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COMBINED FLUORESCENCE DICHROISM
AND POLARIZATION MICROSCOPY
OF COTTON FIBRES UNDER STRESS

by

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Combined fluorescence dichroism and polarization microscopy of cotton fibres under stress¹⁾

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Samenvatting

Gefluorochromeerde katoenvezels vertonen fluorescentie-dichroïsme. Dit verschijnsel werd bestudeerd met behulp van een speciaal voor dit doel aangepaste fluorescentie-microscoop.

Een verschil tussen de vezeldelen met een S- en die met een Z-spiraal is meestal goed zichtbaar. Bij de gebruikte fluorochromering zijn slechts de buitenste groeilagen duidelijk gekleurd. Deze methode kan, indien hij gecombineerd wordt met polarisatie-microscopisch onderzoek en micro-rekproeven, gebruikt worden om slipverschijnselen tussen groeilagen aan te tonen. Deze slipverschijnselen zijn duidelijk zichtbaar in die vezeldelen, waar in de buitenste lagen de fibrillen-spiraal goed ontwikkeld is, maar waar in de binnenste groeilagen de spiralen voor de verschillende lagen niet samenvallen. De beschreven verschijnselen kunnen een rol spelen bij merceriseren onder spanning.

Summary

With the aid of fluorescent staining it is possible, in a microscope specially adapted to this purpose, to investigate the fluorescence dichroism of the cotton fibre.

As a rule, the S and Z spiral parts can thus clearly be seen. The staining is clearly visible in the outer growth layers. Combination of fluorescence microscopy and polarization microscopy with micro-stress experiments shows slip phenomena between the growth layers during stress. These slip phenomena are clearly visible in fibre parts in which a part of one spiral direction is well developed in the outer layers, while in the inner layers this spiral part does not coincide for the different layers.

The phenomena described are probably important for mercerization under tension.

Introduction

The role of the structural architecture of the cotton fibre on the mechanical properties is especially important, when it is tried to improve the fibre properties by chemical treatment.

Light microscopical investigation of the cotton fibre shows that cotton fibres consist of 25-40 concentric cellulose layers (growth layers) encased in a skin or primary wall which contains non-cellulosic materials such as wax and pectic substances [1, 2, 8].

Each growth layer consists of a sheet made up of parallel oriented fibrils which spiral about the fibre at an angle of approximately 45 degrees to the fibre axis [4, 9, 10, 11]. According to the spiral direction there is the distinction between S spirals, clockwise, and Z spirals, anti-clockwise.

While the spiral direction in one place of the fibre is the same for all growth layers, there are frequent reversals of the spiral direction along the fibre axis.

In a fibre length of 1 cm, 20-25 spiral reversals can be found [10]. These reversals are as a rule, for all growth layers in the same place. Figure 1 shows

a schematical picture of the described structure. Although all layers of the secondary wall are identical as to the spiral direction, differences between the layers are possible in spiral angle (pitch). All layers can have the same pitch or the same period [10]. Figure 2 illustrates the two possibilities.

Which one of these two possibilities is present is important for the mechanical properties [10]. When, along the fibre axis, in one place the layers have the same pitch while in other places the principle of the same period is found during stress, stress concentrations can be found in the transition zone.

In the structural investigation of cotton up to now much use has been made of polarization microscopy, electron microscopy, X-ray diffraction and swelling experiments in Cuoxam or other swelling agents [3, 5, 6, 9, 10, 11].

With the aid of these methods, much information has been gained about the architecture of the fibre, as well as about the influence of chemical treatments on the structural elements. In this respect are worth mentioning the experiments of Ziegenspeck [12] who observed in Ramie fibres, oriented absorption of Coriphosphine HK, a fluorescent dye. For fibre technology a fundamental study of weak

¹⁾ Lezing gehouden op het Symposium voor toegepaste microscopie, „Micro 65”, Sheffield, juli 1965.

spots in the textile fibres, of the mechanisms of fibre fracture and of the interaction of structural details of the fibres and the mechanical properties is important.

