Gradient in Dissolution Capacity of Successively Deposited Cell Wall Layers in Cotton Fibres

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Summary: The dissolution of cotton fibres has been studied at different development stages before and after the onset of secondary wall deposition in solvents of varying quality. We show that the dissolution of the primary wall is inefficient even in good solvents. In moderately good solvents, the inside of the secondary wall dissolves by fragmentation, whereas the outside of the secondary wall swells. These data demonstrate the existence of a centripetal radial gradient in the dissolution capacity within the fibre, which must be related to age-dependent structural variation in the cell wall layers.

Keywords: biosynthesis; cellulose; dissolution; fibres; swelling; walls

Introduction

Cellulose is the most important skeletal component in plants. For many industrial applications, cellulose must be dissolved. When placed in a swelling agent or a solvent, natural cellulose fibres show a non homogeneous swelling. The most spectacular effect of this non homogeneous swelling is the ballooning phenomenon where swelling takes place in some selected zones along the fibres (Figure 1). This heterogeneous swelling has been observed and discussed long ago by Nägeli in 1864,[1] Pennetier in 1883,[2] Fleming and Thaysen in 1919,[3] Marsh et al in 1941,[4] Hock in 1950[5] or Tripp and Rollins in 1952.[6,7] One explanation for this phenomenon is that the swelling of the cellulose present in the secondary wall is causing the primary wall to extend and burst. According to this view, the expanding swollen cellulose pushes its way through the tears in the primary wall, the latter rolls up in such a way as to form collars, rings or spirals which restrict the uniform expansion of the fibre and forms balloons as described by Ott.[8] This explanation assumes that cellulose is in a swollen state in each of the balloons, which remains to be demonstrated. In addition, no data exist on the respective roles of the primary and secondary wall layers in this process. Finally, the different stages that lead to the ballooning phenomenon have not been well described.

Further studies of Chanzy and *al* in 1983[9] and Cuissinat and Navard in 2006[10] have shown that the dissolution mechanism is strongly dependent on the solvent quality. Cuissinat and Navard performed observations by optical microscopy of free floating fibres between two glass plates for a wide range of solvent quality (as an example *N*-methylmorpholine-*N*-oxide, NMMO, with various amounts of water w/w). They identified four main dissolution modes for wood and cotton fibres as a function of the quality of the solvent (the quality of the solvent decreases from mode 1 to mode 4):

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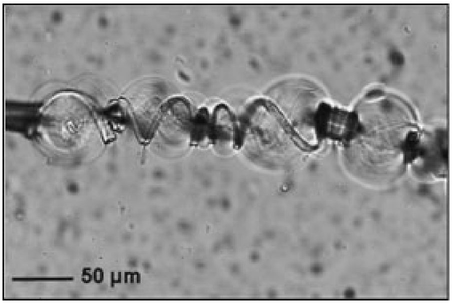

Figure 1.
Gossypium hirsutum cotton fibre swollen by ballooning in *N*-methylmorpholine-*N*-oxide with 20% of water w/w.

Mode 1: Fast dissolution by fragmentation, occuring in good solvent (e.g. in NMMO with less than 17% of water, 90 °C)

Mode 2: Swelling by ballooning and full dissolution, occuring in moderately good solvent (e.g. in NMMO with 19 to 24% water, 90 °C)

Mode 3: Swelling by ballooning and no complete dissolution, occuring in bad solvent (e.g. in NMMO with 25 to 35% water, 90 °C)

Mode 4: Low homogeneous swelling and no dissolution, occuring in very bad solvent (e.g. in NMMO with more than 35% water, 90 °C)

These mechanisms also have been observed with NaOH-water with or without additives,[11] ionic liquids[12] and other solvents[13] for a wide range of plant fibres[14] and some cellulose derivatives that had been prepared without dissolution.[15] From all these studies, it is shown that the key parameter in the dissolution mechanism is the morphology of the fibre. Indeed, as long as the original wall structure of the native fibre is preserved, the dissolution mechanisms are similar for wood, cotton, other plant fibres and some cellulose derivatives.

