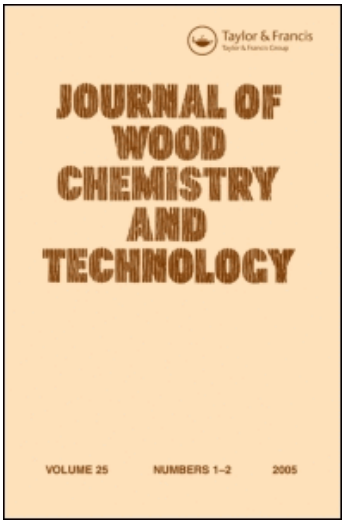

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DETERMINATION OF THE MOLECULAR WEIGHT DISTRIBUTIONS OF XYLANASE-TREATED PULPS

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ABSTRACT

The molecular weight distributions (MWD) of unbleached kraft pulps of Pinus radiata and Populus tremuloides were determined by size exclusion chromatography (SEC) of the carbanilated derivatives. The applicability of this method for the study of the effects of xylanase treatments on these substrates is discussed.

INTRODUCTION

Biobleaching is being studied on several types of wood pulp with xylanases from a variety of sources.[1-8] A prerequisite for this operation is for the xylanase preparation to be free of contamination by cellulases, or the cellulases to be inactivated by inhibitors. The effects of these treatments are normally judged by carbohydrate analysis of substrate and sugars released, measurement of pulp viscosity, kappa number, and handsheet strength properties. A method of determining the effects of purified enzymes on pulps at a small scale and on the integrity of the cellulose component would be beneficial. Size exclusion chromatography (SEC) of the tricarbanilate derivative of cellulose (CTC) has been used to characterize various types of pulps.[9-13] Applicability of this method was examined for the study of the effects of xylanase treatments on the molecular weight distributions (MWD) of hardwood and softwood kraft pulps.

EXPERIMENTAL

Unbleached radiata pine kraft pulp (cellulose 83.4%, xylan 7.45%, mannan 6.89%, lignin 3.16%) was a gift from Dr. Tom Clarke (FRI, NZ), and the unbleached aspen kraft pulp (cellulose 73.39%, hemicellulose 19.32%, lignin 2.33%) was obtained from Weyerhaeuser (Prince Albert, Saskatchewan). A sample (150 g O.D.) of each pulp was treated at 4% (w/v) consistency in 50 mM sodium acetate buffer, pH 4.8, with bulk xylanase at a loading of 500 IU/g O.D. pulp. Xylanase activity was 2270 IU/mL. Carboxymethylcellulase activity was 0.307 IU/mL. The pulps were treated for 24 h at 40°C, filtered and washed with distilled water (5 L).

The carbanilation procedure was similar to that used by Schroeder and Haigh[9], but was done at a microscale. The cellulosic substrates (10 mg) were dried in a vacuum desiccator over P_2O_5. Anhydrous pyridine (3 mL) and phenylisocyanate (200 μL) were added and the mixture was heated at 80°C in 5 mL reacti-vials on a reacti-therm block heater. Aliquots (0.2 mL) were removed after different reaction times, evaporated to dryness, and the resulting residues were then dissolved in tetrahydrofuran (THF) to give 0.02 - 0.03% (w/v) solutions.

Size exclusion chromatography was performed on a series of four Ultrastyragel columns, 10^4, 10^5, linear, 10^6, held at 35°C, with THF (1 mL min^{-1}) as the eluting solvent. A UV detector was used at a wavelength of 278 nm. Universal calibration was used with narrow polystyrene standards and the Mark-Houwink coefficients published by Wood et al.[14]

RESULTS AND DISCUSSION

Reproducibility of Method

Sample-to-sample reproducibility of the analysis by SEC was examined using softwood pulp samples (Table 1). Reproducibility of the technique is good with standard deviations for the means for sets of six results (3 replicates, duplicate injections) for both number-average (DP_N) and weight-average (DP_W) degree of polymerization varying between 2.6 and 1.6% of the means.

