JOURNAL OF APPLIED POLYMER SCIENCE VOL. 15, PP. 1425–1435 (1971)

Electron-Microscopic Investigation of Cerium–Cotton Cellulose Reactions

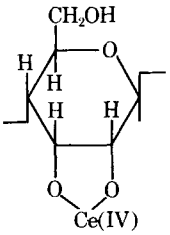
LINDA L. MULLER, JACQUES J. HEBERT, and MARY L. ROLLINS, *Southern Regional Research Laboratory, Southern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, Louisiana 70119*

Synopsis

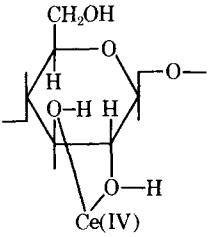
Cotton fibers were treated with an aqueous solution of ceric ammonium nitrate and examined with the electron microscope. The greatest deposition of cerium occurred in the primary wall due largely to the reaction with noncellulosic constituents in this area of the fiber. The use of ceric ions for an electron-microscopic stain was found ineffective for producing the desired contrast in the cotton fiber.

INTRODUCTION

Uses of the ceric ion for initiating graft polymerization of vinyl monomers and methacrylate on cellulose are numerous.[1,2] The mechanism of copolymerization reactions has been reported as either a free-radical process in which the transfer of electrons from the hydroxyl groups of the cellulose to the ceric ion results in the formation of a free radical on the cellulose molecule[3] or a process involving a ceric-coordinated monomer.[4,5] Ogiwara and Kubota[6–9] and Kulkarni and Mehta,[10] after examining various cellulosic materials treated with ceric ammonium nitrate, found evidence that the ceric ion complexed with the cellulosic substances rather than forming ionic bonds with the acidic hydroxyl groups as previously reported.[11] Arthur and co-workers[12] proposed the following as a possible structure for the ceric–cellulose complex, A, while Pottenger[5] gives structure B:



A



B

Further substantiating formation of the ceric–cellulose complex, Ogiwara and co-workers[7–9] reported that ceric ions adsorbed on cellulose were very

stable to dilute acid and dilute alkali and that the combination was very strong and not simply ionic. Furthermore, the amount of ceric ion adsorbed on cellulose was greatly affected by temperature, time, concentration, and the carbonyl group content.[7,9,13]

The purpose of the present study was to determine (1) the location of reacted cerium within the fine structure of cotton fibers and (2) the possibility of using the ceric ion as a stain for electron-microscopic studies of cellulose.

MATERIALS AND METHODS

Deltapine cotton fibers in both native unpurified state and purified state were used as the cellulosic samples. Purification was by the conventional method of ethanol extraction[14] followed by boiling in 1% NaOH under a nitrogen atmosphere.[15,16] The scoured and mercerized samples were commercially prepared. The nitrogen content was determined prior to treatment with ceric ions by micro-Kjeldahl analysis, the carbonyl content was determined by hydroxylamine method, and the carboxyl content, by Davidson's methylene blue absorption method.

One tenth-gram samples of fibers were treated with 50 ml of an aqueous ceric ammonium nitrate, $Ce(NO_3)_4 \cdot 2NH_4NO_3$, solution 0.04*M* for Ce^{+4} at 45°C for 60 min according to the method of Ogiwara et al.[9] After treatment, the samples were washed three times with 100 ml distilled water, allowed to stand overnight in 400 ml distilled water, suction filtered, and air dried. The treated fibers were embedded in a 3:2 methyl-butyl methacrylate mixture, ultrathin sections were cut, the embedding medium was removed with methyl ethyl ketone, and sections were shadowed with platinum and examined in the electron microscope.

Ultrathin sections of native Deltapine were also treated with the same Ce^{+4} solution for 60 min at 45°C on the grid and washed with distilled water to remove the excess reagent. After platinum shadowing, electron micrographs were taken.

Cross sections of the unpurified Deltapine from the above two treatments were placed in 0.5*M* cupriethylenediamine hydroxide (cuene) for 30 min, then thoroughly washed with distilled water, platinum shadowed, and photographed.

Data on the amount of cerium present in the cotton samples were collected using the x-ray fluorescence method with a General Electric XRD-6 x-ray spectrometer.[17]

RESULTS AND DISCUSSION

The relationship between adsorption of ceric ions on cellulose and the extent of impurities in the cellulose is shown in Figures 1 and 2, which clearly indicate that the amount of cerium adsorbed decreased as the purity of the fiber increased. Therefore, the ceric ion appears to be associated with the accessible acidic hydroxyl groups[13,18] but also with impurities such as protein, pectic materials, and wax in raw cotton cellulose.

