



Experiment title: Encapsulation by vesicles	Experiment number: SC-2206
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Local contact(s): Dr Kristina Kvashnina	<i>Received at ESRF:</i>

Names and affiliations of applicants (* indicates experimentalists):

- * Dr. Nadejda Tzokova, Dept of Chemistry, Sheffield University, Sheffield, S3 7RH
- * Dr. Olexander Mykhaylyk Dept of Physics and Astronomy, Sheffield University, Sheffield, S3 7RH
- * Dr. Dave Adams, Unilever Corporate Research, Colworth, Bedford, MK44 1LQ
- * Dr. Michael Butler, Unilever Corporate Research, Colworth, Bedford, MK44 1LQ

Report:

Studies of the self-assembly of synthetic block copolymers in dilute solution have opened up the possibility of using such systems as a medium for encapsulation and delivery. Of the structures formed in solution, vesicles, which are hollow spheres consisting of an amphiphilic bilayer or membrane, are particularly promising for hydrophilic active compounds. 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 [1]. It would be preferable, however, to trigger encapsulation in a bulk solution by changing the solvent conditions – by changing pH [2] or solvent exchange [3] 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 [4].

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 to micelles to worms to rods and finally to vesicles.

For this experiment 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 1a. To prevent interaction between the scatterings species dilute solutions of PEO-PCL block copolymer were used to perform the experiment.

Four sets of dynamic experiments were carried out during the granted beam time. The block copolymer used was PEO_{1k}-PCL_{3k}. Initially the simultaneous Turbidity/SAXS/WAXS experiment was carried on following the protocol of UV/VIS measurements. Polymer was dissolved at 10mg/ml initial concentration in THF and transferred into the cell. Then 10 μ l water droplets were added via automatic pipette every 30 sec. The turbidity increased with adding water and a minimum in transmission was observed after 1ml of water added (similar to the turbidity at the original UV/VIS experiment) but we could see some spikes at the turbidity reading indicating increasing turbidity followed by fast system recovering. The SAXS patterns were normalised and background corrected data showed no information – no scattering development could be seen, just noise. Further the experiment was repeated by increasing polymer concentration – at 50 mg/ml and 25 mg/ml initial concentration in THF. SAXS patterns for 50 mg/ml concentration showed increasing of scattering at the beamstop correlated to the increasing turbidity of the sample. Further the scattering intensity at the beamstop decreased due to the sample dilution when more water added. No peak was seen in the turbidity measurement. The data corresponding of the sample prepared at 25 mg/ml concentration have not been analysed.

To ensure better mixing and equilibrating as well as acquiring longer SAXS/WAXS frames, further experiment has been performed at the followed conditions – initial polymer concentration 10 mg/ml, 20 μ l water droplets added every 12 min for 25 hrs. The turbidity measurement (recorded every 30 sec) showed the first distinctive minimum followed by dropping of intensity – Figure 1c. Second peak have not been observed. The SAXS data showed increasing of background scattering (system shape changing or rearrangement, small structures) corresponding to the minima in transmission measurement. Further the background scattering lowered and the scattering at the beamstop increases, which corresponds to increasing turbidity of the sample, followed by decreasing of scattering at the beamstop when more water was added Figure – 1b. We have seen good correlation between the scattering and turbidity pattern – Figure 1d.

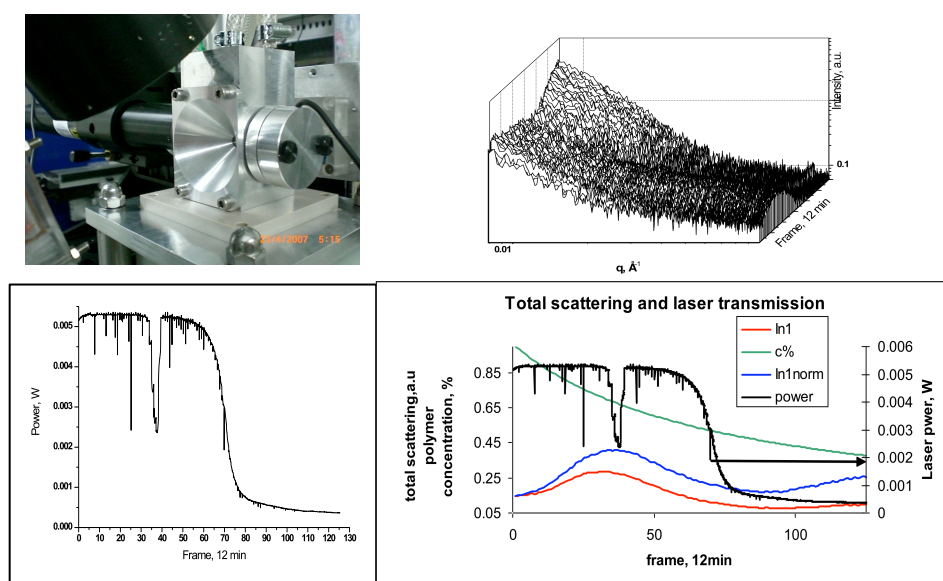


Figure1: a. Cell used for simultaneous SAXS/WAXS/Turbidity measurements; b. SAXS pattern showing development of the scattering intensity with time; c. Transmission trace changes with time; d. Combined graph showing the comparison between the turbidity (black), integrated intensity (red) and normalised to the concentration (green) intensity (blue)

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2. Adams, D. J., M. F. Butler, and A. C. Weaver "Effect of Block Length, Polydispersity, and Salt Concentration on PEO-PDEAMA Block Copolymer Structures in Dilute Solution" *Langmuir* 2006 **22** p.4534-4540.
3. He, X., and F. Schmid "Dynamics of Spontaneous Vesicle Formation in Dilute Solutions of Amphiphilic Diblock Copolymers" *Macromolecules* 2006 **39** p.2654-2662.
4. Luo, L., and A. Eisenberg "Thermodynamic Size Control of Block Copolymer Vesicles in Solution" *Langmuir* 2001 **17** p.6804-6811.