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Dynamically arrested micelles in a supercooled sugar urea melt

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Micelles are nanoscopic, dynamic, equilibrium structures formed by the association of amphiphiles in a liquid. To date, freezing of disordered micelles typically requires cryogenic quenching. This avoids crystallization of the solvent or surfactant, mitigating against micelle destruction. Here we describe a method to create disordered, dynamically arrested water-free micelles, trapped in a glass-forming solvent at ambient temperature. The micelles are formed by dissolving a surfactant in a molten mixture of sugar (fructose or glucose) and urea. These micelles are trapped in a supercooled state upon cooling the mixture, forming an amorphous micro-heterogeneous material driven by hydrogen bonding interactions. Since all components used in this formulation are solid at room temperature, the supercooled micelle formation is analogous to biphasic alloy formation in metals. This method may provide a way to prepare microphase separated organic solids and to control diffusion-limited aggregation of nanoparticles.

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A specific form of self-assembly is micellization, a process by which amphiphilic molecules spontaneously self-organize above a critical concentration into nanoscopic aggregates. The formation of cell membranes from lipids, digestion of fat by pancreatic juice and bile salts, and stabilization of fat globules in milk are examples of natural self-assembly processes driven by the hydrophobic effect^{1–3}. By virtue of its extensive hydrogen bonding network, water is the most preferred solvent for micellar self-assembly. Considering micelle formation in several non-aqueous solvents, it is argued that hydrophobic interaction is not unique to water, but rather a special case of a more general solvophobic effect⁴. Non-aqueous solvents such as glycerol, ethylene glycol, formamide, molten salts, ionic liquids, and deep eutectic solvents have all been explored as alternative media for micelle formation^{4,5}.

Micelles are used in diverse fields including drug delivery, oil recovery, extraction etc, and to control order or porosity in inorganic materials^{6,7}. Due to their colloidal size, they undergo random Brownian motion in liquids. The Brownian motion of colloids such as polymer micelles, microgels, emulsion droplets, or proteins can be arrested by increasing the volume fraction of the colloid above ~0.58 such that the particles are in contact with each other^{8–10}. However, to our knowledge there is no report on arrested state of dilute micellar solutions or micelles dispersed in a solid.

To achieve the goal of preparing dynamically arrested micelles from dilute solutions, we considered the use of a supercooled solvent as the dispersion medium. A molten mixture of sugar (fructose or glucose) and urea is used as the solvent for micellization. The choice of sugar and urea arose from the opposing roles they play in the association behavior of amphiphiles: sugars can stabilize the association structure while urea weakens the hydrophobic interaction^{11,12}. Unlike other additives, sugars and urea can be incorporated at very high concentration in aqueous micellar solutions. The presence of both hydrogen bonding donor and acceptor centers in urea contribute to extensive hydrogen bonding with water, and thus micelle formation is reported even in 10 M aqueous urea solutions⁴. Hydrogen bonding interactions involving urea is used in the formation of thermo-reversible supramolecular assemblies^{13,14}. Self-assembly of amphiphiles has likewise been reported in supersaturated sucrose solutions¹⁵ and sugar–oil mixtures¹⁶. The large number of hydroxyl groups present in sugars is conducive for the formation of a solid solution with urea through intermolecular hydrogen bonding. Such association of the two components in the mixture is expected to slow down sugar crystallization, thereby forming a supercooled solvent to kinetically trap the self-assembled structures present in them.

In this study we report the formation of micelles comprising sodium dodecyl sulfate (SDS) or cetyl trimethyl ammonium bromide (CTAB) in a molten mixture of sugar and urea. Using scattering techniques and electron microscopy, we demonstrate that the water-free micelles formed in the melt are retained in a supercooled state upon cooling the mixture. Dynamic light scattering (DLS) studies show that the Brownian motion of micelles typically observed in liquids is arrested in the supercooled matrix. Hydrogen bonding interactions between sugar and urea make this an attractive matrix to trap self-assembled structures in the solid state.

Results

Microstructure of micelles in fructose–urea mixture. We observed supercooled micelle formation in different sugars and sugar alcohols in the presence of additives such as urea. A typical three component system that forms dynamically arrested micelles consists of fructose, urea and SDS, at a weight ratio of 54:36:10. The micelles are first prepared at a temperature above the matrix melting point and subsequent cooling of the melt leads to the formation of trapped micelles. The supercooled liquid formed by melting and subsequent cooling of the above mixture is optically transparent and viscous enough to hold its own weight.

Evidence for micelles formation in the supercooled state is gained from small-angle X-ray scattering (SAXS), contrast variation small-angle neutron scattering (SANS) and high-resolution transmission electron microscopy (HR-TEM). Using SAXS, we followed the evolution of structures as a function of temperature (Fig. 1a). At 25 °C, the SAXS pattern of the sample (fructose–urea–SDS 54:36:10 w/w) shows characteristic lamellar peaks of SDS. The peaks occur at scattering vector magnitude (q) values of 0.1595, 0.3199 and 0.4799 Å^{−1}. The ratio of the peak positions is close to 1:2:3, with lattice parameter of 3.93 nm, as expected for lamellar crystals¹⁷. Upon sample heating to 50 °C, the lamellar peaks intensity decrease and a broad peak appears at q value close to 0.105 Å^{−1}. At 70 °C, the contribution from the lamellar structure becomes negligible and the system mostly comprises micellar aggregates as shown by the broad peak at q value 0.115 Å^{−1}. This peak arises from the short-range inter-micellar correlations, as observed for micelles in liquids¹⁸. At 80 °C, the lamellar structure completely disappears and the SAXS pattern is reminiscent of pure micelles. Moreover, upon cooling these preformed micelles to 15 °C (within a span of 30 s), the micelles SAXS pattern is retained, indicating kinetically trapped supercooled micelles.

