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Freezing and Sublimation Effects on Cryo-SEM Imaging and Microanalysis

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With high water contents, soft materials [1] and biological samples [2] are impossible to be studied by traditional electron microscopy without dehydration. However, dehydration inevitably introduces dramatic artifacts. By the aid of cryo-fixation, both morphological and compositional information can be obtained with high spatial resolution using cryo-SEM/EDS. Here, we explore the effects of freezing and sublimation on imaging and microanalysis of polyelectrolyte microgels in buffers with varying ionic strength ([NaCl]).

A homogeneous aqueous solution containing 0.01 M sodium phosphate was used to test the freezing effect. The specimens were 200 μm in thickness. They were frozen by either plunging in liquid nitrogen at 1 atm or high-pressure freezing using a Leica HPM-100 HPF [3-4]. In another set of experiments, the effect of sublimation was studied with a colloidal suspension of poly(acrylic acid) (PAA) microgels in sodium phosphate buffer. Cross-sectional specimens were created by cryo-fracture and imaged at -130 °C using a Zeiss Auriga Cross-Beam FIB-SEM equipped with a Leica VCT-100 cryo-transfer system. Some specimens were imaged and analyzed after sublimation at -95 °C for 0 min, 5 min, 10 min, and 15 min. Energy-dispersive spectroscopy (EDS) was performed using 5 keV incident electron via an Oxford Max-80 SDD EDS system.

Figure 1 shows cryo-SEM images of 0.01 M sodium phosphate buffer frozen using different methods. The wavelike morphology showed in Figure 1A stems from the water crystallization when plunged into liquid nitrogen. A compositional heterogeneity is created due to crystallization where the buffer is forced to separate into a solute-rich phase and a solute-depleted phase. In contrast, high-pressure freezing (Figure1B) preserves the homogeneity of the buffer by vitrifying the water. In the same buffer solution, the sublimation effect on topographic contrast is evaluated with PAA microgel suspension (Figure 2). Longer sublimation time leads to higher topographic contrast and reveals the microgel structure initially embedded in buffer. Another consequence of sublimation is the solute enrichment since water is removed. As showed in Figure 3A, when 0.5 M NaCl contained buffer experienced sublimation, more characteristic X-ray signals are detected for both Na and Cl. This indicates the enrichment effect of solutes during sublimation, which should be taken into consideration during microanalysis. Only specimens with the same sublimation can be compared for their compositional differences. With background subtraction and peak fitting (Figure 3B), the X-ray intensities of Na and Cl are linearly related to sublimation extent. This implies constant rates of sublimation and solute enrichment. Overall, the extent of sublimation largely influences the X-ray microanalysis. The extent of sublimation can be estimated by $\delta = nMvt/N_A$ and $P_L = (2.85 \times 10^{23})\,(MT)^{0.5} n$ [5], where v is the specific volume (1.06 $cm^3 \cdot g^{-1}$), t is the time of sublimation, N_A is 6.02 × 10^{23} molecules·mol^{-1}, P_L is the vapor pressure (Torr), M is the molecular weight (18 g·mol^{-1}), n is the sublimation rate (molecules·cm^{-2}·s^{-1}), and T is temperature (K) [6].

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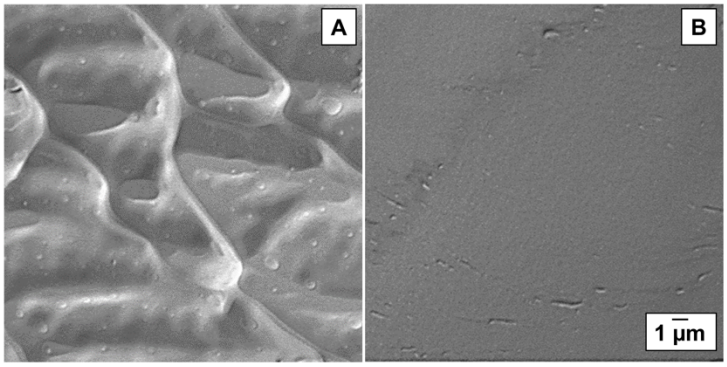

Figure 1. Cryo-SEM images of 0.01 M sodium phosphate buffer frozen by (A) liquid nitrogen plunge-freezing (B) high-pressure freezing.

