Characterization of Non-wood Lignin Precipitated with Sulphuric Acid of Various Concentrations

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Lignin is an attractive, renewable raw material provided by all types of agricultural and silvicultural vegetation. The precipitation of lignin fractions through acidification of the black liquor was performed and the products characterized for the following parameters: C, H, N, and S elemental composition; zeta potential; electrophoretic mobility; heating value; molecular weight; content of non-conjugated, conjugated, and total phenolic hydroxyl groups; and total yield of oxidation products. Lignin was isolated from black liquor by adding sulphuric acid at four levels of concentration (5, 25, 50, and 72 wt%) and subsequently adjusting the pH to 5. A comparison study of the physico-chemical and surface properties was also performed. The acid concentration influenced the yield of precipitated lignin and had an effect on the properties of precipitated lignin and the content of non-conjugated, conjugated, and total amount of phenolic hydroxyl groups. However, the concentration of acid had no relevant effect on the heating value, molecular weight, polydispersity, total yield of oxidation products, or the elemental composition of isolated lignin.

Keywords: Selectively precipitated lignin; Black liquor; Sulphuric acid; Renewable raw materials

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INTRODUCTION

The worldwide increase in the price and cost of petroleum and coal has created an interest in alternative sources of raw materials. Biomass is an attractive renewable raw material comprising all types of agricultural and silvicultural vegetation. This renewable resource is considered to be a major alternative raw material for the chemical industry. For many years, wood chemistry has tried to find applications for lignins other than their use as fuel and to valorize lignin byproducts. Lignin in wood and annual plants may be considered to be a random three-dimensional network polymer composed of phenylpropane units linked together in different ways (Rosas *et al.* 2004). When lignocellulose materials are delignified, the properties of the macromolecules reflect the properties of the network from which they are derived. Most of this biomass is burned as fuels. A small portion is applied (Vishtal and Kraslawski 2011) as carriers, e.g. for fertilizers and pesticides, carbon fibers, blends with thermoplastic polymers, ion-exchange resins, activated carbons. Part is transformed into chemical compounds such as vanillin, hydroxylated aromatics, quinones, aldehydes, aliphatic acids. Lignocellulose is also used in production of phenolic resins, dispersants, mew polymers, detergents, glues, binders and resins, adhesives, feeds, and cement additives.

Larger fractions of material could be used in other industrial and commercial processes if an economical method could convert lignin into a marketable product with sufficient profit margin. Lignin valorisation is a key factor for an economical lignocellulosic biorefinery. Many possible structures could be derived from the guaiacyl and syringyl units present in lignin (Holladay *et al.* 2007).

Currently, only about 2% of the lignins available in the pulp and paper industry are commercially used, comprising about 1,000,000 tons/year of lignosulphonates originating from sulphite pulping and less than 100,000 tons/year of kraft lignins produced in the kraft process (Gosselink *et al.* 2004). Dissolution and fractionation of lignocellulosic material is a critical step in the valorisation of biomass (Zakzeski *et al.* 2010; Vishtal and Kraslawski 2011). Fractionation of biomass, however, is not easy. Many treatment strategies have been developed to isolate lignin with a high yield. These methods (Sun *et al.* 2011) cover physical (pulverisation, irradiation, ultrafiltration (Toledano *et al.* 2010a; 2010b)), chemical (alkali, acid (Tejado *et al.* 2007; Garcia *et al.* 2009; Minu *et al.* 2012), organosolv, ionic liquid (Sun *et al.* 2013), hot water (CO_2 explosion, wet oxidation), biological (enzymolysis), and electrical methods or some combinations of these techniques (Mosier *et al.* 2005).

Black liquor is a highly complex mixture, and the composition of the precipitated lignin depends on the conditions of precipitation (Ibrahim *et al.* 2004; Tejado *et al.* 2007; Garcia *et al.* 2009; Toledano *et al.* 2010a; 2010b; Minu *et al.* 2012) such as the type of acid, temperature, method of isolation (filtration, ultrafiltration, centrifugation, and extraction), and the properties of black liquor. This paper is aimed at verifying the hypothesis that the yield and properties of precipitated lignins depend on concentration of sulphuric acid used as a precipitating agent. A variety of works related to the study of methods for the isolation and concentration of by-products from black liquors have been published (Tejado *et al.* 2007; Garcia *et al.* 2009; Toledano *et al.* 2010a,b; Sun *et al.* 2011). Different types of mineral and organic acids differing in concentration and treatments have been considered (Ibrahimet *et al.* 2004; Garcia *et al.* 2009; Toledano *et al.* 2010a, 2010b; Minu *et al.* 2012).

Several papers describe a selective precipitation of lignins by a precipitating agent differing in pH. Isolated lignin for each pH condition was determined and the physico-chemical properties of the individual lignin fractions registered. Minu *et al.* (2012) found that by maintaining a constant pH value, the yield of lignin was higher when using H_3PO_4 than that obtained by applying H_2SO_4, HCl, or HNO_3. The work of Ibrahim *et al.* (2004), however, led to different results, achieving the highest yield using 20% H_2SO_4. The difference using various acid concentrations was not, however, significant, reaching only 4% at the 1.4% measuring error. This effect was observed for 20% HCl, HNO_3, and H_3PO_4. Taking the technological aspects into account, namely variations in composition of batch pulping, delignification processing, and mode of precipitation, the differences in the measured values are negligible.

