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Glycol Methacrylate, an Embedding Medium to Study the Ultrastructure of Cotton Treated with Swelling Agents

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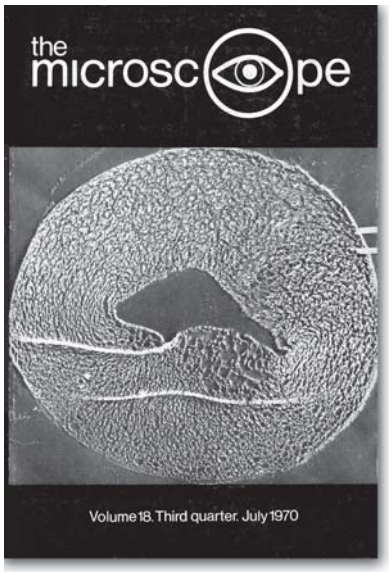
ABSTRACT

Glycol methacrylate, a water-soluble monomer, was used for embedding cotton swollen in sodium hydroxide solution, and the potential of this medium in the study of the ultrastructure of the swollen fiber has been indicated by comparing the results with those obtained by the conventional layer-expansion technique employing methyl and butyl methacrylates.



INTRODUCTION

The layer-expansion technique has been extensively used by Rollins and co-workers (1) in the electron microscopical evaluation of mercerized, crosslinked, grafted and other chemically modified cottons. Recently, Betrabet and Rollins (2) made use of this technique in the evaluation of cotton treated with various inter- and intra-crystalline swelling agents and demonstrated a gradation in morphological changes in the swollen and decrystallized methyl and butyl methacrylates is used in this technique. Water-soaked cotton fibers, on embedding appropriately in this mixture of methacrylates, swell enormously and the secondary cell wall forms concentric layers. Dry fibers, however, do not swell or layer and have an almost solid structure. This layering has been viewed by many as an artifact caused by differential rates of polymerization and concentration of the methacrylates inside and outside the cotton fiber (3). Moreover, fibers treated with various organic and inorganic swelling agents must be thoroughly washed free of the reagent before embedding, perhaps causing the original swollen state and morphology at the microfibrillar level to be altered. To overcome these shortcomings, it was considered worthwhile to explore the possibility of using water-soluble embedding agents in which the chemically treated cotton could be embedded directly in the swollen state without washing. Polyvinly alcohol, a water-soluble embedding medium, has been successfully used by Cannizzaro et al. (4) for embedding chemically substituted cottons, but the preparation of the embedding block was time-consuming and it was not known whether direct embedding of cotton swollen in different alkalies and acids was possible. Glycol methacrylate (ethylene glycol monomethacrylate), which is almost completely water-miscible, was therefore tried as an embedding medium to obviate some of the drawbacks of previous methods and to study the morphological changes in the swollen fiber in the least altered state.

Rosenberg et al. (5) have reported in detail the use of glycol methacrylate for electron microscopy in gen-

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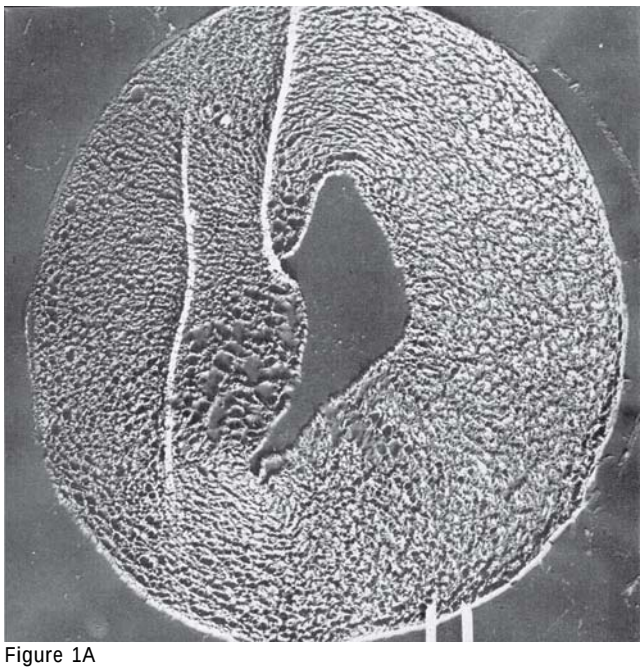

