

FLUORESCENT MOLECULAR PROBES FOR THE CHARACTERISATION OF FIBRE STRUCTURE AND DISTRIBUTION OF TEXTILE RESIN FINISHING ON LYOCELL

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The main topic of this work is to develop a method to characterise the pore structure of cellulosic fibres applying fluorescent dyes. Furthermore, fluorescent dyes were also applied to label and quantify additives on the textile surface, as well as to visualise their distribution within the fibres and the yarns. A combination of virtual cross-sections by confocal microscopy, and real cross-sections from microtomy could deliver valuable and detailed qualitative and semi-quantitative information on the distribution of dye inside the fibre cross-section. Quantitative information

on dye distribution relative to cellulose was obtained by Raman microscopy. The fibre pore structure was probed by the optical brightener Calcofluor, approaching a fibre structure model for the influence of production and treatment parameters on the fibre structure during fibre formation and modification. Application examples in textile finishing are the distribution of chitosan and resin finishing agents in fibres and fabrics.

Keywords: *confocal microscopy, fibre, fluorescence microscopy, Raman spectroscopy*

Introduction

The typical surface morphology and the structure of Lyocell fibres are a result of the parameters of the spinning process [1],[2],[3] and have an important impact on the physical properties and the further behaviour treatment of the textile. To adapt Lyocell to the requirements of the market and to avoid technical problems in the further finishing steps, it's necessary to be able to control and to vary these properties. The classical textile tests deliver the effect of these changes on the physical and textile properties, but are not able to supply the link to the internal fibre structure. For this reason, a deep analysis

on the structure formation during the process and the following treatments is indispensable.

The use of dyes for probing pore structure has been known for long time [4],[5]. Of particular interest are rather large molecules which penetrate the fibre completely only under certain conditions, and fluorescent dyes as they can be detected in very low concentrations and in microtome cross-sections. In earlier experiments, calcofluor was found to be a promising dye.

The distribution of textile finishing agents is an important topic in development of

textile products. Uneven distribution on large scale leads to uneven dyeing. Uneven distribution of resin finishing on the fabric cross-section, yarn and fibre level (the microscopic scale) leads to poor mechanical performance. Fluorescent probes selective for textile finishing have been described. As known from earlier works [6] on cotton fabrics rhodamine B can be utilised to as a selective marker of the resin finishing agent on yarns and fabrics.

One of the main limits of conventional light and fluorescence microscopy is out-of-focus blur degrading the image. Defocused information often obscures important structures of interest, particularly in thick specimens. The out-of-focus light leads to a reduction in image contrast and a decrease in resolution. An alternative was found in confocal microscopy. The illumination in a confocal microscope system is sequential in nature. The specimen is not uniformly illuminated throughout its depth, the light being focused on a spot on one volume element of the specimen at a time. It is possible, to obtain illumination spots as small as 0.25 μm in diameter and 0.5 μm deep. Confocal microscopy applied to transparent samples could deliver highly resolved images. Virtual cross-sections can be delivered by a summation of line scans in different depths in the z-axis.

An appropriate quantification method is Raman spectroscopy, a vibrational spectroscopy which can provide multivariate information on the chemical composition of samples. Integrated in a light microscope system, confocal spectra of small objects can be obtained at a spatial resolution of about 1 μm .

Methods

Fluorescence microscopy

The fluorescence on fibres and fibre cross-sections was observed using an Olympus BX microscope equipped with a mercury burner lamp, monochromatic filters and a

digital camera system. The applied dyes on the respective substances are shown in table 1.

Extended focal images were reconstructed from several images of limited depth of focus using the *Analysis* software.

