-2.0	100	100	100
	$[M + H - CH_2O - H_2O]^+$	273.1120	-0,4	73	98	10
	$[M + H - C_7 H_{10} O_4]^+$	163.0750	-1,9	28	25	42
SILR	[M+H] ⁺	363.1802	0	0	0	0
	$[M + H - H_2O]^+$	345.1691	-1.6	17	27	15
	$[M + H - 2H_2O]^+$	327.1586	-1.5	48	68	23
	$[M + H - 2H_2O - CH_3OH]^+$	295.1324	-1.5	35	27	5
	$[M + H - C_7 H_{11} O_4]^+$	203.1070	1.9	46	40	51
	$[M + H - C_{12}H_{16}O_4]^+$	137.0601	2.6	100	100	100
DDDI	[M+H] ⁺	329.1742	-1.7	100	100	100
	$[M + H - C_7 H_8 O_2]^+$	205.1224	0.7	5	11	7
PR	[M+H] ⁺	359.1489	-1.2	5	5	5
	$[M + H - H_2O]^+$	341.1378	-1.5	100	100	100
	$[M + H - 2H_2O]^+$	329.1274	-1.3	18	17	20
	$[M + H - H_2O - CH_2O]^+$	311.1274	-1.4	21	22	43
	$[M + H - C_8 H_{11} O_3]^+$	203.0707	2.0	4	8	18
	$[M + H - C_{12}H_{13}O_4]^+$	137.0601	2.8	0	30	17

dopants, and in the positive ion mode they are 20-30% lower than in the negative one. Interestingly that due to the discrimination of signals of high molecular weight fractions and an increase in the probability of macromolecules destruction with an increase in their degree of polymerization, the M_n value obtained for the HDL preparation was only slightly lower than that for SDL. This was

Table 3

Number of peaks with relative intensities >0.1% (N_{0.1%}) and >1% (N_{1%}) in APPI-Orbitrap mass spectra and MS-based number-average molecular weights of HDL and SDL lignin preparations obtained in negative and positive ion modes using acetone, 1,4-dioxane and THF as dopants.

Parameter	Acetone		1,4-Dioxane		THF	
	APPI (-)	APPI (+)	APPI (-)	APPI (+)	APPI (-)	APPI (+)
HDL						
M _n (Da)	650	610	700	520	620	540
N _{1%}	1559	1089	1743	980	960	1321
N _{0.1%}	2682	2167	2666	1820	2467	2446
SDL						
M _n (Da)	600	460	620	470	490	460
N _{1%}	1750	840	1764	770	1100	620
N _{0.1%}	3090	1410	2890	1580	2100	1420

despite the fact that the latter, according to size-exclusion chromatography analysis, has an average molecular weight of almost twice as compared to HDL.

Regardless of the used dopant and the ionization mode, the obtained APPI mass spectra consist of large groups of peaks corresponding to hundreds of different elemental compositions of lignin oligomers, combined by the degree of polymerization - dimers, trimers, etc. Accordingly, the distances between groups are close to the mass of the most common guaiacylglycerol (194–196 Da) moieties in the preparation of coniferous lignin [8] and syringylglycerol moieties (224-226 Da) in the case of HDL. Within each group, the distances between intense peaks are 14.0156 Da (methylene group), 2.0157 Da (double bond), as well as 1.9792 Da (replacement of the methyl group with hydroxyl). This forms the fine structure of the mass spectrum, represented by clusters of isobaric ion peaks at each nominal mass (Fig. 5). The distance between the peaks within the cluster is 36.5 mDa (2.0157-1.9792 Da). Another type of isobaric ion pair is 4C/30 $(\Delta M = 15.3 \text{ mDa})$, which can be formed by adding four methylene groups to the structure of the molecule with the simultaneous loss of three hydroxyls and the formation of four double bonds.