SAXS profiles of supercooled samples indicate that micelles exclusively exist up to at least 10% SDS (Fig. 1b). Moreover, as

Fig. 1 Supercooled micelle formation using all solid reagents. **a** Temperature-dependent SAXS patterns of SDS in fructose–urea mixture. Disappearance of the lamellar peaks in SDS crystals upon heating and evolution of the micelle-like SAXS pattern in fructose–urea melt is clearly evident. The micelle-like SAXS pattern is retained upon cooling the melt to 15 °C. **b** SAXS pattern of micelles formed in fructose–urea melt at different SDS concentrations. The blue arrow in **(b)** indicates evolution of the correlation peak with increase in surfactant concentration. SAXS curves were shifted vertically for clarity

Fig. 2 Morphology of supercooled SDS micelles in fructose-urea mixture. **a** Background-subtracted SAXS pattern of SDS micelles in fructose-urea melt and the corresponding fit (magenta lines) using core-shell ellipsoid micelles. Error bars represent standard deviation of the mean value. SAXS curves were shifted vertically for clarity. **b** Relative electron density variation of micelles as obtained from indirect Fourier transformation of SAXS pattern (5% SDS) followed by deconvolution. **c, d** SANS pattern of deuterated SDS micelles in fructose-urea melt (70 °C, **c**) and at supercooled state (30 °C, **d**) along with fitted curves (magenta lines). Error bars represent standard deviation of the mean value. **e, f** HR-TEM (**e**) and digitally zoomed (**f**) images of supercooled SDS micelles at ambient temperature (scale bar 20 nm)

expected for globular micelles, the position of the correlation peak is shifted to higher q values with increasing SDS concentration (see the trend line). This is because the increase in the number density of the micelles decreases the inter-particle distance ($d = 2\pi/q_{\text{max}}$) between micelles, provided that the aggregation number of the micelles remains practically unchanged. At 15% surfactant, the SAXS pattern shows features of micelles superimposed with characteristic peaks of lamellar structures, indicating coexistence of micelles and lamellar SDS crystals in the melt. This is different from the phase behavior of SDS in water¹⁹, where globular micelles exist up to a concentration of 36% w/w, and lamellar crystals form only above ~70% w/w. Thus, we demonstrate that SDS can be solubilized in an organic melt up to a concentration of at least 10% w/w, without any phase separation or transition to lamellar crystals.

The background-subtracted SAXS curve is fitted to a polydisperse core-shell ellipsoidal micelle, interacting via a screened Coulomb potential (Fig. 2a and Table 1). The scattering

length densities (SLDs) of the core, solvent and shell are kept constant throughout data analysis. The SLD of hydrocarbon core of SDS micelles is taken as that of undecane, considering that one CH_2 group of the dodecyl chain is attached to the sulfate head group. From the known density of undecane, the SLD of the core (for Cu- K_α) is obtained as $7.21 \times 10^{-6} \text{ \AA}^{-2}$.

To obtain the SLD of supercooled sugar-urea melt, the density of aqueous solutions of sugar-urea mixtures (60:40) was measured at different solid contents (up to 70% w/w). A linear extrapolation of this plot to 100% solid content yields a density of 1.379 g cm^{-3} for fructose-urea mixture. Using this density, the SLD of the solvent (60:40 mixture of fructose-urea) is obtained as $12.5 \times 10^{-6} \text{ \AA}^{-2}$. Based on electron paramagnetic resonance (EPR) experiments, Griffiths et al reported that the shell of SDS micelles in water is composed of around 52% solvent²⁰. Though the volume fraction of solvent in the shell can be different for the present system, we fixed the SLD of the shell as $13.1 \times 10^{-6} \text{ \AA}^{-2}$, assuming 50% contribution from the solvent. This degree of

Table 1 Structural parameters of SDS micelles

SDS (wt %)	Temperature (°C)	Semi-minor core (Å)	Semi-major core (Å)	Shell thickness (Å)	Charge (e.u.)	SLD (10 ⁻⁶ Å ⁻²)			Reduced χ^2
						Core	Shell	Solvent	
2	25	12.7 ± 0.2	16.5 ± 0.1	6.9 ± 0.1	4.9 ± 0.1	7.21	13.1	12.5	5.2
5	25	13.5 ± 0.1	17.2 ± 0.2	5.8 ± 0.2	13.1 ± 0.2	7.21	13.1	12.5	9.2
10	25	14.1 ± 0.2	17.0 ± 0.1	5.6 ± 0.1	13.7 ± 0.3	7.21	13.1	12.5	14.1
5	30	12.0 ± 0.5 ^a	19.0 ± 1.0 ^a	5.6 ± 0.4 ^a	5.8 ± 0.3 ^a	6.64	3.0	1.87	1.1
5	70	12.0 ± 0.5 ^a	17.5 ± 1.0 ^a	5.2 ± 0.3 ^a	7.6 ± 0.6 ^a	6.64	3.0	1.87	1.4

Structural parameters of SDS micelles in fructose-urea melt, as obtained from SAXS/SANS analysis, using core-shell ellipsoidal micelle with a polydispersity (0.1) in the semi-minor core

^aObtained from SANS

solvation is also taken into account while calculating the volume fraction of the micelles. Moreover, we restricted to same value of shell thickness for both minor and major axes of the ellipsoid. A constant value of polydispersity index (0.1) in the semi-minor axis is included, using Schultz distribution. The semi-minor and semi-major axes of the core of the ellipsoidal micelle in the melt are ~13 Å and ~17 Å respectively with a shell thickness of ~6 Å (Table 1). The shell thickness is attributed to the distance over which the counter ions are distributed from the micelle surface.

