ESRF	**Experiment title:** Investigation of beta-sheet formation during shearing of regenerated silk solutions by small-angle X-ray scattering	**Experiment number**: SC884
Beamline: ID02	**Date of experiment**: from: 28.6.03 to: 30.6.02	**Date of report**: 27.2.04
Shifts: 9	**Local contact(s)**: V. Urban, P. Panine	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

C. Riekel, ESRF

M. Roessle, ESRF (now EMBL c/o Hasylab, Hamburg)

Report:

The structural evolution of regenerated Bombyx mori silk fibroin during shearing with a Couette cell has been studied in-situ by combined SAXS/WAXS experiment.(Roessle, Panine et al. 2004) The configuration of the SAXS/WAXS set-up used in this study provided a SAXS-range of $0.05 < Q(nm^{-1}) < 0.6\ nm^{-1}$ and a WAXS-range of $7 < Q(nm^{-1}) < 50\ nm^{-1}$. The SAXS data and the WAXS data were recorded simultaneously by means of two CCD-cameras. Fig.1 shows change of the viscosity (η) on increase of the shear rate ($\dot{\gamma}$). Characteristic SAXS-patterns recorded in the 4 zones (see text) are also shown above.

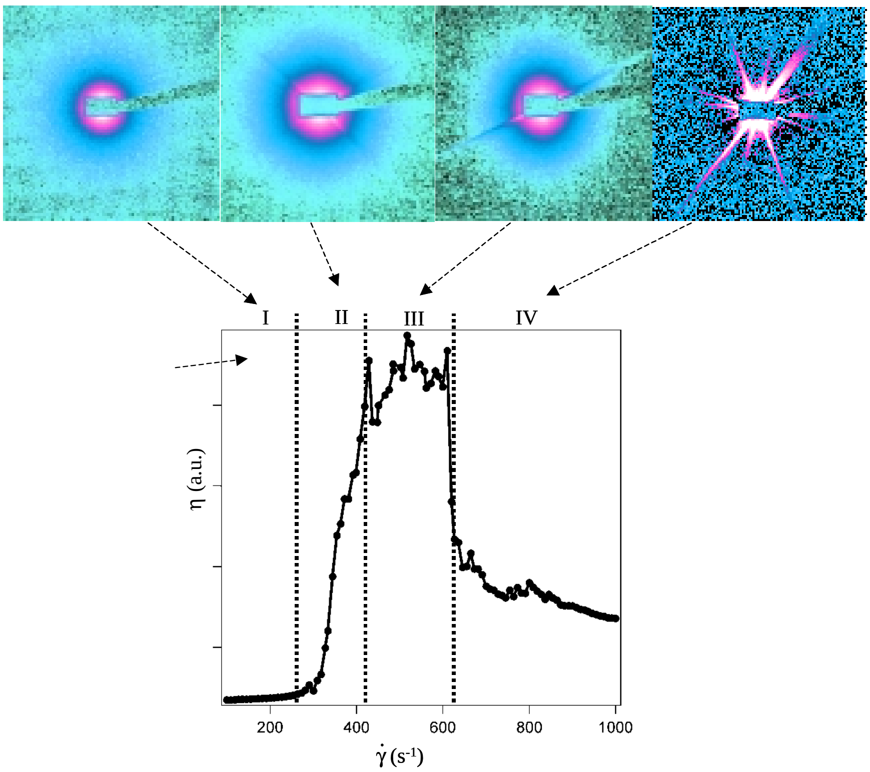

Fig.1

The pre-formation zone (I) starting from $\dot{\gamma}$ =100s^{-1} to ≈250s^{-1} with a nearly Newtonian flow where the viscosity η is practically independent of the shear rate $\dot{\gamma}$. The second zone (II) from $\dot{\gamma}$ ≈250s^{-1} to ≈450s^{-1} shows a non-Newtonian behavior with a fast increase of η by a factor of about 4. In the subsequent zone (III) up to $\dot{\gamma}$ ≈600s^{-1} the appearance of wiggles can be related to rheological instabilities due to the appearance of a solid phase in coexistence with the liquid phase. The formation of solid particles was also optically visible. In zone IV the viscosity drops by a factor of 2 within a small increase of the shear rate.

SAXS data showed different behavior for the different zones of the viscosity change. For the zones I/II the scattering patterns are isotropic and characteristic for independent, randomly oriented particles in solution. (Fig.2) The formation of aggregates at higher shear rates leads to refraction effects at the particle-water interface. The resulting intensive streaks in the scattering patterns impede further analysis of the SAXS data for the subsequent following regions III and IV.

Solution scattering data were analyzed through the radius of gyration, Kratky plot and low-resolution solution scattering analysis using GNOME and DAMMIN program packages. An elongation of fibroin molecules was observed with increasing shear rate, followed by an aggregation phase. WAXS data showed that the aggregated material obtained during shearing was amorphous. The aggregates were found to be with β̃-conformation according to IR-spectroscopy.

Complimentary scanning X-ray microdiffraction on ID13 with a 5 µm beam on aggregated material, which had solidified in air, showed silk II reflections and a material with equatorial reflections close to the silk I structure reflections but with strong differences in reflection intensities. This silk I-type material shows up to two low-angle peaks suggesting the presence of water molecules which might be intercalated between hydrogen-bonded sheets.

Roessle, M., P. Panine, et al. (2004). "Structural evolution of regenerated silk fibroin under shear: combined wide- and small-angle X-ray scattering experiments using synchrotron radiation." *Biopolymers* in press.