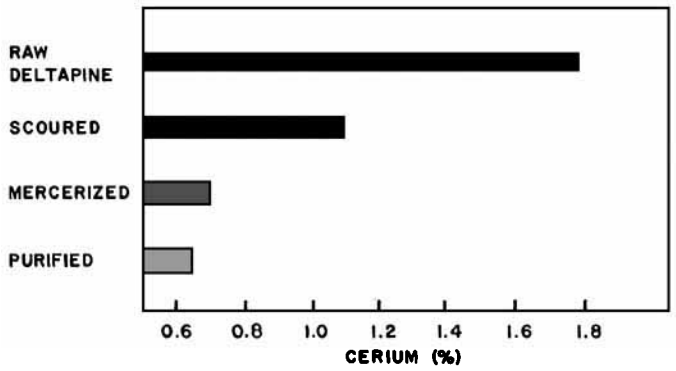


Fig. 1. Per cent Ce in cotton fibers as determined by x-ray fluorescence.

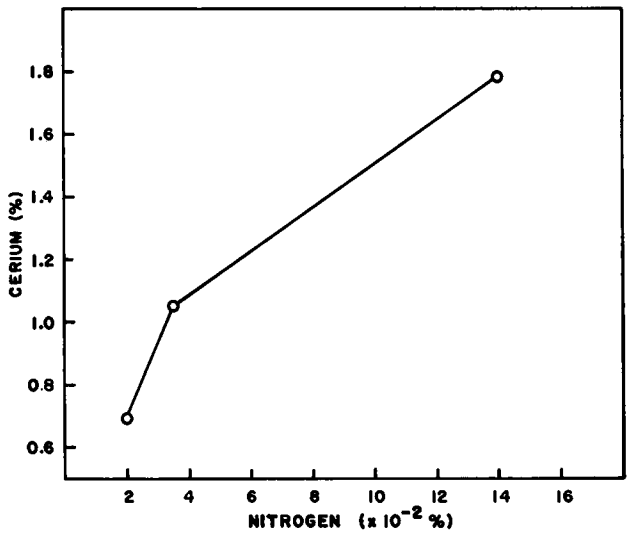


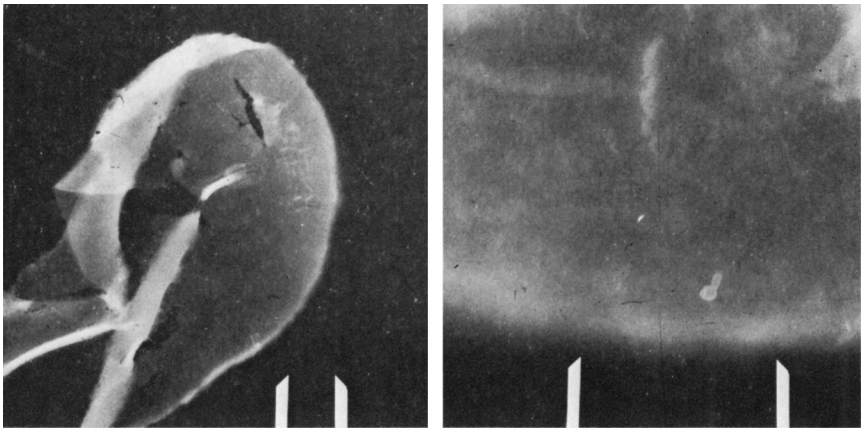
Fig. 2. Relationship between per cent Ce as determined by x-ray fluorescence and per cent N in cotton fibers.

The sample of raw cotton which exhibited the highest sorption of Ce was high in carboxyl and carbonyl contents as well as nitrogen; these functional groups may reflect the presence of the impurities noted above, or they may actually be involved in the binding of the Ce. Table I shows that the cerium content was greater in native cotton which contained more nitrogen, carbonyl, and carboxyl groups than in the purified cotton.