TABLE 1

Molecular Weight Distribution Data for Carbanilated Radiata Pine Kraft Pulp

Sample[a]	DP_W	DP_N	Poly-dispersity	% < MW 100,000
	Ave. ± σ_{n-1}	Ave. ± σ_{n-1}		Ave. ± σ_{n-1}
Control	1750 ± 44	268 ± 7	6.5	16.4 ± 0.4
Enzyme treated	1733 ± 28	230 ± 5	7.5	18.7 ± 0.4

[a]After derivatization for 48 h.

Determination of MWD for Xylanase-treated Hardwood Pulps

The effect of xylanase treatment on the residual xylan in samples of two aspen kraft pulps can readily be observed (Figure 1, Table 2). The peak in the chromatogram assigned to hemicellulose is shifted towards lower molecular weight and the proportion of the distribution below molecular weight 70,000 is reduced. The observed changes are consistent with hydrolysis and partial solubilization of xylan.

Figure 2 shows the effect of reaction time of the derivatization on the MWD. With longer reaction time, the area of the low molecular weight portion decreases and the whole distribution shifts towards higher molecular weights. Prolonged reaction time, however, can cause degradation of the carbanilate derivative resulting in a decrease in the values of molecular weight data. Ideally, MWD data should be determined at reaction times corresponding to completion of derivatization and before degradation of the sample occurs. Inspection of Figure 2, shows that distributions obtained after reaction for 43 and 49 h are very similar in the case of the untreated pulp, but quite different for the enzyme-treated substrate. This is reflected in the calculated values for DP_N and DP_W (Table 2). These results indicate that the degree of completion of the carbanilate reaction at a given reaction time may differ between substrates. The derivatization reaction is taken to be complete when the high

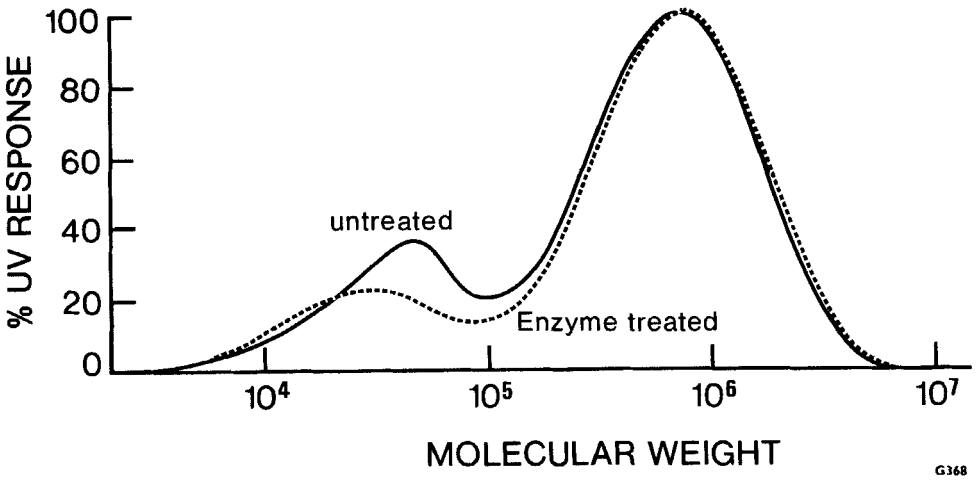

FIGURE 1. Effect of xylanase treatment on the MWD of aspen Kraft pulp (sample A, 49 hour reaction time).

TABLE 2

Molecular Weight Distribution Data for Carbanilated Aspen Kraft Pulp

Sample (h)	Reaction Time	DP_W	DP_N	Polydispersity	% < MW 70,000
Pulp A					
Control	25	808	164	4.9	20
	43	1060	217	4.9	15
	49	1060	205	5.2	17
Enzyme treated	25	870	158	5.5	28
	43	1070	187	5.7	16
	49	1160	201	5.8	14
Pulp B					
Control	49	1440	216	6.7	16
Enzyme treated	49	1590	198	8.0	15

