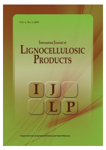

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Applicability of GPC, GC-MS and ^{13}C NMR techniques and DFRC method in comparative structural studies of Lignin

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Abstract

In this research, the characteristics of CMP dioxane lignin (hardwood origin) and soda bagasse lignin (ethanol soluble fraction) and dioxane lignin of bagasse were studied using gel permeation chromatography, gas chromatography-mass spectrometry and ^{13}C NMR spectroscopy and DFRC degradation procedure. The results indicated that the main structural units in lignins are phenyl propane skeletons bonded at β-O-4 positions.

Keywords: Bagasse, Chromatography, CMP dioxane lignin, Derivatization followed by reductive cleavage (DFRC), Soda lignin, Spectroscopy.

1. Introduction

In papermaking applications, lignin has almost a negative role and good-quality papers are made from fibers almost free of lignin. Lignin has a negative impact on physical and mechanical properties of paper. For example, because of light induced oxidation of residual lignin and formation of chromophore groups, the phenomenon of yellowing of paper takes place.

Chemi-mechanical pulp (CMP) is a high yield pulp which is used in newsprint paper and other short-term applications. Despite the importance of CMP paper and its huge consumption, little information exists about the specifications, structure and behavior of the residual lignin in its pulp. Also, the specifications and structure of bagasse lignin has not yet enough been studied.

Knowing the fact that for a country like Iran which is poor regarding industrial forests, bagasse is considered to be a valuable source of papermaking fibers. Therefore, it is worthwhile the chemistry of bagasse and its fibers to be thoroughly studied. The other challenge in this domain is the methods of preparing lignin and the origin of lignins that are studied and compared.

Regarding laboratory methods for lignin preparations, previous studies have shown that a modified (mild) dioxane lignin preparation procedure leads to a product that seems to be similar to native lignin, while its extraction from fibers is simple and practical in an ordinary laboratory (Brauns and Brauns, 1960).

On the other hand, soda lignin derived from black liquor of soda bagasse pulping operations is a representative of degraded lignin which its comparison with dioxane lignin can produce valuable information about the changes of lignin during soda pulping (Mirshokraie, 2011).

In this study, different lignin preparations have been studied using DFRC degradation method, chromatography and spectroscopy techniques and the results have been compared.

2. Material and Methods

2.1. *Materials*

Depithed bagasse from Khuzestan Province, soda bagasse lignin (ethanol-soluble fraction), shredded CMP paper, dioxane, hydrochloric acid, acetyl bromide, acetic acid, zinc powder. Chemicals with laboratory grade purity were used as received without any changes.

2.2. *Instruments*

Gel permeation chromatography: Shimadzu 6-A Chromatograph, Column Type: polystyrene, Detector: RI, Flow rate: 1 ml/min, Liquid phase: Tetrahydrofurane (THF).

Gas Chromatography-Mass spectrometry: Agilent 6890GC, 5973MS, Column: HP-5 MS, Flow rate: 1 ml/min. Sample was dissolved in dichloromethane.

^{13}C NMR Spectroscopy: Bruker 300 MHz NMR, solvent: deuterated chloroform, pulse width 90°, scan time (data collection) 0.911 second, delay time, 2 second. A total of 512 scans were performed on the samples.

For better solubility, samples used in GPC and ^{13}C NMR experiments were acetylated.

2.3. Preparation of dioxane CMP lignin

In order to extract lignin, 10 gr of shredded unprinted newsprint CMP paper (dry weight basis) was mixed with 120 ml dioxane and 13.3 ml of distilled water (volume ratio of 9 to 1) and 8.8 ml hydrochloric acid (37% , 0.8 mol/lit). To avoid undesirable optical reactions, extraction container was covered with aluminum foil (Partovi and Mirshokraie, 2013).

