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VITRIFICATION OF PURE WATER FOR ELECTRON MICROSCOPY

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Vitrified ice (Iv), may be obtained by slow deposition of vapour on a cold substrate or rapid freezing of concentrated solution of cryoprotectant. It was recently claimed that if the cooling rate is high enough, the vitreous state can be obtained from dilute solutions or even pure water (Brügeller & Mayer, 1980). We have devised a method for preparing vitrified ice or any frozen aqueous solution for direct observation in the electron microscope.

Triple distilled water is sprayed from a clean commercially available nebulizer (Satagraph, Sanitaria GmbH, 7140 Ludwigsburg, F.R.G.) pressurized by nitrogen gas at 2.2 atmospheres. This results in the formation of a jet of droplets, 50 % of them being smaller than 30 μm^3. A hydrophilic thin carbon film mounted on a standard specimen grid (200-400 mesh) is allowed to fall through the jet. The geometry is chosen so that the film receives about one droplet per grid square. After passing through the jet, the specimen descends into the cryogen. For typical experimental conditions, the speed of descent is 2 m/sec and the time between crossing the jet and immersion in the cryogen is 50 msec. After freezing, the specimen is transferred to liquid nitrogen, mounted in the standard cryo-specimen holder of the Philips 400 and rapidly introduced into the microscope. Observations are made in the EM at a temperature between 100-130 K or higher for devitrification and evaporation experiments. The temperature is read from a thermocouple situated close to the specimen and calibrated to $\pm 5^o$ by using the devitrification temperature of Iv (Dowell & Rinfret, 1960) and the evaporation rate of ice (Talmon & Thomas, 1977) as references.

When the specimen is cooled in liquid propane or ethane at 100 K, water layers up to 1 µm thickness are vitrified. This is the case for the droplet shown in the figure which has a maximum thickness of 3000 Å. The electron diffractogram of the circled region is shown in insert and is characteristic of Iv (Dowell & Rinfret, 1960). Vitrified droplets devitrify into Ic around 135 K. They evaporate at a rate of about 10 Å/sec at 173 K. These are values expected for pure water (Dowell & Rinfret, 1960; Talmon & Thomas, 1977). No residual material remains after evaporation of the droplets.

When nitrogen slush is used as a cryogen, thin layers of Ic (<1000 Å) are obtained, whereas after freezing in boiling nitrogen, only hexagonal ice has been observed. The jet-freezing method can be used without modifications and with similar results for any aqueous solutions or biological suspensions.

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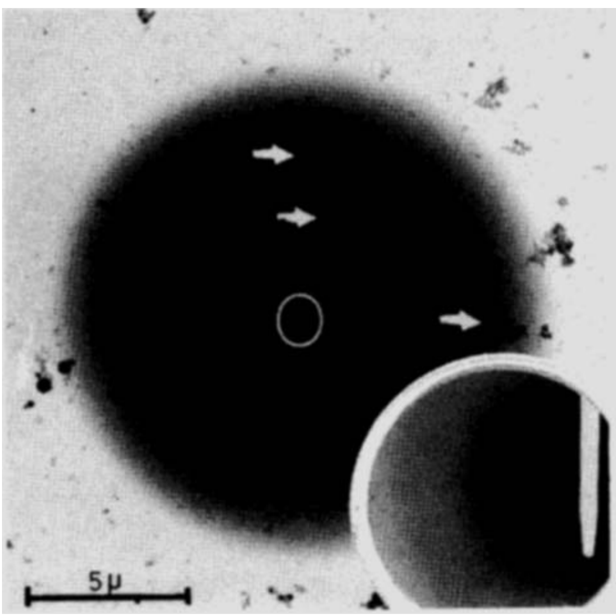

Vitreous droplet of pure water spread on a carbon film. Some crystals produced by condensation of atmospheric water vapour are marked (arrow). Magnification: 3900 x. Insert: electron diffractogram from the circled area. 1 cm = 0.2 $Å^{-1}$.