For the investigation of the mechanism of cross-linking agents and resins on crease resistance and permanent set, it is important to know to what extent slip phenomena between growth layers, occur. As part of the research being carried out under a grant from the Agricultural Research Service, US Department of Agriculture, the influence of torsion and longitudinal stress on single fibres of a number of American cotton varieties was carried out. Several microscopical and micro-mechanical techniques were used for this investigation [7]. In this paper, attention will be focussed on a combined fluorescence and polarization microscopical method for the investigation of the influence of stress on single cotton fibres.

Experimental

Material

For the investigation, mainly fibres of the cotton varieties Bobshaw and Deltapine were used. Accurate data on the physical, mechanical and morphological properties of these varieties are known. Some of these data have been published [10].

Pre-treatment of the fibres

The samples were boiled off for 5 hrs. in a 10% sodium hydroxide solution. After washing in aqua dest. and drying, the fibres were stained for 1 hr. in an 1% Euchrysine 2GNX solution at 60° C. After the staining, the fibres were washed in aqua dest. till the water remained clear. Subsequently the fibres were dried.

Method of investigation

The fibres were put under stress beyond a Leitz fluorescence microscope equipped with a Biopol analyser tube for the investigation of the fluorescence dichroism.

The microscope is, moreover, equipped with a high pressure mercury lamp for the fluorescence microscopy and with a low voltage lamp and polarizer for the polarization microscopy.

Description of the micro-stress device

A fixed clamp is mounted on a base in which a movable clamp is provided in a central opening. This movable clamp is so fitted that it can move without play in the length-wise direction. Motion is effected with the aid of a transmission mechanism. For a length between the clamps of 1.5 cm, one rotation of the knob produces a strain of $1/40\%$. The device can be mounted on the square mechanical stage of the microscope.

Swelling experiments

Fibre parts of approximately 4 mm were swollen at a very low speed in diluted Cuoxam. The dilution was chosen to give a swelling of about 800% in 20 minutes.

The experiments were carried out with the fibre

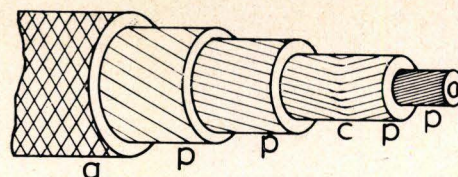


Fig. 1. Model of the cotton fibre with spiral structure and resersal. (a) primary wall, (b) growth layers, (c) reversal (Orr 1961).

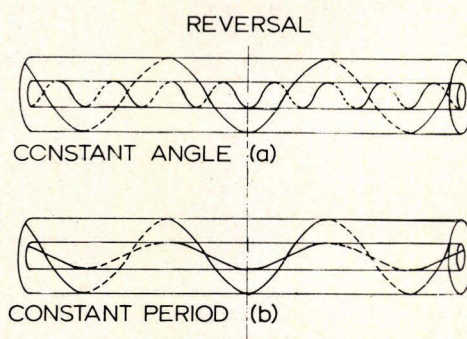


Fig. 2. Diagrams of the fibrillar spiral in the secondary wall of a cotton fibre assuming a constant angle (a) and a constant period (b) (Orr 1961).

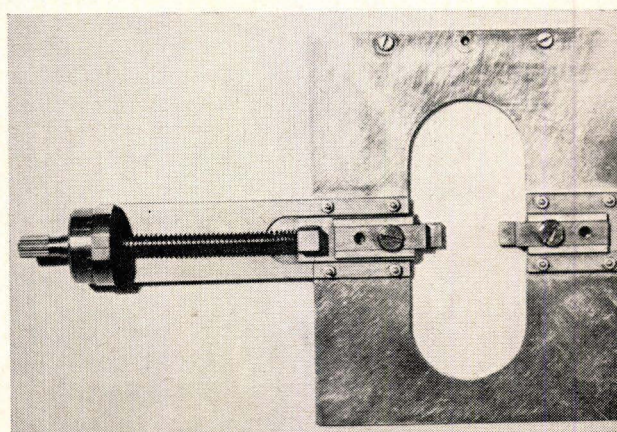


Fig. 3. Micro-stress device.



