

Arresting amphiphilic self-assembly

Carlos C. Co*

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Amphiphilic self-assembly has become the basis for a wide gamut of materials and commercial product applications. In many situations however, the best use of self-assembling complex fluids comes when their microstructures can be made permanent. The impetus for a static microstructure can often be such that an alternative non-aqueous media is preferable. Highlighted here is a new approach to capturing self-assembly through replacement of water in traditional complex fluids with sugars to form room temperature complex glasses. Combining solid and liquid properties at the nanoscale, complex glasses have broad potential applications in encapsulation and materials template synthesis.

Introduction

Thermodynamic in nature, the micellar, rod-like, bicontinuous, and liquid-crystalline structures amphiphiles form are in constant flux. In many applications however, it is highly desirable to be able to lock-in these structures and eliminate sensitivity to temperature or compositional changes. From early cryo-TEM studies to visualize micelles, bicontinuous microemulsions, and liquid-crystalline phases to current transcriptive templating efforts for preparing nanostructured materials, the fixation of self-assembled complex fluids has proven an elusive target. While resisting arrest, self-assembled structures may crystallize when frozen, form gradients when dehydrated, or phase separate when polymerized.

Department of Chemical and Materials Engineering, University of Cincinnati, 497 Rhodes Hall, Cincinnati, OH 45221-0012, USA; Fax: +1 (513) 556-3473; Tel: +1 (513) 556-2731

In most restless structures, water conspires with amphiphiles to make structural arrest difficult. Almost without exception, water appears at the root of every failed attempt in arresting self-assembly. While the technical challenges of vitrifying aqueous self-assembled structures by rapid freezing have largely been overcome for thin sample films, large-scale vitrification remains infeasible for most commercial applications. From this perspective, it is unfortunate that water has become the universal solvent for amphiphilic self-assembly. This highlight traces an alternative historical path where polyhydroxy compounds, such as glycerol and sugars, could have become the dominant media for self-assembly particularly in pharmaceutical, food, and transcriptive templating/materials synthesis applications, where the low-cost, water-solubility, low toxicity, and stabilizing properties of these glassy compounds make them ideal water-replacements.

Arrest by cooling

The story on arresting the structure of soft materials by rapid cooling started from early efforts by Bachmann, Costello, Dubochet, Talmon and their co-workers in the late 1970s, to cryogenically fix samples for electron microscopy.¹⁻⁹ Resolution of the many technical challenges led to rapid progress in the electron microscope imaging of micelles,¹⁰ droplet-type microemulsions,¹¹ vesicles,¹² and ultimately, bicontinuous oil-water microemulsions.^{13,14} The quest to vitrify water or at least limit the size of ice-crystals to be no larger than the features of interest, focused efforts on maximizing sample cooling rates with thin films and small droplets.

When high cooling rates cannot be achieved, *e.g.*, samples larger than ~ 100 μm , it was customary to partially replace water in samples with ice-crystal inhibitors, such as propylene glycol, glycerol, and proteins. While using emulsions to investigate the glass transition in oils, such as chlorobenzene, which was then considered to be a non-glassformer, Angell and co-workers had to suppress ice formation and therefore replaced water with 20 mol% propylene glycol (PG).¹¹ In the process they came across the first examples of droplet-type microemulsions that can be readily cooled into the glassy state.^{15,16} Green¹⁷ and Strey¹⁸ followed suit with systematic studies of the phase behavior of microemulsions wherein water is replaced with glycerol to facilitate cryogenic fixation of samples for freeze-fracture electron microscopy. While these initial efforts focused only on the partial replacement of water to



Carlos Co is an Assistant Professor of Chemical & Materials Engineering at the University of Cincinnati, and joined the faculty in July 2002. He received a BS in Chemical Engineering and Chemistry from the University of British Columbia in 1995, and a PhD in Chemical Engineering from the University of Delaware under the direction of Eric Kaler in 2000. His current research programs are focused on complex fluids in the glassy state, new synthetic routes to liquid-core nanoparticles, and cell-biomaterial interfaces for directing the simultaneous assembly and directional migration of multiple cell types. These research programs are supported by the ACS Petroleum Research Fund, Givaudan Flavors Corporation, and a NSF CAREER award.

form low-temperature eutectics that aid vitrification, they were certainly a provocation to go all the way and replace water in complex fluids entirely to form room temperature complex glasses, unlimited in size and with robust nanostructures that permit verbatim transcription.

