ESRF	**Experiment title:** Fibre diffraction studies of new alginic acid copolymers of bacterial origin.	**Experiment number**: 01 02 649
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Shifts: 6	**Local contact(s)**: **Dr. Philip PATTISON**	*Received at ESRF:*

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Report:

The fibre X-ray diffraction technique was used to study crystallinity and crystal structure of a group of alginic acid copolymers (alginates). We have investigated fibre samples with varying degree of crystalline order. We have studied the effect of humidity on the unit cell parameters and sample crystallinity. A chamber with control of both temperature and relative humidity of the sample environment was used.

Wide angle X-ray diffraction patterns of alginate samples at room temperature and at different relative humidity were recorded using focussed beam (diameter 300 and 500 μm) with λ = 0.0875nm and a MAR345 image plate detector operating in the 2300x2300 pixels mode. Typical exposure time were between 30s - 10 min. The samples were glued to the top of thick glass rods or mounted in 0.3 mm capillaries.

The first shift of the beamtime was used for changing the optics setup from parallel to focussed beam, alignment of the beamline, calibration of the wavelength using a Si powder standard, calibration of the sample-detector distance.

The next 2 shifts were used to collect diffraction patterns of different alginate samples. During these 2 shifts we have tested and selected samples to be used in the experiments with controlled humidity (see Figure 1) Different experimental parameters like sample-detector distance and sample-beamstop distance were adjusted to optimise the signal to noise ratio and reduce the background level as much as possible. It was planned to subtract the major part of the background produced by air-scatter in the data processing stage. Therefore, patterns without sample and but with all the other exposure parameters constant were also recorded.

In the next stage of the experiments (last 3 shifts), a sample chamber with adjustable humidity was used. The sample was first placed in the stream of dry N_2 gas and the diffraction pattern was recorded. Next the relative humidity was increased to 90%. Data were collected repeatedly for a period of around 2h. In two runs a crystalline sample of pure poly-manuronic acid (polyM) and an alginate with 67% content of guluronic acid (HiG) were examined. In addition diffraction patterns of samples sealed in capillaries and saturated with water vapour (RH ~100%) were collected.

Difficulties.
Within the first 16h of the beam time a periodic fringe-like noise on the recorded diffraction patterns was discovered (see insert in Figure 1B). Some tests indicated that these fringes are an artefact produced by the MAR image plate detector. They are not clearly visible for strongly scattering samples yielding to low background and sharp diffraction signals. For small and weakly scattering samples with broad diffraction features, the variation in the background due to that noise is comparable to the intensity of the diffraction signals recorded. The relative level of noise is amplified when background is subtracted. It was observed that the fringe-like noise in not reproducible between different diffraction patterns and therefore it is not possible to eliminate it by subtracting patterns recorded without sample.
It was also noticed that when the standard operating procedure of the detector was followed, the erasure of the recorded image was not completed. This was especially noticeable when a series of similar diffraction patterns were recorded. After 20 exposure/erase cycles when there was only a small change in the scattering from the sample, substantial amount of unerased information was accumulated. This could only be erased by a additional series of extra erase cycles.

Results
Due to the detector problems mentioned above we were not able to correct for background as planned. The detector noise was particularly visible for the weakly scattering samples with broad peaks. The diffraction signals appeared split (into few signals, see insert in Figure 1B) making the accurate determination of their position and intensity impossible. Therefore, small sample size and low crystallinity, made it not possible to record sufficient quality diffraction patterns from bacterial poly(MG) alginate which we have planned to study in details.
For the series of experiments with the humidity control, within the accuracy obtained, initial data analysis indicates the following changes in the diffraction patterns. For the HiG sample, increase perfection of the crystal structure with increasing humidity and time of exposure to moisture is observed. This is manifested in increasing sharpness of the diffraction signals. This result indicates that water molecules must play an important role within the crystal structure. However, no changes in the signal position or their intensity could be detected. Detailed analysis is seriously hindered by problems with the detector. For the poly(M) sample no detectable changes in the diffraction pattern could be observed.
This exploratory experiment shows, that the changes in the recorded fibre X-ray diffraction patterns from alginates induced by changing humidity are small and mainly can be attributed to crystal perfection. For studied alginate samples it was not possible to observe any structural changes. Main experimental difficulties include weak scattering signal from the sample resulting from sample low crystallinity. Small sample size and insufficient degree of fibre orientation are also contributing factors. Some problems could be avoided if the performance of the detector is improved.

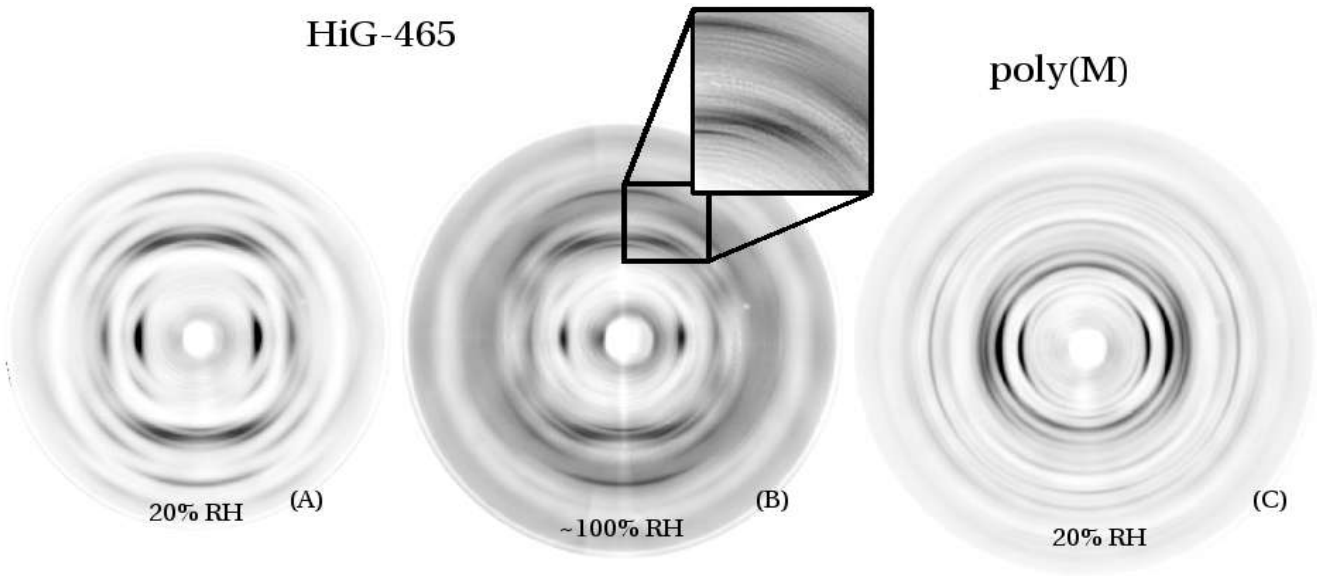

Figure 1: Fibre X-ray diffraction pattern of free acid form of HiG alginate recorded at ambient conditions (A) and at ~100% relative humidity (B). Fibre X-ray diffraction pattern of free acid form of poly(M) recorded at ambient conditions. (C) Insert in (B) shows the fringe-like detector noise.