The mixture was shaken slowly for 24 h. Then the mixture was filtrated and washed by 100 ml fresh dioxane. The filtrate was evaporated under reduced pressure at 40-45 °C to reach to 10% of its initial volume. Then 300 ml distilled water was added and lignin was precipitated. The mixture was centrifuged at 5,000 rpm for 15 min at room temperature and then the lignin was separated from liquid phase. Separated lignin, was washed several times with distilled water and collected. Collected lignin was left in a dark place for an overnight in evacuating desiccator to dry. The yield of dried lignin was determined as 0.69%.

2.4. Preparation of dioxane bagasse lignin

The milled depithed bagasse with particle size of about 20 mesh, was extracted firstly with distilled water and then with ethanol by Soxhlet apparatus. In order to extract the lignin from bagasse, 10 g extracted bagasse flour (dry weight basis) was mixed with 120 ml dioxane and 13.3 ml distilled water (volume ratio of 9 to 1) and 2.2 ml hydrochloric acid (37%, 0.2 mol/lit). To avoid undesirable optical reactions, extraction container was covered with aluminum foil (Partovi and Mirshokraie, 2013).

The mixture was shaken slowly for 24 h. Then the mixture was filtrated and washed by 100 ml fresh dioxane. The filtrate was evaporated under reduced pressure at 40-45 °C to reach to 10% of its initial volume. Then 300 ml distilled water was added and lignin was precipitated. The mixture was centrifuged at 5,000 rpm for 15 min at room temperature and then the lignin was separated from liquid phase. Separated lignin, was washed several times with distilled water and collected. Collected lignin was left in a dark place for an overnight in evacuating desiccator to dry. The yield of dried lignin was determined as 1.1%.

2.5. Preparation of soda bagasse lignin (ethanol soluble fraction)

Soda bagasse lignin was prepared from black liquor of Pars paper Mill (Khuzestan province). For lignin precipitation, black liquor was acidified with dilute hydrochloric acid (0.1 N) to pH 2.5-3.0. The precipitate (impure lignin), was centrifuged and washed several times with distilled water to the point that the pH of filtrate to be almost 7.0.

Impure lignin was dissolved in warm ethanol (50-60 °C) and filtrated. Then the bulk portion of ethanol (about 75%) was evaporated at reduced pressure using an evaporator. Enough distilled water was added to the resulting viscous liquid to obtain a colloidal solution. The mixture was then centrifuged at 4,500 rpm. Significant amount of lignin (yield 42%) was separated and collected (Mirshokraie, 2011).

2.6. *Derivatization followed by reductive cleavage (DFRC)*

DFRC degradation method proposed by Lu and Ralph (1997a, 1997b, 1998) was performed on lignin samples using the following steps (Figure 1):

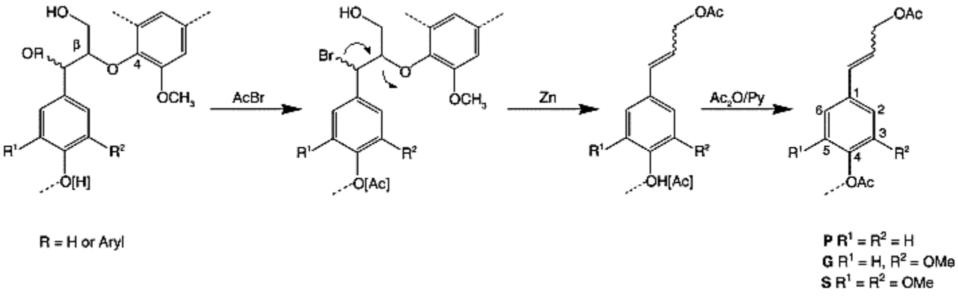


Figure 1. Reactions in DFRC method (Lu and Ralph, 1997a, 1997b, 1998).

2.6.1. *The acetyl bromide step*

To 10 mg of lignin, 2.5 ml of acetyl bromide (AcBr) was added (Acetyl bromide solution contains a mixture of acetyl bromide and acetic acid with the ratio of 8:92 by volume, which remains stable for weeks). The mixture was shaken at room temperature for 24 h. Then the solvent was evaporated under reduced pressure at 40-45 °C.

