Characterization and Optimization of the Glyoxalation of a Methanol-Fractionated Alkali Lignin using Response Surface Methodology

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The glyoxalation of a methanol-fractionated alkali lignin was executed at 60 °C for 8 h with different amounts of glyoxal (40% in water) and 30% NaOH. The weights of the lignin and water were fixed at 10.0 and 15.0 g, respectively. The gel permeation chromatography (GPC) results indicated that depolymerization of lignin molecules occurred during the glyoxalation process. However, a higher polydispersity index (M_w/M_n) of all glyoxalated lignins compared to the unmodified lignin (ML) showed that lignin polymers with a variety of chain lengths were generated through the crosslinking and through the repolymerization of lignin molecules *via* methylene (CH_2) bridges and new, strong C-C bonds after the condensation reaction. This was confirmed by thermogravimetry analysis (TGA). Optimum amounts of glyoxal and NaOH to be used in the glyoxalation process were ascertained by quantifying the intensity of relative absorbance for the CH_2 bands obtained from FT-IR spectra and by using response surface methodology (RSM) and central composite design (CCD), which facilitated the development of a lignin with appropriate reactivity for wood adhesive formulation. The experimental values were in good agreement with the predicted ones, and the model was highly significant, with a coefficient of determination of 0.9164. The intensity of the relative absorbance for the CH_2 band of 0.42 was obtained when the optimum amounts of glyoxal and NaOH, *i.e.*, 0.222 and 0.353, respectively, were used in the glyoxalation process.

Keywords: Lignin; Glyoxalation; Response surface methodology; Glyoxal; Wood adhesive

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INTRODUCTION

Lignin is an industrially manufactured organic by-product of the pulp and paper industry and of the biomass conversion processes that produce biofuel. Large quantities of isolated lignin can be recovered from various pulping or biomass conversion processes. Furthermore, the polyphenolic nature of lignin makes it a potential substitute for phenol, obtained from non-renewable petrochemicals, in the production of ordinary formaldehyde-based resin. However, one of the major challenges to using lignin in such an application is its low reactivity, which can be attributed to its small number of ortho and para reactive sites, as well as their poor accessibility (Alonso *et al.* 2004). Therefore, lignin must undergo some chemical modifications to enhance its reactivity if it is to be used as a raw material in the synthesis of biobased resins.

Hydroxymethylation, or methylolation, is one of the chemical modification methods applied to enhance the reactivity of lignin toward crosslinking agents such as

formaldehyde (Zhao *et al.* 1994; Malutan *et al.* 2008; Mu *et al.* 2009; Lin *et al.* 2010). Hydroxymethylation enhances the reactivity of lignin by introducing reactive functional groups to the lignin molecules. Zhao *et al.* (1994) revealed that under the optimum reaction conditions, 0.36 moles of the CH_2OH/C_9 unit could be successfully introduced into pine kraft lignin molecules. The majority of the CH_2OH reactive functional groups, which constitute about 0.33 moles, were introduced at the C_5 position of the lignin aromatic rings by means of the Lederer-Manasse reaction. An adhesive made from the incorporation of 50% hydroxymethylated pine kraft lignin and 50% phenol formaldehyde resin (w/w) was used to fabricate laboratory boards made with sweetgum flakes, and the resulting bond strength was about 448.16 kPa.

In another study, in contrast, rather than carry out the hydroxymethylation using toxic and volatile aldehydes such as formaldehyde, the same process was conducted using non-toxic and non-volatile dialdehydes such as glyoxal. El Mansouri *et al.* (2011) investigated the influence of reaction conditions, such as the molar ratio of sodium hydroxide to lignin and reaction time, on the properties of the glyoxalated alkaline lignin. They found that a reaction time of 10 h and a pH of 12.0 represented the optimum operating conditions for the glyoxalation of alkaline lignin, as under these conditions the glyoxalated alkaline lignin exhibited superior structural properties for wood adhesive formulation.

Navarrete *et al.* (2012) elucidated the lignin-glyoxal reactions using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and CP-MAS ^{13}C-NMR. They found that lignin depolymerization and repolymerization occurred concurrently during the glyoxalation of lignin. Condensation reactions occurred between the lignin and glyoxal to form glyoxalene bridges linking lignin units and especially linking fractions of lignin units derived from degradation during depolymerization. As some of the degraded lignin units were recombined *via* glyoxalene bridges through the condensation reaction, hence, the reactive hydroxyglyoxalated lignin is the one species still available for co-reaction for use in wood adhesives. Navarrete *et al.* (2013) revealed that 60 and 90 days old glyoxalated lignin solution shows increased viscosity if compared to fresh glyoxalated lignin solution. This is due to the monomers and lower molecular mass oligomers such as dimers polymerized into structures of higher molecular weight as a consequence of their condensation with glyoxal and the formation of glyoxylene bridges between them.

Glyoxalated organosolv lignin has been used to incorporate with tannin/hexamine to develop synthetic resin free wood panel adhesives (Navarrete *et al.* 2010). The authors reported that the particleboards and plywood bonded with tannin/hexamine and glyoxalated organosolv lignin (50:50 by weight) adhesive were able to pass the relevant interior standards regardless the usage of either cheaper non-purified or more expensive purified organosolv lignin.

The glyoxalation of lignin has been used to introduce hydroxymethyl units (CH_2OH) to lignin molecules (El Mansouri *et al.* 2011). The introduction of CH_2OH into lignin molecules is a way to enhance the lignin's reactivity of lignin (Hu *et al.* 2011). The hydroxymethylated (or glyoxalated) lignin can be further crosslinked *via* the formation of methylene bridges under a condensation reaction in an alkaline medium (Chakar and Ragauskas 2004). A reaction mechanism has been diagrammed in the cited work. Hence, the intensity of the methylene bridges formed in the glyoxalated lignin following the condensation reaction could be used to determine the effectiveness of the glyoxalation process and to enhance the reactivity of the lignin.