A certain selectivity of the lignin oligomers detection in APPI (+) and APPI (-) modes is clearly seen using the van Krevelen diagrams (Fig. 6) reflecting the elemental compositions of ions corresponding to all peaks in the mass spectra. The region of typical lignin structures III (H/C = 0.6-1.2, O/C = 0.2-0.4), which also includes the elemental compositions of lignin preparations as a whole, differs only slightly for the positive and negative ion modes, as well as for various dopants. The main difference between APPI (+) and APPI (-) is observed in region IV (H/C = 1.2-1.8, O/C = 0.4-1.0), which corresponds mainly to lignin-carbohydrate complexes. While in the negative ion mode a given area contains a great number of medium intensity signals, registration of positive ions does not allow observing such structures at all. This is apparently due to the destruction of lignin-carbohydrate bonds and carbohydrate residues under APPI (+) conditions. Regardless of the selected ionization mode and dopant, the van Krevelen diagrams for HDL and SDL contain regions I and II with intense signals originating from structures with a low oxygen content (O/C = 0-0.05 and 0.05-0.20, respectively) and an increased degree of unsaturation (H/ C = 0.4-0.7 and 0.6-0.8, respectively). Their formation occurs in APPI mode during side processes in the ion source of the mass spectrometer and ion optics due to the partial degradation of lignin oligomers with the elimination of some functional groups (II) and the formation of condensed structures (I) up to polycyclic aromatic hydrocarbons.

This assumption is supported by the predominance of radical ions with integer RDB values (odd electron particles) in regions I and II. Their formation is not typical for doped APPI (section 3.1). This phenomenon is clearly seen in the m/z – RDB diagrams, where such components form a separate region, which differs from typical lignin structures by higher RDB values and lower molecular weights of the detected compounds (Fig. 7, S8). The use of such diagrams makes it possible to easily isolate signals from intact or minimally degraded lignin oligomers in their mass spectra, both for obtaining general characteristics of the sample and for solving problems of subsequent mass spectrometric sequencing.

4. Conclusions

Highly basic aprotic solvents 1,4-dioxane, acetone, and tetrahydrofuran, possessing a high dissolving power towards lignin, are effective dopants for the photoionization of this biopolymer allowing generating both protonated and deprotonated molecules of lignin oligomers in the molecular weight range up to 1.4-1.8 kDa.



Fig. 5. Thin structure of APPI-Orbitrap mass spectra of lignin.



Fig. 6. Van Krevelen diagrams for SDL and HDL lignin preparations obtained from positive and negative ion mode APPI-Orbitrap mass spectra (dopant – 1,4-dioxane). The color coding indicates the relative intensity of the signals in the mass spectrum. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



Fig. 7. Visualization of positive ion mode APPI-Orbitrap mass spectrum of SDL lignin preparation in m/z–RDB coordinates (dopant – 1,4-dioxane). The color coding indicates the relative intensity of the signals in the mass spectrum. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The use of 1,4-dioxane as a dopant is preferred due to the low level of background signals of this solvent in the mass spectra. The dependences of the temperature and the solution (dopant) flow rate on the ionization efficiency of lignin oligomers are extreme, passing through a maximum at 500 °C and 200–250 μ L min⁻¹. APPI in the negative ion mode provides a higher sensitivity in the region of high molecular weights compared to APPI (+), however, it is characterized by an increased fragmentation of the most common

 β -O-4 ether bonds between phenylpropane moieties of lignin. The combination of APPI with Orbitrap high-resolution mass spectrometry allows obtaining mass spectra of coniferous and deciduous wood lignins with resolved fine structure and containing signals of up to 3000 oligomers (dimers-decamers). This can be used for comprehensive characterization of lignins at molecular level and tracking changes in biopolymer chemical composition in various processes. Subsequent studies should focus on the application of the developed methodology for structural studies of lignins of various origins using tandem mass spectrometry techniques.