To determine the site of reaction of ceric ions in cotton fibers, aqueous ceric ammonium nitrate treatments of cotton fibers were examined with the electron microscope. Figure 3 represents a cross section of an untreated cotton control photographed in the electron microscope, while Figure 4 is a cross section of a cotton fiber after ceric ion treatment. Neither fiber was shadowed with platinum prior to being photographed in the electron

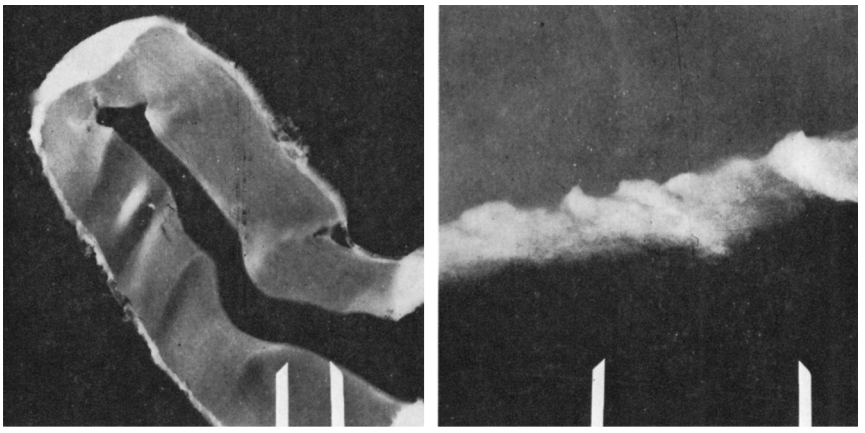
TABLE I
Chemical Analysis of Cotton Fibers

Sample	Nitrogen, %	Carboxyl, mmole/100 g cotton	Carbonyl, mmole/1 g cotton	Reacted cerium, %
Raw Deltapine	0.14	2.37	0.57	1.78
Scoured	0.035	—	—	1.05
Scoured-mercerized	0.02	—	—	0.69
Purified	0.02	0.40	0.08	0.63



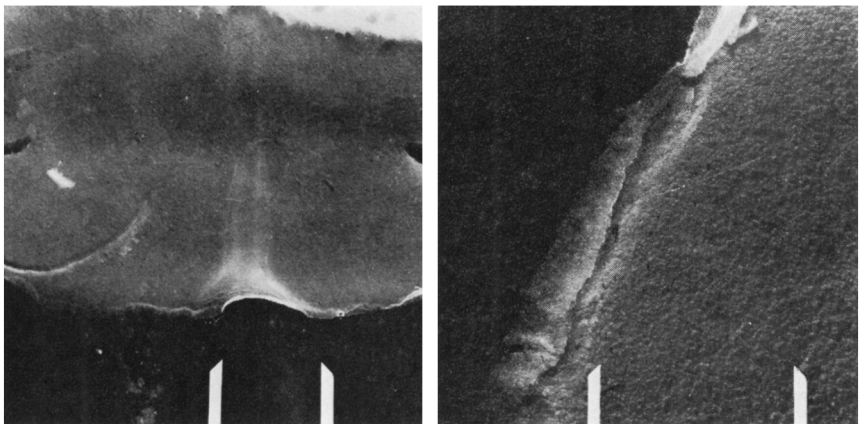
(a) (b)

Fig. 3. Electron micrographs of unshadowed cross sections of raw cotton fiber. Distance between markers, here and in Figs. 4–15, represents (a) 1 μ; (b) 0.5 μ.



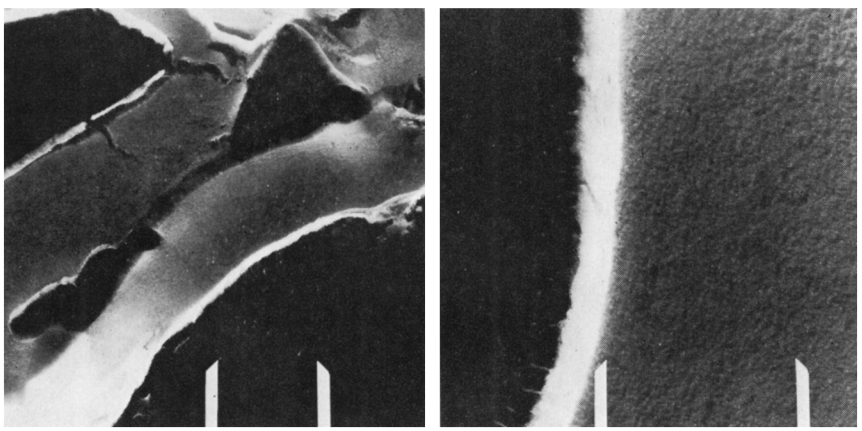
(a) (b)

Fig. 4. Electron micrographs of unshadowed cross sections of raw cotton fiber treated with ceric ion.