Since the reduced χ^2 was greater than unity, attempts were also made to fit the data with different models such as polydisperse core-shell spheres, and core-shell ellipsoids with varying shell thickness for the minor and major axes. The χ^2 values were similar for polydisperse core-shell spheres and core-shell ellipsoids. Although the quality of fits could be improved by using the shell SLD as a variable and invoking different shell thicknesses for the major and minor axes, the value of thickness in the major direction is found to be much larger than that expected and hence is not considered further.

From assessing the various models considered here it is noted that the dimensions of the micelles core are similar in all models while the shell thickness varies depending on the model and the value of shell SLD used in the calculation. Thus, exact measurement of the shell thickness is difficult due to limited knowledge about the solvation of the shell in the present system. More detailed experiments combining SAXS and SANS with contrast variation along with other complementary methods are needed to obtain better insight into the solvation of the shell.

Further, a model-independent analysis of the SAXS pattern was carried out using the indirect Fourier transformation (IFT) procedure²¹. The IFT method gives the pair-distance distribution function (PDDF) of the micelles, $p(r)$, which represents the electron density weighted distribution of paired set of all distances between points within the object. The electron density difference profile of the micelles with respect to the solvent obtained from deconvolution of the $p(r)$ function is shown in Fig. 2b. The negative electron density difference corresponds to the micelle hydrocarbon core, while the positive electron density difference is from the hydrophilic shell composed of counter ions. The hydrophobic core radius, estimated from the position of inflection point, is found to be around 2 nm. Further, the variation in electron density of the shell region indicates that the counter ions are distributed over a distance of about 2 nm from the micelles surface.

The formation of micelles in the melt and supercooled matrix is further probed by SANS. To assess micelle formation in a water-free system of fructose-urea mixtures by SANS, we used deuterated SDS (d-SDS) so that sufficient contrast can be achieved between the micelles and the dispersion medium. SANS studies of 5% w/w d-SDS in fructose-urea melt (70 °C) as well as in the supercooled liquid (30 °C) show a clear signature of interacting micelles (Fig. 2c, d). The SANS data have been fitted using a form factor of core-shell ellipsoid as employed to analyze

the SAXS data. The neutron scattering length density (n-SLD) of the core, dry-shell and the solvent are calculated from respective molecular formulae and molar volumes. The core SLD in this case is also calculated considering core as constituted by undecane, while the shell SLD is computed by taking into account 50% solvent penetration. The parameters of the fit along with χ^2 values are included in Table 1. This approach provides a fairly acceptable value of shell thickness which is consistent with the volume of the sulfate group and the values reported by others^{22,23}. It may also be added that if both shell thickness and the shell contrast are used as fitting parameters, the uniqueness of the fitted values may be lost. Alternatively, SANS data can also be fitted well to a prolate ellipsoid without assuming separate contributions from the core and the shell parts. The n-SLD of the scatterer, in this case, is calculated for the full molecular formula (C₁₂D₂₅SO₄⁻) without considering any level of the solvent penetration. Such an analysis, though not perfect, provides average dimensions of the micelles while restricting the number of parameters in the fit. Interestingly, both approaches yield more and less similar values of the overall micellar dimensions. Thus, quantitative SANS and SAXS analysis showed that the dimensions of the supercooled micelles are in the same range as that were observed in water, without large variation in it either with change in temperature or surfactant concentration.

Direct evidence for micelle formation in the supercooled sugar-urea melt was also obtained from HR-TEM. Micelles were prepared at 80 °C, and then quenched to -196 °C with liquid nitrogen. The quenched specimens were examined at an ambient temperature, using HR-TEM and phase plate TEM. As shown in Fig. 2e, f, SDS micelles have spheroidal shape, with a diameter of 4 nm, in excellent agreement with the scattering findings. Similar micelles were detected at 2% and 5% SDS in fructose-urea melt (figures not shown). One notable feature in the TEM images is the narrow polydispersity in size, as is observed in the case of equilibrium structures formed via self-assembly²⁴. It may be noted that such direct images of micelles were previously observed only at cryogenic temperature, upon vitrification of suspensions. Here, all observations are made at an ambient temperature, and imaging of the micelles was possible due to the supercooled nature of the system.

