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Title: Rapid quantification and characterization of the pyrolytic lignin fraction of bio-oils by Size Exclusion Chromatography coupled with Multi-Angle Laser light Scattering detector (SEC-MALS).

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Abstract

A rapid method was developed to characterize the Pyrolytic Lignin (PL) fraction of flash pyrolysis bio-oils, based on size exclusion chromatography (SEC) coupled to differential refractive index (DRI) and multi-angle laser light scattering (MALS) detectors. Two beech wood bio-oils with different PL content were used in the study. The first was produced with a single-stage condensation system (BO), while the second was an organic fraction collected in the first stage of a two-stage condensation system (F1). PL was isolated from both the BO and F1 bio-oils by the water precipitation method. Our results suggested that quantification of the pyrolytic lignin fraction of bio-oils can be performed by the SEC-MALS-DRI method provided that the specific refractive index increment (dn/dc) is known, and the integration interval is carefully chosen. Average molar mass (M_n) values for the BO and F1 samples were 580±50 Da and 890±50 Da, respectively. Our results indicated that the condensation system and the water precipitation method affect the average molar mass of isolated PL oligomers.

Keywords

Biomass, Flash Pyrolysis, Bio-oil, Fractional Condensation, Pyrolytic Lignin, Polymer characterization, SEC-MALS, MALS

1 Introduction

Flash pyrolysis bio-oils are CO_2 friendly energy and material vectors with a high potential to replace petroleum in the emerging biomass-based economy. Conventional bio-oils are complex liquids, whose composition and morphology depend on the nature of the parent feedstock, the reactor conditions and the condensation system [1]. Extensive research conducted in the last 20 years has highlighted the need to combine several analytical methods to fully characterize the wide range of molecules present in bio-oils [2]. In contrast to the well-elucidated chemical composition of the low molar mass GC-MS detectable fraction of bio-oils, the chemical composition and structure of the high molar mass fraction (>200 $g.mol^{-1}$) is not completely understood. This fraction is mainly composed of oligomer nanostructures originating from the partial decomposition of the parent lignin and holocellulose polymers [3–9].

Size Exclusion Chromatography (SEC), coupled with a Differential Refractive Index (DRI) detector, has been widely used to characterize the molar mass distribution of the oligomer fraction of bio-oils [10]. Nevertheless, previous work [11] highlighted several limitations of this technique, such as: (i) the lack of an appropriate calibration standard that represents the chemical heterogeneity of bio-oils and (ii) the dependency of the DRI detector response factors on the functionality of chemical compounds. SEC+DRI should therefore be used as a qualitative comparison tool for samples expected to have a similar chemical composition [3,9,11].

Other analytical tools have been used to characterize the structure and functionality of the oligomer fraction of bio-oils, such as: wet chemical methods [4], chromatography and/or spectrometry methods [10,12–16] and advanced NMR techniques [7,9,17,18]. However, interpretation of the data obtained from sophisticated chromatographic and spectrometric techniques can be a time-consuming task, especially when dealing with a considerable number of samples. Developing rapid and simple analytical tools is therefore essential for advancing our understanding of the impact of process conditions, upgrading treatments and aging phenomena on the oligomer fraction of bio-oils.

In this work, we propose a quick way of quantifying and characterizing the oligomer fraction of bio-oils in terms of absolute molar mass based on SEC separation combined with a DRI and Multi-Angle Laser light Scattering (MALS) detector. SEC-MALS-DRI has been extensively used to characterise synthetic polymers [19,20], or bio-polymers such as: natural rubber [21], proteins [22], polysaccharides [23] and lignins [24–29]. Nevertheless, to the best of our knowledge, SEC-MALS-DRI has never been used to characterize bio-oils.

2 Materials and methods

This study was conducted in several steps depicted in Figure 1. Briefly, two bio-oil samples with different PL contents were produced using two condensation systems: a standard and a fractional condensation chain. PL content was obtained from the PL precipitation yields by the dropwise water precipitation method. After determining the differential refractive index increment (dn/dc) value of the PL solution with THF, the PL content was estimated from the SEC-MALS-DRI method and then compared to the PL content determined by the precipitation method. Finally, absolute molar mass values were determined for all samples.

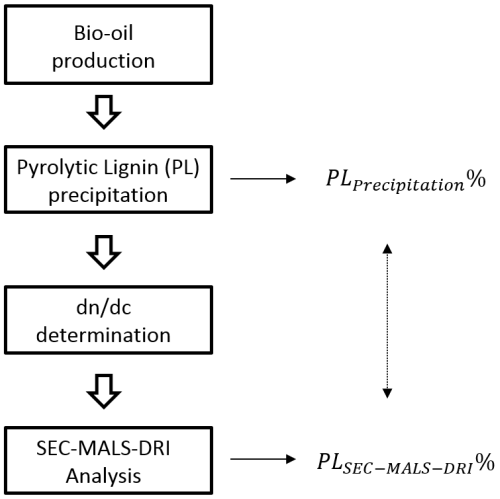

Figure 1: Diagram of the experimental methodology followed in this study.

