

DEVELOPMENT OF FT–IR, UV AND FLUORESCENCE BASED ANALYTICAL METHODOLOGY FOR LIGNIN CHARACTERISATION

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Abstract The development of the novel analytical methodologies for lignin characterisation is presented. Lignin fractions were characterized by NMR, SEC/GPC as well new analytical methodologies were implemented such as FT–IR, UV and fluorescence. FT–IR and fluorescence results show a promising correlation to the NMR and SEC/GPC analysis. Main findings from the SEC/GPC and NMR results indicates that by adding more water to the organosolv spent liquor isolated lignin contains lower amount of β -O 4 linking motif and also has a lower molecular weight. The following is also confirmed by the straightforward analysis using FT–IR and fluorescence giving the opportunity to replace time-consuming and complex lignin characterisation methods with a simple and quick analytics and the possibility to be applied for the in-process control in continuous production processes.

Keywords:
lignin,
molecular
weight,
FT–IR
spectroscopy,
fractionation,
UV

1 Introduction

Lignocellulosic biomass, one of the renewable resources representing an alternative to fossil oil and gas, is composed of cellulose (50 %-source of carbohydrates), hemicellulose (25 %-source of carbohydrates) and lignin (25 %) which could be used in production of biofuels, chemical, biopolymers and sugars (Liao et al., 2020; Liu et al., 2018). Lignin is a by-product of pulp and paper industry processes (kraft, sulfite and soda) and is usually burned to produce energy for the pulping but it could be used to produce value added chemicals and fuels by reducing its heterogeneity and overall molecular weight (Liao et al., 2020; Pang et al., 2021; Sadeghifar et al., 2020; Yáñez-S et al., 2014). More environmentally friendly organosolv process coupled with a certain lignin recovery/fractionation protocol is a promising way to isolate lignin with a particular properties (molecular weight and structural features). Several fractionation processes using different solvents have been developed (Liu et al., 2018).

Lignin is synthesized via radical polymerization using three monolignols, specifically sinapyl alcohol (syringyl (S)), coniferyl alcohol (guaiacyl (G)) and p-coumaryl alcohol (H), inside the plants such as softwood (mostly G units are present), hardwood (G and S units are present), and some grasses (all three of the units are present (H, G and S)) (Lahive et al., 2020; Liao et al., 2020; Liu et al., 2018; Pang et al., 2021). During the polymerization monolignols are coupled forming specific lignin motifs such as β -O-4, β -5 and β - β (Lahive et al., 2020).

Conventionally used analytics in lignin chemistry Nuclear Magnetic Resonance (NMR) and Size-Exclusion Chromatography (SEC)/ Gel-Permeation Chromatography (GPC) are complex and time-consuming. NMR provides information on lignin structural properties, but the sample preparation and analysis by itself are quite long. SEC/GPC provides the molecular weight of lignin, but again sample preparation takes even more time, because of the derivatization procedure. New analytical methodologies has to be developed for lignin characterization to get particular information faster and easier (Zevallos Torres et al., 2020).

The aim of this paper is to characterize lignin fractions with new analytical methodologies such as UV-vis, fluorescence and Fourier-transform infrared spectroscopy (FT-IR) and to establish a relationship with the conventionally used analytics in lignin chemistry such as NMR and SEC/GPC.

2 Methods

Delignification of beech wood was made in 300 mL reactor (Autoclave engineering, Figure 1) at around 150 °C using mixture of solvents (ethanol and water) and diluted sulfuric acid. Filtration of the cold reaction mixture was then applied followed by drying solid residue in the oven and fractionation of the filtered liquid by adding various volumes of water to recover lignin with specific molecular weight. Recovered lignin was then freeze-dried and characterized using NMR, SEC/GPC, FT-IR, UV and fluorescence.

FT-IR spectra were recorded on FT-IR-ATR spectrophotometer (PerkinElmer, Spectrum Two), in the region 4000-400 cm^{-1} with resolution of 4 cm^{-1} and accumulation 64. The average spectrum of the ten parallel measurements of each sample was considered as a representative spectrum. Molecular weights of derivatized lignin samples were determined using size-exclusion chromatographic system (Thermo Scientific Ultimate 3000, ThermoFisher) equipped with UV detector set at 280 nm using THF as an eluent and Plgel 5 μm MIXED D 7.5 \times 300 mm column. Calibration was performed using PS standards. NMR spectra were recorded using a Bruker AVANCE NEO 600 MHz NMR spectrometer following the protocol reported by Tran et al. (Tran et al., 2015). Fluorescence spectra in spectral range of 300 nm to 530 nm with emission step 2 nm was recorded on a Synergy H1 microplate reader (Biotek).



