Characterization of lignins by high-resolution Orbitrap mass spectrometry with atmospheric pressure photoionization

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ABSTRACT

It is shown that use of atmospheric pressure acetone-doped photoionization in combination with high-resolution Orbitrap MS provides the detection of about 2000 oligomers with masses up to 2 kDa in softwood dioxane lignin preparation and up to 10 000 oligomers in the products of lignin solvolysis. The study of the elemental compositions of lignin oligomers allows a rapid assessment of the features and nature of various lignin preparations. The involvement of chemometric methods for high-resolution mass spectra processing opened the possibilities for comparing lignins of various origin and studying their transformation in natural and technical processes.

Keywords: *Lignin, High-resolution mass spectrometry, Orbital ion trap, Atmospheric pressure photoionization*

INTRODUCTION

The most promising method for structural studies of lignin, underlying the new scientific direction – lignomics, is high resolution mass spectrometry, which allows to investigate the most complex objects consisting of hundreds or thousands of components. Due to the limited elemental compositions of natural and some industrial lignins (CHO-class) the reliable determination of elemental compositions of lignin oligomers does not need the very expensive Ion cyclotron resonance technique and can be successfully done by the method of Orbital ion trap mass spectrometry. It allows achieving the spectral resolution up to 500 000 FWHM and mass accuracy better than 1 ppm using the benchtop instruments. Earlier, we have shown that in combination with Orbitrap MS atmospheric pressure photoionization (APPI) can be considered as a preferred method for studying lignin (Kosyakov *et al.*, 2016). This ionization technique is distinguished by a significantly higher intensity of signals and a lower sensitivity to contaminants present in the lignin preparations when compared to other ionization methods. The aim of the present study is to demonstrate the capabilities and advantages of APPI Orbitrap MS for characterization of lignin and its transformations in different processes.

EXPERIMENTAL

Lignin preparations

The alkali lignin isolated from spruce (*Picea Abies*) wood in laboratory conditions was chosen as an object of the study. The elemental composition of the preparation studied was 63.1% C, 6.9% H and 30.0% O. The average molecular weights M_n and M_w determined by size exclusion HPLC were 1050 and 6000 Da, respectively. The ash content was below 0.1%. The depolymerization of lignin was performed in the medium of supercritical isopropanol during 4 h at 450°C using TI-LabS-100 reactor system (TOP Industrie, France) with Inconel reaction cell.

Mass spectrometry

Mass spectra were recorded using a Q Exactive Plus hybrid mass spectrometer (Thermo Scientific, USA) equipped with an orbital ion trap mass analyzer with a resolution of 70,000 FWHM and an Ion Max APPI ion source with a krypton discharge lamp (photon energy of 10.0 eV) as a radiation source. We applied a direct loop injection (5 μL) of the test solution in acetone with a concentration of 50 mg L^{-1} into the ion source of the mass spectrometer at an acetone flow rate of 100–300 μL min^{-1} generated by an LC-30AD chromatographic pump (Shimadzu, Japan). Mass spectra were recorded in the m/z range 300–3000 with subsequent averaging of the results of at least ten measurements and subtracting the solvent background signal. The peaks were detected using a threshold value of the relative intensity of 0.1%. Optimal parameters of the ion source were applied, ensuring the maximum intensity of the mass spectra of the test lignin preparation, found in preliminary experiments: sheath gas pressure, 20 psi; Aux and Sweep gas flow rates, 5 and 2 arbitrary units, correspondingly; desolvation capillary temperature, 250°C; vaporizer temperature, 500°C; S-lens RF level, 55%. In determining the elemental composition of the ions corresponding to the peaks in the mass spectra, a value of 3 ppm was taken as a permissible relative deviation of the calculated *m/z* values from the measured.

RESULTS AND DISCUSSION

APPI-Orbitrap mass spectra of lignin

High-resolution APPI mass spectra of a lignin (Fig. 1) differ by exclusive complexity and contain about 2000 peaks of deprotonated molecules $[M–H]^-$ with relative intensities higher than 0.1%. As typical for the majority of lignins, peaks in the mass spectrum form groups corresponding to oligomers with various degrees of polymerization. The largest detected molecules were decamers with molecular weights up to 1800 Da. The distances between the groups varied in the range Δ m/z from 160 to 200, which corresponds to the abundant guaiacylpropane structural units of coniferous lignins.

The fine structure of the mass spectrum is presented by clusters containing from 5 to 15 peaks of isobaric ions, grouped near integer (in the low-molecular region) m/z values. Their formation is determined mainly by pairs of element combinations with equal integer weights, CH4/O (ΔM =

36.4 mDa) and, much more rare, C4/O3 (ΔM = 15.3 mDa). For example, the peak cluster of trimers with *m/z* 477.05–477.30 corresponds to ten compounds containing from 25 to 31 carbon atoms. In each group, clusters form subgroups of peaks covering mass range of about 10 Da, spaced from each other by 14–16 Da (methylene group or oxygen atom).