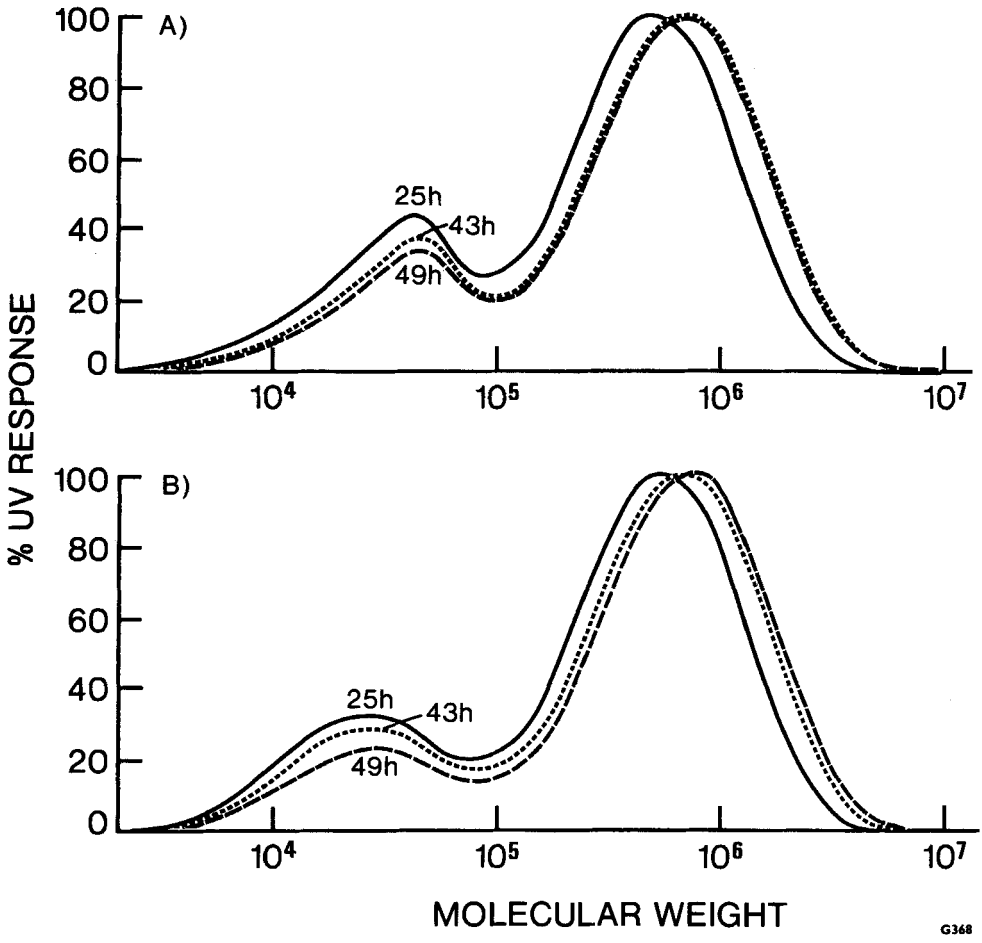

FIGURE 2. MWD of aspen Kraft pulps at 25, 43, and 49 hours carbanilation reaction time: A. untreated control B. xylanase treated.

molecular weight portion of the distribution is at its maximum value since prolonged reaction times cause degradation of the CTC. In the low molecular weight region the results indicate a difference in reactivity of the contributing component polymers or a difference in the stability of their carbanilate derivatives as compared to the high molecular weight cellulose. Comparison of MWD curves of carbanilated samples at any given reaction time without knowledge of the degree of completion of the derivatization may lead to erroneous interpretations.

Determination of MWD for Xylanase-treated Softwood Pulps

The MWD curves obtained for enzyme-treated radiata pine kraft pulp show an increase in the low molecular weight portion of the distribution (Figure 3). This is reflected in a decrease in DP_N and an increase in the proportion of the distribution below molecular weight 100,000 (Table 1). This effect was consistently observed in all softwood samples studied. This observed increase in the low molecular weight portion after enzyme treatment of the softwood pulp may be explainable in two ways. As a result of the enzyme action, either more sites are available for derivatization and therefore more material is detected in this region, or, material previously left in the reaction residue is now soluble with or without derivatization.

The problem of variation in the MWD with reaction time observed for aspen substrates was observed again with softwood pulps. The distribution for the enzyme-treated substrate changed significantly in the low molecular weight region between reaction times of 40 and 48 h, but no corresponding change was observed in the control samples (Figure 4). As for the aspen pulps this may reflect degradation of the low molecular portion.