(*a*) (*b*)

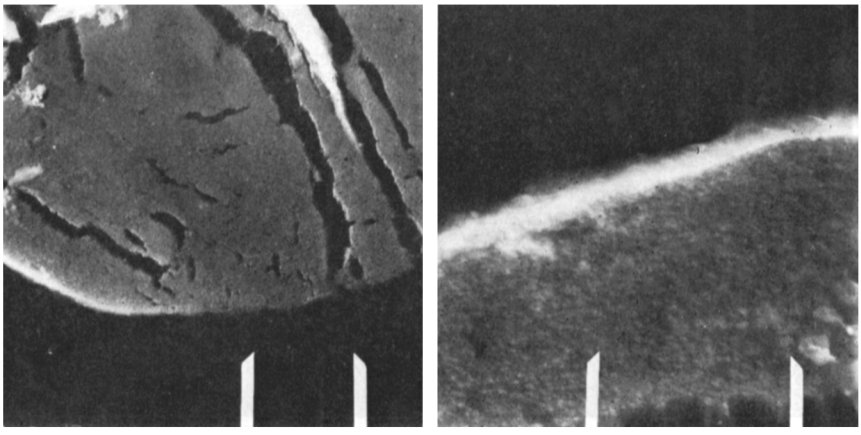
Fig. 5. Electron micrographs of Pt-shadowed cross sections of raw cotton fiber.



(*a*) (*b*)

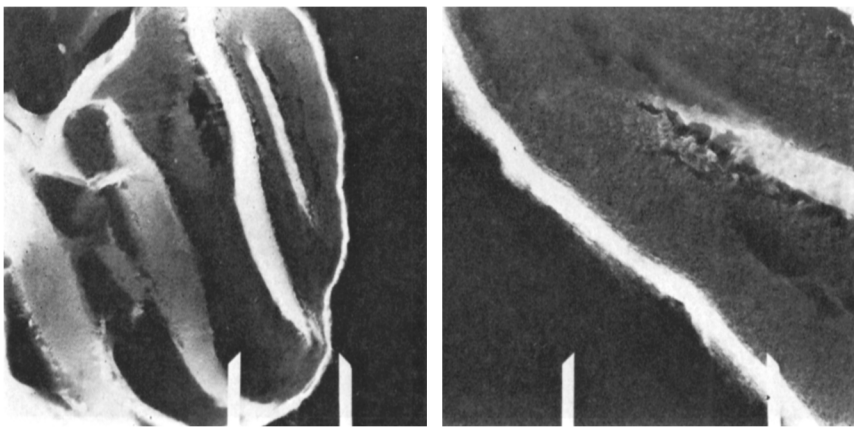
Fig. 6. Electron micrographs of Pt-shadowed cross sections of raw cotton fiber treated with ceric ion.

microscope. The obvious lack of contrast here can be rectified only by the use of a metal shadow on the specimen. Therefore, all remaining electron micrographs were platinum shadowed, and it was decided that Ce^{+4} ion staining was not effective as an electron-microscopic stain. Paired Figures 5 and 6, 7 and 8, 9 and 10, and 11 and 12 show the marked differences in the primary wall between the untreated and ceric ion-treated samples. Figures 6, 8, 10, and 12 illustrate that the site of reaction of ceric ions was mainly within the primary wall of the cotton fibers. The primary wall was heavily stained, whereas the secondary wall was at best very lightly stained. The cerium-treated fiber in Figure 8 and the untreated fiber in Figure 7 readily illustrate the difference in density in the primary wall. After the cellulose–cerium complex was formed in the primary wall, the secondary wall prob-



(*a*) (*b*)

Fig. 7. Electron micrographs of Pt-shadowed cross sections of scoured cotton fiber.



