
Extraction of lignins using a modified dioxane method and an ionic liquid and comparative molecular weight and structural studies by chromatography and ^{13}C NMR spectroscopy, techniques

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Abstract: In this study, lignins were extracted from three different plant species including poplar (*populus nigra*), cypress (*Cupressus sempervirens*), and bagasse (*Gramineae Saccharum officinarum L.*) by dioxane method and ionic liquid method and their molecular weight distributions (GPC) and their structural features (^{13}C NMR) were compared to conclude which of lignins (DL or IL) is more unchanged and similar to the original lignin. The results indicate that there is considerable differences between dioxane lignins and ionic liquid lignins of three different species regarding average molecular weights (M_n^-). Meanwhile, ^{13}C NMR spectroscopy, showed that dominant structural units of the three kind of lignins are phenyl propanes, and reveals the known differences between the lignins of hardwood, softwoods and non-woods. It seems that from this point of view, there are no considerable differences between dioxane lignins and ionic liquid lignins.

Keywords: Populus Nigra, Cupressus Sempervirens, Bagasse, ^{13}C NMR, Dioxane Lignin (DL), Ionic Liquid Lignin (IL), GPC

1. Introduction

In structural studies of lignin, the main concern is changes of structure of lignin during its isolation from lignocellulosic materials. Too much attempts have been made to extract lignin from wood or other lignocellulosic materials with the least physical and chemical changes in lignin, so that extracted lignin to be acceptably representative of original lignin.

Then, the results of experiments and analyzes would be reliable and applicable in scientific and technical studies.

One of the first and important methods to extract lignin from wood meals was developed by Bjorkman (1954) [1]. The lignin obtained is called Bjorkman lignin or Milled Wood Lignin (MWL). Of course the method is almost difficult and time consuming procedure. [2]

Another method for preparing almost unchanged lignin is performed by using carbohydrate consuming enzymes which is referred to as enzymatic or Cellulytic lignin. It is believed

that this lignin is unchanged and similar to intact lignin. [3]

This method has noticeable advantages too, but seems to be an expensive procedure.

Preparation of lignin using a mixture of dioxane, water and HCl (Dioxane Lignin) is almost a short route for extracting lignin from lignocellulosic materials. But using hydrochloric acid and heating the extracting mixtures, probably causes structural changes in lignin. A number of researchers have tried dioxane method in lower temperature (and consequently lower yields) to minimize structural changes in extracted lignins. [4]

1.1. Ionic Liquid

In recent years, ionic liquids have been used to dissolve carbohydrates and remaining lignin as a residual which is hoped to be relatively unchanged. [5]

Ionic liquids are defined as salts with melting points less than 100°C , and are frequently liquid at room temperature. Alternative solvents including supercritical fluids and room

temperature ionic liquids form a significant portion of research in green chemistry [6]. Interest in ILs stems from their potential application as "green solvents" [7]. Given the delocalized nature of charge over stable, bulky organic or inorganic ion, they tend to have good to excellent thermal and electrochemical stability and virtually nil volatility, implying a case of recycling with virtually no VOC (volatile organic compounds) release. [8]

Major interest in ionic liquid for biomass dissolution and processing was triggered by seminal paper of [9], which rediscovered and significantly advanced Graenacher's observation that the organic salt N-ethyl pyridinium chloride mixed with pyridine could dissolve cellulose up to 25 wt % [10]. The same group subsequently demonstrated that [Bmim] Cl can partially or fully dissolve wood. [11, 12]

Several reviews and chapters are already available exclusively on the topic of ionic liquids as "solvent" for biomass with a strong or sole focus on cellulose or lignin dissolution and processing. [5]

Another review will only briefly cover the dissolution of whole (lignin-containing) biomass, before focusing on the selective dissolution or isolation of lignin.

Numerous researchers have investigated the dissolution of whole biomass in ILs followed by addition of anti-solvents to yield distinct "cellulose-rich" and lignin components. [5]

Two of the most commonly encountered ILs associated with biomass dissolution are 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) and 1-butyl-3-methylimidazolium chloride ([Bmim]Cl).

