Experimental Report

Proposal: **9-13-469** **Council:** 4/2012

Title: Nanoscale deformation mechanisms underlying the strength of cellulose-based materials

This proposal is a new proposal

Researh Area: Chemistry

Main proposer: **JARVIS Michael**

Experimental Team: JARVIS Michael
THOMAS Lynne

Local Contact: FORSYTH Trevor
MARTEL Anne
GRILLO Isabelle

Samples: cellulose, (C6H11O5)20000, partially deuterated

Instrument	Req. Days	All. Days	From	To
D33	3	2	16/11/2012	18/11/2012
D19	9	8	15/11/2012	23/11/2012

Abstract:

Plants and materials derived from them, such as wood and natural fibres, are nanocomposites that derive their strength largely from cellulose microfibrils. Each cellulose microfibril contains ordered and disordered domains that co-operate in determining its mechanical properties. The accessibility of cellulose is the key to its suitability as a substrate for biofuel manufacture. Hydroxyl groups in the disordered domains are deuterium-exchangeable, providing SANS contrast and permitting the WANS contributions of the ordered and disordered domains to be separated. By carrying out these experiments under mechanical tension we will characterise the relative importance of reorientation, stretching and shear slippage of the microfibrils in the deformation of Sitka spruce wood. These experiments will be extended to non-coniferous plant materials.

Experiment Number 9-13-469: Nanoscale deformation mechanisms underlying the strength of cellulose-based materials
Beamlines D19 and D33, December 2012

Background

Utilising wood is one of the best carbon-neutral ways to reduce the consumption of fossil fuels and carbon-intensive building materials. Wood comprises more than half of the biomass on the planet and globally, wood synthesis provides more long-term carbon sequestration than any other process in the biosphere. Wood is a sophisticated material with complex nanoscale structure that critically influences its mechanical properties [1].

The wood cell wall is a nanocomposite of cellulose microfibrils in a matrix of other polysaccharides and lignin [2]. Through a combination of spectroscopic and scattering experiments we have shown that about half of each microfibril of higher-plant cellulose consists of the crystalline cellulose allomorphs Iα and Iβ [3-5]. The structures of these crystalline forms are well established from neutron diffraction [6-8]. The remainder of each microfibril is less ordered and partially hydrated, but relatively well-oriented. Water modulates the structure of both disordered [3] and crystalline [9] domains in wood and influences its mechanical properties [10,11]. Wood in a living tree is water-saturated and its ability to dissipate strain energy and impede fracture is dependent on the association of water with the disordered polymers. Combinations of ordered and disordered domains are a key feature of other strong biological materials like spider silk [12], but in wood the mechanisms appear to be different.

Vibrational spectroscopy and neutron scattering, if they can be carried out on samples under mechanical stress, are very effective ways in which to probe the mechanical behaviour of the domains accessible to water and hence to deuteration. We have developed the use of bandshifts in the vibrational spectra to identify the way in which tensile loads are distributed between individual polymers. WANS coupled to specific deuterium exchange of hydroxyl groups in disordered regions permits the behaviour of these regions under tension to be separated from the behaviour of the crystalline regions inaccessible to D_2O. SANS under similar conditions, and in the presence of additional D_2O, permits changes in the orientation and spacing of the microfibrils to be evaluated. An integrated programme of WANS and SANS experiments, on beamlines D19 and D33 respectively, was therefore planned.

Methods

A chamber capable of controlling relative humidity in either H_2O or D_2O was already available on D19 and was thought to be capable of being adapted for use on other beamlines at ILL, although it had not previously been tried on D33. Using this system we had shown that it is possible to control the moisture content of small wood samples with good precision at any desired point within a range from approximately 3% to 20% by mass, and with any H:D ratio.

For preliminary trials in 2011 a tensile testing device was constructed fit inside the ILL humidity chamber. This device allows for samples with lateral dimensions 8 mm x 500 μm, to put as much sample volume as possible into a 9 mm diameter neutron beam. The device needed to be not only very compact but also robust enough to cope with the relatively large loads (up to 1 kN) required to stretch these large samples to the point of fracture. In general the whole diffraction pattern was not recorded, only segments corresponding to the intense 004 axial reflection and the principal equatorial reflections (1-10, 110, 200) so that axial extension, reorientation and any lateral contraction on the unit cell could be measured. For static experiments on beamline D19 the foil excapsulation method described in the report on experiment 9-13-300 was used to control moisture content and deuteration.

Results and Discussion

SANS Experiments on Beamline D33. In previous SANS experiments on spruce wood we had found that coherent scattering from whole, loosely arrayed cellulose microfibrils gave an equatorial Bragg peak with an approximate *d*-spacing of 3 nm. Increasing the level of hydration with D_2O increased the intensity of the Bragg peak and also increased the *d*-spacing, as the microfibrils were forced apart [3,13]. Experiments of this kind had not previously been carried out under mechanical tension.