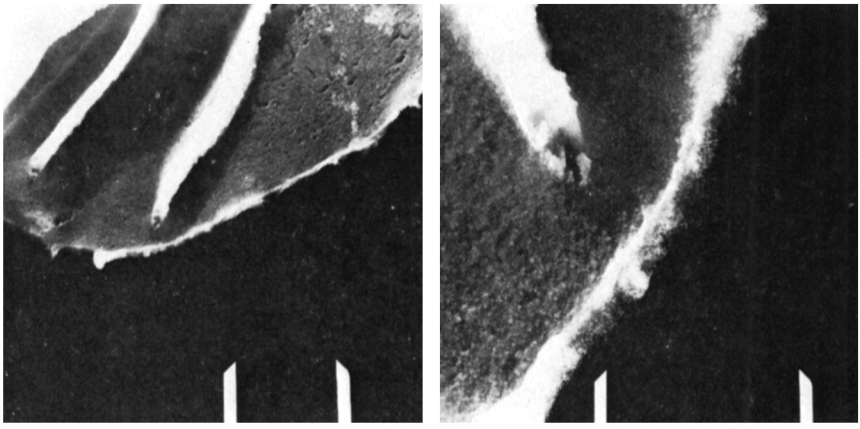
(*a*) (*b*)

Fig. 8. Electron micrographs of Pt-shadowed cross sections of scoured cotton fiber treated with ceric ion.

ably became inaccessible to the reagent. However, cerium might have complexed with substances other than α-cellulose (hemicellulose, pectins, waxes, and proteins); and since the secondary wall of cotton is approximately 95% α-cellulose, the reaction might have occurred only in the primary wall.

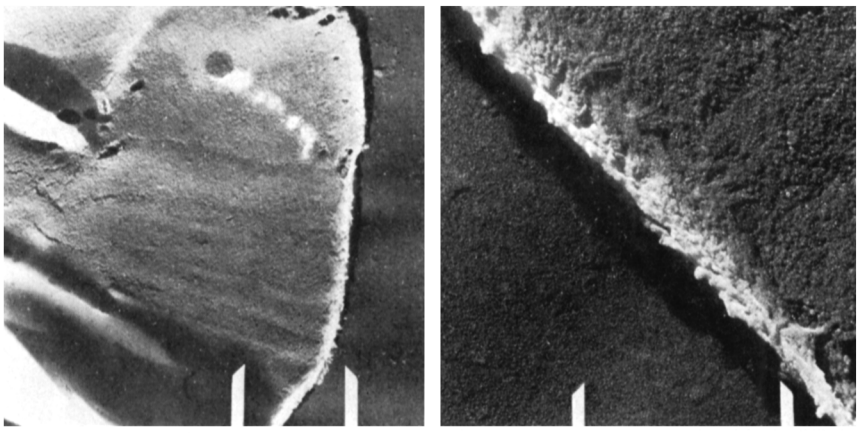
To ascertain whether any differences exist between the primary and secondary walls of untreated cottons, cross sections of fibers without contact with aqueous ceric ammonium nitrate were examined with the electron microscope. In Figures 5, 7, 9, and 11, where the samples contained no cerium, the fibers showed no sharp differentiation between primary and secondary walls.

To further determine the inaccessibility of the secondary wall to the re-



(a) (b)

Fig. 9. Electron micrographs of Pt-shadowed cross sections of mercerized cotton fiber.

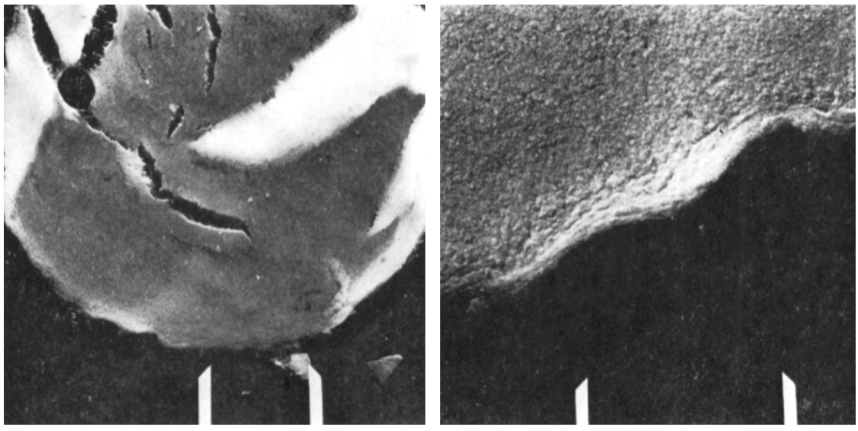


(a) (b)

Fig. 10. Electron micrographs of Pt-shadowed cross sections of mercerized cotton fiber treated with ceric ion.

