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Gemini-Induced Columnar Jointing in Vitreous Ice. Cryo-HRSEM as a Tool for Discovering New Colloidal Morphologies

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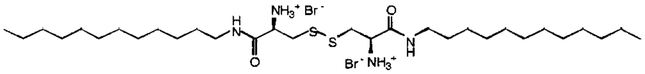
A joint is a fracture of geological origin along which no appreciable displacement has occurred. Impressive jointing is seen in basaltic structures such as the Devil's Post Pile (U.S.A.) and the Giant's Causeway (Northern Ireland), where massive polygonal columns stand vertically in a close-packed arrangement.[1] The columns originally formed from lava which, as it cooled, experienced thermal stresses and inwardly propagating fractures. It can be shown that the polygonal arrays are favored when the two principal horizontal stresses are equal but smaller than the vertical compressive stress in the shrinking rock.[2] The reasons for regularly oriented joint systems (often with pentagonal or hexagonal cross-sections) are obscure,[3,4] although the spacing is apparently related to rock type. In the present Communication, we describe a remarkable parallel between jointing in geological formations and jointing in vitreous ice containing a surfactant (Figure 1) when observed by cryo-high-resolution scanning electron microscopy (cryo-HRSEM). As will be shown, cryo-HRSEM can be a particularly useful and noninvasive method for discovering new modes of self-assembly in water.

The jointing is induced by a double-chained surfactant, namely the L-cystine derivative (**1**) whose structure is drawn below. Cryo-HRSEM was the method of choice for investigating the colloidal system because the samples were fully hydrated, unfixed, and uncontaminated with a cryoprotectant (e.g., sucrose) or buffer, and (in contrast to the more common freeze-fracture TEM) the images were not indirect metal replicas. Since our cryo-HRSEM images of pure water, and those published previously,[5] are entirely featureless, ice crystals (if present at all) must be smaller than 2−3 nm, the approximate instrument resolution.



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The didodecylamide of L-cystine was synthesized from the dicarbobenzoxy derivative of L-cystine, which was converted into the diacid chloride with PCl_5 (ether, 0 °C), and then into the diamide with *n*-dodecylamine (ether, −78 to 0 °C). Removal of the carbobenzoxy protecting group (HBr/HOAc/CH_2Cl_2, 25 °C) gave the final product **1**, which was purified by crystallization from MeOH/acetone and characterized by ^{1}H and ^{13}C NMR, FAB−HRMS, and elemental analysis.

The structure of **1** classifies it as a gemini surfactant, an increasingly important family of compounds[6] that has recently been applied to a variety of uses, including DNA transfection[7] and the construction of new materials.[8] Owing to its amphiphilic nature, **1** should self-assemble in water, and this indeed was found to be the case. An aqueous solution of 2.0−3.5 wt % forms a sticky, viscous suspension. Gentle warming at greater than 3.5 wt % (>0.049 M) creates a transparent gel which, upon cooling, becomes translucent. Dynamic light scattering (Coulter N4) on 0.24 mM solutions of **1** revealed the presence of 200−400 nm particles. Both the size of the particles and known properties of related gemini surfactants[9] are consistent with vesicle formation. Differential scanning calorimetry (Hart Scientific) showed no phase transition between 10 and 100 °C. Occasional groups of entangled fibers from a hydrated solid were detectable by optical microscopy (Nikon Diaphot-TMD).

In an attempt to learn more about the gel structure,[10] we made use of cryo-HRSEM in which ca. 10 μL of gel was placed in a gold planchet, plunge-frozen in ethane (−183.2 °C), and stored in liquid nitrogen.[5] The sample was loaded into a prechilled cryo-preparation chamber (cooled to ca. −170 °C) attached to an Oxford CT-3500 cryostage, where it was fractured with a precooled blade to remove a dome-shaped "cap" and to expose a fresh horizontal surface. Chromium sputter deposition was accomplished by transferring the cryostage to a Denton DV-602 coater which plasma-deposited a l nm Cr layer onto the sample. Samples were observed at 25 kV in the upper in-lens stage of a Schottky field-emission SEM (DS-130F), where brief warming to −110 °C allowed any rime frost (i.e., moisture condensed on the Cr-coated surface during the transfer from coater to microscope) to sublime.

Plunge-cooling ("quench-freezing") was carried out in liquid ethane, a much more efficient coolant than liquid nitrogen and well known to vitrify pure water.[11,12] Thus, the appearance of patterned vitreous ice (Figure 1, photo 1), reminiscent of jointing in well-known geological formations, came unexpectedly. Polygonal columns of vitreous ice, roughly 1 μm in cross section, were obtained repeatedly in multiple experiments. The presence of **1** is essential for the appearance of this columnar jointing. A close-up of photo 1 showing the striking polygonal shapes assumed by the vitreous water containing 3.5 wt % **1** is given in photo 2. The picture reveals the presence of surfactant-induced ridging of the column surfaces as well as occasional vesicular structures.