Issue	Label	$\lambda_{\text{Excitation}}$ [nm]	$\lambda_{\text{Emission}}$ [nm]
Resin-finishing	Rhodamine B	500 – 570	590 -
Chitosan	Fluoresceine Isothiocyanate (FITC)	449 – 498	510 -
Accessibility	Calcofluor	210 – 250	410 -

Table 1. Fluorescent labels

Fibre cross-sections were produced using a Reichert (model: 1140/Autocut) microtome after being embedded in a 2-hydroxyethylmethacrylate resin (Technovit7100TM, Kulzer). It was possible to prepare cross-sections down to 5 μm thickness. Cross-sections were prepared at 8 μm thickness from yarns and fibres, and 20 μm thickness from fabrics.

Confocal microscopy

The confocal observation was performed at the Institute of Ecology and Conservation Biology of the University of Vienna using a confocal laser scanning microscope (Leica TCS SP2 in combination with an inverted DM IRB microscope). The system has three lasers: HeNe (633nm/10mW, red), He/Ne (543nm/1,2mW, green) and Ar (458nm/5mW, 476nm/5mW, 488nm/20mW, 514nm/20mW, blue) [7]. FITC fluorescence was excited with the blue lines, rhodamine fluorescence using the green lines. The scans were performed at 0.5 μm of resolution.

Yarns were taken out of the fabrics and evacuated in a water-filled syringe to eliminate air pockets. The yarns were presented on a microscope slide and immersed in water.

Raman spectroscopy

The Raman measurements were performed with a HoloLab Series 5000 Modular Raman Spectrometer (HL5R) from Kaiser Optical Systems Inc. (USA) equipped with f/1.8 optics, transmission grating, multichannel CCD array detector (optimized for NIR), and a 785 nm diode laser (500 mW) coupled via single mode fibre to the microscope (approximately 80 mW on the sample). Between microscope and detector, a confocal fibre with a pinhole (silver coated) of 20 μm was used. For the measurements a 100X (0.9 N.A.) objective was used.

Cross-sections of calcofluor-dyed Lyocell fibres were prepared. The Raman intensity of Calcofluor relative to cellulose was measured.

Resin labelling with Rhodamine B [8]

Resin-finished woven fabrics of Lyocell cloth were dyed with a 10^{-4} M Rhodamine B (MW = 480) solution. The fibres were dyed for 24 h in a 10^{-3} M solution of rhodamine B in a pH 9.3 buffer solution ($\text{NH}_4\text{Cl}/\text{NH}_3$). The dye shows high affinity to the finishing resin. Unfinished fabrics showed a very weak autofluorescence. The fluorescence distribution over the cross-section is a direct representation of the resin distribution.

Dying with Uvitex BHT

Lyocell fibres were treated for various times with an aqueous 1g/L technical Uvitex BHT (Ciba, Basel, CH; an optical brightener, contains Calcofluor, molecular mass 960 Da) solution and dried at room temperature.

Chitosan labelling with FITC [9]

Chitosan-impregnated fibres were dyed with a 10^{-4} g/L fluorescein isothiocyanate (FITC) solution at pH 7 (Acetate puffer) for 1 h at room temperature, washed with

water and ethanol and dried at 60°C. FITC forms a covalent bond with chitosan through the amino groups.

Results and discussion*Observation of Chitosan distribution on cellulosic fibres*

Three types of Chitosan-containing cellulosic fibres were observed.

Chitosan on cellulosic fibres produced by various routes was labelled using the fluorescent dye Fluorescein isothiocyanate (FITC). The distribution of Chitosan on fibres and within the fibre cross-section was observed by classical fluorescence microscopy (Figure 1 – Figure 3). Confocal microscopy was used to obtain depth resolved image data. A reconstruction image over the thickness of the fibre gives an impression of the overall distribution of chitosan in the water-swollen state (Fig. 4).

Calcofluor as a fluorescent molecular probe for fibre structure analysis

The depth and the intensity of dye intrusion into fibres depend on the properties of the dye such as molecular weight, and affinity / substantivity. For a given dye, the distribution in a fibre can serve as a molecular probe for structural properties of the fibre porous system.