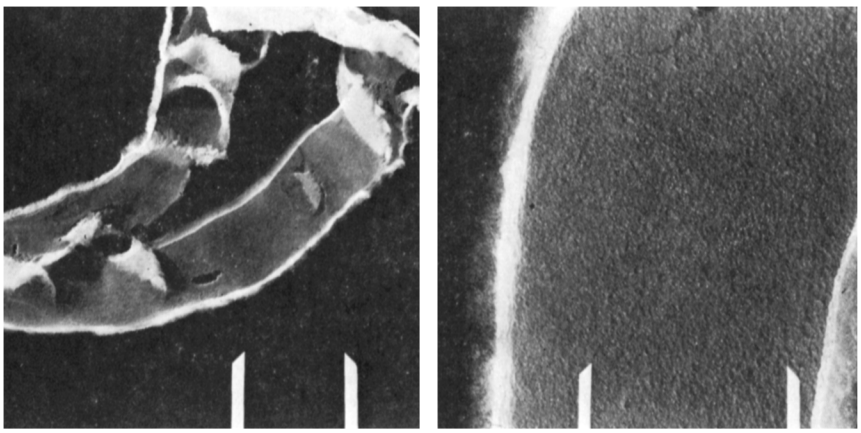
agent, examinations with the electron microscope were made of cross sections of native Deltapine cotton which had been treated with the solution of Ce^{+4} for 60 min on the grid at 45°C. Figure 13 shows some cerium on the secondary wall as well as a heavier adsorption on the primary wall. A penetration problem may have existed in the treatment of whole fibers; that is, once the complex was formed in the primary wall, the reagent molecules were possibly too large to reach the secondary wall.

Electron micrographs (Fig. 14) of cross sections after cuene treatment depicted only the heavily stained primary wall remaining in those treated with Ce^{+4} in the fiber form. In Figure 15, after cuene, remnants of cerium on both the primary and secondary walls of the cross sections treated on the grid with Ce^{+4} were present.



(*a*) (*b*)

Fig. 11. Electron micrographs of Pt-shadowed cross sections of purified cotton fiber.

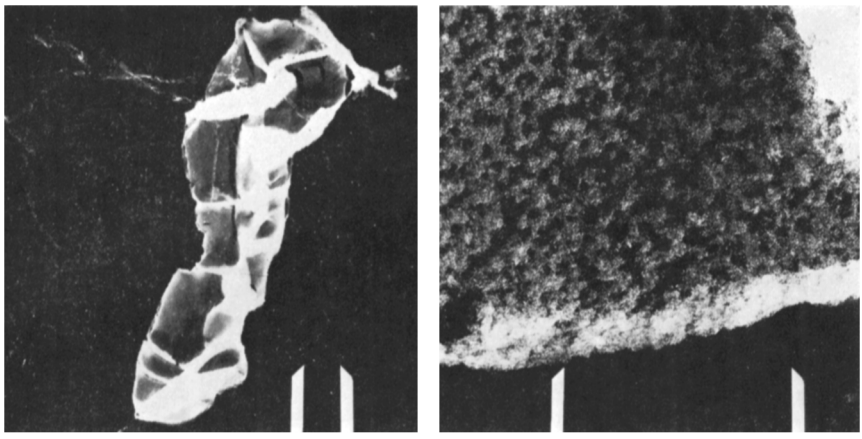


(*a*) (*b*)

Fig. 12. Electron micrographs of Pt-shadowed cross sections of purified cotton fiber treated with ceric ion.

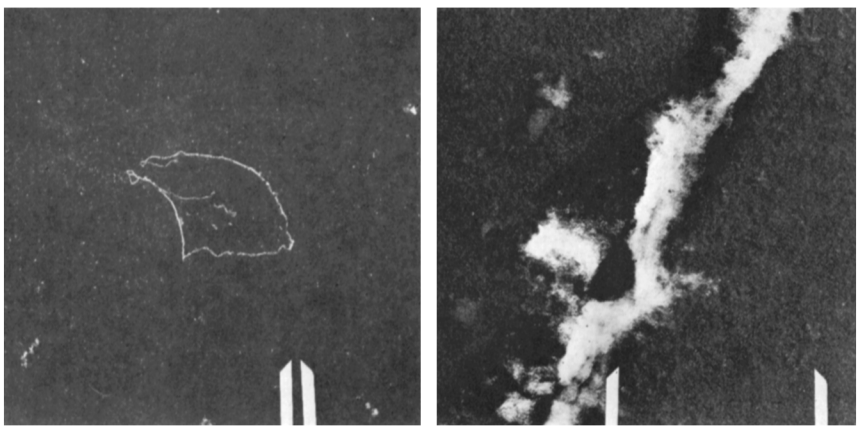
The primary wall of raw cotton contains a much larger percentage of noncellulosic constituents than the secondary wall, but still consists of approximately 54% cellulose. Presence of these noncellulosic substances in the primary wall modifies the dyeing properties, rates of reaction, and wettability of the fiber, since in such operations reagents reach the fiber body by passage through the primary wall. Of the noncellulosic materials found in raw cotton, wax, pectin, and protein are the most prevalent.