Fig. 4. Fluorescence dichroism in cotton fibre.



Fig. 5. The same fibre in the polarizing microscope.

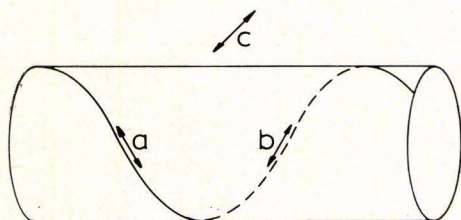


Fig. 6. Orientation of the stretched stain molecules in the fibre. (a) axis of the staining molecule at the upper side of the fibre; (b) axis of the staining molecule at the underside of the fibre; (c) direction of polarization of the analyser.

S			in the Polarizing Microscope			S	Z	S
S	Z	S	in the Fluorescence Microscope			S	Z	S

Fig. 7. A Z part originating during the stress experiment.

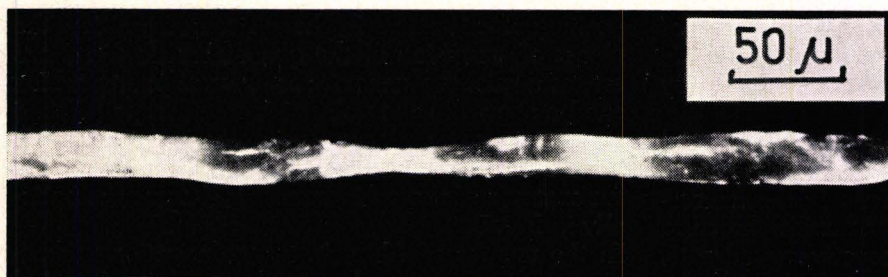
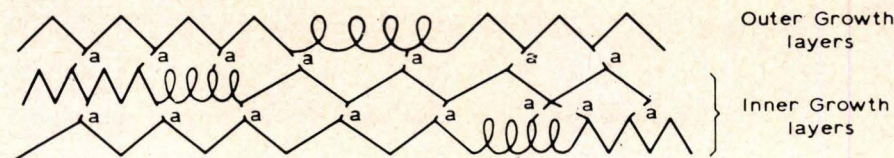


Fig. 8. Cotton fibre, fluorescence dichroism. Maximum extinction for S spiral parts. In the narrow part a small Z region can be seen.



Fig. 9. The same fibre in the polarizing microscope. The Z part in the narrow region of the fibre is not present.

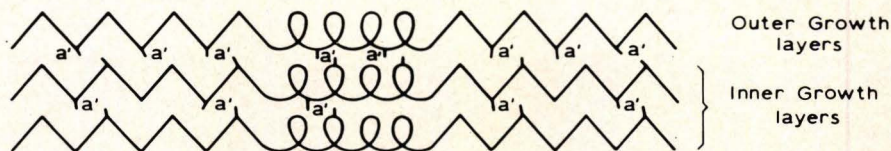


= S Spiral

= Z Spiral

a = link between growth layers

Fig. 10. Fibre in unstressed condition.



= S Spiral

= Z Spiral

a' = severed link

Fig. 11. Fibre under stress.

axis in the orthogonal position beyond a Leitz polarizing microscope.

Results

The fibres, stained with Euchrisine 2GNX, were investigated for fluorescence dichroism. In the unstressed position, as well as in different stages of increasing stress, the angle at which the dichroitic extinction reached its maximum was determined by rotating the analyser.

It was shown that the following two preferential extinction angles could be found in the fibres: 1) according to the positive quadrants in the microscope under an angle of $40^\circ \pm 5^\circ$ with the fibre axis, and 2) according to the negative quadrants, the maximum extinction being reached with the analyser direction making an angle of $130^\circ \pm 5^\circ$ with the fibre axis. Direct comparison with the polarization microscopical results showed that the dichroitic direction mentioned in 1) was found in fibre parts with a Z spiral structure, while the direction mentioned in 2) was found in the S spiral parts.

