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Electron Diffraction Technique for the Determination of Cellulose Crystallinity

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Synopsis

An electron-microscopic diffraction technique has been described to record with ease diffraction patterns of cotton cellulose with 3–5 sec exposure time, which is suited to determine the crystallinity of cellulose. The % crystallinity index for cotton and ramie determined by this technique amounted to 81% and 85%, respectively.

INTRODUCTION

Electron diffraction techniques have been used only in recent years by a few investigators for the study of the structure of cellulose materials such as cotton and ramie, even though their versatility was demonstrated by Honjo and Watanabe[1] in 1958 by their low-temperature diffraction study of *Valonia*. Hebert et al.[2] have reported the crystallite orientation within the fibrils of cotton to be around 10° irrespective of variety, and about 6° in the case of ramie. Hebert and Muller[3] further provided evidence to support the existence of at least two different unit cells, one for cotton and ramie, and the other for *Valonia ventricosa* and *Acetobacter xylinum*. Dobb et al.[4] have also demonstrated the presence of a large number of reflections by electron diffraction which were not discerned by x-ray diffraction. But to date, no one has reported the use of electron diffraction for the determination of cellulose crystallinity. A major constraint has been the sensitivity of cellulose fibrils to electron beam which results in the deterioration of lateral order.

Earlier investigators[5] have used an exposure time of about 30 sec and above for recording electron diffraction pattern to obtain intense reflections. In the present study, a simple technique has been standardized to overcome the deterioration of specimens due to the electron beam, by minimizing the exposure time to as little as 3 sec.

EXPERIMENTAL

Purified samples of cotton and ramie were thoroughly beaten in a laboratory blender to obtain discrete microfibrillar bundles in a slurry. A drop of diluted slurry was placed on uncoated 400-mesh copper grid and dried at room temperature.

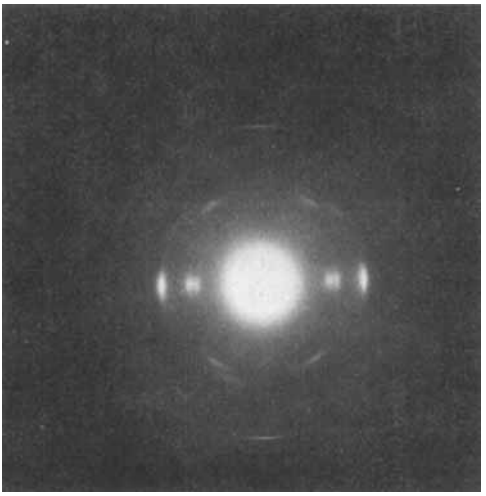

Fig. 1. Electron diffraction pattern of cell wall fragment of cotton cellulose taken at 5 sec of exposure for determining crystallinity. Note 101, 10$\bar{1}$, 002, 021, and 040 reflections which are discernible.

A Hitachi HU 11-E electron microscope at an accelerating potential of 100 kV and extremely low beam current was used; liquid nitrogen was used throughout the experiment to cool the specimen.

A grid supporting freshly prepared thin film of purified Al was inserted in the electron microscope; the microscope was set in the diffraction mode and focussed to get a typical Al diffraction pattern. This served two purposes, one to determine the camera constant of the electron microscope, and the other, to take rapid electron diffraction pattern of cellulose by replacing the Al supporting grid by the grid supporting microfibrillar bundles of cotton or ramie still keeping the microscope in the focussed state in the diffraction mode. Scanning of grids supporting cellulose microfibrils in the bright-field transmission mode was thus avoided, and thereby any degradation due to the electron beam was minimized. The diffraction pattern of selected area of cooled specimen of cellulose formed in the back focal plane of the objective lens, magnified by appropriate lenses, was then recorded with ease in as little time as 3 to 5 sec. Fuji orthochromatic electron microscopic film was used to record the diffraction pattern. The film was developed with continuous agitation for 6 min at 20°C in Kodak Universal Developer. The film, after brief washing, was treated with fixer for 5 min and finally washed thoroughly in running water for 30 min and air dried.

RESULTS AND DISCUSSION

Typical electron diffraction patterns of cotton taken at 5 sec exposure (Fig. 1) revealed at least nine reflections. Of these, the 101, 10$\bar{1}$, 002, 021, and 040 reflections were easily discernible. To get more intense reflections as observed by earlier workers, high exposure time of about 30 sec was needed as is evident from Figure 2.

