ESRF	**Experiment title:** Influence of pressure on hydrogen bonded polymers; polyamides and biopolymers	**Experiment number**: Sc1279
Beamline: ID11	**Date of experiment**: from: November 4th 2004 to: November 9th 2004	**Date of report**: January 14th 2005
Shifts: 15	**Local contact(s)**: Silvia Capelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Sanjay Rastogi
Ann Terry
Esther Vinken
Denka Hristova

Report:

In two previous allocations of beamtime as part of this longterm proposal, we confirmed that nylon could indeed be dissolved in water at moderate pressures. There is great potential in such an environmentally friendly processing route avoiding the typical use of strong acids to dissolve nylons. Since it is the disruption by water of the hydrogen bonding inherent in the formation of the beta-sheet structure of nylons that leads to this dissolution process, it must be possible to dissolve other hydrogen bonding polymers. For this reason, this work has been expanded to include naturally occurring, hydrogen-bonding bio-polymers such as keratins from feathers and wool, silks and celloses with most of our studies concentrating on keratin.

Small quantities of the barbs of contour feathers from a crow were placed in a thin walled capillary and water added such that the ratio of feather to water is <25vol.%. The capillary was placed in a specifically designed copper block containing a heating element as well as a platinum resistance thermocouple which is placed as close as possible to the beam position in the sample. A small entrance hole and exit cone was cut through the block to allow passage of the X-ray beam and diffracted signal. The top of the capillary is sealed using an o-ring. The heating element was controlled with the Linkam control unit TMS94. The sample was heated and cooled from room temperature to 220ºC (typical heating rates are 10-20ºC/min. The water in the capillary is unable to evapourate and so the vapour pressure increases leading to superheated water (but below the supercritical point) in contact with the sample. Eventually the design will allow the pressures developed within the cell to be monitored and perhaps even controlled.

Fig.1 shows the initial WAXD pattern for feather and water at room temperature before heating. A background pattern of the capillary and water has been subtracted. The data still requires correcting for multiple scattering due to the wall thickness of the glass but clearly a very weak oriented pattern is observed. A strong reflection is seen at $2\theta=1.2$ (corresponding to a d-spacing of 23.7Å) and further weaker signals at higher angles. The exact crystallographic structure is still being determined. No changes are observed in the pattern until a temperature of 116ºC when the intensities begin to drop. Above 147ºC, it is very difficult to

determine the reflections about the noise. Heating was continued until 180ºC and then the sample cooled at 10ºC/min. The diffraction pattern observed prior to dissolution is not observed again, only a high intensity close to the beamstop. The final solution is very red in colour. Previously we have seen that complete dissolution of the feather is possible if held at high temperatures (180-220ºC) for 30-90 minutes.

To confirm that the conformational changes were not just due to the heating, a sample without water was subjected to the same heating and cooling regime. Even at 220ºC, the feather still diffracts with no apparent change in form or degree of crystallinity.

If the solution after dissolution of feather is extracted and a droplet is placed on a cleaned glass slide, it is possible to grow very fine, needle-like crystals (fig.2). The re-crystallization is very slow (of the order of days) and appears to require some impurity to initiate it; indeed no crystalline structure is observed by WAXD of a solution left at room temperature for 10 hours.

The exact nature of the dissolution (or denaturing) is the focus of continuing study. We now know that the final solution consists of residues of 5-6 amino acid sequences in length although the structure from circular dichroism measurements may be random coil rather than beta-sheet.

We have also demonstrated that the same dissolution technique is successful for silks (both insectide and aranid) and hydroxy(propyl) cellulose. Work is continuing on these materials.

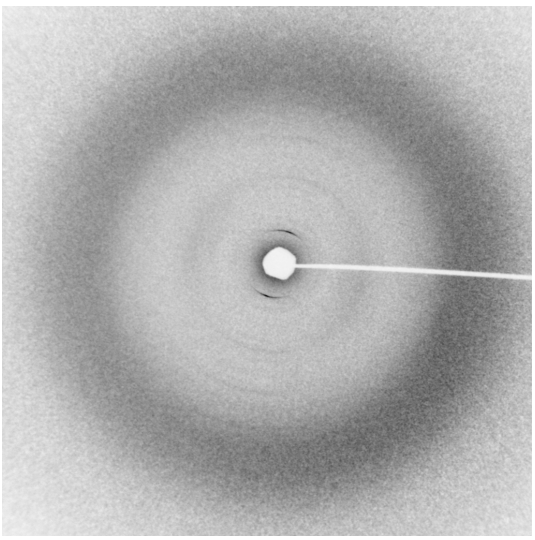

Figure 1: WAXD pattern of feather keratin in water after partial background subtraction of water and glass. Taken using the Frelon CCD at ID11, (λ=0.4956Å, beamsize = 300x300µm)

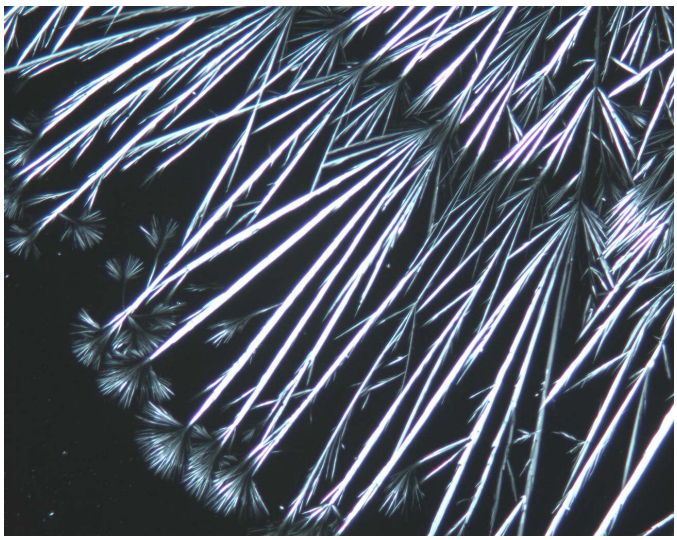

Figure 2: Optical micrograph between crossed polars of the crystals grown from feather keratins dissolved into water at 220ºC for 90 minutes in a sealed reactor.