CRediT authorship contribution statement

Dmitry S. Kosyakov: Conceptualization, Supervision, Methodology, Writing – review & editing. **Ilya I. Pikovskoi:** Writing – original draft, preparation, Investigation, Methodology, Data curation. **Nikolay V. Ul'yanovskii:** Investigation, Methodology, Data curation, Visualization, Resources, Project administration.

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.

Acknowledgements

This study was performed using instrumentation of the Core Facility Center "Arktika" of the Northern (Arctic) Federal University and supported by Russian Science Foundation (grant 21-73-20275). The work of I. Pikovskoi on lignin mass spectra analysis was also supported by Russian Foundation for Basic Research (Ph.D. student grant 19-33-90043). Authors are grateful to Prof. Albert T. Lebedev for English editing and proofreading the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.aca.2021.338836.

References

- R.C. Sun, Lignin source and structural characterization, ChemSusChem 13 (2020) 4385–4393.
- [2] M. Balakshin, E.A. Capanema, X. Zhu, I. Sulaeva, A. Potthast, T. Rosenau, O.J. Rojas, Spruce milled wood lignin: linear, branched or cross-linked? Green Chem. 22 (2020) 3985–4001.
- [3] K. Morreel, O. Dima, H. Kim, F. Lu, C. Niculaes, R. Vanholme, R. Dauwe, G. Goeminne, D. Inze, E. Messens, J. Ralph, W. Boerjan, Mass spectrometrybased sequencing of lignin oligomers, Plant Physiol. 153 (2010) 1464–1478.
- [4] K. Morreel, H. Kim, F. Lu, O. Dima, T. Akiyama, R. Vanholme, C. Niculaes, G. Goeminne, D. Inzé, E. Messens, J. Ralph, W. Boerjan, Mass spectrometrybased fragmentation as an identification tool in lignomics, Anal. Chem. 82 (2010) 8095–8105.
- [5] D.V. Evtuguin, F.M.L. Amado, Application of electrospray ionization mass spectrometry to the elucidation of the primary structure of lignin, Macromol. Biosci. 3 (2003) 339–343.
- [6] Q. Yulin, F. Pingqing, D.A. Volmer, Analysis of natural organic matter via Fourier transform ion cyclotron resonance mass spectrometry: an overview of recent non-petroleum applications, Mass Spectrom. Rev. (2020) 1–15.
- [7] R.A. Zubarev, A. Makarov, Orbitrap mass spectrometry, Anal. Chem. 85 (2013) 5288-5296.
- [8] D.S. Kosyakov, N.V. Ul'yanovskii, E.A. Anikeenko, N.S. Gorbova, Negative ion mode atmospheric pressure ionization methods in lignin mass spectrometry: a comparative study, Rapid Commun. Mass Spectrom. 30 (2016) 2099–2108.
- [9] J. Prothmann, P. Spégel, M. Sandahl, C. Turner, Identification of lignin oligomers in Kraft lignin using ultra-high-performance liquid chromatography/ high-resolution multiple-stage tandem mass spectrometry (UHPLC/HRMSn), Anal. Bioanal. Chem. 410 (2018) 7803-7814.
- [10] A. Kubátová, A.A. Andrianova, J. Hatton, E.I. Kozliak, Atmospheric pressure ionization mass spectrometry as a tool for structural characterization of lignin, Rapid Commun. Mass Spectrom. 34 (2020), e8813.
- [11] D.S. Kosyakov, S.S. Hviyuzov, N.S. Gorbova, K.G. Bogolitsyn, Protolytic properties of lignin in binary mixtures of water with aprotic solvents, Russ. J. Appl. Chem. 86 (2013) 1064–1069.
- [12] N.V. Shorina, D.S. Kosyakov, K.G. Bogolitsyn, Acidity of guaiacol derivatives in water-acetone mixtures, Russ. J. Appl. Chem. 78 (2005) 127–131.
 [13] D.V. Evtuguin, P. Domingues, F.M.L. Amado, C. Pascoal Neto, A.J. Ferrer Correia,
- [13] D.V. Evtuguin, P. Domingues, F.M.L. Amado, C. Pascoal Neto, A.J. Ferrer Correia, Electrospray ionization mass spectrometry as a tool for lignins molecular weight and structural characterization, Holzforschung 53 (1999) 525–528.
- [14] S.O. Asare, F. Huang, B.C. Lynn, Characterization and sequencing of lithium cationized β-O-4 lignin oligomers using higher-energy collisional dissociation mass spectrometry, Anal. Chim. Acta 1047 (2019) 104–114.
- [15] S.O. Asare, P. Kamali, F. Huang, B.C. Lynn, Application of chloride adduct ionization tandem mass spectrometry for characterizing and sequencing synthetic lignin model compounds, Energy Fuels 32 (2018) 5990–5998.
 [16] A.A. Andrianova, T. DiProspero, C. Geib, I.P. Smoliakova, E.I. Kozliak,
- [16] A.A. Andrianova, T. DiProspero, C. Geib, I.P. Smoliakova, E.I. Kozliak, A. Kubátová, Electrospray ionization with high-resolution mass spectrometry as a tool for lignomics: lignin mass spectrum deconvolution, J. Am. Soc. Mass Spectrom. 29 (2018) 1044–1059.
- [17] J.H. Banoub, M. Delmas, Structural elucidation of the wheat straw lignin polymer by atmospheric pressure chemical ionization tandem mass spectrometry and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, J. Mass Spectrom. 38 (2003) 900–903.
- [18] J.H. Banoub, B. Benjelloun-Mlayah, F. Ziarelli, N. Joly, M. Delmas, Elucidation of the complex molecular structure of wheat straw lignin polymer by