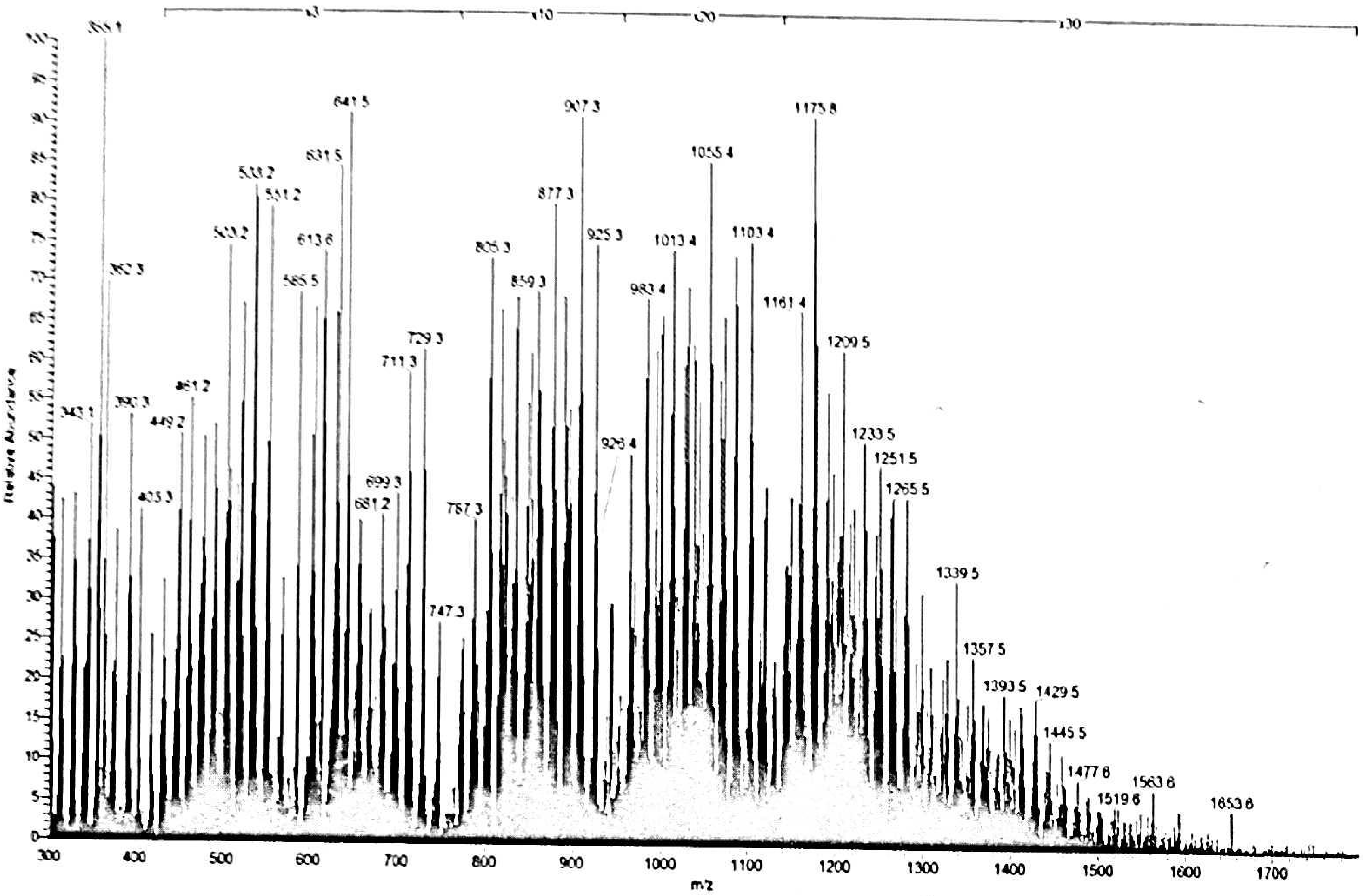

Fig. 1. APPI-Orbitrap mass spectrum of alkali lignin

Elemental compositions of lignin oligomers and their transformation during solvolysis

The calculation of elemental compositions of all detected oligomers using their exact masses allows obtaining the images of lignin samples in van Krevelen coordinates based on elemental ratios. The region of H/C ranging from 0.7 to 1.2 and O/C of 0.2 to 0.5 (Fig. 2) contains the maximum number of peaks, including the most intense. It corresponds to an average gross formula of the structural unit of $C_{10}H_{10}O_3$, which is closed to result of elemental analysis of sample studied. The considerable number of oligomers belongs to the region 0–0.2 O/C and 0.4–0.8 H/C, which corresponds to more condensed and unsaturated structures. During different chemical treatments the transformation of the whole sample can be studied as an image change in van Krevelen plot. For example, the combination of pyrolysis and solvolysis in supercritical isopropanol leads to the shift of main elemental compositions area to the lower oxygen content (0–0.35 O/C) and disappearing the group of condensed unsaturated structures. This means the course of the processes of reduction by isopropanol, as well as the hydrogenation and destruction of condensed structures.