The costs of the glyoxalation of lignin could be reduced by developing an understanding of which amounts of glyoxal and sodium hydroxide (NaOH) would optimize

the process. In addition to lowering costs, the establishment of the optimal proportions of glyoxal and NaOH would also allow glyoxalated lignin to be obtained with a level of reactivity suitable for its utilization as a raw material for wood adhesive formulation. Therefore, the aims of the present study were to characterize and to establish the optimal amounts of glyoxal and NaOH for use in the glyoxalation of lignin, by means of response surface methodology (RSM) and central composite design (CCD), to obtain a glyoxalated lignin with an appropriate intensity of relative absorbance for the CH_2 band (*i.e.*, with appropriate reactivity).

EXPERIMENTAL

Materials

The chemicals used in this study included a low-sulfonate content softwood alkali lignin (96%), which had a molecular weight (M_w) of 11,646 g/mol. This lignin was further fractionated into particles of lower M_w using methanol (average M_w: 4179 g/mol). The fractionated lignin was then used as the starting material for the glyoxalation process. The lignin, sodium hydroxide (NaOH) solution (50% in H_2O), and glyoxal solution (40% in water) were purchased from Sigma-Aldrich (M) Sdn. Bhd. The NaOH solution was further diluted with distilled water until its concentration was 30%, and the glyoxal solution was used as received.

Methods

Response surface methodology and central composite design

In the present study, a central composite design (CCD) using response surface methodology (RSM) was used to determine the optimum amounts of glyoxal and NaOH to be used in the glyoxalation of the methanol-fractionated lignin model compound. This method was conducted to determine the effect of variables on the response in the region of investigation (Zaidon *et al.* 2012). The CCD was carried out using Design Expert 8 software (Stat-Ease, USA). The test variables were then coded according to the following regression equation (Eq. 1),

$$x_i = \frac{\left(x_{\mathrm{i}} - x_{\mathrm{i0}}\right)}{\Delta x_{\mathrm{i}}} \quad (1)$$

where x_i is the independent variable in terms of the coded value, x_{i} is the independent variable in terms of the real value, x_{i0} is the independent real value on the center point, and Δx_{i} is the interval, i = 1, 2, 3, 4.

The range and the levels of the variables under investigation are given in Table 1.

Table 1. The Range and Levels of the Variables

Factor	Variables	Unit	Range and level of actual and coded values				
			-α	-1	0	1	A
x_1	Glyoxal	mol	0.073	0.102	0.171	0.239	0.268
x_2	NaOH	mol	0.111	0.153	0.253	0.353	0.419

Each of the independent variables was studied at five different levels containing two axial points (α = 1.41) and five replications of the center point for a total of 13 experiments. The combined effects of the two independent variables, corresponding to the effects on the response variables, were studied in their specific ranges, that is, amounts of glyoxal and NaOH. The response variable used was the intensity of relative absorbance for the CH_2 band obtained from the FT-IR spectra. The plan of the CCD, with respect to the coded and actual levels of the two independent variables, is shown in Table 2.

Table 2. Experimental Condition of Central Composite Design (CCD)

Run	Coded Value		Actual Value	
	X_1	X_2	Glyoxal (mol)	NaOH (mol)
1	0	0	0.171	0.253
2	-1	1	0.102	0.353
3	0	1.41	0.171	0.419
4	-1.41	0	0.073	0.253
5	0	0	0.171	0.253
6	0	0	0.171	0.253
7	0	0	0.171	0.253
8	1.41	0	0.268	0.253
9	0	-1.41	0.171	0.111
10	1	-1	0.239	0.153
11	0	0	0.171	0.253
12	1	1	0.239	0.353
13	-1	-1	0.102	0.153

Glyoxalation of lignin

The procedure for the glyoxalation of lignin was carried out in accordance with the procedure of El Mansouri *et al.* (2007a).

Lignin powder (10.0 g) was added slowly to 15.0 g of distilled water. Different amounts of 30% NaOH, as shown in Table 2, were added from time to time to achieve better dissolution of the lignin powder, followed by vigorous stirring with an overhead stirrer. A 250-mL flat-bottom flask equipped with a condenser, thermometer and magnetic stirrer bar was charged with the above mixture and then heated to 60 °C. When the mixture was heated to 60 °C, different amounts of glyoxal, as shown in Table 2, were added to the mixture. The mixture was then continuously stirred with a magnetic stirrer/hot plate for 8 h. At the end of this process, the mixture was collected and left to cool at ambient temperature. Then, the pH value of the mixture was measured using a digital pH meter.

Determination of glyoxalated lignin solid content

To determine the solid content of the glyoxalated lignin, approximately 2 g of the glyoxalated lignin was heated in oven at 120 °C for 3 h, followed by reweighing. The solid content for all the glyoxalated lignins was approximately 37±2%. The samples used in this test were kept in a dessicator for further analysis.

Gel permeation chromatography (GPC)

The M_n, M_w, and polydispersity index (M_w/M_n) of each of the glyoxalated lignin powder samples were determined using gel permeation chromatography (GPC) conducted on a Waters Ultrahydrogel 250 PKGD column (USA). The column was operated at 50 °C

and eluted with a mobile phase (80/20 water/acetonitrile with 0.1 M sodium nitrate buffer adjusted to pH 11.0 or higher with 50% sodium hydroxide) at a flow rate of 0.3 mL/min and an injection volume of 20 µL. The standard samples employed for calibration were narrow polyethylene oxide (PEO, MWs of 5200, 11,600, 24,000, 48,600, 79,000, 148,000, 273,000, 410,000, and 668,000). The M_n and M_w values of the lignin fractions were computed from their chromatograms.