The substantive fluorescent dye Calcofluor (MW 960) was used as a molecular probe to explore the surface and the internal pore structure of Lyocell, viscose, modal, and cotton fibres. It's intrusion into the fibre cross-section is limited and depends on time and on the porous structure of the textile fibres. Fluorescence microscopy could deliver qualitative and semi-quantitative information about the dye penetration into different fibres.

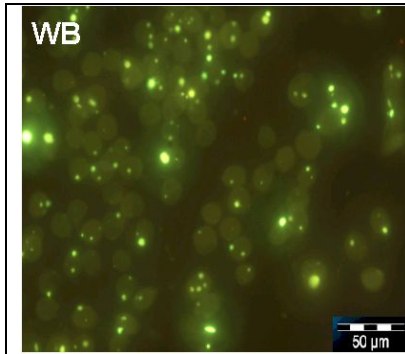


Figure 1. Cross-sections of chitosan-incorporated lyocell fibres

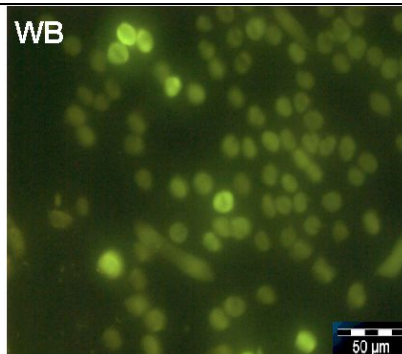


Figure 2. Cross-sections of chitosan-impregnated lyocell fibres

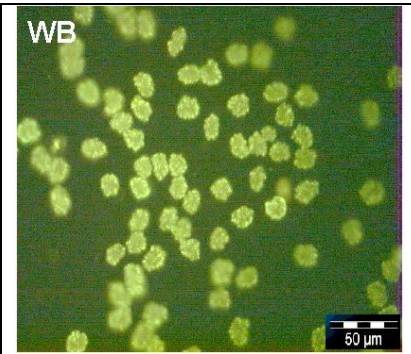


Figure 3. Cross-sections of commercial chitosan-incorporated viscose fibres (Crabylon®)

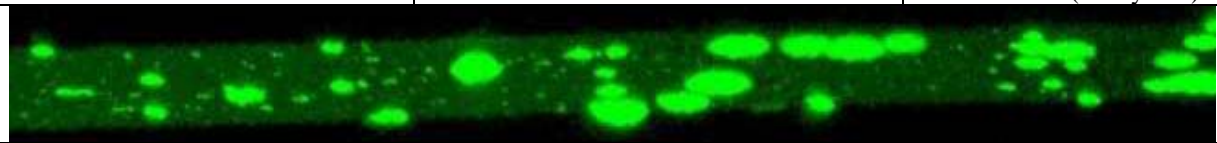


Figure 4. A reconstructed confocal image of a chitosan-incorporated Lyocell fibre in water



- Thinner skin
- Less and thinner macropores
- Appearance of core

Figure 5. Structure model of the Lyocell fibre [10]

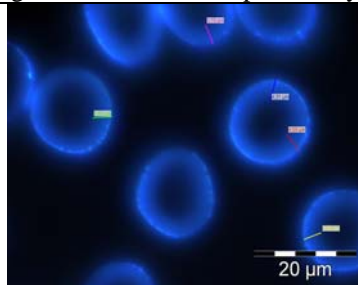


Figure 6. Calcofluor intrusion into Lyocell fibres. Cross-sections after 4 h (left) and 24 h (right)

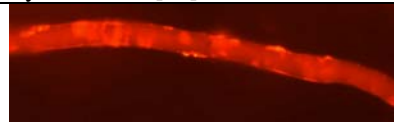
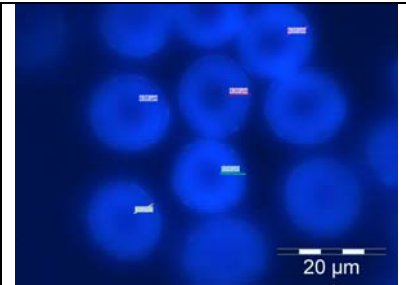