In initial experiments there were problems in obtaining equilibration with D2O vapour, but we were able to demonstrate that the orientation distribution of the microfibrils became narrower under tension, reaching a minimum width of orientation distribution $\sigma = 5°$. When the equilibration problem was eventually overcome, a small increase in *d*-spacing under was observed immediately when tension was applied. When the sample was then maintained at constant extension for 30 min the *d*-spacing relaxed towards the initial value with kinetics similar two one of the two phases of stress-relaxation observed in mechanical and FTIR-bandshift experiments. This is a very interesting result that will help us to understand the mechanism of deformation of wood under tension. We wondered if this sequence of events might trap some D_2O between the microfibrils and give rise to some deuteration resistant to equilibration with the atmosphere, so one sample that had been part of the previous experiment was checked when exposed to the open air. It showed no Bragg peak so there was no permanent D_2O uptake under strain.

Static SANS experiments were also carried out, without mechanical stress. In this case hydration with D_2O or H_2O was maintained using the foil encapsulation method developed previously [3]. The aim of these experiments was to find out lignocellulosic materials from flowering plants showed the same hydration-dependent Bragg peaks as cellulose in the conifer Sitka spruce. Bamboo, related to grasses and cereals, gave a strong Bragg peak at similar *q* values to Sitka spruce, indicating contact between 3.0 nm microfibrils in the dry state. However in contrast to spruce cellulose the intensity of the Bragg peak from bamboo cellulose remained considerable even in the dry state, possibly indicating that microfibril surfaces in bamboo were more accessible to deuteration.

Cellulose from the hardwoods birch and cherry, and from the very strong vascular tissue of sunflower stems, gave only weak Bragg peaks even when hydrated. In each of these materials some Bragg intensity was retained in the dry state but the *d*-spacing was greater than for spruce cellulose, primary-wall cellulose from celery or bamboo cellulose. There is no other evidence that cellulose microfibrils from hardwoods are greater in diameter than those of conifer wood or primary cell walls so it seems possible that the aggregated hardwood microfibrils were separated by lignin. The cellulose from the setae (spore stalks) of the moss *Polytrichum*, which gives rather similar WAXS patterns to celluloses from higher plants, showed no sign of any Bragg scattering and would appear not to form regular microfibril aggregates.

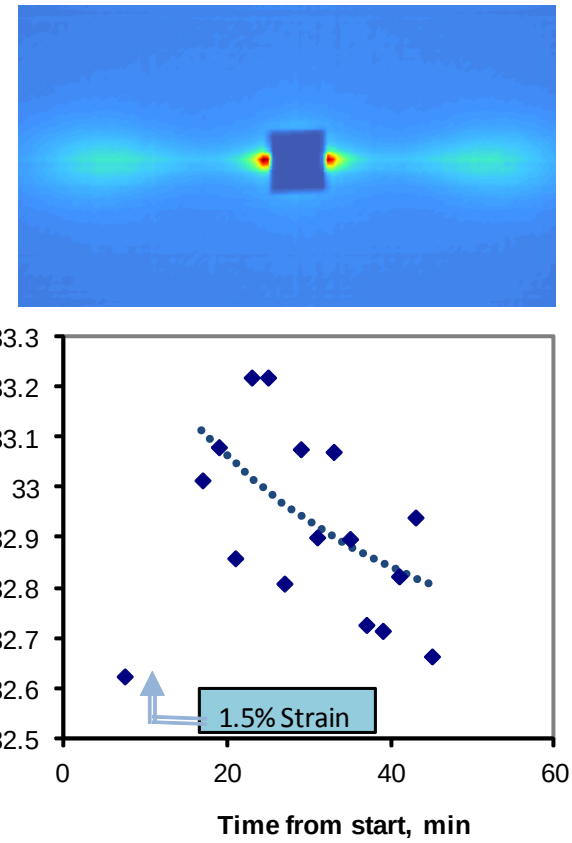

Figure 1. **A**: SANS pattern (from hydrated spruce wood. The fibre axis is vertical. **B:** Under sustained tension the *d*-spacing corresponding to the equatorial SANS Bragg peak (microfibril spacing) first expanded and then slowly relaxed towards the original value.

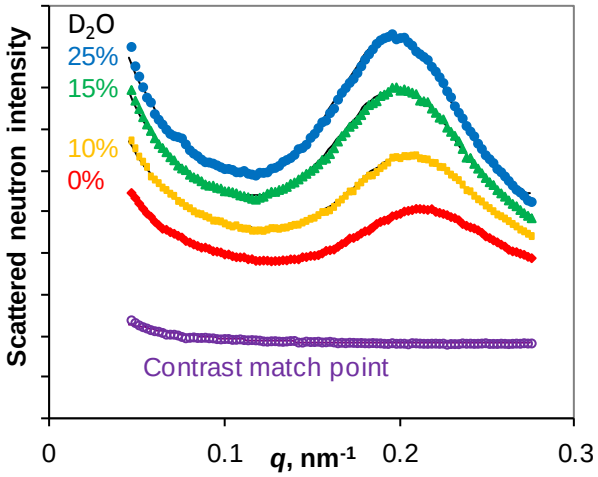

Figure 2. Equatorial SANS Bragg peak from bamboo fibres, increasing in intensity and in *d*-spacing as cellulose microfibrils separate on hydration with D_2O.