Each electron diffraction pattern was equatorially scanned by a microphotometer. The intensity curve was corrected for background scattering (inelastic

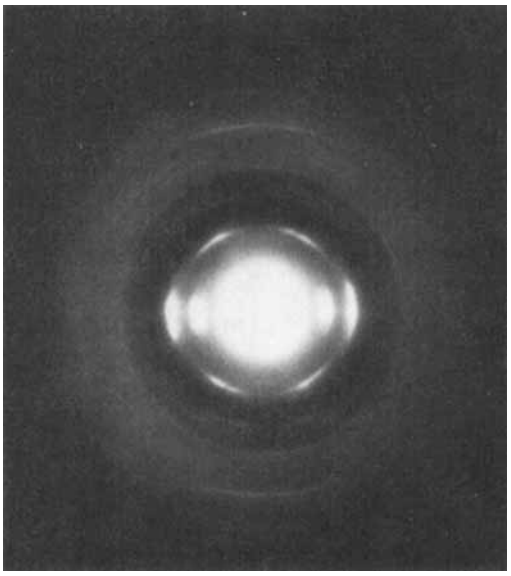

Fig. 2. Electron diffraction pattern of cell wall fragment of cotton cellulose taken at 30 sec of exposure. Note the higher intensity of reflections but broadening of arcs due to degradation.

scattering as well as scattering due to the direct beam) by employing the standard baseline technique.[6] Figure 3 illustrates the corrected intensity tracing along the equator of the electron diffraction pattern of cotton taken at 5 sec exposure.

Based on the empirical procedure of Segal et al.[7] for determining the crystallinity index %, from x-ray diffractograms, the crystallinity index was calculated from the electron diffractogram (Fig. 3) adopting the formula

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

where CrI represents crystallinity index, %; and I_{002} and I_{am} represent intensity, in arbitrary units, of the 002 interference peak and amorphous scatter, respectively.

For the determination of the crystallinity index %, from Figure 3 the intensity at $\sin \theta/\lambda = 0.1216$ represented I_{002}, and the intensity at $\sin \theta/\lambda = 0.1054$ represented I_{am}. The % crystallinity index for cotton amounted to 81%, and the value for ramie was 85%. These values may not be strictly comparable to those reported by Segal et al.[7] (74%), Wakelin et al.[8] (70%), and Patil et al.[9] (73%), who had determined crystallinity of cotton by x-ray diffraction from powder diagrams, the latter two having used external standards.

Ideally, the cellulose fibrils may be considered 100% crystalline. However, in the present study, the maximum value for crystallinity index obtained was 85% for ramie. The lower value could be attributed to the presence of distortions in the lattice, mainly of the paracrystalline type.[10] Such distortions may be present on the surface and in the lateral direction[11] as well as in the axial direction of the microfibrils.[12] Though liquid nitrogen was used for cooling the cellulose cell wall specimen and the exposure time was only 3–5 sec, decrystallization or

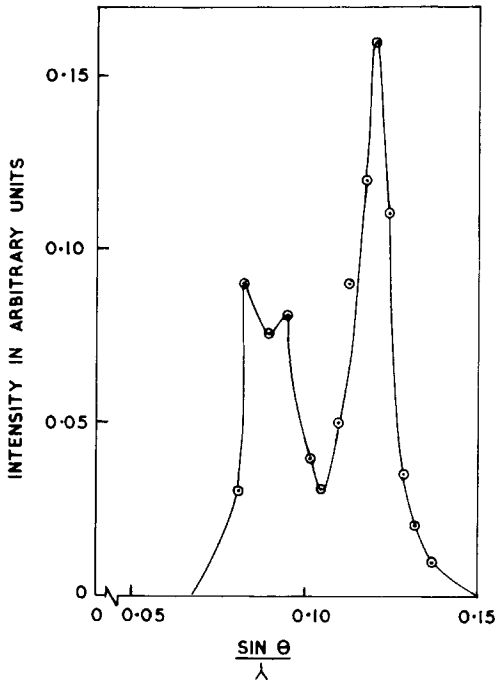

Fig. 3. Intensity tracing along the equator of the electron diffraction pattern of cotton taken at 5 sec of exposure.

lattice distortion due to inelastic scattering of electrons cannot be altogether precluded, but these are expected to be very negligible.

Electron diffraction has many advantages over x-ray diffraction for elucidating the fine structural parameters of cotton fiber, primarily because one can obtain diffraction patterns uncomplicated by convolutions and reversals, with the added advantage of recording larger numbers of reflections. The present-day sophisticated electron microscopes have overcome the earlier shortcomings in the application of electron diffraction technique for the study of cellulose. Besides determining crystallite orientation within fibrils and unit cell dimensions, it should now be possible to determine the crystallinity and crystallite dimensions, etc., by this technique.

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