2.6.2. *Reductive cleavage step*

The product from previous step was dissolved in 2.5 ml of a mixture containing dioxane/acetic acid/water, with volume ratio of 5:4:1. By adding 50 mg of zinc powder (as reducing agent), the mixture was shaken for 30 min, then 0.5 mg anthracene as internal standard (dissolved in dichloromethane), 10 ml dichloromethane and 10 ml saturated ammonium chloride was added. The mixture was poured into separator funnel and shaked vigorously. Then the pH of aqueous phase was adjusted to below 3 by adding few drops of dilute hydrochloric acid and left for 30 min. Then the organic phase was separated. The extraction was repeated with 2×5 ml dichloromethane. All organic phases were collected in a container and dried by adding 0.5 gr magnesium sulfate. After 30 min, the mixture was filtrated and washed with 2×5 ml additional dichloromethane. Then the solvent was evaporated under reduced pressure at 40-45 °C.

2.6.3. *The acetylation step*

Remaining oil from previous step was dissolved in 1.5 ml dichloromethane and 0.2 ml acetic anhydride and 0.2 ml pyridine and the mixture was shaken slowly for 40 min. Then the solvent and volatile components were removed in reduced pressure at 40-45 °C. The dissolution and evaporation were repeated three times, each time by using 5 ml ethanol. The obtained material was dried in a glass plate while keeping away from light.

3. Results and Discussion

3.1. *Gel permeation chromatography (GPC)*

Gel permeation chromatography was performed on the acetylated samples of soda (ethanol soluble fraction) and dioxane lignins of bagasse and dioxane lignin of CMP paper, before and after DFRC experiments and the chromatograms obtained were examined and compared (Table 1). The results indicate that the average molecular weights of lignins after DFRC are less than before of this operation, which is due to lignin degradation in DFRC reactions.

Table 1. Gel permeation chromatography data of acetylated lignin samples before and after DFRC experiments.

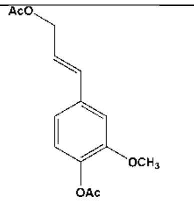
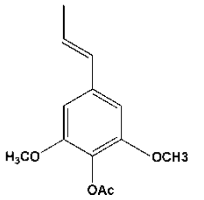
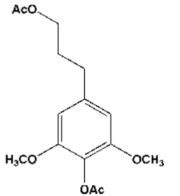
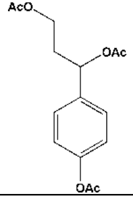
Sample	weight average molecular weight ($\overline{Mw}$)	number average molecular weight ($\overline{Mn}$)	polydispersity index ($\overline{DP} = \overline{Mw}/\overline{Mn}$)
Dioxane bagasse lignin before DFRC	4280	1350	3.16
Dioxane bagasse lignin after DFRC	865	226	3.82
Soda bagasse lignin* before DFRC	3964	1518	2.50
Soda bagasse lignin* after DFRC	713	182	3.91
Dioxane CMP lignin before DFRC	1430	1082	1.32
Dioxane CMP lignin after DFRC	1071	853	1.25

* ethanol soluble fraction

3.2. Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-Mass spectrometry technique was used to identify the degradation products of DFRC experiments (Rio et al., 2001). This is a rapid technique for the analysis of monomeric components obtained from lignin degradation. For this purpose, 0.05 g of reaction product was dissolved in 0.5 ml dichloromethane and 1 µl of this sample was injected at 1 ml/min rate into the GC-MS device. This injection was done at 275 °C. The ionized components were detected and identified. Table 2 has summarized some of the important monomeric degradation products which were identified by GC-MS technique.

Table 2. Some of the important monomeric products, observed in the soda (ethanol soluble fraction) and dioxane bagasse lignins and dioxane CMP lignin after DFRC.