Arrest by transcription

The story on arresting self-assembled structures by chemical fixation follows two streams – transcriptive and synergistic templating. The synergistic templating of self-assembled structures formed by polymerizable amphiphiles typically results in verbatim fixed structures.¹⁹ The more general and desirable transcriptive templating of self-assembled structures formed by conventional surfactants using typical hydrophobic monomers is fraught with challenges however. Transcriptive templating of self-assembled structures is made difficult by the contradictory demands of a template that self-assembles to form over a reasonable period of time and yet robust enough to retain its structure over the course of polymerization. With few exceptions, transcriptive templating results in phase separation or rearrangement of the starting template and yields final structures containing macroscopic features absent in the starting template.^{20–36}

The thermodynamic forces driving rearrangement are complex and practically unavoidable as they arise from spatially non-uniform initiation and polymer growth, localized swelling and depletion of monomer, changes in the spontaneous curvature as monomer is converted to polymer, and incompatibility of the surfactant chains with the resulting polymer. The key to successful transcriptive templating thus lies in kinetically trapping the template by retarding its dynamics relative to the rate of polymerization. From this, the convergence of structural arrest by vitrification and polymerization comes naturally. Although transcription of self-assembled structures can sometimes be achieved by replacing low-molecular weight surfactants with amphiphilic block copolymers,^{37,38} the extremely slow dynamics of vitrified templates eliminates entirely concerns about the relative rates of polymerization to structural rearrangement.

Arrest by desiccation

Typical monomers are unreactive below room temperature. Therefore the glycerol and PG-based microemulsion glasses studied by Strey and Angell are unsuitable. Glass forming sugars, in particular, equimolar mixtures of sucrose and trehalose, which are almost uncrystallizable, are a better choice. Because the idea of mixing anhydrous sugar powder directly with oil was deemed impossible initially, experiments began with replacing water in traditional microemulsions with progressively more concentrated sugar solutions while noting its effect on the phase behavior³⁹ (Fig. 1). While the overall shape of the one phase region resembles the “fish-tail” observed for aqueous systems, lamellar phases that typically intrude between the upper and lower one-phase boundaries are absent until much higher surfactant concentrations (>35 wt%). Although the phase behavior studies were often terminated at 80% sugar replacement by rational impatience, the weak dependence of the phase behavior on sugar concentration and absence of lamellar phases suggested that it may be possible to desiccate microemulsions to the anhydrous glass state without phase separation. To accomplish this, ~0.5 mm films were transferred to sealed chambers whose vapor phase is saturated with the hydrophobic monomer and continuously dehydrated with anhydrous calcium sulfate. One night of controlled desiccation is typically sufficient to yield transparent and brittle microemulsion glasses (Fig. 2a) that can be photopolymerized without phase separation.⁴⁰ The sugar–surfactant template can then be subsequently removed by dissolution in excess water to yield membranes (Fig. 2b).

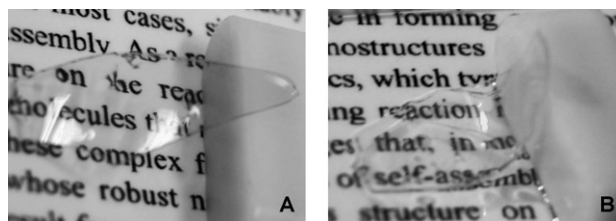


Fig. 2 (a) Optically clear microemulsion sugar glass, containing ~50 vol% divinylbenzene is indistinguishable before and after photopolymerization. (b) Dissolution of the sugar glass template in water leaves behind an optically clear and flexible polydivinylbenzene membrane. Reprinted with permission from ref. 40, Copyright 2006, American Chemical Society.