Thermogravimetry analysis (TGA)

Approximately 8 to 10 mg of glyoxalated lignin powder was weighed into an aluminum pan and placed in the TA-TGA Q500 instrument manufactured by TA Instruments, USA. The heating was conducted at a rate of 10 °C/min and ranged from room temperature to approximately 700 °C. The test was performed in a nitrogen atmosphere. A curve of weight loss against temperature was constructed. A derivative of this curve (DTG) was produced to indicate the temperatures at which the maximum rates of weight loss occurred.

Fourier transformed infrared (FT-IR) spectroscopy

A FT-IR spectrophotometer was used to determine the functional groups in each of the samples of glyoxalated lignin powder. FT-IR spectra tests were run at ambient temperature using pure samples within the wave number range of 400 to 4000 cm^{-1} and at a resolution of 4 cm^{-1}. The infrared spectra of the samples were measured on a Perkin-Elmer FT-IR (model spectrum 100 series, USA). The absorption bands were assigned as suggested by Tejado *et al.* (2007).

RESULTS AND DISCUSSION

Molecular Weight Distributions of Glyoxalated Lignins

The M_n, M_w, and M_w/M_n values for glyoxalated lignins with various amounts of glyoxal and sodium hydroxide (NaOH) are presented in Table 3. Generally, the results showed that the M_n and M_w values of the glyoxalated lignins were lower than those of the unmodified lignin (ML), regardless of the amounts of glyoxal and NaOH used. This shows that the lignin molecules were degraded during the glyoxalation process. This finding was in close agreement with previous studies (Malutan *et al.* 2008; Navarrete *et al.* 2012). Malutan *et al.* (2008) conducted a hydroxymethylation of lignins with formaldehyde in an alkaline medium and found that the lignins exhibited a loss in molecular weight following their modification *via* the hydroxymethylation process.

The M_n for the R1, R5, R6, R7, and R11 (glyoxal: 0.171 mol, NaOH: 0.253 mol) samples were 360, 361, 335, 355, and 350 g/mol, respectively. The M_w value for these samples were 1243, 1250, 1204, 1232, and 1220 g/mol, respectively. Meanwhile, the M_n and M_w values for ML were 2205 and 4179 g/mol, respectively. The fact that the values of M_n and M_w in these samples were lower than those of ML reveals that the lignin molecules were degraded during the glyoxalation process. This may be attributed to the fact that the glyoxalation of lignin, when executed in a hot and alkaline medium for several hours, can lead to the cleavage of linkages between parts of the lignin molecules (Lei 2009).

The M_w/M_n values for these glyoxalated lignin samples were 3.45, 3.46, 3.59, 3.47, and 3.49, respectively, while the M_w/M_n value for ML was 1.90. The increase in the M_w/M_n values in these samples indicated that the lignin polymer chain lengths had become

heterogeneous as a result of the glyoxalation process. Since the heterogeneity of alkali lignin used in this study was reduced through the organic solvent fractionation, one of the factors that may contributing to an increase of the M_w/M_n is the lignin molecules were degraded in non-uniform way during the degradation process which yielded some very small fragments and some larger fragments. In addition, the increasing in the M_w/M_n is also likely attributed to the crosslinking, through condensation reactions during the solid content test, of the lignin molecules *via* methylene (CH_2) bridges to form lignin polymers of various chain lengths. This indicates that the partial substitution of a CH_2OH group into a free C_5 position of the lignin aromatic group enhanced the reactivity of the lignins and linked the lignin molecules together by CH_2 bridges to form a compound having a lower molecular weight (Lei 2009; Navarrete *et al.* 2012; Zhao 2013).

Table 3. Molecular Weight Distributions of ML and Glyoxalated Lignin Samples

Runs	M_w (g/mol)	M_n (g/mol)	M_w/M_n
ML	4179	2205	1.90
R1	1243	360	3.45
R2	680	231	2.95
R3	877	262	3.35
R4	1076	412	2.61
R5	1250	361	3.46
R6	1204	335	3.59
R7	1232	355	3.47
R8	879	272	3.23
R9	1039	299	3.48
R10	1116	300	3.72
R11	1220	350	3.49
R12	1300	367	3.54
R13	1246	364	3.42

For both the R4 (glyoxal: 0.073 mol, NaOH: 0.253 mol) and R8 (glyoxal: 0.268 mol, NaOH: 0.253 mol) samples, the values of M_n and M_w were also found to be lower than those of ML, *i.e.*, 412 and 1076 g/mol for R4 and 272 and 879 g/mol for R8, respectively. These results indicated that lignin molecules in both of these samples had been degraded following the glyoxalation process. The M_w/M_n value of the R4 was slightly higher than that of the ML, *i.e.*, 2.61 compared to 1.90, whereas the M_w/M_n value of R8 (3.23) was higher than both R4 and ML samples. This suggests that, following the glyoxalation process, lignin polymers of different chain lengths were produced by means of a condensation reaction. The lower M_w/M_n value of the R4 compared to the R8 suggests that the lower amount of glyoxal used did not favor the glyoxalation of lignin, as fewer CH_2OH groups were introduced into free C_5 positions of the lignin aromatic group.

Therefore, the reactivity of the lignin molecules was not strong enough to crosslink the lignin molecules *via* the CH_2 bridges during the condensation reaction. The fact that the M_w/M_n value of R4 was greater than that of ML may have been due to the self-repolymerization of the lignin molecules, as less stable carbon-centered radicals generated during the glyoxalation process (in alkaline medium) tend to recombine to form new, strong carbon-carbon bonds (Li *et al.* 2007). As in the cases of the R1, R5, R6, R7, and R11 samples, the higher amount of glyoxal used in sample R8 led to the crosslinking during the condensation reaction of lignin molecules via CH_2 bridges, which produced lignin polymers with various chain lengths.