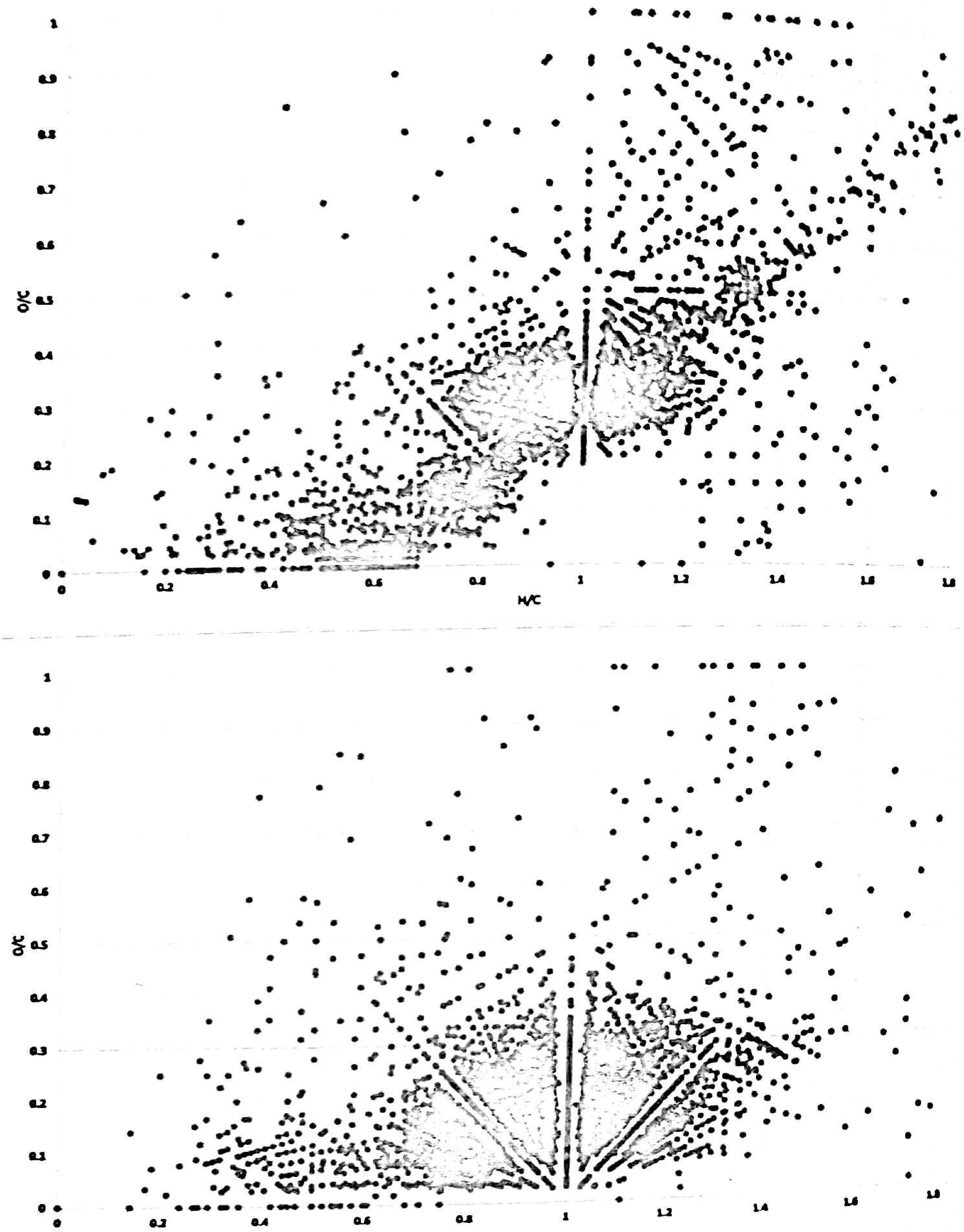

Fig. 2. Van Krevelen plots for alkali lignin (top) and its depolymerization products (bottom).

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