2.1 Bio-oil production

The bio-oils used in this study were produced at CIRAD using a 0.1 kg/h bench-scale fluid bed reactor described elsewhere [30]. Beech wood sawdust (Lignocel Grade HBK 750-2000 µm) purchased from Rettenmaier & Söhne GmbH (Germany) was used for the experiments as feedstock. Controlled storage conditions resulted in a stable moisture content (≈6%wt) low enough to allow use of the feedstock as received (a.r). Pyrolysis vapours of beech wood were produced at 500°C and online filtered with a ceramic Hot Gas Filter (HGF) placed downstream of the pyrolysis reactor, kept at 450°C. The low fine particle content of beech sawdust minimized char accumulation on the filter surface and thus secondary heterogeneous reactions of pyrolysis vapours inside the HGF unit.

Two tests were conducted in this study, with different condensation systems shown in Figure 2. On the one hand, run 1 was conducted using system A, which corresponded to a single-stage condenser chain. The bio-oil recovered with system A, called BO, was representative of a typical homogeneous bio-oil. On the other hand, run 2 was conducted using system B, which corresponded to a fractional condensation chain composed of two stages. The first stage included a heat exchanger and an electrostatic precipitator, both maintained at 80°C. The bio-oil fraction (F1) recovered at the first stage of system B corresponded to a heavy and almost

water-free bio-oil, whereas the bio-oil fraction (F2) recovered in the second stage was mainly composed of water. To avoid misinterpretation, the organic, water and lights yields were used instead of the whole bio-oil yield. The organic yield was calculated by subtracting the mass of water determined for each stage by Karl Fischer titration (Mettle Toledo, ASTM E 203) from the mass of bio-oil. The standard deviation (SD) for water content was less than 1.5%wt. Lights yield was calculated from the mass gain of the lights trap. Experimental uncertainty was demonstrated in three repeated tests described elsewhere [30]. Before analysis, all samples were stored in a sealed glass container, kept in a refrigerator at 3°C and finally homogenized (magnetic stirring 300 rpm, 15 minutes) prior to analysis. The ultimate analysis was conducted using the complete oxidation method described elsewhere [31]. Oxygen was determined by percentage difference.

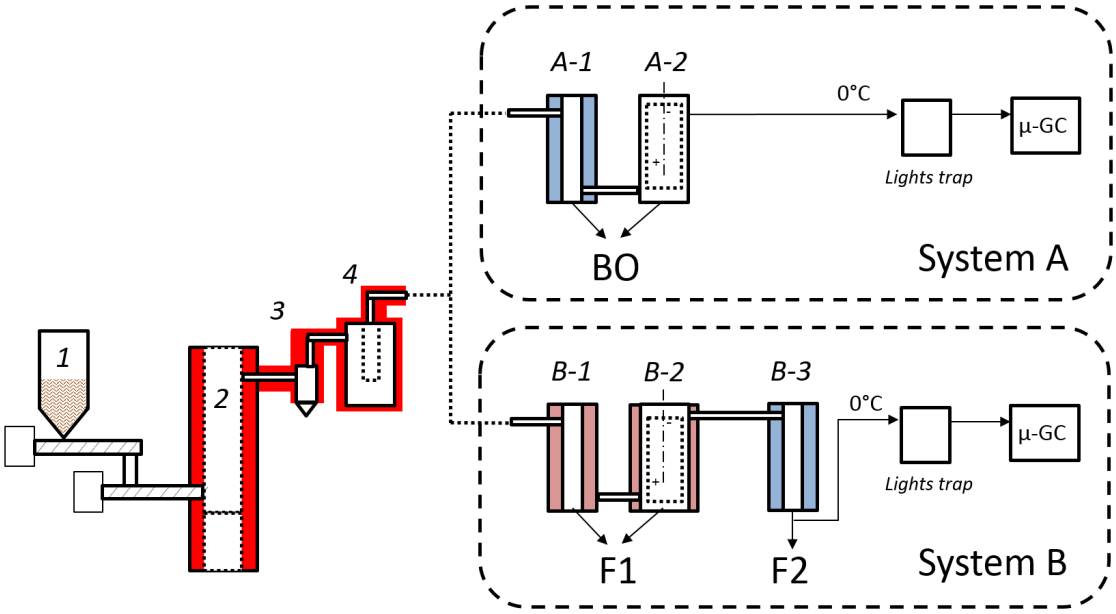

Figure 2: Flash pyrolysis experimental rig at CIRAD. 1) Biomass feeding system, 2) Fluid bed reactor, 3) Cyclone separator, 4) Hot gas filter. Condensation system A: A-1) Heat exchanger, A-2) Electrostatic precipitator. Condensation system B: B-1) Heat exchanger, B-2) Electrostatic precipitator, B-3) Heat exchanger.