Figure 1: Autoclave Engineering.

Source: own.

3 Results

SEC/GPC chromatogram profiles (Figure 2a) show higher molecular weights at the fraction 1 (lowest amount of water was added) compared to fraction 5 (the highest amount of water was added). Using water as an anti-solvent it is possible to separate lignins with the specific properties, for instance average molecular weight. Initial lignin was used as a reference and was isolated adding the excess volume of the anti-solvent.

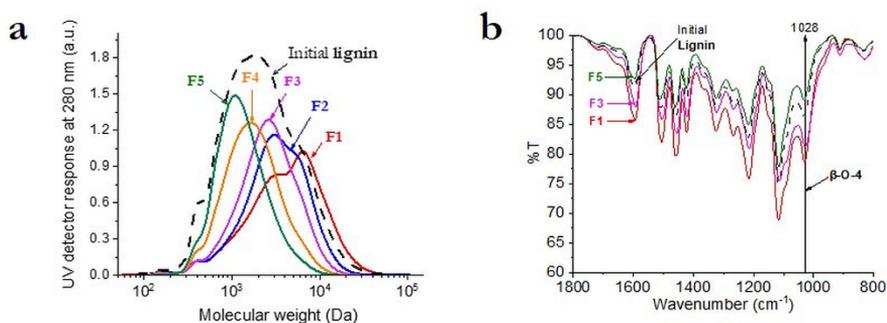


Figure 1: a) molecular weight distributions of the isolated lignins, b) enlarged FT-IR spectrum between 800 cm^{-1} and 1800 cm^{-1} of the initial lignin and three fractions.

Source: own.

FT-IR analysis identifies β -O-4 linkage with a corresponding signal around 1030 cm^{-1} from which the assumption of the number/quantity of it was made (Yáñez-S et al., 2014). The comparison of FT-IR spectra (Fig. 2b) shows a clear increase of the signal at 1026 cm^{-1} from last (F5) to first (F1) fraction which indicates higher content of this linking motif in F1 then in F5 (Yáñez-S et al., 2014). The comparison of the transmittance perversely mentioned signal with integrated surface of signal in NMR spectra which are 14.0, 12.9 and 8.5 per 100 C9 units, for F1, F3 and F5 fractions respectively, show similar pattern (lower integrated number higher transmittance) meaning those two analyses can be correlated.

Combination of fluorescence and SEC/GPC (Fig. 3) analysis indicates that the maximum intensity at 320 - 330 nm is align with the molecular weight which could be used to calculate the approximate molecular weight of initial or any other lignin isolated using EtOH/water organosolv pulping.

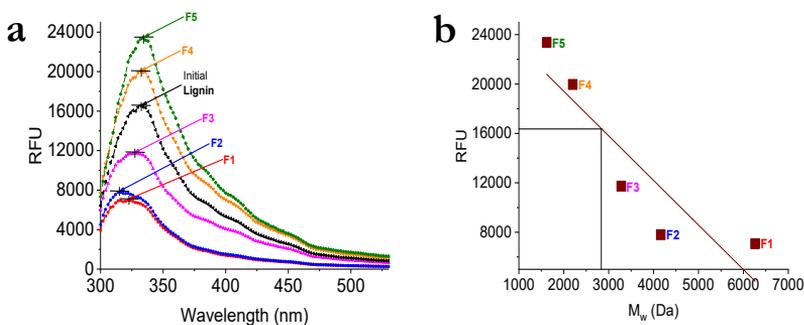


Figure 3: a) fluorescence measurements of initial lignin and all fractions. b) highlighted maximum values are used to correlate fluorescence and SEC analytical data

Source: own.

4 Conclusion

Straightforward FT-IR, fluorescence analysis show a promising correlation with NMR and SEC analytical data when used for lignin characterisation. The outstanding relationship between fluorescence and SEC analysis could be used for the in-process control in continuous production processes, for instance isolating specific lignin/fractions. Further, the SEC-fluorescence relationship will certainly be applied

for the development of more complex analytical methodologies including UV, FT–IR and NMR analytical data.

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