Figure 8. Resin-finished Lyocell fibre (Rhodamin B - labelled, extended focal imaging))

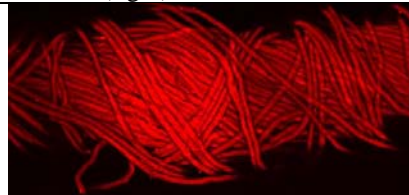


Figure 7. A resin-finished Lyocell yarn (confocal image after Rhodamin B- labelling)

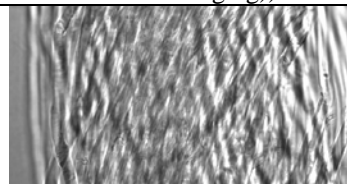


Figure 9. A comparison between a longitudinal internal view of a lyocell yarn interior, using normal light (left) and confocal fluorescence (right)

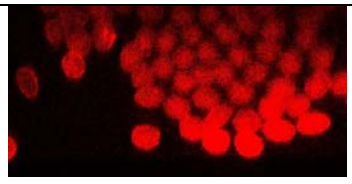
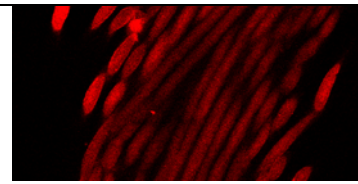


Figure 10. A confocal cross-section of a fluorescence-labelled, resin-finished Lyocell yarn

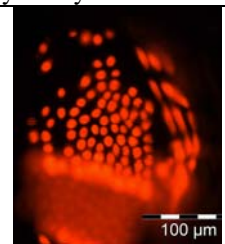
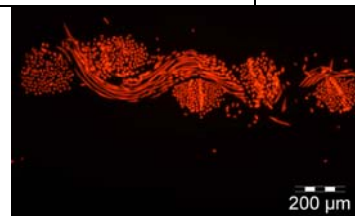
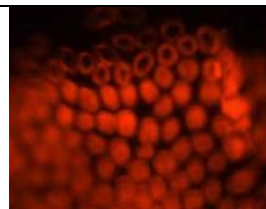
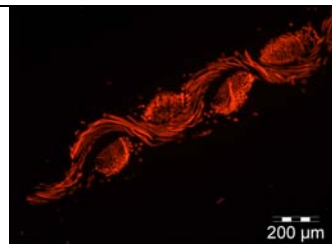


Figure 11. Microtome cross-sections of fabrics and yarns of woven fabric samples A (left) and B (right) (see text for details)

The impact of modifying the spinning and treatment parameters could be observed by the kinetics of the penetration of calcofluor. A correlation of the dye penetration kinetics with the fibre physical properties is also possible and helps to derive the relationship between fibre structure and fibre properties.

Characterisation of the fibre pore structure

Preliminary experiments applying calcofluor show that the never-dried Lyocell fibres are much more accessible to the dye than dried fibres. The intrusion of calcofluor could even reach the centre of the cross-section in some cases revealing a fairly open porous system, which collapses irreversibly after the first drying. This phenomenon wasn't observed on viscose and modal fibres, where the calcofluor dyeing remained superficial.

In earlier works [10] on cellulose crystallisation following the Lyocell process, a model of crystalline core, a porous zone and a membrane fibre skin was suggested (Figure 5). Although the model was made following experiments on the crystallisation out of dope blocks and not in the dynamic spinning process, some parallel characteristics can be seen also in the structure of Lyocell fibres.

Cross-section of fibres dyed with Calcofluor for 4 and 24 hours (Figure 6) were prepared. The fluorescence microscopy observation gave a dye distribution profile which fits with this model.