Collectively, HR-TEM together with neutron and X-ray scattering provide unequivocal evidence for the existence of globular micelles in a supercooled organic melt. We further examined if these micelles can exist below 0 °C. The similarity of SAXS patterns recorded upon cooling the melt containing micelles at temperatures ranging from 80 °C to -25 °C (Fig. 3a) confirm that the aggregates are kinetically trapped and remain in micellar form at least up to -25 °C. This is contrary to what is observed for SDS micelles in water, where the surfactant starts crystallizing at ~15 °C, and diffraction peaks corresponding to SDS crystals are found already at 5 °C (Fig. 3b). Micelle formation in supercooled sugar matrix is found to be a more general case in

Fig. 3 Micelles in organic melt remain disordered below 0 °C **a** Temperature-dependent SAXS patterns of 10% SDS in fructose-urea melt (60:40) at 80 °C and subsequent cooling of the sample to −25 °C. **b** Temperature-dependent SAXS patterns of 10% SDS in water indicating crystallization of the surfactant below 15 °C. SAXS curves were shifted vertically for clarity

Fig. 4 Arrested diffusion of supercooled micelles. **a** Temperature-dependent SAXS pattern of CTAB in glucose-urea mixture. Micelles formation is evident from the change in the SAXS pattern upon melting the solvent. **b** Evolution of DLS pattern (intensity correlation function) upon dilution of micelles formed in sugar-urea melt at different water concentrations (20% to 95%) The solid lines are fit to a double exponential decay. **c** Time-dependent SAXS pattern of supercooled SDS micelles (10%) in fructose-urea mixture (0 and 72 h). SAXS curves in (**a**, **c**) were shifted vertically for clarity. **d** Time-dependent SAXS pattern of supercooled CTAB micelles (5%) in glucose-urea mixture (0, 2, 4, 8, 24 and 30 h)

which a variety of other matrices can be investigated. We found that cationic and anionic surfactants can self-assemble in other sugars and sugar alcohols in the presence of urea, and produce supercooled micelles. Preliminary work in our laboratory suggests that similar behavior can be observed with non-ionic surfactants as well. For example, a molten mixture of sorbitol and urea (60:40) can be used to dissolve Triton X-100 or tween-80 (10% w/w) which upon supercooling forms trapped micelles. Similarly, cetylpyridinium chloride (10%) can form trapped micelles in xylitol-urea mixture.

Arrested dynamics of micelles in glucose-urea mixture. Temperature-dependent SAXS patterns of a CTAB, glucose and urea ternary mixture in which CTAB concentration is 10% (w/w) and the glucose to urea weight ratio is 60:40 (w/w) indicate the

formation of micelles upon melting (Fig. 4a). The lamellar peaks seen at 70 °C disappear completely at 90 °C, and are replaced by a broad scattering pattern reminiscent of micelles in water (Fig. 4a and Supplementary Fig. 1). Concentration-dependant SAXS pattern indicate that CTAB micelles are formed in glucose-urea melt even up to 25% surfactant (Supplementary Fig. 2). The SAXS data of CTAB micelles were analyzed using a core-shell ellipsoid model, as discussed for SDS micelles and the parameters of the fit are summarized in Table 2. For CTAB micelles, the SLD of shell is calculated assuming 30% solvent penetration.

One of the important implications of having micelles in a supercooled state is its ability to arrest their global motion. Colloids have been employed as model systems to understand glass transition and have shown to undergo dynamical arrest upon approaching glass transition²⁵. DLS studies on CTAB

Table 2 Structural parameters of CTAB micelles

CTAB (wt %)	Temperature (°C)	Semi-minor core (Å)	Semi-major core (Å)	Shell thickness (Å)	Charge (e.u.)	SLD (10^{-6} Å^{-2})			Reduced χ^2
						Core	Shell	Solvent	
5	25	18.4 ± 0.1	58.2 ± 0.1	10.6 ± 0.1	15.3 ± 0.1	7.49	12.0	12.7	1.6
10	25	21.1 ± 0.1	46.7 ± 0.1	11.4 ± 0.1	17.5 ± 0.2	7.49	12.0	12.7	4.3
20	25	18.6 ± 0.1	33.6 ± 0.1	10.8 ± 0.1	14.8 ± 0.3	7.49	12.0	12.7	8.9
25	25	18.2 ± 0.1	29.4 ± 0.1	9.8 ± 0.2	13.3 ± 0.2	7.49	12.0	12.7	28.3

Structural parameters of CTAB micelles in glucose-urea melt, as obtained from SAXS analysis, using core-shell ellipsoidal micelle with a polydispersity (0.1) in the semi-minor core

micelles formed in glucose-urea mixture at different water concentrations (20% to 95%) indicate arrested diffusion of the micelles in supercooled state (Fig. 4b). To obtain a correlation function in DLS, the presence of water was necessary, presumably due to the poor scattering contrast and frozen dynamics of the micelles in the melt. As compared to micelles formed in 95% water, a six order of magnitude decrease in the diffusion coefficient of the micelles is observed when the micelles are present in 20% water, the rest being glucose, urea and surfactant. The evolution of the micelles' correlation function with a decrease in water concentration is analogous to that observed in colloids under jammed state^{25,26}. In particular, the correlation function changes from a single exponential to a double exponential function as is observed in the case of arrested macromolecules in a crowded environment²⁷.

Further, SAXS analysis of supercooled SDS micelles (10%) in fructose-urea melt as a function of time reveals that the micelles are kinetically stabilized. SAXS patterns obtained after 72 h of aging in a SAXS sample holder at 25 °C shows the same features as that observed in a freshly prepared (0 h) sample (Fig. 4c). Depending on the surfactant concentration and storage temperature, the micelles transformed to lamellar crystals after about 1 week. However, in the case of CTAB micelles (5%), SAXS analysis show progressive changes in the structure of micelles with time (0, 2, 4, 8, 24 and 30 h), without lamellar crystal formation (Fig. 4d).

