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Cryo-Ultramicrotomy of Complex Molecular Fluids

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Complex molecular fluids (CMFs, e.g., liquid crystals, crude oil, and detergents) have tremendous impacts on the modern world, but the detailed understanding of CMF behaviors at the molecular level is often surprisingly limited. This can be attributed to their complicated structure and the lack of effective nanoscale structural probes for CMFs in general. In this paper, we summarize our recent effort to apply cryo-ultramicrotomy to cryo-TEM studies of a representative and challenging CMF group − liquid crystals (LCs). Our results show that cryo-ultramicrotomy can be used as a reliable specimen preparation technique allowing subnanometer resolution direct imaging of CMFs in general. We also discuss on the advantages and disadvantages of the technique based on comparative studies using different techniques including thin film plunge freezing and replica techniques.

Liquid crystals (LCs) are mesophases with orientational order only (nematic mesophases) or both orientational and 1D (or 2D) positional orders (smectic mesophases) [1]. LC structures are very sensitive to surface and interface, making it difficult to preserve the native structure in electron beam transparent thin films. In addition, due to the very weak intermolecular interaction, LC materials normally show severe radiation damage under electron beam. As a result, a replica TEM technique, freeze fracture TEM (FFTEM), has been the dominating electron microscopy technique for LCs despite its relatively low resolution (a few nanometer).

We use cryo-ultramicrotomy in both thermotropic and lyotropic LCs. A thermotropic LC typically consists of single- or multiple-component complex molecules with phase transitions driven mainly by temperature; while a lyotropic LC is a solution of complex molecules in certain solvent (most often, water) and the amount of order is predominantly controlled by the solution concentration. We applied the so-called cryo-electron microscopy of vitreous section (CEMOVIS) technique to lyotropic LCs, i.e., high pressure freezing is employed to obtain vitrified "bulk" lyotropic LCs, followed by cryo-ultramicrotomy and cryo-TEM. While for thermotropic LCs, plunge freezing is used to quench the desired structure stabilized at a higher temperature. Two examples are shown here: lyotropic disodium cromoglycate (DSCG), known also as an anti-asthmatic drug (Fig. 1); and Au nanoparticle doped 5CB, a commonly used thermotropic LC (Fig. 2). We compare the "bulk" cryo-ultramicrotomy results with those obtain using "thin film" plunge-freezing [2].

Figure 1 shows comparative study of the DSCG solutions. The uniform contrast of the suspended 6.2% DSCG and the stripes with bright/dark contrast in 15% DSCG match the isotropic and uniaxial nematic structures, respectively. The dark stripes can be understood as the elongated chromonic aggregates formed by face-to-face packing of the DSCG molecules in water. In general, the cryo-ultramicrotomy "bulk" method yield similar results to the "thin film" plunge-freezing. But the bulk approach minimize the surface anchoring effect played by the LC/air and LC/carbon interfaces. We also present our results on the influence of cryoprotectant, which is often needed for low concentration solutions.

Figure 2 shows comparative study of Au nanoparticle doped 5CB. The nanoparticles of 1-3 nm in size seem to be distributed randomly in 5CB without agglomerations. However, Fig. 2a clearly shows that

the Au nanoparticles are strongly attracted by the carbon supporting film used in "thin film" plunge freezing and can decrease the concentration of Au nanoparticles in the suspended 5CB. In addition, we show that STEM Z-contrast imaging improves the visibility of the nanoparticles, which is especially useful for cryo-sectioned TEM specimens due to the damages from the sectioning [3].

References:

[1] PG De Gennes and J Prost, "The Physics of Liquid Crystals", (Oxford University Press, New York).
[2] M Gao *et al*, Microsc Res Tech **77** (2014), 754.
[3] The TEM-related experiments were carried out at the cryo-TEM lab of the Liquid Crystal Institute, Kent State University. The author thanks Dr. Oleg D. Lavrentovich, Dr. Chenming Xue, and Dr. Quan Li for providing the samples.

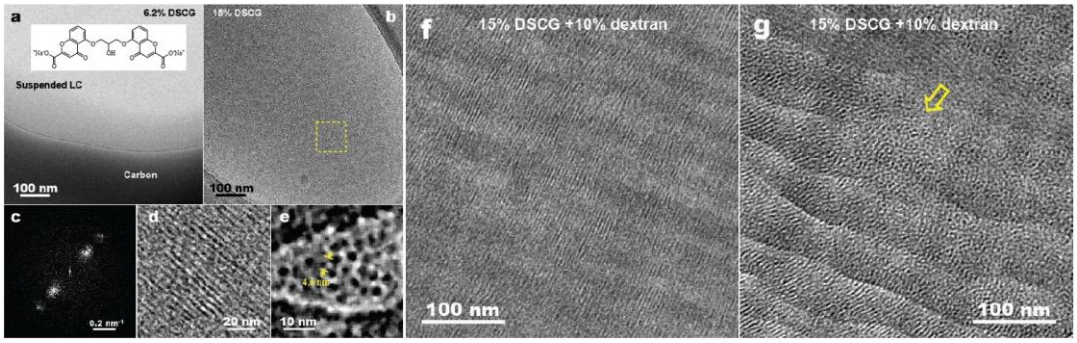

Figure 1. Comparative cryo-TEM study of DSCG lyotropic chromonic LCs. (a) – (e) Cryo-TEM results of DSCG solutions prepared by the "thin film" approach (plunge frozen specimens). (a) A typical image of 6.2% DSCG, showing uniform contrast corresponding to the isotropic phase. (b) A typical image of 15% DSCG. (c) Corresponding FFT pattern of the nematic structure shown in Fig. 1b. (d) A magnified image of the marked local area in Fig. 1a. (e) An image of the aggregates perpendicular to the thin film surface observed in 15% DSCG. (f) and (g) CEMOVIS images of nematic regions in 15% DSCG with 10% dextran. The hollow arrow in (g) points out a domain of aggregates perpendicular to the specimen surface.

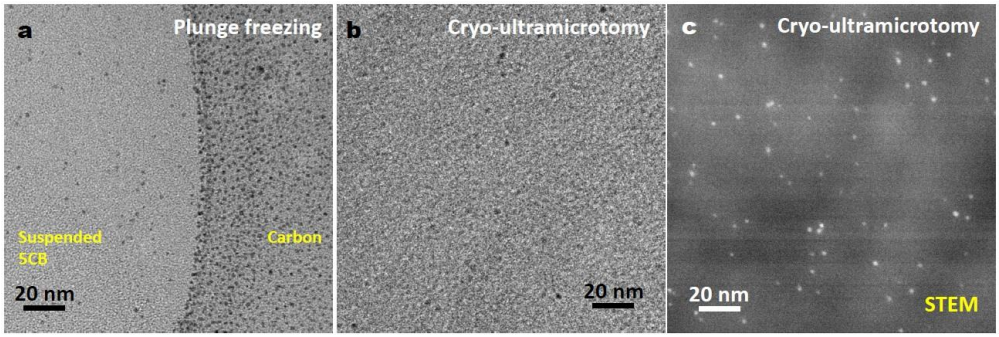

Figure 2. Comparative cryo-(S)TEM studies of Au nanoparticle doped 5CB prepared by "thin film" plunge-freezing (a) and cryo-ultramicrotomy (b and c).

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