All the mentioned factors may influence the properties of lignin, including the character and ratio of its functional groups. When using lignin as a biofuel, knowledge of its composition is not important. When utilizing lignin at a higher level (*e.g.,* as an antioxidant for rubber blends), such information may be of crucial importance. The knowledge of the concentration and/or ratio of the individual groups may be used to optimize the antioxidant activity of lignin and its derivatives. This importance focused our efforts on characterization of lignin fractions precipitated by H_2SO_4 with different initial concentrations of H_2SO_4, and the subsequent adjustment of the pH to 5, through gel permeation chromatography (GPC), ultraviolet-visible (UV-VIS) spectroscopy, nitrobenzene oxidation, and higher heating value determination.

EXPERIMENTAL

Materials

Black liquor (soda-AQ pulping) characterization

The annual plants, hemp and flax, used for obtaining black liquor were kindly supplied by OP Papírna Ltd. (Olšany, Czech Republic). The cooking conditions: active alkali sodium hydroxide and the presence of anthraquinone (AQ). The black liquor obtained had the following characteristics (Table 1): pH of 12.9 ±0.3 (determined by a digital Jenway (3510 pH-meter, UK) and density 1.242 g/mL (determined by measuring the mass with the known volume of the black liquor).

Table 1. Characterization of black liquor

Dry matter (wt%)	36.80 ± 0.62
Elemental analysis of black liquor	
C (wt%)	36.24 ± 0.09
H (wt%)	4.93 ± 0.05
N(wt%)	1.13 ± 0.01
S(wt%)	0.24 ± 0.04
Ash (wt%)	45.75 ± 0.32
Lignin (wt%)*	21.23 ± 0.64
Carbohydrates (wt%)	33.02

*Klason lignin was determined according to TAPPI T 222 om-02

Lignin recovery from black liquor

The precipitation of lignin from black liquor was initially studied as a single step process in which a dilute solution of sulphuric acid (5 wt% (1.05 N), 25 wt% (6.01 N), 50 wt% (14.22 N), and 72 wt% (23.99 N)) was added to the black liquor with the pH adjusted to the desired value at a temperature of 50 °C. Then, 100 mL of the black liquor was treated with different amounts of diluted acid to obtain a final pH value of 5 while keeping the temperature constant at 50 °C. After precipitation, the content of each flask was filtered through a pre-weighed oven-dried filter paper using a vacuum filtration unit. The precipitated lignin was washed twice with hot water (total volume 400 mL, pH= 6.8) to remove impurities. The lignin was then dried at 25 °C under a pressure of 0.5 mbar using lyophilisation equipment (LYOVAC (GT2, Germany) until reaching a constant weight.

Methods

Lignin analysis

Different techniques were used to establish the physicochemical characteristics of the obtained lignins.

C, N, H, and S elemental analysis

Total nitrogen (N), carbon (C), hydrogen (H), and sulphur (S) content of all samples were determined by dry combustion using a Vario Macro Cube C/H/N/S-analyser (Elementar, Hanau, Germany). Two replicates were measured, and the mean standard errors were 0.35% for C, 0.04% for N, 0.05% H, and 0.01% for S.

Nitrobenzene oxidation

Oxidation of lignin samples by nitrobenzene was carried out using 2 M NaOH in 10-mL stainless steel vessels at a temperature of 180 °C for 2 h, and oxidation products were analysed by isocratic high-performance liquid chromatography under the following conditions: LiChrospher 100 RP-18, 5 μm, 4 × 100 mm ID column (Merck KGaA, Darmstadt, Germany), mobile phase water:methanol:acetic acid (850:150:1), flow rate of 1.0 mL min^{-1}, column temperature of 35 °C, and detection *via* diode array detector in the 210-360-nm region. The quantities of nitrobenzene oxidation products were determined using the method of direct calibration (Kačík *et al.* 1995). Measurements were performed on four replicates per sample. An S/G ratio was calculated using the formula (Eq. 1):

$$S/G = (\text{syringyl aldehyde} + \text{syringic acid}) / (\text{vanillin} + \text{vanillic acid}) \qquad (1)$$

Gel permeation chromatography (GPC)

The molecular weight distribution (MWD) of lignin was measured by GPC using a Separon Hema S-300 column. The precipitated lignin sample was dissolved in dimethylformamide (DMF) (5 mg/mL) and the sample was filtered (pore size: 0.45 mm, material: PTFE) to avoid plugging of the columns from bulky impurities. The temperature of the column oven was 35 °C, and a volume of 10 µL of each sample was injected. Lithium bromide (0.005 M) in DMF was used as eluent with a flow rate of 1 mL/min. A differential refractometer (RI) and a diode array detector (DAD) at 280 nm were used as detectors (Kacik *et al*. 1992). For calibration, polystyrene samples with various molecular weights (500 to 98,900) were used. Molecular weight was calculated from the experimental data using the software Clarity (version 2.3.0.163, Czech Republic).