The M_n and M_w values for the R10 sample (glyoxal: 0.239 mol, NaOH: 0.153 mol) were 300 and 1116 g/mol, respectively, while the M_n and M_w values for the R13 sample (glyoxal: 0.102 mol, NaOH: 0.153 mol) were 364 and 1246 g/mol, respectively. As with other samples, the lignin molecules in both of these samples were also found to have been degraded during the glyoxalation process. The M_w/M_n values of both of these samples were 3.72 and 3.43, respectively, making them higher than those of the ML sample. These results suggested that even though a lower amount of NaOH was used in these samples, the introduction of a CH_2OH group into a free C_5 position of the lignin aromatic group still occurred, as lignin polymers of various chain lengths were observed following the condensation reaction.

For the R2 (glyoxal: 0.102 mol, NaOH: 0.353 mol) and R12 (glyoxal: 0.239 mol, NaOH: 0.353 mol) samples, the values of M_n and M_w were 231 and 680, and 367 and 1300 g/mol, respectively. Again, the lignin molecules in both of these samples were also observed to have been degraded following the glyoxalation process. The M_w/M_n value of R2 was 2.95, whereas the M_w/M_n value of R12 was 3.54. The wide molecular weight distribution of the glyoxalated lignin samples may be attributed to the lignin molecules degraded in non-uniform way. In addition, the fact that both of these values were higher than those of the ML sample suggested the occurrence of either crosslinking or repolymerization, by means of the condensation reaction, of the lignin molecules to form polymers of various chain lengths. As in the R4 sample, the fact that the M_w/M_n value observed in the R2 sample was higher than that of the ML sample but lower compared to that of the R12 sample may have been due to a repolymerization reaction instead of a crosslinking *via* CH_2 bridges. The lignin molecules may have tended to repolymerize instead of crosslink because of i) the usage of less glyoxal, ii) the more basic environment (more NaOH) during the glyoxalation process, and iii) the usage of greater amounts of NaOH, which also caused undesirable side reactions, such as the Cannizzarro reaction in which glyoxal reacts with itself. The Cannizzarro reaction becomes more severe when more NaOH is used (Malutan *et al.* 2008). Hence, no or fewer CH_2OH groups were introduced into the free C_5 positions of the lignin aromatic group in the R2 sample in which the CH_2 bridges were unable to form *via* the condensation reaction. When the amount of glyoxal used was increased, as in the R12 sample, lignin polymers of various chain lengths were formed during the condensation reaction *via* the formation of CH_2 bridges, as more CH_2OH groups were introduced into free C_5 position of the lignin aromatic groups during the glyoxalation process.

When the amount of glyoxal was held constant, as in the R2 and R13 samples, it was found that the glyoxalation process was more favorable if less NaOH was used because of the crosslinking of the lignin molecules *via* CH_2 bridges to form lignin polymers of various chain lengths. As discussed above, the use of more NaOH, as in the R2 sample, was not favorable to the glyoxalation process because of the undesirable side reaction, *i.e.*,

the reaction of glyoxal with itself. This is similar to the R10 and R12 samples, despite the different amounts of glyoxal used, in that lower amounts of NaOH also tended to cause crosslinking of the lignin molecules *via* CH_2 bridges to form lignin polymers of a variety of chain lengths, although the M_w/M_n value was higher in the R10 than in the R12 sample. The lower M_w/M_n value in the R12 sample was probably attributable to the occurrence of the Cannizzarro reaction.

When 0.171 mol of glyoxal was used, a similar effect was imparted to the glyoxalated lignin, regardless of the amount of NaOH used, in which crosslinking of the lignin molecules *via* CH_2 bridges to form lignin polymers of a variety chain lengths occurred. The M_n and M_w values for the R3 sample (glyoxal: 0.171 mol, NaOH: 0.419 mol) were 262 and 877 g/mol, respectively, while for the R9 sample (glyoxal: 0.171 mol, NaOH: 0.111 mol), these values were 299 and 1039 g/mol, respectively. The occurrence of the crosslinking of the lignin molecules *via* CH_2 bridges to form lignin polymers of various chain lengths was supported by the M_w/M_n values of the R3 and R9 samples, which were 3.35 and 3.48, respectively.

Thermal Stability of Glyoxalated Lignin

The thermogravimetric (TG) curves reveal the mass loss of substances in relation to the temperature of thermal degradation, while the first derivative of that curve (DTG) shows the corresponding rate of mass loss. The peak of this curve (DTG_{max}) may be expressed as a single thermal decomposition temperature and can be used to compare the thermal stability characteristics of different materials.

The DTG_{max} and mass loss of ML and glyoxalated lignin samples are summarized in Table 4. The TGA thermograms of the ML, R2, and R3 samples are shown in Figs. 1 through 3. The results show that three events were taking place during the TGA test, as three DTG_{max} peaks were recorded. This TGA result was different from the results reported by other related studies that conducted the same tests, in which the authors only reported that two events had occurred during the test, such that the first DTG_{max} peak corresponded to the evaporation of moisture and volatiles and the second DTG_{max} peak corresponded to the thermal decomposition of the lignins (Malutan *et al.* 2008; El Mansouri *et al.* 2011). The TGA results in this study suggested that new lignin polymers were generated after the glyoxalated lignins had undergone the solid-content test.

It was found that all lignin samples, including the ML and glyoxalated lignin samples, showed the first DTG_{max} peak as recorded in the range of 60.0 to 141.8 °C. This peak corresponded to the mass loss of the samples resulting from the evaporation of moisture and volatiles. The mass loss was recorded as 2.73 to 9.27%. The second DTG_{max} peak was considered an extra peak, as this peak could not be found in the ML or in a few of the glyoxalated lignin samples. The recorded temperatures for this peak ranged from 218.05 to 266.43 °C, and the mass loss rate ranged between 12.49 and 19.08%. The emergence of this peak was also supported by the data on molecular weight distribution as discussed in the previous section, in which lignin polymers of various chain lengths were observed after the solid content test. The second DTG_{max} peak indicated that the lignin polymers with lower molecular weights, which are less thermally stable, had been formed *via* the condensation reaction during the solid content test. The third DTG_{max} peak was found in all ML and glyoxalated lignin samples. This peak corresponded to the mass loss of the samples as a result of the decomposition of the remaining lignin polymers, which was not involved in the formation of the lignin polymers that caused the emergence of a

second DTG_{max} peak. The recorded temperatures for this peak ranged from 313.0 to 369.56 °C, and the mass losses ranged from 7.41 to 29. 94%.

