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Colloidal Assemblies of Branched Geminis Studied by Cryo-etch-HRSEM

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The mundane micelle, still elusive after decades of research,[1] has taught chemists a lesson: Unless a small organic compound assembles into a crystalline array, the task of determining its colloidal structure at the molecular level often presents a major challenge. Gross morphology (i.e., whether the assembly is a sphere, tube, sheet, etc.) is less of a problem thanks largely to the microscope. Even if the assembly is dynamic, one can hope to capture a picture of a transient morphology by using rapid freezing methods in conjunction with microscopy. Thus, colloid chemistry has made wide use of transmission electron microscopy (TEM and cryo-TEM) in structural investigations of molecular assemblies.[2,3] We, however, have moved on to a quite different method called cryo-etch-high-resolution scanning electron microscopy (cryo-etch-HRSEM). Since the method is not widely known, we introduce it to the world of self-assembly. The information-content of cryo-etch-HRSEM, pictorial but distinctive in nature, is illustrated with gel and coacervate systems formed from a new series of gemini surfactants.

In TEM, the electrons (which scatter as they pass through a sample) are collected by a detector below the sample. In conventional SEM, the sample and detector lie below the electron-lens; secondary electrons emitted from the sample's surface are collected (as are spurious electrons reflected from the chamber walls). In HRSEM, the sample lies within the lens, while the detector is positioned above the lens. This arrangement minimizes spurious reflections of electrons from reaching the detector, thereby greatly improving the resolution and providing magnifications up to a million.

Colloidal assemblies prepared in water can be rather fragile. We deal with this situation by high-pressure freezing a 1 mm^3 sample of aqueous colloid to −105 °C in 6−7 ms, a process that creates noncrystalline (vitreous) ice into which the colloid is embedded.[4] This step is followed by "etching" a freshly cleaved surface of ice for 1−20 min under high vacuum (2×10^{-7} Torr) to remove, by sublimation, some or all of the ice surrounding the colloidal structure. Finally, a 1−2 nm chromium coating (chromium giving a smaller grain size than any of the precious metals) completes the sample preparation.[5]

Geminis surfactants are compounds composed of: hydrocarbon−ion−spacer−ion−hydrocarbon.[6,7] Their structural versatility,[8] unusual properties,[9] practical applications,[10] and appearance as articles of commerce[11] have combined to stimulate a great deal of current interest in such compounds. We have recently synthesized a family of branched-chain geminis, three representative examples of which are shown in Table l. It is seen that the compounds are zwitterionic ("fraternal twins"). They were synthesized according to a method already published,[12] shown to be pure by NMR, HRFAB-MS, and EA, and examined in water via cryo-etch-HRSEM.

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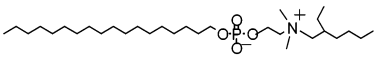
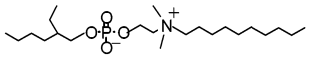
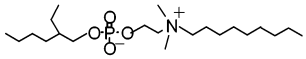
Table 1. Branched Geminis and Their Colloidal Assemblies

Name	Structure	Properties
A		1 & 5 wt-%, clear gel
B		1 & 5 wt-%, coacervate
C		1 & 10 wt-%, clear solution

Visual inspection (Table 1) shows that 1% aqueous solutions of the geminis are either a clear gel (**A**), a coacervate (**B**), or clear solutions (**C**). A gel is defined (arbitrarily) as a phase that does not flow from an inverted vial at room temperature. Gemini **A** is a "physical" gel in the sense that its molecular framework impeding water flow is held together by noncovalent forces. A coacervate is defined as a fluid phase that is water-immiscible despite being water-rich (typically 85−98% water).[13] Coacervate **B** forms when, seconds after dissolving the solid gemini in water, oily droplets (visible under the light microscope) fall out of the water. These coacervate droplets slowly coalesce into a bottom layer, leaving a layer of almost pure water on top. The question, of course, is what type of aggregate morphology corresponds to the gel and coacervate, and it is here where cryo-etch-HRSEM provides valuable information. Unless the gemini molecules can rearrange into new macrostructures at sub-freezing temperatures within 6−7 ms, the photomicrographs should faithfully represent the self-assemblies as they actually exist in liquid water.

Gemini **A** forms a clear gel overnight after it has dissolved in pure water at room temperature; no fibers or other structures are visible by light microscopy. A 1 and 5 wt % gel is thermally and mechanically reversible (i.e. both heating to 70 °C and vigorous shaking soften the gel which stiffens quickly upon cooling or standing, respectively). No syneresis (water separation) was observed from the gels after standing for weeks. Figure 1A shows a cryo-etch HRSEM picture of this gel (1 wt % surfactant; 50000×; 3-min etch-time). Unbound surface ice has been removed, leaving a molecular network sheathed in ice. By increasing the etch-time to 10 min (Figure 1B), the gelator was depleted more extensively of its hydration shell, thereby creating a delicate and porous assembly. We presume Figure 1B closely depicts the gel structure in water. At the higher concentration in Figure 1C (5%; 50000×; 5-min etch-time), the network density is increased while the average pore size is decreased.

