Effect of Accelerating Potential on the Determination of Structural Parameters of Cellulose by Electron Diffraction Technique

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Synopsis

An accelerating potential of 75 kV combined with very low beam current and exposure time of 5 sec seem ideal for recording electron diffraction pattern of cooled specimens of cellulose microfibrils. Over 16 reflections could be seen distinctly. The structural parameters of ordered regions were: 89% crystallinity index, 72 Å crystallite width, and 209–230 Å crystallite length. Even the diffraction pattern of the uncooled specimen at 75 kV had a large number of reflections and was superior to those recorded on the cooled specimen at 50 or 100 kV.

INTRODUCTION

Electron diffraction has not been routinely used as yet as has been x-ray diffraction to elucidate the fine structure of cellulose and modified cellulose in spite of certain advantages. The major constraint has been the destructive effect of the electron beam on the specimen. Specimen thickness, accelerating potential, beam density, specimen cooling, and exposure time are the main factors that need to be standardized and controlled to minimize this degradation. Skilful use of ultrasonicator or high-speed microhomogenizer can give desired specimen thickness, and liquid nitrogen can be used to cool the specimen. In our earlier publication,[1] we have described the technique to record the diffraction pattern within 3–5 sec exposure time using extremely low beam current, unlike previous workers who had adopted 30–50 sec exposure time. This leaves only one factor that needs to be examined in depth, viz., the accelerating potential.

Ever since Honjo and Watanabe[2] demonstrated some 400 reflections in the cooled specimen of *Valonia* microfibril and advocated the use of accelerated voltage at 100 kV in preference to lower accelerating voltage of 50 kV, or 25 kV used earlier by Preston and Ripley,[3] later investigators[4–7] have generally used an accelerating voltage of 100 kV. Even Dobb and Murray,[7] who had otherwise analyzed all aspects, theoretical and experimental, to obtain meaningful high-resolution electron diffraction diagrams of ramie cellulose, did not critically investigate the effect of accelerating potential. This paper describes how the proper choice of accelerating voltage further improves the quality of diffraction pattern.

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EXPERIMENTAL

The experimental procedure was largely the same as that described in detail in the earlier publication,[1] except for the varying accelerating potential.

The diffraction patterns were recorded on thin and discrete microfibrillar bundles both in the cooled and uncooled state at the accelerating voltages of 50, 75, and 100 kV using extremely low beam current. Liquid nitrogen was used to cool the specimen, and the diffraction pattern of a selected area formed in the back focal plane of the objective lens was recorded at an exposure time of 5 sec using a Hitachi HU 11-E electron microscope. The electron diffraction patterns thus obtained were scanned along the equatorial and meridional directions using a sensitive microphotometer. The intensity distribution obtained in the two cases was corrected for background scattering by the standard baseline technique.[8]

Degree of crystallinity was determined adopting Segal's[9] formula

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100$$

where CrI is the crystallinity index %, and I_{002} and I_{am} represent intensity in arbitrary units of the 002 interference peak and amorphous scatter, respectively. In the intensity tracings obtained from electron diffraction patterns, the intensity at $\sin \theta/\lambda = 0.126$ represented I_{002} and the intensity at $\sin \theta/\lambda = 0.1054$ represented I_{am}.

The lateral crystallite dimensions were estimated from the width at half-maximum intensity of the recorded 002 interference by the Scherrer[10] line-broadening relationship, viz.,

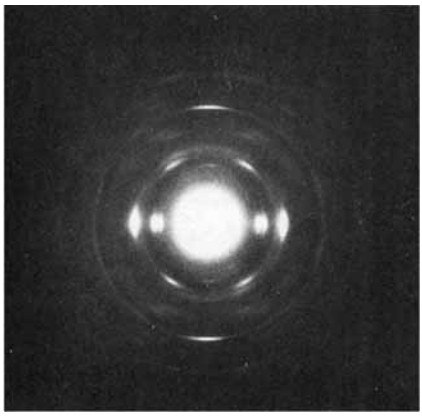
$$Dhkl = \frac{K\lambda}{\beta \cos \theta}$$

where K is considered to be unity. Since the contribution to half-maximum width due to the instrumental broadening as determined by the standard procedure[11] was found to be negligible, no correction was applied to the observed values. The crystallite length was determined from meridional reflections 020 and 040 by replotting the profiles of the two reflections after applying corrections due to background scatter. Width at half-maximum for both 020 and 040 interferences were then calculated using the Scherrer formula to arrive at the crystallite length $\perp$ to 020 and 040 reflections.