Visualising the fibre skin

The membrane skin on the fibre surface can be seen clearly after being illuminated with the fluorescent dye. The dye diffusion through the fibre inside is faster than through the fibre skin, which seems to serve as a semi-permeable membrane.

Relative quantification of Calcofluor on fibres by Raman spectroscopy

Complementary to the optical observations, the concentration of Calcofluor through the cross-section could be quantified applying Raman spectroscopy on fibre cross-section. It can be measured relatively to the cellulose amount, which serves here as an internal reference.

A comparison of the dye intensity on the fibre surface and the fibre core was made. Dried and never-dried Lyocell fibres produced under two different spinning conditions were dyed with calcofluor (Figure 14). The intrusion of calcofluor was observed scanning along the cross-sections. Calcofluor was quantified integrating its specific band of the aromatic ring system at 1600 cm^{-1} relative to the cellulose band at 1090 cm^{-1} (Figure 12).

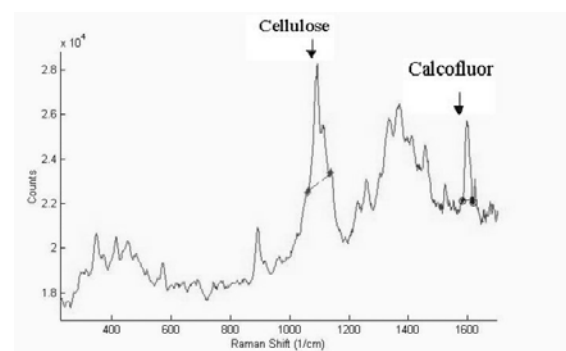


Figure 12. Raman spectrum of calcofluor on a Lyocell fibre, and bands used for quantification

It's obvious, that the kinetic of calcofluor penetration into the fibres differs depending on the spinning and drying conditions. Additionally to the qualitative and the semi-quantitative information obtained from the fluorescence microscopy, Raman spectroscopy can supply quantitative and localised information on calcofluor concentration within the fibre. Cellulose serves here as an internal reference.

Effect of caustisation (lying)

Standard and lyed (14 °Bé) CLY fabrics were dyed with Uvitex BHT for 10 min, 4

and 24 h. The penetration of dye was measured on the fibre cross-sections (Figure 13).

Although initially similar, the intrusion was deeper into the lyed than into the normal fibres. In both cases the fibre

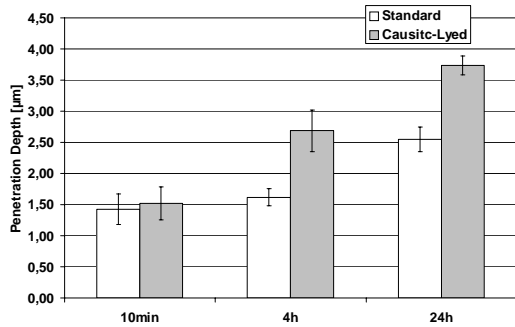


Figure 13. Time scale of the penetration of calcofluor into standard and causticised Lyocell fibres

centre couldn't be reached by the dye.

On the other hand, the fluorescence intensity was in all cases higher on the lyed than on the normal fibres.

Distribution of finishing resin

Resin-finishing on Lyocell stained with rhodamine B was observed in fluorescence microscopy of longitudinal views on fibres (Figure 8), confocal imaging (Figure 7) and fabric cross-sections. The distributions on the surface of fibres were sometimes uneven (Figure 8).

Virtual cross-sections of yarn were obtained by summing up line scans in different depths of the yarn. The conductor effect of the fibres could be eliminated, delivering highly resolved images (Figure 9).

The main problem was the depth of detection on yarns, where the decreasing signal could lead to wrong conclusion. Only the outer 3 – 4 layers of fibres could be observed accurately (Figure 10). This can be avoided combining the observations with the ones from microtomy and conventional microscopy. Any how, preparation-free virtual cross-sections were produced and gave the first approach to the distribution of fluorescent agent in the fibres.

