Comment on "Critical Behavior of a Binary Mixture of Protein and Salt Water"

Ishimoto and Tanaka[1] report a critical point in water:lysozyme:0.5*M*-NaCl. The physical nature of the separate phases (indeed, phase separation as opposed to conditions at which ". . . the mixture suddenly becomes opaque . . .") was not noted. Reference 1 is used as a model for eye cataracts.[2,3]

In a programmatic study[4] on probe diffusion in complex solvents, we reexamined this system. To obtain separated phases, warm lysozyme:0.5*M*-NaCl solutions were quenched in ice. The solutions became reversibly opaque, but only one phase was readily apparent. At 250 g/L lysozyme, the opaque system appeared solid; it could be cut with a chilled spatula. A consolute critical point cannot link a liquid to a solid phase, which forces reinterpretation of the opacity phenomenon.

Ishimoto and Tanaka[1] report slow growth of lysozyme crystals in the mixture. This phenomenon and the critical opacification were both observed; they are easily distinguished. Over several hours the slow growth creates a lawn of crystals on exposed surfaces; this growth is not reversed by heat. The critical opacification occurs swiftly (seconds to minutes) in bulk solution, and is immediately reversed by gentle heating.

The opacification was reexamined with an Ernst Leitz (Berlin) model KM microscope (384×) with temperature-control stage ($\pm 0.1\,°\mathrm{C}$). Lysozyme :0.5*M*-NaCl solutions on glass slides were quenched to temperatures T_q. For T_q below the cloud temperature T_{cl}, the solution became cloudy, filling with clusters of microscopic crystals. The microclusters are demonstrably solid. When the cover slip is prodded and fluid flow observed, clusters visibly translate and rotate as rigid assemblies. Microcrystals from quenched slides and from opaque bulk phases are identical. For $T_q > T_{\mathrm{cl}}$, the solutions remain clear, but microcrystals were seen in solution, especially near T_{cl}. Microcrystal growth is enhanced by slower cooling. Larger microcrystals scatter less light; by the cooling of a solution more slowly, the apparent cloud temperature can be reduced. This time-dependent effect could account for the differences between our data and Ref. 1.

Figure 1 shows (vertical bars) the highest temperatures at which each sample clouded. Solutions become *and remain* opaque at all protein concentrations, *not only on the critical isopleth,* unlike critical opalescence, which only occurs near the critical isopleth close to T_c.

The reversible opacity of lysozyme solutions, being due to scattering from crystals rather than from evanescent concentration fluctuations, is properly a cryoprecipitation effect, not a consolute critical point. The reported changes in solution viscosity, light-scattering intensity, and quasielastic scattering linewidth near the cloud curve might be due to microcrystals seen in solution above T_{cl} or to the formation of large oligomers as a precursor to cryoprecipitation. While the solid line of Fig. 1 could be interpreted as a metastable liquid-liquid phase separation underlying a stable solubility ("liquid-solid") curve, it would at least be unusual that our rapid quenching locates the stable transition, while the slower cooling (5–10 min) of Ref. 1 is able to reach an underlying metastable state.

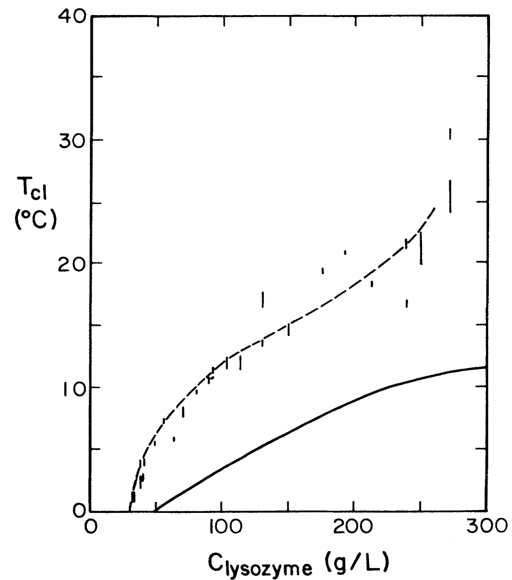

FIG. 1. Measured cloud temperatures (vertical bars) and inferred cloud curve (dashed line) for water:lysozyme :0.5*M*-NaCl. Solid line: coexistence curve of Ref. 1.

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