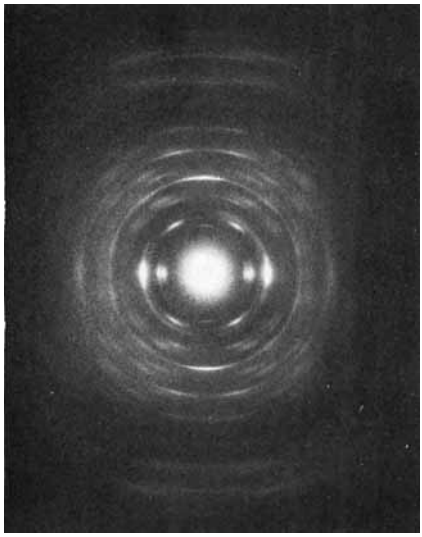
RESULTS AND DISCUSSION

The typical electron diffraction patterns of cooled specimen of cotton cellulose microfibril taken at 75 kV at 5 sec and longer exposure time are illustrated in Figure 1. The characteristic equatorial tracings of the electron diffraction patterns of cooled specimens recorded at 50, 75, and 100 kV are presented in Figure 2, and Table I summarizes the data on both cooled and uncooled specimens at the three accelerating voltages with respect to crystallinity index and crystallite dimensions.

At the accelerating voltage of 75 kV and exposure time of 5 sec in Figure 1, at least 16 sharp reflections are discernible per quadrant. Of these, 101, $10\bar{1}$, 002, 021, 221, $22\bar{1}$, 130, 131, 020, 040, 050, and 060 are easily indexed. In comparison,



(a)



(b)

Fig. 1. Electron diffraction pattern of cooled cell wall fragment of cotton cellulose at 75 kV: (a) exposure time 5 sec; (b) long exposure time. Note the sharp reflections even at 5 sec exposure and a large number of reflections out to the 10th layer line at higher exposure time.

as reported earlier,[1] the diffraction pattern at 100 kV, though satisfactory, had revealed about nine reflections.

In Figure 1(b), however, due to longer exposure time the reflections were visible out to the 10th layer line, and over 70 reflections per quadrant were recorded. But such a pattern, though suited for determining unit cell dimension, is not suited for line-broadening analysis since the specimen is exposed to the electron beam for a long period.

The Figure 2 as well as Table I clearly demonstrate that the electron diffraction diagram recorded at 75 kV excels over those observed at 50 or 100 kV. In fact, it was observed that even the diffraction pattern of uncooled specimen at 75 kV had almost the same number of reflections as the cooled specimen and was superior to those obtained at 50 or 100 kV in many ways. The peaks (Fig. 2) of all

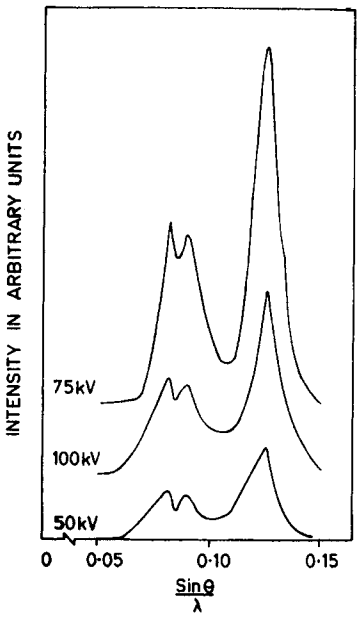


Fig. 2. Intensity tracings along the equator of the electron diffraction patterns of cooled specimens of cotton cellulose taken at accelerating voltages of 50, 75, and 100 kV.