Structure Name	Structure	Dioxane CMP lignin	Dioxane bagasse lignin	Soda bagasse lignin
A		✓	✓	✓
B		—	✓	✓
C		✓	✓	✓
D		✓	✓	✓

3.3. ^{13}C-Nuclear Magnetic Resonance Spectroscopy (^{13}C NMR)

^{13}C-Nuclear Magnetic Resonance Spectroscopy technique was used for comparative study and characterization of the structure of lignin samples before and after DFRC degradation process. To obtain the ^{13}C NMR spectra, the acetylated lignin samples were dissolved in deuterated chloroform. ^{13}C NMR spectroscopy provided some useful information about the structure of lignins and degradation products.

3.3.1. *Acetylated dioxane lignin of bagasse*

The results derived from ^{13}C NMR spectra of acetylated dioxane lignin of bagasse before and after DFRC (Figures 2 and 3) have been collected in Table 3.

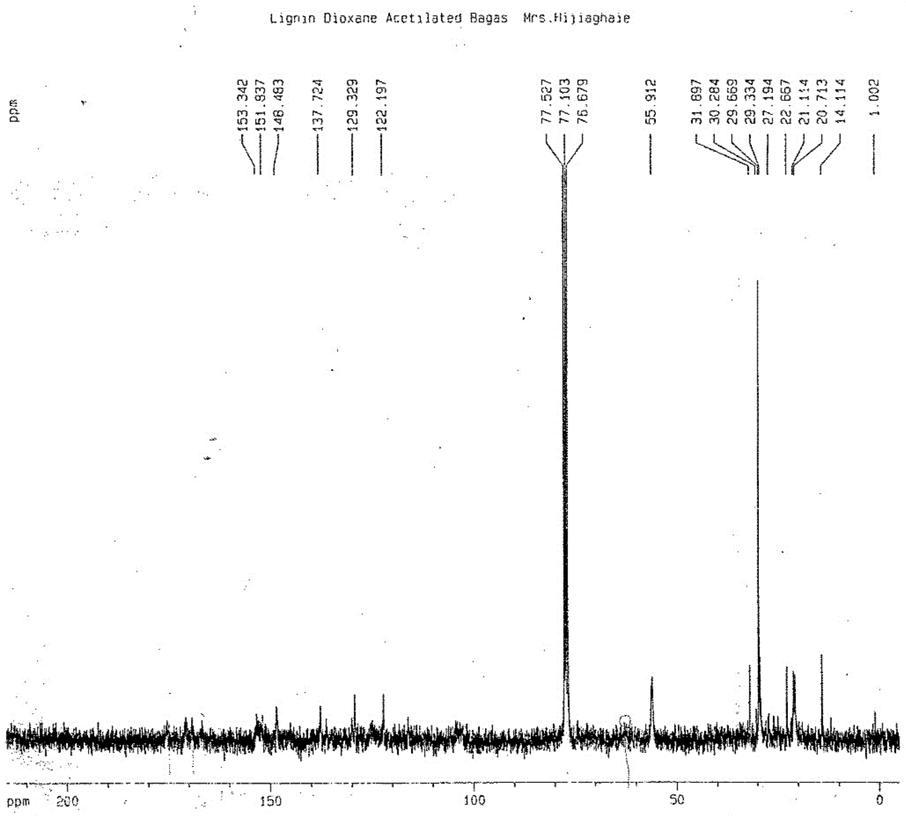


Figure 2. ^{13}C NMR spectra of acetylated dioxane lignin of bagasse before DFRC.