Precipitation is frequently used as a method of purifying the CTC in MWD determination of the cellulose. This method of preparation of the derivative was examined for the softwood pulp, using water - methanol (30:70) as the precipitation solvent.[15] Figure 3 shows the effect of precipitation on the MWD curve. As previously observed in analysis of chemical pulps[14] low molecular weight material is lost. In the case of the xylanase treatment the effect on the substrate is no longer readily apparent.

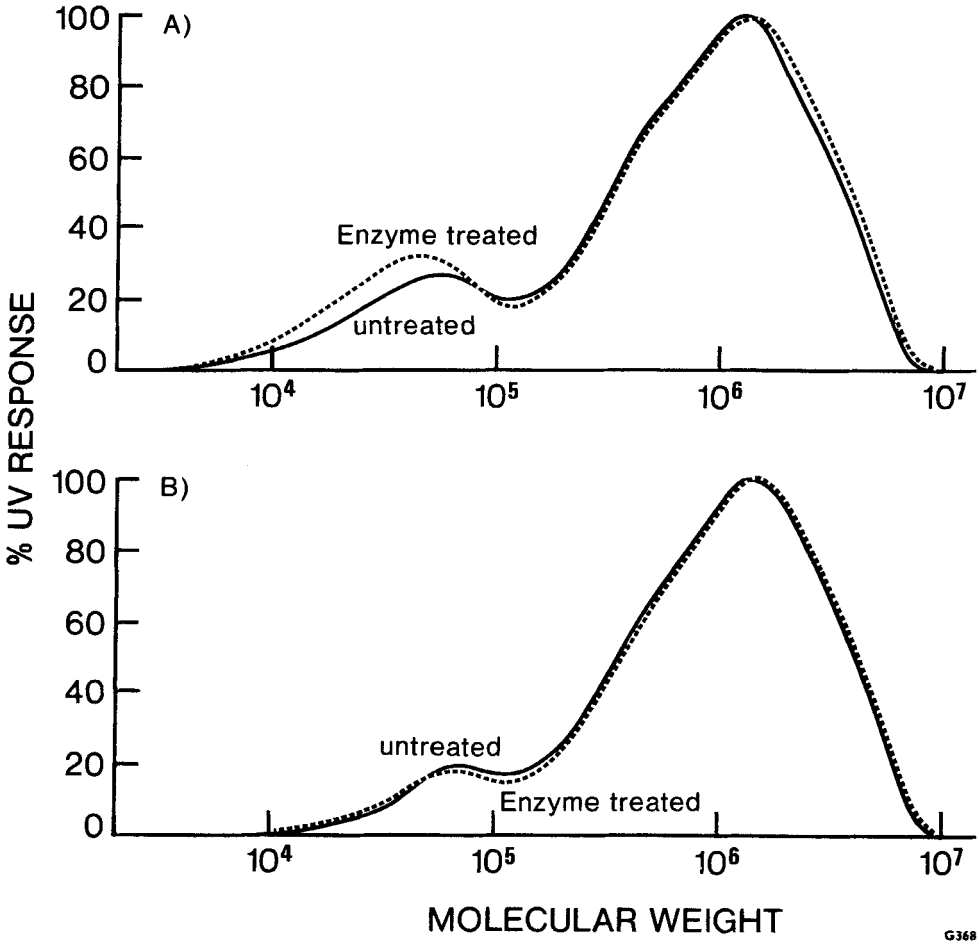

FIGURE 3. MWD of untreated and xylanase-treated radiata pine Kraft pulps: A. unprecipitated carbanilate derivative B. precipitated carbanilate derivative.