The wax content of raw cotton averaging 0.6% is presumably located in the primary wall of the fiber.[19,20] Much of the pectic substances in raw cotton is localized in the primary wall as demonstrated by ruthenium red staining,[21] but there is evidence that pectin exists elsewhere in the mature fiber.[19,20] Protein occurs in the primary wall and the lumen; however,



(*a*) (*b*)

Fig. 13. Electron micrographs of Pt-shadowed cross sections of raw cotton treated on the grid with ceric ion.



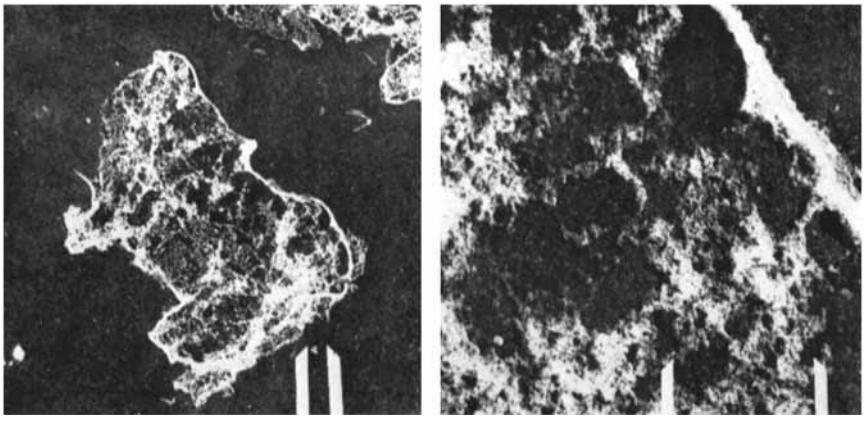
(*a*) (*b*)

Fig. 14. Electron micrographs of Pt-shadowed cross sections of raw cotton fiber treated with ceric ion; after cuene.

some of the nitrogen is possibly derived from other sources, and the actual protein content is less than the calculated amount 1.3–1.9%.[19,22]

The major impurities can be easily removed so that cotton of 99% cellulose, instead of only 94% in raw cotton, can be obtained. Pectin and nitrogenous material can be removed from fibers by kier boiling with 1% sodium hydroxide for as little as 30 min.[15] After scouring, the nitrogen content is reduced to 0.035%.[16] Soxhlet extraction with 95% ethanol for 6–12 hr has been found effective in the removal of wax.[14]

Much of the work of ceric ion-initiated graft polymerizations was done using wood pulps as the cellulose source.[7,9,13,23] These wood pulps were not pure cellulose, that is, α-cellulose. Softwoods and hardwoods are com-



(a) (b)

Fig. 15. Electron micrographs of Pt-shadowed cross sections of raw cotton treated on the grid with ceric ion; after cuene.

posed of approximately 50% cellulose, 25% hemicellulose or noncellulosic polysaccharides, and 25% lignin.[24,25] In the pulping procedures, only part of the lignin and hemicelluloses was removed.[24] It has been reported[6,7,9,13] that the gram atoms of adsorbed ceric ion were approximately equal to the moles of total carbonyl and carboxyl groups in the cases of cotton and sulfite pulp, which are low in hemicellulose content, and were several times larger in the cases of Kraft pulp and semichemical pulp, which contains about 22% hemicellulose. One can, therefore, draw a parallel between the above observations and the data in Table I, to conclude that the ceric ions also reacted with impurities in cotton fibers in addition to α-cellulose.

