W0115

Protein / Polyethylene Glycol Phase Behavior: Implications for Protein Crystallization. D. Vivares, E.W. Kaler, A.M. Lenhoff, CMET, Dept. of Chemical Engineering, Univ. of Delaware, Newark, DE 19716.

Polyethylene glycol (PEG) favors the formation of protein crystals by decreasing the protein solubility, but it can also induce other phase changes, including liquid-liquid phase separation, protein aggregation, and the formation of gels, each of which may slow or accelerate the crystallization step. Here we report the phase behavior of mixtures composed of a large model protein, glucose isomerase (Mw = 173kDa), and PEGs of different molecular weights from 1.5kDa to 100kDa. The phase diagrams include the solubility curve, the liquid-liquid phase boundary and the aggregation curve. Only the position of the liquid-liquid boundary depends strongly on the PEG molecular weight. Within the liquid-liquid domain, we observed, by optical microscopy, heterogeneous crystal growth, starting with a few crystals and progressing from droplet to droplet, and also homogeneous crystal growth, which occurred independently and simultaneously in numerous droplets. Fluorescence labeling allowed confocal fluorescence microscopy observations of the partitioning of the protein and PEG between the liquid phases and showed the two steps in the crystallization process. The liquid-liquid phase separation appeared to accelerate the nucleation of protein crystals.