Zeta potential of lignin particles

The zeta potential of the lignin particles was determined in water solutions with a lignin concentration of 10 mg/mL, using a Zetasizer Nano ZS (Malvern Instruments, UK) at 23 °C. The samples were prepared by dissolving the dry lignin powder. In our study, zeta potential and electrophoretic mobility were determined at pH 6.6 ± 0.1. To achieve a good colloidal dispersion, the samples were ultrasound-treated for 10 min at room temperature prior to the measurements.

UV/VIS spectroscopy

For each type of isolated lignin from black liquor (hemp and flax; modified alkaline anthraquinone cooking) precipitated by sulphuric acid (5, 25, 50, and 72 wt%), a stock solution was prepared by dissolving lignin powder (4 mg) in 50 mL of NaOH (0.2 M). The UV-VIS absorption spectra were registered using a solution of lignin on a Cecil spectrophotometer (Cecil Instruments, UK), absorption region 200 to 450 nm, scan speed 5 nm/s, and 1 nm resolution. Measured values of absorbance were normalized by absorption maxima at 222 nm.

Difference UV/VIS spectroscopy

After being dried overnight at 80 °C, a precisely weighed amount (5 mg) of lignin was dissolved in 5 mL of dioxane and 5 mL of 0.2 M NaOH. Some of the solutions were not quite clear and were filtered using a 0.45-µm PVDF membrane filter. From each lignin solution, 2 mL was further diluted to 25 mL using either a pH 6 buffer solution (citrate/NaOH, Merck) or 0.2 M NaOH (Gartner and Gellerstedt 1999). This gave each solution a final lignin concentration of about 0.04 g/L. The UV-VIS spectra were recorded on a Cecil spectrophotometer in the absorption region from 200 to 450 nm, scan speed 5 nm/s, and 1-nm resolution. The lignin solution with pH 6 was used as a reference, and the alkaline solutions were measured against it. From the difference spectra, the absorbance values at 300 and 350 nm, measured against the solution containing 0.2 M NaOH, were recorded. According to the original work of Gartner and Gellerstedt (1999), six structural types of phenolic structures exist (Fig. 1). Maxima at 300 nm and 350 to 360 nm can be assigned to unconjugated phenolic structures (I and III), and those at 350 to 370 nm can be assigned to conjugated structures (II and IV). According to Zakis (1994), the maximum at 360 nm is attributed only to IIa and IVa types of phenolic structures in lignin.

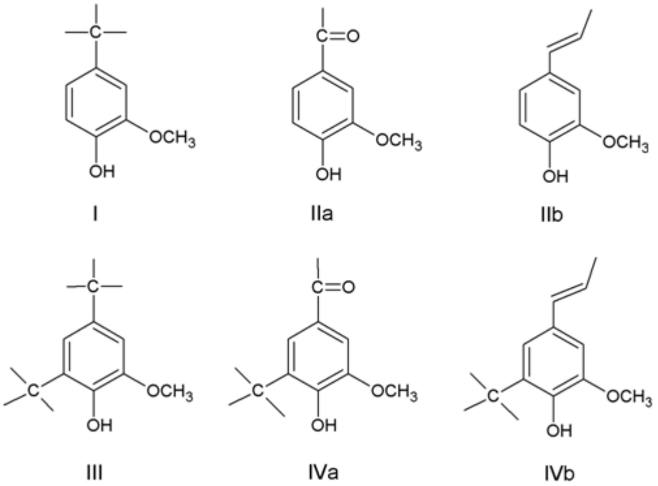

Fig. 1. Type of phenolic structures determined by the UV method (Zakis 1994; Gartner and Gellerstedt 1999)

To obtain the concentration of individual phenolic structures in mmol/g, the following absorbance-based formulae were developed (Zakis 1994; Gartner and Gellerstedt 1999) and are applied in their original form also in this work (Eqs. 2-4):

a) Non-conjugated phenolic structures (I+III)

$$\text{OH (I+III)} = \{(0.250\times A300\text{nm (NaOH)} + 0.0595\times A350\text{ nm (NaOH)}\} \times 1/(c\times d) \qquad (2)$$

b) Conjugated phenolic structures (II+IV)

$$\text{OH (II+IV)} = \{0.0476\times A350\text{nm (NaOH)}\} \times 1/(c\times d) \qquad (3)$$

c) Total amount of phenolic hydroxyl groups

$$\text{OH (I+II+III+IV)} = \{0.250\times A300\text{nm (NaOH)} + 0.107\times A350\text{ nm (NaOH)}\} \times 1/(c\times d) \qquad (4)$$

where $\Delta\lambda$ is the absorbance at a given wavelength divided by the corresponding molar absorptivity, c is mass concentration in g/L, and d is path length through the sample in cm.