Figure 1A

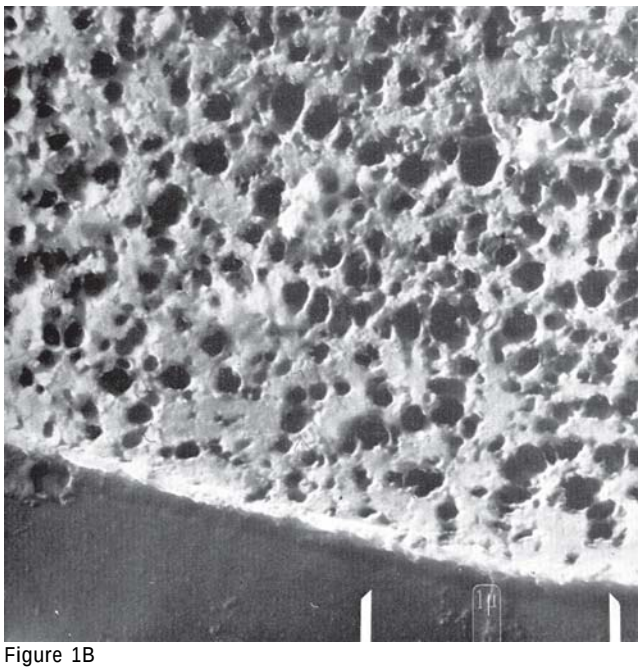

Figure 1B

Figure 1. Cross-section of sodium hydroxide-treated cottom fiber by layer-expansion. Note the spongy-honeycomb structure under low magnification (1A), and compactnes and fusion of microfibrils under high magnification (1B). Distance between the vertical lines represents one micron.

eral, and it was specifically used by Leduc et al. (6) for ultrastructural cytochemistry. They used a prepolymerized, highly viscous, embedding solution of 70% glycol methacrylate and 30% butyl-methyl (85:15) methacrylates in gelatin capsules, and the polymerization was carried out in a cold room at 3 °C under a UV lamp. The polymerization time varied from overnight to several days, depending upon the intensity of the UV light. Polcin and Karhanek (7) used glycol methacrylate for embedding swollen cellulosic wood fibers. They carried out their embeddings in gelatin capsules containing 95% glycol methacrylate and 3-5% methyl methacrylate. The curing period was 24 hours at 45-50 °C.

TECHNIQUES AND RESULTS

In our investigation on cotton treated with swelling agents, a flat embedding technique was standardized for ease of preparation and sectioning with the ultramicrotome. The addition of 3-5% methyl methacrylate or a mixture of 30% butyl and methyl methacrylates to the glycol methacrylate did not impart satisfactory cutting properties to the embedding blocks. The temperature range of 45-50 °C and a curing period of 24 hours or beyond were also unsuited for the flat embedding of the fibers. After carrying out several preliminary experiments by adding to the glycol methacrylate various proportions of methyl and butyl methacrylates, individually or together, a satisfactory embedding technique was developed. The results with respect to cotton treated with sodium hydroxide of mercerizing strength are presented as a typical example. It may be mentioned that mercerization is a very important commercial process which is used to improve the luster, strength and dyeability of cotton. It consists of treating cotton yarn or fabric usually under tension with an aqueous 18-25% solution of sodium hydroxide.