The cotton fruit is a capsule (commonly called cotton boll) composed of 4 or 5 carpel, each of them bearing about 10 seeds. Each cotton fibre is produced by the outgrowth of a single epidermal cell of the seed coat. A cotton fibre is a single cell, mainly made of cellulose microfibrils arranged in concentric walls. Fibre development can be divided in five main growth stages, initiation, elongation, transition, development and maturation.[16]

- The initiation stage corresponds to the differentiation of epidermal cells into fibre cells and takes place around 2 or 3 days preanthesis.
- The elongation stage corresponds to the synthesis of the primary wall and takes place between 1 and 15 days postanthesis (DPA)
- The transition stage corresponds to the end of the primary wall synthesis and to the beginning of the secondary wall deposition (S1 wall) and takes place between 15 and 25 DPA. At this stage, secondary wall deposition is initiated while the cell is still elongating.
- The development stage corresponds to the massive deposition (without elongation) of cellulose forming the main body of the secondary wall (S2 wall) and takes place between 25 and 50 DPA.
- After about 50 days the cotton bolls is mature and it opens. After opening, the cotton fibres dry out. This stage is called maturation.

The focus of this paper is to study the swelling and dissolution mechanisms of cotton fibres in order to clearly identify and separate the behaviour of the primary and the secondary walls in different solvents. To this end, cotton fibres were collected at successive growth stages, from 7 DPA to maturity and their dissolution mechanisms were studied in very good, good and moderately good solvents.

Materials and Methods

Cotton Production:

Cotton, *Gossypium hirsutum, plants* were grown in a greenhouse of the Cell Biology laboratory at the INRA research centre in Versailles. Unopened cotton bolls were collected on plants at various growth stages

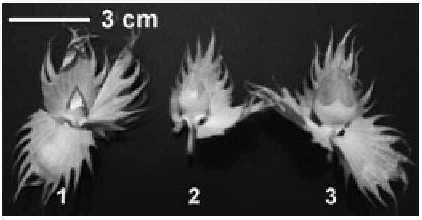

Figure 2.
Cotton bolls at elongation stage (1) 7 DPA, (2) 11 DPA, (3) 14 DPA.

(counted as DPA or days postanthesis) and immediately sent to Cemef in Sophia Antipolis to be studied.

Solvent Preparation:
The solvent mixtures were based on NMMO provided by Sigma-Aldrich and various amount of distilled water were added (w/w), i.e. 20% of water corresponds to 0.2 g of water with 0.8 g of NMMO. The monohydrated form of NMMO-water mixtures contains 13.3% water. As was shown by Chanzy et al.[9] and Cuissinat and Navard,[10] the quality of the NMMO-water solvent decreases with the increase of water content (for 13.3% of water very good solvent, for 16% of water good solvent and for 20% of water moderately good solvent). All the swelling and dissolution experiments were carried out at 368 K to avoid the crystallisation of the NMMO-water system.

Fibre Extraction and Observation:
Cotton fibres were carefully extracted from their bolls (see Figures 2 and 3) at various growth stages, isolated from the seeds and slightly dried with a blotting paper. The swelling and dissolution treatments were immediately performed in NMMO with 13.3, 16 and 20% of distilled water, w/w (noted NMMO- 13.3, 16 or 20% water).

Samples were observed between two glass plates by optical microscopy in transmission mode with a Metallux 3 (Leitz) equipped with a Linkam TMS 91 hot stage at 368 K. The solvent contained in a pipette was injected by capillarity between the two glass plates.

Results and Discussion

A. Elongation Stage Fibres
Cotton fibres taken at elongation stage were studied under the optical microscope. To facilitate the isolation of the fibres, which are wet and adhering one to another and only about 100 μm long, they were dried on a blotting paper and separated in small clusters. The dissolution of these fibres turned out to be impossible in both good and moderately good solvents. In NMMO- 16 and 20% water, the reaction is very slow and leads to a uniform gel like material with no measurable swelling. The elongation stage fibres dissolve only in the very good solvent NMMO monohydrate (13.3% of water). The reaction leads to a uniform gel like material. A subsequent regeneration in water shows that this material was partially dissolved.