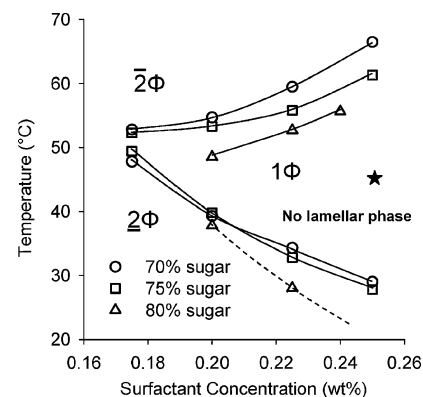


Fig. 1 Phase diagram of sugar-based microemulsions containing equal masses of sugar (equimolar mixture of sucrose and trehalose to prevent crystallization during desiccation) and isobutylacrylate oil. Water used in traditional microemulsions is replaced with super-saturated 70, 75, and 80 wt% aqueous sugar solutions. The concentration axis represents the overall concentration of a 35 : 65 weight ratio of octyl and dodecyl glucoside surfactants (C₈G₁ and C₁₂G₁), with 9 wt% (relative to the two surfactants) of octanediol co-surfactant, in the entire microemulsion sample. Phase boundaries delineate two-phase emulsion regions from one-phase microemulsion regions. Microemulsions with 80% sugar in their “aqueous” phase are highly viscous and require several hours to noticeably phase separate, making it impractical to locate the lower phase boundary accurately. Reprinted with permission from ref. 39, Copyright 2004, American Chemical Society.

Although small-angle scattering (Fig. 3) revealed only minimal rearrangement before and after polymerization that can be accounted for by the increased density, the scattering spectra is not always consistent with that of typical aqueous microemulsions.⁴⁰ Only at very high oil loadings (oil–sugar ratio of 0.7) do the scattering spectra become comparable to that of liquid microemulsions

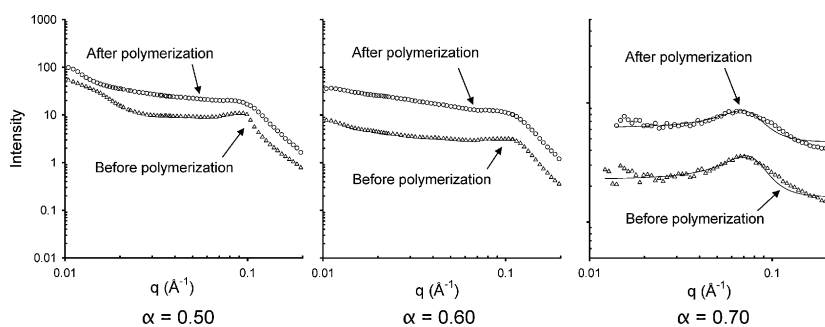


Fig. 3 SANS spectra of sugar-based microemulsion glasses before and after polymerization. Spectra are not on absolute intensity scale and were scaled for comparison. Microemulsion glass samples correspond to precursor microemulsions with initial oil-to-sugar mass loadings of $\alpha = 0.50, 0.60,$ and 0.70 . The solid lines for $\alpha = 0.70$ are model fits to the Teubner–Strey model. Reprinted with permission from ref. 40, Copyright 2006, American Chemical Society.

and permit rational analysis with the Teubner–Strey model.⁴¹ The scattering of the precursor non-glassy microemulsions are in all cases classical however. The increasing viscosity during dehydration impedes relaxation of compositional gradients that are inevitable while drying. With increased loading of liquid oil, the compositional and accompanying structural gradients become locked-in only at later stages when the samples are almost completely dry. These gradients, visible in SEM images of the membranes following polymerization, explain the anomalous scattering of the microemulsion glasses that with increasing oil loading, fade away to yield spectra consistent with the scattering from liquid microemulsions.