The temperatures for the first and third DTG_{max} peak in the ML sample were 91.73 and 313.0 °C, respectively, while the mass losses for the first and third DTG_{max} peaks were 4.0 and 29.94%. It was found that the temperatures of the third DTG_{max} peak for all glyoxalated lignin samples were higher than that of ML. This reveals that all the glyoxalated lignin samples underwent a partial repolymerization reaction during the condensation reaction in the solid content test; as a result, more thermally stable lignin polymers were generated (Sun *et al.* 2000). The highest temperature for the third DTG_{max}, that is, 369.56 °C, which was associated a mass loss of 20.76%, was found in the R2 sample. No second DTG_{max} peak was found in this sample. This suggests that the weights of the glyoxal and NaOH (glyoxal: 0.102 mol, NaOH: 0.353 mol) used in this sample did not promote the crosslinking reaction of the lignin molecules to form lignin polymers of lower molecular weights *via* CH_2 bridges, but rather led, during the condensation reaction of the solid content test, to a repolymerization reaction of the lignin molecules to form lignin polymers that were more thermally stable.

For some of the glyoxalated lignin samples, the second DTG_{max} peak was attributed to the partial crosslinking of the lignin molecules *via* CH_2 bridges to form lignin polymers of lower molecular weights *via* the condensation reaction of the solid content test. The highest temperature of the second DTG_{max} peak, which was 266.43 °C and was associated with a mass loss of 15.25%, was found in the R3 sample. This indicated that a higher degree of crosslinking of lignin molecules *via* CH_2 bridges was achieved in this sample, which was attributed to the great extent of substitution of CH_2OH groups into free C5 positions for the lignin aromatic group during the glyoxalation reaction. The weights of the glyoxal and NaOH for this sample were 0.171 moles and 0.419 moles, respectively.

Table 4. TGA Data for ML and Glyoxalated Lignin Samples

Runs	Peak 1		Peak 2		Peak 3	
	DTG_{max}, °C	Mass loss, %	DTG_{max}, °C	Mass loss, %	DTG_{max}, °C	Mass loss, %
ML	91.73	4.0	-	-	313.0	29.94
R1	141.25	4.22	255.0	17.52	343.05	15.83
R2	96.67	8.26	-	-	369.56	20.76
R3	106.66	3.38	266.43	15.25	353.33	7.41
R4	60.0	9.27	-	-	362.55	24.55
R5	141.80	3.51	255.56	17.80	343.20	15.60
R6	140.82	4.54	255.88	17.50	342.88	15.73
R7	141.18	4.04	255.96	18.10	342.75	15.48
R8	103.33	2.73	239.12	18.77	333.76	16.95
R9	110.0	2.84	218.05	16.01	325.87	21.82
R10	121.45	4.62	225.11	18.63	325.44	16.75
R11	140.88	3.85	255.70	17.95	342.97	15.55
R12	140.86	3.78	250.78	19.08	350.58	12.98
R13	89. 91	5.99	250.30	12.49	320.41	19.26