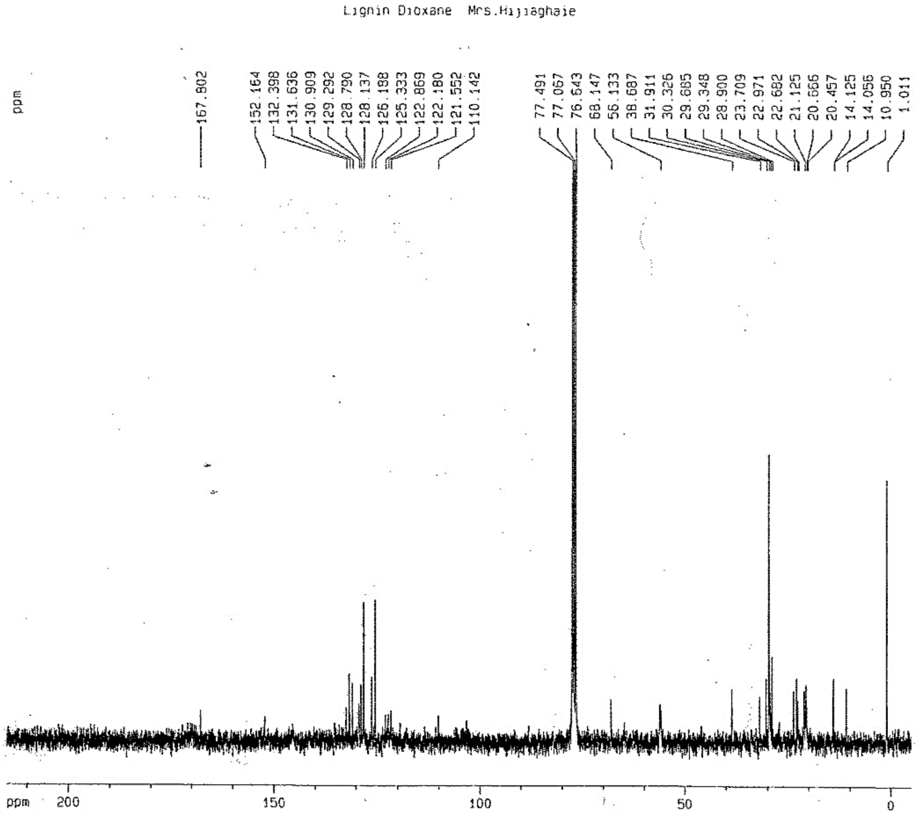


Figure 3. ^{13}C NMR spectra of acetylated dioxane lignin of bagasse after DFRC.

Table 3. Results derived from ^{13}C NMR spectra of acetylated dioxane lignin of bagasse before and after DFRC.

Structural Groups	Chemical shifts (ppm)	
	before DFRC	after DFRC
CH_3 of acetoxy group in C_4 of aromatic ring	14.1	14-14.1
CH_3 of acetoxy group in $C\gamma$	20.7	20.4-20.6
CH_3 of acetoxy group in C_α	22.6-29.6	22.6-29.6
C of double bond between C_α and C_β (phenylpropene structure)	———	125.3-128.1
CH_3 of methoxy groups in aromatic rings	55.9	56.1
C of aromatic rings	122.1-153.3	121.5-152.1
C of Carbonyl in acetoxy groups	___	167.8

3.3.2. *Acetylated soda lignin of bagasse (ethanol soluble fraction)*

The results derived from ^{13}C NMR spectra of acetylated soda lignin of bagasse before and after DFRC (Figures 4 and 5) have been collected in Table 4.

