

**Studies of *Eucalyptus grandis* Lignin. Part II:  
High-Performance Size-Exclusion Chromatography of Milled  
Wood Lignin, Kraft and Organosolv Lignins**

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**Received: october 14, 1991.**

A distribuição de peso molecular da lignina no licor "kraft" durante o processo de deslignificação permite conhecer as melhores condições para processos de deslignificação. Para comprovar isso foram realizados quatro análises de cromatografia líquida de alta eficiência com lignina de madeira moída, com lignina "organosolv", e com ligninas "kraft", de um processo suave (número de kappa = 20) e de um processo mais agressivo (número de kappa = 15). Os pesos moleculares médios em peso ( $\bar{M}_w$ ) das quatro amostras decresceram na ordem indicada acima. Os conteúdos em lignina de Klason foram 7,6% (polpa "organosolv"), 3,1 e 2,0% (polpas "kraft") dos processos suave e agressivo, respectivamente. Estes resultados indicam que o  $\bar{M}_w$  pode ser usado como parâmetro do grau de deslignificação de polpas uma vez que ele decresce com o aumento da quantidade de lignina extraída na polpação. Além disso, o  $\bar{M}_w$  da lignina de madeira moída é por volta de 8.400 Dalton com exclusão de 8% da fração molecular de alto peso.

The molecular weight distribution (MWD) of lignin from pulping liquor allows to know the best conditions for delignification processes. To prove that, four lignins of *Eucalyptus grandis* wood: milled wood lignin (MWL), organosolv lignin (OEL), and kraft lignins obtained under mild conditions (target kappa number = 20, KL1) and under vigorous conditions (target kappa number = 15, KL2) were submitted to high performance size-exclusion chromatography (HPSEC) analysis. The weight-average molecular weights ( $\bar{M}_w$ ) of the four lignins decreased in the order: MWL > OEL > KL1 > KL2. The Klason lignin contents were 7.6% for organosolv pulp, and 3.1 and 2.0% for kraft pulps (mild and vigorous processes, respectively). These results indicate that the  $\bar{M}_w$  can be used as parameter for degree of delignification since it decreases with increasing amount of extracted lignin in pulping processes. Moreover, the  $\bar{M}_w$  of *Eucalyptus grandis* MWL is around 8,400 Dalton after exclusion of 8% of the high molecular weight fraction.

**Key words:** lignin; delignification.

## Introduction

The present work is part of our investigations on *Eucalyptus* lignin. In this paper we will present the results from MWD analysis of the lignin by HPSEC. In addition, the relationship between degree of delignification in sulphate and organosolv pulping and degradation of the lignin macromolecule has also been investigated. Because *Eucalyptus* lignin is sparingly soluble in THF, the samples were acetylated to alleviate this problem.

It was not possible to find in the literature anything about the molecular weight distribution of lignin macromolecule of *Eucalyptus* wood grown in tropical regions. Bland and Menshyn<sup>1</sup> had referred to the work of Yean, Rezanowich and Goring who determined the  $\bar{M}_w$  of *Eucalyptus regnans* lignin by the sedimentation equilibrium technique. Milled wood lignins from three successive extractions of this non-tropical wood yielded MWL's with  $\bar{M}_w$  values of 35,000; 52,000 and 90,000 Daltons. These results are far greater

than those usually mentioned in the literature<sup>2-8</sup> for MWL and kraft lignins, respectively.

## Materials and Methods

**HPSEC analysis:** the samples were injected in a Waters Associates HPSEC-Chromatograph, equipped with three PL-gel columns (1,000 Å, 5 µm; 500 Å, 10 µm; and 100 Å, 10 µm) connected in serie in order of increasing pores size. The effluent was monitored at 254 nm with an ultraviolet detector (Mod. 440/010 - Waters Associates). The columns were calibrated using polystyrene standards in the range of 106 to 68,000 Dalton. The flux of THF was 1ml/min and the loading was 5 mg/2ml. The data were processed in a Waters Associates Data Module.

**Milled wood lignin:** the MWL was extracted from wood according to the procedure of Bland Menshun adapted to tropical *Eucalyptus grandis*<sup>9</sup>.

**Pulping processes:** the mild pulping process was a modification kraft cooking to achieve a target kappa number near 20 (Klason lignin content ~ 3%); and the vigorous pulping conditions were obtained in a normal kraft cooking and led to a target kappa number near 15 (Klason lignin content ~ 2%). In the organosolv process a mixture of

acetic acid, ethyl acetate and water was employed. The ratio of solvent/wood was 10 (volume/dry mass) and the reactor was heated for 1 hour at 150°C.