Discussion

The unusual observation of supercooled water-free micelles in sugar-urea mixture can be explained on the basis of intramolecular association of sugar and urea via hydrogen bonding. Sugars are one of the widely studied classes of molecules that influence the structure of water²⁸. This is due to the fact that the presence of numerous hydroxyl groups on sugars makes it amenable for hydrogen bonding. Hydrogen bonding interactions in molten sugars can drive the amphiphiles into self-assembly via solvophobic effect, similar to the hydrophobic effect in water. The presence of urea enhances this hydrogen bonding network due to the amide bonds present in urea and prevents crystallization of glucose/fructose upon cooling below its melting point. This is conducive for the stabilization of micelles, as it was observed that phase separated CTAB crystal adopts lamellar morphology while in liquids it forms globular aggregate. Moreover, addition of urea significantly decreases the melting point of glucose and hence conducive for solubilization of the surfactant without any decomposition of the sugar²⁹.

The ability of sugars to remain as supercooled liquid is well known. Mixtures of sugars are known to form supercooled glass at room temperature and this property has been widely exploited in candy making³⁰. The ability of urea molecule to take part in hydrogen bonding is also well known. Urea and its analogs were found to be excellent precursors for cooperative assembly of small molecules to form three-dimensional networks. Low molecular

weight hydrogelators based on amphiphilic tris (urea) and other urea derivatives were reported recently^{31,32}. It is proposed that halogen bonding interaction between the pyridyl group in bis (pyridyl urea) and diiodotetrafluorobenzene is responsible for the gelation in aqueous methanol or aqueous dimethylsulfoxide³². Alkaline urea solution was found to induce gelation in biopolymers such as chitosan as well³³. Hydrogen bonding and π - π interaction between aryl pyridyl urea motifs have been demonstrated in a variety of substituted pyridyl urea derivatives. Crystal structure studies indicate that the molecules adopt a planar structure in the solid state with the urea protons in *cis* conformation. These protons are conducive for hydrogen bonding interaction with the N-atom on the pyridyl group leading to supramolecular gel formation³⁴. Various sugars are known to be stabilizers of proteins and help to retain the activity of enzymes even in freeze dried state³⁵. Urea on the other hand is a denaturing osmolyte that destabilizes the protein by forming hydrogen bonds to the peptide group³⁶. The counteracting effect of urea and small molecule osmolyte trimethylamine N-oxide on structural change of α -synuclein was demonstrated³⁷. Thus, the effect of urea and glucose is complementary in inducing self-assembly. Such complementary action of the two ingredients helps in trapping the micelles in a supercooled state, without phase separation.

Self-assembly in high temperature molten fluids and low temperature supercooled liquids is a rather unexplored domain. Here, we demonstrate that we can form molecular assemblies like micelles in an organic melt that when supercooled yield dynamically arrested micelles at room temperature. The formation of micelles in water-free environment and their trapping in an amorphous matrix suggests a new and exciting area in surfactant research. An important advantage of this method is the ability to create nanostructured solids by freezing equilibrium structures. Furthermore, the present approach yields micelles, and potentially additional self-assembled structures, in an amorphous matrix by using primarily solid ingredients. The use of the self-assembly to control mesoscale structures of solid-state materials has been previously limited to microphase separated block copolymers or inorganic-organic composites, e.g., non-centrosymmetric micelles from block copolymers³⁸, surfactant templated mesoporous silicates^{39,40} and liquid crystalline structure of metallosurfactants in molten metal halides⁴¹. Also, organized structures at the air-water interface were used as a substrate to grow freestanding metallic thin films and for the growth of mesostructured organic-inorganic composites^{42,43}.

To the best of our knowledge, here we present the first example of dispersing one organic solid in another solid through self-assembly and nanostructure formation. Collision-mediated agglomeration of primary particles during nanoparticle synthesis depends on the viscosity of the dispersion medium and diffusion of colloidal particles⁴⁴. Arresting the dynamics of micelles or microemulsion droplets in a supercooled state may offer an opportunity to modulate diffusion-limited aggregation phenomena, and hence to engineer mesoscale structures of materials. For

example, microemulsion droplets of molten lipids may potentially be supercooled in a sugar matrix and successive dissolution of the sugar could yield organic nanoparticles of controlled size. This may offer a new avenue for investigating self-assembly over a wide temperature range using glass-forming matrices.

Methods

Materials. SDS and deuterated SDS were obtained from Sisco Research Labs and Sigma-Aldrich respectively. CTAB, fructose, glucose and urea (all AR grade) were obtained from SD Fine Chemicals, India. All chemicals were used as received.

Sample preparation. To prepare water-free micelles in a supercooled liquid, first a mixture of sugar (fructose or glucose) and urea at weight ratio of 60:40 is placed in a mortar and ground well to form a homogenous powder. Appropriate amount of solid surfactant (SDS or CTAB) was also added before grinding using a mortar and pestle (for example, to prepare 10% SDS micelles, a mixture of 0.54 gm fructose, 0.36 g urea and 0.1 g SDS are taken in a mortar). The well-ground powder is transferred to a glass vial, sealed and placed in an oil bath maintained at 90 °C. The melting started at around 68 °C and complete homogenous solution was obtained at 90 °C (fructose-urea mixture becomes homogenous at 80 °C while glucose-urea needs 90 °C). Care should be taken not to raise the temperature high to avoid charring. Occasional stirring helps to dissolve easily. Once a homogenous solution is formed the vial is placed in a temperature-controlled water bath kept at 15 °C. The mixture reached a temperature of 15 °C within a span of 30 s. The supercooled melt is sticky initially which gets hardened with time. For in situ SAXS analysis, the well-ground powder is loaded in a metallic paste cell (Anton Paar, Austria) with Kapton window and heated/cooled using the Peltier sample holder.