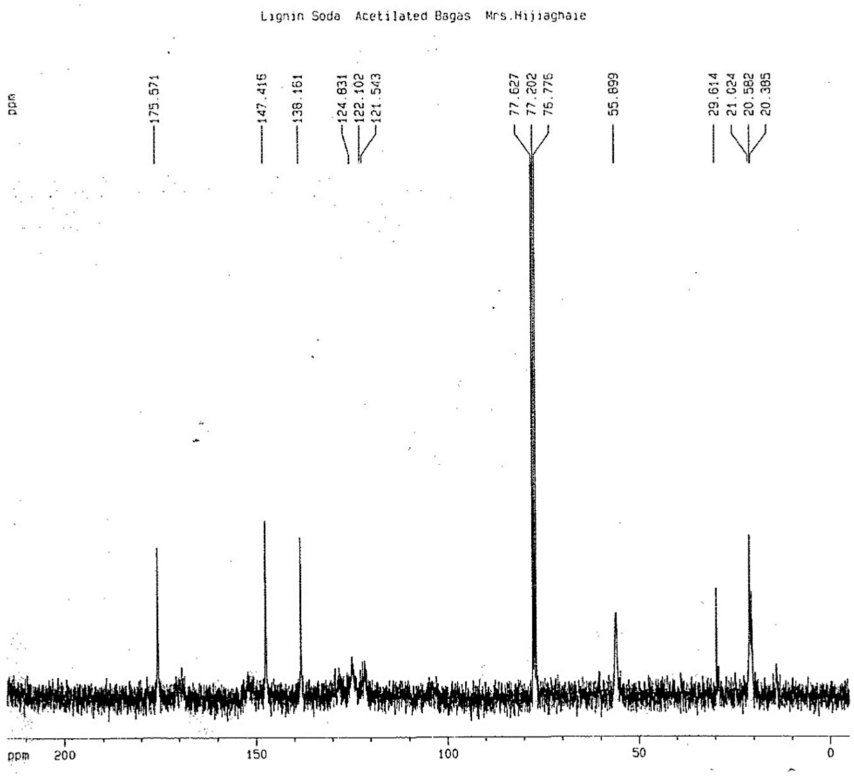


Figure 4. ^{13}C NMR spectra of acetylated soda lignin of bagasse before DFRC.

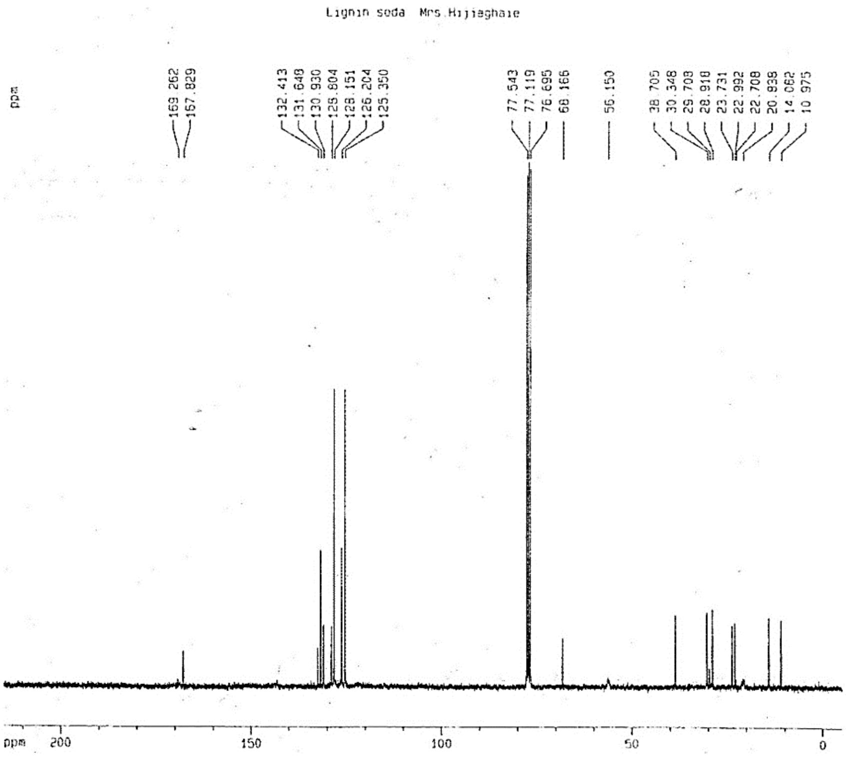


Figure 5. ^{13}C NMR spectra of acetylated soda lignin of bagasse after DFRC.

Table 4. Results derived from ^{13}C NMR spectra of acetylated soda lignin of bagasse (ethanol soluble fraction) before and after DFRC.