We found [Bmim] Cl to be enough suitable and effective solvent that can partially or fully dissolve wood. The ionic liquid can dissolve the biomass owing to the strong hydrogen-bonding of certain ions such as the weakly solvated chloride anion in pure [Bmim]Cl. These anions can extensively disrupt the hydrogen-bonding in tractions present in the three-dimensional network of lignocellulosics, leading to either dissolution of wood or selected individual components based on the nature of the anion. [5]

2. Experimental

2.1. Materials and Apparatuses

2.1.1. Materials

Hardwood (*Populus nigra*) from Tehran, softwood (*Cupressus sempervirens*) from northern Iran and bagasse (*Gramineae Saccharum officinarum L.*) from southern Iran were prepared and used. 1-butyl-3-methylimidazolium chloride (Sigma-Aldrich), dioxane, dimethylsulfoxide (DMSO), Pyridine, acetone, hydrochloric acid from Merck were used as received.

2.1.2. Gel Permeation Chromatography (GPC)

GPC chromatograms were generated on a Shimadzu-6A instrument with a polystyrene column (10000 Å), refractive index detector (RID), Eluent: THF, and at the solvent flow rate of 1.0 ml min⁻¹.

2.1.3. FT-IR Spectroscopy

FT-IR spectra were obtained using a Shimadzu IR prestige-21 spectrometer. Lignin samples were dispersed in a matrix of KBr, and then compressed to form pellets.

2.1.4. ¹³C NMR Spectroscopy

¹³C NMR spectra (125 MHz in CDCl₃) were obtained using a Bruker (Avance300 MHz) spectrometer, solvent: deuterated chloroform, delay time, 2 second, number of scan: 10240, to characterize the specifications of IL and DL.

2.2. Methods

2.2.1. Preparation of Dioxane Lignin (DL)

The samples (poplar, cypress and bagasse chips) were grinded (Wiley mill), sieved (140 μm) and were extracted (Soxhlet apparatus) using water, ethanol and benzene respectively. The extractive-free samples were oven dried (100°C) over night. [13]

Dioxane lignins were prepared according to a modified method. [4]

In this method, 0.3 g, 0.7g and 0.4 g of DL were prepared from 100g flour of bagasse, poplar and cypress, respectively. Thus, the yields of prepared dioxane lignins for bagasse, poplar and cypress were 1/1%, 2/8% and 1/3% respectively.

2.2.2. Preparation of Ionic Liquid Lignin (IL)

10g of ionic liquid ([Bmim] Cl) was charged into a 50 ml dried flask equipped with a mechanical stirrer (under an inert atmosphere of argon). The temperature of the dissolution process was controlled by an oil bath at the specified temperature (80-85°C).

Then, 0.8-1 g of fully-dried, extractive free of lignocellulosic materials (bagasse, poplar and cypress) was gradually added into the ionic liquid. After 2.5 hours, 4-5 drops of anhydride acetic and 2ml DMSO was added into the solution and the dissolution proceeded for 24 hours [14]. After 24 hours, the brownish wood solution was added into an excess of rapidly stirred dioxane and distilled water (90-10 V/V), and this solution was allowed to stir for another 24 hours. Then, the precipitated bulky material was filtered using a Buchner funnel, washed thoroughly with a solution of dioxane and water (90-10ml). Then, solvents were evaporated under reduced pressure (temperature: 50-60°C) until the total volume of solution was 2-3 ml (turbid brownish solution).

The lignin was removed from the brownish solution by addition of distilled water and diethyl ether and precipitated with cooled distilled water by centrifuging for 20 minutes at 6000 rpm.

In this method, from 100 g of wood flour, 0.7 g, 1.5 g and 1 g of IL were prepared from bagasse, poplar and cypress respectively. Thus, the yield of IL was about 2.3%, 5.7% and 2.8%, respectively.