2.2 Pyrolytic Lignin precipitation

PL was isolated from the bio-oils with a cold water precipitation method adapted from elsewhere [14]. Briefly, bio-oil samples of BO and F1 were added dropwise to a volume of tap water at 6°C while stirring at 9000 rpm (Ultra-Turrax, IKA). Dropwise adding of bio-oil samples to water was performed with a syringe pump at a rate of 5 mL/h. The bio-oil to water ratio used for each sample is indicated in Table 1. After bio-oil addition was finished, the stirring speed was decreased to 7000 rpm and maintained for 15 minutes. Subsequently, the PL aggregates were filtered through a Buchner funnel using Whatman glass microfibre filter disks (Grade 934-AH). Because of filter plugging, four filters were used for each precipitation. Lastly, the filters were dried under vacuum at room temperature for 48 h until the mass was stabilised. In order to verify repeatability, three precipitations were conducted for each bio-oil

sample. The PL precipitation yield ($PL_{Prercipitation}$ %) was calculated as the average of the three replicates. The mass of product remained stuck in the glassware was measured after drying the Buchner funnel in an oven at 110°C and calculated by difference. The PL precipitated from BO was called "PL-BO", while the PL precipitated from F1 was called "PL-F1".

Table 1: Bio-oil to water ratio used for Pyrolytic Lignin (PL) precipitation and nomenclature of PL extracts

Bio-oil sample	BO	F1
Bio-oil to water ratio (g/ml)	3/500	1,5/500
Nomenclature of PL extracts	PL-BO	PL-F1

2.3 Pyrolytic lignin analysis by the SEC-MALS-DRI method

A SEC-MALS-DRI device is divided into three main parts: a SEC system combined with a Multiangle Laser Light Scattering detector (MALS) followed by a Differential Refractive Index detector (DRI). Analytical grade Tetrahydrofuran (THF) was used as the solvent and eluent for all analyses. The THF was stabilised with 2,6-di-tert-butyl-4-methylphenol (BHT, 250 mg.L^{-1}) and filtered through a 0.1 µm filter.

SEC was performed using a Shimadzu HPLC system equipped with a pump (LC-20AD), a solvent degasser (DGU-20A3R), an auto sampler (SIL20AHT) and a SEC-column (PSS polymer – SDV, 10^3 Å, 8 x30 mm) placed in an oven (CTO-20A), kept at 45°C. The flow-rate of the mobile phase was fixed at 0.65 mL/min. The refractive index measurements were taken using a Wyatt Optilab T-rEX DRI detector at 658 nm and 30°C. Multi-angle light scattering measurements were taken using a Wyatt DAWN-HELEOS II MALS instrument equipped with 18 diode detectors placed at fixed angles about the scattering volume, alternating on either side of the cell. Laser polarized light was set at 663 nm. All diode detectors were normalised using a THF solution of a low-polydisperse polystyrene standard (M_w=30.3 kg/mol, Wyatt technology). The same solution was used to determine the interconnection volume between the two detectors (0.091 mL). The basic theory for determining the weight average molar mass and the radius of gyration for a dilute solution of polymers has already been described in the literature [32,33]. In this study, the average molar masses for each slice of the chromatogram were calculated with Zimm formalism using the first order polynomial for data fitting. Data acquisition and calculations were performed with ASTRA software version 6.1.7.16 (Wyatt technology). The fluophore nature of lignin aromatic systems can lead to an overestimation of the apparent molar mass [24,25,27,29,34]. To overcome this issue, even-number detectors of the MALS analyser were equipped with fluorescence-blocking filters (Wyatt technology) with a pass band of 20 nm [35,36]. The effect of interference filters on LS signals is shown in Figure 3. For all the calculations, only the even-numbered detectors equipped with interference filters were used. In the rest of the text, elution profiles obtained with the MALS analyser will be simply called "LS profiles". Identically, the elution profiles obtained with the DRI-detector will simply be called "DRI profiles".