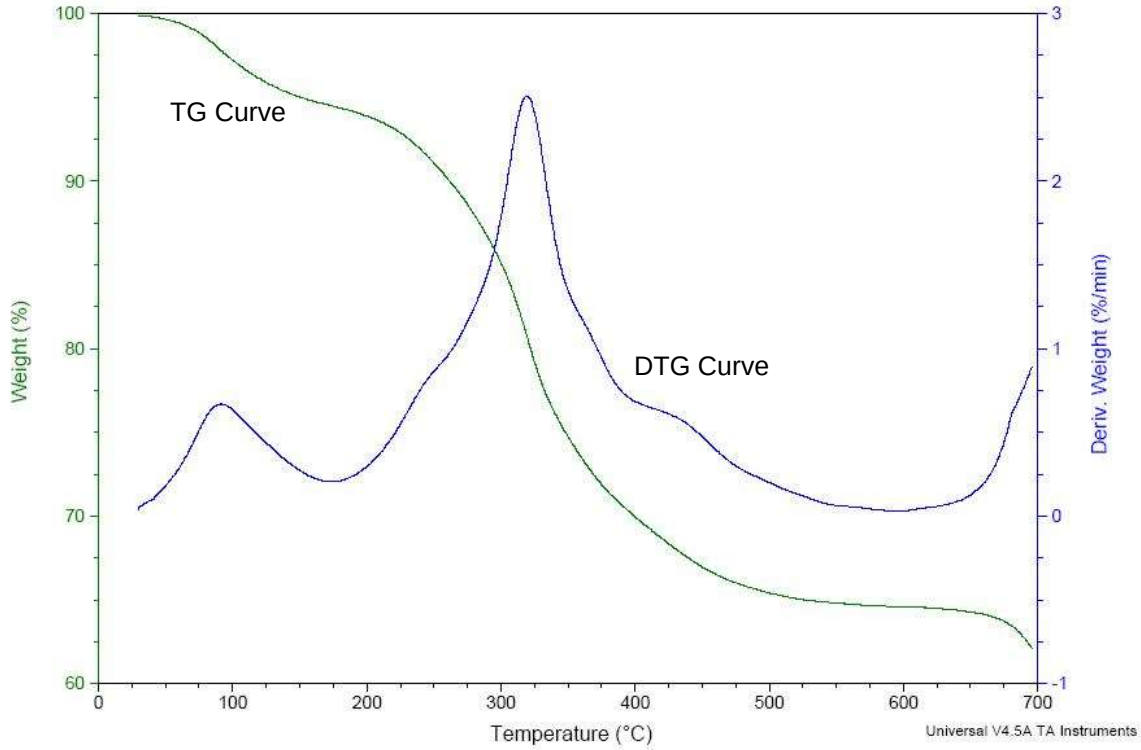

Fig. 1. TGA thermogram of ML

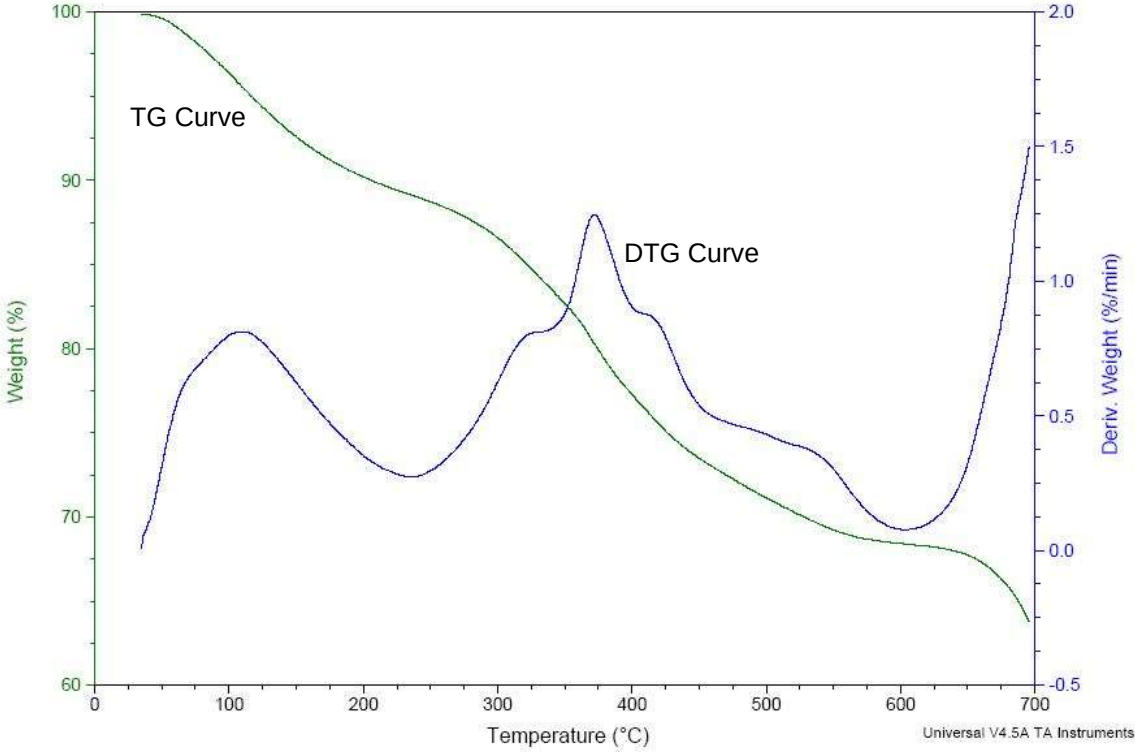

Fig. 2. TGA thermogram of R2 sample

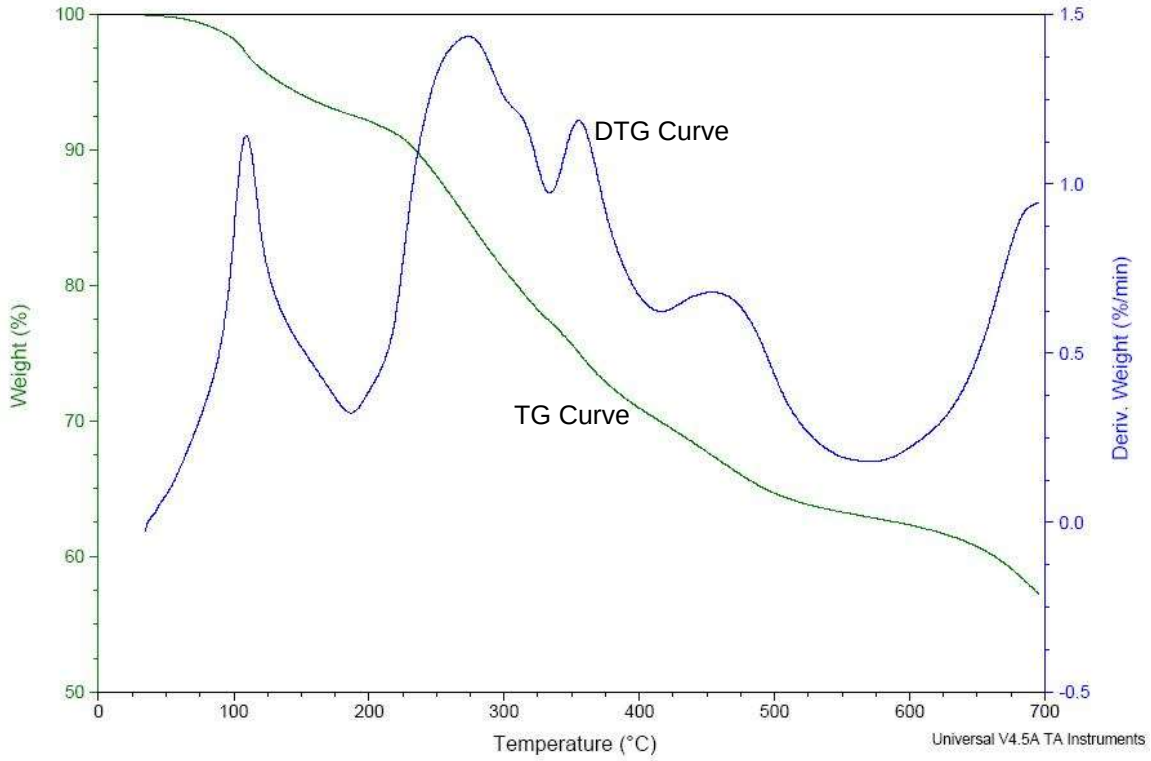

Fig. 3. TGA thermogram of R3 sample

Optimization of reactant proportions for glyoxalation of lignin

Quantitative analysis based on FT-IR spectra referred to previous studies (Malutan *et al.* 2008; El Mansouri *et al.* 2011; Mancera *et al.* 2011). The absorption intensities for each band were normalized based on the vibration of the aromatic ring appearing at the wavenumber at 1600 cm^{-1}. Table 5 shows the band assignments for the FT-IR spectrum and the relative absorbance of bands for the CH_3 + CH_2 stretching vibrations at 2960 to 2925 cm^{-1} and the CH_3 + CH_2 deformation vibration at 1460 cm^{-1}, respectively.

Table 6 presents the actual and predicted intensities of relative absorbance for the CH_2 bands as obtained through RSM analysis. The actual values corresponded to the measured response data for a particular run, and predicted values were evaluated based on the fitted model. A reduced quadratic model was used to explain the mathematical relationship between the independent factors and the dependent response. In the first stage of analysis, the analysis results showed that the coefficient, B^2, was statistically insignificant. Therefore, it was deleted from the equation and added to the lack of fit. As a result, new regression models were obtained for both coded and actual factors (Eqs. 2 and 3, respectively):

$$\text{Intensity of relative absorbance for } CH_2 \text{ band} = 0.33 + 0.025A + 0.057B + 0.045AB - 0.047A^2 \quad (2)$$

$$\text{Intensity of relative absorbance for } CH_2 \text{ band} = 0.12598 + 0.035307\text{Glyoxal} - 0.013563\text{NaOH} + 0.0028125\text{GlyoxalNaOH} - 0.00290761\text{Glyoxal}^2 \quad (3)$$

Table 5. Band Assignments for FT-IR Spectra and Intensity of Relative Absorbance for CH_2 Bridge in Glyoxalated Lignin Samples

Band (cm^{-1})	Group	Runs												
		1	2	3	4	5	6	7	8	9	10	11	12	13