Small-angle X-ray scattering. SAXS experiments were carried out using Anton Paar SAXSpace instrument which employs line collimated monochromatic X-ray source (Cu K α , $\lambda = 0.1542$ nm) operated at 40 kV, 50 mA. A Peltier controlled sample holder unit (TCS 150, Anton Paar) was used to control the temperature of the specimen within ± 0.1 °C. To measure the SAXS pattern of micelles in sugar-urea melt, a paste cell with Kapton window was employed while aqueous samples were measured using 1 mm diameter quartz capillary. The scattering intensities were monitored in transmission geometry by using fast read-out, low-noise two-dimensional (2D) charge-coupled device (CCD) detector (pixel size 24 micron) to span a q (momentum transfer) range of $0.01 \text{ \AA}^{-1}$ to $0.65 \text{ \AA}^{-1}$. The sample holder is positioned at a distance of 305 mm from the CCD detector and temperature controlled at 25 °C. The detector is operated at -40 °C to reduce the thermal noise. The collimation system, sample chamber and beam path were enclosed in vacuum at a pressure below 3 mbar. A semitransparent beam stop is employed to measure transmittance and zero q position. For each measurement, 400 frames were obtained at 5 s exposures and averaged. The 2D SAXS images were processed into one-dimensional scattering profiles and measured SAXS intensities were calibrated for transmission by normalizing with zero- q attenuated primary beam intensity. For quantitative analysis of the SAXS pattern, the intensities were subtracted by dark counts, empty cell and solvent scattering using standard protocols in the SAXSquant software (Anton Paar). Instrumental smearing was taken into account during data analysis using the measured beam profile.

Small-angle neutron scattering. SANS experiments were carried out using the SANS diffractometer at the Dhruva Reactor, Bhabha Atomic Research Centre, Trombay, India. The experiments were performed using a neutron beam of mean wavelength (λ) 5.2 Å and the angular distribution of the scattered neutrons was recorded using a one-dimensional position-sensitive detector (PSD). The accessible q range of the diffractometer is $0.017\text{--}0.35 \text{ \AA}^{-1}$. The PSD allows simultaneous recording of data over the full q range. The samples were held in a quartz sample holder of 2 mm thickness. The measured SANS data were corrected and normalized to a cross-sectional unit using standard procedures.

Dynamic light scattering. DLS experiments were performed in a Malvern 4800 instrument equipped with helium-neon laser operated at 632.8 nm and a digital correlator. All experiments were performed at a scattering angle of 90°. The samples were placed in 10 mm square cuvettes and thermostated using Peltier controlled sample holder.

Transmission electron microscopy. HR-TEM experiments were done using an FEI Talos F200C equipped with phase plates. Images were recorded on both a CETA 16M camera and a falcon 3 direct detector.

SAXS/SANS data analysis. The scattering intensity $I(q)$ is the complex square of the scattering amplitude, which represents the Fourier transform of the scattering length density difference $\Delta\rho(r)$, and thus describes the structure of the particle in real space. The general equation treating the scattered intensity versus q

(momentum transfer/scattering vector) is,

$$I(q) = nP(q)S(q) + \text{background}, \quad (1)$$

where n is the number density of scatterers, $P(q)$ is intra-particle interference factor also called the form factor and is decided by shape and size of particles and $S(q)$ is inter-particle structure factor, which depends on spatial arrangement of particles.

The simulation of the SAXS/SANS profiles for the determination of micelle size and shape parameters has been made with the model function core-shell ellipsoid form factor in the SasView 4.1.2 computer program developed by the University of Tennessee. The analytical expressions of the form factor $P(q)$ of a core-shell ellipsoid of revolution is

$$P(q) = \frac{\text{scale}}{V} \int_0^1 |F(q, r_i, \alpha)|^2 d\alpha + \text{background}, \quad (2)$$

where scale is the proportionality constant that accounts for the volume fraction of the scattering objects, V is volume of individual scattering particle, ρ is the electron density (for SAXS) or scattering length density (for SANS). $F(q, r_i, \alpha)$ is the single particle scattering amplitude, given by

$$|F(q, r_i, \alpha)| = V_{\text{core}}(\rho_{\text{core}} - \rho_{\text{shell}})(3j_1(u_{\text{core}})/u_{\text{core}}) + V_{\text{shell}}(\rho_{\text{shell}} - \rho_{\text{solv}})(3j_1(u_{\text{shell}})/u_{\text{shell}}), \quad (3)$$

where $V_{\text{core}} = (4\pi/3)r_{\text{maj,c}}^2 r_{\text{min,c}}$

$$V_{\text{shell}} = (4\pi/3)r_{\text{maj,s}}^2 r_{\text{min,s}}$$

$$u_{\text{core}} = q \left[r_{\text{maj,c}}^2 \alpha^2 + r_{\text{min,c}}^2 (1 - \alpha^2) \right]^{\frac{1}{2}}$$

$$u_{\text{shell}} = q \left[r_{\text{maj,s}}^2 \alpha^2 + r_{\text{min,s}}^2 (1 - \alpha^2) \right]^{\frac{1}{2}}$$

$$j_1(u_i) = (\sin x - x \cos x)/x^2,$$

here $j_1(u_i)$ is the first-order Bessel function of first kind; r_{maj} is the semi-major radius along the rotational axis of the ellipsoid and r_{min} is semi-minor radius perpendicular to the rotational axis of the ellipsoid and α is the angle between the axis of the ellipsoid and vector q .