A small tuft of cotton fibers was combed to make the fibers parallel, and tied at the two ends with thread to form a small bundle. The bundle was soaked in an aqueous solution of 20% sodium hydroxide for 1 hour at 25 °C. The fiber bundle was then given successive 1 hour soaks at 25 °C in 50, 75 and 90% aqueous solutions of glycol methacrylate, and finally soaked overnight in a mixture of glycol, methyl and butyl methacrylates (90:6:4), containing 1% Luperco CDB* [*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 my also be suitable.] (50% of 2, 4-dichlorobenzoyl peroxide in dibutyl

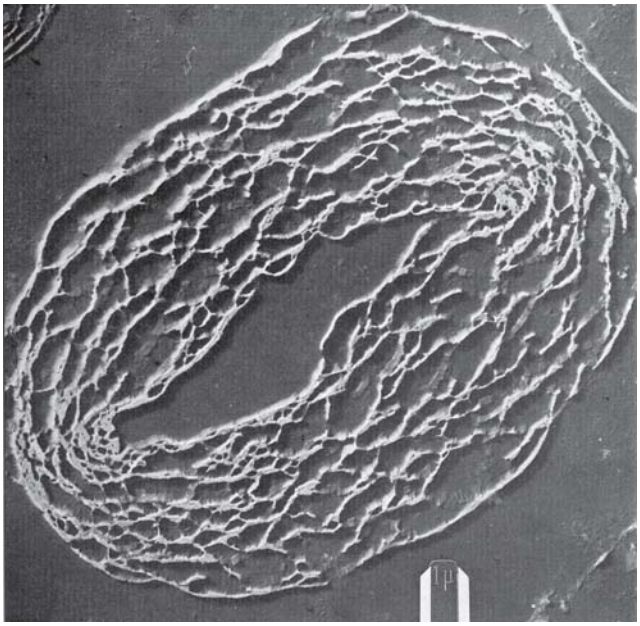

Figure 2A

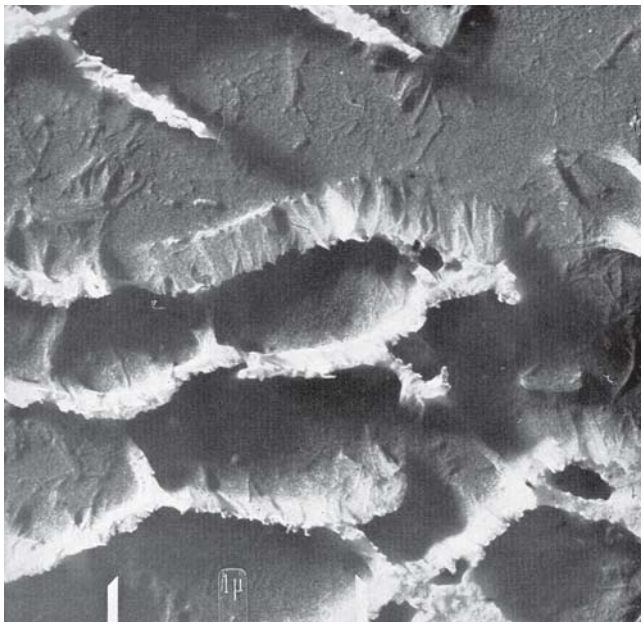

Figure 2B

Figure 2. Cross-section of untreated cotton fiber by layer-expansion. Note the characteristic concentric layers (2A) and the microfibrils (2B).