2960 to 2925	CH_3 and CH_2 stretching vibration	0.18	0.18	0.31	0.13	0.17	0.18	0.20	0.27	0.18	0.11	0.19	0.18	0.13
1460	CH_3 and CH_2 deformation vibration	0.46	0.32	0.57	0.31	0.49	0.52	0.42	0.25	0.28	0.37	0.53	0.64	0.39
Mean Value		0.32	0.25	0.44	0.22	0.33	0.35	0.31	0.26	0.23	0.24	0.36	0.41	0.26

Table 6. Actual and Predicted Intensity of Relative Absorbance for CH_2 Band

Run Order	Response		Internally Studentized Residual	Externally Studentized Residual
	Actual Value	Predicted Value		
1	0.23	0.25	-1.225	-1.271
2	0.26	0.28	-1.044	-1.051
3	0.41	0.41	-0.314	-0.295
4	0.32	0.33	-0.637	-0.611
5	0.22	0.21	0.824	0.806
6	0.33	0.33	-0.206	-0.193
7	0.24	0.21	1.856	2.300
8	0.35	0.33	0.656	0.630
9	0.25	0.28	-1.635	-1.875
10	0.26	0.25	0.534	0.509
11	0.31	0.33	-1.068	-1.078
12	0.44	0.42	1.247	1.300
13	0.36	0.33	1.086	1.101

The results of the analysis of variance (ANOVA) are shown in Table 7. The model and each term were significant at the p-value of 0.05. The equation was highly reliable, as reflected by the high coefficient of determination (R^2), 0.9164. The ANOVA results also revealed a statistically insignificant lack of fit ($p = 0.2776$) at the 5% level. Because this value was more than 0.05, the model was deemed statistically appropriate for further analysis. The effects of various factors on the intensity of relative absorbance for the CH_2 band are shown in Fig. 4. From the 3D graph, it can be observed that the intensity of relative absorbance for the CH_2 band reached a value of 0.42 when 0.222 mol of glyoxal and 0.353 mol of NaOH were used in the glyoxalation process.

Table 7. ANOVA for Response Surface Reduced Quadratic Model

Source	Sum of Squares	df	Mean Square	F Value	Prob > F
Model	0.054	4	0.014	21.93	0.0002
A-Glyoxal	4.830E-003	1	4.830E-003	7.79	0.0235
B-NaOH	0.026	1	0.026	42.13	0.0002
AB	8.100E-003	1	8.100E-003	13.07	0.0068
A^2	0.015	1	0.015	24.72	0.0011
Residual	4.957E-003	8	6.196E-004		
Lack of Fit	3.237E-003	4	8.093E-004	1.88	0.2776
Pure Error	1.720E-003	4	4.300E-004		
Cor Total	0.059	12			
R^2	0.9164		Adjusted R^2	0.8746	

