Mass Loss of Wood and Its Components During Transmission Electron Microscopy

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Synopsis

The loss of mass of wood and its polymeric constituents in transmission electron microscopy has been determined by measurement of the decrease in the continuum x-ray intensity for various doses of irradiation. It was found that for doses higher than 5×10^{-8} C/μm^2, 42% of the original mass of the wood remained on the grid. The corresponding percentages for cellulose, xylan, and lignin were 32, 45, and 70, respectively. The significance of these results in the use of transmission electron microscopy for imaging and for quantitative microbeam analysis is discussed.

INTRODUCTION

When a sample of biological material is under observation in a transmission electron microscopy (TEM), it is severely damaged by electron irradiation.[1] The most important result of this damage is a rapid loss of mass, which can range from 10 to 90% depending on the chemical composition of the specimen.[2-4]

Wood is a composite of three biological macromolecules, and the extent of its damage during electron microscopy is not known. The goal of the work described in the present paper is to quantify the damage by measuring the decrease in weight of the whole wood as well as of its three major constituents when they are in a TEM for imaging or for microbeam analysis. This information is important for the interpretation of the photomicrographs obtained as well as for quantitative elemental analysis. In the present study the technique used was the change in continuum x-ray intensity obtained by energy dispersive analysis of x-rays during electron irradiation of the specimens. This continuum intensity is proportional to the local specimen mass per unit area.[5]

EXPERIMENTAL

Principle of the Mass Loss Measurement

As electrons pass through a specimen, characeristic x-rays are emitted corresponding to the different elements. In addition, a continuum of x radiation (also called bremsstrahlung, or "white" radiation) is generated. The decrease in intensity of this x-ray continuum while the electronic irradiation proceeds corresponds to the change in the specimen mass per unit area.[5]

Journal of Applied Polymer Science, Vol. 31, 957–963 (1986)
 CCC 0021-8995/86/040957-07$04.00

Thus the mass loss of a speicmen may be measured by use of an x-ray spectrometer on a transmission electron microscope.

Suppose that after a total electron irradiation dose D the intensity of the x-ray continuum generated by the sample is I_c. By extrapolation of the curve obtained by plotting I_{c0} against D to D = 0, the intensity I_{C0} corresponding to zero dose may be obtained.

If M represents the total mass of the sample after an irradiation dose D, then

$$I_c = kM \tag{1}$$

If M_0 represents the total mass of the sample before irradiation, then

$$I_{c0} = kM_0 \tag{2}$$

From equations (1) and (2),

$$\frac{I_c}{I_{c0}} = \frac{M}{M_0} \tag{3}$$

which gives the proportion of the mass remaining after an irradiation dose D.

Experimental Conditions Used

A Philips EM400T electron microscope was used with an EDAX 9100/60 analyzer, a liquid nitrogen anticontamination trap, and a beryllium specimen holder to reduce the background noise. The microscope was operated at an accelerating voltage of 100 kV. The tilt angle of the specimen holder toward the detector was 21°. The total intensity of the continuum x radiation was measured from 2 to 20 keV by a 100-s analysis (living time). The characteristic peaks present in the spectra were avoided by using appropriate windows.

The diameter of the spot corresponding to the area of the specimen analyzed was from 40 to 150 μm. It was not possible to use spots much smaller than this. At the low beam currents required for the initial stages of mass loss ($\leq 10^{-13}$ A/μm^2), the signal-to-noise ratio would be too small for accurate measurement. Therefore, mass loss measurements on the individual morphological elements of the cell, such as the cell corner area or the secondary wall, were not possible.

The continuum x-ray intensity due to the instrumental environment and the supporting film was determined for each series of measurements and subtracted from the total continuum intensity in order to obtain the intensity I_c coming from the sample itself. Values of I_c ranged from 5000 counts to 50,000 counts. The reproducibility of I_c was on average 500 counts.

The electron beam current was measured using the exposure meter connected to the phosphor screen. The reading of the exposure meter was converted to the screen current by means of a conversion chart supplied by Phillips.