Arrest at room temperature

Drying slower or starting dry can both minimize gradients. The discovery that microemulsions could be formed using 80% sugar solutions, encouraged phase-behavior studies with anhydrous molten sugar. Since the final sugar–oil glasses would be candy-like, there was also every reason to make them edible. Persisting with sucrose ester surfactants, 99.5% dry sucrose–trehalose powder, and limonene (orange oil), conditions were found wherein pre-mixed powders of sugar and surfactants will “dissolve” into oil (Fig. 4). The spontaneous solubilization of molten sugar–surfactant in oil is phenomenologically identical to the spontaneous microemulsification of water and oil. However, when cooled below their glass transition (~ 60 °C), these sugar–oil microemulsions become

entirely solid-like (0.7 mohs at room temperature) whilst containing over 50 vol% liquid oil. With annealing, crack and bubble-free samples over 5 grams can be easily prepared that have exhibited no signs of phase separation or crystallization after over 18 months at room temperature. The samples can also be repetitively cycled between molten and solid states with no signs of crystallization or alteration in phase behavior.

The patterns of phase behavior and microstructure of sugar-based microemulsion glasses in the molten state and traditional aqueous microemulsions are remarkably similar.⁴² Variations to the interfacial curvature and total interfacial area brought about by changes to the chemistry and loading of surfactant yields a one-phase microemulsion region resembling the Kahlweit–Strey “fish-tail” found in aqueous microemulsions.¹⁸ Variations in oil-loading also reveal a one-phase channel that begins from the molten sugar–surfactant solution at positive interfacial curvatures (sugar-in-oil) to

almost approach the sugar-free oil–surfactant solution at negative interfacial curvatures (oil-in-sugar). However, molten microemulsion glasses form monolithic solids by cooling only when the oil–sugar volume ratio is less than $\sim 75 : 25$.

The phase behavior of microemulsion glasses in the molten state, like that of aqueous microemulsions of sugar–surfactants, is temperature insensitive.^{43–48} Thus, cooling into the glassy state induces no observable composition or structural gradients. Unlike microemulsion glasses prepared by desiccation, the SANS spectra of room temperature microemulsion glasses prepared by cooling from the molten state always follow the Teubner–Strey model with domain sizes and correlation lengths of ~ 30 nm and ~ 10 nm, respectively.⁴² The phase behavior is also rather insensitive to the ratio of sucrose and trehalose, therefore the glass transition temperature of the final microemulsions can be tuned easily by varying the ratio of these disaccharides, whose glass transitions differ by ~ 60 K.

For solid microemulsions, the glassy sugar sub-phase must have a space-spanning connected structure. The liquid oil however can exist either as discrete oil-swollen micelles or connected channels. Magnetic resonance imaging (MRI) experiments revealed that the oil in the microemulsion glasses has liquid-like self-diffusion coefficients over millimetre length-scales possible only with a sponge-like glass structure continuous in both sugar and oil. With increasing positive interfacial curvature and structural approach to oil-swollen micelles in sugar, the diffusion coefficients decrease

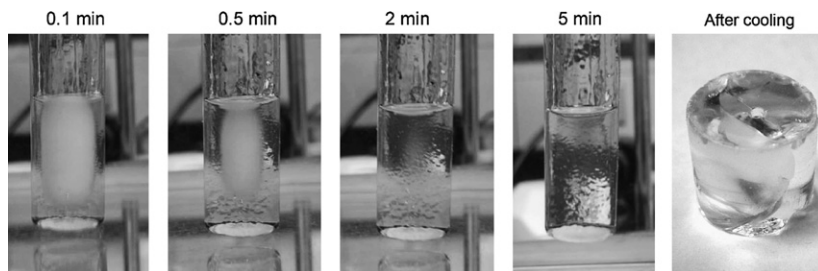


Fig. 4 Spontaneous formation of a microemulsion glass. Sugar and surfactant powder dried to 99.5% dryness dispersed in oil at room temperature “dissolves” upon heating at 365 K to form a one phase molten microemulsion glass. Gradual cooling of the molten glass to room temperature yields a solid microemulsion glass (right) containing ~ 52 vol% liquid oil with a Mohs hardness of 0.7. Reprinted with permission from ref. 42, Copyright 2007, Nature Publishing Group.