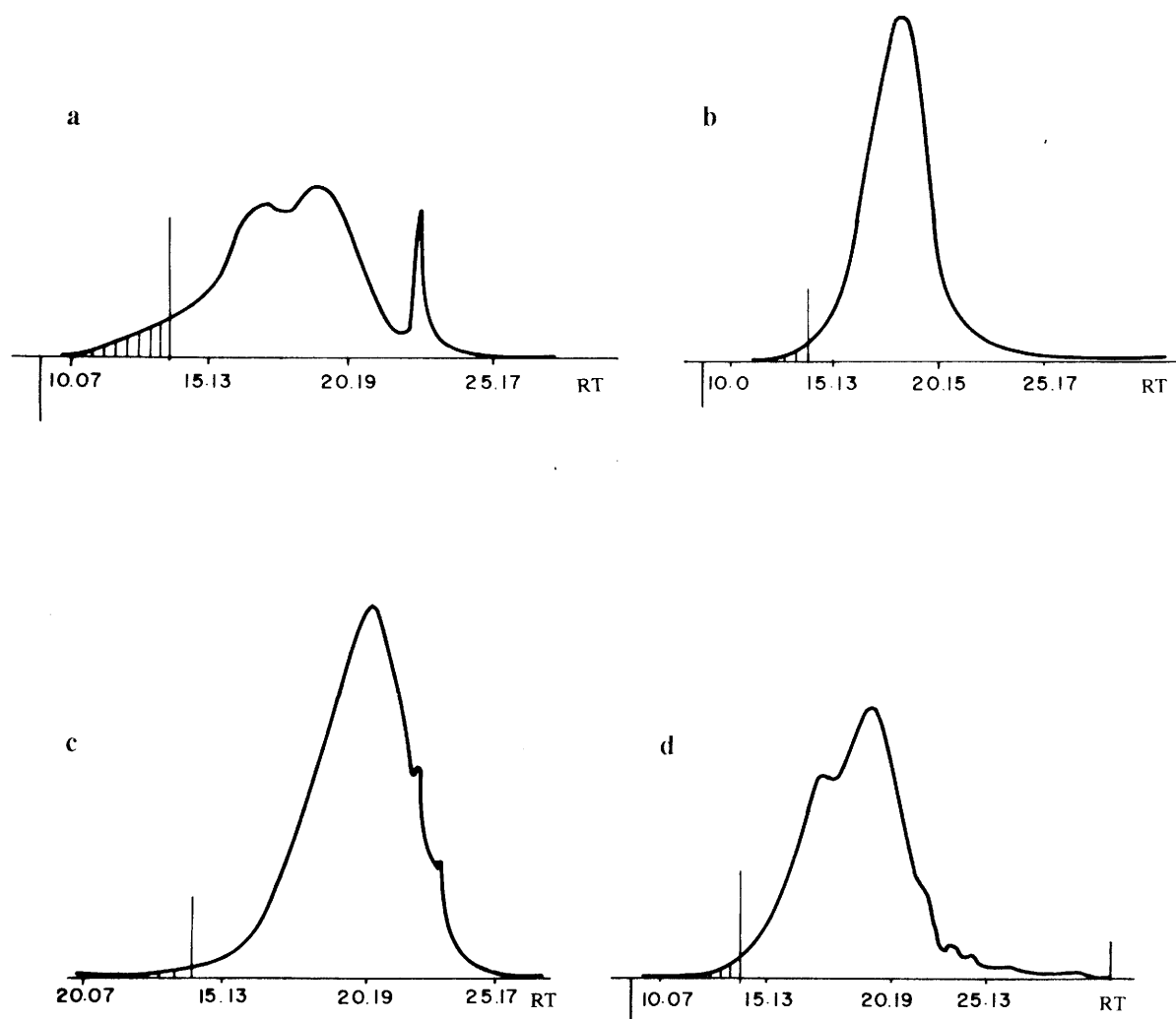
**Kraft lignins:** the kraft lignins were precipitated from liquor with concentrated HCl. The sulphur compounds were evaporated in the air under normal conditions and the residual lignin was washed with water until neutral pH was achieved.

**Organosolv lignin:** the organosolv lignin was obtained after removal of the extraction liquor and subsequent purification.