2.2.3. Acetylation of Lignin

Acetylation of lignin samples (10-15mg) was conducted with 2 ml of acetic anhydride-pyridine (1:1,v/v) at 50°C for 50 minutes in a 50 ml flask by stirring vigorously. Then,

ethanol (5ml) was added. After 10 minutes, the solvents were removed by evaporation under reduced pressure at 45-50°C. Addition and removal of ethanol were repeated more than 5 times to result in a complete removal of acetic acid and pyridine from the sample. After 48 hours, drying the acetylated samples were used for GPC and ¹³CNMR experiments. [5]

3. Results and Discussion

In this study, after preparation of DL and IL, we recognized the functional groups by FT-IR spectroscopy, and after acetylation of lignin samples, they were analyzed and compared using GPC and ¹³C NMR techniques. [15]

3.1. Molecular Weight Measurements

Figures 1a, 2a and 3a show the GPC chromatograms of acetylated DLs of bagasse, poplar and cypress, respectively. Figures 1b, 2b and 3b show the GPC chromatograms of acetylated ILs. The weight average molar mass, (M_w^-), number average molar mass (M_n^-) and polydispersity coefficient ($D = M_w^- / M_n^-$) are given in Table 1.

Table 1. GPC results of DLs and ILs of bagasse, poplar and cypress.

D	M_n^-	M_w^-	DL or IL	Lignocellulosic material
2/5096	1116	2802	7a(DL)	Bagasse
1/4133	3522	4978	7b(IL)	
3/5727	1113	3978	8a(DL)	
1/2834	3147	4039	8b(IL)	Poplar
2/2402	1708	7625	9a(DL)	Cypress
2/0547	4501	10015	9b(IL)	

The results show that M_w^- of DLs of the three samples are

lower than those of ILs. Molecular mass dispersion (D) of DLs of all samples are higher than ILs. These observations show that higher DLs dispersity results in higher molecular mass dispersion, hence, a higher inhomogeneity was observed in the DLs of the samples than the ILs and higher degradation for DLs compared to the ILs.

3.2. FT-IR Spectroscopy

FT-IR spectral datas of DLs and ILs of bagasse, Poplar and cypress have been summarized in Table 2.

Table 2. Characteristic bands of FT-IR spectra of DL and IL.

Wavenumbers (cm ⁻¹)	Assignment
1220-1270	C—O stretching of syringyl
1300-1350	Syringyl/guaiacyl ring breathing with C—O stretching
1460-1470	Asymmetric bending in CH ₃
1500-1600	Aromatic skeletal vibration
1650-1750	C=O stretching of carbonyl or carboxylic acid
3400-3500	OH stretching

All of the IR spectras include the stretching and bending bands characteristics of lignin, including functional groups and aromatic rings as part of phenyl propane skeleton. However, these spectras showed that no clear differences were detected in the shapes of bands except for a slight shift in the position of some bands. But the intensity of some bands between DLs and ILs were different. [16]

3.3. ¹³CNMR Spectroscopy

¹³CNMR spectras of acetylated Ionic and Dioxane lignin of bagasse, poplar and cypress the corresponding assignments have been shown in table 3.

Table 3. ¹³CNMR data of AcDL and AcIL of bagasse, poplar and cypress.