Electron beam currents from 10^{-14} to 10^{-13} A/μm^2 were used for analysis. The range of beam currents for irradiation was from 10^{-13} to 10^{-11} A/μm^2.

Specimen Preparation

Wood

Small pieces of black spruce wood were soaked in water and pressed in the radial direction at about 260° K in the wet condition. The purpose of this treatment was to compact the wood and close the lumina.[6,7] The pressed wood was embedded in methyl methacrylate resin and sectioned with a Porter Blum MT-2 ultramicrotome. Sections of 150–500 nm thickness were obtained and deposited on nylon grids. After removal of the resin with acetone, they were lightly coated with carbon to suppress charge effects. The carbon film was 3–5 nm thick.

Lignin

Periodate lignin[8] was used. After wet pressing at a tempereature of 260° K, the block was sufficiently compact to allow direct sectioning without any embedding material. Sections of about 200-nm thickness were deposited on a carbon-coated beryllium grid and lightly coated with carbon.

Xylan

Xylan was extracted from white birch wood with a solution of NaOH in DMSO.[9] A drop of a solution of xylan in DMSO was deposited and dried on a carbon-coated beryllium grid. The film thus formed was then lightly coated with carbon.

Cellulose

Because of its ease of preparation, cellulose from *Valonia ventricosa* was used instead of cellulose from wood. Samples were purified successively in aqueous sodium hydroxide, distilled water, hydrochloric acid, and distilled water, as described elsewhere.[10] By careful delamination of the cell wall, two to three lamellae were deposited on a carbon-coated beryllium grid and lightly coated with carbon.

RESULTS AND DISCUSSION

Figure 1 shows the mass loss of the whole wood plotted against the dose for doses smaller than 10^{-9} C/μm^2. The trend is linear, and extrapolation to the original mass of the sample is precise. The vertical dashed line represents the dose at which the crystallinity of the cellulose as detected by electron diffraction disappears and corresponds to a total dose of about 2×10^{-11} C/μm^2. The mass loss at this level of irradiation is negligible.

In Figure 2, the mass loss is plotted against the dose for much higher levels of irradiation. The decrease of the mass is quite rapid until it reaches

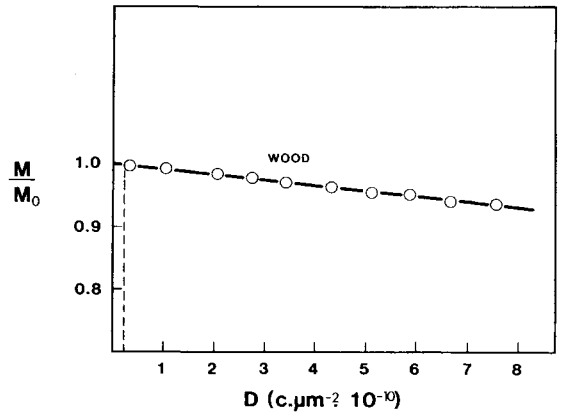

Fig. 1. Mass loss of black spruce wood due to an irradiation with 100 keV electrons at room temperature. The thickness of the sample is ≤ 500 nm.

an approximately constant value for doses higher than 5×10^{-8} C/μm^2. The percentage of mass remaining in the whole wood at this level of irradiation is only 42%, as shown in Table I.

When the electron beam current (flux of the electrons) was varied, essentially no change was found in the curve of mass loss versus dose. This is in agreement with the results of other workers.[4,11-13] Furthermore, no change was noted when the thickness of the sample was varied from 150 to 500 nm. Thus, the loss of mass in a TEM operated at 100 kV is independent of the dose rate and of the specimen thickness up to 500 nm.