Structural Groups	Chemical shifts (ppm)	
	before DFRC	after DFRC
CH_3 of acetoxy group in C_4 of aromatic ring	14.0	10.9-14.0
CH_3 of acetoxy group in $C\gamma$	20.3-20.5	20.8-22.7
CH_3 of acetoxy group in C_α	21.0-29.6	22.9-29.7
C of double bond between C_α and C_β (phenylpropene structure)	———	125.3-128.8
CH_3 of methoxy groups in aromatic rings	55.8	56.1
C of aromatic rings	121.5-138.1	125.3-132.4
C of Carbonyl in acetoxy groups	175.5	169.2

3.3.3. Acetylated dioxane CMP lignin

The results obtained from ^{13}C NMR spectra of acetylated dioxane CMP lignin before and after DFRC (Figures 6 and 7) have been collected in Table 5.

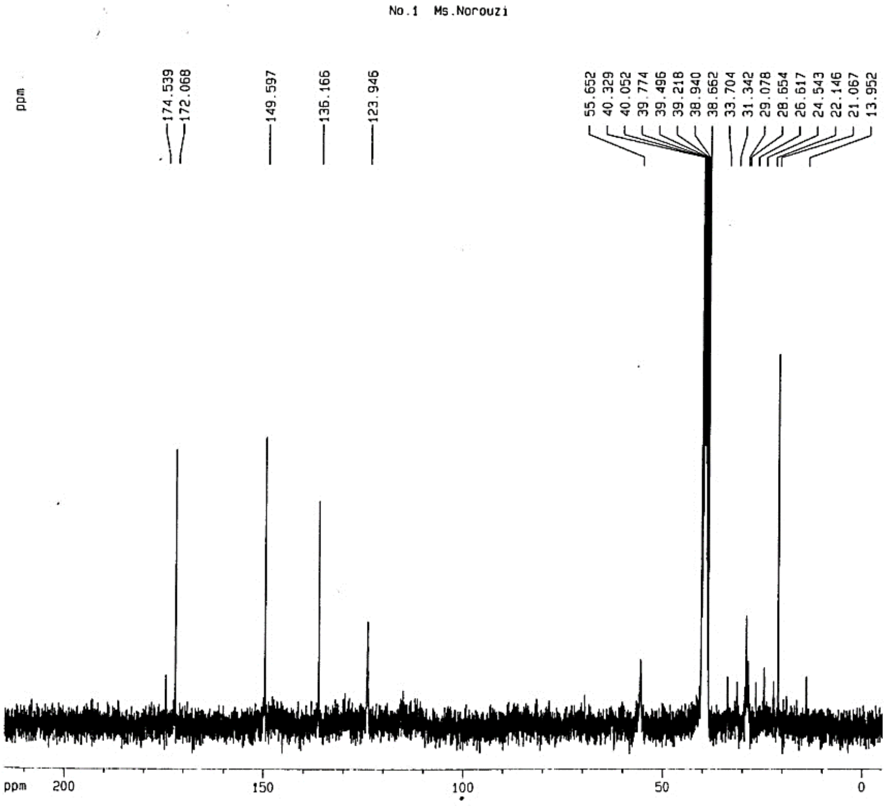


Figure 6. ^{13}C NMR spectra of acetylated dioxane CMP lignin before DFRC.