Chemical shift (ppm) s: high intensity, m: medium intensity, w: low intensity			
Bagasse	<i>P. nigra</i>	<i>C. sempervirens</i>	Assignment
14.1(DL)	14.1(DL)	14.1(DL)	CH ₃ (Ac O at C4)
14.1(IL)	13.7(IL)	14.1(IL)	
20.6-21.1(DL)	19-20.6(DL)	20.9(DL)-18.9	CH ₃ (Ac O at Cα)
20.7(IL)	19.2(IL)	19.1-20.9(IL)	
29.3-31.2(DL)	29.6-31.8(DL)	29.1-31.8(DL)	CH ₃ (Ac O at C _γ)
22.6-29.6(IL)	29.4-31.7(IL)	29.1-31.5(IL)	
55.96(DL),w	55.98, (DL),m	56.1(DL), m	CH ₃ (OMe at C3,5)
-----	49.1(IL),m	49.1(IL), m	
76.5-77(DL), s	76.5-77(DL), s	76.5-77(DL), s	CH ₃ Cα
76-77(IL), w	76-77(IL), w	76-77(IL), w	
77-77.4(DL), s	77-77.4(DL), s	77-77.4(DL), s	CH ₃ Cβ
77-77.4(IL), w	77-77.4(IL), w	77-77.4(IL), w	
69(IL), w	69(DL), m	69-70(DL), m	CH ₃ C _γ
70.5(IL), w	70-70.5(IL), s	70-70.5(IL), s	
122-153(DL), w	104-152(DL), w	104-152(DL), w	C of Arom.
123.1(IL), s	122-146(IL), s	122-146(IL), s	
168(DL), m	169.7(DL), m	169.7(DL), m	C=O (Ac O at C4)
169(IL), w	168.5(IL), w	168(IL), w	
170(DL), m	171.6(DL), m	171.6(DL), m	C=O (Ac O at Cα)
170.5(IL), w	170.3(IL), w	170(IL), w	
170(DL), m	170.6(DL), m	170.6(DL), m	C=O (ACO at C _γ)
170.5(IL), w	170.1(IL), w	169.6(IL), w	

Considering the spectra and related spectral data, it can be concluded that the functional groups, main structural features of lignin is observed in all lignins extracted from different species by two different methods (dioxane lignins and ionic liquid lignins). Therefore it can be concluded that considerable degradation reactions have not been taken places. Guaiacyl and especially syringyl structures are usually found abundant in the lignin of hardwoods (*P. nigra*) but the lignin of softwoods, such as *C. sempervirens*, contains almost guaiacyl structures. In hardwoods these structures are derived from coniferyl alcohol and synapyl alcohol units; however, in softwoods they are found mainly from coniferyl alcohol units. In non-woody resources such as bagasse structural units are derived from *p*-coumaryl alcohol. In extracted lignins cleavage occurred in the β-O-4 position and the formation of double bond between α and β carbon atoms resulting from the phenylpropane structures.

4. Conclusions

The results obtained from GPC, FT-IR and ¹³CNMR analyses are in good agreement regarding the less structural changes and degradations of ionic lignins of *P. nigra* (Poplar), *C.sempervirens* (Cypress) and *Gramineae Saccharum officinarum L.* (bagasse) compared to the dioxane lignins. GPC analyses shows higher molecular weight and lower molecular dispersity for ionic liquid lignins of all samples compared to dioxane lignins. It can be concluded that less degradation of ionic liquid lignins has taken place. The FT-IR spectra of all lignin samples are typical spectra of lignin representing the characteristic functional groups of lignins such as OH groups, C=O and C-O stretching bands, etc .

The results of ¹³C NMR proves noticeable structural differences between the lignins of three different species (hardwood, softwood and non-wood) and also, to some extent, between ionic liquid lignins and dioxane lignins. The latter, of course is attributed to different extraction and separation conditions of lignins from lignocellulosic resources.

