Imaging new transient nanostructures using a microfluidic chip integrated with a controlled environment vitrification system for cryo-TEM

JINKEE LEE[†], ASHISH K. JHA[‡], ARIJIT BOSE[*,‡] AND ANUBHAV TRIPATHI[*,†]

[†]Division of Engineering, Brown University, Providence, RI 02912
[‡]Department of Chemical Engineering, University of Rhode Island, Kingston, RI 02881
[*] E-mail:anubhav_tripathi@brown.edu, bosea@egr.uri.edu

Materials

Aqueous stock solutions of Cetyltrimethylammonnium bromide (CTAB, >99% purity , Sigma–Aldrich (St. Louis, MO)) and dodecyl benzene sulfonic acid (HDBS, Stepan Company (Northfield, IL)) were prepared using 18.2MΩ ultra pure water at room temperature, and then stored at 25°C until used. The CTAB and HDBS samples were kept above their Kraft points[1] to avoid any precipitation. The holey carbon electron microscope grid is bought from Ted Pella, INC (Redding, CA).

Method

Each surfactant solution is primed in the reservoir of the microfluidic chip and air pressure is applied to force the liquid through the channel and capillary. A typical experiment consists of passing the two micellar solutions from their reservoirs through the T-junction, and allowing them to mix before ejecting the solution onto the grid. The volumetric flow rate of the mixed solution was kept constant at 100 l/min. At this flow rate the residence time through the channels and capillary was about 12 seconds. To increase the residence time for surfactants, the flow was stopped in the channel for the desired time, and then reinitiated to supply the solution on to the grid. Any excess liquid on the grid was blotted out. The deposition of droplets exiting from the microfluidics capillary was performed within the CEVS unit, preventing water evaporation and temperature changes in the sample. Subsequently, the grid bearing the sample was plunged into a liquid ethane reservoir, cooled by liquid nitrogen to a temperature close to its freezing point. The rapid heat transfer away from the grid vitrified the sample. The specimen was transferred to the cooled tip of a cryo transfer stage (CT3500J; Oxford Instruments). Finally, the stage was inserted under positive dry nitrogen pressure into a JEOL 1200-EX TEM and imaged at slight under focus (1-3 μm). The sample temperature was maintained at -165 °C at all times during imaging to prevent the amorphous-to-crystalline phase transformation in ice.

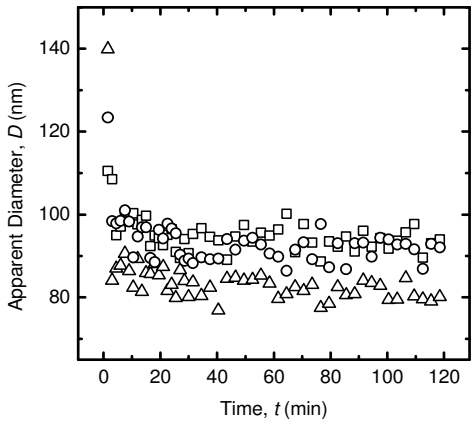

Figure A1. Mean hydrodynamic diameter of the aggregates formed in the solution after mixing of 0.86 wt% CTAB and 0.78wt% HDBS in a 7/3 ratio at 25°C. The symbols represent different experimental runs. (Reprinted from Xia *et al.*[2])

Insights from Dynamic Light Scattering Experiments

Dynamic light scattering (DLS) experiments can show some signatures via hydrodynamic diameter measurements as a function of evolution time[2] for slow to fast growing material. For example, the slowly evolving CTAB/SOS structures show an increase its hydrodynamic diameter with time before the sample reaches a steady state after several hours. The diameter increase has been

explained through a vesicle formation mechanism. where the SOS/CTAB mixture forms disc shape structures before closing to form spherical shaped vesicles. This mechanism has been confirmed by Cryo-TEM imaging, showing a mixture of discs and vesicles as a function of growth time.

But a rapidly evolving system, such as CTAB/HDBS, shows a different trend by DLS. Figure A1 shows a an initial increase in the measured hydrodynamic diameter of aggregate structures, rapidly decaying to a steady state value subsequently. There was no explanation[2] offered so far for the initial rise and then rapid reduction of size. The manually prepared cryo-TEM samples could only supply images at time points after the initial transition, and always showed spherical vesicles. Our experiments provide data that shows that the initial size increase is the result of tubule formation, and the drop in size is the result of the breakup of these tubules to vesicles.

Additional Experiments

We have conducted experiments with the CTAB/SOS system using our µf-cryo-TEM setup. We see only disks and vesicles in that system, and no tubules. We confirm that the tubules are a material property of the CTAB/HDBS system.

References

1. Van Os, N. M.; Haak, J. R.; Haak, L. A. M., *Physicochemical Properties of Selected Anionic, Cationic, and Nonionic Surfactants*. Elsevier: New York, 1993.
2. Xia, Y.; Goldmints, I.; Johnson, P. W.; Hatton, T. A.; Bose, A., Temporal evolution of microstructures in aqueous CTAB/SOS and CTAB/HDBS solutions. *Langmuir* **2002,** 18, (10), 3822-3828.