**Acetylation:** the lignins were acetylated with pyridin/acetic anhydride 1:1 in volume at room temperature for 24 hours. The derivative was precipitated with water, then it was separated by filtration and washed with water until free of pyridin and acetic acid.

## Results and Discussion

Figure 1 present the elution of the MWL, KL1, KL2 and OEL. Figure 2 presents the respective MWD. Here lignin fractions of low molecular weights and injections solvent were not taken into account. Furthermore, due to the low accuracy of polystyrene standards over 68,000 Dalton, the fraction of lignin macromolecules in this range was ex-



**Figure 1.** High-performance size exclusion chromatography of four acetylated lignins: a) MWL; b) KL1; c) KL2; and d) OEL.

cluded to estimate the apparent molecular weights.

From Figure 2 it is possible to follow pulping process qualitatively. The lignin macromolecule, which has a wide molecular weight distribution, is selectively degraded to smaller molecules during the pulping. With the more vigorous kraft treatment, the lignin was degraded into fractions whose  $\bar{M}_w$  is near to 3,000 Dalton, while with mild conditions the  $\bar{M}_w$  is near to 5,000 Dalton (Table 1).

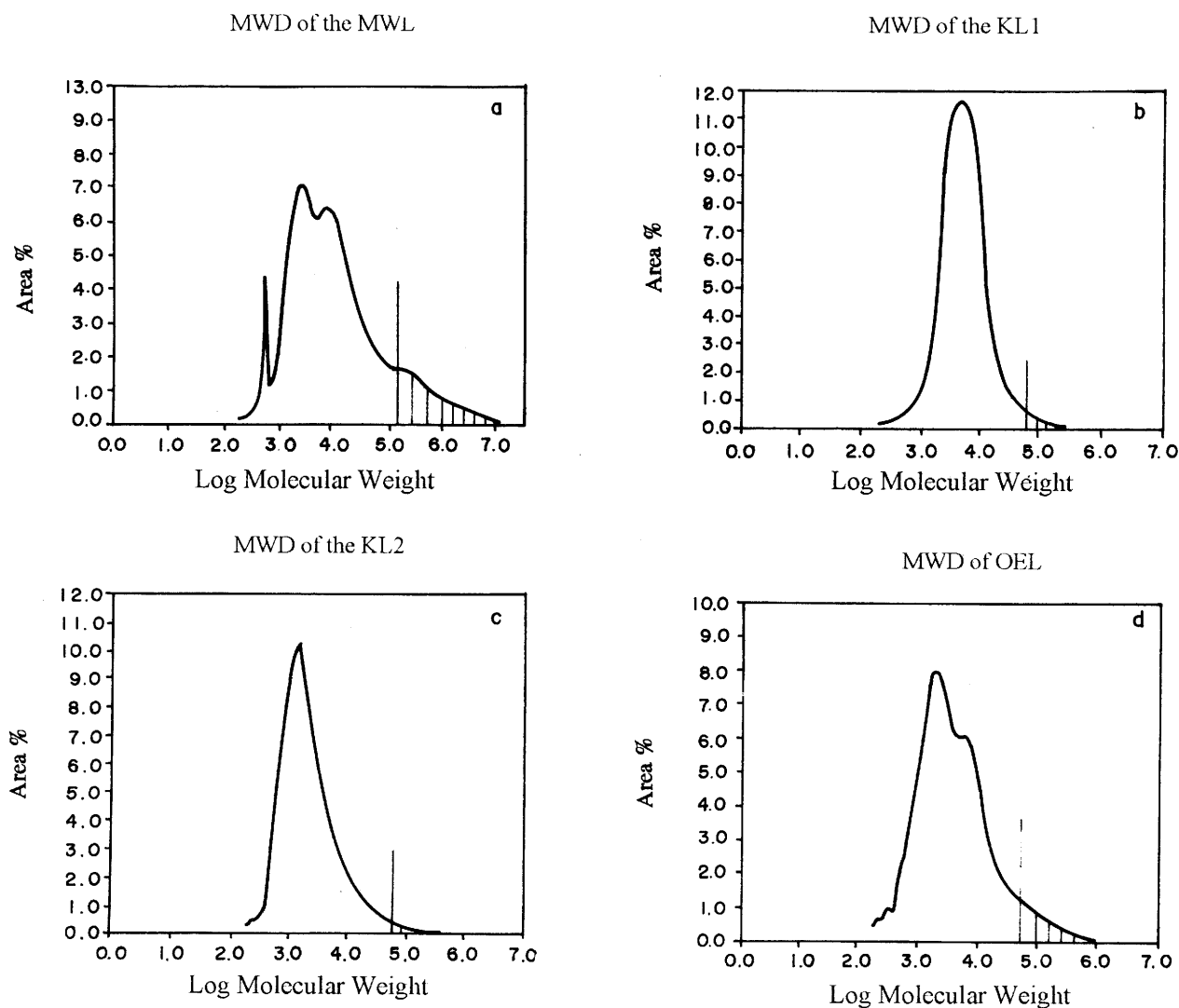
The MWD pattern of OEL shown in Figure 2d is similar to that of MWL (Fig. 2a), except the higher molecular weight part which was excluded. Both curves are wide and have two maxima. Evidently, the fraction of high molecular weight molecules has decreased in OEL. This means that the delignification by the organosolv method degraded the lignin but less effectively than the kraft processes.