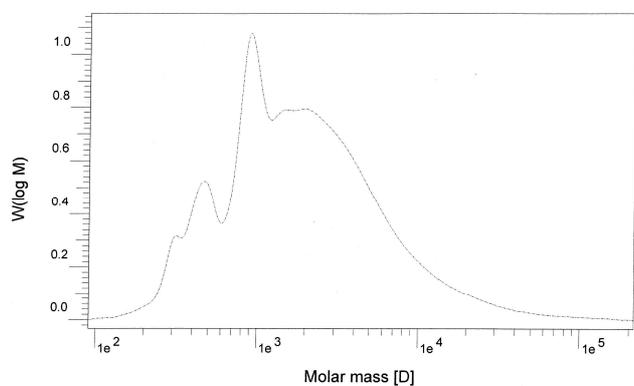


Fig. 1a. GPC chromatogram of ACDL of bagasse

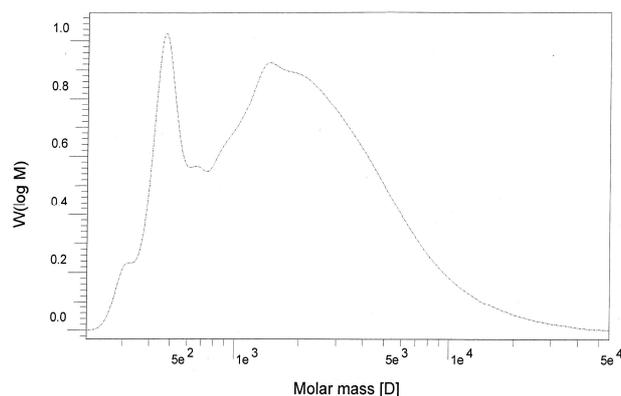


Fig. 2a. GPC chromatogram of ACDL of *P. nigra*

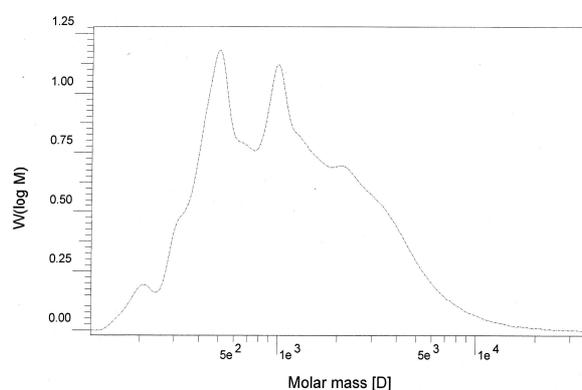


Fig. 3a. GPC chromatogram of ACDL of *C. sempervirens*

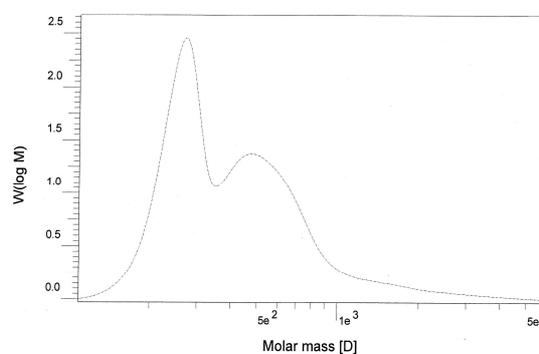


Fig. 1b. GPC chromatogram of ACIL of bagasse

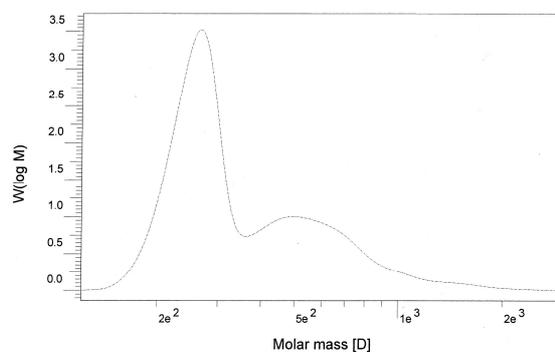


Fig. 2b. GPC chromatogram of ACIL of *P. nigra*

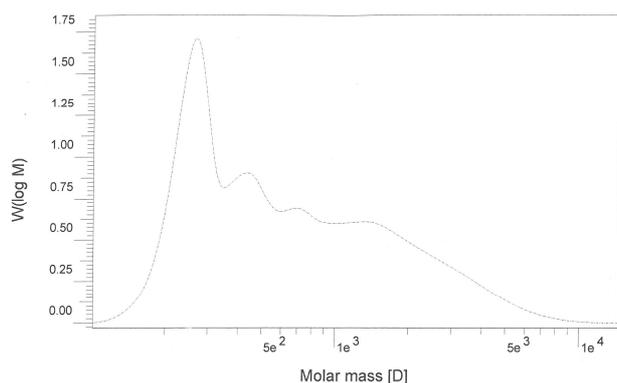


Fig. 3b. GPC chromatogram of ACIL of *C. sempervirens*

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