The inter-particle structure factor $S(q)$ is related to the total correlation function $h(r) = g(r) - 1$ as

$$S(q) = 1 + 4\pi n \int_0^\infty [g(r) - 1] r^2 \frac{\sin(qr)}{qr} dr, \quad (4)$$

where $g(r)$ is their pair-correlation function. The structure factor is captured using a screened Coulomb repulsion within the rescaled mean spherical approximation closure relation.

Data availability. All data generated and analyzed during this study are available within the paper and supplementary information, or are available from the corresponding author upon reasonable request

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References

- Capito, R. M., Azevedo, H. S., Velichko, Y. S., Mata, A. & Stupp, S. I. Self-assembly of large and small molecules into hierarchically ordered sacs and membranes. *Science* **319**, 1812–1816 (2008).
- Mezzenga, R., Schurtenberger, P., Burbidge, A. & Michel, M. Understanding foods as soft materials. *Nat. Mater.* **4**, 729–740 (2005).
- Patton, J. S. & Carey, M. C. Watching fat digestion. *Science* **204**, 145–148 (1979).
- Ray, A. Solvophobic interactions and micelle formation in structure forming nonaqueous solvents. *Nature* **231**, 313–315 (1971).
- He, Y., Li, Z., Simone, P. & Lodge, T. P. Self assembly of block copolymer micelles in an ionic liquid. *J. Am. Chem. Soc.* **128**, 2745–2750 (2006).
- Li, W., Liu, S., Deng, R. & Zhu, J. Encapsulation of nanoparticles in block copolymer micellar aggregates by directed supramolecular assembly. *Angew. Chem. Int. Ed.* **50**, 5865–5868 (2011).
- Xiao, C., Fujita, N., Miyasaka, K., Sakamoto, Y. & Terasaki, O. Dodecagonal tiling in mesoporous silica. *Nature* **487**, 349–353 (2012).
- Chen, S., Chen, W. & Mallamace, F. The glass-to-glass transition and its end point in a copolymer micellar system. *Science* **300**, 619–622 (2003).