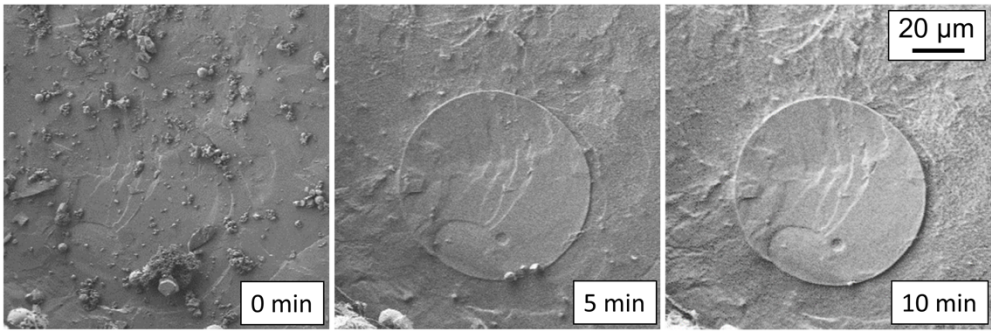

Figure 2. Cryo-SEM images of PAA microgel suspended in 0.01 M sodium phosphate buffer after sublimation at -95 ˚C for 0 min, 5 min and 10 min.

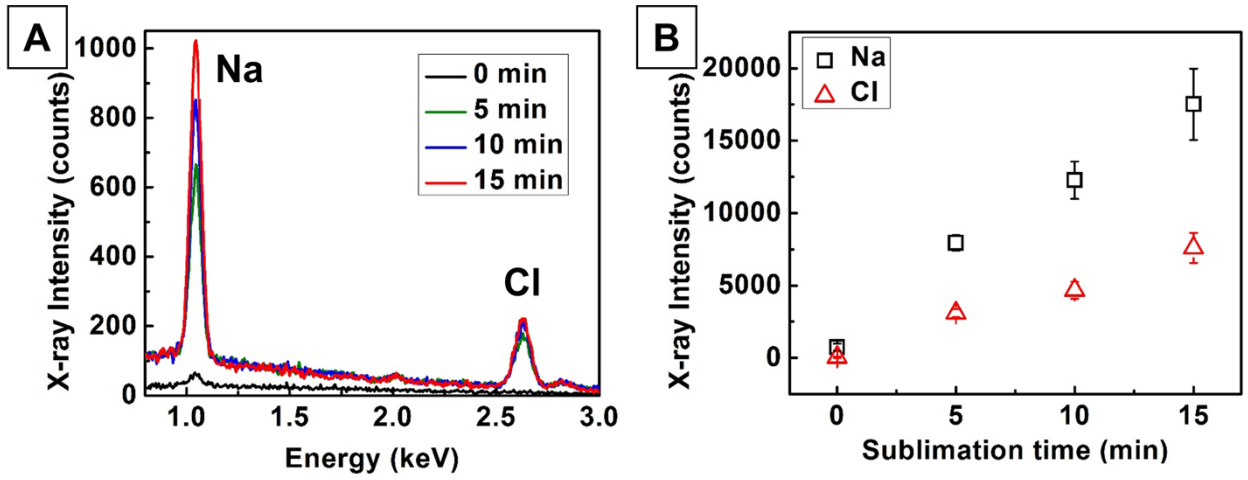

Figure 3. Na and Cl peak intensities of a 0.5 M NaCl buffer solution are extracted from typical x-ray spectra (A); replotted over sublimation time after digital filter and least-square fitting (B). The specimens are sublimed at -95 ˚C for 0 min, 5 min, 10 min and 15 min.

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