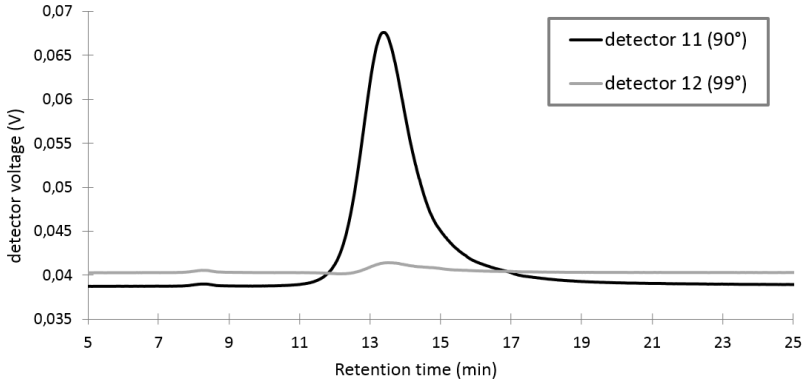

Figure 3: Effect of interference filter on LS signals.

2.3.1 Determination of dn/dc

A necessary condition for determining molar mass with a MALS detector is to know precisely the differential refractive index increment (dn/dc) value of the material in solution. In this study, the dn/dc value was determined for two samples of liquid bio-oil (BO and F1) and one sample of a solid pyrolytic lignin extract (PL-F1). The dn/dc value was calculated using THF as the solvent and the injector rig directly connected to the DRI detector following the method described elsewhere [37]. Briefly, serial solutions were prepared by dissolving approximatively 350 mg of liquid bio-oil in 120 mL of THF. In the case of the solid PL extract, approximately 350 mg of PL was re-solubilised in 120 mL of THF. For this purpose, several glass filter disks containing PL aggregates were placed in a sealed flask and mixed with THF for 15 min in a rotary mixer. All solutions were prepared using a similar range of concentrations (0.6-3 $mg.ml^{-1}$). The samples were filtered through 1 µm syringe filters (Acrodisc glass fibre, Pall) directly into closed glass vials, gently homogenized for 15 min in a rotary mixer and kept in a water bath at 30°C prior to analysis. Under the range of concentrations used, sample solutions remained clear with no evidence of precipitation. The samples were loaded into a WISH Injection Module (Wyatt technology) using a syringe. Once the loop was loaded, the injector was switched to the inject mode, and the sample was allowed to flow into the DRI detector. The determination was repeated twice to assure accuracy of the result. Finally, because the dn/dc value of lignin solutions can change over time [29], we additionally took several measurements at different time intervals, corresponding to t_0+2h, t_0+48h and t_0+7 days.

2.3.2 SEC-MALS-DRI analysis

Samples for SEC-MALS-DRI analysis were prepared following two procedures depending on whether the sample was liquid (BO and F1), or solid (PL-BO and PL-F1).

To prepare liquid BO and F1 samples, 150 mg of raw bio-oil was dissolved in 30 ml of THF in a sealed flask. Samples were homogenized for 15 min in a rotary mixer, kept in a water bath at 30°C for 30 min and finally filtered through 1 µm syringe filters (Acrodisc glass fibre, Pall) directly into glass vials.

For the solid PL-BO and PL-F1 extracts, approximately 300 mg of PL was re-solubilised in 30 ml of THF. For this purpose, 2 glass filter disks containing PL aggregates were placed in a sealed flask and mixed with THF for 15 min in a rotary mixer. Samples were kept in a water bath at 30°C for 30 min and finally filtered through 1 µm syringe filters (Acrodisc glass fibre, Pall) directly into glass vials. The efficiency of PL re-solubilisation was about 99.9%, checked gravimetrically by measuring the mass of the 2 filter disks after THF evaporation.

Three solutions were prepared for each sample and each solution was injected once (50 µl). The PL percentage determined by the SEC-MALS-DRI method (hereinafter called $PL_{SEC-MALS-DRI}$ %) was calculated using equation (1), defined as the mass of sample quantified by the SEC-MALS-DRI method divided by the mass of sample in the solution. For all the samples, the values of $PL_{SEC-MALS-DRI}$ % and average molar masses were calculated as the mean of the three injections.

$$PL_{SEC-MALS-DRI}\ \% = \frac{mass\ of\ quantified\ sample}{mass\ of\ sample\ in\ solution} * 100 \qquad (1)$$

3 Results and Discussion

3.1 Product yields and physicochemical properties of bio-oil samples

The product yields of the two experimental runs are shown in Figure 4. The bio-oil yield obtained for Run 1 was in line with values typically obtained from the flash pyrolysis of beech wood [38]. In the case of Run 2, fraction F1 contained significantly less water than fraction F2. It is noteworthy to mention that overall yield of products remained unchanged. Further analysis of the F1 and F2 samples with gas chromatography coupled with a mass spectrometry detector (GCMS) indicated that F2 was exclusively composed of low M_w compounds, such as alcohols, aldehydes, carboxylic acids and ketones (results not shown). Therefore, the F2 fraction was excluded from this study.