Examples of determination of non-conjugated and conjugated phenolic structures are expressed in Eqs. 5-7.
The data for the sample precipitated lignin with 5 wt% solution of sulphuric acid:
$A_{300nm} = 0.230646$, $A_{350nm} = 0.208924$, $c = 0.0424$ g/L, $d = 1.02$ cm

a) Non-conjugated phenolic structures (I+III)
$\mathrm{OH\ (I+III)} = \{(0.250 \times \mathrm{A300nm\ (NaOH)} + 0.0595 \times \mathrm{A350\ nm\ (NaOH)}\} \times 1/(c \times d)$ (5)
$\mathrm{OH\ (I+III)} = \{(0.250 \times 0.230646 + 0.0595 \times 0.208924\} \times 1/(0.0424 \times 1.02)$
$\mathrm{OH\ (I+III)} = 1.621\ \mathrm{mmol/g}$

b) Conjugated phenolic structures (II+IV)
$\mathrm{OH\ (II+IV)} = \{0.0476 \times \mathrm{A350nm\ (NaOH)}\} \times 1/(c \times d)$ (6)
$\mathrm{OH\ (II+IV)} = \{0.0476 \times 0.208924\} \times 1/(0.0424 \times 1.02)$
$\mathrm{OH\ (II+IV)} = 0.229\ \mathrm{mmol/g}$

c) Total amount of phenolic hydroxyl groups
$\mathrm{OH\ (I+II+III+IV)} = \{0.250 \times \mathrm{A300nm\ (NaOH)} + 0.107 \times \mathrm{A350\ nm\ (NaOH)}\} \times 1/(c \times d)$ (7)
$\mathrm{OH\ (I+II+III+IV)} = \{0.250 \times 0.230646 + 0.107 \times 0.208924 \times 1/(0.0424 \times 1.02)$
$\mathrm{OH\ (I+II+III+IV)} = 1.850\ \mathrm{mmol/g}$

Higher heating value

Higher heating value (HHV) was determined with a FTT Calorimetric Bomb (FTT Scientific, UK) using a standard method (Sivaramakrishnan and Ravikumar 2011; Telmo and Lousada 2011).

Lignin purity determination

Isolated lignin is a raw material containing other substances along with pure lignin. Polysaccharides as accompanying pollutants were eliminated by a 1 h boiling treatment in 5 wt% H_2SO_4. The lignin (%) was determined based on the mass loss at acidic hydrolysis.

RESULTS AND DISCUSSION

The fractionation process

Some of the results obtained are graphically depicted in Fig. 2, which represents the lignin precipitation and the properties of the precipitate observed during the treatment of black liquor from annual plants, such as flax and hemp. Recovery of lignin is influenced by the concentration of added sulphuric acid. The yields of lignin precipitated by sulphuric acid were in the range of 9.3 to 10.5 g for every 100 mL of black liquor used. The concentration of sulphuric acid played a crucial role in this process. The results indicated that the yield is dependent both on the hydrogen ion concentration in the solution and the ionic strength. These observations are in agreement with earlier investigations (Helander *et al.* 2013). Quantity and purity of isolated lignin are listed in Table 2. Using 25% H_2SO_4 as a precipitating agent, the yield of precipitated lignin reached 9.4 g/100 mL liquor, while with 72 wt% H_2SO_4, the yield was higher (10.5 g/100 mL liquor) but the purity was lowest (82.4%). This result could be caused by concentration gradients at sulphuric acid addition, different hydrodynamic conditions, or a different ionic strength and protonation. Protonation depends on the pKa value of the lignin structures (Wada *et al.* 1962; Helander *et al.* 2013) and reaches about 50%. Using H_2SO_4 of a higher concentration, the present lignin-saccharide complexes undergo a local lower-degree decomposition that is reflected by lower purity of precipitated lignin. Lignin is precipitated at the same time as sulphuric acid approaches an even distribution in the system. This is why the purity and yield of the formed lignin cannot be unambiguously displayed. The most positive of our results in this connection is finding that the lignin of highest purity is formed when treating the liquor with 25 wt% H_2SO_4.

Table 2. Purity of isolated fractions of lignin

Precipitated lignin with solution of sulphuric acid	Purity (%)	Yield of pure lignin (g/100 mL)
5 wt%	85.1 ± 0.2	7.9
25 wt%	92.8 ± 0.1	9.4
50 wt%	90.6 ± 0.4	9.3
72 wt%	82.4 ± 0.4	8.7

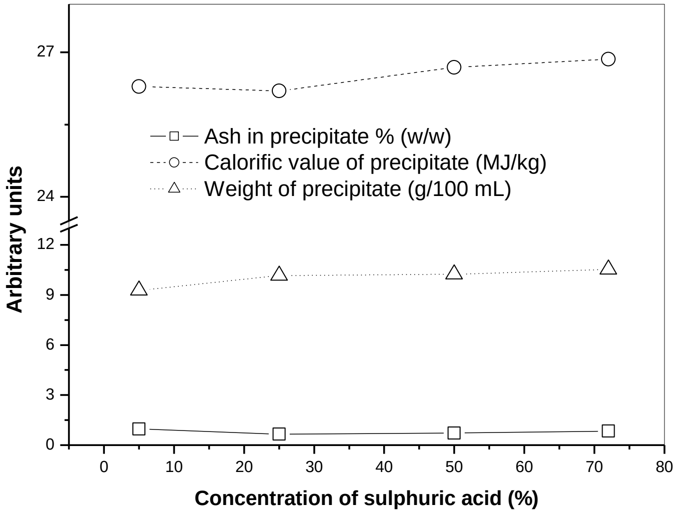

Fig. 2. Effect of the concentration of sulphuric acid on the properties of precipitated lignin from the black liquor of flax and hemp