The question arose as to whether the columnar patterns would vary with concentration. Photo 3 shows an image at 5.0 wt % **1**, and photo 4 shows an image at 6.5 wt % **1**. No discernible concentration dependence of columnar dimensions on concentration is seen within the limits examined. The rate of freezing, on the other hand, had an effect: When the sample was plunge-frozen in liquid nitrogen, which has a smaller thermal capacity than ethane and thus lowers the sample temperatures less rapidly,[12] fracturing occurred, but polygonal columns were not as prevalent. We did, however, observe on the solid surface an interesting "honeycomb-

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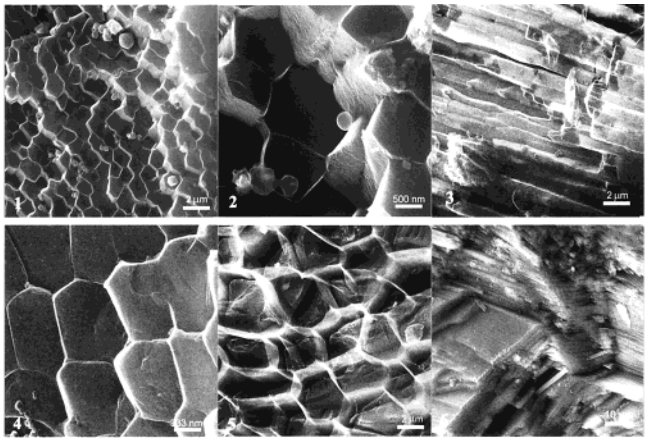

Figure 1. Photos 1−4 and 6: plunge-frozen in ethane. Photo 5: plunge-frozen in nitrogen. Labeled scale bars are given with each photo. Photo 1: cryo-HRSEM of ice containing 3.5 wt % **1**. Photo 2: close-up of photo 1. Photo 3: 5.0 wt % **1**. Photo 4: 6.5 wt % **1**. Photo 5: 3.5 wt % **1**. Photo 6: 3.5 wt % of analogue of **1** with two 9-carbon chains instead of two 12-carbon chains. Note the lamellar rather than columnar morphology.

like” network, devoid of ice, seen in photo 5. When the samples were cooled very rapidly (7 ms) in a high-pressure freezer (HPM 010),[13] the structure was more heterogeneous, with only occasional columnar features of width identical to those obtained by plunge-freezing (not shown). This could be related to the fact that the high-pressure method uses a totally enclosed “double planchet” so that the sample was no longer exposed at the top, and freezing took place more evenly. As with geological structures, unequal directional stresses might be favorable for observing the columnar jointing. These stresses presumably arose from ice increasing its density from 0.917 at 0 °C to 0.934 at −180 °C.

The following observations lead us to believe that the columnar jointing is not an “artifact”:[14] (a) As already mentioned, cryo-HRSEM of pure water invariably gives featureless images. In the presence of **1**, however, reproducible columnar jointing occurs at several concentrations. (b) After several years of cryo-electron microscopy, working with aqueous proteins, DNA, and phospholipids, we have yet to encounter a substance that could promote formation of a similar ice morphology. (c) An analogue of **1**, with two 9-carbon chains instead of two 12-carbon chains, yields a cyro-HRSEM image having a lamellar rather than a columnar structure (Figure 1, photo 6). Clearly, the jointing phenomenon is imposed onto the sample by the surfactant, and it is highly structure-specific. (d) Cryo-SEM images of self-assembled lamellar ribbons in water have been obtained by others[15] with no hint of “artifacts”.

At least two mechanisms seem possible for the columnar jointing. The gemini surfactant might self-assemble into a submicroscopic network of polygonal columns which are filled first with water and later with ice. The columns, invisible by light microscopy, should contribute to the viscosity of the gel at higher temperatures. The textured column walls, uncharacteristic of vitreous ice and evident in photo 2, suggest that gemini surfactant **1** is, indeed, occupying the column interfaces. Moreover, photo 5 provides an important clue in that hexagonal “fences” devoid of ice are clearly observed. This supramolecular skeleton, presumably composed of self-assembled **1**, provides a template for the unusual fracturing of the vitreous ice. We cannot exclude the possibility that the columnar assemblies actually formed during the freezing process, but such an intricate self-organization would have had to occur at low temperatures within a subsecond time frame.