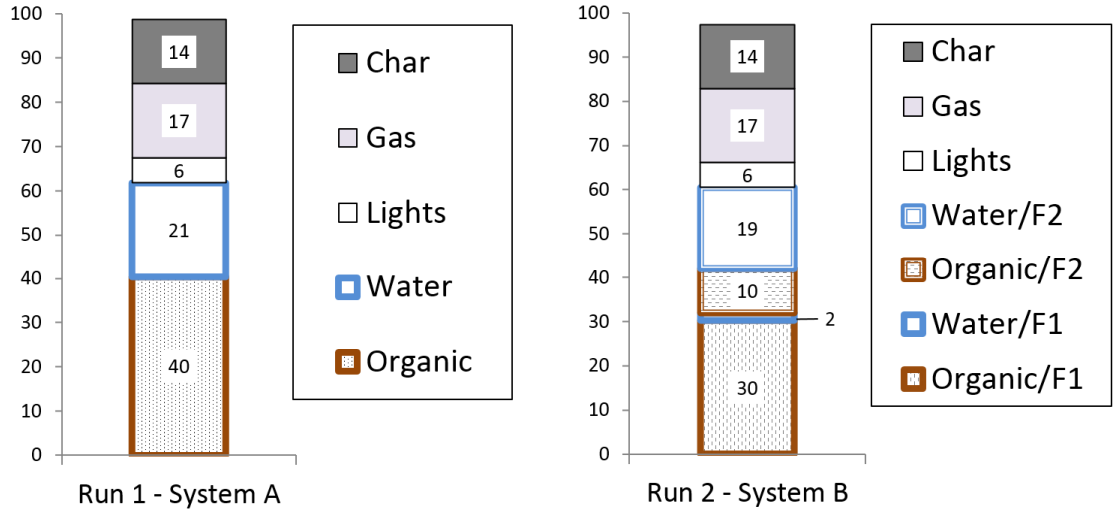

Figure 4: Product yields (wt. % feedstock a.r.) for the flash pyrolysis experiments.

The water content and elemental composition of the BO and F1 samples are detailed in Table 2. F1 contained less oxygen and more carbon than BO, due to the elimination of low M_w oxygenates and the diminution of water content, respectively. The high heating value of F1 increased about 60% compared to BO, while the organic yield decreased by only about 25%. This result showed that the fractional condensation of flash pyrolysis vapours is a simple way of upgrading the heating value of bio-oil, while minimising the loss of organic yield. Moreover, the separation of highly reactive low M_w oxygenates from holocelluloses and lignin-derived oligomers in bio-oils has been shown to undermine ageing phenomena [39]. Nevertheless, in order to limit the reactions between oligomer nanostructures as a result of the elimination of indigenous polar solvents, such as low M_w oxygenates and water, the addition of other solvents, such as ethanol, is expected to have a positive effect [40,41].

Table 2: Physicochemical properties of bio-oil samples.

Bio-oil sample	BO	F1
Water content (wt.% bio-oil w.b.)	33.1 ± 0.1	5,6 ± 0.2
Elemental composition (wt.% bio-oil d.b.)		
%C	56.4 ± 0.6	60,9 ± 0.1
%H	6.6 ± 0.02	6,2 ± 0.01
%O	36.9 ± 0.7	32,6 ± 0.1
LHV (MJ/kg bio-oil w.b.)[a]	14,1	22,7

[a] Estimated from Channiwala's equation [42], wet base (w.b.), dry base (d.b.).

3.2 Pyrolytic Lignin content from the precipitation method

Table 3 shows the average values of PL precipitation yields ($PL_{Precipitation}\%$) for the BO and F1 samples.

Table 3: Pyrolytic Lignin precipitation yields (wt.% bio-oil wet base).

Sample	$PL_{Precipitation}$ (%)	Product in glassware (%)
PL-BO	14.4 ± 2.1	3.9 ± 1.6
PL-F1	34.0 ± 2.2	14.6 ± 2.1

The PL precipitation yield obtained for BO was in line with typical values reported for bio-oils produced from beech wood [38,43–45]. In contrast, the PL precipitation yield obtained for F1 was more than twice as high. As expected, the elimination of most of the water and low M_w oxygenates from F1 resulted in a higher PL precipitation yield. Later value was similar to the water insoluble content obtained by Pollard et al. [46] for the first and second stages of a fractional condensation system. Nevertheless, substantial differences in the water precipitation method prevented further comparison. In any case, the global PL yields (expressed as wt.% biomass a.r.) were nearly the same within experimental uncertainty and resulted in 7.8% and 10.8% for BO and F1, respectively. Moreover, although the bio-oil-to-water precipitation ratio was decreased for F1, the formation of a viscous phase that stuck to the internal walls of the recipient during precipitation increased the mass of product in the glassware. These results indicated that the precipitation method should be optimised according to the type of bio-oil. Sample dilution with a polar solvent (i.e. methanol) or sample extraction with a non-polar solvent (i.e. hexane) prior to dropwise water precipitation was shown to be an effective alternative to minimise product losses in glassware [5,7].