the three interferences, viz., 101, $10\bar{1}$, and 002, are distinctly sharp and more in height at 75 kV as compared to those recorded at 50 or 100 kV. Regarding the state and size of ordered regions, the results (Table I) obtained on cooled specimens at 75 kV are: crystallinity index, 89%; crystallite width $\perp$ to 002 plane, 72 Å; and crystallite length $\perp$ to 040 and 020 planes, 230 and 209 Å, respectively. These values are comparable to the values reported in the literature for these parameters based on x-ray diffraction studies[12] if K is taken as 0.9 instead of unity as in the present investigation, where the shape of the microfibril is assumed to be cylindrical. However, the possibility of some decrystallization and lattice distortion due to electron irradiation, even if negligible, may not be overlooked despite cooling the specimen and use of extremely low beam current and exposure time of only 5 sec.

The likely reasons why the results obtained at 75 kV are superior to those obtained at 50 or 100 kV need some explanation. There are two types of electron scattering due to the interaction between electrons and the specimen. The first

TABLE I
Crystallinity Index and Crystallite Dimensions of Cotton Cellulose Determined by Electron Diffraction at Different Accelerating Voltages

Accelerating voltage, kV	Crystallinity index, %		Crystallite dimensions $\perp$ to planes *hkl*, Å					
			*D*002 (width)		*D*040 (length)		*D*020 (length)	
	Cooled	Uncooled	Cooled	Uncooled	Cooled	Uncooled	Cooled	Uncooled
50	75	73	55	44	230	106	203	–
75	89	88	72	67	230	220	209	200
100	82	79	67	59	217	115	185	123

type is elastic scattering, where there is negligible loss of energy by electrons. The second type, which is of more interest to us, is inelastic scattering that involves appreciable energy loss. The energy loss due to inelastic scattering is (i) proportional to the number of electrons per square cm, (ii) inversely proportional to the square of the electron beam potential (kV), and (iii) proportional to the specimen thickness.[13,14] Of these three factors, the specimen thickness in all the experiments could be expected to be more or less the same, as they were taken from the same source, viz., homogenized slurry of microfibrillar bundles of cotton cellulose. Therefore, at the relatively low accelerating potential of 50 kV, the energy loss due to inelastic scattering is much more than at higher accelerating potential, which results in the rise of temperature leading to chain scission, crosslinking, and eventual loss of order. This is apparent from Figure 2 and Table I which reveal that at 50 kV, considerable degradation occurs in most of the parameters of crystalline region as compared to the results obtained at 75 or 100 kV; the uncooled specimen degraded far more rapidly and the meridional reflection 020 could not even be recorded.

At 75 kV, the intensity of the beam is much reduced as compared to that at 100 kV, and the inelastic scattering is also reduced to a great extent as compared to one at 50 kV thereby minimizing the two major factors causing specimen degradation. Thus, a fortuitous compromise seems to have been struck at 75 kV. In fact, we observed that the electron diffraction pattern of both cooled and uncooled specimen persisted for a longer period at 75 kV than at 50 or 100 kV. This may also be the reason why Fisher and Mann[15] could obtain satisfactory results on uncooled specimens of *Valonia* at 75 kV, which was comparable to the results obtained by Honjo and Watanabe[2] on cooled specimens at 100 kV. Another observation of interest was that the meridional interferences 040 and 020 seem to decay faster under high-energy electrons at 100 kV than at 50 or 75 kV (Table I).

Earlier investigators[2,4–6] operating the electron microscope at an accelerating voltage of 100 kV and exposure time of about 30 sec had primarily demonstrated the use of the electron diffraction technique for determining unit-cell dimensions more accurately from its large number of reflections, and for crystallite orientation within the fibrils. From our results, it can now be concluded that the electron diffraction diagram recorded on the selected area of a very thin cooled specimen of cellulose microfibrils at the accelerating potential of 75 kV, at an extremely low beam current, and at an exposure time of about 5 sec is also suited for the quantitative interpretation of intensities for structural parameters such as crystallinity, crystallite width, and crystallite length.

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