This behavior of *Eucalyptus* lignin in pulping can also be followed by means of the weight-average molecular weights ( $\bar{M}_w$ ) of the samples. Table 1 shows the apparent average molecular weights and dispersivities for the various lignins. The  $\bar{M}_w$  for MWL is the greatest and that for KL2 from the vigorous kraft pulping is the lowest; lignin

from the organosolv process possesses a  $\bar{M}_w$  greater than that of KL1 obtained by the mild kraft pulping. It is important to emphasize that excluded macromolecular fraction for other lignins. This means that the  $\bar{M}_w$  of MWL is much larger than that shown in Table 1; and similarly, the  $\bar{M}_w$  of OEL might be also larger than that shown in the same table.

**Table 1.** Apparent average molecular weights (in Dalton) and dispersivities for the studied lignins.

Lignin	area % of			
	macromolecules excluded	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
MWL	8.0	8,400	1,960	4.3
OEL	2.5	5,600	1,523	3.7
KL1	0.7	4,980	1,193	3.7
KL2	0.5	2,865	1,311	2.2



**Figure 2.** Molecular weight distribution of the four lignins shown in Fig. 1.

The course of delignification could also be followed by means of the dispersivities ( $\overline{M}_w / \overline{M}_n$ ) but in the present case the accuracy is not as good as in the case of  $\overline{M}_w$  because the effect of the excluded macromolecular fractions is more pronounced. However, for the kraft lignins, which have similar exclusion fraction, the dispersivities indicate great degradation from mild to more vigorous pulping as do the  $\overline{M}_w$ .

Definitively, the number-average molecular weight ( $M_n$ ) can not be used to follow delignification during pulping. It is apparent from Table 1 that  $M_n$  of lignins does not decrease significantly with their degradation. Moreover, the  $M_n$  of KL1 is smaller than that of KL2. Perhaps, in this case, the small difference between the excluded macromolecular fractions is decisive.

The MWD profile presented by the *Eucalyptus grandis* lignin shows a accentuated predominancy of high molecular fraction and differs from others milled wood lignins<sup>4,8</sup>. Unfortunately, the calibration with polystyrene standards is not enough accurate over 68,000 Dalton to allow a precise  $\overline{M}_w$  determination. Certainly the use of universal calibration by means of on-line viscosimetric detectors will provide best MWD for *Eucalyptus grandis* lignin. However, the results obtained in this work are sufficient to characterize this lignin as a very condensed one, as sedimentation equilibrium studies had indicated for *Eucalyptus regnans* lignin.

It is well established that *Eucalyptus* lignin is easily solublized into cooking liquor during the kraft pulping. Thus, it is likely that the constituents of high molecular weight fraction of the lignin do not contribute significantly to condensation with carbohydrates either in wood and during the pulping.

### Conclusions

The HPSEC is a good tool to determine the endpoint of pulping.  $\overline{M}_w$  around 4,000 Dalton corresponds to low residual lignin contents in the pulp from tropic *Eucalyptus*.

*Eucalyptus grandis* MWL presents a molecular weight distribution shifted to large macromolecules in agreement with results obtained for *Eucalyptus regnans* lignin by sedimentation equilibrium.

### Aknowledgments

The authors are grateful to FINEP, CNPq and FAPEMIG for financial supports and Aracruz Celuloses S.A. for the modified kraft lignin sample; to Physical Organic Chemistry Group, IFQSC-USP, for HPSEC measurements; to Dr. Chen-Loung Chen for helpful discussions, and suggestions.

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