These results show that the primary wall of cotton fibres is very resistant to dissolution in solvents that, as will be seen below, are dissolving the secondary wall. Another important observation is that the balloon-

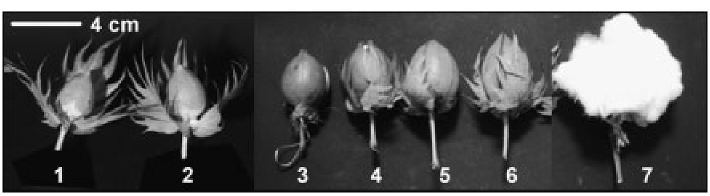

Figure 3.
Cotton bolls at transition stage. (1) 20 DPA, (2) 23 DPA; development stage, (3) 26 DPA, (4) 29 DPA, (5) 30 DPA, (6) just before opening and (7) mature fibres.

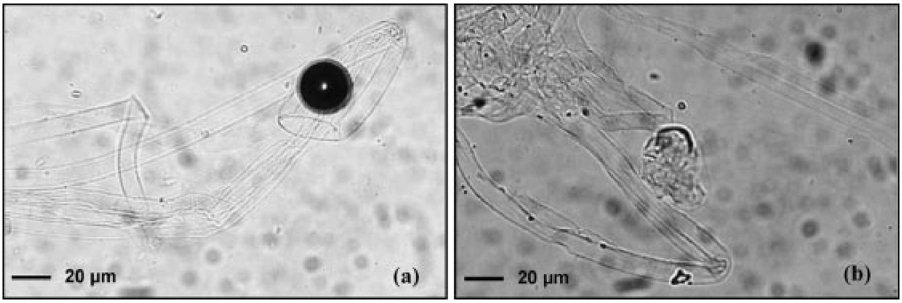

Figure 4.
Swelling and dissolution mechanisms of cotton fibres at the elongation stage. (a) in NMMO- 16% water; (b) in NMMO- 20% water. No ballooning phenomenon is observed.

ing phenomenon is not present with these fibres as shown in Figure 4. This is fully in agreement with the common explanation which attributes the balloons to the swelling of the secondary wall causing the extension and the bursting of the primary wall. Without secondary wall, there are no balloons (note that mature fibres without primary walls are also not showing balloons[3,6,13]).

B. Transition Stage Fibres

At this stage a small number of balloons can be observed as shown in Figure 5a. These balloons are much smaller (swelling ratio around 200–300%) than those observed for mature cotton fibres (swelling ratio around 450–600%). Balloons may appear in areas of the fibre with more pronounced secondary thickening as suggested by the increased contrast (Figure 5b).

This result shows that the balloons are indeed linked to the existence of a secondary wall layer under the primary wall. During the transition stage, the rate of cellulose deposition increases up to 100-fold[15] compared to the elongation stage. It is presumably the swelling of this cellulose located in the S1 wall that causes the extension and the bursting of the primary wall, leading to the ballooning aspect. In the good and moderately good solvent (NMMO- 16 and 20%), the S1 wall cellulose was not dissolving efficiently.

In conclusion, the good and moderately good solvents did not dissolve the primary wall and poorly dissolved S1 wall cellulose.

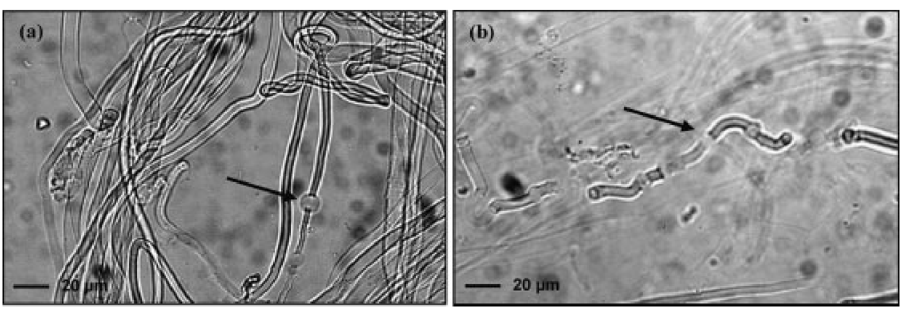

Figure 5.
Swelling and dissolution of cotton fibres at transition stage in NMMO- 16 and 20% water; (a) First balloons appear at the transition stage, (b) outer layer around the inside part that is undergoing ballooning (black arrows).

