



	Experiment title: Encapsulation by vesicles using a solvent switch mechanism	Experiment number: SC-2319
Beamline: BM26B	Date of experiment: from: 5 October 2007 to: 8 October 2007	Date of report: 1 September 2008
Shifts: 9x8hrs	Local contact(s): Dr Kristina Kvashnina	<i>Received at ESRF:</i>
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Report:

Block copolymer vesicles, which are hollow spheres consisting of an amphiphilic bilayer or membrane, are promising encapsulation devices for water soluble molecules. This is because of the large internal volume of the water core and the higher stability and tenability of the polymer membranes compare to those prepared from lipids [1]. For polymer vesicles to be of commercial importance as delivery vehicles, reliable encapsulation techniques are required. The process of rehydration of vesicles either with a trapped active compound, or in the presence of an active in solution has been shown to allow encapsulation [2]. It would be preferable, however, to trigger encapsulation in a bulk solution by changing the solvent conditions – by changing pH [3] or solvent exchange [4] for example. Little is known of the mechanism required for vesicles formation, although theoretical studies are now indicating that it may occur via spinodal decomposition prior to the formation of spherical micelles which fuse together to yield vesicles [5]. Recently, Adams et al. published experimental result showing that for vesicle formation via pH switch the most probable mechanism is the one involving phase separation followed by re-structuring and that this mechanism leads to highly inefficient encapsulation on vesicle formation [6].

We investigated the possible intermediates formed during the preparation of poly (ethylene oxide)-b-poly (caprolactone) (PEO-PCL) vesicles. Because the hydrophobe, PCL, is crystalline it is expected to decrease the permeability of the vesicle membrane thus improving encapsulation properties. However, the formation of vesicles via rehydration of a thin film can prove difficult when the hydrophobic block is either glassy or crystalline. A more successful method is the addition of water to solution of vesicle-forming block copolymer where the solvent is good solvent for both blocks and also miscible with water. Such procedure was employed for the formation of the above block copolymer. Previously measurements in the laboratory have been performed on the slow addition of water to THF solution of PEO-PCL and structural changes identified via turbidity measurements. Cryo-TEM have been performed on extracted samples and they showed progression in the structural organisation from lamellar (or spinodal structures) to micelles to worms to rods and finally to vesicles. We aimed to repeat this experiment using SAXS to try to detect the intermediate structures during the water addition.

For the previous experiment carried out in April 2207, experiment number SC-2206, a cell has been designed which allowed the simultaneous measurement of SAXS/WAXS/turbidity under controlled temperature conditions from pre-made solution of PEO-PCL in THF. The cell is based on the cuvette used in the original UV/VIS experiments i.e. 1cm² at the base, allowing vigorous stirring, but with a narrow section included enabling X-rays to pass through. Water was added drop wise using an automatic pipette to ensure a precise rate of addition. Use of a laser and photomultiplier tube will allowed turbidity measurements to be performed simultaneously with the SAXS/WAXS study –Figure 1. This experiment was an extension of the one mentioned above using higher polymer concentration to eventually improve the scattering contrast.

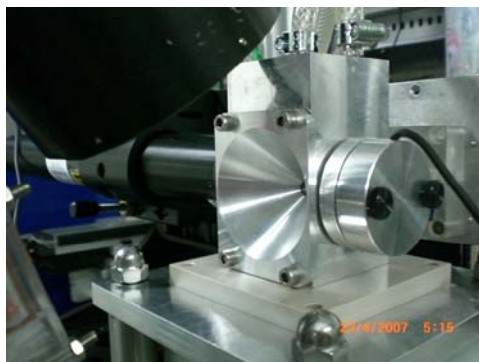


Figure1. Experimental cell

Three sets of dynamic experiments were carried out during this granted beam time using three different polymers with the same hydrophilic length and varying hydrophobic length - PEO_{1k}-PCL_{3k}, PEO_{1k}-PCL_{4k} and PEO_{1k}-PCL_{5k}. The experiments have been performed at the followed conditions – initial polymer concentration 50 mg/ml, 50 μ l water droplets added every 10 min for 10 hrs. The turbidity measurement was recorded every 20 sec and the SAXS measurement – 5 min frame at 25.0°C temperature (measured with thermocouple dipped in the solution) controlled by circulating water from water bath . The same procedure has been applied when the background was recorded. The SAXS patterns were normalised and background corrected.

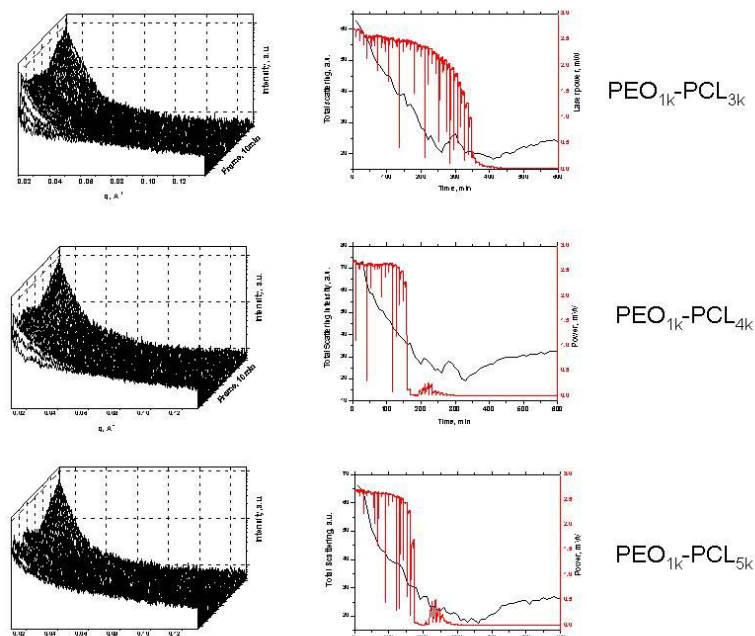


Figure2. Scattering curves (left) and corresponding total scattering intensity and laser transmission (right) for the three block copolymers analysed.

For all three polymers the total scattering and transmission showed a decrease with water addition. The polymer PEO_{1k}-PCL_{3k} (Figure2, top) behaved differently compare to the PEO_{1k}-PCL_{4k} (Figure2, middle) and PEO_{1k}-PCL_{5k} (Figure2, bottom), which showed similar trend in transmission and SAXS. Laser transmission showed initially a plato followed by drop almost to zero, the drop being sharper and earlier (at lower water amount) for the high molecular weight polymers. In the case of the high molecular weight polymers SAXS curves showed increased background scattering at the begging, when the water amount was low, followed by drop in background scattering and slight increase followed by decrease of the scattering at the beamstop. This primary increase of the scattering at the beamstop has not been observed for the low molecular weight polymer. For all polymers with further water addition the scattering at the beamstop increased more, corresponding to

the low transmission of the sample. The total scattering intensity decreased with water addition followed by slight increase from the big structures present in the sample. There were no good agreement in the transmission and total scattering intensity patterns – one possible explanation is that the corresponding background subtracted is not correct due to the preferential solvent partitioning in the membrane. Other possibility is that the sample although in the right range to carry out the SAXS experiment was too concentrated to give reliable result for the laser transmission measurements.

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