atmospheric pressure photoionization quadrupole time-of-flight tandem mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 2867–2888.

- [19] J. Banoub, G.H. Delmas, N. Joly, G. Mackenzie, N. Cachet, B. Benjelloun-Mlayah, M. Delmas, A critique on the structural analysis of lignins and application of novel tandem mass spectrometric strategies to determine lignin sequencing, J. Mass Spectrom. 50 (2015) 5–48.
- [20] T.K.F. Dier, K. Egele, V. Fossog, R. Hempelmann, D.A. Volmer, Enhanced mass defect filtering to simplify and classify complex mixtures of lignin degradation products, Anal. Chem. 88 (2016) 1328–1335.
- [21] A. Mikhael, T.D. Fridgen, M. Delmas, J. Banoub, Top-down lignomics analysis of the French pine lignin by atmospheric pressure photoionization quadrupole time-of-flight tandem mass spectrometry: identification of a novel series of lignin-carbohydrate complexes, Rapid Commun. Mass Spectrom. 34 (2020), e8910.
- [22] D.S. Kosyakov, K.G. Bogolitsyn, N.S. Gorbova, Specific features of solvation of lignin related phenols in the binary mixtures of water with dimethyl sulfoxide, 1,4-dioxane, and acetonitrile, Russ. Chem. Bull. 63 (2014) 2045–2050.
- [23] D.S. Kosyakov, E.V. Ipatova, S.M. Krutov, N.V. Ul'yanovskii, I.I. Pikovskoi, Study of products of the alkaline decomposition of hydrolysis lignin by atmospheric pressure photoionization high-resolution mass spectrometry, J. Anal. Chem. 72 (2017) 1396–1403.
- [24] A.D. Ivakhnov, I.S. Shavrina, D.S. Kosyakov, I.I. Pikovskoi, I.O. Dumanskii, Depolymerization of alkaline lignin in the medium of supercritical 2propanol, Russ. J. Appl. Chem. 93 (2020) 99–107.
- [25] I.I. Pikovskoi, D.S. Kosyakov, I.S. Shavrina, N.V. Ul'yanovskii, Study of nettle (Urtica dióica) lignin by atmospheric pressure photoionization Orbitrap mass spectrometry, J. Anal. Chem. 74 (2019) 1412–1420.
- [26] I.I. Pikovskoi, D.S. Kosyakov, A.V. Faleva, I.S. Shavrina, A.Y. Kozhevnikov, N.V. Ul'yanovskii, Study of the sedge (Cárex) lignin by high-resolution mass spectrometry and NMR spectroscopy, Russ. Chem. Bull. 69 (2020) 2004–2012.
 [27] G.F. Zakis, Synthesis of Lignin Model Compounds, Zinatne, Riga, 1980.
- [27] G.T. Zakis, synthesis of lightly model compounds, Zhathe, Nga, 1980.
 [28] J.M. Pepper, P.E.T. Baylis, E. Adler, The isolation and properties of lightly behavior of lightly behavior of a spen woods in dioxane-water metained by the acidolysis of spruce and aspen woods in dioxane-water me-