in direct analogy to the structure–diffusivity relationships observed in aqueous microemulsions.⁴⁹

With bicontinuous sugar–oil microemulsions that are already solid glasses at room temperature, preparing samples for electron microscopy involves only direct vaporization of the oil under vacuum and sputter coating of the sugar structure, which remains wholly intact (Fig. 5). The size and shape of the interconnected tubular structures observed in the SEM images are consistent with those from freeze-fracture electron microscopy (FFEM) of bicontinuous aqueous microemulsions by Jahn, Strey and co-workers.^{13,14} However, the approximate diameter of the tubular structures of sugar (~60 nm) is considerably larger than the domain size (~30 nm) extracted from Teubner–Strey analysis of SANS data.^{41,42} Real-space computer simulations show that these dimensions are equivalent when the volume fractions of sugar and oil are equal.⁵⁰ Nonetheless, a comparable discrepancy is also present in the FFEM images of Jahn, Strey and co-workers,^{13,14} which has been regarded as an artifact of the sample-preparation process.¹⁷ With sugar–oil microemulsions that can be readily cooled to the glassy state however, we have no explanation.

Nonetheless, following vaporization of the oil, the interstices within the glassy sugar scaffold can be easily refilled by immersion, under vacuum, with any

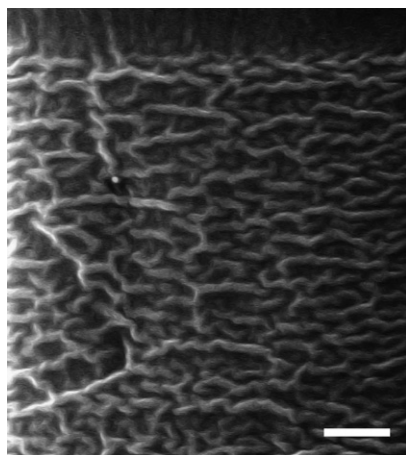


Fig. 5 SEM image of the microemulsion glass shown in Fig. 4 following vaporization of the limonene oil and sputter coating with ~1.5 nm Pt. Scale bar represents 300 nm. Image courtesy of Hiteshkumar Dave and Chris Frethem.

liquid that does not dissolve or plasticize the sugar. The ease with which the limonene oil can be replaced is important for many applications where different functional oils are desirable. After all, phase-behavior studies with sugar glasses can be painstakingly slow, and there is no guarantee on the existence of a surfactant to enable the spontaneous mixing of an oil with sugar.

Reloading with liquid or gaseous monomers, *e.g.*, tetrafluoroethylene, enables transcriptive templating of the sugar scaffold, which can be subsequently removed by dissolution in water and recycled. Crystallizing fats, wax, and other solidifying oils may also be incorporated into the edible sugar structure to control the texture and sensory properties of foodstuffs. A variety of optically active, flavor, and fragrant oils, may also be entrapped in optically clear sugar monoliths by rapid melt-sealing of exposed surfaces. Alternatively, the urethanes can be used to cross-link and seal exposed surfaces of the sugar scaffold or permeated into the structure to render it completely insoluble in water.