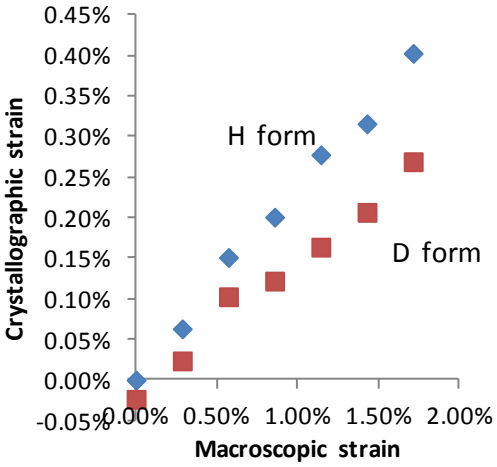

Figure 3. Crystallographic strain measured from the 004 reflection in the neutron diffraction pattern of cellulose from spruce wood, under tension with and without deuteration of accessible cellulose chains

WANS Experiments on Beamline D19. A set of experiments on spruce wood under tension had been carried out in the previous year but there had been problems in processing the data. These problems were solved during the present series of experiments the data sets from both sets of experiments were considered at the same time. In both cases the focus was on the 004 axial reflection which responds to stretching of the lattice within the microfibrils, and is intensified when the accessible surface hydroxyls of the microfibrils are deuterated.

Because of the relatively long time (several hours) required for data collection there was considerable stress relaxation during each experiment, and the crystallographic strain recorded from the 004 reflection was only about 20% of the macroscopic strain applied to the wood samples. As a result the crystallographic strain was quite difficult to measure. The procedure adopted was to use the corresponding area of the equatorial images (from the same pixels of the detector) to carry out a very precise background correction and then use a bandshift fitting method developed for vibrational spectroscopy to estimate changes in the q value of the 004 reflection.

The results contained a considerable amount of scatter but the general pattern was that less movement of the 004 reflection under tension was observed after deuteration. The orientation distribution could not be measured as precisely as by SANS but did not appear to differ significantly between the D and H forms. These experiments did not lead to an unambiguous conclusion but the most straightforward implication is that stress transmission to D_2O-accessible microfibrils is less effective than stress transmission to inaccessible microfibrils within larger aggregates, and the D_2O-accessible microfibrils therefore stretch less when the whole structure is under tension. An alternative explanation might be that there is incomplete stress transmission *within* microfibrils, but this would go against most currently accepted models.

References

[1] Burgert I. & Fratzl, P, *Integrative and Comparative Biology* 49, 69-79 (2009)
[2] Xu, P., Donaldson, L. A., Gergely, Z. R. & Staehelin, L. A.. *Wood Science and Technology* 41, 101-116 (2007).
[3] Fernandes, A.N., Thomas, L.H., Altaner, C.M., Callow, P., Forsyth, V.T., Apperley, D.C., Kennedy, C.J. & Jarvis, M. C. *Proceedings of the National Academy of Sciences of the USA* **108,** E1195-E1203 (2011).
[4] Sturcova, A., His, I., Apperley, D. C., Sugiyama, J. & Jarvis, M. C. *Biomacromolecules* 5, 1333-1339 (2004).
[5] Kennedy, C. J., Sturcova, A., Jarvis, M. C. & Wess, T. J. *Cellulose* 14, 401-408 (2007).
[6] Nishiyama, Y., Sugiyama, J., Chanzy, H. & Langan, P. *Journal of the American Chemical Society* 125, 14300-14306 (2003).
[7] Jarvis, M. *Nature* 426, 611-612 (2003)
[8] Nishiyama, Y., Johnson, G. P., French, A. D., Forsyth, V. T. & Langan, P.. *Biomacromolecules* 9, 3133-3140 (2008).
[9] Zabler, S., Paris, O., Burgert, I. & Fratzl, P. *Journal of Structural Biology* 171, 133-141 (2010).
[10] Keckes, J. et al. Nature Materials 2, 810-814 (2003).
[11] Altaner, C. M. & Jarvis, M. C. Journal of Theoretical Biology 253, 434-445 (2008)
[12] Vollrath, F. & Porter, J. Soft Matter 2, 377-382 (2006).
[13] Thomas, L.H., Forsyth, V.T., Sturcova, A., Kennedy, C.J., May, R.P., Altaner, C.M., Apperley, D.C., Wess, T.J. & Jarvis, M. C. *Plant Physiology* 161, 465-476 (2013).
[14] Jarvis, M. C. *Plant Physiology Plant Physiology* 163, 1483-1484 (2013).