The use of lignin as a fuel in different applications requires a knowledge of its heating value. Several studies investigating the heating values of different biomass species (Demirbas 2003; Vargas-Moreno *et al.* 2012) and isolated lignins (Minu *et al.* 2012) have been published. Cellulose and hemicellulose have heating values ranging from 14.7 to 19.0 to MJ/kg, whereas lignin has values ranging from 22.3 to 27.3 MJ/kg (Minu *et al.* 2012). Pure lignin has a rather high heating value, which makes it valuable for use as a bio-fuel. On average, the heating value of precipitated lignins was 26.5 ±0.3

MJ/kg of dry lignin. This information indicates that the calorific value of precipitated lignin is independent on the used concentration of acids.

Elemental analysis

The weight percentages of elements C, H, N, O, and S of the isolated precipitate of lignins are listed in Table 3. The atomic O/C ratios of the isolated lignin were approximately located between 0.29 and 0.33, and their atomic H/C ratios range from 1.18 to 1.23. The average weight percentage of elements for four samples was C: 64.78 ± 0.87%, H: 6.44 ± 0.09%, N: 1.13 ± 0.03%, S: 0.06 ± 0.01%, and O: 26.79 ± 0.95%. The results indicate that the lignin elemental composition of is not markedly affected by the concentration of sulphuric acid. The relatively low content of sulphur required for the precipitation process makes this biofuel attractive as an additive to fuels.

Table 3. Elemental Analyses and O/C and H/C Atomic Ratios

Precipitated lignin with solution of sulphuric acid	Elemental analysis (%)					H/C	O/C
	N	C	H	S	O		
5 wt%	1.11	65.75	6.46	0.06	25.59	1.18	0.29
25 wt%	1.12	63.64	6.54	0.07	26.95	1.23	0.33
50 wt%	1.12	64.84	6.33	0.04	27.96	1.17	0.31
72 wt%	1.18	64.90	6.42	0.07	25.66	1.19	0.31

UV/VIS and Difference UV/VIS spectroscopy

Normalized UV/VIS spectra of preparations are shown in Fig. 3. Free and etherified hydroxyl groups contribute to the characteristic absorption maximum of lignin near 280 nm. The lignin fractions showed a characteristic absorption band at 284 nm, which could be assigned to the non-conjugated phenolic groups of lignin. Absorption maxima of non-conjugated guaiacyl and 3,4-dimethoxy-phenyl model compounds fall within the narrow wavelength range from 277 to 282 nm. In an alkaline solution, ionization of the hydroxyl groups shifts the absorption bands of compounds with free phenolic groups towards longer wavelengths. Ultraviolet spectroscopy was used to further investigate the structural components of lignin. The different spectra of alkaline and neutral solutions of lignins enable interpretation of the chemistry of these substances. Differential UV-Vis spectroscopy allows the identification of the ionization of phenolic hydroxyl groups in alkaline solutions while the absorption of etherified lignin units is unchanged in neutral and alkaline media. Figure 4 shows that all of the precipitated lignin displayed the characteristic ionisation difference spectra of lignin, with local maximum intensities centered at 222, 251, 299, and 350 nm; minima were found near 279 and 318 nm.

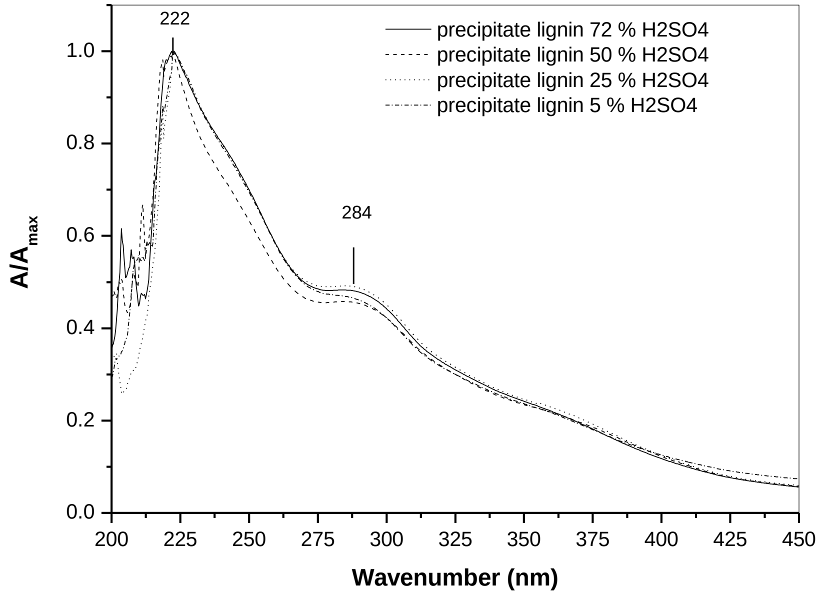

Fig. 3. Normalised UV/VIS spectra of isolated preparations

Table 4. Content of non-conjugated, conjugated, and total phenolic hydroxyl groups