A second possible mechanism for jointing involves “pore pressure”, a well-recognized source of geological fracturing.[16−18] Under certain environmental conditions, hydrostatic pressure is created when water has filled vesicles in volcanic rocks and intergrannular spaces in sedimentary rock. The pressure promotes faulting that would not occur in dry rock. Similarly, the gemini surfactant may exist as vesicles, micelles, or submicroscopic aggregates that serve as nuclei for hexagonal fracturing of the vitreous ice. Stress formation could arise, for example, if gemini vesicles are solvated by structured water that is unable to increase its density as rapidly as can the bulk vitreous ice during the plunge-freezing in the liquid ethane. This jointing mechanism is similar to its geological counterpart in that both depend on the presence of small foreign bodies to exert stress within the solidified fluid. The fact that other vesicle-forming compounds we have screened (e.g., phospholipids) failed to create columnar jointing speaks against the mechanism. Likewise, the observation of “fences” in photo 5 favors discrete surfactant self-assembly within the ice.

Cryo-HRSEM, a method that has not been extensively applied to colloidal suspensions, would seem to have a promising future in discovering as yet unimagined modes of self-assembly in water.

In the first line of his *One Hundred Years of Solitude*, Gabriel García Márquez writes, “Many years later, as he faced the firing squad, Colonel Aureliano Buendía was to remember that distant afternoon when his father took him to discover ice.” Apparently, there is still much to discover about ice and how it responds to colloidal additives.

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References

(1) Green, J.; Short, N. M. *Volcanic Landforms and Surface Features*; Springer: Berlin, 1971.
(2) Sengupta, S. *Evolution of Geological Structures in Micro- to Macro-scale*; Chapman & Hall: London, 1997.
(3) Ramsay, J. G.; Huber, M. I. *The Techniques of Modern Structural Geology, Vol. 2, Folds and Fractures*; Academic Press: London, 1987.
(4) Ollier, C. *Volcanoes*; Basil Blackwell: London, 1988.
(5) Apkarian, R. P.; Caran, K. L.; Robinson, K. A. *Microsc. Microanal.* **1999**, *5*, 197−207.
(6) Menger, F. M.; Keiper, J. S. *Angew. Chem., Int. Ed.* **2000**, *39*, 1906−1920.
(7) Camilleri, P.; Kremer, A.; Edwards, A. J.; Jennings, K. H.; Jenkins, O.; Marshall, I.; McGregor, C.; Neville, W.; Rice, S. Q.; Smith, R. J.; Wilkinson, M. J.; Kirby, A. J. *Chem Commun*. **2000**, 1253−1254.
(8) Benjelloun, M.; Van Der Voort, P.; Cool, P.; Collart, O.; Vansant, E. F. *Phys. Chem. Chem. Phys*. **2001**, *3*, 127−131.
(9) Menger, F. M.; Peresypkin, A. V. *J. Am. Chem. Soc*. **2001**, *123*, 5614−5615.
(10) Menger, F. M.; Caran, K. L. *J. Am. Chem. Soc.* **2000**, *122*, 11679−11691.
(11) Dubochet, J.; McDowall, A. W. *J. Microsc.* **1981**, *124*, RP3−RP4.
(12) Ryan, K. P.; Purse, D. H.; Robinson, S. G.; Wood, J. W. *J. Microsc.* **1987**, *145*, 89−96.
(13) Moor, H. *Cryotechniques in Biological Electron Microscopy*; Springer−Verlag: Berlin, 1987.
(14) An irregular polygonal pattern, obtained from a 0.5% aqueous solution of the 14-2-14 gemini surfactant, was interpreted as an “artifact”. See: Talmon, Y. In *Modern Characterization Methods of Surfactant Systems*; Binks, B. P., Ed.; Marcel Dekker: New York, 1999; pp 161−163. This work is not directly comparable to ours because it is a TEM image, with tiny two-dimensional cells, which was obtained with relatively slow cooling. It is possible, in any case, that the image does, in fact, portray an observable propensity of the compound to self-assemble.
(15) Liang, J.; Ma, Y.; Zheng, Y.; Davis, H. T.; Chang, H.-T.; Binder, D.; Abbas, S.; Hsu, F.-L. *Langmuir* **2001**, *17*, 6447−6454.
(16) Hobbs, B. E.; Means, W. D.; Williams, P. F. *An Outline of Structural Geology*; Wiley: New York, 1976.
(17) Timur, A.; Hempkins, W. B.; Weinbrandt, R. M. *J. Geophys. Res.* **1971**, *76*, 4932−4948.
(18) Davis, G. A. *Geol. Soc. Am. Bull*. **1965**, *76*, 463−468.

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