In Fig. 4, a micrograph of a fibre is shown for which the analyser was fixed for maximum extinction for the Z regions. Principally this extinction can only be determined for the upper side of the fibre. The underside does not show extinction at the same position of the analyser as the upper side, as the stain molecules, due to the spiral architecture of the fibre, are oriented in different directions for

the upper and the underside of the fibre (Fig. 6).

By accurate focussing on the upper side of the fibre, the disturbing influence of the fluorescence of the underside is low. Only in places where the fibre is twisted, or the ribbon-like fibre is curled up on the edges, fluorescence can be seen in the position of extinction. In the unstressed position, the places of transition of the two dichroitic directions are, as a rule, the same as the reversal points, which can be shown in the polarizing microscope (Figs. 4 and 5). In some places, especially in long S or Z parts of the fibres, differences were found. An example of these differences is shown schematically in Fig. 7.

In the unstressed fibre, in the polarization microscope only a nearly homogeneous, long S spiral part is present. In the fluorescence microscope, in this S part, a little region of Z dichroism is found. Even at very accurate polarization optical investigation, nothing of such a Z part can be found. In some cases, somewhat lower birefringence could be seen. Figure 8. shows in the fluorescence investigation, a little Z part. The same fibre part, in the polarizing microscope, does not show a Z spiral part in the same region (Fig. 9).

At a strain of 2-4%, the position of the right half of Fig. 7. originated. In the polarizing as well as in the fluorescence microscope, the little Z part was visible. To explain these observations, cross sections of Euchrisine-stained cotton fibres were made. In the fluorescence microscopical investigation, it was

evident that an outer layer of approximately $\frac{1}{4}$ of the thickness of the fibre wall was stained. The rest of the fibre cross-section contained only very little staining material. In some places, fluorescent material was found in the lumen. When fibres were treated with NaOH or Na₂CO₃ solutions, or were mounted in fluormount (Gurr), an even distribution of the staining in the fibre cross section was obtained.

In the swelling experiments in Cuoxam, in several cases in long fibre parts of one spiral direction it was seen that suddenly at a fairly high degree of swelling (approximately 800%), a small part of the reversed spiral direction originated. In small S or Z parts, this phenomenon is sometimes seen, but these cases, as a rule, only in fibre parts which clearly show growth irregularities.

Discussion

For the explanation of the phenomena described above, it is necessary to keep in mind that in the fluorescence experiments, only the outer growth layers of the fibres were stained intensely, while the inner growth layers contained only very little staining material. In the polarization microscopical investigation, the influence of this thin outer layer is slight.

From the experiments, it is furthermore evident that the small Z part was essentially present in the S part (or a small S part in a long Z part), before the stress experiment. From the fluorescence dichroism observations, it was clear that this small part was well developed in the outer layers.

The results of the polarization microscopical investigation show that in the inner growth layers, the largest part of the fibre diameter, these Z parts did not coincide for the different growth layers.

The situation present in the fibre before the stress experiments is shown schematically in Fig. 10.

The S and Z spirals are partially in the stretched, partially in the compressed position.

This condition of the fibre is stable because of the inter growth layer links (Fig. 1a).

When these links are severed by stress of swelling,

(a' Fig. 11) an equilibrium of stress will develop, due to contraction of the stretched spiral parts. Thus the condition presented in Fig. 11 arises; the Z part becomes visible, both fluorescence microscopically and polarization microscopically.

The links between the growth layers which can be broken by stretching can also be broken by swelling, the process being similar to that of stress. As was the case in the stress experiments, a small part of a reversed spiral direction can originate in a long S or Z part.

At this stage of the experiments, this was only seen at a swelling of about 800%. In mercerization without tension this phenomenon, as a rule, will not be important, the fibre being swollen to a lesser extent. When mercerization is followed by stress, these links between the growth layers will probably be broken, after which because of relaxation, the camouflaged parts, as described above, will become visible.

Conclusions

In cotton fibres, slip phenomena between growth layers occur during stress. In some cases indications were obtained that the fibre structure differed from the structure described in the literature. Slip phenomena occurring in the dry fibre only at fairly high strain, occur in swollen condition without strain or at low strain.

The process of slippage between growth layers is probably important for the chemical modification of cotton fibres.

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