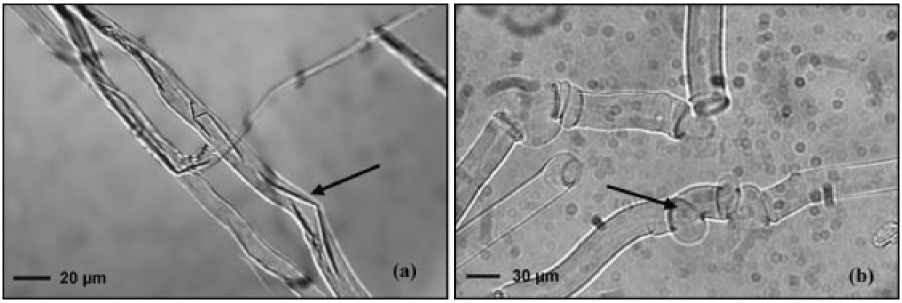

Figure 6.
Swelling and dissolution of cotton fibres at development stage; (a) Dissolution by fragmentation in NMMO- 16% water; (b) Swelling by ballooning in NMMO- 20% water (black arrows).

C. Development and Mature Stage Fibres

• *NMMO-16% Water*

In this good solvent, the fibres dissolved starting from the inside by fragmentation (Figure 6a). Interestingly, the remaining fragments were solid (most probably very crystalline) rod-like pieces, elongated in the fibre direction (Figure 7). This shows that the weak areas, most probably corresponding to less crystalline parts, were also oriented in the fibre direction. It should be interesting to investigate how this heterogeneity in crystallinity arises during the deposition of the cell wall.

The rod-like fragments formed inside the fibre at the beginning of the dissolution process penetrated the undissolved outer layer of the fibre (Figure 6a). The dissolution process occured without ballooning (mode 1) and the fragments eventually dissolved totally. The outer layer dissolved more slowly. It should be noticed that this outer layer was not always visible.

• *NMMO-20 % Water*

In this moderately good solvent, the ballooning phenomenon also occurred. However, balloons were much larger (swelling ratio around 450–600%) than those observed during the transition stage (swelling ratio around 200–300%). The ballooning phenomenon is thus directly linked to the existence of the secondary wall. Balloons, formed by the extending secondary S1 wall, break through the outer layer (Figure 6b). The outer layer rolls up and forms helices and unswollen sections as shown in Figure 1.

With the appearance of the S2 wall, an important phenomenon is observed. The inside of the balloons dissolves by frag-

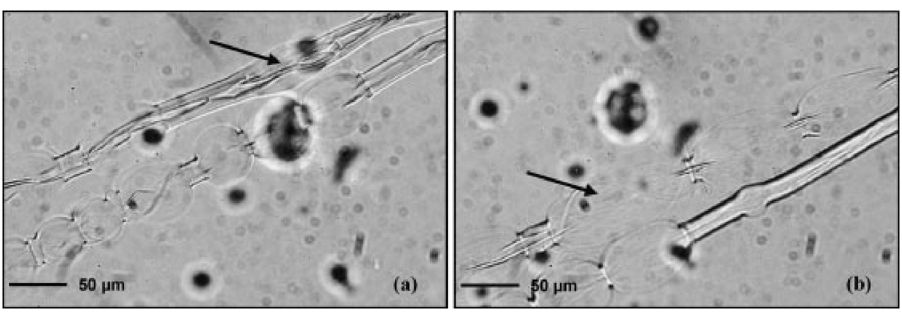

Figure 7.
Mature cotton fibre in NMMO-20% water. Fragments inside the balloons are dissolving and balloons are growing (black arrows). Time is progressing from picture (a) till picture (b).

mentation. A fraction of the cellulose chains inside of the balloons is dissolving and balloons are growing due to the intake of solvent (osmotic pressure). Eventually (Figure 7a and b), all visible fragments are fully dissolved. The inside of the balloons is thus a cellulose solution. The fragmentation is the result of the dissolution of the inner part of the fibre. This event is similar to the fragmentation that is occurring in good and very good solvents as NMMO- 16% water.