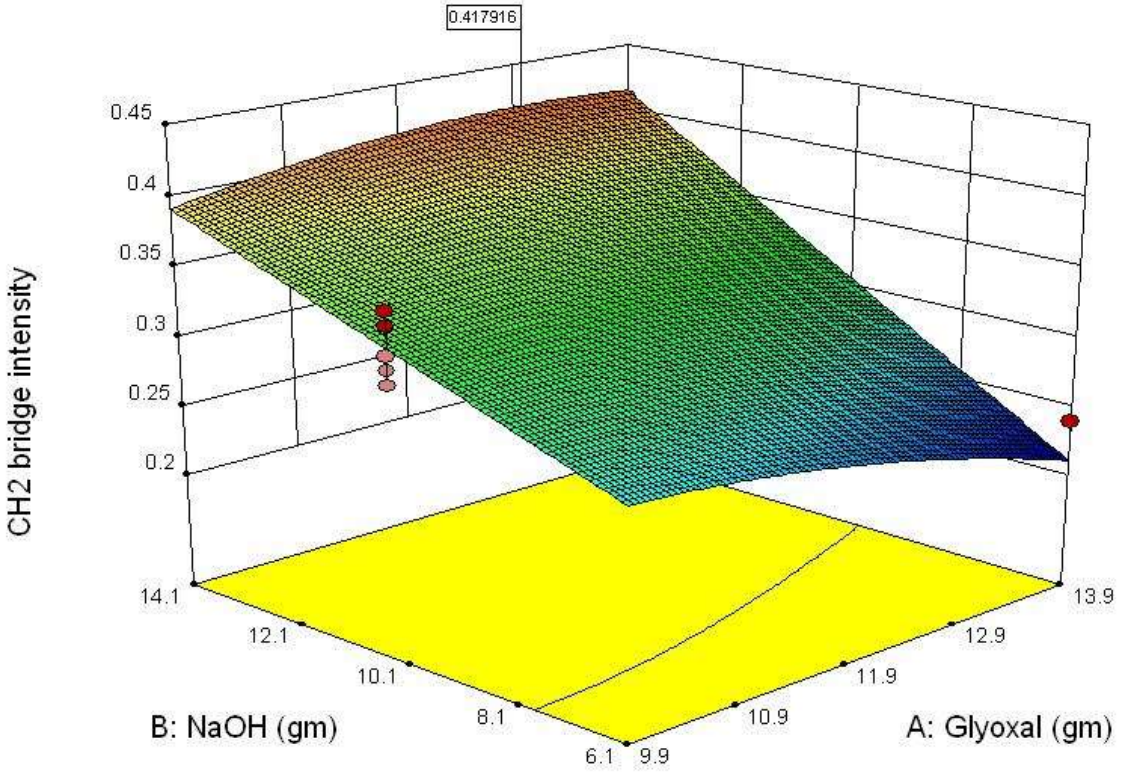

Fig. 4. 3D-surface plot of intensity of relative absorbance for CH_2 bridges in glyoxalated lignin as a function of weights of glyoxal and NaOH

To obtain a higher value of intensity of relative absorbance for the CH_2 band, higher amounts of glyoxal had to be used (between the centre and upper levels), and the amount of NaOH had to always be higher than the amount of glyoxal used, which kept the glyoxalation process in a more basic environment. These finding were in good agreement with previous studies (El Mansouri *et al.* 2007b), in which the authors found that when

higher amounts of glyoxal were used in the glyoxalation process, the adhesives produced were of higher joint strength compared to the adhesives produced with lower amounts of glyoxal. The introduction of the CH_2OH group into the lignin molecules was more favorable when the glyoxalation process was conducted under basic conditions rather than more acidic conditions (El Mansouri *et al.* 2011).

Adequacy of the Model

The fitted model needed to be assessed to ensure that it gave a sufficient approximation of the results obtained in the experimental conditions. The coefficient of multiple regressions, R^2, is a global statistical parameter used to assess the fit of a model (Myers and Montgomery 2002). In this model, R^2 was 0.9164, which indicated the good fitness of the model (Table 6). For further validation of the model, an adjusted R^2 was used for confirming the model adequacy. The adjusted R^2 was calculated to be 0.8746, which indicated a good model for use in field conditions. A residual analysis was also carried out for validating the model accuracy (Myers and Montgomery 2002). An identification of the outliers was performed by examining the internally Studentized residuals. The residuals should be approximately normal with mean zero and unit variance. To validate the model further and check the outliers, the r-Studentized values were calculated (Table 6). All the values were within -2 to +2 (values between -3 and +3 were the acceptable limit), thereby validating the model.

CONCLUSIONS

1. The glyoxalation process was successfully executed to enhance the reactivity of methanol-fractionated lignin model compounds.
2. Depolymerization of the lignin molecules was found to have occurred during the glyoxalation process, as the molecular weights of all glyoxalated lignin samples were lower than those of unmodified lignin (ML).
3. The higher polydispersity index (M_w/M_n) for all the glyoxalated lignins compared to ML revealed that the lignin may be degraded in non-uniform way during the glyoxalation process which yielded smaller and larger lignin fragments. Apart from this, lignin polymers of a variety of chain lengths were also generated by the condensation reaction.
4. Thermogravimetry analysis (TGA) confirmed that lower-molecular weight lignin polymers were formed in all glyoxalated lignin samples except the R2 (glyoxal: 0.102 mol, NaOH: 0.353 mol) and R4 (glyoxal: 0.073 mol, NaOH: 0.253 mol) samples. The lignin molecules that were not involved in the formation of the lignin polymers of lower molecular weights underwent repolymerization through the condensation reaction.
5. FT-IR spectroscopy revealed that the formation of lignin polymers of lower-molecular weight was attributed to the crosslinking of lignin molecules *via* methylene (CH_2) bridges.
6. RSM and CCD were successfully applied to ascertain the optimum amount of glyoxal and NaOH to be used in the glyoxalation of lignin for obtaining lignin with an appropriate level of reactivity. A reduced quadratic model, in terms of moles of glyoxal and NaOH, was developed. The experimental values were in good agreement with the

predicted ones, and the model was highly significant, having a coefficient of determination of 0.9164.

7. Values for the intensity of relative absorbance for the CH_2 bands (0.42) were obtained based on the optimum amounts of glyoxal and NaOH to be used, *i.e.*, 0.222 and 0.353 moles, respectively.

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