3.3 Specific refractive index (dn/dc) values and evolution

Table 4 summarizes the dn/dc values obtained for BO, F1 and PL-F1 and their evolution over a 7-day period.

Table 4: dn/dc values (expressed in mL/g). Standard deviation was determined from two measurements.

	t_0+2h	t_0+3days	t_0+7days
BO	0.055±0.001	0.052±0.001	0.048±0.0025
F1	0.125±0.002	0.124±0.001	0.125±0.0025
PL-F1	0.184±0.004	0.188±0.001	0.757±0.0020

The dn/dc value obtained for PL-F1 at t_0+2h was 0.184±0.004 mL/g. This result was in line with the dn/dc values typically reported for wood lignin, which range between 0.12 and 0.22 [24,26–29]. The later wide distribution could be attributed to several factors, such as the parent biomass, the extraction method used to isolate lignin from the biomass matrix, or the solvent used [27]. Regarding the evolution of dn/dc over time, our results did not show any significant changes for the BO and F1 samples. The dn/dc value of PL-F1 was constant after 3 days of incubation and increased thereafter. In line with the study by Contreras et al.[29], this result indicated that pyrolytic lignin solutions can undergo chemical modifications during incubation. SEC-MALS-DRI analyses must therefore be conducted within a short period of time after sample preparation.

3.4 Pyrolytic lignin content from the SEC-MALS-DRI method

Figure 5 shows the DRI elution profiles corresponding to (A) PL-BO and BO and, (B) PL-F1 and F1.

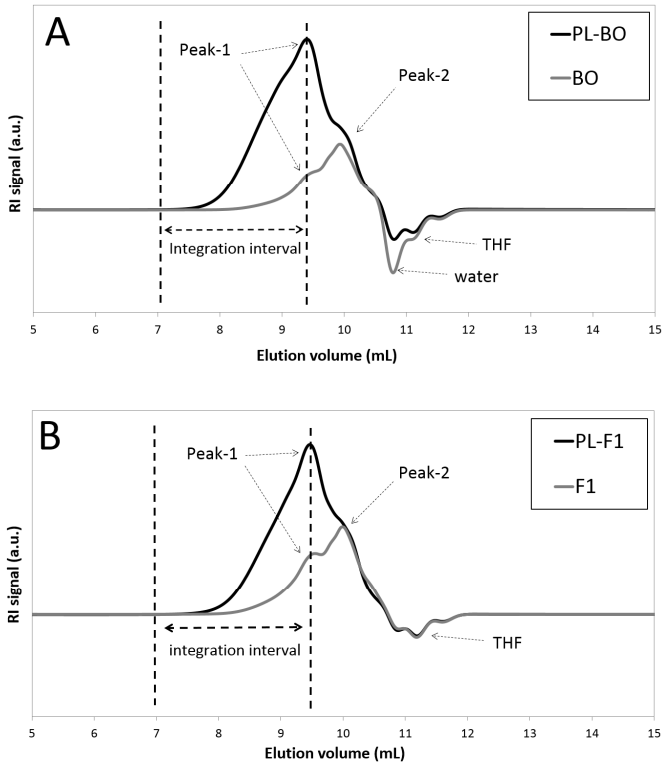

Figure 5: DRI elution profiles of: (A) PL-BO and BO samples and, (B) PL-F1 and F1 samples. Each chromatogram is normalised against the greatest magnitude over all chromatogram data.

The DRI profiles obtained for PL samples PL-BO and PL-F1 showed a single peak (peak-1) with a maximum located at an elution volume value of 9.4 mL and a small shoulder at 10 mL. The elution volume value of 9.4mL was further used to identify the elution volume of PL oligomers in our system. In contrast, the DRI profiles obtained for the BO and F1 samples showed two closely superposed peaks (Peak-1 and Peak-2) with a maximum at 9.4 and 10 mL, respectively. Modifying the SEC separation parameters, such as the number of columns and/or the column packaging, could be an alternative to minimise peak overlapping. Moreover, the lack of peak-2 in the PL-BO and PL-F1 samples confirmed that most of the holocellulose-derived oligomers/molecules and of the lignin-derived low M_w molecules were solubilised in water during PL precipitation. Nevertheless, the small shoulder at peak-2 for PL-BO and PL-F1 indicated that some of these oligomers/molecules are still present in PL extracts probably due to (i) incomplete separation during PL precipitation and (ii) the lack of a purification step for the PL-BO and PL-F1 extracts after water precipitation [5,9].