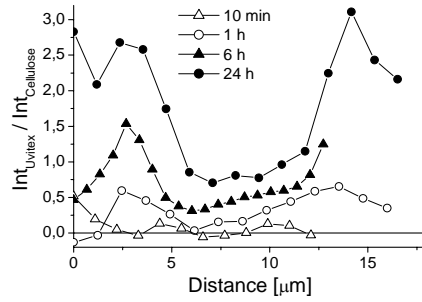


Figure 14. Raman intensity of Calcofluor relative to cellulose through fibre cross-sections over dyeing time. The rims of the fibre were at about 1 µm and 13 µm along the distance scale.

Figure 14a. never-dried Lyocell (type 1)

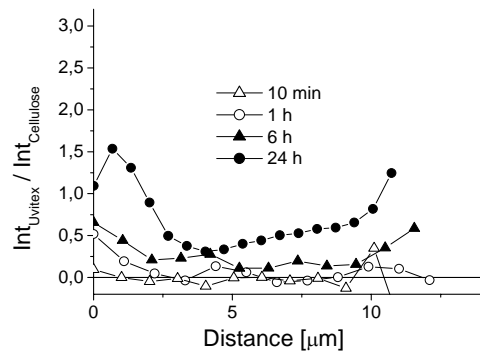


Figure 14b. dried Lyocell (type 1)

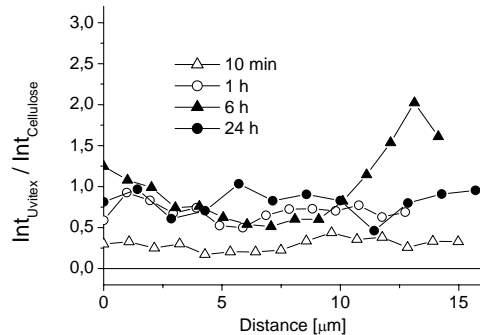


Figure 14c. never-dried Lyocell (type2)

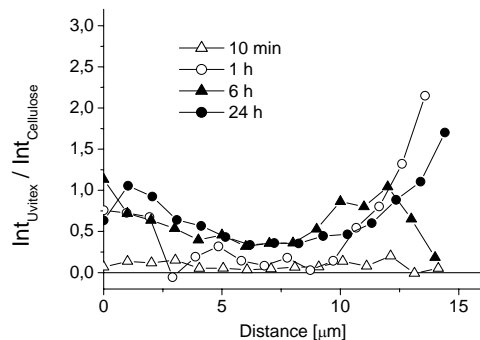


Figure 14d. dried Lyocell (type2)

Effect of drying on resin distribution

Lyocell fabrics were resin-impregnated (Fixapret ECO, BASF), dried under two different conditions and polymerised at 180°C. The samples were dried after the impregnation under the following conditions:

A: Pad - Dry at 180 °C - Cure at 180 °C for 40 s.

B: Pad - Dry at Room Temperature - Cure at 180 °C for 40 s

The samples dyed with rhodamine-B and the fluorescence images were taken on real microtome and virtual confocal cross-sections (Figure 11).

Non-finished fibres showed a very weak rhodamine B fluorescence although the cross-section. On the quickly dried sample A, the dye occurs as a ring around the fibre cross-section. The resin monomer seems either to migrate outwards in both yarn and fibre levels during the drying, or not to be able to diffuse into the fibre due to oligomer formation at the high temperature. In the second case, the diffusion is mostly limited in the more exposed zones and the monomer doesn't have enough time before the polymerisation.

On the slowly dried sample B, the fluorescence occurs through the whole cross-section. This indicates that the resin distribution remains homogenous through the whole fibre/yarn cross-section.

Correlation with serviceability: Sample B with even resin distribution had significantly better serviceability regarding crease resistance, weight loss in the Martindale abrasion test and yarn strength. Yarn elongation remained unaffected.

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