Precipitated lignin with solution of sulphuric acid	Non-conjugated phenolic structures (I+III) (mmol/g)	Conjugated phenolic structures (II+IV) (mmol/g)	Total amount of phenolic hydroxyl groups (OH I+II+III+IV) (mmol/g)
5 wt%	1.62 ± 0.5	0.23 ± 0.2	1.85 ± 0.4
25 wt%	1.67 ± 0.3	0.24 ± 0.3	1.91 ± 0.3
50 wt%	1.37 ± 0.6	0.17 ± 0.6	1.54 ± 0.6
72 wt%	1.03 ± 0.4	0.17 ± 0.4	1.19 ± 0.4

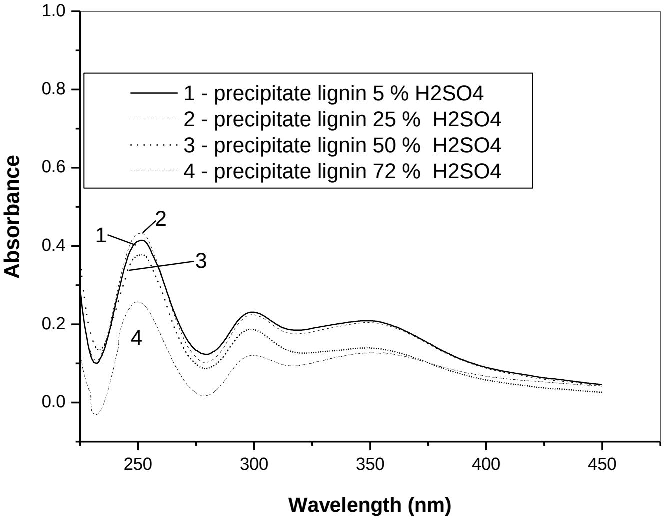

Fig. 4. Ionisation difference spectrum of precipitated residual lignin

The content of non-conjugated, conjugated, and total phenolic hydroxyl groups for these preparations is presented in Table 4. It is known that when lignin is precipitated from kraft black liquor, the methoxyl content decreases, while the carboxyl and phenolic hydroxyl contents increase at a lower pH (Wada *et al.* 1962). Lowering the pH of the black liquor using different agents (organic or mineral acids, CO_2) protonates the phenolic hydroxyl groups in lignin (Helander *et al.* 2013). Isolation of lignin, effectiveness of precipitation, and properties of obtained lignin (the presence and concentration of individual functional groups) depend both on the precipitation conditions (temperature, lignin concentration, ionic strength), and lignin protonisation, as well as acid used (*i.e.*, its acidity constant) (Helander *et al.* 2013). The content of non-conjugated phenolic structures (I + III) ranged from 1.03 to 1.67 mmol/g with the following trend: higher H_2SO_4 concentration led to lower structural content. The same trend was found for the total amount of phenolic hydroxyl groups (OH I + II + III + IV). The content of conjugated phenolic structures (II + IV) was 0.17 to 0.24 mmol/g. The concentration of H_2SO_4 thus influenced both the content of individual groups and their total content. Free and etherified hydroxyl groups contributed to the characteristic absorption minimum of lignin near 280 nm (difference spectrum). The spectral intensity at 280 nm was influenced to a substantial extent by the presence of unsaturated substituents on the α-carbon of a side chain (*e.g.*, carbonyl group, double bond). Such unsaturated groups cause absorption in the near UV region (300 to 400 nm) in the form of broad peaks or shoulders and thus affect lignin colour (Polčin and Rapson 1969). Absorption minima of non-conjugated guaiacyl and 3,4-dimethoxy-phenyl model compounds fall within the narrow wavelength range from 277 to 282 nm. The lignin absorption at 300 to 400 nm is due to carbonyl groups and/or double bonds conjugated with an aromatic ring. Evidence for phenolic conjugated α-keto groups was provided by the difference method. Ionisation difference spectra for certain lignin samples showed a shoulder or a maximum near 350 nm, which agrees well with the maxima in the spectra of alkaline solutions of model compounds such as vanillin (λ_{max}= 353 nm). The intense band near 340 to 350 nm is consistent with the formation of extensive conjugated structures, such as the $C_\alpha=C_\beta$ double bond, β-C=O structure, and the conjugated carbonyl group in side chains of lignin such as coniferyl aldehyde (Polcin and Rapson 1969). The peak centered at 355 nm in the ΔA spectrum of the preparations was composed of peaks due to p,p-dihydroxystilbenes (λ_{max} = 356 nm) and phenolic α-carbonyl units (λ_{max} > 350 nm).