One expected advantage of the SEC-MALS-DRI method over typical SEC-DRI analysis is the possibility of quantifying PL directly in the bio-oil sample provided that the dn/dc value of the sample is accurately known. The principle is based on integrating the DRI profile. Since flash pyrolysis bio-oils are complex liquids (with hundreds of different molecules and a wide variety of chemical functionalities) use of a single dn/dc value to integrate the whole DRI profile could lead to systematic error [47]. Hence, to avoid error caused by chemical heterogeneity, the PL percentage ($PL_{SEC-MALS-DRI}$%) was calculated by setting the integration boundaries between 7 mL and 9.4 mL for all samples. Indeed, this integration interval covered the left half of peak-1 mostly composed of pyrolytic lignin. Therefore, a dn/dc value of 0.184 mL/g was used to process the selected integration interval. Table 5 shows the $PL_{SEC-MALS-DRI}$ % values calculated for the selected integration interval.

Table 5: Pyrolytic lignin percentage ($PL_{SEC-MALS-DRI}$%) for each sample calculated by the SEC-MALS-RI method. Pyrolytic Lignin percentages are expressed as (wt.% bio-oil wet base)

Sample	$PL_{SEC-MALS-DRI}$%
BO	7.2 ± 0.3
PL-BO	57 ± 6
F1	16.1 ± 0.1
PL-F1	54 ± 5

For PL-BO and PL-F1, the $PL_{SEC-MALS-DRI}$ % values were between 50% and 60%, whereas for BO and F1 the values were 7% and 16%, respectively. Furthermore, since the selected integration interval contained practically half of the area of peak-1, and under the hypothesis of a Gaussian distribution, the $PL_{SEC-MALS-DRI}$% corresponding to the whole of peak-1 was estimated by simply doubling the values detailed in Table 5. As a result, the estimated PL content for PL-BO and PL-F1 was about 100%, whereas in the case of BO and F1, the PL content amounted to 14.4% and 32.2%, respectively. A comparison of the latter estimated PL content values with the PL content determined from the precipitation yields (14.4% for PL-BO, 34% for PL-F1, Table 3), indicated that PL quantification can be conducted directly on raw bio-oil samples by the SEC-MALS-DRI method provided that the dn/dc value is known. The SEC-

MALS-DRI method therefore allows rapid PL quantification instead of the dropwise water precipitation method, which is labour-intensive and can entail high inter-laboratory uncertainty. However, more experimental work is needed to fully validate this PL quantification method.

3.5 Determination of PL average molar masses in bio-oil

Table 6 summarizes the number-average molar mass (M_n), the weight-average molar mass (M_w) and the polydispersity index (Đ) calculated from the selected integration interval (7 mL to 9.4 mL) for the BO, F1, PL-BO and PL-F1 samples.

Table 6: Results for the number-average molar mass (M_n), the weight-average molar mass (M_w) and the polydispersity index (Đ).

Reference	M_n (Da)	M_w (Da)	Đ=M_w/M_n
BO	580 ± 50	980 ± 120	1.7 ± 0.2
PL-BO	1190 ± 100	1640 ± 130	1.4 ± 0.1
F1	890 ± 50	1490 ± 80	1.7 ± 0.2
PL-F1	1100 ± 100	1530 ± 100	1.4 ± 0.2

The M_n measured for BO was about 580 Da. This value is in line with the values reported by Bayerbach et al. [3], obtained by a combination of separative SEC and MALDI-TOF-MS analyses for a PL sample isolated from a beech wood bio-oil. Assuming that PL oligomers are mainly composed of Guaiacyl and Syringyl moieties with an individual molar mass between 180 and 200 Da, it be can concluded that the PL oligomers present in BO are mostly formed by 3 to 4 monomer units. In the case of F1, the M_n was approximatively 890 Da, indicating that the PL oligomers present in F1 are mostly composed of 4 to 6 monomer units. The higher M_n of F1 in comparison with BO suggested that re-polymerisation reactions took place to some extent, probably promoted by: (i) the high wall temperature of the first stage of condensation and (ii) more severe aging catalysed by the lower water content of this sample.