Figure 1 can be better appreciated in context of other known small-molecule gelators, the vast majority of which function only in organic solvents.[14] Often the gelation appears to be a case of

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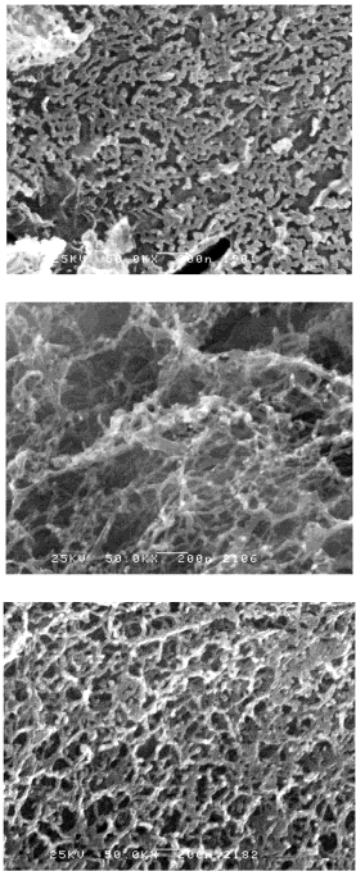

Figure 1. Cryo-etch-HRSEM of gel **A** imaged at 50000× and −115 °C. Top (1A): 1 wt %, 3-min etch-time; middle (1B): 1 wt %, 10-min etch-time; bottom (1C): 5 wt %, 5-min etch-time. Scale bar (5 mm) = 200 nm.

"arrested crystallization" where hair-like crystallites randomly fall out of solution and, in so doing, entrain the organic solvent within the interstices.[15−17] Frequently the fibers, visible under a light microscope, render the gel opaque. The hierarchical structure of such self-assembled soft materials in organic solvents (fibers-to-bundles-to-sheets) has been discussed recently.[18] With regard to aqueous systems, the number of small-molecule gelators is limited. According to their published TEM and SEM pictures, the gels are also of the "long fiber" type.[19−21] (Dehydration inevitably accompanies TEM and SEM, but presumably the systems were sufficiently stable to withstand this abuse.) The cryo-etch-HRSEM of the gemini hydrogel in Figure l is noteworthy because it shows a continuous 3-D network of short interconnected and branched segments. Water viscosity is elevated here not by long entangled hairs but by a delicate, fractal-like molecular web. In contrast to many fiber-based physical gels, the web can, as mentioned, regenerate itself if mechanically disrupted.

Previous work carried out in the Holmberg laboratory[22] on straight-chain analogues of **A**−**C** demonstrated an unusually efficient packing density of the molecules on solid surfaces. Presumably, an alternating "(+−)(+−)(+−)" molecular arrangement within the adsorbed monolayer allows electrostatic attraction of the ionic headgroups, and van der Waals attraction of the chains, to operate concurrently. It is reasonable that these same forces favor the assembly of **A** into interdigitated bilayers that constitute the primary structural unit of the gel.

Figure 2 shows the cryo-etch-HRSEM of coacervate **B** (5 wt %; 50000×; 10-min etch-time). Although the coacervate (like the gel) is porous, there are major differences between the two: The coacervate more effectively retains its bound and occluded water

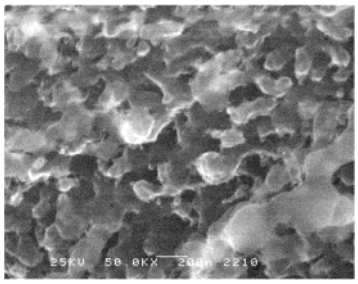

Figure 2. Cryo-etch-HRSEM of coacervate **B** (50000×, −115 °C, 5 wt %, 10-min etch-time). Scale bar (5 mm) = 200 nm.

even after a prolonged 10-min etching. Being far less hydrophobic than **A**, **B** assembles into less robust bilayers. If **B**'s coacervate structure accords with that proposed previously for related geminis,[13] weak bilayer sheets of **B** link up with one another at various points, resulting in a spongy but water-insoluble mass. Thus, gel and coacervate are related and differ mainly according to the structural integrity of their component bilayers.

Gemini **C** forms neither a gel nor coacervate but, instead, dissolves in water as a clear, free-flowing isotropic solution. Since the chain lengths of **B** seem barely able to sustain a lamellar phase (and a labile one at that), reducing the chain length by only one methylene, to create **C**, suffices to prevent sheet formation altogether. Cryo-etch-HRSEM pictures of **C** (not shown) reveal a rough surface consistent with a colony of spherical vesicles projecting from the ice. Polydisperse particles with hydrodynamic diameters of 100−250 nm were detected in solution by dynamic light scattering. The one-carbon difference between **B** and **C** emphasizes the super-sensitivity of colloidal configuration to molecular structure.

If colloid chemistry, and indeed all of science, travels down the road of new instrumental methods, then cryo-etch-HRSEM will be a helpful companion along the way.

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