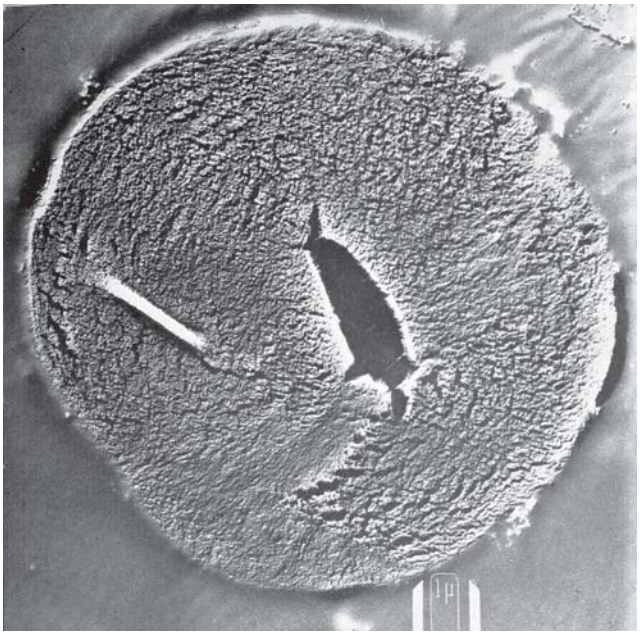

Figure 3A

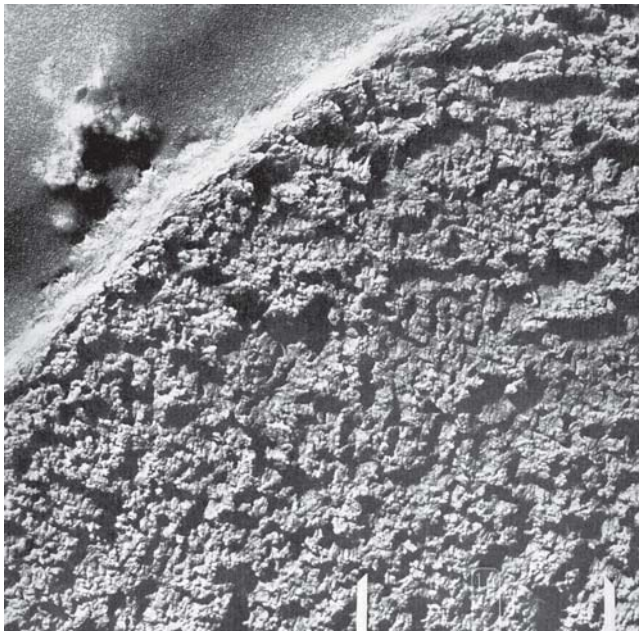

Figure 3B

Figure 3. Cross-section of sodium hydroxide-swollen cotton fiber embedded in glycol methacrylate. Note the spongy-honeycomb structure (3A), fusion of microfibrils (3B) and similarity to Figure 1.

phthalate) as a catalyst. The fiber bundle was then placed in an 18 x 12 x 12 mm aluminum foil trough, which was filled with the mixture of methacrylates. After the air bubbles were removed in a vacuum desiccator, the curing was carried out for about 10 hours at 65 °C on a warming table. The embedding block was covered with a Petri dish to prevent rapid evaporation of the medium and to help in uniform curing. A