PL-BO and PL-F1 samples shown similar M_n values within experimental uncertainty. Furthermore, the higher M_n and lower dispersity index values of the PL-BO and PL-F1 samples compared with BO and F1 was probably linked to permanent attachment of oligomer moieties during PL precipitation. However, to the best of our knowledge, this effect has not been reported previously since most of the studies regarding the structure and chemical composition of the PL fraction of bio-oils were performed after PL isolation by the solvent fractional method [3–5,7–9]. Therefore, more research should be conducted to unveil the impact of precipitation and precipitation parameters on the structure and composition of PL oligomers.

Figure 6 shows the DRI and LS profiles obtained for BO, F1, PL-BO and PL-F1. In the case of BO, the signal-to-noise ratio was slightly below the ten-fold recommended limit [37]. This result suggested that sample concentration should be increased when dealing with conventional bio-oil samples. Note that a negative peak was systematically observed at the beginning of the LS

profiles of the PL-BO and PL-F1 samples. Elution slices that had negative values were automatically removed by ASTRA software before calculating the averages. One explanation for this abnormal profile is linked to the absorption of the incident light by fluorophore molecules present in the PL-extract samples. In this case, a decrease in sample concentration could be a simple way of mitigating this phenomenon.

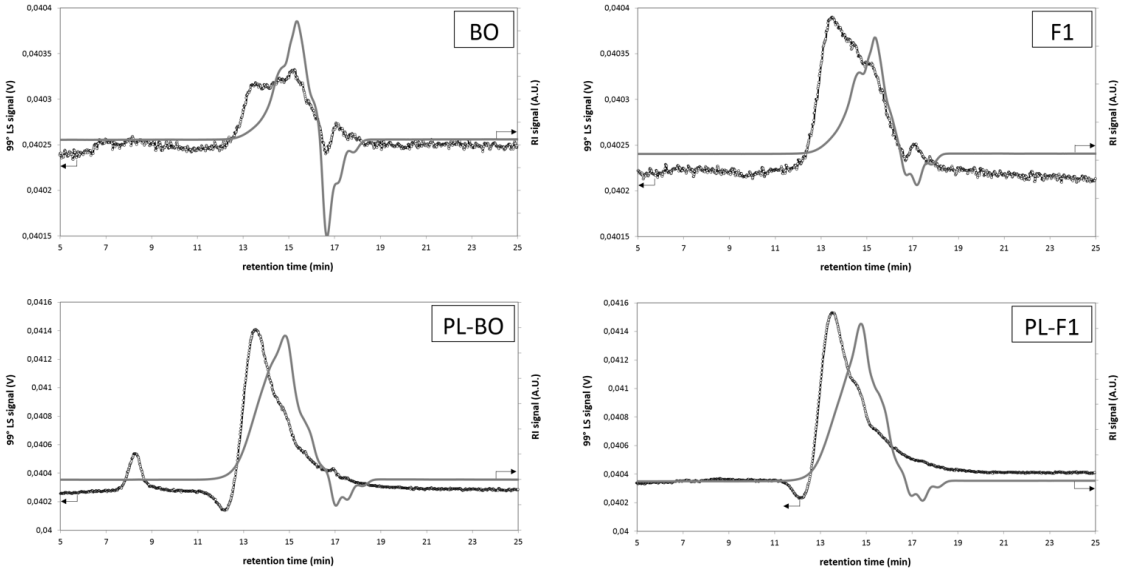

Figure 6: Elution profiles of the LS (dotted line) and DRI (continuous line) detectors for BO, F1, PL-BO and PL-F1 samples. Note that for the sake of clarity only results for one of the three repeated injections were illustrated. In the case of PL-BO, the LS and DRI elution profiles shown correspond to the excluded sample.

4 Conclusions

In this work we developed a rapid method based on SEC-MALS-DRI to quantify and characterize the oligomeric fraction of bio-oils in terms of molar mass. The following major conclusions were drawn from this study:

- The fractional condensation system used in this study was successful in producing a heavy oil rich in holocellulose and lignin-derived oligomers with a very low water content.
- The PL content of bio-oils can be determined by the SEC-MALS-DRI method provided that the precise value of the dn/dc parameter is known and the integration interval is carefully chosen. Optimisation of SEC separation parameters, such as the number of columns and the column packaging, is suggested to avoid peak overlapping.
- The average M_n of the PL oligomers of a standard beech wood bio-oil was 580±50 Da. In the case of a heavy fraction obtained at the first stage of a fractional condensation system, the average M_n of the PL oligomers was 890±50 Da.
- PL oligomers obtained after water precipitation of bio-oil showed a higher M_w and lower dispersity than their corresponding bio-oil samples.
- In order to avoid a lack of LS signal resolution and minimise fluorescence, sample concentration should be carefully selected.

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