Figure 3 shows a series of curves representing the mass loss of wood, cellulose from *V. Ventricosa*, periodate lignin, and xylan. The curves are similar in shape, decreasing to a constant value of mass loss after a dose of about 5×10^{-8} C/μm^2. However, as shown in Table I, this constant value is different for each type of sample. The proportion of mass remaining after long irradiation is 32% for cellulose, 45% for xylan, and 70% for periodate lignin. By assuming that these three samples are representative of the cellulose, hemicellulose, and lignin in black spruce wood in the proportions 50:25:25, respectively, it is possible to calculate the mass loss of

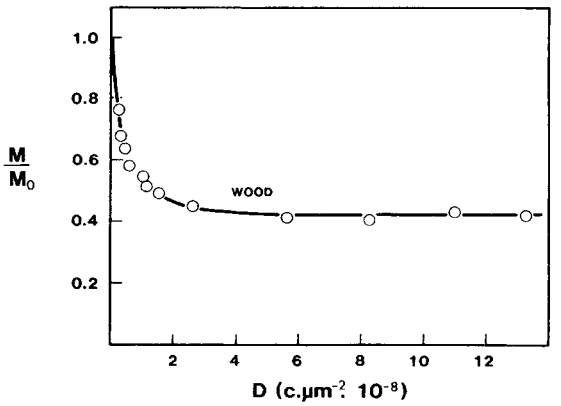

Fig. 2. Same as Figure 1 but for higher electron irradiation doses.

TABLE I
Mass Loss of Wood and Its Components After an Irradiation[a]

Specimen	M/M_0[b]
Cellulose (*Valonia*)	0.32
Xylan	0.45
Periodate lignin	0.70
Wood (black spruce)	0.42

[a] Dose $\geq 10^{-7}$ Cμm^2 with 100 keV electrons at room temperature. The thickness of the sample is $\leq$500 nm.

[b] M/M_0 = Proportion of mass remaining after an irradiation dose $D \geq 10^{-7}$ C/μm^2.

the whole wood. The value thus obtained is 44.7%, which is in good agreement with our result obtained by direct measurement, that is 42%.

The lower mass loss for lignin is not surprising. It has already been established that aromatic compounds are less sensitive to irradiation damage than linear polysaccharides, because the energy is spread over the whole benzene ring.[2,4] The reason for the higher resistance of xylan compared with cellulose is not known. It may be because xylan is a branched polymer and cellulose is linear. It should be noted that the mass loss in wood cellulose would be expected to be greater than that from *Valonia* cellulose, because, as observed in our labortory, the crystal structure of *Valonia* cellulose is more stable than that of wood cellulose in the electron beam.

The difference in mass loss between lignin and the carbohydrates shown in Table 1 will lead to differences in contrast between the TEM images of the middle lamella and the secondary wall of the cell. For low doses of iradiation, the diffraction contrast effect due to the cellulose crystallinity[14] will also affect the contrast. This behavior is illustrated in Figure 4. Figure 4a is an image of a cross section of wood at a dose of about 0.5×10^{-11} C/μm^2 using a conventional objective aperture. The diffraction contrast effect causes the crystalline cellulose to produce a darker image of the secondary wall. For much higher doses, as in Figure 4b, the crystallinity of the cellulose is destroyed and mass loss will occur to constant values. Based on the composition of the secondary wall and middle lamella found

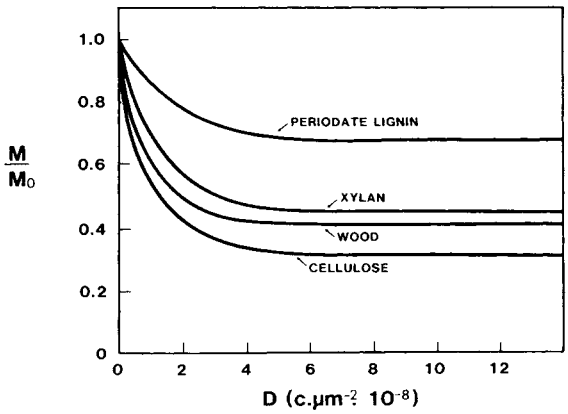

Fig. 3. Mass loss of black spruce wood and its components due to an irradiation with 100 keV electrons at room temperature. The thickness of the samples is $\leq$500 nm.