Outlook on complex glasses

Investigations of sugar-based complex glasses are still in their infancy. Studies on the phase behavior and structure of liquid-crystalline phases, vesicles, and reverse sugar-in-oil micelles for example, have begun in earnest only recently. Experimental challenges arise principally from the high viscosity, thermal decomposition, and optical rotation of the chiral sugars. Nonetheless, initial indications are that their patterns of phase behavior and structure, set by interfacial curvature and excluded volume, will parallel that of aqueous complex fluids. Extending the science of complex fluids to the solid state with complex glasses promise many new avenues for research and commercial applications that make their study especially rewarding. Particularly interesting for example, would be the crystallization and stabilization⁵⁰ of membrane proteins in micellar and liquid-crystalline phases where diffusion and nucleation rates may be decoupled to improve crystal quality by tuning of the glass transition and crystallization temperatures. Beyond sugar-based complex glasses are other room-temperature ionic and metallic

complex glasses, based on urea/choline halides and gallium for example, that would likely have unique tunable electric and optical properties.

References

- 1 J. Dubochet, F. P. Booy, R. Freeman, A. V. Jones and C. A. Walter, *Annu. Rev. Biophys. Bioeng.*, 1981, **10**, 133.
- 2 E. Knapek and J. Dubochet, *J. Mol. Biol.*, 1980, **141**, 147.
- 3 J. Dubochet and E. Knapek, *Chem. Scr.*, 1979, **14**, 267.
- 4 M. J. Costello, R. Fetter and M. Hochli, *J. Microsc.*, 1982, **125**, 125.
- 5 M. J. Costello, *Scan. Electron Microsc.*, 1980, 361.
- 6 M. J. Costello and J. M. Corless, *J. Microsc.*, 1978, **112**, 17.
- 7 T. Gulik-Krzywicki and M. J. Costello, *J. Microsc.*, 1978, **112**, 103.
- 8 L. Bachmann and Y. Talmon, *Ultramicroscopy*, 1984, **14**, 211.
- 9 Y. Talmon, *Rev. Sci. Instrum.*, 1979, **50**, 698.
- 10 L. Bachmann, W. Dasch and P. Kutter, *Phys. Chem. Chem. Phys.*, 1981, **85**, 883.
- 11 D. R. Macfarlane and C. A. Angell, *J. Phys. Chem.*, 1982, **86**, 1927.
- 12 A. H. Falls, H. T. Davis, L. E. Scriven and Y. Talmon, *Biochim. Biophys. Acta*, 1982, **693**, 364.
- 13 W. Jahn and R. Strey, *J. Phys. Chem.*, 1988, **92**, 2294.
- 14 M. Kahlweit, R. Strey, D. Haase, H. Kunieda, T. Schmeling, B. Faulhaber, M. Borkovec, H. F. Eicke, G. Busse, F. Eggers, T. Funck, H. Richmann, L. Magid, O. Soderman, P. Stilbs, J. Winkler, A. Dittrich and W. Jahn, *J. Colloid Interface Sci.*, 1987, **118**, 436.
- 15 J. Dubochet, M. Adrian, J. Teixeira, C. M. Alba, R. K. Kadiyala, D. R. Macfarlane and C. A. Angell, *J. Phys. Chem.*, 1984, **88**, 6727.
- 16 C. A. Angell, R. K. Kadiyala and D. R. Macfarlane, *J. Phys. Chem.*, 1984, **88**, 4593.
- 17 J. L. Green, *J. Phys. Chem.*, 1990, **94**, 5647.
- 18 R. Strey, *Kolloid Z.*, 1994, **272**, 1005.
- 19 D. L. Gin, W. Q. Gu, B. A. Pindzola and W. J. Zhou, *Acc. Chem. Res.*, 2001, **34**, 973.
- 20 J. D. Morgan, K. M. Lusvardi and E. W. Kaler, *Macromolecules*, 1997, **30**, 1897.
- 21 J. H. Burban, M. T. He and E. L. Cussler, *Aiche J.*, 1995, **41**, 907.
- 22 C. C. Co, R. de Vries and E. W. Kaler, *Macromolecules*, 2001, **34**, 3224.
- 23 R. de Vries, C. C. Co and E. W. Kaler, *Macromolecules*, 2001, **34**, 3233.
- 24 C. C. Co, P. Cotts, S. Burauer, R. de Vries and E. W. Kaler, *Macromolecules*, 2001, **34**, 3245.
- 25 C. C. Co and E. W. Kaler, *Macromolecules*, 1998, **31**, 3203.
- 26 K. D. Hermanson and E. W. Kaler, *J. Polym. Sci., Part A: Polym. Chem.*, 2004, **42**, 5253.
- 27 T. H. Chieng, L. M. Gan, W. K. Teo and K. L. Pey, *Polymer*, 1996, **37**, 5917.
- 28 V. Challa, K. Kuta, S. Lopina, H. A. Cheung and E. von Meerwall, *Langmuir*, 2003, **19**, 4154.