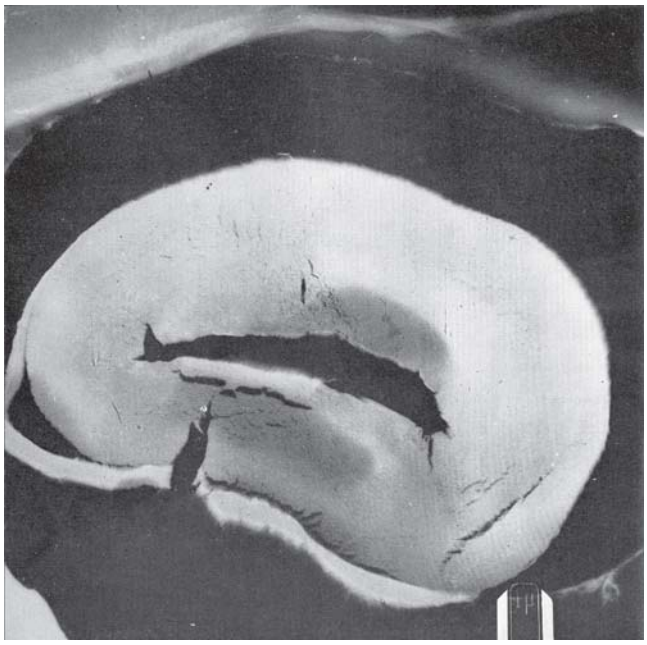

Figure 4A

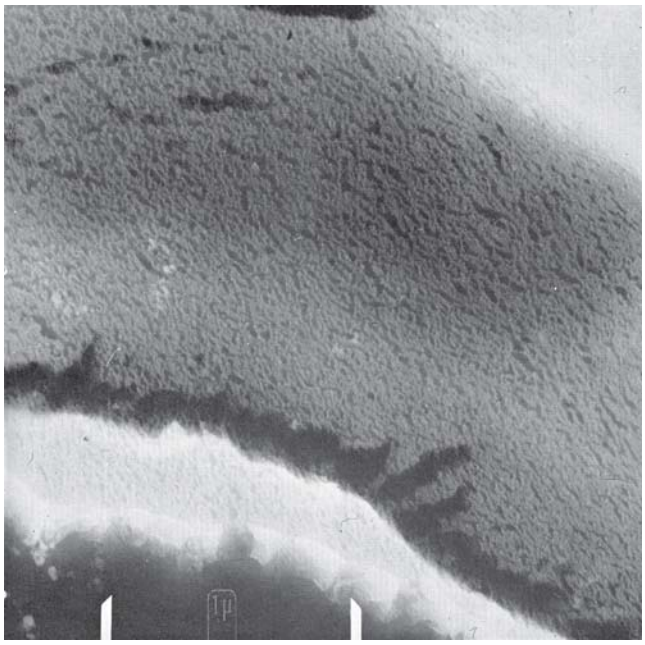

Figure 4B

Figure 4. Cross-section of water-swollen cotton fiber embedded in glycol methacrylate. Note the compactness of the structure (4A) and no layering (4B) as seen in Figure 2.

fiber bundle of native cotton was soaked in distilled water and embedded in a similar manner to serve as a control. Simultaneously, a bundle of cotton fibers, swollen in 20% sodium hydroxide, washed free of alkali, and air dried after solvent exchange in methanol and water, was examined by the conventional layer-expansion technique (1).

The blocks were ground to a suitable thickness and trimmed in the form of a pyramid and ultrathin cross-sections were cut with a Porter Blum* microtome equipped with a diamond knife. [*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 my also be suitable.] Sections were floated in a trough containing aqueous 10% ethanol. Since glycol methacrylate embedding blocks are difficult to cut, a slightly concave meniscus was maintained at the knife edge to prevent the sections from being pulled away from the liquid in the trough during continuous sectioning. A nichrome loop was used to transfer the sections to carbon-coated grids which were placed on filter paper strips saturated with water to facilitate flattening out of the sections on the grid. The grids were allowed to dry on filter paper before shadowing them with platinum at an angle of about 25°. The cross-sections were then examined in an electron microscope.

Cotton fibers treated with sodium hydroxide of mercerizing strength when examined by the layer-expansion technique are known to produce a randomly honeycombed or spongy structure (Figure 1) as compared to the concentrically layered structure of the untreated cotton fiber (Figure 2). The radical lateral disorder due to high swelling in a strong solution of alkali seems to cause this morphological change because of the fusion of several layers of fibrils which can be seen through the openings between capillary spaces. This characteristic morphology of mercerized cotton is indeed confirmed by the glycol methacrylate embedding technique (Figure 3). The crusty appearance of the glycol methacrylate embedded cross-section, especially at the high magnification (Figure 3B), is due to the presence of polymerized glycol methacrylate. Polymerized glycol methacrylate is almost impossible to dissolve without affecting the cross-section of the cotton fiber. In contrast, the layer-exapansion pictures (Figure 2) of sodium hydroxide-treated cotton were taken after dissolving the polymerized methyl-butyl methacrylates in methyl ethyl ketone. The water-swollen control sample embedded in glycol methacrylate, however, does not layer in contrast to the fiber examined by the layer-expansion technique. This would perhaps indicate the absence of any "polymerization explosion" in glycol methacrylate (Fig-

ure 4). The glycol methacrylate flat-embedding technique has been used extensively by us with success to study the morphology of cotton treated with different inter- and intra-crystalline swelling agents. The results obtained form the subject matter of another publication (8).

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