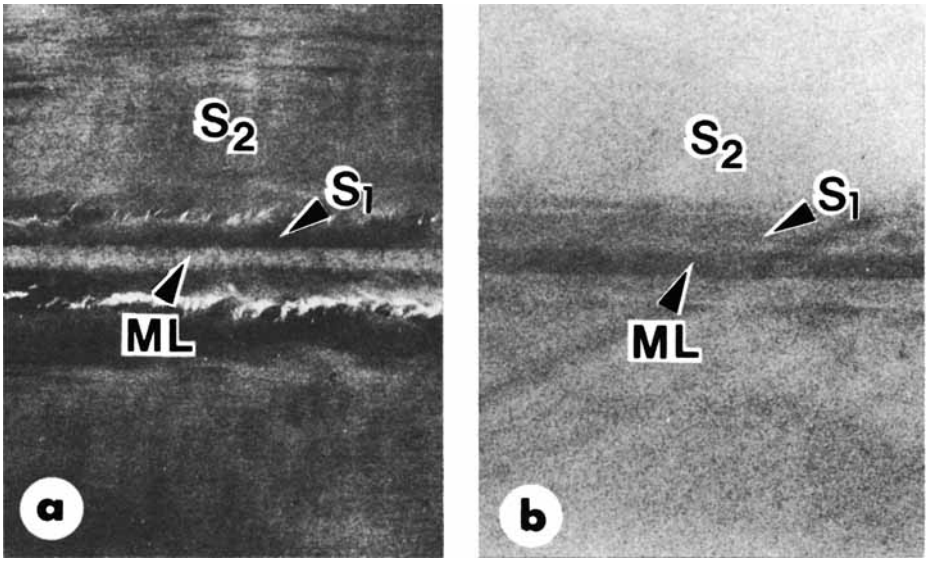

Fig. 4. Electron micrographs of 50-nm black spruce wood transverse sections taken with 100 keV electrons at room temperature. (a) the total irradiation dose was 0.5×10^{-11} C/μm2 (b) the total irradiation dose was $\geqslant 10^{-7}$ C/μm^2.

by Fergus et al[15] and as discussed by Rydholm,[16] about 44% of the secondary wall and 65% of the middle lamella will remain after such a dose. This is illustrated by the markedly darker image of the middle lamella in Figure 4b. Thus a more accurate interpretation of the images becomes possible if mass loss is taken into account.

Several papers have recently been published from this laboratory[17-20] and elsewhere[21-26] in which the microscopic distribution of certain elements in wood has been measured by electron microscopy coupled with an energy-dispersive x-ray analyzer (SEM-EDXA or TEM-EDXA). In some cases, the sensitivity of the signal to the time of irradiation was measured and no change in intensity was found.[18,23] However, in general, irradiation greater than 10^{-7} C/μm^2 is required for quantitative microbeam analysis. For such high doses, it is likely that mass loss will occur even before the first measurement can be made. All observations will correspond, therefore, to the level-off intensities shown in Figures 2 and 3. Clearly, such effects should be taken into account in the interpretation of quantitative results.

Unfortunately, with the sensitivity available at present, it is difficult to make measurement of initial mass loss (as shown in Fig. 1) with a spot size less than 40 μm in diameter. This resolution is several orders of magnitude too coarse to allow measurements in morphological regions in wood, such as the middle lamella or the torus. No doubt the loss in weight could be reduced by keeping the sample at low temperatures in a cooling stage.[5,27] However, even at very low temperatures, such as that of liquid helium, some damage is still present.[28,29], It seems likely, therefore, that, in the high-resolution microbeam analysis of woody material, the effects of mass loss will be elucidated only when the sensitivity of the method has been improved considerably. In the meantime, caution should be exercised in the interpretation of the results obtained.

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Received May 5, 1985
Accepted July 5, 1985