9. Mason, T. G., Bibette, J. & Weitz, D. A. Elasticity of compressed emulsions. *Phys. Rev. Lett.* **75**, 2051–2054 (1995).
10. Stradner, A., Sedgwick, H., Cardinaux, F., Poon, W. C., Egelhaaf, S. U. & Schurtenberger, P. Equilibrium cluster formation in concentrated protein solutions and colloids. *Nature* **432**, 492–495 (2004).
11. Zangi, R., Zhou, R. & Berne, B. J. Urea's action on hydrophobic interactions. *J. Am. Chem. Soc.* **131**, 1535–1541 (2009).
12. Andersen, D. H., Wang, C., Arleth, L., Peters, H. G. & Westh, P. Reconciliation of opposing views on membrane–sugar interactions. *Proc. Natl. Acad. Sci. USA* **108**, 1874–1878 (2011).
13. Cordier, P., Tournilhac, F., Soulié-Ziakovic, C. & Leibler, L. Self-healing and thermoreversible rubber from supramolecular assembly. *Nature* **451**, 977–980 (2008).
14. Ying, H., Zhang, Y. & Cheng, J. Dynamic urea bond for the design of reversible and self-healing polymers. *Nat. Commun.* **5**, 3218 (2014).
15. Gawali, S. L., Kumar, S., & Hassan, P. A. Jammed micelles in high solid content sucrose solutions. *J. Nanofluids* **6**, 596–602 (2017).
16. Hiteshkumar, D., Gao, F., Lee, J. H., Liberatore, M., Ho, C. C. & Co, C. C. Self-assembly in sugar–oil complex glasses. *Nat. Mater.* **6**, 287–290 (2007).
17. Battaglia, G. & Ryan, A. J. The evolution of vesicles from bulk lamellar gels. *Nat. Mater.* **4**, 869–876 (2005).
18. Yaghmur, A. & Glatter, O. Characterization and potential applications of nanostructured aqueous dispersions. *Adv. Colloid Interface Sci.* **147**, 333–342 (2009).
19. Kekicheff, P., Grabielle-Madellmont, C. & Ollivon, M. Phase diagram of sodium dodecyl sulfate–water system. *J. Colloid Interface Sci.* **131**, 112–132 (1989).
20. Griffiths, P. C., Paul, A., Heenan, R. K., Penfold, J., Ranganathan, R. & Bales, B. L. Role of counterion concentration in determining micelle aggregation: evaluation of the combination of constraints from small-angle neutron scattering, electron paramagnetic resonance, and time-resolved fluorescence quenching. *J. Phys. Chem. B* **108**, 3810–3816 (2004).
21. Popela, J. B. & Glatter, O. Small-angle scattering of interacting particles. I. Basic principles of a global evaluation technique. *J. Appl. Cryst.* **30**, 431–442 (1997).
22. Sanchez-Fernandez, A. et al. Micelle structure in a deep eutectic solvent: a small-angle scattering study. *Phys. Chem. Chem. Phys.* **18**, 14063–14073 (2016).
23. Mehan, S., Chinchalikar, A. J., Kumar, S., Aswal, V. K. & Schweins, R. Small-angle neutron scattering study of structure and interaction of nanoparticle, protein, and surfactant complexes. *Langmuir* **29**, 11290–11299 (2013).
24. Zanten, R. V. & Zasdzinski, J. A. Using cryo-electron microscopy to determine thermodynamic and elastic properties of membranes. *Curr. Opin. Colloid Interface Sci.* **10**, 261–268 (2005).
25. Mattsson, J. et al. Soft colloids make strong glasses. *Nature* **462**, 83–86 (2009).
26. Sentjabrskaja, T. et al. Anomalous dynamics of intruders in a crowded environment of mobile obstacles. *Nat. Commun.* **7**, 11133 (2016).
27. Zhao, K. & Mason, T. G. Shape-designed frustration by local polymorphism in a near-equilibrium colloidal glass. *Proc. Natl. Acad. Sci. USA* **112**, 12063–12068 (2015).
28. Bruździak, P., Panusko, A. & Stangret, J. Influence of osmolytes on protein and water structure: a step to understanding the mechanism of protein stabilization. *J. Phys. Chem. B* **117**, 11502–11508 (2013).
29. Imperato, G., Eibler, E., Niedermaiera, J. & König, B. Low-melting sugar–urea–salt mixtures as solvents for Diels–Alder reaction. *Chem. Commun.* **9**, 1170–1172 (2005).
30. Saavedra-Leos, M. Z. et al. Glass transition study in model food systems prepared with mixtures of fructose, glucose, and sucrose. *J. Food Sci.* **77**, E118–E126 (2012).
31. Jinno, Y. & Yamanaka, M. Ionic surfactants induce amphiphilic tris(urea) hydrogel formation. *Chem. Asian J.* **7**, 1768–1771 (2012).
32. Meazza, L., Foster, J. A., Fucke, K., Metrangolo, P., Resnati, G. & Steed, J. W. Halogen-bonding-triggered supramolecular gel formation. *Nat. Chem.* **5**, 42–47 (2013).
33. Wang, Z., Nie, J., Qin, W., Hu, Q. & Tang, B. Z. Gelation process visualized by aggregation-induced emission fluorogens. *Nat. Commun.* **7**, 12033 (2016).
34. Pandurangan, K., Kitchen, J. A., Blasco, S., Paradisi, F. & Gunnlaugsson, T. Supramolecular pyridyl urea gels as soft matter with antibacterial properties against MRSA and/or E. coli. *Chem. Commun.* **50**, 10819–10822 (2014).
35. Kaushik, J. K. & Bhat, R. Why is trehalose an exceptional protein stabilizer? An analysis of the thermal stability of proteins in the presence of the compatible osmolyte trehalose. *J. Biol. Chem.* **278**, 26458–26465 (2003).
36. Lim, W. K., Rosgenc, J. & Englander, S. W. Urea, but not guanidinium, destabilizes proteins by forming hydrogen bonds to the peptide group. *Proc. Natl. Acad. Sci. USA* **106**, 2595–2600 (2009).
37. Ferreón, A. C. M., Moosa, M. M., Gambin, Y. & Deniz, A. A. Counteracting chemical chaperone effects on the single-molecule α -synuclein structural landscape. *Proc. Natl. Acad. Sci. USA* **109**, 17826–17831 (2012).
38. Paul, A. R., Laurent, C., Mitchell, A. W. & Ian, M. Non-centrosymmetric cylindrical micelles by unidirectional growth. *Science* **337**, 559–562 (2012).
39. Han, L. & Che, S. Anionic surfactant templated mesoporous silicas (AMSs). *Chem. Soc. Rev.* **42**, 3740–3752 (2013).
40. Han, L., Miyasaka, K., Terasaki, O. & Che, S. Evolution of packing parameters in the structural changes of silica mesoporous crystals: cage-type, 2D cylindrical, bicontinuous diamond and gyroid and lamellar. *J. Am. Chem. Soc.* **133**, 11524–11533 (2011).
41. Martin, J. D., Keary, C. L., Thornton, T. A., Novotnak, M. P., Knutson, J. W. & Folmer, J. C. W. Metallotropic liquid crystals formed by surfactant templating of molten metal halides. *Nat. Mater.* **5**, 271–275 (2006).
42. Edler, K. J. & Yang, B. Formation of mesostructured thin films at the air–liquid interface. *Chem. Soc. Rev.* **42**, 3765–3776 (2013).
43. Jin, J., Wakayama, Y., Peng, X. & Ichinose, I. Surfactant-assisted fabrication of free-standing inorganic sheets covering an array of micrometre-sized holes. *Nat. Mater.* **6**, 686–691 (2007).
44. Thanh, N. T. K., Maclean, N. & Mahiddine, S. Mechanisms of nucleation and growth of nanoparticles in solution. *Chem. Rev.* **114**, 7610–7630 (2014).

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Author contributions

P.A.H. conceptualized the idea. S.L.G. and P.A.H. together designed the SAXS experiments, performed, and analyzed the data. M.Z. and D.D. designed the TEM experiments, performed, and analyzed the data. S.K. and V.K.A. performed and analyzed SANS data. All authors together wrote the manuscript.

Additional information

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