CONCLUSION

Electron-microscopic examination of fibers treated with ceric ammonium nitrate showed that the primary walls of intact fibers were selectively stained. The major reaction probably occurred with noncellulosic substances within the primary wall. Such reactions, along with the relatively few cellulose–cerium reactions which do occur in the primary wall, restrict the accessibility of the pure α-cellulose in the secondary wall. When this treatment was applied to cross sections of cotton fibers, some deposition of cerium did appear in the secondary wall.

The use of ceric ions for an electron-microscopic stain was found ineffective, since Pt shadowing was necessary to obtain the desired contrast.

The authors are grateful to Rosalie Babin for her assistance in preparing the illustrations, to Lawrence Brown for the chemical analyses, and to Biagio Piccolo for the x-ray fluorescence analyses.

Use of a company or product name by the department does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

References

1. G. Mino and S. Kaizerman, *J. Polym. Sci.*, **31**, 242 (1958).
2. Y. Ogiwara, H. Kubota, and K. Arai, *J. Appl. Polym. Sci.*, **14**, 1049 (1970).
3. E. H. Immergut, in *Encyclopedia of Polymer Science and Technology*, Vol. 3, H. F. Mark, N. G. Gaylord, and N. M. Bikales, Eds., Interscience, New York, 1965, p. 242.
4. T. Takahashi, Y. Hori, and I. Sato, *J. Polym. Sci. A-1*, **6**, 2091 (1968).
5. C. R. Pottenger and D. C. Johnson, *J. Polym. Sci. A-1*, **8**, 301 (1970).
6. Y. Ogiwara, H. Kubota, and Y. Ogiwara, *J. Polym. Sci. A-1*, **6**, 3119 (1968).
7. Y. Ogiwara, Y. Ogiwara, and H. Kubota, *J. Polym. Sci. A-1*, **6**, 1489 (1968).
8. Y. Ogiwara, Y. Ogiwara, and H. Kubota, *J. Appl. Polym. Sci.*, **12**, 2575 (1968).
9. Y. Ogiwara and H. Kubota, *J. Polym. Sci. A-1*, **7**, 2087 (1969).
10. A. Y. Kulkarni and P. C. Mehta, *J. Polym. Sci. B*, **5**, 209 (1967).
11. S. A. Rydholm, *Pulping Processes*, Interscience, New York, 1965, pp. 1050, 1115.
12. J. C. Arthur, Jr., P. J. Baugh, and O. Hinojosa, *J. Appl. Polym. Sci.*, **10**, 1591 (1966).
13. Y. Ogiwara, Y. Ogiwara, and H. Kubota, *J. Polym. Sci. A-1*, **5**, 2791 (1967).
14. C. M. Conrad, *Ind. Emg. Chem., Anal. Ed.*, **16**, 745 (1945).
15. R. L. Whistler, A. R. Martin, and M. Harris, *J. Res. Nat. Bur. Stand.*, **24**, 555 (1940).
16. J. H. Kettering and C. M. Conrad, *Ind. Eng. Chem., Anal. Ed.*, **14**, 432 (1942).
17. D. Mitcham, B. Piccolo, V. W. Tripp, and R. T. O'Connor, *Advan. X-Ray Anal.*, **8**, 456 (1965).
18. A. Hebeish and P. C. Mehta, *J. Appl. Polym. Sci.*, **12**, 1625 (1968).
19. V. W. Tripp, A. T. Moore, and M. L. Rollins, *Text. Res. J.*, **21**, 886 (1951).
20. E. I. Valko, in *Chemistry and Chemical Technology of Cotton*, K. Ward, Ed., Interscience, New York, 1955, pp. 121, 127.
21. C. W. Hock and M. Harris, *Text. Res. J.*, **10**, 323 (1940).
22. M. S. Catlett, R. Giuffria, A. T. Moore, and M. L. Rollins, *Text. Res. J.*, **21**, 880 (1951).
23. R. Y. M. Huang and P. Chandramouli, *J. Polym. Sci. A-1*, **7**, 1393 (1969).
24. A. H. Nissan, G. K. Hunger, and S. S. Sternstein, in *Encyclopedia of Polymer Science and Technology*, Vol. 3, H. F. Mark, N. G. Gaylord, and N. M. Bikales, Eds., Interscience, New York, 1965, pp. 131ff.
25. K. Kratzl, in *Cellular Ultrastructure of Woody Plants*, W. A. Côté, Jr., Ed., Syracuse University Press, Syracuse, N.Y., 1965, p. 157.

Received January 22, 1971