At this stage of the dissolution, the fibre was composed of two main zones, balloons (highly expanded zones that broke through the primary wall) and unswollen sections. In the balloon, two zones could be distinguished, the inside corresponding to the dissolved cellulose (obtained by the fragmentation mechanism) and the surface of the balloon, composed of a part of the secondary wall that is not dissolved. We showed by using fibres in their transition stage that the dissolution of the S1 wall is very slow, going through a swollen gel phase before dissolving. The balloons in the mature fibres therefore must correspond to the dissolved S2 wall inside the swollen S1 wall. This swollen S1 wall is what we previously referred to as "the membrane".[10–15] This part is a swollen gel that will dissolve slowly. After the total dissolution of the balloons, the unswollen sections and remainings of the primary wall

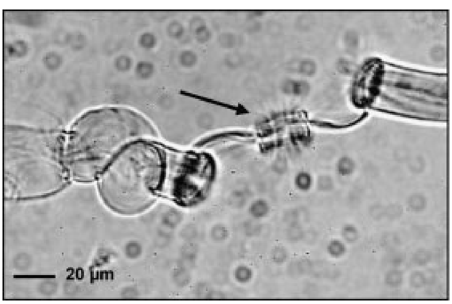

Figure 8.
Swelling and dissolution of a cotton fibre in NMMO-20% water. The S2 wall dissolves first, followed by the membrane of the balloons (S1). The thick helices (remains of the primary wall) and the unswollen sections (black arrow) dissolve later.

(Figure 8) will also dissolve. The sequence of dissolution is thus the following: first the inside of the fibre (S2 wall) by fragmentation, then the S1 wall, then the unswollen sections and remainings of the primary wall.

Conclusion

These dissolution experiments on cotton fibres at different growth stages show that there is a gradient of dissolution capacity from the inside to the outside of the fibre.

1- For fibres with enough cellulose inside the secondary wall, the inside of the fibre is the easiest part to dissolve. The dissolution mechanism is fragmentation where weak parts of the wall are quickly dissolved, leaving rod-like fragments floating, which completely dissolve later. The fact that the dissolution kinetics of crystalline parts is slower than that of the amorphous parts backs the hypothesis that the rod-like fragments are highly crystalline. The finding that these fragments are oriented along the fibre direction while the deposition of the cellulose chains occurs at 35–45° might provide meaningful information on the mechanism of cellulose synthesis and deposition in cotton fibres. The availability of tools for the *in vivo* visualisation of cellulose synthase complexes and the cytoskeleton[17] should facilitate the elucidation of this question.

2- Ballooning appears in fibres having a secondary wall, at least being at the transition stage. Balloons are formed by the expansion of the secondary wall due to the dissolution. It must be surrounded by a membrane (otherwise there would be no balloon). The membrane is clearly formed by the swollen S1 layer of the secondary wall, formed during the transition stage, which is more difficult to dissolve than the S2 layer. Why this layer is more difficult to dissolve is not known, but it must be related to differences in composition and/or architecture.

3- When the secondary wall is swelling by ballooning, the primary wall breaks in localized places and rolls up to form helices

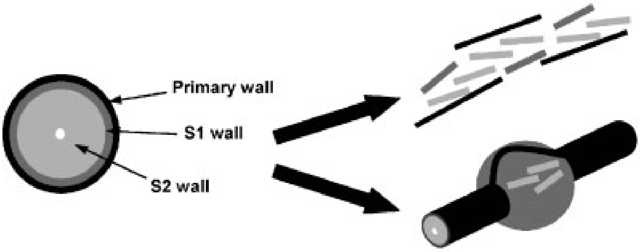

Figure 9.
Schematic representation of the S2, S1 and primary wall behaviour in good (e.g. NMMO- 16% water) and moderately good solvent (e.g. NMMO- 20% water).

and surround fibre sections that cannot be swollen (called unswollen sections[10–15]). The primary wall does not dissolve easily, and even sometimes does not dissolve at all as it is occurring in bad solvents like NaOH-water, where only the inside of the fibre can dissolve.[11]

The mechanisms of swelling and dissolution are schematically represented on Figure 9 in function of the solvent quality.

The study of well characterized cotton fibres in terms of growth stage have shown that most recent deposited cell wall layers (S2 wall) are most easily dissolved. Since the degree of polymerization and the crystallinity increase during the fibre development,[16,18] the dissolution capacity depends, in a first instance, not on thermodynamic (molar mass) or kinetic (crystalline or amorphous) parameters, but on the composition and the architecture of the fibre walls. The absence of noncellulosic polysaccharide networks in younger wall layers may explain their higher dissolution capacity. Considering that the same mechanisms have been observed for wood-based fibres, the same conclusion about the influence of the composition of the cell wall layers on the dissolution should also hold for these fibres.

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