	Experiment title: Time-resolved SAXS study of EDTA-induced tomato bushy stunt virus swelling	**Experiment number:** LS1557
Beamline: ID02	**Date of experiment:** from: 26/04/00 to: 29/04/00	**Date of report:** 29/08/00
Shifts: 9	**Local contact(s):** Stéphanie Finet	*Received at ESRF:*

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Report:

The aim of the experiment LS1557 (April 2000, 26th 7 am to 29th 7 am) was to perform a kinetic study of the swelling process of TBSV previously initiated on the SAXS beamline D24 at LURE, Orsay, France. The high flux of the ESRF coupled to the use of bidimensional detectors was expected to improve considerably the statistics of the data in the early time-range of the swelling process and more specifically in the larger s-range ($s > 0.01$ Å^{-1}) which is essentially out of reach using the instrument at LURE. Unsatisfactory test measurements on compact and swollen virus solutions performed before the experiment LS1557 using the CCD detector on ID2 led us to ask for the gas-filled delay-line detector also available on ID2.

During the allocated beam-time an accumulation of technical problems were encountered, ranging from storage disk breakdown to various mechanical and electronic problems which, in spite of the involvement and efforts of the beam-line responsible and our local contact, as well as of several technical staff members, prevented us from collecting data before the last shift or so. This remaining period could be extended into the afternoon of the 29th thanks to the beam-line responsible. This allowed to collect a complete data set at a given temperature and EDTA concentration. Although conditions were not optimal since the measuring cell could not be placed under vacuum, thereby increasing the parasitic scattering, the data collected were of a much improved quality as compared to those previously obtained at LURE (see Fig. 1). Clearly, a wealth of biologically relevant information should be retrieved from such measurements.

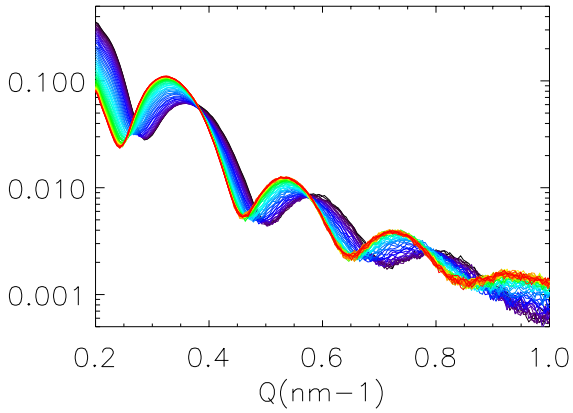

Figure 1
64 frames of 2s each representing the first 128 s of the swelling kinetics. The figure represents the average of 12 times the same experiment. Among the new observations with respect to what we could see at LURE, there is an alternation between isoscattering points and spread crossing points.

However, the comparison of the normalised and integrated patterns with the reference curve of the compact virus (initial state), which was only possible during the last hours, showed that the first kinetic frame was significantly different from the compact state, showing that part of the virus in the beam had been in contact with EDTA for longer than the few ms or so between mixing and the beginning of data collection. This could be attributed to either (or both) of two causes. First, some EDTA could have diffused away from the corresponding reservoir into the virus containing reservoir during data recording, thereby triggering the reaction before the next mixing. Second, the volume mixed at each time might not have been large enough to ensure a complete replacement of the "old mixture" by freshly mixed solutions. The total volume mixed represented a three-fold excess with respect to the dead volume (from the mixer to the end of the measuring cell), which, in general, has been found to be adequate. The fact that the difference between the first kinetic frame and the compact pattern was much stronger after a "long kinetic measurement' (about 20 minutes) than after a 'short one' (about 2 minutes data recording) seems to favour the first explanation, but is certainly not exclusive of the second.

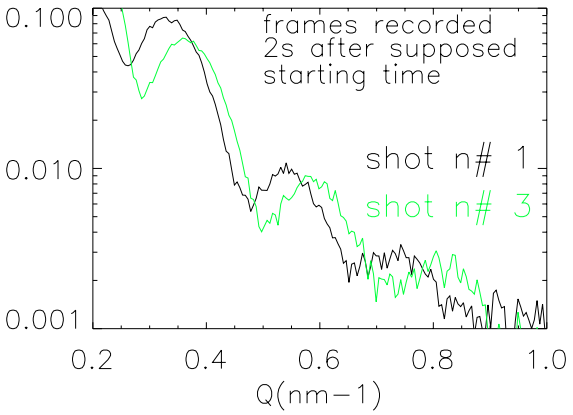

Figure 2
To get better statistics, the same kinetic experiment was repeated 20 times (or 20 "shots"). The figure shows the first frame of the 1st and 3rd shots. Clearly, in the first shot, the virus had already initiated its swelling process, while at first sight, this is not the case in the third shot. Note that the first shot was performed about 1 hour after the virus solution in the reservoir was pushed for the last time, while only two minutes lasted between the second and the third shot.

Whatever the reason, the problem, once identified, can easily be circumvented by increasing the volume of injected reactants during each shot, so as to be sure that only freshly mixed solutions fill up the measuring cell. This will correlatively increase the amount of virus spent for each measurement, but fortunately this is not a severe problem in the case of TBSV.

Last minute addition

Thanks to the beamline scientist, T. Narayanan, we benefited from an extra day of measurements on the first of September during which a series of tests and some actual experiments using the CCD camera were performed. Some persistent problems with the stopped-flow apparatus have been better understood and could be circumvented. Some others have been solved. The data sets we obtained look extremely promising : the statistics are very good, even at high angles, for exposures as short as 0.1 s, and no radiation damage could be detected after 110 exposures of 0.1 s each over a period of 30 minutes. The data will be analysed in the oncoming weeks, but the preliminary assessment we could perform online makes us confident that a wealth of information will be retrieved from such data and all the more eager to obtain the required beamtime.