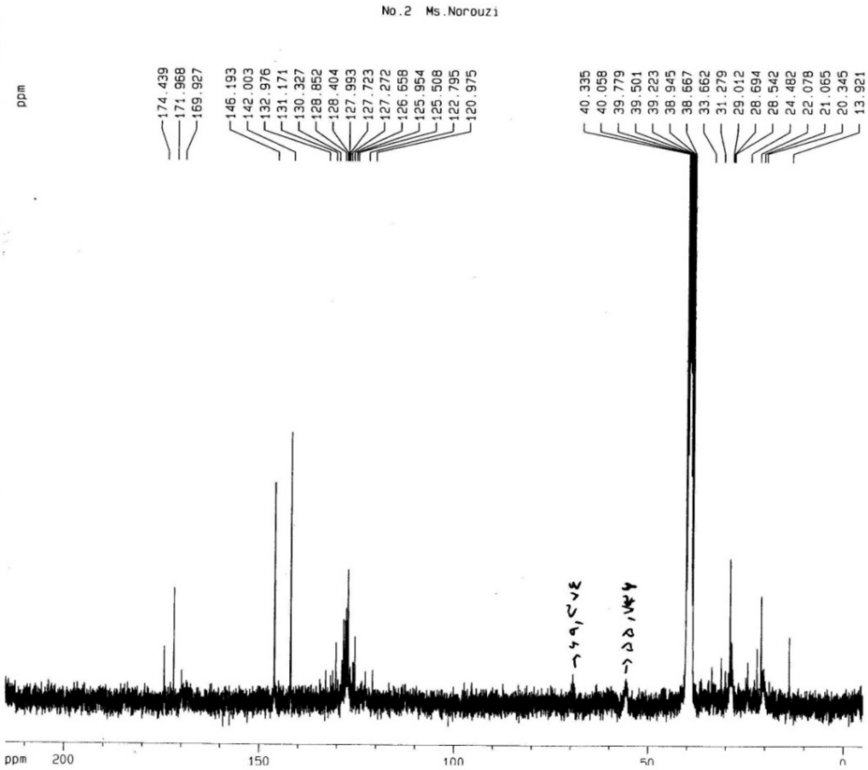


Figure 7. ^{13}C NMR spectra of acetylated dioxane CMP lignin after DFRC.

Table 5. Results derived from ^{13}C NMR spectra of acetylated dioxane CMP lignin before and after DFRC.

Structural Groups	Chemical shifts (ppm)	
	before DFRC	after DFRC
CH_3 of acetoxy group in C_4 of aromatic ring	13.9	13.9
CH_3 of acetoxy group in $C\gamma$	21.0	20.3-21.0
CH_3 of acetoxy group in C_α	22.1-29.0	22.0-29.0
C of double bond between C_α and C_β (phenylpropene structure)	———	125.5-128.8
CH_3 of methoxy groups in aromatic rings	55.6	55.7
C of aromatic rings	123.9-149.5	120.9-146.1
C of Carbonyl in acetoxy groups	172.0	171.9

4. Conclusions

4.1. Analysis of GPC chromatograms

The following information could be derived from GPC chromatograms:

1. Average molecular weights ($\overline{Mw}$ and $\overline{Mn}$) of dioxane CMP lignin, soda and dioxane lignins of bagasse before DFRC treatment are much greater than those of after DFRC treatment which confirms the fact that the DFRC treatment is a suitable method for the cleavage of ether bonds in lignin.
2. Average molecular weights ($\overline{Mw}$ and $\overline{Mn}$) of dioxane and soda lignins of bagasse are more than dioxane CMP lignin. This could be assigned to extended degradation of lignin in CMP pulping process in which lignin is partially solfunated by using sodium sulfite.
3. Average molecular weight ($\overline{Mw}$ and $\overline{Mn}$) of dioxane lignin is more than soda lignin which is due to extensive chemical degradation of soda lignin during the process of soda pulping. In other words, in the mild conditions of extraction of dioxane lignin, extensive structural changes does not occur.

4.2. Analysis of GC-MS spectra

Some important GC-MS spectrometry results could be summarized as following:

1. In all three lignin samples, cleavage of β-O-4 bonds have been occurred.
2. In all three lignin samples, creation of phenylpropene structures were confirmed.

4.3. Analysis of ^{13}C NMR spectra

The ^{13}C NMR results could be summarized as following:

1. DFRC process has not been caused destruction of phenylpropane structures but cleavages mainly have been occurred in β-O-4 ether positions.
2. DFRC process has caused degradation of lignin into monomeric compounds.
3. Methoxy groups (methyl ether units) as substituents have been left unchanged during DFRC experiment.

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