Electrokinetic properties

A knowledge of the electrokinetic properties of lignin is therefore of fundamental and practical importance. Zeta potential of lignin solutions shows that charge of lignin colloid can be an indicator of the tendency for the colloid to precipitate (Table 5) (Rudatin *et al.* 1989; Wang and Chen 2013). Dong *et al.* (1996) found that the pH strongly affects the zeta potential of kraft lignin. In our study, zeta potential and electrophoretic mobility at pH 6.6 ±0.1 were determined. The highest value of electrophoretic mobility (-1.62 ±0.04 μmcm/Vs) and zeta potential (-20.64 ±0.53 mV) were determined for lignin precipitated by 5 wt% H_2SO_4. Applying acid of a higher concentration reduced both the electrophoretic mobility and zeta potential (Table 4). The differences in both the electrophoretic mobility and zeta potential may result from the fact that even at the same final pH value the nature of charge distribution reflects a mode of solution formation. This issue will be treated in detail elsewhere. Different separation procedures may result in different functionality or different functional group on the surface of lignin. Zeta potential is important in the air flotation of slurry waste water. In this case, the zeta potential and electrophoretic mobility of the lignin particles decreased from -20.64 mV and -1.62 μm cm/Vs (sample 5 wt%) to -17.54 mV and -1.37 μm cm/Vs (25 wt%). This may indicate strengthening of the association/aggregation interactions, leading to the formation of lignin particles with less charge and larger in size. It follows from the zeta potential and electrophoretic mobility values that the lignin precipitated with 25 wt% sulphuric acid exhibited the highest yield of pure lignin.

Table 5. Zeta potential and electrophoretic mobility of lignin particles in lignin aqueous solution, pH 6.6 ±0.1

Precipitated lignin with solution of sulphuric acid	Zeta potential, (mV)	Standard deviation, (mV)	Electrophoretic mobility, (μmcm/Vs)	Standard deviation, (μmcm/Vs)
5 wt%	-20.64	0.53	-1.62	0.04
25 wt%	-17.54	0.52	-1.37	0.04
50 wt%	-18.30	1.19	-1.43	0.09
72 wt%	-18.42	0.39	-1.44	0.03

Nitrobenzene oxidation

Nitrobenzene oxidation is one of the standard procedures for analysing lignin by the chemical degradation technique to gain information about the composition of the original polymer. The composition of the three monomeric lignin units, p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S), which are capable of producing degradation products such as vanillin, vanillic acid, syringaldehyde, syringic acid, p-hydroxylbenzadehyde, and p-hydroxybenzoic acid, were analysed, and the results are shown in Tables 6a and 6b. Vanillin was found to be the predominant compound followed by syringaldehyde as a second major degradation product. The total yield of the oxidation products ranged from 6.64% to 6.76%. The presence of a lesser amount of p-hydroxybenzaldehyde and p-hydroxybenzoic acid is considered most likely to be indicative of non-condensed p-hydroxyphenyl units. H:G:S ratios were also calculated for precipitated lignins. The results are displayed in Table 7. The values of the G-units for lignin samples were above 63%. The values for S-units and H-units of precipitated lignin samples were in the range of 25.2 to 28.1% and 7.4 to 8.8%, respectively. For precipitated lignin samples, the ratio of G:S:H was 7 to 9:3 to 2:1 which fell in the same category as straw or grass type lignin (Scalbert and Monties 1986; Ibrahim *et al.* 2004).

Table 5a. Yield of degradation products of the precipitated lignin samples by nitrobenzene oxidation

Precipitated lignin with solution of sulphuric acid	Vanillin		Vanillic acid		Syringaldehyde		Syringic acid	
	(%)							
	AVG	SD	AVG	SD	AVG	SD	AVG	SD
5 wt%	3.217	0.005	1.072	0.003	1.481	0.003	0.456	0.002
25 wt%	3.236	0.003	1.021	0.007	1.504	0.007	0.449	0.003
50 wt%	3.199	0.012	1.005	0.006	1.438	0.017	0.438	0.004
72 wt%	3.204	0.071	0.881	0.026	1.680	0.098	0.498	0.040

Table 5b. Yield of degradation products of the precipitated lignin samples by nitrobenzene oxidation

Precipitated lignin with solution of sulphuric acid	p-hydroxybenzaldehyde		p-hydroxybenzoic acid		Total (Yield) %	
	(%)					
	AVG	SD	AVG	SD	AVG	SD
5 wt%	0.057	0.001	0.352	0.003	6.635	0.010
25 wt%	0.074	0.001	0.373	0.006	6.658	0.017
50 wt%	0.067	0.002	0.333	0.002	6.480	0.044
72 wt%	0.072	0.007	0.424	0.006	6.760	0.235