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- 29 T. H. Chieng, L. M. Gan, C. H. Chew, L. Lee, S. C. Ng, K. L. Pey and D. Grant, *Langmuir*, 1995, **11**, 3321.
- 30 J. Santhanalakshmi and K. Anandhi, *Langmuir*, 1996, **12**, 3320.
- 31 T. H. Chieng, L. M. Gan, C. H. Chew, S. C. Ng and K. L. Pey, *Polymer*, 1996, **37**, 4823.
- 32 T. D. Li, L. M. Gan, C. H. Chew, W. K. Teo and L. H. Gan, *Langmuir*, 1996, **12**, 5863.
- 33 Y. I. Gonzalez, M. Stjern Dahl, D. Danino and E. W. Kaler, *Langmuir*, 2004, **20**, 7053.
- 34 F. P. Hubbard, G. Santonicola, E. W. Kaler and N. L. Abbott, *Langmuir*, 2005, **21**, 6131.
- 35 C. A. McKelvey, E. W. Kaler, J. A. Zasadzinski, B. Coldren and H. T. Jung, *Langmuir*, 2000, **16**, 8285.
- 36 M. A. DePierro and C. A. Guymon, *Macromolecules*, 2006, **39**, 617.
- 37 D. Y. Zhao, J. L. Feng, Q. S. Huo, N. Melosh, G. H. Fredrickson, B. F. Chmelka and G. D. Stucky, *Science*, 1998, **279**, 548.
- 38 N. Zhou, F. S. Bates and T. P. Lodge, *Nano Lett.*, 2006, **6**, 2354.
- 39 F. Gao, C.-C. Ho and C. C. Co, *J. Am. Chem. Soc.*, 2004, **126**, 12746.
- 40 F. Gao, C. C. Ho and C. C. Co, *Macromolecules*, 2006, **39**, 9467.
- 41 M. Teubner and R. Strey, *J. Chem. Phys.*, 1987, **87**, 3195.
- 42 H. Dave, F. Gao, M. Liberatore, J. H. Lee, C. C. Ho and C. C. Co, *Nat. Mater.*, 2007, **6**, 287.
- 43 K. Kluge, C. Stubenrauch, T. Sottmann and R. Strey, *Tenside, Surfactants, Deterg.*, 2001, **38**, 30.
- 44 L. D. Ryan and E. W. Kaler, *Langmuir*, 1997, **13**, 5222.
- 45 L. D. Ryan and E. W. Kaler, *Langmuir*, 1999, **15**, 92.
- 46 L. D. Ryan and E. W. Kaler, *Colloids Surf., A*, 2001, **176**, 69.
- 47 L. D. Ryan, K. V. Schubert and E. W. Kaler, *Langmuir*, 1997, **13**, 1510.
- 48 T. Sottmann, K. Kluge, R. Strey, J. Reimer and O. Soederman, *Langmuir*, 2002, **18**, 3058.
- 49 U. Olsson, K. Shinoda and B. Lindman, *J. Phys. Chem.*, 1986, **90**, 4083.
- 50 S. H. Chen, S. L. Chang and R. Strey, *J. Appl. Crystallogr.*, 1991, **24**, 721.