- dium, Can. J. Chem. 37 (1959) 1241–1248. [29] D.S. Kosyakov, E.A. Anikeenko, N.V. Ul'yanovskii, O.Y. Khoroshev, I.S. Shavrina, N.S. Corboya, Jonic Jiquid matrices for MALDL mass spectrometry of lignin.
- N.S. Gorbova, Ionic liquid matrices for MALDI mass spectrometry of lignin, Anal. Bioanal. Chem. 410 (2018) 7429–7439.[30] A. Nzihou, Handbook on Characterization of Biomass, Biowaste and Related
- By-Products, 2020.
- [31] S.B. Turnipseed, W.C. Andersen, C.M. Karbiwnyk, J.E. Roybal, K.E. Miller, Nodischarge atmospheric pressure chemical ionization: evaluation and application to the analysis of animal drug residues in complex matrices, Rapid Commun. Mass Spectrom. 20 (2006) 1231–1239.
- [32] J.A. Syage, Mechanism of [M+H]⁺ formation in photoionization mass spectrometry, J. Am. Soc. Mass Spectrom. 15 (2004) 1521–1533.
- [33] T.J. Kauppila, J.A. Syage, T. Benter, Recent developments in atmospheric pressure photoionization-mass spectrometry, Mass Spectrom. Rev. 36 (2017) 423–449.
- [34] I. Marchi, S. Rudaz, J.-L. Veuthey, Atmospheric pressure photoionization for coupling liquid-chromatography to mass spectrometry: a review, Talanta 78 (2009) 1–18.
- [35] M.V. Kuznetsova, D.S. Kosyakov, N.S. Gorbova, K.G. Bogolitsyn, Acidity constants of lignin model compounds in the electronically excited state in water – NN-dimethylformamide mixtures, Russ. J. Phys. Chem. A. 94 (2020) 1587–1595.
- [36] T.J. Kauppila, T. Kotiaho, R. Kostiainen, A.P. Bruins, Negative ion atmospheric pressure photoionization-mass spectrometry, J. Am. Soc. Mass Spectrom. 15 (2004) 203–210.
- [37] T.J. Kauppila, H. Kersten, T. Benter, Ionization of EPA contaminants in direct and dopant-assisted atmospheric pressure photoionization and atmospheric pressure laser ionization, J. Am. Soc. Mass Spectrom. 26 (2015) 1036–1045.
- [38] D.B. Robb, M.W. Blades, Effects of solvent flow, dopant flow, and lamp current on dopant-assisted atmospheric pressure photoionization (DA-APPI) for LC-MS. Ionization via proton transfer, J. Am. Soc. Mass Spectrom. 16 (2005) 1275–1290.
- [39] T.J. Kauppila, A.P. Bruins, Effect of the solvent flow rate on the ionization efficiency in atmospheric pressure photoionization-mass spectrometry, J. Am. Soc. Mass Spectrom. 16 (2005) 1399–1407.