Molecular weight

The molecular weight distributions (MWDs) of the four investigated precipitated lignins are presented in Fig. 5. From the GPC results, the weight-average (Mw), number-average (Mn), and polydispersity (Mw/Mn) values were calculated and are shown in Table 6. Lignin isolated by sulphuric acid (concentration 5 wt%, 25 wt%, 50 wt%, and 72 wt%), showed no significant difference in Mw, ranging from 9367 to 9823. In general, the distribution curves of lignin preparations present reasonably symmetrical peaks, whereas those of lignin fractions are asymmetric to a greater or lesser degree, dependent on the type of fractionation. It is obvious that there is a connection between the lignin and polysaccharide fractions (Zoia *et al.* 2011). All of the samples showed a bimodal molecular weight distribution. On the other hand it is known that some association processes due to different lignin branching and cross-linking can occur influencing the shape of distribution curve (Cathala *et al.* 2003). Such associations may result from random distribution of OH, CO, and COOH groups in lignin and their mutual interactions, *e.g.*, by van der Waals forces, due to ionisation of the groups and formation of intermediates of lignin oxidation (Bikova *et al.* 2004). As pointed out by Guerra *et al.* (2007), an unambiguous correlation of the groups' content and their ionisation is not statistically proven. It should be stressed that, here, polysaccharide-containing lignins, *i.e.*, raw lignin, were isolated and investigated (Tables 1 and 7). The yield of pure lignin ranged from 7.9 to 9.4 g/mL; the higher value was reached at precipitation by 25 wt% H_2SO_4. Our results are in accordance with those published by Ibrahim *et al.* (2004) who realized the highest yield of lignin using 20 wt% sulphuric, hydrochloric, phosphoric or nitric acid. Ibrahim *et al.* (2004) ascribed this fact to localised acidification or a non-uniform precipitation effect when high acid concentrations were used.

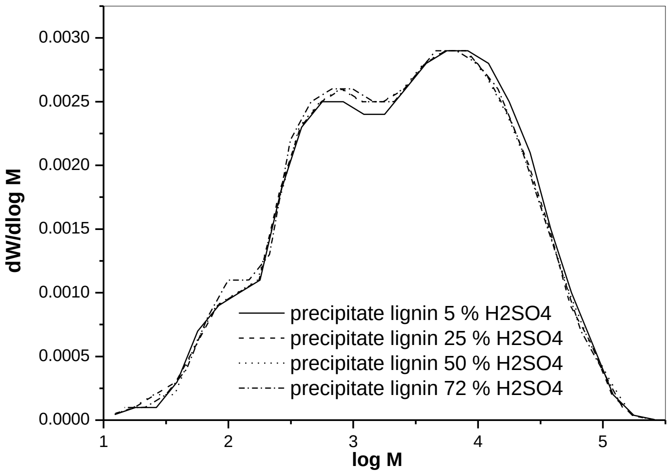

Fig. 5. GPC chromatograms of different preparations obtained by acid precipitation using sulphuric acid of different concentrations

Table 6. Molar Ratio of Degradation Products of the Precipitated Lignin Samples by Nitrobenzene Oxidation and Molecular Weights and Polydispersity of the Lignin Fractions

Precipitated lignin with solution of sulphuric acid	G	S	H		Mn	Mw	Mz	Mz+1	Mw/Mn
5 wt%	67.2	25.5	7.4	AVG	478	9823	41451	74653	20.56
				SD	8.49	24.75	232.64	589.02	0.39
				RSD	1.78	0.25	0.56	0.79	1.91
25 wt%	66.4	25.6	8.0	AVG	455	9440	40483	73036	20.76
				SD	2.12	29.70	85.56	116.67	0.04
				RSD	0.47	0.31	0.21	0.16	0.19
50 wt%	67.4	25.2	7.4	AVG	458	9367	40533	73136	20.46
				SD	5.66	31.82	178.19	311.13	0.31
				RSD	1.24	0.34	0.44	0.43	1.50
72 wt%	63.1	28.1	8.8	AVG	480	9593	42427	77395	20.01
				SD	15.56	130.81	1489.17	4010.00	0.90
				RSD	3.24	1.36	3.51	5.18	4.50

Note: Mn = number-average molecular weight, Mw = weight-average molecular weights, Mz = Z-average molecular weight, Mz+1 = Z+1 average molecular weight (Mz+1), (Mw/Mn) = polydispersity

CONCLUSIONS

1. According to the distinct acid precipitation behaviors, fractionation of lignin with various concentrations of sulphuric acid (5 wt%, 20 wt%, 50 wt%, and 72 wt%) at pH 5 was established in this study. Physicochemical and surface characterization of

precipitated lignin samples was performed. There were clear differences between the precipitates obtained at pH 5 with differing concentrations of sulphuric acid, especially considering the content and properties of obtained lignin, such as the content of non-conjugated, conjugated, and total phenolic hydroxyl groups.

2. The acid concentration influenced the yield of precipitated lignin. The concentration of acid had an effect on the electrokinetic properties of solutions containing precipitated lignin. The zeta potential and electrophoretic mobility for the lignin precipitated with 25 wt% sulphuric acid exhibited the highest yield of pure lignin. However, the concentration of this acid did not have an effect on the elemental composition and on the higher heating value of precipitated lignin.

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