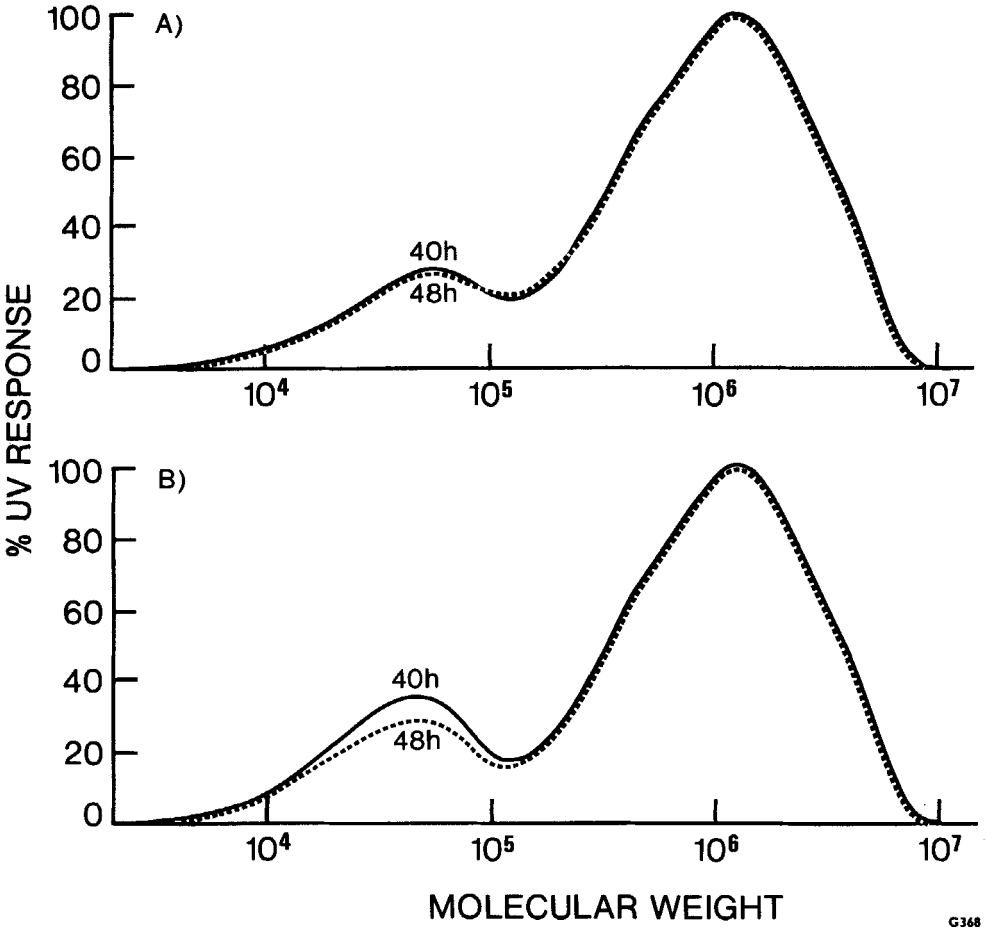

FIGURE 4. MWD of radiata pine Kraft pulp at 40 and 48 hours carbanilation reaction time: A. untreated control B. xylanase-treated.

Validity of Method

Determination of MWD by SEC relies on the ability to relate retention time to molecular weight, and detector response to the concentration of eluting polymer. The validity of universal calibration using polystyrene standards for the determination of the molecular weight of CTC has been demonstrated.[16] Hardwood xylan is reported to have the same relationship between intrinsic viscosity and molecular weight as cellulose.[17] However, the accuracy of calibration for CTC when applied to the determination of the molecular weight of xylan dicarbanilate is unknown. The values determined by this calibration are therefore CTC equivalents, and not true values for the DP of the xylan component. Additionally, the number of chromophores per unit mass of carbanilated xylan is 6% less than for CTC, and therefore detector response is similarly reduced. With these inaccuracies in calibration and detector response for the xylan component, the values obtained from SEC for the MWD data for pulps containing xylan will not accurately reflect their absolute molecular weight. Values for DP_N will be especially affected by these inaccuracies.

Reproducibility of the technique will allow the effects of enzyme-action, i.e xylan removal and the integrity of the cellulose component, to be indicated with reasonable accuracy; with the proviso that complications resulting from the presence of other hemicelluloses, lignin, or degraded cellulose of low molecular weight can be taken in to account.

CONCLUSIONS

Determination of MWD of pulps by SEC analysis of the carbanilate derivative gives reproducible results. The relative effect of xylanase treatments can be obtained with this method. These results can be interpreted in the case of hardwood pulps in which xylan is the only major hemicellulose and there are less interferences from other components. Care must be exercised in the interpretation of results due to changes in the measured MWD with reaction time and to interferences especially in softwood pulps. Precipitation of the carbanilate derivative is undesirable if the low molecular weight portion of the distribution is of interest.

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