Use of Sorbyl Chloride–Osmium Staining for Fibrillar Measurements in Cotton Fibers

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Synopsis

The technique for staining unsaturated additives within cotton fibers reacts osmium tetroxide with a sorbyl moiety which has been attached to the cellulose chain. Resulting electron micrographs indicate that contrast is considerably enhanced. Measurements of fibrillar size averaged 0.30 nm, closely approximating the values in the literature.

INTRODUCTION

Electron microscopy of nearly all organic material requires some method to improve contrast because of low atomic masses present. This is especially true in the study of the substructure of cotton fibers which is made up of concentric cellulose layers. Each of these layers is an aggregate of fibrils,[1] and these fibrils can be further divided into basic or elementary fibrils.[2,3] Exact measurement of fibrillar size is extremely difficult because of the inherent lack of contrast within the cellulose polymer due to low atomic weight. Attempts to enhance this contrast have followed two paths: metal shadow casting or electron staining techniques.[4]

Metal shadowing or vacuum coating adds a thin film of metal to the surface of the fibril and may very well affect the dimensions to be measured. Other disadvantages of this method include possible heat damage to the specimen during coating and the necessity of subdividing the fibrillar aggregates prior to shadowing.

Electron staining encompasses two general approaches: (a) positive staining, where the heavy metal is chemically bound to the material being stained, and (b) negative staining, where the electron-dense substance is deposited around the material, creating a "negative" contrast.

The purpose of this paper is to report a positive, though indirect stain for use in measuring cellulose fibrillar dimensions.

EXPERIMENTAL

The method involves reacting the cellulose chain with an unsaturated acid chloride, then further reacting the double bonds in the acyl derivative with osmium tetroxide.

In a previous study, acrylic acid was used to introduce the double bonds.[5] The present work was done with sorbyl chloride. Like acrylic acid, its reaction with

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cellulose produces osmiophilic sites in close proximity to the surface of the elementary fibril. Sorbyl chloride, however, contains one more unsaturated bond than acrylic acid and therefore possesses a greater propensity for osmium staining.

In esterification,[6] the acid chloride (0.06 mole) and 160 ml dimethylformamide (DMF) were heated to 105°C. Twenty milliliters pyridine, heated separately, was mixed with the DMF solution. Ten grams cellulose fibers (0.06 anhydroglucose moles) was immersed in the combined solutions for various time periods, all of which were less than the 20 hr needed for the acrylic acid–cellulose reaction.[5] Esterified cellulose fibers were stained with 2% osmium tetroxide in 0.1*M* phosphate buffer, pH 6.8, overnight. After thorough washing with distilled water and dehydration with acetone, fiber bundles were embedded in Maraglas according to standard procedures.[7] Sections cut parallel to the fiber axis with a Porter–Blum MT-1 microtome equipped with a diamond knife were collected on 500-mesh copper grids having no substrate. Section thickness was approximately 60 nm. Electron micrographs were taken with a Philips Em 300 electron microscope and were subsequently enlarged photographically to facilitate measurement of fibrillar dimensions.

RESULTS

Photomicrographs of stained fibers that contained 8.3% sorbyl groups are typical of our results (Figs. 1 and 2). The distance between marks in each represents 0.5 μm. The striated appearance which is absent in the osmium-treated control fibers is evidence of the efficiency of this technique.

Measurements of the striations in several micrographs yielded a distribution

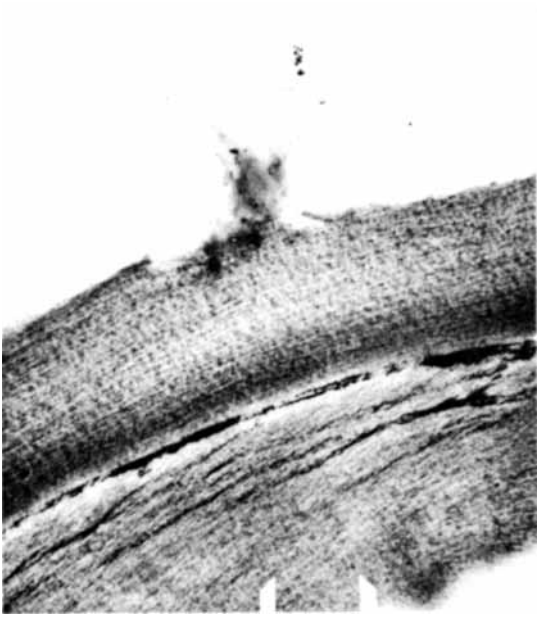

Fig. 1. Electron micrograph of stained material. Distance between markers represents 0.5 μm.

Fig. 2. Electron micrograph of stained material at a higher magnification. Distance between markers represents 0.5 μm.

of values with a mean of 0.292 ± .004 nm. This value is well within the range normally accepted for cellulose fibrillar diameters[8] and appears to corroborate the value of 0.33 nm obtained in the previous study.[5]

Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

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